

EVALUATION OF ROOIBOS WASTE PLANT MATERIAL FOR THE DEVELOPMENT OF A HIGH-VALUE HERBAL TEA PRODUCT

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.

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ABSTRACT

Rooibos (*Aspalathus linearis*) is a South African fynbos plant species that is predominantly harvested for consumption as herbal tea. The demand for this herbal tea has grown significantly along with the industry over the years. However, declining production yields and export volumes, partially as a result of drought, are a threat to the industry. Rooibos processing generates a noteworthy volume of waste plant material in the form of fine dust and coarse stems. Therefore, the purpose of this study was to determine the sensory profiles of fermented rooibos waste plant material, separately and as blends, to gauge the feasibility of possibly reutilising rooibos waste for the production of rooibos products of acceptable quality for the herbal tea market.

Firstly, three commercial enzymes (Rapidase, Validase and Filtrase) were tested for their effectivity to increase the soluble solids content of rooibos dust extract. Enzyme-assisted extraction (EE) of rooibos dust resulted in a minor increase in the extract yield. Rapidase at the highest dose of 10% (1000x dosage recommended by supplier) resulted in the largest increase in extract yield (8.4%). EE of rooibos dust is therefore impractical. Hot water extraction (HWE) conditions were therefore optimised using response surface methodology. Preliminary “one-factor-at-a-time” experiments demonstrated that extraction time, extraction temperature and plant material-to-water ratio had significant effects ($P \leq 0.05$) on the extract yield. A central composite design was used to optimise these three variables, followed by identification of the optimal extraction conditions using desirability profiling, but taking cost-efficiency and practicality into consideration. Satisfactory predictive ability for the extract yield ($R^2_{adj} = 0.988$) was verified confirming suitability of the prediction model. Extract yields varied between 16.4% and 27.9% when the practically optimal extraction conditions (94 °C, 20 min and 1:20 plant material-to-water ratio (m.v⁻¹)) were applied to different batches (n=20) of rooibos dust.

Secondly, sensory attributes (aroma, flavour, taste and mouthfeel) associated with diluted dust extracts (at “cup-of-tea” strength) and stem infusions individually, as well as diluted dust extract and stem infusion combinations (50/50 and 75/25 ratios), were characterised using descriptive sensory analysis. Diluted dust extracts, as well as diluted dust extract and stem infusion combinations, produced infusions of similar sensory quality as normal rooibos infusions. In contrast, stem infusions produced weak infusions, indicating that the use of stem plant material alone would result in rooibos infusions with decreased quality. Additionally, unusual “planky/pencil shavings”, “raisin” and “almond” aroma attributes were perceived in the stem infusions. The “planky/pencil shavings” aroma note was perceived as non-typical and undesirable. This attribute was carried through into all dust extract and stem infusion combinations. A reduction of the stem plant material content did not adequately decrease the undesirable “planky/pencil shavings” aroma. If it is possible to eliminate the latter by

blending with good quality rooibos tea, reutilisation of the waste plant material could be feasible to address the shortages in the rooibos industry.

UITTREKSEL

Rooibos (*Aspalathus linearis*) is 'n Suid-Afrikaanse fynbos plantspesie wat grotendeels gebruik word as 'n kruie-tee, en aanvraag daarvoor het oor die jare aansienlik toegeneem tesame met 'n groeiende bedryf. Dalende opbrengste en uitvoer-volumes, gedeeltelik toegeskryf aan heersende droogte, bedreig egter die industrie. Verwerking van rooibos plantmateriaal vir tee produksie lewer 'n aansienlike hoeveelheid afvalmateriaal in die vorm van fyn stof en growwe stingels ("stok") op. Die doel van hierdie studie was om die sensoriese profiel van gefermenteerde rooibos afvalmateriaal, apart en as mengsels, te ondersoek om die vatbaarheid van hul moontlike hergebruik as rooibos produkte vir die kruie-tee mark te bepaal.

Eerstens is drie verskillende kommersiële ensieme (*Rapidase*, *Validase* en *Filtrase*) getoets vir hul effektiwiteit om die oplosbare vastestofinhoud van rooibos stof-ekstrak te verhoog. Ensiembehandeling het die ekstrakopbrengs van rooibos stof effe verhoog. *Rapidase* teen 'n dosering van 10% (1000× meer as die vervaardiger se aanbeveling) het die hoogste toename in opbrengs (8.4%) bewerkstellig. Ensiembehandeling van rooibos stof is dus onprakties. Gevolglik is warm water ekstraksie (WWE) van rooibos afvalmateriaal geoptimeer deur toepassing van respons-oppervlak metodiek.

Voorlopige enkel faktor eksperimente het getoon dat ekstraksietyd, -temperatuur en plantmateriaal-tot-water verhouding 'n beduidende effek ($P \leq 0.05$) op ekstrakopbrengs het. 'n Sentraal saamgestelde ontwerp is eers gebruik om die drie veranderlikes te optimiseer. Die optimale ekstraksie toestande is gevolglik deur middel van multi-respons optimisering geïdentifiseer, inaggenome praktiese oorwegings en koste-doeltreffendheid. Die goeie voorspellingsvermoë van die kwadratiese model vir ekstrakopbrengs is geverifieer ($R^2_{adj} = 0.988$), wat op die toepaslikheid van die model dui. Ekstrakopbrengste van verskillende lotte rooibos stof ($n = 20$) het gewissel tussen 16.4 en 27.9% toe die prakties optimale ekstraksie parameters (94 °C, 20 min en 1:20 plantmateriaal-tot-water verhouding, m/v) daarop toegepas is.

Tweedens is die geassosieerde sensoriese eienskappe (aroma, geur, smaak en mondgevoel) van verdunde stof-ekstrak (teen die sterkte van 'n koppie rooibostee) en stok-infusie, apart, sowel as mengsels van 50/50 en 75/25 verdunde stof-ekstrak/stok-infusie, bepaal m.b.v. beskrywende sensoriese analise. Die sensoriese profiele van die verdunde stof-ekstrak en stof-ekstrak/stok-infusie mengsels was soortgelyk aan normale gefermenteerde rooibostee. Daarenteen was stok-infusies flou, wat dui daarop dat die gebruik van slegs rooibos stok 'n flou tee van 'n laer kwaliteitsgraad sal lewer. Verder was buitengewone "plankagtige/potloodskaafsel", "rosyntjie" en "amandel" aromas teenwoordig in die stok-infusies. Die "plankagtige/potloodskaafsel" aroma is beskou as nie-tipies en onwenslik. Dit is ook waargeneem in beide die stof-ekstrak/stok-infusie mengsels. Verlaging van die hoeveelheid stok het die intensiteit van die onwenslike "plankagtige/potloodskaafsel" aroma voldoende verlaag nie. Indien

laasgenoemde geëlimineer kan word deur die byvoeging van goeie kwaliteit rooibostee, sou die hergebruik van rooibos afvalmateriaal moontlik vatbaar wees om sodoende tekorte in die rooibosbedryf aan te spreek.

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“If you want to go fast, go alone. If you want to go far, go together”

– African proverb

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 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 and conclusion. 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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I. General introduction

Rooibos (*Aspalathus linearis*), an indigenous fynbos shrub of South Africa, is largely cultivated for the production of rooibos tea which is well known and loved by consumers of all ages all over the world. The increasing demand for rooibos tea has led to the tremendous growth of the rooibos tea industry over the years (Joubert & De Beer, 2011). The unique traits of rooibos endowed it Geographical Indication (GI) protection which is only awarded to products in possession of qualities attributable to a particular place of origin (Anon., 2014b). Therefore the name “rooibos” and any other names related to it (e.g. “red bush”, “rooitee”, etc.) are protected from being used elsewhere and strictly belong to the South African rooibos industry, unless the product is originally from South African rooibos growing areas (Anon., 2014a). Granting the rooibos industry with GI status has led to economic growth and social development in rooibos production regions.

Rooibos is being exported in significant amounts to the global marketplace (Anon., 2014a) where it represents 10% of the global herbal tea market (Anon., 2015). Approximately 15 000 tons of rooibos are consumed globally and export volumes have reached up to more than 7000 tons per annum. Of the 6560 tons exported in 2015, the export market continues to be dominated by Germany (31%), the Netherlands (16%), Japan (15%), the United Kingdom (11%) and the United States of America (7%). The rooibos export market has grown to include other countries such as Poland (3%), Sri Lanka (2%), France (2%) and China (1%) (SARC, 2016). Therefore, if both the export and local volumes are sold and consumed as pure rooibos, this would equate to 6 billion cups of tea which is close to one cup per person globally (SARC, 2016).

While rooibos is enjoyed mainly as a hot beverage with a unique aroma, flavour and taste profile, it has an enduring reputation as a health-promoting beverage, which has also contributed largely to its rise of demand and consumption. Koch *et al.* (2012) and Jolley *et al.* (2017) described the characteristic sensory profile of rooibos as “honey”, “rooibos-woody”, “fynbos-floral” notes coupled with a slightly sweet taste and astringent mouthfeel. In addition to this primary aroma characteristic profile, “fruity-sweet”, “caramel”, “apricot” and “hay/dried grass” aromas were considered to be part of the secondary aroma characteristic profile of rooibos. Rooibos is highly valued for its caffeine-free and low tannin quality in conjunction with antioxidant activity (Joubert & De Beer, 2011).

Lately, ongoing droughts have contributed largely to the unpredictable tea production currently experienced by the rooibos tea market (E. Joubert, Agricultural Research Council, Stellenbosch, South Africa, 2016, personal communication). In addition, the production area suitable for rooibos production is shrinking due to climate changes (Lotter & le Maitre, 2014). These circumstances have thus resulted in a significant decrease in production yields and export volumes (SARC, 2016). As a result, the price stability and commitment of tea traders to the continuous promotion and marketing of the product is affected by this uncertainty in the market place. Moreover, the increasing production prices and shortages in supply have a negative impact on rooibos extract producers supplying the food ingredient and nutraceutical industries.

During rooibos production a significant amount of waste ($\pm 10\%$ per production batch), in the form of fine dust and coarse stems, is generated. Currently, most of the rooibos waste plant material is disposed in compost heaps (E. Joubert, Agricultural Research Council, Stellenbosch, South Africa, 2016, personal communication). Recently, however, rooibos wood chips have been used as an alternative to oak wood for the production of wooded Merlot wine by Audacia Wines (in Stellenbosch, South Africa) (De Wet, 2015). Conversion of this waste material into quality rooibos products provides an option to increase the existing annual production. Several options such as the production of rooibos extracts exist. However, rooibos stems are considered as poor quality due to their low extract yield (Joubert, 1984), making extract production very costly. Addition of a small percentage of stems to the leaf material of the product sold as herbal tea is the normal practice; however, a large percentage is associated with a poor quality product. Alternative uses for the stems are therefore required to convert this waste material into a product of good and acceptable sensory quality at “cup-of-tea” strength.

The second waste product, rooibos dust, is composed of mainly fine leaf material and therefore contains valuable extractable matter such as polyphenols, which are responsible for health-promoting properties and sensory characteristics of rooibos. To date the use of the rooibos dust in extraction has been limited as the overly fine plant material poses challenges during filtration. Moreover, its addition in teabags would be inappropriate as it would percolate through the teabag and produce hazy infusions (E. Joubert, Agricultural Research Council, Stellenbosch, South Africa, 2016, personal communication). As a result no research on its use as a source material for extract preparation has been conducted. In addition, no data exists on the rooibos dust extract yield and its properties, in particular its phenolic composition, colour and turbidity when reconstituted to “cup-of-tea” strength. However, extracts prepared from dust could be used either as food ingredient extract or to enhance the “tea value” of rooibos stems. Different options for the extraction of rooibos dust e.g. hot water extraction or enzyme-assisted extraction are possible. However, the application of enzymes to rooibos has not been studied extensively and limited information is available (Pengilly *et al.*, 2008; Coetzee *et al.*, 2014; Zwane, 2014). A number of studies have documented the extraction of rooibos solids and phenolic compounds (Joubert, 1984, 1988, 1990a, 1990b; Joubert & Hansmann, 1990; Von Gadow *et al.*, 1997; Jaganyi & Wheeler, 2003; Joubert & De Beer, 2012), however, a limited amount of published literature, is available on rooibos extraction optimisation (Miller *et al.*, 2017). The variation in overall quality is a noteworthy challenge involved in the use of plant material for the production of extracts, with some raw materials containing suboptimal levels of extractable compounds for commercialisation (Takeuchi *et al.*, 2009). Baseline data for soluble solids and phenolic content of hot water extracts prepared from fermented rooibos (Joubert & De Beer, 2012) and rooibos infusions (equivalent of ‘cup-of-tea’) (Joubert *et al.*, 2012) have been generated previously. This

data will serve as benchmark for comparison of extracts made from rooibos dust, and infusions of rooibos dust and stems individually and in combinations at “cup-of-tea” strength.

According to market research based on consumer acceptance of healthy products, flavour and taste are the most important deciding factors for the consumers to purchase such products (Olivo, 2015). However, no information is available on the flavour and taste of extracts (or infusions) prepared from rooibos stems and dust nor combinations thereof. Moreover, the colour, turbidity and phenolic composition of infusions made from individual rooibos waste products or combinations of rooibos dust and stems when reconstituted to “cup-of-tea” strength has not been established. Whether the sensory attributes of waste plant material will be similar or different to those of normal rooibos tea, when used individually or in combination, is unknown vital information. Therefore, profiling the rooibos waste plant material will provide valuable information regarding sensory attribute similarities and differences in comparison to the primary and secondary characteristic profiles of rooibos infusions. The continuous growth and market expansion of rooibos stresses the importance of guaranteeing that both the consumers and bulk purchasers of rooibos have a constant supply of rooibos and rooibos-derived products.

In view of the above, the main aim of this study was to evaluate the sensory characteristics of fermented rooibos waste plant material (dust and stems) individually and in combinations to ultimately assess the commercial viability of potentially reusing rooibos waste for the production of quality rooibos products. The first objective was to optimise extraction of soluble solids from rooibos dust. Commercial enzymes were evaluated, and thereafter response surface methodology was applied for the optimisation of the hot water extraction conditions. The second objective was to characterise the sensory attributes (aroma, flavour, taste and mouthfeel) associated with diluted dust extracts and stem infusions individually, and dust extract and stem infusion combinations at “cup-of-tea” strength.

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2. Literature review

2.1. Rooibos (*Aspalathus linearis*)

2.1.1. General overview

Rooibos (*Aspalathus linearis*) is an indigenous South African fynbos plant originating from the Cederberg area, including the Citrusdal, Clanwilliam and Nieuwoudtville regions of the Western and Northern Cape (Figure 2.1). The *Aspalathus* (Fabaceae, Tribe Crotalariaeae) genus is inclusive of an approximate minimum of 270 species which are habitually endemic to the Cape Peninsula region (Dahlgren, 1968). Of these, *Aspalathus linearis* and more specifically the red/Rocklands type, is presently being cultivated and harvested for the production of herbal tea on a commercial scale (Van Heerden *et al.*, 2003; Malgas *et al.*, 2010; Hawkins *et al.*, 2011; Kotina *et al.*, 2012). Other rooibos types such as red-brown, grey and black were cultivated and harvested in the past, but due to their inconsistent and substandard quality, their use was discontinued in 1966 (Joubert *et al.*, 2008). The name “rooibos” is an Afrikaans term for “red bush” used to describe and refer to the colour of the processed leaves, as well as the water infusions prepared from the dried leaves of the plant (Wilson, 2005). Its characteristic red-brown colour is a consequence of “fermentation” (fermentation is an oxidation process involving the phenolic fraction of the leaf) that the tea undergoes during production (Joubert & Schulz, 2006).

With more than 2000 different types of teas available in the tea market to date, product differentiation has proven to be the key to success in such industries. For this reason, it has been of essence that rooibos tea is clearly distinguishable from other teas and herbal infusions especially in terms of flavour and aroma (Koch, 2011). The fragrant traditional version of rooibos tea has gained global popularity over the years and is consumed by many, young and old. Moreover, it is popular due to its caffeine-free status and relatively low tannin levels in combination with health-promoting properties, specifically antioxidant activity (Joubert & De Beer, 2011). Most of the rooibos on the local market is available in teabag form instead of loose-leaf form, largely due to the fact that it is convenient and easy to dispose. Generally, a cup of rooibos tea is prepared by infusing ca. 2 g rooibos for 2-5 minutes in freshly boiled water (Joubert & De Beer, 2011).

Rooibos possesses unique traits which are closely related to its geographical location and has led to rooibos being a recipient of geographical indication (GI) certification. This is a formal recognition of the fact that rooibos occurs in the Fynbos biome of the Cape Floristic region which is one of 25 locations previously recognised as “diversity hot spots”. Thus, the function of GI certification is the protection of intellectual property to ascertain that rooibos cultivation does not occur outside South African borders, and that its given name is not exploited commercially (Biénabe *et al.*, 2009; Anon., 2014b). Rooibos spanning from the Western and Northern Cape provinces was shown to be no different in terms sensory characteristics, however, rooibos from different production years could be distinguished based on differences in perceived aroma attribute intensities (Jolley *et al.*, 2017).

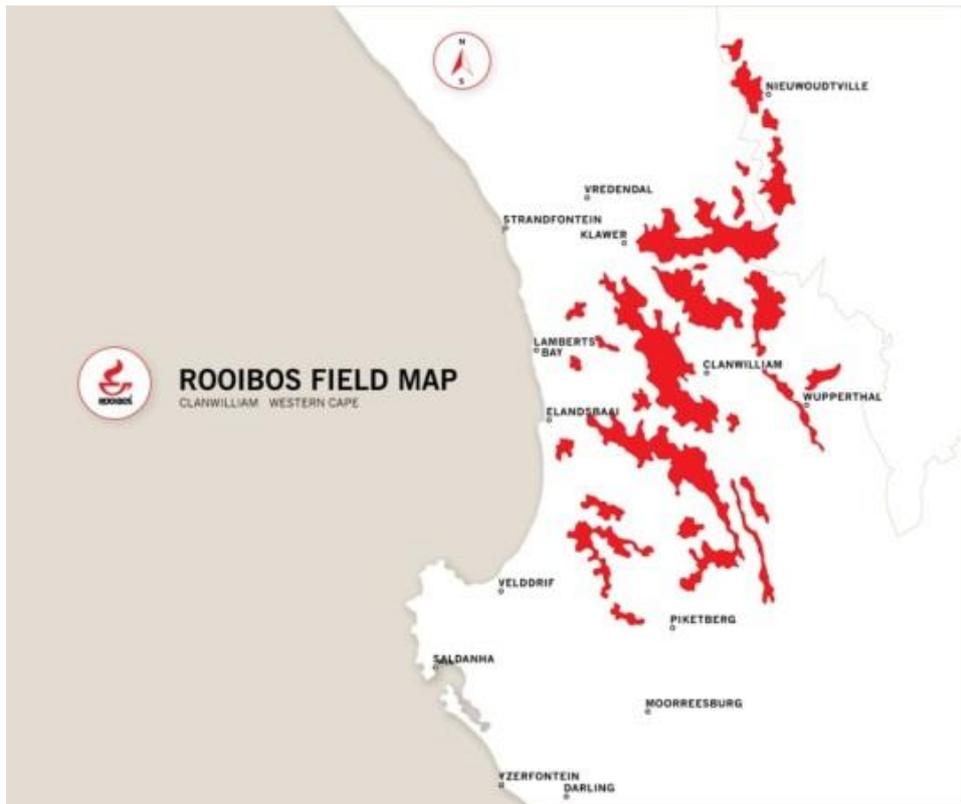


Figure 2.1 Distribution of *Aspalathus linearis* (Map supplied by the South African Rooibos Council, 2016).

The rooibos tea market is valued at approximately R550 million annually and is representative of 10% of the global herbal tea market and less than 0.3% of the global tea market (Anon., 2015a). In addition, rooibos tea mainly competes in the same segment as black tea and has an 18% market share of the domestic tea market. The rooibos industry has proven to be sustainable and will continue doing so, provided that consistent quality is maintained to meet the demand by consumers. It is estimated that the global consumption of rooibos reached 15 000 tons in 2015 (SARC, 2016). In the past ten years, production has varied between 10 000 and 18 000 tons a year (SARC, 2016; Figure 2.2). Lately, the rooibos tea market has experienced market inconsistencies largely due to persisting droughts which have resulted in a decrease in production yields and export volumes (E. Joubert, Agricultural Research Council, Stellenbosch, South Africa, 2016, personal communication).

Cycles of shortage in supply, along with high prices, followed by production expansion resulting in over-supply, accompanied by low to incredibly low prices are characteristic of the rooibos industry (Joubert & De Beer, 2011). Back in 2004, rooibos was sold for an average all time record of R16.00/kg, however it was followed by a continual decline to R4.50/kg in 2010, a farm gate value last received in 1999 (data supplied by SARC). As a result, the economic viability of the crop was affected negatively. Despite these challenges, rooibos tea continues to be exported globally with Germany, the Netherlands, the United Kingdom, Japan and the United States of America being the biggest importers of rooibos currently (SARC, 2016).

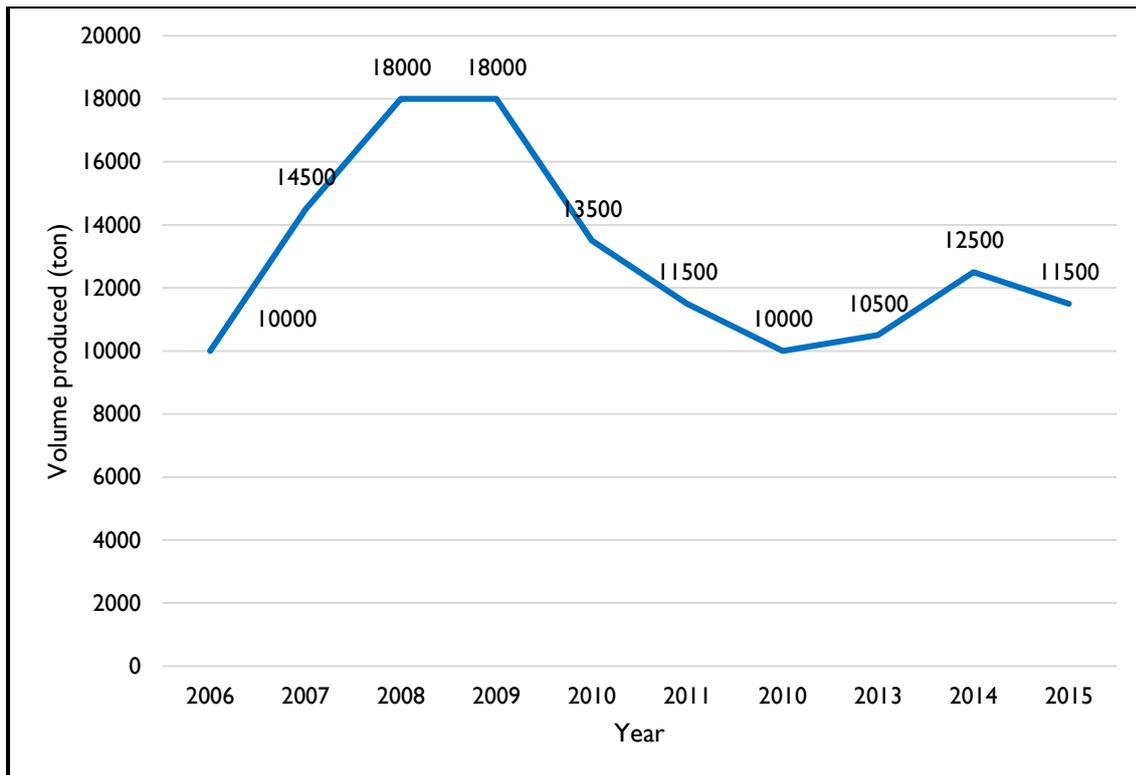


Figure 2.2 Roobos production in South Africa from 2006-2015 (South African Rooibos Council, 2016).

2.1.2. Rooibos cultivation, harvesting and processing

The commercial potential of rooibos as a herbal tea was first realised in 1904 by Benjamin Ginsberg, a merchant of Clanwilliam. He observed that the descendants of the Khoi were already processing the plant material during the hot summer months (Morton, 1983). Processing entailed chopping the plant material into small pieces with an axe, where after, cut leaves and stems were allowed to “sweat” in the hollows of stone reefs and were sun-dried (Joubert *et al.*, 2008). This process laid the foundation for the production process that is used to date.

In the Cederberg area of South Africa, approximately 350 to 550 farmers produce rooibos using seedlings (Anon., 2014a). About 8 000 – 10 000 plants are planted per hectare of land during the winter season and after about eight months, the plants are stimulated to branch by being trimmed. However, this young plant material does not produce good quality tea. Full production is reached in the following year. Before the harvest can be taken, eighteen months of growth are needed, and double the amount of time is needed to acquire a production that is fully feasible (Joubert & Schulz, 2006). The average dry yield per hectare of rooibos is approximately 300 kg (Anon., 2014a).

The harvesting of the rooibos plant takes place during the hot summer months and the beginning of autumn, usually from January until April (Cheyney & Scholz, 1963). No harvesting takes place during spring as the presence of flowers often results in a weak, mild tasting tea of lower quality

(Joubert *et al.*, 2008). During harvest, the bush is topped to about a height of 45 cm or just above the level of the previous harvest.

The processing of plant material at on-farm or central processing yards entails the following basic steps: Shredding into small pieces, placing the shredded plant material in “fermentation heaps”, bruising and wetting, mixing, “fermentation”, spreading a thin layer of the plant material on a flat surface for completion of fermentation and sun-drying. The characteristic red-brown colour of rooibos is a result of the “fermentation” (oxidation) process that contributes to development of the unique rooibos flavour and aroma (Joubert & De Beer, 2011). Bruising and wetting of the plant material assists with the release of polyphenols along with the development of colour to ensure a uniform product. Moreover, adequate aeration, by turning over the plant material several times, is necessary so that uniform oxidation can occur throughout the heaps of rooibos plant material. Insufficient aeration results in a product of low quality due to under-fermentation (Joubert, 1998). The fermentation process is conducted at a temperature range between 38°C and 42°C, usually overnight between 12 and 14 hours (Joubert & Schulz, 2006). However, factors such as bush age, young growth and the area of cultivation may have an effect on fermentation times such that they could vary from 8 to 24 hours (Joubert, 1994). After the fermentation period, the rooibos plant material heaps are spread out in the sun in thin layers to dry. Depending on the weather, drying may take up to 24 hours which may sometimes lead to tea of low quality. Drying usually commences immediately after fermentation due to the fact that fermentation continues when the plant material is still moist (Joubert, 1994). After drying, the fermented rooibos is sieved, graded and steam-pasteurised before it is mostly bulk packaged. Processed plant material is sold to secondary processors for further production and development of consumer products. Eight large processors currently dominate the secondary processing of rooibos; as a collective they are responsible for 90% of the market share (Biénabe *et al.*, 2009; Anon., 2014a).

In an attempt to address the problem of microbial contamination of rooibos tea which had resulted in significant losses to the industry in previous years, a steam-pasteurisation process was introduced in 1986 by the Rooibos Tea Board to “de-contaminate” the final product before packaging (Snyman, 2000). Since freshly harvested plant material from various batches is usually combined for the standardisation of product quality, the possibility of the introduction of contamination is increased. When rooibos is fermented, the ideal conditions for the growth of bacteria exist. Therefore, microbial contamination is unavoidable and steam pasteurisation at 99.5 °C for 2 min before packing has been suggested to achieve reduction of microbial load to tolerable levels (Du Plessis & Roos, 1986). However, steam pasteurisation at 96 °C for 60 s has been found adequate and is employed by South Africa’s largest rooibos processor (Koch *et al.*, 2013). After steam pasteurisation an extra drying step is needed to reduce the moisture content to 10% or less in accordance with the official South African regulations relating to rooibos quality standards (Anon., 2010). Therefore, processed rooibos tea is a

well-preserved product due to its low final moisture content which allows it to be considered as microbiologically safe under recommended storage conditions (Joubert & De Beer, 2011). Anecdotal evidence suggested that steam pasteurisation changed the aroma of a cup of rooibos tea, but it was only recently investigated. Koch *et al.* (2013) showed that steam pasteurisation of rooibos decreased the intensity of its aroma and flavour attributes. Additionally, steam pasteurisation resulted in a decrease in the soluble solids, total polyphenol, aspalathin contents and “total colour” of infusions.

2.1.3. Rooibos historical and modern medicinal use

Botanists, MacOwan and Marloth, who went on botanical missions to the Clanwilliam, Wupperthal, Gifberg and Cederberg areas between 1897 and 1901 did not mention rooibos (*A. linearis*) in their reviews of Cape medicinal products (Van Wyk & Gorelik, 2017). However, Watt & Breyer-Brandwijk (1932) first documented rooibos as a medicinal plant native to South Africa, but no specific medicinal uses were noted (Joubert *et al.*, 2008). Therefore, rooibos has been enjoyed for decades in South Africa as a herbal tea made into a strong brew with the addition of milk and sugar in the same manner Oriental tea is consumed. Its use saw the evolution from being used as a medicinal source to that of a non-medicinal source, i.e. herbal tea consumed for pleasure purposes, to the current day situation where “food as medicine” has increased the desire for the consumption of foods with medicinal properties, driven by the rise of health problems of an ageing population (Joubert *et al.*, 2008). Rooibos tea was first deemed to be healthy due to the absence of caffeine and its low tannin content (Cheney & Scholtz, 1963). However, a discovery made by Annetjie Theron in 1968 revealed that a rooibos infusion had the ability to cure chronic restlessness, vomiting and stomach cramps of her colicky infant. As a result, her discovery led to a larger interest and consumer base (Joubert *et al.*, 2008). Since then, babies have been fed rooibos either in their milk or as a weak brew. This led to rooibos being marketed under a label specifically for babies, named “Rooibos baby”. In addition to its conventional use as herbal tea, rooibos extracts were later developed for the production of a cosmetic product range under the trademark *Annique* (Morton, 1983). Topical applications of rooibos are believed to treat dermatological problems such as nappy rash, acne and eczema (Joubert *et al.*, 2008).

Rooibos remains marketed as a remedy for various ailments with anecdotal reports suggesting that rooibos acts as an effective reducer of nervous tension, heartburn and nausea, as an allergy treatment, digestive aid, appetite stimulant and even a sleep remedy due to a mild sedative effect (Van Wyk *et al.*, 1997; Joubert & De Beer, 2011; Street & Prinsloo, 2013). However, in recent years, the health-promoting properties of rooibos have been accredited to its phenolic content with benefits such as antioxidant, anticarcinogenic, antidiabetic, hepatoprotective, anti-inflammatory and hepatoprotective properties. Other benefits including alleged anti-ageing, antimicrobial, immunoprotective and antihemolytic properties have been studied thoroughly (Joubert & Ferreira,

1996; Joubert *et al.*, 2008; Joubert & De Beer, 2011; Muller *et al.*, 2016). Moreover, the potential of rooibos flavonoids and rooibos extracts to prevent or alleviate metabolic syndrome recently received substantial interest. It has been demonstrated that aspalathin has the ability to enhance the uptake of glucose *in vitro* and *in vivo* (Kawano *et al.*, 2009; Muller *et al.*, 2012; Son *et al.*, 2013), enhance insulin resistance (Mazibuko *et al.*, 2013; Mazibuko *et al.*, 2015), control oxidative stress (Uličná *et al.*, 2006; Kondo *et al.*, 2013; Hong *et al.*, 2014), reduce high glucose-induced inflammation (Ku *et al.*, 2015) and inhibit adipogenesis (Sanderson *et al.*, 2014). Aqueous extracts of fermented rooibos have also demonstrated a protective effect on cultured cardiomyocytes from diabetic rats (Dludla *et al.*, 2014; Dludla *et al.*, 2017), and significantly decreased the amount of serum cholesterol, triglycerides and free fatty acid concentrations in hyperlipidaemic mice (Beltrán-Debón *et al.*, 2011). A recent review by Miller *et al.* (2017) covered the optimisation of extraction conditions for maximising the aspalathin content of aqueous green rooibos extracts. According to Marnewick *et al.* (2011), the daily consumption of six cups of traditional rooibos has the potential to improve the lipid profile and redox status, which are both relevant to heart disease, in adults at risk for the development of cardiovascular disease. Therefore, extracts that have the ability to deliver the equivalent of six cups of rooibos have now become a “gold standard” in industry as they relate to the amount perceived as stimulating a quantifiable valuable health effect (Joubert & De Beer, 2012).

2.1.4. Rooibos extracts for food and nutraceutical applications

Rooibos extracts, produced locally and globally, are regarded as intermediate value-added products in the value chain (Joubert & De Beer, 2011). The development of an “instant” rooibos tea powder took place in 1980s (Joubert, 1984; 1988a) to provide the consumer with a more convenient form of rooibos as the brewing process of a cup of tea was a time-consuming process. However, it was only in 2000 that the commercial application of the concept of soluble rooibos products in South Africa received serious consideration with the production of powdered extracts for the beverage, food and dietary supplement markets (Anon., 2005a, b). The majority of rooibos extract manufactured on an annual basis is prepared from fermented rooibos and can be tailored to suit a number of applications (Joubert & De Beer, 2011). Green or unfermented rooibos, first produced on an experimental basis during the 1990s for the achievement of higher antioxidant levels (Von Gadow *et al.*, 1997), has since been commercialised as a herbal tea and for the preparation of extracts (Joubert & De Beer, 2012). Therefore, aspalathin-enriched extracts can also be prepared from green rooibos as this compound is present substantially higher levels in the unfermented plant material (Manley *et al.*, 2006; Schulz *et al.*, 2003; Joubert & De Beer, 2012). However, the extent of enrichment is highly dependent on the extraction conditions, the level of purification (Joubert & De Beer, 2012) and the ratio of leaf-to-stem material (Miller *et al.*, 2017). Therefore, the addition of fermented rooibos extract to food products

provides not only the characteristic flavour of rooibos, but the polyphenol content of rooibos, whether produced from the unfermented or fermented plant material that may potentially contribute toward overall health benefits of the product.

Many rooibos-derived products are currently available on the market. The herbal tea is available in various flavours (such as honey, lemon, blackcurrant and vanilla) and tea mixtures (e.g. honeybush, fennel and buchu) from a number of local brands. Moreover, a range of ready-to-drink rooibos iced teas with different flavours has been introduced into the iced tea market (Food and Beverage Reporter, 2006) as a result of iced tea becoming a highly popular beverage. A market for green unfermented rooibos, although still small, has also developed locally and globally due to its higher antioxidant activity and associated health benefits (Food and Beverage Reporter, 2004). In a bid to create a completely new and innovative product, a rooibos espresso called Red Espresso® was developed by refining the rooibos into an espresso grind similar to that of coffee. This product created a new beverage category for itself as it was the first tea espresso ever to be produced and is now also available in various flavours (Food and Beverage Reporter, 2007). Moreover, the rooibos industry has recently seen the use of rooibos extracts as an ingredient in Albany's Ultima "Rooibos and Rye" whole-wheat bread variant which is promoted for its high antioxidant content and is marketed as a health-promoting product (Anon., 2015b). Unfermented rooibos plant material has also been utilised in the production process of the range of sulphite-free wines (Anon., 2015c) and an innovative niche craft beer known as Stellenbrau Governor's Red (Anon., 2015c). Other rooibos derived products include slimming products and cosmetics, dietary supplements, instant rooibos cappuccino, rooibos-flavoured yogurts and breakfast cereals (Biénabe *et al.*, 2009; Wynberg *et al.*, 2009; Joubert & De Beer, 2011; Joubert & De Beer, 2014).

2.2. Rooibos quality

2.2.1. Rooibos quality grading and development of quality control tools

In order to achieve effective product standardisation and commercialisation through control improvement, which translates to customer satisfaction, grading systems are put in place. The development of such systems entails the identification, definition and measurement of quality parameters. However, the effectiveness of the measurement of parameters is highly dependent on measurement simplicity, time, scientific validation and correlation to how consumers would perceive product quality (Feria-Morales, 2002). These principles are applicable to many products including rooibos.

The quality grading of rooibos has encountered many changes for its improvement over the years (Joubert, 1994). During the early years of rooibos production, the first attempt at quality grading entailed subjective grading of rooibos tea into six grades based on the aroma, cut and colour of dried

rooibos leaves and stems. A mechanical sieving system was introduced in 1965 to classify rooibos according to cut length, in addition to the aroma and colour of dried rooibos leaves and stems. It was only in 1985 when the evaluation of rooibos tea infusions in terms of taste, aroma and colour was considered, which resulted in additional quality grades, i.e. “Super”, “Choice” and “Standard” (Joubert, 1994). Minor changes were made over the years and in 1992, “Selected” was added as one of the grades. Thereafter, three rooibos tea categories (A, B, C) were introduced to group the tea according to strong, medium and poor typical taste and aroma characteristics. Moreover, coarse and fine tea particles were separated according to their size of cut for utilisation in tea bags or loose tea packaging.

With the abolishment of the one channel marketing system, and thus the Rooibos Tea Control Board, rooibos tea processing companies make use of their own sensory evaluation procedures and standards in order to grade rooibos. According to Koch (2011), the most structured evaluation system used in industry to date (employed by Rooibos Ltd., South Africa) includes mechanical sieving of plant material received from producers with grading being conducted by experienced tasters thereafter. The appearance of the dry and wet tea leaves is assessed followed by the appearance (colour and brightness) and flavour (aroma, taste and mouthfeel) of infusions prepared from the tea leaves. Poor quality and processing practices can be indicated by the appearance of the dry and wet leaves because over-fermentation may result in dull-brown coloured leaves and bland infusions with a woody aroma. Ideal rooibos infusions, made from tea of high quality, are clear and possess a brick-red-brown colour with an orange yellow tint at the cup’s rim where under-fermented infusions, which are of low quality, are often orange-yellow in colour (Koch, 2011). Infusions made from over-fermented plant material are brown and turbid and they may have a negative effect on the visual quality of infusions (Joubert, 1994). Moreover, the use of tristimulus colour measurements for potentially predicting rooibos quality was explored by Joubert (1995). The red colour (a^* value) of rooibos infusions was shown to play a vital role in the visual grading of rooibos quality, i.e. infusions with higher colour grading possessed higher a^* values (according to CIELAB).

The flavour of rooibos can be considered the most important quality element since it ultimately has an effect on whether the product is liked by the consumer. The aroma of a rooibos infusion is just as important and must not contain any foreign notes (old honey, rotting plant water, seaweed, musty/mouldy, medicinal or dusty), and furthermore green notes must be absent. The intensity of the “characteristic”, honey-like, sweet aroma also determines the grade given to the aroma of rooibos tea. Therefore, tea of high quality is supposed to possess a full-bodied, strong, sweet, “characteristic” taste and possess no bitter, musty, sour, salty or foreign notes. However, a slightly grassy flavour is acceptable for certain grades. Infusion aroma, flavor, taste and mouthfeel therefore have the greatest impact on grades given to rooibos tea (Koch, 2011).

Currently, no specific guidelines within legislation exist which state the manner in which rooibos tea quality should be regulated. The only regulation pertaining to rooibos quality standards

states that “All rooibos shall have the clean, characteristic taste and aroma and clear, distinctive colour of rooibos” (Anon., 2002). However, South African consumers may be more accustomed to the term “characteristic” aroma and flavour as they are more familiar with rooibos tea than foreign consumers are. In an attempt to address rooibos grading inconsistencies, a generic sensory wheel and lexicon were developed for industry by Koch *et al.* (2012). However, the latter sensory wheel and lexicon were developed based on data gathered from only one production season (2009) and one production area (Western Cape). As a result, a follow-up study was undertaken to validate both the sensory wheel and lexicon by using a larger data set. By including data from a number of production areas, grades and years, all possible variations were covered. From the data it was evident that rooibos infusions possess a primary (“rooibos-woody”, “fynbos-floral” and “honey”) and secondary (“fruity-sweet”, “caramel” and “apricot”) characteristic aroma profile. Moreover, the “hay/dried grass” aroma note, although perceived at low intensities, was present in 99% of the rooibos samples, which made it to be considered a part of the “characteristic” aroma profile of rooibos. The production area did not affect the sensory profile of rooibos tea, but the production year played a role – production years were distinguishable based on differences in perceived aroma attribute intensities (Jolley *et al.*, 2017). Further discussion of the rooibos sensory lexicon and wheel is provided in sections 2.4.3 and 2.4.4.

2.2.2. Quality control and regulation

Long-term market growth is achieved through the production of consistent quality products and effective quality control procedures. The export regulations for rooibos tea only state that all rooibos should have a “clean, characteristic taste and aroma and clear, distinctive colour of rooibos” and “may contain no more than 10% white sticks” (Anon., 2002). No evaluation of the colour and flavor of rooibos is undertaken (Snyman, 2000). Furthermore, there are no reference standards or definitions provided for the terms “distinctive” and “characteristic”. Rooibos processors are therefore free to set their own flavour, colour and mouthfeel quality standards of rooibos infusions. All other specifications are in line with food safety standards. Pesticide residues, moisture content and microbial contamination are limited according to set levels and are included in regulatory control procedures.

The quality standard of rooibos intended for sale outside South Africa is regulated by the Perishable Products Export Control Board (PPECB), however, it does not evaluate the colour and the flavour of the end product (Snyman, 2000). Specifications regarding the polyphenol content, antioxidant capacity or composition of rooibos tea also do not exist. Extract manufacturers, on the other hand, set minimum levels for the total polyphenol content and antioxidant capacity of standardised extracts (Joubert and De Beer, 2011).

2.2.3. Processing and effect on rooibos quality

Variation and quality of the composition of plant tissue is a result of numerous factors such as seasonal effects, climate, seedling genetic make-up, drought, light intensity and plant distribution (Aherne & O'Brien, 2002). Many studies have been done on *Camellia sinensis* teas, in particular black tea, to understand factors contributing to its quality. Growing environment, variety, manufacturing conditions, particle size, age of tea leaves and season were found to have an effect on the tea leaf composition and thus the tea quality (Astill *et al.*, 2001; Lin *et al.*, 2003; Yao *et al.*, 2005). Studies trying to establish a link between rooibos sensory attributes and composition, production area and harvest year are limited to those of Koch *et al.* (2013) and Jolley *et al.* (2017).

A major contributor to variation in quality, however, remains the traditional open-air processing method that is still employed to date as limited control over processing parameters is possible. As a result, no two rooibos batches are identical in terms of aroma, flavor, taste and mouthfeel, and this makes grading, product differentiation and defining the term “characteristic” a challenging endeavor. The experience of the producer plays an important role in guiding processing variables such as fermentation and drying times. The fermentation period depends not only on the composition of the plant material, but also external factors such as ambient temperature and air movement. Ideally, fermentation should be terminated when a sweet aroma has developed. When processed under controlled conditions the best sensory quality is achieved between 10 to 14 hours of fermentation and at temperatures between 38°C and 42°C (Joubert & De Villiers, 1997). Over-or-under fermentation has a large effect on the quality of the final product, and it is usually a result of a farmer's inexperience, bad weather or low night temperatures. Furthermore, it was found that drying at higher temperatures was detrimental to the aroma of rooibos (Joubert, 1994). Therefore, it could be expected that ambient temperatures during open-air drying of rooibos tea could also affect aroma and thus quality. A factory-based fermentation and drying process, however, would not be feasible due to the capacity of the processing needed, along with the energy requirements for the tea to be dried (Joubert & De Beer, 2011).

2.3. **Rooibos chemical composition**

2.3.1. Phenolic composition

Rooibos is comprised of a number of compounds that are responsible for the characteristic colour, flavour, aroma and functional properties of the popular herbal tea. A number of researchers have studied its chemical composition, as well as the changes that occur in the chemical profile during fermentation, extensively. Rooibos is considered a low tannin beverage despite dimeric, trimeric and

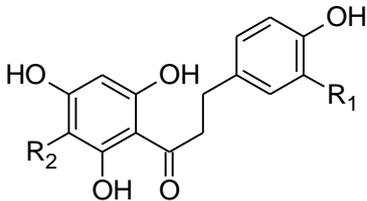
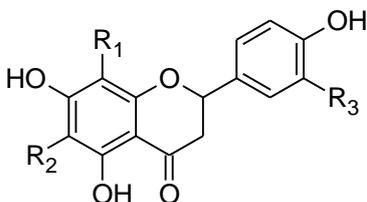
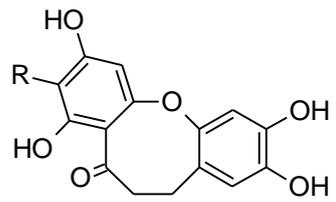
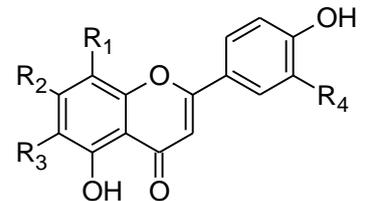
pentameric proanthocyanidin compounds being identified in the past. However, limited information is available about the structure of the tannins in rooibos tea (Joubert & De Beer, 2011).

The most significant compounds in fermented rooibos tea that have a substantial effect on the organoleptic properties of the tea are the phenolic components especially the flavonoids and their oxidised polymeric products that are formed during fermentation (Joubert, 1994). The unique status of rooibos tea is attributed to the presence of two phenolic compounds known as aspalathin, a C-C dihydrochalcone glucoside (Koeppen & Roux, 1965), and the cyclic dihydrochalcone aspalalinin (Shimamura *et al.*, 2006). Another rare compound, nothofagin which is a 3-dehydroxy dihydrochalcone glucoside is also found in rooibos which was previously only identified in two other species *Nothofagus fusca* (Hillis & Inoue, 1967) and *Schoepfia chinensis* (Huang *et al.*, 2008). Other major phenolic compounds found in rooibos include flavones (orientin, isoorientin, vitexin isovitexin, luteolin, chrysoeriol), flavanones (dihydro-orientin, dihydro-isoorientin, hemiphlorin) and flavonols (quercetin, hyperoside, isoquercitrin, rutin) (Ferreira *et al.*, 1995; Koeppen *et al.*, 1962; Marais *et al.*, 2000; Rabe *et al.*, 1994; Shimamura *et al.*, 2006) (Table 2.1). Other compounds that have been identified include phenolic acids, lignans, flavone diglycosides, (+)-catechin, a phenylpyruvic acid glycoside, the flavonol quercetin-3-O-robinobioside and the coumarins, esculetin and esculin (Beltrán- Debón *et al.*, 2011; Breiter *et al.*, 2011; Krafczyk and Glomb, 2008; Marais *et al.*, 1996; Shimamura *et al.*, 2006). Joubert *et al.* (2012) gave the first report consisting of representative quantitative data of detectable monomeric phenolic compounds in rooibos infusions at “cup-of-tea” strength. Aspalathin, orientin, isoorientin and quercetin-3-O-robinobioside (flavonoids) as well as Z-2-(β-D-glucopyranosyloxy)-3-phenylpropenoic acid (PPAG), a phenylpropenoic acid (present at > 5 mg/L), were present at the highest concentrations. Vitexin, isovitexin and hyperoside (quercetin-3-O-galactoside) were other compounds that were detected at levels > 2 mg/L. Nothofagin, isoquercitrin (quercetin-3-O-glucoside), rutin (quercetin-3-O-rutinoside) and ferulic acid were present at > 0.9 mg/L. More recently, three compounds previously reported in *Cyclopia* spp (honeybush), namely the dihydrochalcone phloretin-3',5'-di-C-β-D-glucopyranoside, the flavanone hesperidin and the flavone scolymoside were by identified Walters *et al.* (2017b) for the first time in rooibos extracts. According to Joubert (1996), the amount of aspalathin and nothofagin present in rooibos tea is usually dependent on the degree of oxidation of the plant material. In addition to the health benefits that are linked to the phenolic content of rooibos tea (Joubert *et al.*, 2008), the presence of phenolic compounds is vital for the taste and mouthfeel attributes of rooibos (Joubert *et al.*, 2013; Koch *et al.*, 2013). The “sweet” taste of rooibos infusions has been found to be associated with PPAG (Koch *et al.*, 2012), yet when tested as a pure compound it was perceived as “bitter”, suggesting that taste modulation occurred when it was present in the infusion (Joubert *et al.*, 2013). Moreover, the “bitter” taste has also been previously associated with rutin and isoquercitrin when tested in water (Scharbert *et al.*, 2004).

It was demonstrated by Koeppen and Roux (1965) that during the fermentation of rooibos plant material for the production of the herbal traditional tea (oxidised form), aspalathin is converted to dihydro-iso-orientin and dihydro-orientin. This oxidation phenomenon was confirmed by Marais *et al.* (2000) in the presence of heat (30°C) and light. Fermentation, however, results in the alteration of the phenolic composition of rooibos, further resulting in a decrease in the average total polyphenol and soluble solids content (Schulz *et al.*, 2003). A further investigation of the oxidation of rooibos was conducted by Krafczyk and Glomb (2008) who demonstrated the conversion mechanisms of aspalathin into dihydro-iso-orientin and dihydro-orientin. Furthermore, Krafczyk *et al.* (2009) identified aspalathin, amongst other compounds, to be an important compound that is partly responsible for the browning that occurs during oxidation. Enzymes, however, were considered to be responsible for the initiation of colour change during oxidation. It was suggested by Joubert & De Villiers (1997) that the bruising of rooibos leaves that results in the rapid formation of the characteristic red-brown colour of fermented rooibos was in fact enzyme-mediated oxidation. This suggestion was further supported by the fact that the treatment of green, unfermented rooibos with steam inactivates enzymes, thereby retaining its green colour. However, Krafczyk *et al.* (2009) concluded that the browning reactions occurring during oxidation were non-enzymatic.

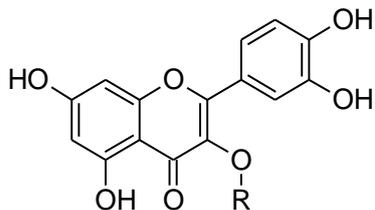
Various high-performance liquid-chromatography methods have been developed and used for the quantification of rooibos phenolic compounds, requiring run times ranging from 16 to 125 min per sample. Recently, an improved method which targets rooibos phenolic compound changes due to fermentation was developed by Walters *et al.* (2017a). This method aimed to further quantify compounds such as eriodictyolglucopyranoside isomers in a reasonable time. These compounds have not been quantified in rooibos plant material to date due to separation challenges.

Table 2.1 Major phenolic compounds identified in fermented *A. linearis* plant material (as reviewed by Joubert *et al.*, 2008)

General structure	Compound type, names and substituents
	<p>Dihydrochalcones</p> <p>Aspalathin: $R_1 = \text{OH}$, $R_2 = \beta\text{-D-glucopyranosyl}$ Nothofagin: $R_1 = \text{H}$, $R_2 = \beta\text{-D-glucopyranosyl}$</p>
	<p>Flavanones</p> <p>Hemiphlorin: $R_1 = \beta\text{-D-glucopyranosyl}$, $R_2 = R_3 = \text{H}$ (<i>R</i>)/(<i>S</i>)-eriodictyol-8-<i>C</i>-glucoside: $R_1 = \beta\text{-D-glucopyranosyl}$, $R_2 = \text{H}$, $R_3 = \text{OH}$ (<i>R</i>)/(<i>S</i>)-eriodictyol-6-<i>C</i>-glucoside: $R_1 = \text{H}$, $R_2 = \beta\text{-D-glucopyranosyl}$, $R_3 = \text{OH}$</p>
	<p>Cyclic dihydrochalcone</p> <p>Aspalalinin: $R = \beta\text{-D-glucopyranosyl}$</p>
	<p>Flavones</p> <p>Orientin: $R_1 = \beta\text{-D-glucopyranosyl}$, $R_2 = R_4 = \text{OH}$, $R_3 = \text{H}$ Iso-orientin: $R_1 = \text{H}$, $R_2 = R_4 = \text{OH}$, $R_3 = \beta\text{-D-glucopyranosyl}$ Vitexin: $R_1 = \beta\text{-D-glucopyranosyl}$, $R_2 = \text{OH}$, $R_3 = R_4 = \text{H}$ Isovitexin: $R_1 = R_4 = \text{H}$, $R_2 = \text{OH}$, $R_3 = \beta\text{-D-glucopyranosyl}$ Luteolin: $R_1 = R_3 = \text{H}$, $R_2 = R_4 = \text{OH}$ Luteolin-7-<i>O</i>-glucoside: $R_1 = R_3 = \text{H}$, $R_2 = \beta\text{-D-glucopyranosyloxy}$, $R_4 = \text{OH}$ Chrysoeriol: $R_1 = R_3 = \text{H}$, $R_2 = \text{OH}$, $R_4 = \text{OCH}_3$</p>

Flavonols

Quercetin: R = H

Isoquercitrin: R = β -D-glucopyranosyloxyHyperoside: R = β -D-pyranosyloxyRutin : R = α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranosyloxyQuercetin-3-O-robinoside: R = α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-galactopyranosyloxy**2.3.2. Non phenolic composition**

Although rooibos health benefits are mainly linked to its phenolic content, numerous non-phenolic compounds have been identified and investigated in a review by Joubert *et al.* (2008). No caffeine has been documented in rooibos, and rooibos is thus renowned for its caffeine-free status. However, the related alkaloid sparteine has been reported in rooibos by Van Wyk & Verdoorn (1989). The mineral content of fermented rooibos plant material and fermented rooibos infusions has been investigated by Touyz and Smit (1982), Mokgalaka *et al.* (2004) and Joubert *et al.* (2008). It was found that the highest mineral concentrations were obtained for sodium and potassium in both the plant material and infusions, followed by magnesium, calcium and phosphorus, whereas only the infusions contained traces of iron. However, according to data reported by Morton (1983), a cup of tea has been found to contain iron, calcium, magnesium, phosphate and potassium.

Volatile compounds, made up of ketones, aldehydes, alcohols, esters, hydrocarbons, phenols and ethers, contribute to the characteristic aroma and flavour of rooibos. Examples of volatile constituents which have been identified include breakdown products of β -carotene, i.e. β -damascenone and β -ionone (Habu *et al.*, 1985; Kawakami *et al.*, 1993), which are present in relatively high concentrations and have prompted further investigation by Sefton *et al.* (2011). Moreover, other major fermented rooibos volatile compounds that have been found include guaicol, dihydroactinidiolide, 5,6-epoxy- β -ionone, 6-methyl-3,5-heptadien-2-one, β -phenylethyl alcohol, benzaldehyde, 2-phenylethanol, geranylacetone and 6-methyl-5-hepten-2-one (Habu *et al.*, 1985, Kawakami *et al.*, 1993). The aroma profile of these compounds can be found in Table 2.2. Compounds such as cis-3-hexenal and trans-3-hexenal, which are associated with the green/grassy aroma, were also present in the rooibos volatile fraction (Koch, 2011). When analysed individually, none of these compounds incorporate the full “characteristic” aroma of rooibos tea. A combination of volatiles is usually used to explain the aroma of foodstuffs (Chambers & Koppel, 2013).

Table 2.2 The aroma profiles of chemical compounds found in rooibos infusions

Chemical compound	Aroma
Guaicol	Woody Smokey ^a
B-damascenone	Floral, violet ^b
Dihydroactinidiolide	Sweet, tea-like odor ^a
B-ionone	Rose-like ^a
5,6-epoxy-ionone	Fruity, floral ^a
6-methyl-3,5-heptadien-2-one	Spicy ^b
β-phenylethyl alcohol	Floral, rose/dried rose ^b
Benzaldehyde	Almond ^a

^aHabu *et al.*, 1985; ^b Kawakami *et al.*, 1993

2.3.3. Factors affecting phenolic composition of rooibos products

All plant material has a large phenolic content variation due to genetic variation, however, numerous other factors such as climate, seasonal effects, diurnal cycles, development stage of shoots and post-harvest processing methods also have a significant effect on the phenolic content variation (Aherne & O'Brien, 2002; Yao *et al.*, 2005; Joubert *et al.*, 2008). Therefore, the same applies to cultivated rooibos and this has been confirmed through a number of studies which made use of large sample sets (Joubert & Schulz, 2006; Manley *et al.*, 2006; Joubert & De Beer, 2011; Joubert *et al.*, 2012; Joubert *et al.*, 2013). Seasonal variation was demonstrated in a study conducted by Yao *et al.* (2005) where Australian-grown *Camellia sinensis* with fresh shoots harvested in warmer months contained significantly higher levels of antioxidant flavonoids than those harvested during cooler months ($P \leq 0.05$). The effect of season was also shown by the variation in aspalathin and nothofagin content of rooibos plant material harvested in summer, winter and mid-spring (De Beer *et al.*, 2017). In another study where the phenolic content and antioxidant activity of hot water infusions of batches of fermented rooibos ($n = 114$) from different production seasons (2009, 2010 and 2011) was investigated, significant variation in the individual content values of phenolic compounds ($P \leq 0.05$) was reported (Joubert *et al.*, 2012). Moreover, a large sample set ($n = 209$) of fermented, unpasteurised rooibos collected from two major production areas (Northern and Western Cape Provinces, South Africa) over the production period of 2011 to 2013 was analysed by Joubert *et al.*, 2016. A significant production area x production year interaction ($P \leq 0.05$) was seen for the flavonol subclass of phenolic compounds, whereas the dihydrochalcone content was significantly affected by the production area, with samples from the Western Cape having higher aspalathin ($P \leq 0.0001$) and nothofagin ($P = 0.0207$) content than those originating from the Northern Cape. Moreover it was shown that fermented rooibos plant material

of the same production season varied naturally in terms of the concentration of two major compounds, aspalathin and quercetin-3-O-robinobioside, although there was no significant effect on the total polyphenol content and total antioxidant capacity. This study also revealed that higher quality grade rooibos samples are often associated with higher levels, confirming that large variation within quality grade exists.

The phenolic composition of rooibos may also be affected by processing methods, especially the uncontrolled fermentation process that causes the dihydrochalcones, aspalathin and nothofagin, to be subjected to enzymatic oxidative degradation (Joubert, 1996). The oxidative degradation of orientin, iso-orientin and nothofagin is slow compared with that of aspalathin, suggesting that the C-ring configuration and the hydroquinone moiety of the B-ring plays a key role in the oxidation process (Krafczyk *et al.*, 2009). In 1996, Joubert demonstrated that as soon as rooibos leaves were cut into small pieces, rapid browning and a decrease in dihydrochalcone content occurred. Less than 80% of the initial aspalathin content of the rooibos leaves remained 15 minutes after the oxidation process was initiated, and approximately 33% remained after 210 minutes of oxidation. In addition, the effects of fermentation temperature (30-42 °C), drying temperature (40-70 °C) and drying method (sun-drying vs. controlled drying) on the subjective tea quality and objective colour measurement of fermented rooibos were studied by Joubert & De Villiers (1997). It was found that the drying method had no significant effect on the subjective tea quality, however, sun-dried rooibos had a significantly darker colour ($P = 0.003$) than that dried under controlled conditions. The subjective tea quality improved with increasing fermentation temperature and decreased with increasing drying temperature ($P \leq 0.05$). Steam-pasteurisation (1 min at 96 °C) of fermented rooibos resulted in significant decreases in the soluble solids, aspalathin and total polyphenol contents of the corresponding hot water infusions ($P \leq 0.05$) (Koch *et al.*, 2013). The decreases in the total polyphenol and total soluble solids contents were strongly positively correlated, suggesting that the decrease in soluble solids could be strongly attributed to the decrease in the soluble polyphenol content.

2.4. Sensory quality

Sensory analysis is described as a scientific mechanism utilised commonly for evoking, measuring, analysing and interpreting responses to food and beverage attributes as perceived by the five senses (Stone & Sidel, 1993). A panel of judges are usually trained on how to analyse specific products sensorially, primarily to guarantee reliable and consistent results. The manner in which products are analysed are not usually related to the typical way in which the products are consumed since the aim is the determination of product variation (Stone & Sidel, 1993). Therefore, the sensory profiling of food and beverage products can assist with the determination of individual attributes that drive the

sensory quality of a product and market success (Lawless & Heymann, 2010). Individual attributes include the aroma, flavour, texture and appearance of the product in question.

The development of a relevant, reliable grading system is a challenge for a complex product like rooibos tea. In addition, limitations of the grading system exist despite sensory evaluation by graders being quick, cost-efficient and simple. This grading procedure cannot be validated scientifically, primarily due to the fact that quality standards are purely dependent on the subjective opinion of tasters who act as the instruments to measure and quantify particular quality parameters (Koch, 2011). According to Land and Shepard (1984), expert tasters are defined as 'people with considerable experience and proven ability in sensory assessment of a given product under specified conditions'. Therefore expert tasters have the ability to make rapid quality judgements due to long exposure to a single product whereby they develop acute sensitivity to its characteristics. However, an expert taster's judgement may be influenced by external factors and may therefore be biased, and daily perceptiveness of products may differ (Feria-Morales, 2002). The market value awarded to each tea batch is determined by the awarded grade (Joubert, 1995) and thus tea grades awarded to tea have financial implications for both the producer and processor. Black tea (*Camellia sinensis*) also experienced the above-mentioned restrictions as noted by producers and processors. A single, objective and reliable scientific method for the determination of tea quality has not yet been developed although efforts have been made to correlate particular black tea parameters with sensory analysis results. Furthermore, tea tasting is the most used tea quality determination method since instrumental analysis methods are regarded as work-intensive and slow (Cabarello *et al.*, 2003).

Despite this, new methods of analysing foods and beverages that do not require any human elements have also been developed. The electronic tongue and nose, for example, are technologies that allow the accurate measurement of human responses. Moreover, such technologies minimise the effect of any brand identity biasness or any other influential factors. However, existing evidence demonstrates that descriptive sensory analysis carried out by a trained panel of judges provides valid and reliable results, more especially sensory attributes perceived by the human senses (Lawless & Heymann, 2010).

2.4.1. Predicting tea sensory quality using chemical and instrumental data

The use of objective quality parameters for the prediction of the sensory quality of black and green teas has been studied extensively, where the challenge of the correlation of chemical compounds and sensory attributes has been acknowledged. This is mainly due to the fact that the amount of a compound in a food product is not a reflection of its effect on the sensory characteristics due to threshold value differences and food matrix effects (Drake & Civille, 2002). Also, food or beverage volatile compounds are not all odour-active (Friedrich & Acree, 1998). Gas-chromatography (GC)

olfactometry is a technique that is often used to determine the sensory characteristics of particular fractions of different products. The aroma characteristics of individual GC effluent compounds are sniffed and described by a trained panelist, and often, this is the first step of assessing the impact of chemical compounds on sensory quality (Drake & Civille, 2002). However, the effects of the compounds when masticated or swallowed are not considered by this technique and the effects of temperature, pH, saliva and interactions between volatiles are also ignored. Therefore, descriptive sensory analysis and instrumental or chemical analysis are often conducted in unison where univariate and multivariate statistical techniques are used to evaluate results (Drake & Civille, 2002).

In a study conducted by Obanda *et al.* (1997), the correlation between the score given to black tea by a pair of highly skilled tasters and the green leaf chemical components, the thearubigin and theaflavin content of the black tea liquor, as well as the total liquor brightness and colour were analysed. Significant positive correlations were observed between the tasters' scores for the black tea infusions and the levels of epicatechin gallate, epigallocatechin gallate and caffeine in the green leaf. Liang *et al.* (2003) conducted a similar study whereby the colour differences and chemical composition of black tea infusions, and their effect on sensory quality was determined. Sensory quality was a measure of the appearance of the dry (10%) and infused (10%) leaves, infusion colour (15%), aroma (30%) and tea taste (35%). The sensory quality of black tea was significantly correlated with the caffeine, nitrogen, amino acids, polyphenols, theaflavins and total catechins content, and infusion colour. The development of a consistent and speedy model for the establishment of quality of green tea samples was explored by Pongsuwan *et al.* (2007) by means of metabolomics or "chemical fingerprints", and the green tea quality was evaluated by GC data and multivariate statistical techniques. This methodology therefore supplied useful green tea quality information and may be applied as a quick, consistent and informative screening method. The prediction of black tea sensory quality has also been conducted by using artificial neural networks which are based on HPLC profiles of phenolic compounds (Tomlins & Gay, 1994). Certain black, Oolong and green tea samples sensory qualities have been correlated with the GC profiles of their volatile flavour components by the use of multivariate calibration models (Togari *et al.*, 1995). Moreover, due to the fact that tea flavour is largely dependent on the levels, combination and threshold values of volatile compounds found in tea infusions (Dutta *et al.*, 2003), numerous attempts have been made to use various GC peak area ratios as a form of tea quality measurement (Wickremasinghe *et al.*, 1973; Owuor *et al.*, 1986; Baruah *et al.*, 1986; Mahanta *et al.*, 1988; Yamanishi *et al.*, 1989; Owuor, 1992).

In addition, the use of the electronic tongue and nose, and capillary electrophoresis for the classification and quality estimation of tea has been explored (Legin *et al.*, 1997; Horie & Kohata, 1998; Ivarsson *et al.*, 2001; Dutta *et al.*, 2003; Chen *et al.*, 2008; He *et al.*, 2009). The electronic nose has the ability to identify and estimate odourant sample concentrations with gas sensors that have varying sensitivities, together with a signal processing system (Dutta *et al.*, 2003). Dutta *et al.* (2003), Yu and

Wang (2007), and Tudu *et al.* (2009) were therefore able to differentiate between teas produced under different processing conditions (e.g. under-fermentation and over-fermentation). Moreover, satisfactory results for black tea discrimination and classification were obtained by Chen *et al.* (2008), He *et al.* (2009) and Bhondekar *et al.* (2010). The apparatus functions on the basis of an array of non-specific chemical sensors that display fractional specificity to varying components in a solution. Qualitative and quantitative information relating to solution composition are generated by pattern recognition tools such as principal component analysis or artificial neural networks (Legin *et al.*, 1997).

Due to keen interest from researchers, it has been suggested that for this quality determination route partially replace the routine work of expert tasters since it enables the production of objective measurements in a cost-effective, time-efficient and consistent way (Scampicchio *et al.*, 2006). Moreover, this method is useful for eliminating subjectivity and fatigue problems that are often associated with expert tasters. It is evident from the above-mentioned studies that similar, extensive, focused research is needed for rooibos tea for the investigation of whether a reliable, feasible and efficient manner to correlate rooibos sensory quality can be discovered. On the other hand, the studies conducted on black and green tea have produced worthwhile, interesting results, however the quality prediction methods are not applied widely in commercial tea production and marketing due to cost and time implications. The sensory analysis of tea by expert tasters and panelists remains the most common practice to date used to evaluate tea sensory characteristics and qualities.

2.4.2. Descriptive sensory analysis

Descriptive analysis, used regularly during new product development and research, is a very useful tool for conducting sensory analysis. A complete sensory description of a product compiled by a panel of well-trained judges is achieved through the differentiation and description of qualitative (attributes) and quantitative (intensity) sensory characteristics (Meilgaard *et al.*, 1999).

A number of commercial descriptive analysis methods such as quantitative descriptive analysis (QDA) have been developed over the years. Most research institutions use the non-commercial version, generic descriptive sensory analysis (DSA). This method relies on the skills of numerous panel members to characterise the perceptions of products in a consistent, reproducible and reliable manner (Stone, 1992). The panel members are trained so that they are able to recognise particular product attributes, rate their intensities and determine the sequence of detection (Stone *et al.*, 1974). The basic DSA procedure can be summed up as follows (Stone, 1992):

- The selection, screening and training of panel members
- The development of the full aroma, flavour, taste and mouthfeel profile by the trained panel
- The compilation of a list of sensory attributes in order of detection

- The quantification of attribute intensities on a fixed, unstructured line scale
- The use of various statistical techniques for data analysis

When rating product attributes and their intensities, it is vital that panel members make use of the same comparison or frame of reference. Therefore, extensive panel training must be conducted to ensure that the panel is standardised by developing a list of descriptors and/or providing concrete reference standards for each perceived attribute of the product in question. Descriptors must discriminate between different attributes in a clear manner, and must thus be non-redundant (Lawless & Heymann, 2010; Murray *et al.*, 2001). Reference standards may be chemicals, food and beverage products or other substances that are able to communicate the product attribute concept and to ensure that all panel members understand the jargon used to define the attributes (Drake & Civille, 2002). In addition, reference standards may not be identical to the perceived product attribute, but they are beneficial for the calibration of the panel since reference standards do not change throughout training. By having reference standards accompany the descriptor list, panel variability is reduced as the panel members are better able to understand the restrictions of the given attributes. Therefore, it becomes less difficult for the panel to understand terms when analysing samples (Lawless & Heymann, 2010). Reference standards can be quantitative, qualitative or both. Quantitative reference standards, which are not utilised often during DSA training, represent the upper intensity limit for a specific attribute, while qualitative reference standards which are the most vital training component, demonstrate the nature of an attribute. The maximum intensity becomes the reference point that panel members can refer to when rating the intensity of a specific product attribute (Munoz & Civille, 1998).

Panel performance can be improved by careful screening and selection of panel members, comprehensive training sessions and standardisation of scaling (Drake & Civille, 2002). Software packages such as Panelcheck (Nofima Mat, Norway) can be used for the evaluation of parameters relating to the performance of the panel and thus verify the efficiency and reliability of the panel. The internal consistency (Judge*Treatment interaction) and the temporal stability (Judge*Replication interaction) associated with the panel members can also be examined to analyse the judge reliability and reproducibility (Prichett-Mangan, 1992; Carbonell *et al.*, 2007). In this way, unreliable judges can be identified and removed.

During DSA, the final testing phase of a product is performed individually by each panel member in a taste booth where they are unable to be influenced by another panel member (Carlucci & Monteleone, 2001; Lawless & Heymann, 2010). Each of the attributes being evaluated are usually evaluated using an unstructured line scale on a computer with a data capturing software package such as Compusense® five (Compusense®, 2012), which makes data collection and analysis easier. The different attributes are thus evaluated by panel members on anchored numerical scales (Murray *et al.*, 2001; Lawless & Heymann, 2010; Lee & Chambers, 2007). The scale is usually anchored with 0 on the

lower end and 100 on the higher end. Words such as “none” and “extremely” are used as anchors (Lee & Chambers, 2007; Powers, 1984). Panel performance should, therefore, be evaluated and monitored carefully during sensory analysis as it ensures reliability of the results.

Often, certain parameters need to be adhered to when specific products are being analysed. Researchers are thus able to ensure that products are in their exact state of analysis, and that there are no outside factors that can affect and skew the results. Koch *et al.* (2012) conducted an experiment on rooibos to determine the full sensory profile of different commercial rooibos tea batches. In this study, it was demonstrated that keeping the infusions warm at a constant temperature was vital. This ensured that the flavour and aroma attributes were maintained and not affected in any way, as noted when the infusion temperature decreases. Moreover, the flasks and mugs used during the study were preheated to assist with the temperature control of the infusions. Therefore, product knowledge prior to testing is vital to ensure that the sensory profile and results of any study are not compromised in any way possible.

Analysis of data obtained from sensory analysis is critical for the success of research. Data obtained from a sensory panel are always seen as a three-way data table containing the assessors, samples and attributes representing the three different “ways” which need to be taken into account when analysing data correctly. This becomes particularly important for analysing the similarities and differences between both the panelists and the different samples (Luciano & Næs, 2009). However, before the final data analysis, at least one of these dimensions (ways) is usually removed due to the assessor results being averaged. This is done to simplify the data for easier analysis, but on the other hand it makes obtaining information about the individual data amongst the assessors more difficult (Dahl *et al.*, 2008). The aforementioned effect can be eliminated by the use of previously developed methods such as Principal Component Analysis (PCA) and Parallel Factor Analysis (PARAFAC). These methods provide more information about the relationships amongst assessors and samples, but can be complex (Dahl *et al.*, 2008). The PARAFAC method takes into account the fact that panel members have varied sensitivities towards variables and permits improved handling of variations in the scale and variability between the assessors. PCA, however, is based on the assumption that all panel members are equally skilled, meaning that they are all seen as competent and do not exhibit any individual differences (significant) within their individual data (Bro *et al.*, 2008).

After the pre-processing of the final dataset using the multivariate methods above, the dataset is also analysed using Analysis of Variance (ANOVA) (Lawless & Heymann, 2010). Spider diagrams are often used as a graphical representation for the data when ANOVA is applied (Murray *et al.*, 2001). ANOVA is also useful for the determination of significant differences amongst results obtained for the same attribute after replicate testing. It is generally recommended that more than one statistical method should be used to analyse data since each method generates a slightly different picture of certain correlations and relationships that are hidden in the data sets (Palmer, 1974).

DSA has numerous applications such as identification, quantification and documentation of sensory characteristics for research purposes or product maintenance, and the correlation of instrumental or chemical measurements with sensory attributes. Moreover, DSA finds application in the definition of specifications for controlling product quality and consistency, and in the examination of changes in sensory attributes during production processes (Stone, 1992; Meilgaard *et al.*, 1999). Often, no other alternative analytical methods are able to provide the same information generated by descriptive analysis (Stone, 1992). Therefore, the ability of obtaining accurate and reliable quantitative information, as well as a descriptive sensory profile gives this method an advantage over many others (Cartier *et al.*, 2006).

2.4.3. Sensory lexicon

A sensory lexicon is an important food industry tool used by marketers, processors, researchers and consumers alike (Lee & Chambers, 2007). It is defined as a set of terms used for the description of product sensory attributes along with the reference standards and definitions for clarification purposes. Sensory lexicons have been used within numerous industries to assist with the description and discrimination amongst products within the same product category, profiling new products, developing flavours in prototypes, determining drivers of liking when creating new product formulations and assisting with product quality control (Drake & Civille, 2002). DSA is used for the generation and quantification of terms of which sensory lexicons are composed. Sensory lexicons have been developed for numerous food products such as pawpaw pulp (Brannan *et al.*, 2012), spices (Lawless *et al.*, 2012), honey (Galán-Soldevilla *et al.*, 2005) and almonds (Civille *et al.*, 2010). Several aspects need to be taken into consideration in order to generate a reliable and pertinent lexicon: attribute intensities need to be anchored in the same way, terms must be precise and defined appropriately, and reference standards must be provided. Moreover, terms need to be discriminating, relevant, descriptive and non-redundant (Drake & Civille, 2002; Kreutzmann *et al.*, 2007). There has been great success in the development and use of the sensory lexicon in the green tea industry. The green tea flavour lexicon consists of 31 flavour attributes along with reference standards. An excerpt from a sensory lexicon developed for green tea is shown in Table 2.3 (Lee & Chambers, 2007). A sensory lexicon has also been developed in South Africa for rooibos tea infusions (Koch *et al.*, 2012) and is shown in Table 2.4.

Table 2.3 Excerpt from a green tea sensory lexicon (Lee & Chambers, 2007).

DEFINITIONS OF ATTRIBUTES FOR GREEN TEA EVALUATION		
Attributes	Definition	Reference
Green	Sharp, slightly pungent aromatics associated with green plant/vegetable matter, such as asparagus, Brussels sprouts, celery, green beans, parsley, spinach, etc.	Fresh parsley water = 9.0 (flavor) 25 g of fresh parsley, rinse, chop, and add 300 mL of water. Let it sit for 15 min. Filter and serve liquid part
Floral/perfumy	The somewhat sweet aromatics generally associated with fruit and flowers	Geraniol Pure = 8.0 (aroma) Put 1 drop geraniol in 200 mL of distilled water in a large-size snifter. Cover
Fruity	A sweet, floral, aromatic blend, reminiscent of variety of ripe fruits such as apricots, peaches	Blackberry WONF 3RA654 (McCormick & Wild Inc., Hunt Valley, MA) (character reference) Place one drop of chemical on a cotton ball in a medium size snifter. Cover
Astringent	The drying, puckering sensation on the tongue and other mouth surfaces	0.03% "Alum solution" Alum (McCormick & Co., Inc.) = 1.5 0.050% "Alum solution" Alum = 2.5 0.10% "Alum solution" Alum = 5.0 0.15% "Alum solution" Alum = 7.5

Table 2.4 Excerpt from a rooibos tea infusion sensory lexicon (Koch *et al.*, 2012).

Sensory lexicon describing flavor and mouthfeel characteristics of rooibos infusions analyzed by descriptive analysis.		
Attributes	Definitions	Reference standards
Herbal floral	The unique, somewhat sweet aromatics associated with flowers of the fynbos ^a vegetation	α -ionone (1.5 μ L in 100 mL water)
Woody	Aromatics associated with dry bushes, stems and twigs of the fynbos vegetation	2% (v/v) FTNF Rooibos Extract [Rooibos Ltd., Clanwilliam, South Africa]
Honey	Aromatics associated with the sweet fragrance of fynbos honey	Wild Flower Honey [Hillcrest, South Africa]
Caramel	Sweet aromatics characteristic of molten sugar or caramel pudding	0.4% (v/v) Wild® Natural Flavor Type Caramel, [Comhan Products, South Africa]
Apricot jam	An aromatic associated with the sweet smell of fruit especially apricot jam and berries	5 mL each of Superfine Apricot Jam [All Gold] and Strawberry Jam [All Gold] dissolved in 100 mL hot water
Plant-like/green ^b	Slightly sour aromatics characteristic of freshly cut green leaves or plant material	Fresh parsley water (25 g of fresh parsley, rinse, chop, add 300 mL water, allow to stand for 10 min, filter and serve liquid part)
Grassy ^b	Aromatics associated with freshly cut grass	Cis-3-hexenol (5 μ L in 100 mL water); or finely chopped fresh green grass (<i>Pennisetum clandestinum</i>) Dried grass (<i>Pennisetum clandestinum</i>)
Hay/dried grass	Slightly sweet aromatics associated with dried grass or hay	Place a piece of old, dry tree bark (<i>Jacaranda mimosifolia</i>) in
Dusty ^c	Earthy aromatics associated with wet hessian or wet cardboard	100 mL hot water, allow to stand for 10 min, filter and serve liquid part
Musty ^c	Moldy aromatics associated with mildew or damp cellars	0.1% sucrose
Sweet	Fundamental taste sensation of which sucrose is typical	0.035% citric acid
Sour	Fundamental taste sensation of which citric acid is typical	0.03% Caffeine solution
Bitter	Fundamental taste sensation of which caffeine is typical	0.7% Alum solution
Astringent	The drying, puckering sensation on the tongue and other mouth surfaces	

^a Fynbos is natural shrubland vegetation occurring in the Western Cape, South Africa.
^b "Plant-like/green" and "grassy" were grouped together under one attribute during descriptive analysis.
^c "Dusty" and "musty" were grouped together under one attribute during descriptive analysis.

2.4.4. Sensory wheels

Sensory wheels serve as graphical representations of information provided by sensory lexicons. A sensory wheel, which is a simpler, more convenient representation of product characteristics, is formed by sensory attributes that are arranged in a wheel format. The positioning of the attributes within the wheel allows for a clear and rapid understanding (Jolly & Hattingh, 2001). A flavour wheel usually consists of two attribute tiers. Terms near the center of the wheel are the broader, basic characteristics while the more detailed, descriptive characteristics are located on the outer part of the wheel (Lawless & Heymann, 1998). Sensory wheels can be developed for aroma, flavour or even mouthfeel attributes or these attributes can be combined to form one sensory wheel. An example of a wine flavor wheel is shown in Figure 2.3 (Noble *et al.*, 1984). Terms used to describe similar aromas or flavours can be grouped accordingly, mainly to prevent the appearance of redundant terms. Terms such as “musty” and “mouldy” for rooibos tea, for example, are often interpreted equally, and are therefore grouped together as “musty/mouldy”, in order to prevent misinterpretation (Koch *et al.*, 2012).

Sensory wheels have therefore been developed for product development and quality control purposes for the identification of positive product attributes generally associated with the product, but also negative attributes that are usually not associated with acceptable sensory quality (also referred to as taints). The development of a flavour wheel in the beer industry, which is still in use to date, was revolutionary as it provided clear, acceptable terms for beer sensory analysis (Schmelzle, 2009). The communication between winemakers, marketing personnel, wine researchers, wine writers, as well as consumers, was also revolutionised by the development of a wine aroma wheel in 1984 (Noble *et al.*, 1984). Moreover, a mouthfeel wheel summarising terminology used for the description of mouthfeel sensations stimulated by red wines was developed by Gawel *et al.* (2000). A flavour wheel has also been developed for black tea as shown in Figure 2.4 (Bhuyan & Borah, 2001).

A sensory wheel for the rooibos industry was developed and is shown in Figure 2.5 (Koch *et al.*, 2012). Positive and negative attributes, which are the primary descriptors of the sensory attributes, are displayed in the outer tier of the wheel. More detailed descriptors, i.e. a range of attributes describing each of the primary descriptors in the outer tier, are displayed in the inner tier. However, Jolley (2014) deemed the development of a more comprehensive sensory wheel validated by industry as very important, as it could be used to decrease quality inconsistencies that are still experienced in industry to date. Jolley *et al.* (2017) thus developed a revised rooibos aroma wheel using a large sample set, spanning two production areas and three production years, which enabled the capturing of variation in the intensities and occurrence frequency of rooibos sensory attributes (Figure 2.6). A primary (“rooibos-woody”, “fynbos-floral” and “honey”) and secondary (“fruity-sweet”, “caramel” and “apricot”) aroma profile for rooibos was established for the first time from the data collected, making

the aroma wheel more comprehensive and valuable. The aroma wheel consequently provides users with the relative importance of attributes (as determined by intensity and occurrence frequency). The slice width indicates the relative mean attribute intensity, accompanied by bar graphs that indicate relative occurrence frequency of attributes (Figure 2.6). The aroma wheel is, therefore, more user-friendly and can be used by industry to assist with the grading and marketing of rooibos tea on a local and especially global scale.



Figure 2.3 Wine flavour wheel with three sensory attribute levels (Noble *et al.*, 1984).

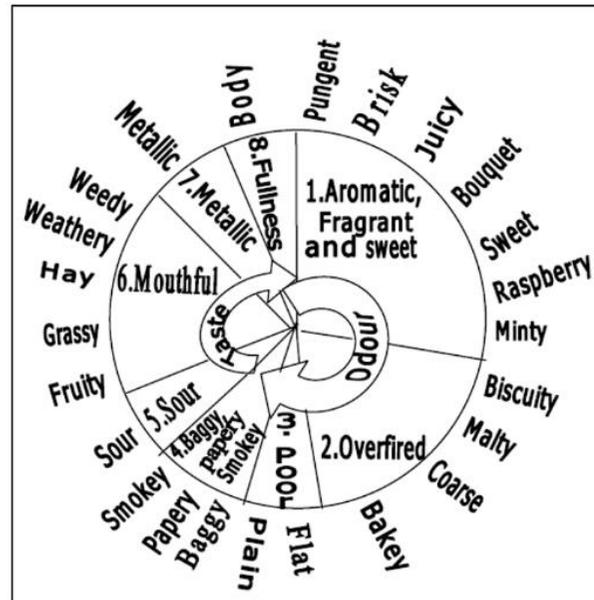


Figure 2.4 Black tea flavour wheel illustrating the international flavour terminology for tea as accepted by the Indian Tea Research Association (Bhuyan & Borah, 2001).

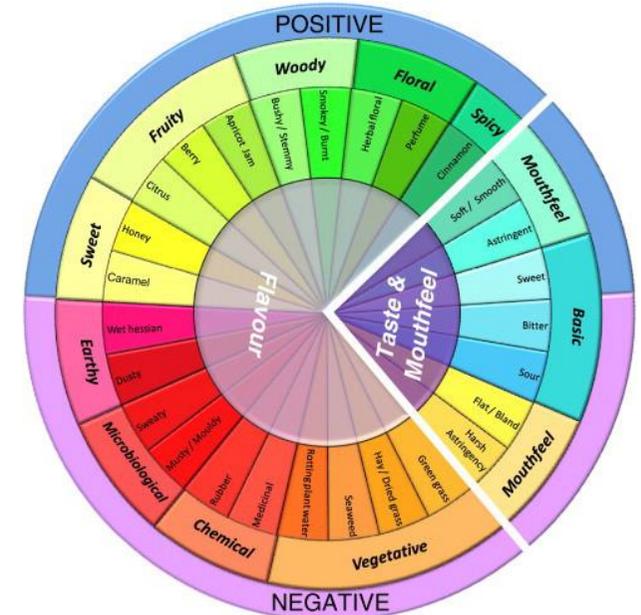


Figure 2.5 Rooibos sensory wheel including (Koch *et al.*, 2012).

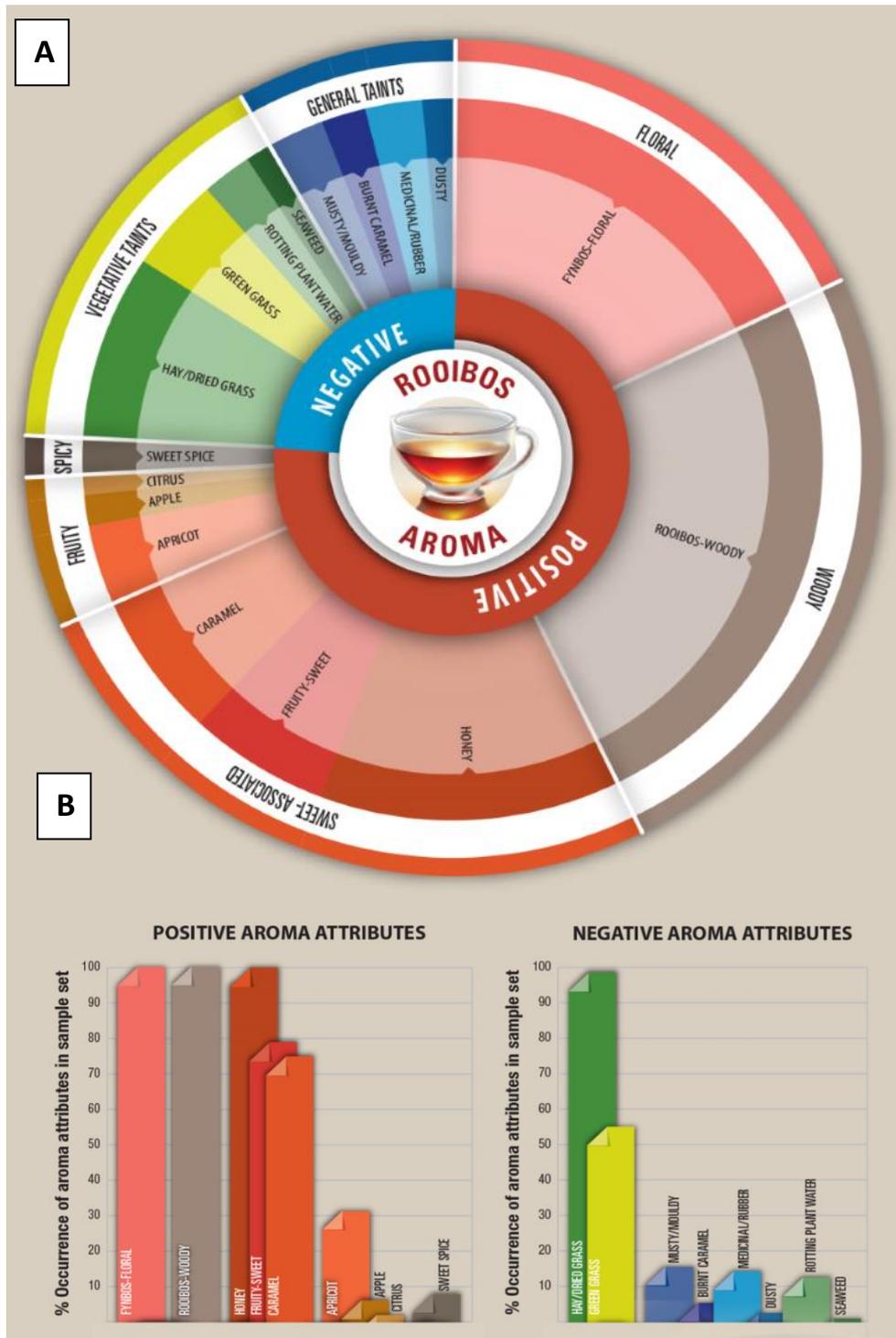


Figure 2.6 Rooibos aroma wheel, showing relative intensities of 17 aroma attributes (a), accompanied by bar graphs (b) indicating percentage occurrence frequency of positive and negative (Jolley et al., 2017).

2.5. Tea waste management

The increase of agricultural production and the expansion of agro-based industries in numerous countries has brought about the production of large amounts of agricultural wastes, where most are managed and used inadequately. Environmentally friendly, energy saving and recycling initiatives within the food industry have been a subject of crucial research for decades. Because tea is one of the most consumed beverages in the world, tea and tea beverage manufacturing companies produce tons of tea leaf waste annually, most of which is discarded into landfills, burned or used to make compost (Kondo *et al.*, 2007). In past years, alternative uses for black tea (*Camellia sinensis*) waste have been investigated in an attempt to implement good environmental practices. Some of these applications will be discussed in the following section.

Uddin *et al.* (2008) demonstrated the potential use of black tea waste for the adsorptive removal of methylene blue, a cationic dye, from aqueous solution. It was found that the adsorption capacity of methylene blue onto tea waste was several folds higher than other potential adsorbents. The plant material is mainly comprised of cellulose materials that absorb heavy metal ions. In another study, decolourised and sized tea waste exhibited very good adsorption of Cu (II) and Cd (II) in synthetic wastewater at pH 5.5 and room temperature (Cay *et al.*, 2004). Amarasinghe & Williams (2007) showed that tea waste is capable of binding appreciable amounts of Pb and Cu from aqueous solutions, optimally at pH range 5-6. The results from these studies therefore indicate that industrial tea waste, which is of low economical value, may be used effectively as a means of metal ion removal for environmental cleaning purposes.

On the other hand, tea waste has been found to contain considerable concentrations of crude protein (22 - 35% of dry matter) which may have high value as a dietary supplement for goats (Kondo *et al.*, 2007). It was found that green tea waste silage could substitute lucerne hay cube as a protein supplement, however, tannins in the black tea waste bound proteins in the digestive tract, lowered nitrogen degradability in the rumen and increased fecal nitrogen output.

Black tea waste in other instances has been used as a supplement for the cultivation of *Ganoderma lucidum* (Peksen & Yakupoglu, 2009), manufacturing of particleboard from (Yalinkilic *et al.*, 1998), the production of a construction brick with improved durability and mechanical properties (Demir, 2006), casing material in mushroom (*Agaricus bisporus* (L.) Sing.) cultivation (Gülser & Pekşen, 2003), production of synthetic fuels (Uzun *et al.*, 2010) and more commonly as fertilizer or compost (Senesi, 1989).

The re-use of rooibos waste has not been well documented in literature and very few studies exist. A first attempt at using the coarse stem material obtained when the processed product is sieved prior to grading and packaging, was to develop an “instant” rooibos tea following the extraction of the soluble fraction of the waste material (Joubert, 1984). The soluble fraction of the rooibos coarse stems

(waste) was 8.8% compared to 20.4% of the refined fraction obtained after removal of the stems. However, the focus eventually fell on the unrefined tea as the rooibos industry experienced an overproduction.

A significant amount of rooibos tea waste in the form of fine dust and coarse stems ($\pm 10\%$ of production) is generated during processing. Mostly, rooibos waste is discarded in compost heaps or the coarse stems are cut into smaller pieces and blended with the refined fraction to produce a cheaper product with a milder aroma (E. Joubert, Agricultural Research Council, Stellenbosch, South Africa, 2016, personal communication). This processing step has become common practice among rooibos tea processors, however, it results in tea of lower quality. Similarly to black tea waste, Safarik *et al.* (2015) showed that spent fermented rooibos tea biomass has considerable potential for the removal of selected xenobiotics, such as dyes, from water and that the adsorption properties of rooibos are dependent on the type of dye.

Recently, rooibos wood chips, prepared from rooibos bushes that are not productive anymore, have been used by Audacia Wines (in Stellenbosch, South Africa) for the production of a wooded Merlot wine. The idea behind this innovative concept was to seek unique wood alternatives to oak derivatives currently used in wine making. Addition of the rooibos wood chips enabled Audacia to produce red wine containing no sulphur dioxide, a widely used preservative in wine, which can cause allergic-type reactions. No rooibos leaves may be added to wines as per regulation. Research by De Wet (2015) further explored this innovative concept by assessing the consumer acceptability of wines treated with rooibos by comparing them to other commercially available wines. The results from the study indicated that the consumers neither liked nor disliked the wines produced with rooibos, and thus no real high degree was indicated. Rooibos possesses a distinctive aroma profile and could potentially aid in the development of wines with unique aromatic profiles. Therefore, further research on the effect of the addition of rooibos waste material to wine may greatly benefit both the wine and rooibos industries as the use of indigenous tea wood is considered as sustainable, cost effective and economically viable.

2.6. Extraction of bioactive compounds

Bioactives are metabolites that are produced by plants for self-defense and other purposes and have the potential to be used for a number of applications (Puri *et al.*, 2012). The development of health-promoting plant-derived compounds has been driven by the demand for new and unique natural compounds, and an increasing interest in and realisation of the value of functional food ingredients (Toledo, 2007). The release of bioactives from plant materials is generally achieved by the disruption of cells and extraction through cell walls by a variety of methods. Essential and non-essential bioactives are found in a diverse range of food products (such as grains, fruits and vegetables) and have been

widely processed for use by the food and nutraceutical industries. Therefore, a great deal of effort has been invested by food and nutraceutical industries into the optimisation of natural plant-bioactive extraction procedures.

Bioactives found in plant materials range from simple to highly complex. Also, the various amounts and types of phenols differ between plant types, where they may have direct interactions with carbohydrates and proteins to develop into insoluble complexes. However, the full recovery of plant bioactives is not always feasible, unless the correct solvent and operating conditions are used in unison with pre-treatment (if necessary) of the sample to enhance the recovery of target bioactives (Takeuchi *et al.*, 2009). The feasibility of extraction procedures in industrial settings relies on the best combination of process factors that are able to decrease costs and increase procedure functionality. Water, ethanol and isopropanol and their combined use have certified GRAS (Generally Recognised as Safe) status by the United States Food and Drug Administration, which means that these solvents are suitable to use in the production of nutraceuticals (Wang & Weller, 2006).

Extraction rate, yield and purity are largely impacted by the selected extraction method which should essentially be appropriate for the desired end use of the product, whilst also considering practicality, economics, the environment and logistics. Moreover, extraction methods should ideally be quantitative and time saving. Several recent reviews cover extraction strategies to recover bioactives from agro-processing waste products (Wijngaard *et al.*, 2012; Galanakis, 2013; Putnik *et al.*, 2016). The following discussion highlights some aspects and technologies

Commonly used methods for the extraction of plant bioactive compounds and the factors affecting their efficiency are also discussed in the following section, including recent green extraction practices, which intend to make use of less chemical solvents and energy (Pasrija & Anandharamakrishnan, 2015).

2.6.1. Solvent-based extraction

Solid-liquid extraction is defined as the use of liquids for dissolving and removing soluble fractions (solute) from insoluble, permeable matrixes (Gertenbach, 2002; Takeuchi *et al.*, 2009). Traditional solvent-based extraction methods such as maceration with alcohol, Soxhlet extraction and hydrodistillation are reliant on the extracting ability of numerous solvents and the use of heat and/or mixing (Takeuchi *et al.*, 2009). A typical example of a solid-liquid extraction is when a cup of coffee or tea is made where the hot water acts as the solvent.

The use of traditional solvent-based extraction methods is often linked to several hindrances including high energy, time and solvent consumption, the use of harsh chemicals, poor extraction selectivity, low product quality, overheating of the plant material and the inactivation of important plant compounds which decrease the quality of the end product, making it sometimes unsuitable for

human consumption. Moreover, the selected chemical solvent and process factors can have an effect on the efficiency of the extraction method (Teo *et al.*, 2010; Azmir *et al.*, 2013). The large volume of solvent used will also have to be removed at some point either before or during the drying of the end product.

It is expected that the extraction efficiency and the solubility of individual compounds will vary with the use of different or combinations of solvents due to the polarities of the different chemical solvents (Liu *et al.*, 2016). Ethanol, methanol and water are polar protic solvents with dielectric constants of 24, 33 and 80, respectively, in comparison to non-polar ethyl acetate and polar acetone aprotic solvents with dielectric constants of 6 and 21, respectively. A higher solvent dielectric constant means that a solvent is more polar and thus translates to higher yields of some extractable total polyphenols (Wang *et al.*, 2011).

Hot water extraction (HWE) is an extraction method that makes use of water only as a solvent and it is therefore not very efficient for the extraction of non-polar compounds. Therefore, to increase the extraction capacity of non-polar target compounds, co-solvents such as ethanol or a chemical modifier are often used (Azmir *et al.*, 2013). Naturally, water-soluble products like sugars, organic acids and proteins, as well as inorganic materials, are more suited to be extracted with water due to the high polarity of water (Chemat *et al.*, 2012).

Solvent-based extraction is currently the standard method used for the commercial production of rooibos extracts and for the analysis of plant material samples. Aspalathin is known to be a hydrophilic, water-soluble molecule (Huang *et al.*, 2008), and hot water extraction is a non-toxic rooibos extraction method especially for the production of extracts that are food-grade. It is generally accepted that hot water extracts would not be contaminated with toxic substances that would not be usually be found in a normal cup of rooibos tea because aqueous infusions of rooibos have been consumed as a household beverage for decades. Moreover, using only water assists with spray-drying as the system can function with an “open configuration” without requiring the removal of organic solvents prior to the process (Büchi, 2009).

An alternative solvent-based extraction method in which carbon dioxide (CO₂) is frequently used is known as supercritical fluid extraction (SFE). CO₂ is used often as a solvent for the extraction of non-polar molecules due to its food-grade status, relatively low cost, widespread availability, low critical temperature (31.1 °C) and low critical pressure (7.4 MPa) (Azmir *et al.*, 2013). Its most well-known application is the decaffeination of coffee (McHugh *et al.*, 2013). Due to its low polarity, pure CO₂ is limited to the extraction non-polar substances, fats and lipids. Therefore, to increase the polarity of CO₂, the addition of co-solvents (e.g. ethanol) or chemical modifiers (e.g. diethylamine or dichloromethane) has been explored (Lang & Wai, 2001; Azmir *et al.*, 2013). Other parameters such as extraction temperature, pressure and time, and CO₂ flow rate can be altered in a typical SFE process to further enhance extraction (Wijngaard *et al.*, 2012). Extraction temperature is a critical factor which

has an effect on the extraction efficiency of SFE systems. It is known that extraction conducted at elevated temperatures may result in the deterioration of chemical compounds that are heat-labile. However, heat-sensitive compounds may be extracted using SFE, but the long processing times and relatively high costs of using this extraction method may be a disadvantage (Shah & Rohit, 2013). The manipulation of extraction pressure brings about changes in the solvent density, which affects the solvating power of the SFE process (Wijngaard *et al.*, 2012). A study conducted by Topal *et al.* (2006) on lycopene extraction from tomato skin demonstrated that an increase in pressure at a constant temperature resulted in an increase in the density of the CO₂, thereby decreasing the intermolecular space between the CO₂ molecules, and hence increasing the interactions between the CO₂ molecules and the target compound (Topal *et al.*, 2006). Therefore, the use of increased operating pressures is beneficial because it enables the same extraction efficiency to be achieved at lower temperatures. Moreover, the solvent remains in one phase but it possesses properties of both liquid and gas, and thus the solvation capacity is improved under SFE operating conditions (Wijngaard *et al.*, 2012).

2.6.2. Green extraction concept

Large amounts of chemical solvents are often required for extraction processes and as a result, energy and time input is high, while a good yield of extracted compounds is not always guaranteed (Chemat *et al.*, 2012). In an attempt to develop technologies that are more eco-friendly, the idea of 'green' chemistry and engineering has been established. Green chemistry is categorised into one of two categories: (i) technologies that recycle food co-products and industrial waste into biofuels and food additives, i.e. ensuring that 'today's waste is tomorrow's resource' and (ii) technologies which strive for the improvement of the sustainability of existing techniques through their adaptation to move towards using eco-friendly processing parameters and solvents (Clark, 2011; Chemat *et al.*, 2012). For "green extraction", the source of the plant should be in constant supply and renewable (to prevent population depletion and inaccessibility which would ultimately increase transport costs), and traditional chemical solvents should be replaced with solvents such as water or other biodegradable solvents (Clark, 2011).

Natural ionic liquids and deep eutectic solvents (NADES), which are made up of natural compounds, have been discovered and they offer numerous advantages such as biodegradability, sustainability, high solvation power for polar and non-polar compounds and favourable toxicity profiles (Du *et al.*, 2009; Paiva *et al.*, 2014). NADES have been successfully applied to the extraction of polyphenols from *Chamaecyparis obtuse* leaves (Bi *et al.*, 2013), green coffee beans (Paiva *et al.*, 2014), green tea (Zhang *et al.*, 2014a), safflower (Dai *et al.*, 2013) and shrimp byproducts (Zhang *et al.*, 2014b), amongst others. Although NADES demonstrate prospective use for the extraction of plant bioactives, the area of research is in need of more attention. Extracts produced should ultimately not contain

any harmful contaminants and abide by applicable regulations such as the ICH guidelines (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) which declares the acceptable levels for remaining solvents in products (post manufacture) according to their toxicity level (Puranik *et al.*, 2009; Chemat *et al.*, 2012). Moreover, the production of green extracts requires the review of the entire supply chain, which includes plant material growth and harvesting, the end-point of waste products and the biodegradability of end products (Mason *et al.*, 2011).

Therefore, the consumption of energy can be reduced greatly through as much solvent recycling as possible and through the recovery and re-use of any produced energy. Furthermore, the use of new or alternative techniques, the modification of existing processes to be more effective and the maintenance of equipment could result in energy savings (Chemat *et al.*, 2012; Grobler, 2013). Energy analysis, which assists with the identification of the areas in which the greatest energy losses occur, can also be applied to determine energy consumption (Van Gool, 1992).

2.6.3. Novel extraction techniques

Alternative novel extraction methods are a subject of on-going research due to their environmental and economic advantages such as lower solvent, chemical and time requirements, improved selectivity and increased yields (as reviewed by Wang & Weller, 2006; Wijngaard *et al.*, 2012; Azmir *et al.*, 2013; Shah & Rohit, 2013).

Ultrasound-assisted extraction (UAE) utilises sound waves (20 kHz-100 MHz) for the induction of acoustic cavitation in liquid mediums which causes disruption of plant cells, enabling more efficient and quicker penetration of solvent into plant material and assists with the recovery of desired compounds (Shah & Rohit, 2013). The extraction efficiency of plant bioactives can be improved significantly through the use of UAE at reduced processing time, solvent and temperature, which is essential for avoiding the thermal damage and loss of volatile compounds (Wu *et al.*, 2001).

Mason and Zhao (1994) applied ultrasound to tea to enhance the yield of extracted solids and found that the extracted solids yield at 60°C was improved by nearly 20% which was comparable to the efficiency of thermal extraction performed at 100°C. An investigation of the effects of UAE on the chemical and sensory quality of green tea infusions, conducted by Xia *et al.* (2006), showed that UAE improved the extraction efficiency of the main chemical components from tea at lower temperatures. In comparison to conventional extraction, the amino acid, polyphenol and caffeine content of green tea infusions was increased, while the extraction of pectin and protein was inhibited. Moreover, an increase in aroma components and glycosidic aroma precursors, as well a change in the Owuor index of green tea infusions, was achieved by UAE, thus improving the sensory quality of the infusions. Otherwise UAE has been widely used in the food industry for the enhancement of extraction of

compounds such as aromatic compounds, anthocyanins, polyphenolics and polysaccharides from many plant and animal sources (Vilkhu *et al.*, 2008). However, ultrasound energy may have a harmful effect on the active components of some medicinal plants due to the formation of free radicals (Kawamura *et al.*, 1999).

Microwave-assisted extraction (MAE) operates on a similar principle to UAE and allows more efficient extraction that is reached at shorter durations and higher rates. Microwaves, typically 300 MHz to 300 GHz, penetrate plant material and break the weak hydrogen bonds in polar molecules (e.g. water) due to their dipole rotation (Wang & Weller, 2006; Shah & Rohit, 2013). MAE also utilises electromagnetic radiation to rupture and create cavitations in structure of polar molecules where active compounds are released in the process (Tatke & Jaiswal, 2011). However, volatile or non-polar molecules are not extracted efficiently by MAE (Shah & Rohit, 2013). MAE has been used for the extraction of tea polyphenols and caffeine from green tea leaves. Results indicated that MAE provides high extraction yields, high extraction selectivity and required less time and labour in comparison to conventional extraction methods (Pan *et al.*, 2003; Nkhili *et al.*, 2009).

Pulsed-electric field extraction (PEF) utilises electrodes for the creation of a transmembrane potential across plant material. As a result, repulsion occurs between the charge carrying molecules that form pores in weak membrane areas, thereby allowing easier extraction of target molecules due to increased permeability of cell membranes. PEF is therefore suitable for compounds that are heat sensitive due to its ability to induce extensive membrane damage without a large temperature increase (Wijngaard *et al.*, 2012; Azmir *et al.*, 2013).

2.6.4. Factors influencing extraction

The recovery of plant bioactives is affected by factors such as particle size, extraction time, extraction temperature, solvent type, pH and solvent-to-solid ratio. These factors have an individual effect on the mass transfer kinetics of plant material and therefore the development of optimised extraction procedures is necessary (Wijngaard *et al.*, 2012). Capability and selectivity considerations with regard to extraction of the target compound, reactivity, toxicity, stability, cost, interfacial tension and viscosity should guide the choice of solvent (Takeuchi *et al.*, 2009).

The extraction of natural compounds within the structure of plant materials is affected by process parameters such as solvent type, target compound solubility, mechanical action (e.g. ultrasonication, shaking, stirring and pressure application) and temperature. Target compounds are situated within various parts of plant material. For example, target antioxidant compounds in sage, oregano and rosemary are found on the leaf surface whereas in other plant materials they may be located in roots and seeds (Takeuchi *et al.*, 2009). Therefore, pre-treatment of the raw plant material

should accompany the appropriate choice of solvent in order to maximise the yield of extracted compounds through the facilitation of optimal extraction processes.

Solvent polarity also has an effect on the efficacy of the extraction process. The use of water as a solvent is appropriate for polar target compounds, however, the use of a less polar solvent (e.g. ethanol) for non-polar target compounds would be more appropriate in order to achieve an equivalent effect (Wang & Weller, 2006). According to Zheng *et al.* (2009), polyphenol extraction and the rate of cell wall degradation may be affected by the pH of the reaction solution. Sodium hydroxide and acetate were used for pH control to study the effect of pH on the total polyphenol content (TPC) of an ethanol extract of unripe apple (*Malus pumila*). It was shown that at a pH of 3.7, an extract with the highest TPC was obtained. However, when the pH was raised above 4.0, a significant decline in TPC was observed. Moreover, a study by Chethan & Malleshi (2007) demonstrated that higher TPC in finger millet (*Eleusine coracana*) was associated with a highly acidic to near neutral pH (6.5) rather than a higher, more alkaline pH. The concentration gradient is increased by higher solvent: solid ratios, which results in a faster extraction process but a more diluted extract, which will require further treatment to get rid of the remaining large solvent volumes (Shah & Rohit, 2013).

Often, the primary factor of importance to consider in industrial unit operations is the preparation of the solid plant material (Takeuchi *et al.*, 2009; Wijngaard *et al.*, 2012; Azmir *et al.*, 2013). Target compounds are usually situated in cell structures, intracellular spaces or capillaries. Therefore, the grinding or crushing of the raw plant material results in an increased contact area between the plant matrix and the solvent, and a decreased diffusion distance between the interior and the solid matrix surface of the target compound. Therefore, the restrictions of mass transfer are reduced by smaller particle size and the rate of extraction is increased due to reduced diffusion distances for solutes within plant matrices. However, extremely fine plant material could cause extract filtering complications or block the extraction equipment due to the agglomeration of the small particles. In rooibos particularly, the polyphenol and soluble solids content of extracts are affected by the particle size and stem content of the plant material. Joubert and De Beer (2012) conducted a study on a large number of production batches of fermented rooibos, each separated by sieving into the three particle size fractions: fractions: >10 (coarse, i.e. mainly stems), $10 > x > 40$ (refined tea) and <40 mesh (dust). Between 4.6 to 23.8% of the unrefined rooibos tea represented the coarse tea fraction. A weak ($r=0.300$), but significant ($P=0.009$) correlation between the soluble solids yield and the percentage coarse fraction in the unrefined rooibos tea observed. Previously, Joubert (1984) showed that rooibos waste material, consisting mostly of stems, delivered a low soluble solids yield (8.9%) in comparison with the refined fraction (20.4%). No studies have been conducted on the extraction of rooibos waste material to determine the soluble solids yield and phenolic content for potential re-utilisation.

In accordance with mass transfer principles, the concentration gradient of the solute between the solid and bulk of the solvent drives extraction processes. When a higher solvent-to-solid ratio is

used, the concentration gradient increases irrespective of the chosen solvent of use (Takeuchi *et al.*, 2009). This suggests that the use of higher solvent-to-solid ratios results in greater soluble solids yields, but would also result in more consumption of solvent which affects the cost efficiency of the extraction process. Therefore, the solvent-to-solid ratio should be considered carefully during the process of the selection of optimal process parameters. High water-to-leaf ratios in rooibos extraction processes have been shown to be associated with higher soluble yields and polyphenol content in end products (Joubert, 1998, 1990a, 1990b; Joubert & Hansman, 1990).

According to Azmir *et al.* (2013), the use of high temperatures for the enhanced extraction of polyphenols increases plant compound solubility, especially polymeric fractions, and increases the mass transfer of solutes. However, the enhancing effect of the use of high temperatures is nullified due to the promotion of oxidative degradation of compounds during exposure to high temperatures for extended periods (Shi *et al.*, 2005; Yang *et al.*, 2010). The hot water extraction of catechins from green tea (*Camellia sinensis*) was optimised by Vuong *et al.* (2011) where the joint influence of pH, time, tea particle size, temperature and tea-to-water ratio was determined. An increase in extraction temperature resulted in a significant increase in all investigated catechin yields. Moreover, the catechin yields were maximised by the optimal combination of treatment levels whilst minimising thermal degradation: particle size of 1 mm, temperature of 80 °C, tea-to-water ratio of 50:1 (mL.g⁻¹), pH <6.0 and an extraction time of 30 minutes. The effects of temperature (23-90 °C), water-to-tea mass ratio (5:1 and 10:1) and water flow rate (0.1 and 0.2 m³.h⁻¹) on the soluble solids yield and polyphenols of fermented rooibos using single stage batch extraction were investigated by Joubert (1988b and 1990a). The polyphenol and soluble solids yields increased in proportion to mass ratio and temperature increases, whereas at a higher water flow rate, both were decreased. Moreover, no significant interaction was observed between the extraction temperature, water: tea mass ratio ($P \leq 0.01$) and flow rate ($P \leq 0.01$), where the temperature effect was less evident at reduced mass ratios and increased flow rates. The temperature, however, had a more visibly positive impact on the extraction of flavonoids than non-flavonoid phenols and it was shown that the increase in the total polyphenol content was a result of an increase in the flavonoid content. In an extraction optimisation study conducted by Miller *et al.* (2017), extraction time, extraction temperature and water-to-plant material ratio were shown to have an effect on the extract yield and aspalathin content of green rooibos extracts, where the extraction temperature demonstrated the greatest effect.

The hot water extraction of flavonoid and non-flavonoid phenols, and total polyphenols from fermented rooibos using a 90 °C fixed-bed system and flow rates of 0.09 and 0.18 m³.h⁻¹ was studied by Joubert (1990b). At a fixed extraction time, an increase in the flow rate associated with a decline in the total polyphenol yield was observed. However, an increase in extraction time to 8 min resulted in an increase of the flavonoid and total polyphenol yield, whereas the increase in extraction time did not significantly affect the non-flavonoid phenols. Depending on extraction time, the total polyphenol

content of the extract mainly consisted of flavonoids (59-68%). An increase in the phenolic content of fermented rooibos extracts, which was achieved through the use of a longer extraction time, was demonstrated by Von Gadow *et al.* (1997), where the antioxidant activity of the extracts was stabilised during extended exposure to heat. Jaganyi and Wheeler (2003) also demonstrated that about 50% of the aspalathin in fermented rooibos is extracted at 80 °C within 5 min in water, and the steady state is reached after ca. 60 min. No other literature has been published on the extraction kinetics of rooibos.

2.6.5. Enzyme-assisted extraction

Enzyme-assisted extraction (EE) is a potential alternative method to conventional solvent-based extraction methods. This method is based on the natural ability of enzymes to speed up chemical reactions with very high specificity, regio-selectivity and the ability to operate under mild processing conditions in aqueous mediums. Enzyme reactions are normally conducted at low temperatures between 15°C and 45°C, which could be vital for compounds that are thermolabile. Any temperature above 60°C results in an irreversible change in the shape of the protein, causing damage and thus severely affects its catalytic ability (Sowbhagya & Chitra, 2010). Moreover, the efficient release and extraction, modification or synthesis of natural complex bioactive compounds is achieved due to the ideal catalytic ability of enzymes (Gardossi *et al.*, 2009) whereby the integrity of cell walls and membranes is disrupted. A particularly beneficial application of enzymes increases the efficiency of solvent pre-treatment and either decreases the amount of solvent needed for extraction or increases the yield of extractable compounds (Puri *et al.*, 2012).

Cellulases and related polysaccharidases have huge potential ability to convert lignocellulose, the most abundant and renewable energy source on Earth, to glucose and soluble sugars (Coughlan, 1985a, b; Mandels, 1985; Reese, 1976; Reese & Mandels, 1984). EE has been shown to achieve high extraction yields for a variety of compounds including oils, flavours, polysaccharides, natural pigments and medicinal compounds (Wu *et al.*, 2005; Passos *et al.*, 2009; Barzana *et al.*, 2002; Sowbhagya & Chitra, 2010; Yang *et al.*, 2010). The concept of improving the yield of phytochemicals through enzyme treatment is mainly applicable to the extraction of phyto-chemicals from a variety of high-value plant substrates, in particular those with potential application in the prevention and/or treatment of health problems (Puri *et al.*, 2012). Other practical applications include the use of enzymes such as cellulases, hemicellulases and pectinases in juice processing and beer clarification to break down cell walls and improve juice extractability (Puri *et al.*, 2012; Bhat, 2000). Phenolic compounds released into fruit juices through the disruption of the cell wall matrices can result in the quality of the end product being improved (Puri *et al.*, 2012). Furthermore, enzymes are also usually applied in red wine production for clarification purposes where improvement in chromatic (colour) and sensory characteristics of

enzyme-treated wine in comparison with control wine is normally observed (Bautista-ortín *et al.*, 2005).

Among many EE studies, Choudhari & Ananthanarayan (2007) demonstrated that EE of lycopene from tomato tissues using pectinases and cellulases under optimised conditions resulted in a significant increase (206%) in lycopene yield versus control experiments. Wilkens *et al.* (2007), hydrolysed cellulose, hemicellulose and pectin in grapefruit peel waste into monomer sugars by using cellulose and hemicellulose enzymes. In addition, the analysis of an EE method proved to be more suitable for the recovery of catechins (~100% yield) from a variety of milk tea beverages instead of an acid precipitation method (~74% yield) (Ferruzzi & Green, 2006).

Studies have also demonstrated that EE achieves reduced solvent usage, faster extraction, higher recovery and lower energy consumption in comparison to non-enzymatic methods (Puri *et al.*, 2012). Because of environmental and regulatory reasons, decreased solvent use during extraction is particularly important as it provides a “greener” option than traditional non-enzymatic extraction methods. A list of some extracted products of industrial importance obtained using enzyme-assisted extraction is presented in Table 2.5.

Enzymes can be obtained from fungi, bacteria, vegetable/fruit extracts or animal organs (Sowbhagya & Chitra, 2010). For optimal use of enzymes for extraction, it is vital to understand the nature of enzymes, the source of enzymes, their active site, their mode of action, catalytic property, optimal operation conditions and which enzyme or enzyme combination is appropriate for the selected plant material. Moreover, prior knowledge of the cell wall composition of the raw material assists with the selection of an enzyme or a combination of enzymes that are useful for pre-treatment (Puri *et al.*, 2012). Processing variables such as time, pH, temperature and enzyme concentration which affect the operation also need to be optimised for the efficient release of bioactives from plant material.

Table 2.5 List of bioactive compounds of industrial importance obtained by enzyme-assisted extraction from plants

Material	Enzyme/s	Extracted compound	Reference
Plant material	Cellulases, hemicellulase, pectinases	Colours (anthocyanins, carotenoids, chlorophylls etc) and flavours (vanilla, pepper, mustard, citrus etc.)	Sowbhagya & Chitra, 2010
Seeds (sunflower, rapeseed, canola, avocado); coconut, olives	Cellulases, hemicellulases, pectinases	Oils	Dominquez <i>et al.</i> , 1995; Cintra <i>et al.</i> , 1986; Burenrosto & Lopez, 1986; Hernandez <i>et al.</i> , 2000
Unripe apples	Viscozyme L , Celluclast 1.5L & Pectinex 5XL (fungal sources)	Polyphenols	Zheng <i>et al.</i> , 2009
Grape residues and pulp	Celluclast 1.5L , Pectinex Ultra, Vinozyme EC, Vinozym G, Novoferm and Irgazyme M-10	Antioxidants, anthocyanins and leucoanthocyanins	Gómez-García <i>et al.</i> , 2012; Mandzhukov & Velichkov, 1979; Munoz <i>et al.</i> , 2004
Citrus peels (lemon, grapefruit, mandarin and orange)	Cellulase MX, Cellulase CL, Kleerase , Xylan-degrading enzymes, pectinase and Citrozyme CEO	Polyphenols; volatile oil; sugar	Li <i>et al.</i> , 2006; Wilkens <i>et al.</i> , 2007; Mishra <i>et al.</i> , 2005; Coll <i>et al.</i> , 1995
Raspberry waste	Pectinase & cellulase cocktails	Antioxidants	Laroza <i>et al.</i> , 2010
Asparagus	Inulinase	Inulin	Singh <i>et al.</i> , 2006
Pumpkin	Xylase, cellulose, β -glucosidase,	Pectin	Ptichkina <i>et al.</i> , 2008

	endopolygalacturonase and pectinesterase		
Cassava	Pectinase	Starch	Dzogbefia <i>et al.</i> , 2008
Lentils and white beans	Glucoamylases	Proteins	Bildstein <i>et al.</i> , 2008
Black current juice and juice press residue	Econase CE, Pectinex Smash, Pectinex BE-3L, Pectinex Ultra SP-L and Biopectinase CCM.	Antioxidants, phenols and anthocyanins	Landbo & Meyer, 2001; Buchert <i>et al.</i> , 2005
Pomegranate peel	Cellulases, pectinases and proteases cocktail	Polyphenols	Mushtaq <i>et al.</i> , 2016
Watermelon	Kemzyme cocktail – pectinase, endo- β -glucanase, α -amylase, endo- β -xylanase and protease	Antioxidant phenolics	Mushtaq <i>et al.</i> , 2015
Marigold flower	Viscozyme, Pectinex, neutrase, corolase, HT-proteolytic, cellulases, hemicellulases and pectinases	Carotenoids	Barzana <i>et al.</i> , 2002; Navarette <i>et al.</i> , 2004; Delgado-Vargas and Pardes-Lopez, 1997; Delgado-Vargas and Pardes-Lopez, 2002
Tomato	Pancreatin, Cellulase, pectinase, pectophoetidum, celloviridin and Rohamet R max	Lycopene, carotene	Dehghan-Shoar <i>et al.</i> , 2011; Gan & Latiff, 2010;
Tea beverage	Pepsin	Catechins	Ferruzzi & Green, 2006
Flax	Cellulase and glycosidase	Lignans	Renourd <i>et al.</i> , 2010

Vanilla green pods	β -glucosidase and pectinase	Vanillin	Ruiz-Teran <i>et al.</i> , 2001; Ramachandra Rao & Ravishankar, 2000
Olives	Cytolase O, Maxoliva and Bolivia	Oil	Ranalli <i>et al.</i> , 2003; Ranalli <i>et al.</i> , 2004
Ginger, chilli and garlic	Cellulase, hemicellulase, pectinase and amylo glucosidase	Capsaicinoids, carotenoids, oil and liquid flavour bases	Santamaria <i>et al.</i> , 2000; Brouard- Fenie, 1998; Tomoyuki, 1999; Kenkyusho, 1993

Enzyme-assisted extraction of bioactive compounds has potential commercial viability but also has technical difficulties associated with it: (i) the processing of large volumes of plant material with enzymes becomes expensive; (ii) current available enzyme preparations cannot fully hydrolyze plant cell walls thus limiting extraction yields of compounds; (iii) it can be difficult to scale enzyme-assisted extraction to industrial scale as the behavior of enzymes is affected by the change in environmental conditions such as nutrient availability, dissolved oxygen and temperature (Puri *et al.*, 2012).

Nonetheless, enzyme-assisted extraction methods are gaining more attention due to the demand for eco-friendly extraction methods and technologies. Should the above limitations be overcome, enzyme-assisted extraction could provide an opportunity to not only increase extraction yields, but also enhance end product quality by enabling the use of milder processing conditions such as lower extraction temperatures. The investigation of the interaction and stability of enzymes with other food ingredients during processing and storage is an important area of research. In addition, a more in-depth understanding of the polysaccharide structure of the plant material and the use of specific enzymes for improved hydrolysis would assist the enzyme to better reach the active site. The further improvement of extraction techniques is needed and can be done if tailored enzymes are produced, either through the screening of available biodiversity, genetic engineering approaches, or a combination of both (Puri *et al.*, 2012). Enzyme-based extractions are, therefore, a subject of continuing research and have the potential to be commercially attractive. Research on enzyme-assisted extraction of rooibos tea is discussed in the following section.

2.6.5.1. Enzyme-assisted extraction of black and green teas

Commercial green and black teas are produced from the *Camellia sinensis* plant where only the tender shoots of the plant are processed due to their rich polyphenol and endogenous enzyme content (Bhatia & Ullah, 1968; Sanderson, 1972; Forrest & Bendall, 1969; Ota *et al.*, 1968; Jain & Takeo, 1984). A study to determine the effect of additional enzymes to the traditional black tea manufacturing process was conducted by Ravichandran & Parthiban (1998) where commercial enzymes made up of pectinase and cellulase were used. A marked enhancement in liquor colour, soluble solids and sensory properties was observed. Therefore tea processing with the supplementation of enzymes was able to enhance the black tea quality markedly in terms of cuppage and creaming properties.

The maceration of black tea leaves has also been enhanced by the application of cellulolytic enzymes such as cellulases, xylanases, pectinases, tannases, proteinases and laccases which are capable of degrading the cell wall of tea leaves (Murugesan *et al.*, 2002; Angayarkanni *et al.*, 2002; Sariri *et al.*, 2006; Lu *et al.*, 2009; Pengilly *et al.*, 2008; Chandini *et al.*, 2011). White-rot fungi have also become of great biotechnological interest due to their renowned ability to produce polysaccharases and laccases which are able to convert insoluble lignocellulosic plant material, which is abundant in tea plant

material, to soluble substances (Morais *et al.*, 2001). A number of black tea quality parameters such as thearubigin and theaflavin levels, total soluble solids, total liquor colour and extractable dry matter have previously been improved by crude enzyme extracts of *Aspergillus flavus*, *Aspergillus niveus* and *Aspergillus indicus* (Angayarkanni *et al.*, 2002). Moreover, the combination of cellulose (from *Aspergillus niger*) treatment with solvent pre-treatment has shown to the ability to further increase the extractability of a number of functional and nutritional components such as polyphenols, reducing sugars and catechins from green tea waste (Kim *et al.*, 2010).

2.6.5.2. Enzyme-assisted extraction of rooibos

Fermented rooibos, exhaustively extracted with hot water, contains ca. 20% soluble solids (Joubert, 1984), making the extract expensive. A decrease in extract production costs could be achieved through higher extraction yields and higher production capacity (Pengilly *et al.*, 2008). Rooibos shortages due to persisting droughts and ever-increasing demands for the product have, therefore, driven the development of new processes and technologies for the improvement of extraction efficiencies of both traditional and green rooibos during the production of extracts (Pengilly *et al.*, 2008). However, the insoluble nature of the cellulosic backbone of the rooibos stalk plant material makes extraction of soluble matter difficult (Zwane, 2014).

The use of enzymes for the hydrolysis of rooibos plant material has not been explored widely. However, a few studies have reported the application of fungal and commercial enzymes for their potential ability to enhance the quality of rooibos plant material, i.e., improving the extraction of soluble solids and aromatic compounds from rooibos, increasing the polyphenol content and antioxidant capacity of rooibos soluble solids, and enhancing the colour formation in green rooibos (Pengilly *et al.*, 2008; Coetzee *et al.*, 2014; Zwane, 2014). Fundamentally, the complex rooibos plant material polysaccharide structure is macerated by cellulose-targeting polysaccharases which are able to cleave chemical bonds which ultimately result in an improvement of the quality of the plant material.

Five food grade fungal species, which are capable of producing oxidative and hydrolytic enzymes during wood decomposition, were screened by Pengilly *et al.* (2008) amongst others to enhance the extraction of soluble matter and polyphenols from fermented rooibos plant material. The five food grade fungi studied included an Asian shiitake mushroom, *Lentinula edodes* (Berk.) Pegler, which is capable of producing high levels of oxidases and hydrolases (including laccases) in the process of lignocellulosic waste bioconversion (Nagai *et al.*, 2003; Silva *et al.*, 2005a; Zhao & Kwan, 1999). It has been reported by Galhaup *et al.* (2002) that laccases (benzenediol:oxygen oxidoreductases: EC 1.10.3.2) utilise polyphenols as substrates and play a vital role in polymerisation reactions. Laccase activity was due to the potential application to enhance browning of rooibos tea to shorten the traditional fermentation process. The valued filamentous fungus, *Rhizopus oryzae*, is used, amongst

others, for the production of fermented foods, industrial enzymes, organic acids and corticosteroids (Skory, 2004). *Pleurotus ostreatus* var. *florida*, commonly known as the oyster mushroom, is a commercially important edible mushroom commonly produced for human consumption. Along with *Pleurotus djamor*, these fungi make up almost 25% of the global production of cultivated fungi (James et al., 2004; Penas et al., 2002). Lastly, *Aspergillus niger*, with GRAS status, is an excellent producer of citric acid and is considered the most biotechnologically important fungal species. One of its many food industry uses include the production of glucoamylase (Silva et al., 2005b). Therefore, fungal strains representing *A. niger*, *L. edodes*, *P. djamor*, *P. ostreatus* var. *florida* and *R. oryzae* were characterised according to their cellulase (endoglucanase), xylanase, pectinase, and laccase activities (Table 2.7).

For an enzyme to work effectively, the composition of the plant material must be well known. Dried fermented rooibos plant material has been found to contain approximately 42% cellulose and 27% lignin (Pengilly et al., 2008). This finding therefore suggested that cellulases would be most effective for the hydrolysis of rooibos plant material for the release of soluble solids. Moreover, a neutral sugar analysis of polysaccharides found in fermented rooibos plant material (Table 2.6) demonstrated that glucose and xylose are in higher abundance, making up 67% and 21% of sugar moieties, respectively (Pengilly et al., 2008). Therefore, the release of glucose could be achieved via the combined action of enzymes, including endoglucanases, whereas xylose showed that xylanases could contribute towards the supplementary role in the maceration of the plant material (Pengilly et al., 2008). The extraction efficiency was affected greatly by the type of culture medium used to produce each enzyme cocktail. It was of utmost importance that the enzyme cocktails produced contained high levels of enzymes distinguished as essential for the addition of value to rooibos (Table 2.7) i.e., endoglucanase, pectinase, xylanase, (improved solubility of plant material) and laccase (improved colour and flavour).

According to Pengilly et al. (2008), some fungal enzyme cocktails were able to improve the soluble solids yield (*Lentinula edodes* and *Rhizopus oryzae* cultured in yeast peptone-wheat straw medium) or the antioxidant yield (*R. oryzae* cultured in potato dextrose or yeast peptone-wheat straw medium) from fermented rooibos. When industrial simulations were performed, *Rhizopus oryzae* extracts were able to increase soluble solid yields from fermented rooibos by 30%, where in comparison to untreated samples, the total polyphenol content was 39% higher. The application of *L. edodes* (cultured in yeast peptone-wheat straw medium) to green rooibos resulted in the enhancement of the release of soluble solids, as well as the formation of colour, expected from the laccase activity. Furthermore, it was evident that the performance of the enzyme cocktails on the different plant materials, i.e. green and fermented rooibos, were determined by the varying fungal strains and culture conditions. Extraction of total polyphenols and flavonoids from fermented rooibos was performed best by the *R. oryzae*-PD cocktail whereas the *R. oryzae*-YP-wheat straw cocktail was more effective for extracting soluble solids, which is most likely attributable to the high levels of endoglucanase and xylanase in the crude enzyme extract. It was therefore concluded that a combination of cellulose and

xylanase activities are needed for maceration of rooibos plant material for the release of additional soluble solids (Pengilly *et al.*, 2008).

Commercial enzymes and their activities applied to green and fermented rooibos by Coetzee *et al.* (2014) are listed in Table 2.8. Coetzee *et al.* (2014) reported that pre-treating fermented and green rooibos plant material with enzymes suitable for food production such as ferulic acid esterase, cellulase and/or pectinase resulted in higher soluble solids yields when extraction were performed with hot water, while the total polyphenol yield was increased by pre-treatment with ferulic acid esterase and β -glucanase/ β -xylanase. An increase of up to 33% in the soluble solids yield of green and fermented rooibos under simulated industrial extraction conditions was achieved when pectinase and β -glucanase/ β -xylanase were combined. However, there was a decline in the antioxidant and total polyphenol content of soluble solids due to the extraction of non-polyphenolic soluble compounds. Furthermore, the sensory attributes of the extracts were not affected and were clear when assessed visually. Enzyme application, however, resulted in the reduction of the extract aspalathin content. Nonetheless, an increase in isoorientin, the aspalathin flavone counterpart, with significant pharmaceutical value was noted. Given the susceptibility of aspalathin to be converted to flavones, their respective decrease and increase in content of the soluble solids is mostly likely responsible.

A recombinant *Aspergillus tubingensis* ferulic acid esterase Type A (FAEA), expressed in *Aspergillus niger* D15#26, was explored by Zwane (2014) for its potential to improve the extraction and release of polyphenolic compounds from green and fermented rooibos plant material. Similar observations noted by Coetzee *et al.* (2014) were noted in this study. An increase in soluble solids yields was achieved for green and fermented rooibos, however, the increase in soluble solids yields was accompanied by a decrease in the total polyphenol and antioxidant content of green and fermented rooibos. Once again, it was suggested that this decrease was attributable to the release of non-phenolic compounds. A significant increase in the levels of ferulic acid (53%), isoquercitrin (33%) and luteolin-7-glucoside (150%) was enabled by the enzyme cocktails used.

From the abovementioned studies, it is evident that the use of hydrolytic enzymes could assist in the extraction of soluble matter and the release of polyphenols from rooibos plant material. Furthermore, there is potential for the application of a wider range of enzymes for value-addition to rooibos plant material. Moreover, enhanced extraction yields will reduce raw material costs, which in turn could create new opportunities in the market.

Table 2.6 Neutral Sugar Analysis of Polysaccharides in Fermented Rooibos (Pengilly *et al.*, 2008).

Monosaccharide	Mole fraction ¹
Glucose	0.668
Xylose	0.206
Arabinose	0.042
Galactose	0.035
Mannose	0.026
Rhamnose	0.020
Fucose	0.003

¹Fraction each monosaccharide represented of the total measurable neutral sugars

Table 2.7 Quantitative and qualitative analysis of selected enzymes in 10-fold concentrated fungal extracts (Pengilly *et al.*, 2008).

Cocktail	Activity (IU/mL)			
	Endoglucanase	Pectinase	Xylanase	Laccase
<i>R. oryzae</i> YP- wheat straw	8.1	1.2	344.5	583.7
<i>A. niger</i> -YP-wheat straw	7.3	1.7	183.1	570.3
<i>P. djamor</i> - YP- wheat straw	4.6	2.2	9.6	594.6
<i>P. ostreatus</i> var. <i>florida</i> - YP- wheat straw	3.9	1.2	14.0	568.9
<i>L. edodes</i> - YP- wheat straw	1.2	3.3	4.1	176.1

*The best two values for each type of enzyme activity are indicated in bold

Table 2.8 Commercial enzymes and activities (as specified by the manufacturers) (Coetzee *et al.*, 2014).

Commercial enzyme	Activities	Concentration (mg total protein/ml)	Source
Depol™ 112L	7000 U/g β -glucanase, 4000 U/g xylanase	82.66	Biocatalysts
Depol™ 670L	1200 U/g cellulase, 800 U/g pectinase, 7 U/g ferulic acid esterase	85.12	Biocatalysts
Depol™ 692L	800 U/g cellulase, 3 U/g ferulic acid esterase	66.93	Biocatalysts
Depol™ 740L	36 U/g ferulic acid esterase	47.84	Biocatalysts
Pectinex® Ultra SP-L	26 000 PG/ml pectinase	55.28	Novo Nordisk

2.7. Quality-by-design (QbD) methodology

2.7.1. General overview of QbD

The concept of quality-by-design (QbD) was first introduced in 1992 and it refers to the effective design of desired quality attributes of a product into the manufacturing process rather than being dependent on post-production quality testing (Juran, 1992). This concept has been authorised and widely applied in the biopharmaceutical industry (ICH, 2009; Rathore & Winkle, 2009). The initial phase of the QbD process is the identification of the critical material attributes (CMAs) and critical process parameters (CPPs) which have an effect on the critical quality attributes (CQAs) of the final product. Ultimately, the end goal is the description of a “design space”, i.e. the ranges of CQAs which would be adequate for a specific unit operation (Huang *et al.*, 2009; Lebrun *et al.*, 2012; Das *et al.*, 2014; Gong *et al.*, 2014a; Gong *et al.*, 2014b; Yan *et al.*, 2014).

QbD provides numerous tools for the evaluation of feasible process inputs in order to determine which of them would have the most significant effect on the output. The Ishikawa (fishbone) diagram, a cause and effect diagram, becomes very useful for identifying and grouping potential factors that are expected to cause a variation within the system (Nagar *et al.*, 2010). Figure 2.7 demonstrates an Ishikawa diagram as presented in a study by Gong *et al.* (2015) in which

process parameters that could affect the extraction of Danhong injection were identified. Major process input categories were identified as materials, equipment, environment and extraction-related factors. Therefore, these categories were populated by specific factors known to have an effect on the active ingredient yields and dry matter yield, based on a review of existing data and prior experience.

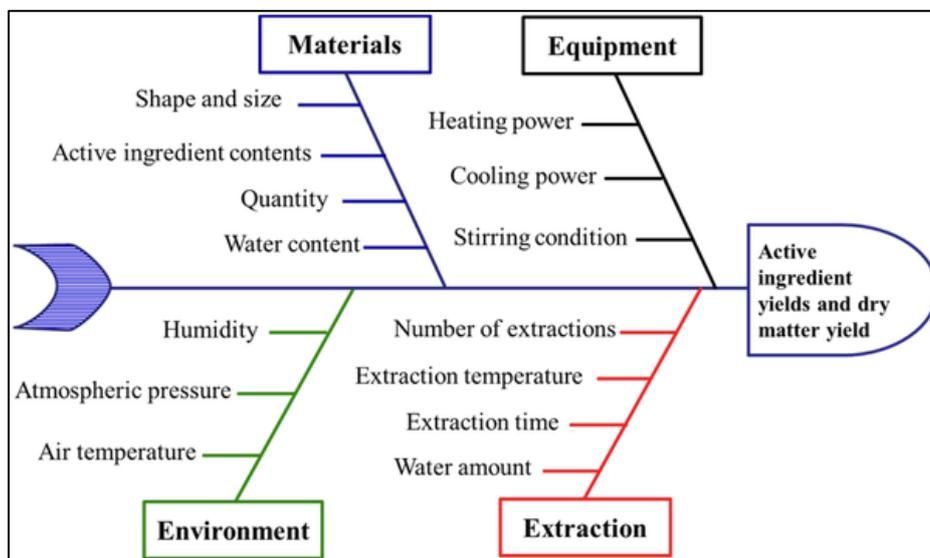


Figure 2.7 Ishikawa diagram demonstrating inputs of extraction process of Danhong injection (Gong *et al.*, 2015).

2.7.2. General overview of Response Surface Methodology (RSM)

Process optimisation aims to increase yields and minimise costs, which ultimately results in improved performance (Baş & Boyacı, 2007; Huang *et al.*, 2009). It can be performed via the use of a one-variable-at-a-time technique (OVAT) whereby a single variable is optimised at a time. However, the OVAT technique is not always feasible for the optimisation of more than one variable, as a large number of time-consuming experiments are needed and result in the consumption of raw material and reagents, which lead to increased expenses (Baş & Boyacı, 2007; Bezerra *et al.*, 2008). For simultaneous multivariate analysis, RSM has become a widely used optimisation method (Baş & Boyacı, 2007). Many recent applications for optimising extraction of phenolics from a number of botanicals can be found in literature. Some examples include the extraction of polyphenols from olive leaf extract (Şahin & Şamli, 2013), phenolic compounds, antioxidant and anthocyanin from sugar beet molasses (Chen *et al.*, 2015), phenolic antioxidants from green tea (Lee *et al.*, 2013), phenolics from onion solid waste (*Allium cepa*) (Kiassos *et al.*, 2009) and soluble solids and aspalathin from green rooibos (*Aspalathus linearis*) (Miller *et al.*, 2017).

2.7.3. RSM Principles

RSM is an effective statistical and mathematical technique that is useful for the development, improvement and optimisation of processes in which various independent variables influence a response of interest that needs to be optimised (Baş & Boyacı, 2007; Bezerra *et al.*, 2008; Dejaegher & Vander Heyden, 2011). RSM application is essential especially in the design, development and formulation of new products and in the improvement of existing product designs. It also defines the effect of independent variables, alone or in combination, on the process (Baş & Boyacı, 2007).

A response is defined as an observed or measured quantity that needs to be optimised (Hibbert, 2012), for example the extraction yield of antioxidants. The response is a result of the interaction of independent experimental factors. On the other hand, a factor is an independent parameter that has an effect on the response, such as solvent composition, extraction time and temperature, which are critical factors for the optimisation of extraction of substances. Therefore, RSM is an efficient substitute for the OVAT technique, which is unable to explain interaction between factors. Time and money are thus saved in the process and a great deal of information is obtained with the minimal number of possible experiments (Baş & Boyacı, 2007; Bezerra *et al.*, 2008; Dejaegher & Vander Heyden, 2011).

2.7.4. RSM experimental design

An optimisation study can be separated into three phases. The primary phase is the phase in which preliminary trials are conducted for the identification of independent factors where their levels are tested as well. The identification of factors that have a large effect on the response occurs at this stage and only two or three factors are usually optimised. A larger number of experiments will be required if more than three factors are chosen and as a result, the entire response surface will not be visualised and it becomes more difficult to determine optimal conditions (Dejaegher & Vander Heyden, 2011). The secondary phase is the selection of an experimental design and the prediction and verification of the model polynomial equation, which describes the relationship between factors and a response. The level of a factor refers to the value ascribed to that factor and the number of factor levels typically used to name the design type, e.g. two or three-level design (Leardi, 2009). A full factorial design contains every possible combination of factors at the desired levels, i.e. there are L^k combinations of k factors at L levels (Hibbert, 2012). The setting of different factor levels, e.g. particle sizes for extraction or different operating temperatures, should be carefully considered as it could complicate the optimisation process if extremes of experimental ranges, or levels which are too close, are used (Das *et al.*, 2014). Therefore, if factors have more than two levels, this can result in a need for a large number of experiments, which is time-consuming. However, a central composite design (CCD) can

be a better option as factors can be tested at five levels instead of three, in a fewer number of experiments. A CCD consists of a two-level full factorial design at factor levels -1 and $+1$, a star design at factor levels 0 , $-\alpha$ and $+\alpha$ and a centre point at factor level 0 (Dejaegher & Vander Heyden, 2011). It can be utilised for the optimisation of two or three factors (Figure 2.8). The selection of factor levels such as different temperatures or solvent concentrations is mandatory as it defines the efficiency of the optimisation (Hibbert, 2012). The tertiary phase of the process is obtaining the response surface plot and contour plot of the response as a function of the independent parameters and determination of optimum points (Baş & Boyacı, 2007).

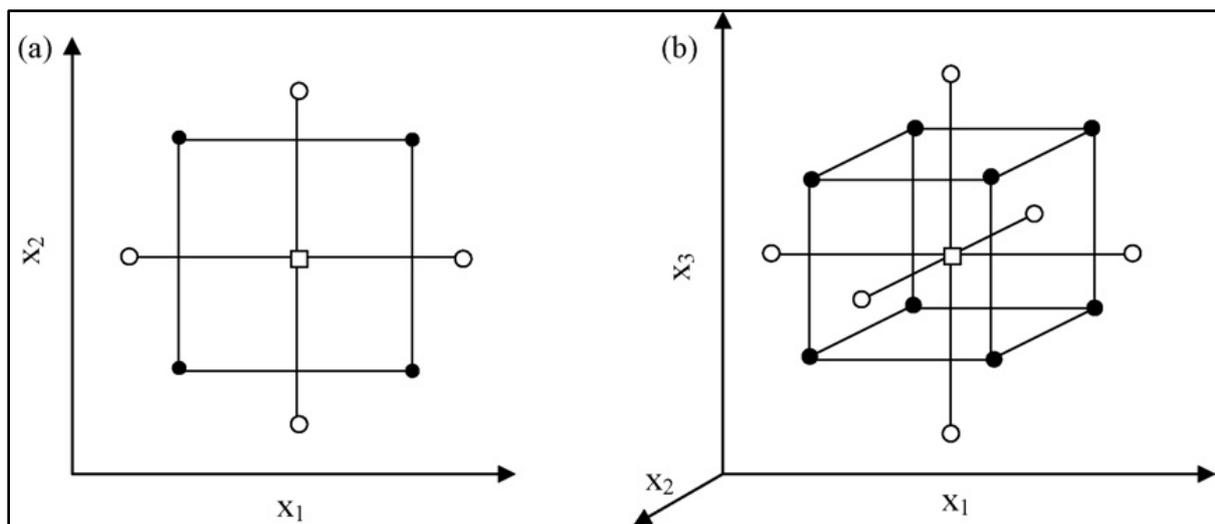


Figure 2.8 Central composite design (CCD) for the optimisation of (a) two variables ($\alpha = 1.41$) and (b) three variables ($\alpha = 1.68$). (●) Points of factorial design, (○) axial points and (□) central point (Bezerra *et al.*, 2008).

2.7.5. RSM data modelling and interpretation

Upon completion of the experiments according to a selected experimental design, a model equation is established and regression coefficients are predicted (Bezerra *et al.*, 2008). In RSM, a second order polynomial model is typically used for fitting data:

$$y = \beta_0 + \sum_{j=1}^k \beta_j X_j + \sum_{j=1}^k \beta_{jj} X_j^2 + \sum_{i < j} \beta_{ij} X_i X_j + \varepsilon$$

where β_0 , β_i , β_{ii} , and β_{ij} represent regression coefficients for intercept, linear, quadratic and interaction terms, respectively. The response variable (dependent variable) is represented by y , and X_i and X_j represent the level of the independent variables (factors). The term k represents the number of factors to be investigated, while ε represents the residual error associated with the experiment (Baş & Boyacı, 2007; Bezerra *et al.*, 2008; Dejaegher & Vander Heyden, 2011). Thereafter, analysis of variance (ANOVA) is applied to determine how well the generated model fits the data. ANOVA

compares the variation due to different treatments with variation due to random errors inherent to the measurements of the generated responses, which is represented by the coefficient of variation (R^2) (Bezerra *et al.*, 2008). Additionally, the statistical significance of the factors and their interactions on the measured responses are estimated by ANOVA. The experimental procedure is repeated to validate the proposed model and to compare experimental results to predicted values.

The regression (predicted model) equation is graphically depicted by two dimensional contour plots or three dimensional response surface plots. They demonstrate the type of interaction (significant vs. negligible) between the factors that are tested and their relationship to measured responses (Baş & Boyacı, 2007; Bezerra *et al.*, 2008). In addition, the response surface plot demonstrates the scale of a response value as a result of the combined effect of two factors at a particular time or condition (Yang *et al.*, 2010). If three or more variables are present, the plot visualisation is only possible if one or more variables are kept at a constant level. If a slope on the response surface plot is steep, showing a maximum response, minimum response or a saddle point, the response is significantly affected by a change in factor levels, whereas a flat surface indicates a non-significant effect. The optimum value in a range of tested parameters is also referred to as the critical/stationary point and it can be determined by using the second order polynomial equation. A single optimum value in RSM cannot always be identified. Therefore, in such cases an optimum region of values can be displayed on the response surface plot instead (Bezerra *et al.*, 2008, Granato & De Araújo Calado, 2014).

Figure 2.9a and Figure 2.9b show response surfaces where the maximum response is found within the space of the experimental design. However, Figure 2.9b differs as it demonstrates a plateau in relation to variable X_2 , which means that the change in its levels has no effect on the level of the response (y). In Figure 2.9c, the maximum response does not lie completely within the experimental region. In this case, the experimental design would have to be altered to get an optimum response, i.e. the experimental design would have to include extended ranges of the independent variable. A minimum point found within the experimental region is shown in Figure 2.9d, and a saddle point, which represents an inflexion point between a relative maximum and minimum, is shown in Figure 2.9e. Saddle point coordinates, however, are not considered as valid optimal values when the aim is to obtain a minimum or a maximum response in a system (Bezerra *et al.*, 2008).

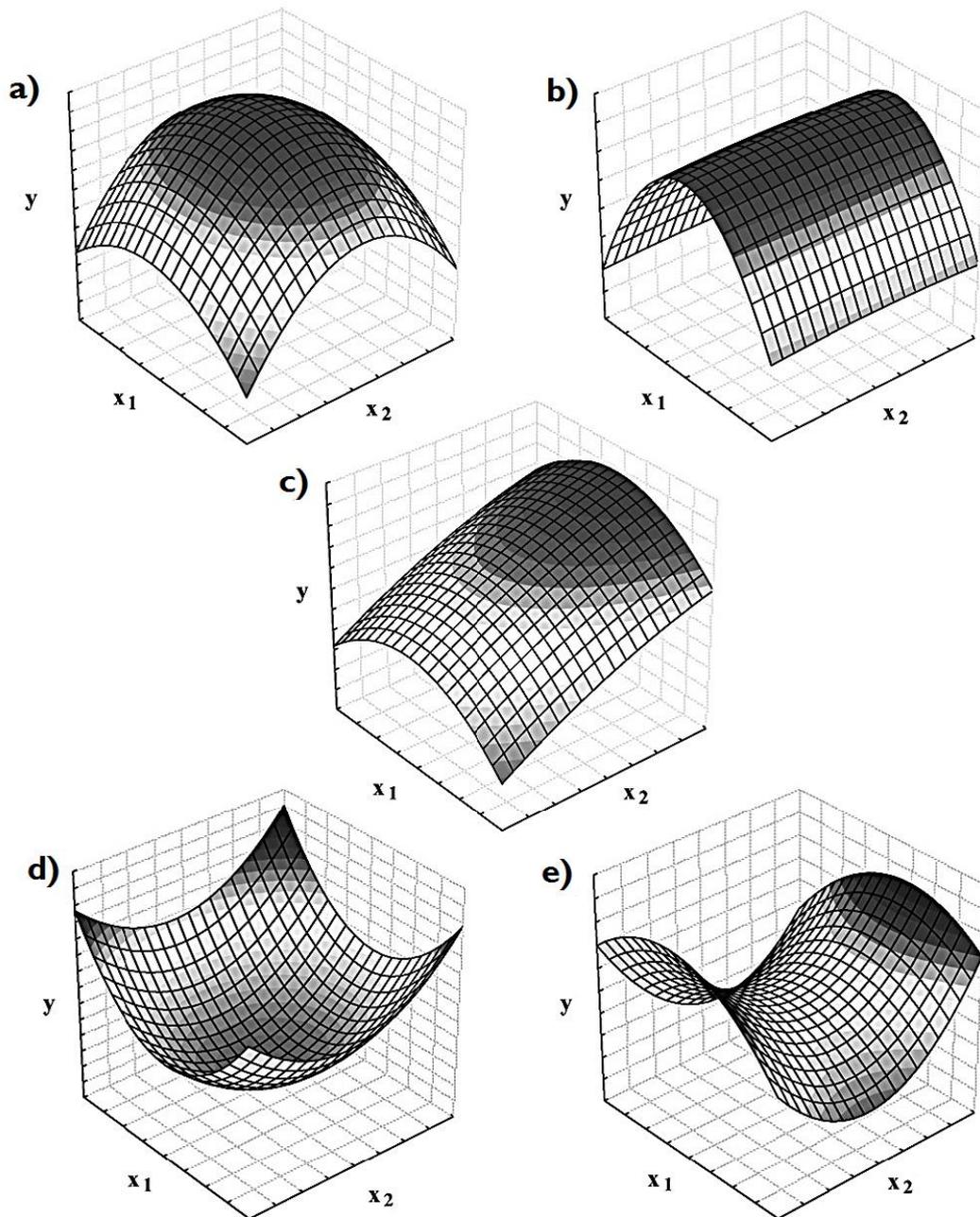


Figure 2.9 Examples of response surface plots obtained in the optimisation of two variables, X_1 and X_2 , showing (a) maximum, (b) plateau, (c) maximum outside the experimental region, (d) minimum, and (e) saddle surfaces (Bezerra *et al.*, 2008).

Zhang *et al.* (2012) successfully used RSM for optimising the extraction of tea polyphenols, (-)-epigallocatechin gallate and theanine from summer green tea. The combined effect of extraction temperature (axis A) and ratio (axis B), amongst others, on the yield of polyphenols (vertical axis/axis) was visualised on a three dimensional response surface plot which demonstrated that a maximum response was attained within the experimental range (Figure 2.10).

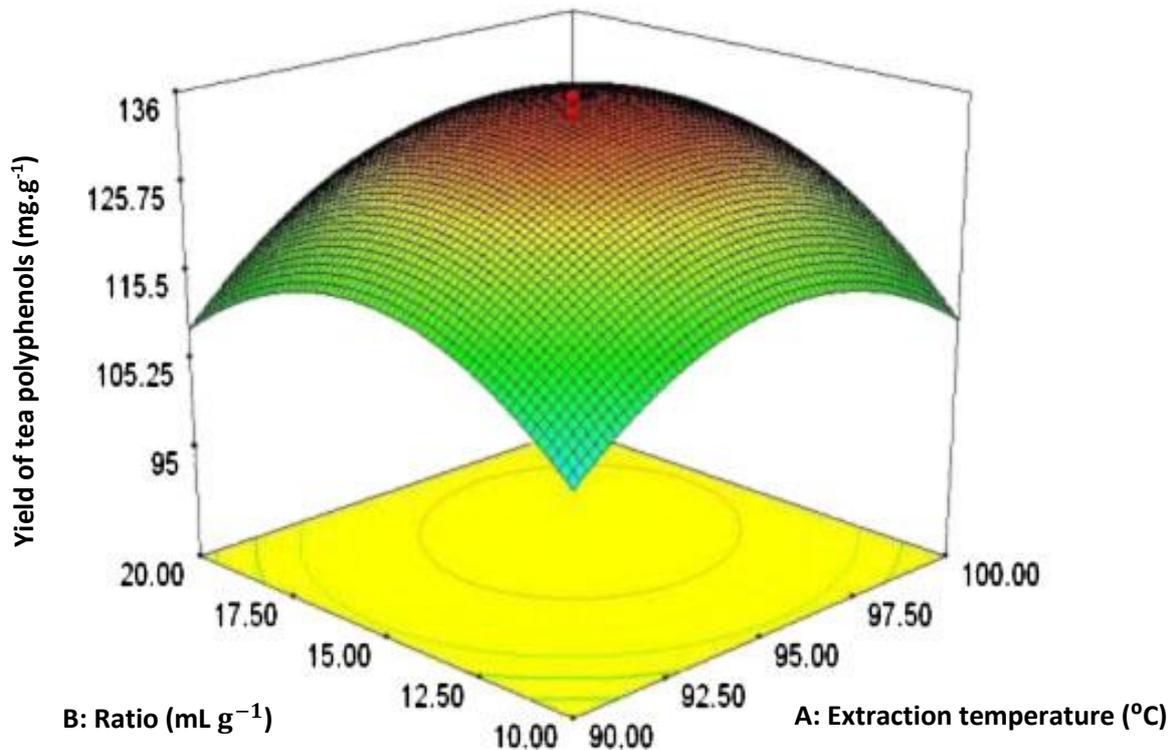


Figure 2.10 Response surface plot demonstrating the effect of two process variables on the yield of summer green tea polyphenols (Zhang *et al.*, 2012).

Two-dimensional contour plots can also be used to illustrate response surfaces, where plotlines that are close together (i.e. darker areas) indicate that minor alterations to the input factors are associated with significant changes in the response value. Significant interactions are represented by elliptical contour plots, while non-significant interactions are represented by circular plots. (Steinberg & Bursztyn, 2010). Zhu *et al.* (2014) made use of RSM for the optimisation of enzyme-assisted extraction and characterisation of polysaccharides from *Hericium erinaceus*, using two-dimensional contour plots for the illustration of the effect of three process variables on the extraction efficiency. Figure 2.11 shows the effect of pH (X_1) and extraction time (X_3) on the polysaccharide yield at a fixed temperature. The elliptical shape of the contour plot indicates a significant quadratic effect where the pH was 5.7 and the extraction time was 33.79 min.

Response surfaces are also typically visually illustrated as three-dimensional (3D) surface plots in combination with their corresponding two-dimensional (2D) contour plots (Figure 2.12). Chen et al. (2012) optimised the ultrasound assisted extraction of water-soluble polysaccharides from *Boletus edulis* mycelia and utilised combined plots to demonstrate the effect of process variables X_1 (ratio of dried mycelia to water) and X_2 (extraction time) on extraction yield. The elliptical shape of the contour plots shows that there was a significant interaction between these two variables, and the optimal ranges were identified as 1:55 (ratio of dried mycelia) and 8.4 min (extraction time).

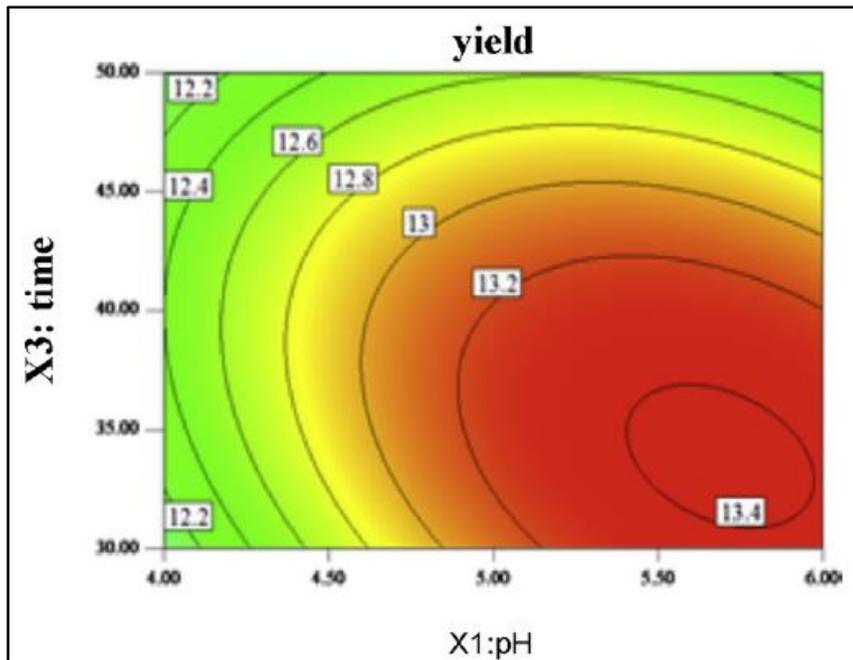


Figure 2.11 Elliptical contour plot demonstrating the combined effect of pH (X_1) and extraction time (X_3) on the extraction yield of polysaccharides from *Hericium erinaceus* (Zhu et al., 2014).

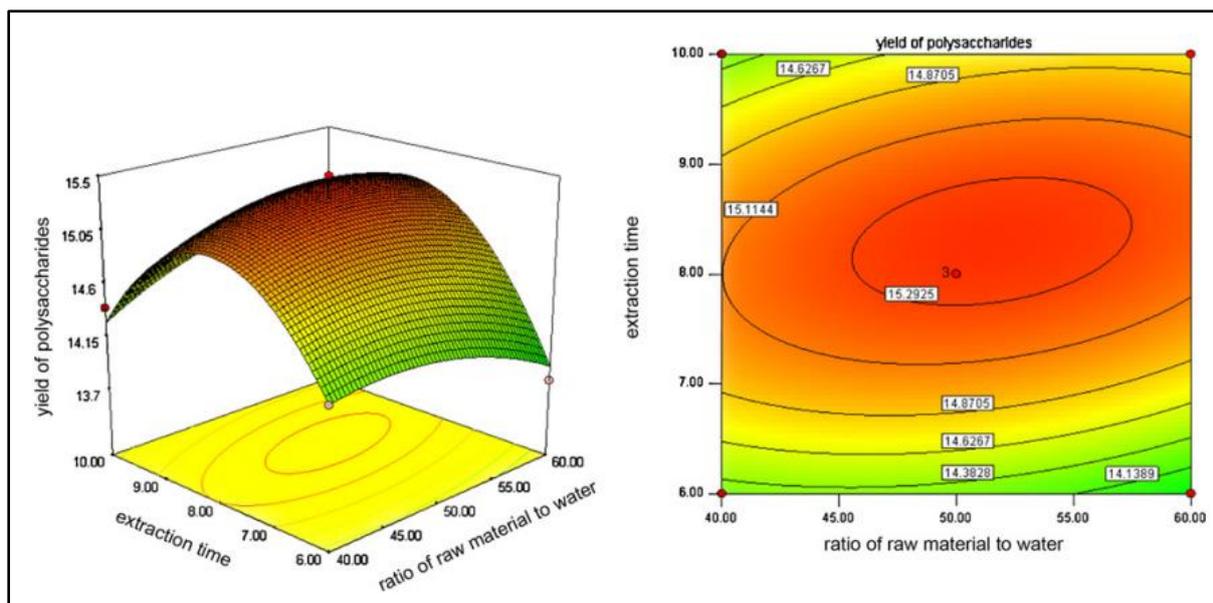


Figure 2.12 Combined response surface plot (A) and corresponding contour plot (B) demonstrating effects of two variables (X_1 = ratio of dried mycelia to water; X_2 = extraction time) on extraction yield (Chen *et al.*, 2012).

A standardised Pareto chart ranks the response, identifying the most critical parameters. It is also used to graphically demonstrate the significance of linear, quadratic and interaction effects. Factors and their interactions are represented by horizontal bars, and those bars which intersect the vertical line represent significant effects at 5% level of significance ($P=0.05$). The magnitude of its effect is proportional to the length of a horizontal bar, where a negative effect on the measured response is indicated by a negative value bar length (Das *et al.*, 2014).

Silva *et al.* (2011) studied the effects of temperature, catalyst concentration, reaction time and molar ratio of alcohol in relation to oil on the transesterification of soybean oil with ethanol, and presented their ANOVA data in a standardised Pareto chart (Figure 2.13). The magnitude and significance ($P<0.05$) of the linear, quadratic and interaction effects of the four tested factors on the ethyl esters produced was depicted graphically in the form of a Pareto chart. Six bars with positive effect estimate values out of the fourteen horizontal bars representing these terms crossed the vertical black line which denotes the 5% significance level. The linear effects of molar ratio (1) and catalyst concentration (2) had the most significant positive effect on the response, as portrayed in the relative size of their bars and the standardised effect values (12.701 – 16.463). The interaction effects of molar ratio and time, molar ratio and temperature and the quadratic effects of temperature and time also had a significant, positive effect on the response, but these factors were considerably smaller than those of the two linear terms. However, the interaction effect of molar ratio and catalyst concentration, as well as the linear effect of temperature had a significant, negative effect on the

response. The six terms represented by the remaining six bars on the Pareto chart did not have significant effects on the response.

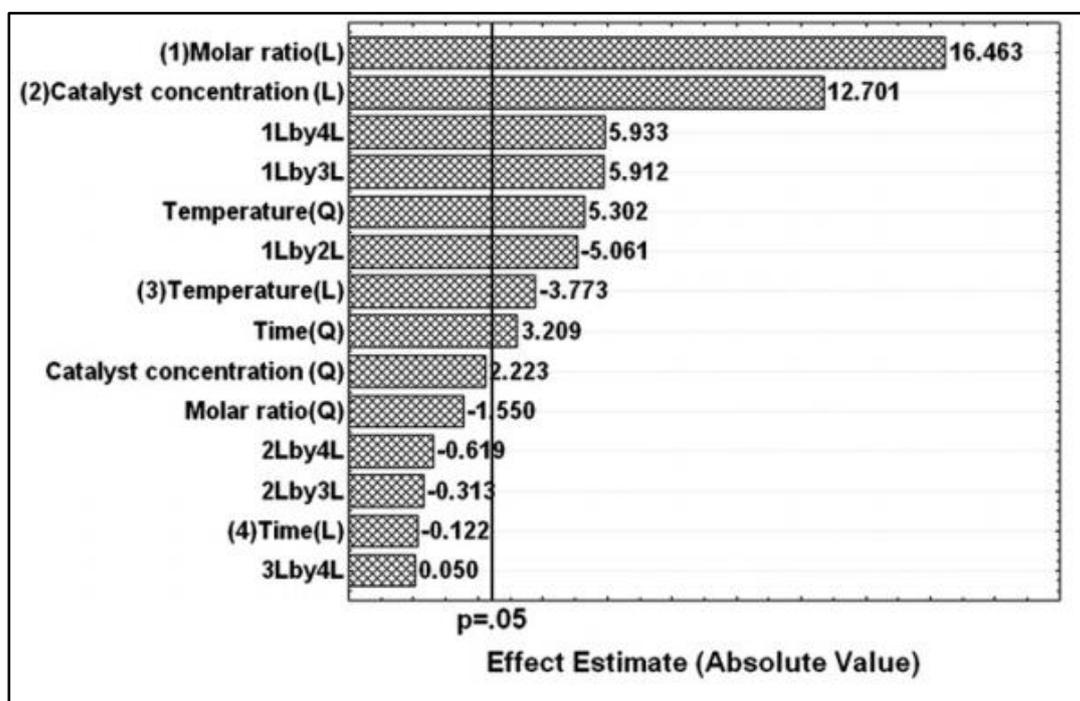


Figure 2.13 Standardised Pareto chart for effects on ethyl esters production. (L) is the linear and (Q) is the quadratic interaction of variables (Silva *et al.*, 2011).

If several responses need to be optimised simultaneously, then multi-criteria methodology such as desirability profiling can be utilised. The levels of factors that result in maximum overall desirability for the process in terms of output are determined by this method (Bezerra *et al.*, 2008). Therefore, an optimal compromise needs to be made since factors can have opposite effects on the measured responses. The individual desirability function for each response is determined by assigning a dimensionless number to the predicted scores, ranging from 0 (very undesirable) to 1 (very desirable), from which an overall desirability function can be obtained. Therefore, this method indicates the level of factors that display optimal overall desirability (Bezerra *et al.*, 2008). Multi-response desirability profiling was utilised by Miller *et al.* (2017) for the optimisation of aspalathin and soluble solids yield in a hot water extraction process for green rooibos (*Aspalathus linearis*). Prediction profiles were generated which demonstrate the effect of the three independent variables under investigation (extraction time, extraction temperature and water-to-plant-material ratio) on the desirability of predicted aspalathin and soluble solids yield (Figure 2.14). The assessment of prediction reliability is aided by the blue horizontal lines on the prediction profiles which indicate 95% confidence intervals. The levels of the independent variables which would result in the most desirable (i.e. optimal) extract (soluble solids) and aspalathin yields are indicated by the vertical red lines that intersect the x-axes and apices of the desirability curves (green). In this study, the optimal levels were an extraction

time of 37 min, an extraction temperature of 93 °C and a water-to-plant material ratio of 23.4:1 (v.m⁻¹). After consideration of operation costs and energy input, the extraction time and water-to-plant material ratio could be reduced to 30 min. and 10:1 v.m⁻¹, respectively, without affecting the desired responses. Therefore a range of values (29-31 min, 90-95 °C and 9-11:1 v.m⁻¹) is more feasible from a process control perspective as it allows for minor deviations in operations.

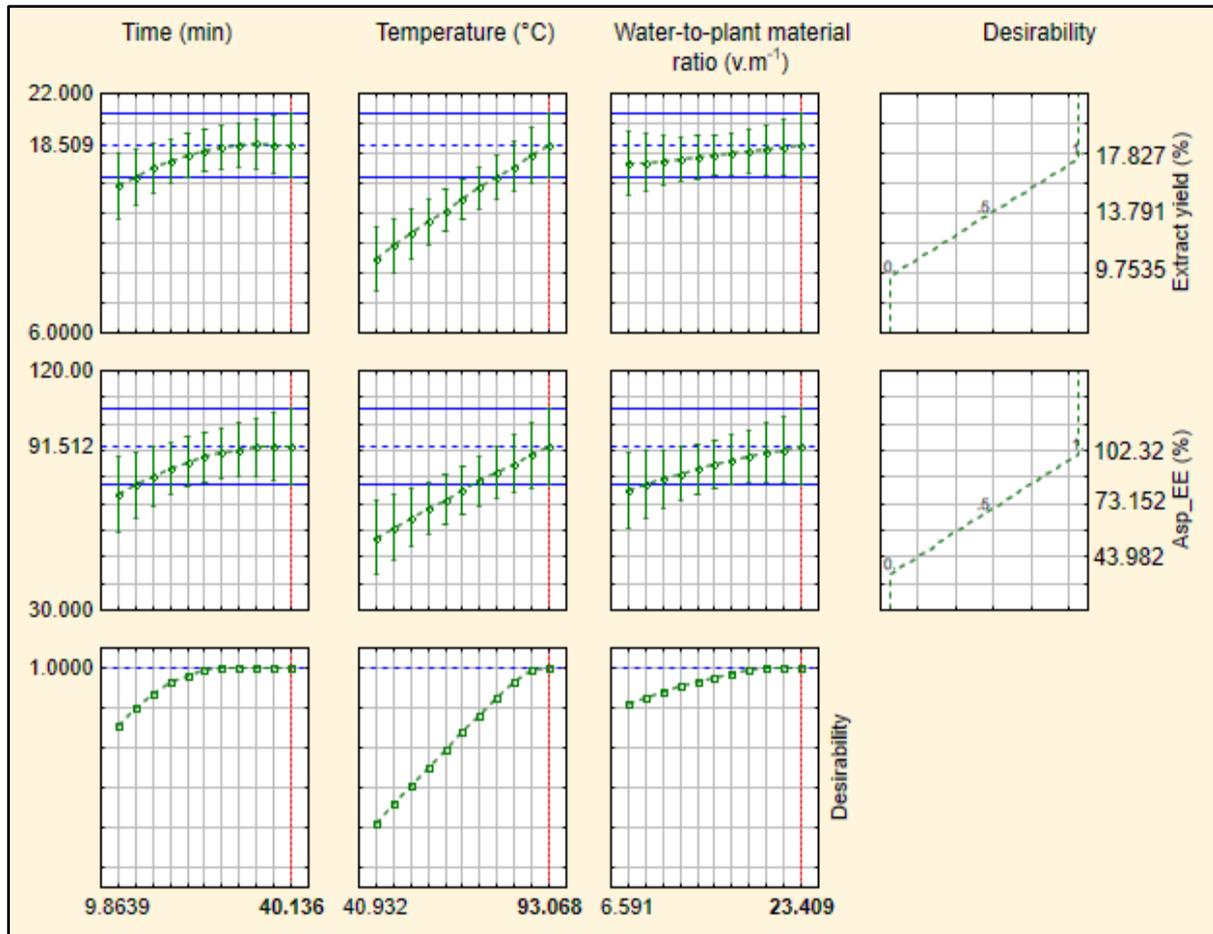


Figure 2.14 Multi-response desirability profiles for maximum extract yield (soluble solids) and aspalathin extraction efficiency in green rooibos (*Aspalathus linearis*) hot water extraction process (Miller *et al.*, 2017).

2.7.6. RSM advantages and disadvantages

As stated before, RSM can generate a large amount of data from a small number of experiments and thus reduce energy and raw material consumption, and wastage of reagents through the optimisation of analytical methods or particular processes. Classical methods require a lot of time and the performance of a system is explained using a large number of experiments (Baş & Boyacı, 2007; Dejaegher & Vander Heyden, 2011). Moreover, RSM allows the observation of the interaction effect between independent parameters on the response, unlike OVAT, which is of vital importance in

biochemical processes due to the occurrence of additive, synergistic or antagonistic reactions (Baş & Boyacı, 2007; Bezerra *et al.*, 2008). On the downside, however, RSM is not capable of fitting all curvature observed in data obtained from different systems to a second order polynomial model. Data can, therefore, be converted to alternative forms such as logarithmic transformation or other linearisation methods (Baş & Boyacı, 2007). Unfortunately, the obtainment of satisfactory results for all systems is not guaranteed, although transformations may be useful. In addition, trying to establish which transformation works best can be a time-consuming, difficult endeavor. Alternatively, a smaller range of independent factors can be selected which can increase the accuracy of the model equation, but decrease the chances of determining the stationary point (Baş & Boyacı, 2007). This thus highlights the importance of effective preliminary work for effective process optimisation.

2.8. Summary

Rooibos tea has found wider local and global consumer appeal with the addition of new markets to the traditional European export markets since the 1990s. Its popularity is attributable to its low tannin and caffeine-free status, along with its associated health benefits. The continuous growth and market expansion of rooibos stresses the importance of guaranteeing that both the consumers and bulk purchasers of rooibos have a constant supply of rooibos and rooibos-derived products.

Despite the challenges that the rooibos industry is currently facing due to rain shortage and unpredictable weather patterns, waste generated during rooibos processing is not re-used to its fullest potential. No research has been conducted on its quality and chemical and sensory attributes, and thus no data is readily available. Moreover, very few studies have documented the use of enzymes for treating rooibos plant for quality enhancement purposes. Therefore, an opportunity to extend annual production using rooibos waste exists. Alternative uses for rooibos waste material would be required to convert the waste material into valuable products of acceptable, if not good, quality.

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3. Optimisation of soluble solids extraction from fermented rooibos dust using response surface methodology

3.1. Abstract

The current shortages of rooibos merited investigation of converting rooibos waste material into a valuable tea product to supply the growing demand. Rooibos “dust” has the potential to be utilised as a source material for the production of extracts, where extracts can be used as a food ingredient or to improve the “tea value” of rooibos stems. Maximising the extraction of soluble matter from rooibos dust was therefore imperative. Two approaches, i.e. enzyme-assisted extraction (EE) and optimisation of the conventional hot water extraction (HWE) process using response surface methodology (RSM), were followed. The dust was treated with three food-grade enzymes (Validase, Rapidase and Filtrase) for two hours, each at varying concentrations (0.05, 0.1, 1, 2, 5 & 10%). The effect of each enzyme on the extract colour, turbidity and phenolic content was also determined. Enzyme treatment resulted only in a slight improvement in the soluble solids (SS) yield with Rapidase being the most effective, delivering an increase of 8.4% SS at the highest dose. The latter extract was significantly lighter and more turbid than the control extract. Therefore, the need to use a high enzyme concentration, combined with the additional treatment time, will increase the extract production costs. Optimisation of the HWE process was thus considered more feasible. From a preliminary investigation, using a one-factor-at-time (OFAT) approach, the individual effects of extraction temperature, plant material-to-water ratio and extraction time on the extraction of SS from rooibos dust were determined. The individual effects of each variable were tested at fixed levels of the remaining variables. Ranges of the selected parameters were identified in which optimal SS yields would most likely be detected. Subsequently multifactorial RSM based on a central composite design (CCD) with three independent variables, extraction temperature (40-94°C), plant material-to-water ratio (1:10-1:30, m.v⁻¹) and extraction time (10-30 min), was used to optimise HWE. Temperature was found to have the largest effect on the extract yield (EY). A prediction model and response surface plots for extract yield (EY: g SS.100 g⁻¹ plant material, %) were generated. Verification of the prediction model displayed satisfactory predictive ability for EY ($R^2_{adj} = 0.988$). Desirability profiling was then applied for the identification of optimal values of the independent variables that would maximise the EY. The optimal dust extraction conditions were: temperature (94°C), time (30 min) and plant material-to-water ratio (1:30 m.v⁻¹). However, the time and plant material-to-water ratio desirability profiles did not increase with each increasing increment as they drew nearer to their experimental range maximum points. The optimal conditions were thus selected on the basis of industrial practicality: temperature (94°C), time (20 min) and plant material-to-water ratio (1:20 m.v⁻¹). Extraction of twenty different production batches of rooibos dust was carried out at the “optimal” conditions to determine the batch-to-batch variation in EY that could be expected. The EY of the twenty different production batches varied between 16.4% and 27.9% due to natural plant material variation and variation introduced during “fermentation”.

3.2. Introduction

Rooibos (*Aspalathus linearis*) has been produced for decades as a herbal tea and more recently, also processed as a food ingredient and nutraceutical extract. The fermented plant material comprises the bulk of rooibos production (Joubert & De Beer, 2011). The growing demand for fermented rooibos, combined with current shortages, provided the motivation for the present investigation into the utilisation of fermented rooibos dust, a waste material, for the eventual production of a valuable rooibos product for the herbal tea market with the same sensory quality parameters as a cup of rooibos tea produced from fermented leaves. The dust waste plant material was used as a plant material source because significant amounts of dust and stems are left over after the production of rooibos tea. Extracts prepared from the dust can be used either as food ingredient extract or to enhance the “tea value” of rooibos stems, another waste product. No data exists on the extract yield from the dust and its properties, in particular its phenolic composition, colour and turbidity when reconstituted to “cup-of-tea” strength. This data are critical for the future utilisation of the waste plant material. Emphasis has been placed on the importance of optimising extraction processes in order to decrease waste generation and limit resource usage; hence sustainability has become a significant differentiation point in the nutraceuticals market (Moloughney, 2016). Extraction time, solvent composition, extraction temperature, particle size and solid:solvent ratio are typical parameters that are usually optimised for extraction of polyphenols from plant material (Liu *et al.*, 2010; Yang *et al.*, 2010; Prasad *et al.*, 2012; Tabaraki *et al.*, 2012; Lai *et al.*, 2013).

A limited amount of published literature, however, is available on fermented rooibos extraction optimisation. The effect of water-to-leaf ratio, extraction temperature and flow rate on the recovery of soluble solids and polyphenol content of extracts from fermented rooibos, through the use of a fixed-bed flow-through batch extraction system, has been evaluated in previous studies in which one-factor-at-a-time (OFAT) analysis was applied (Joubert, 1988; 1990a; 1990b; Joubert & Hansmann, 1990). Higher water-to-leaf ratios, higher extraction temperatures, as well as longer extraction times resulted in improved extraction of polyphenols and soluble matter. More recently, OFAT analysis was applied to green rooibos, using a quality-by-design approach, to evaluate the effects of extraction time, extraction temperature and water-to-plant material ratio on the extract yield and aspalathin extraction efficiency (Miller *et al.*, 2017). Temperature was shown to have the largest effect on both the EY and aspalathin extraction efficiency, followed by extraction time and water-to-plant material ratio.

The variation in overall quality is a noteworthy challenge involved in the use of plant material for the production of extracts, with some raw materials containing suboptimal levels of extractable compounds for commercialisation (Takeuchi *et al.*, 2009). Besides the natural plant material genetic variation, external factors such as seasonal effects, climate, UV-radiation, diurnal cycles and post-

harvest processing techniques may play a role in natural plant material variation (Aherne & O'Brien, 2002; Yao *et al.*, 2005; Di Ferdinando *et al.*, 2013).

The first approach explored for the maximisation of soluble matter extraction from rooibos dust, enzyme-assisted extraction, is a potential alternative method to conventional solvent-based extraction methods as it has been shown to achieve high extraction yields for a number of compounds and plant materials. Moreover, studies have demonstrated that EE achieves reduced solvent usage, faster extraction and lower energy consumption in comparison to non-enzymatic methods (Puri *et al.*, 2012). Solutions for decreasing the amount of water used for extraction purposes is vital taking into account the current water shortages in the Western Cape of South Africa. Therefore, because of environmental reasons, decreased water use during extraction is vital as it provides an eco-friendly extraction option. However, the application of enzymes to rooibos has not been studied extensively and limited information is available. This fact therefore prompted further investigation into the effect of enzymes on rooibos plant material. Existing studies on EE of rooibos have documented that enzymes have resulted in significant increases in extract yields when applied to green and fermented rooibos, enhanced clarity of fermented rooibos extracts and major losses of aspalathin in green and fermented rooibos extracts (Pengilly *et al.*, 2008; Coetzee *et al.*, 2014; Zwane, 2014). In the current study, three commercial enzymes were applied to rooibos dust to investigate their effect on the extract yield, colour, turbidity and phenolic content.

The second approach explored for the maximisation of soluble matter extraction from rooibos dust, response surface methodology, is an effective statistical tool that has successfully been utilised for the optimisation of polyphenol extraction from various plant sources through the use of various extraction techniques (Liu *et al.*, 2010; Yang *et al.*, 2010; Co *et al.*, 2012; Lai *et al.*, 2013; Lee *et al.*, 2013). RSM permits simultaneous optimisation of numerous responses which are a result of the interaction of the independent, controllable experimental parameters (Bezerra *et al.*, 2008). It serves as a very resourceful substitute to OFAT testing, which is exclusive of the interaction between different factors. Furthermore, both time and money are saved as it generates substantial amounts of data with the fewest possible experiments (Bezerra *et al.*, 2008). Energy consumption has become an ever-increasing concern in the food industry and other industries alike. Therefore, optimising extraction processes can greatly reduce the required energy input through the avoidance of extensive extraction times (Chemat *et al.*, 2012; Grobler, 2013). Manufacturing processes affecting both the reproducibility of the process and final product consistency, which contribute to the quality of botanical products, are standardised by the optimisation of extraction procedures (Seeram *et al.*, 2006).

In summary, the objective of this study was to optimise the extraction conditions of soluble solids from fermented rooibos dust. Two approaches were followed to achieve extraction of maximum soluble matter from rooibos dust. The first entailed EE, using commercial enzymes, while

the second approach involved application of RSM to the hot water extraction process to optimise the extraction temperature ($^{\circ}\text{C}$), extraction time (min.) and plant material-to-water ratio (m.v^{-1}). Twenty batches of rooibos dust were extracted using the optimum conditions to provide a measure of natural variation in extract yield that could be expected. The extracts were also characterised in terms of colour, turbidity and phenolic content.

3.3. Materials and methods

3.3.1. Chemicals and reagents

Authentic phenolic reference standards (purity $\geq 95\%$) were obtained from Extrasynthese (Genay, France; iso-orientin and orientin) and the South African Medical Research Council (PROMEC Division, Bellville, South Africa; aspalathin and nothofagin). Glacial acetic acid (98-100%) and HPLC gradient grade acetonitrile were purchased from Merck Millipore (Darmstadt, Germany), and ascorbic acid was purchased from Sigma-Aldrich (St Louis, MO, USA). Deionised water prepared using an Elix Advantage 5 water purification system (Merck Millipore) was purified further to obtain high performance liquid chromatography (HPLC) grade water using a Milli-Q Reference A⁺ water purification system (Merck Millipore). Deionised water was used in all extraction experiments and for the preparation of aqueous solutions. However, tap water was used for all EE experiments as per supplier's recommendation due to the enzymes being unable to function without metal ions (cofactors).

3.3.2. Rooibos waste plant material

A large sample of rooibos dust (≈ 15 kg) from an individual production batch, supplied by Bokkeveld Rooibos (Nieuwoudtville, South Africa), was used for EE extraction, OFAT and RSM experiments. A sample set ($n = 20$) representing individual production batches (batches 1 to 20; ≈ 2 kg per batch) of fermented rooibos dust from various plantations was obtained from Bokkeveld Rooibos (Nieuwoudtville, South Africa). The optimal extraction conditions were applied to the sample set.

3.3.3. Enzymes

Three commercial food-grade enzymes, Validase TRL, Rapidase Fiber and Filtrase NL Fast, were sourced from DSM (Delft, The Netherlands) (Table 3.1). The fine rooibos dust was treated with each enzyme at six concentrations (0.05%, 0.1%, 0.2%, 0.5%, 5% & 10%) (mL enzyme solution "as is" provided by the manufacturer per 100 g) at a fixed plant material-to-water ratio ($1:20 \text{ m.v}^{-1}$), extraction temperature (50°C) and extraction time (2 h). A general extraction procedure was followed and it entailed weighing the dust into 1 L Schott bottles. Hot water extraction commenced by adding the

required amount of deionised water (preheated to the required experimental temperature) to the plant material, and placing the Schott bottle in a preheated water-bath. The liquid enzyme solutions were applied “as-is” to the rooibos dust and water mixture at the start of the extraction procedure. Extraction time was recorded from the moment the water was added to the plant material. The contents of each sealed Schott bottle were agitated for 5 s at 10 min intervals in the water bath for the duration of the extraction period. Once the extraction time had elapsed, the contents were immediately filtered through a polymon mesh cloth using a vacuum-assisted Büchner filtration apparatus. Upon cooling, the filtrate was centrifuged for 10 min (8000 rpm). The supernatant was used to determine the effectivity of each enzyme to increase SS yields. In addition, the effect of each enzyme treatment on the colour (C^* , L^* , a^* , b^* and h^*), turbidity (NTU) and phenolic content of the extract was evaluated.

3.3.4. One-factor-at-a-time (OFAT) experiments

A series of OFAT experiments was carried out to determine the ranges of the independent variables, extraction temperature, extraction time and plant material-to-water ratio in which an optimum response would likely be achievable. These ranges formed the basis for selecting an appropriate experimental design for the subsequent RSM experiments. The general extraction procedure described in section 3.3.3 was followed. The contents of each sealed Schott bottle were agitated for 5 s at 5 min intervals in the water bath for the duration of the extraction period. No enzymes were added to the rooibos dust and water mixture. The supernatant was used to determine the SS content of the dust extracts.

The effect of various extraction times (10, 15, 20, 25 and 30 min) on the extraction of soluble matter (i.e. extract yield) from the dust was determined by extraction at a fixed temperature (50 °C) and plant material-to-water ratio (1:20 m.v⁻¹). The effect of temperature (40, 50, 60, 70, 80 and 90 °C) on the extraction of soluble matter was determined at a fixed extraction time (20 min) and water-to-plant material ratio (1:20 m.v⁻¹). The effect of different plant material-to-water ratios (1:10, 1:15, 1:20, 1:25 and 1:30 m.v⁻¹) on the extraction of soluble matter was determined at a fixed extraction temperature (50 °C) and extraction time (20 min). The ratios were achieved by adding the appropriate volume of pre-heated deionised water to the corresponding amount of plant material (e.g. 200 mL water was added to 10 g of plant material to obtain a 1:20 plant material-to-water ratio). All extractions were performed in triplicate.

3.3.5. Optimisation of extractions

A central composite design (CCD) consisting of 16 experimental runs was used to optimise the extraction process. The independent variables under investigation were extraction temperature (°C), extraction time (min) and plant material-to-water (m.v⁻¹) ratio. The ranges of the independent variables (Table 3.2) were chosen based on the results of the OFAT extractions and industrial practicality. The 16 experimental runs of the CCD were performed in triplicate in a completely randomised order. The same general extraction procedure was followed as with the OFAT extractions.

The experimental data obtained from the CCD experiments were used for the generation of regression coefficients and fitted to a second order polynomial equation:

$$y = \beta_0 + \sum_{j=1}^k \beta_j X_j + \sum_{j=1}^k \beta_{jj} X_j^2 + \sum \sum_{i < j} \beta_{ij} X_i X_j + \varepsilon$$

where β_0 , β_j , β_{jj} , and β_{ij} represent regression coefficients for intercept, linear, quadratic and interaction terms, respectively. The response value (dependent variable; extract yield) is represented by y , and X_i and X_j represent the level of the independent variables (factors). The term k represents the number of investigated factors, while ε represents the residual error associated with the experiment. Verification of the prediction model for the dependent variables was carried out by conducting an additional replication of the CCD. Thereafter, extraction of twenty different batches of fermented rooibos dust was carried out in duplicate and the extract yield determined. The colour (C^* , L^* , a^* , b^* and h), turbidity (NTU) and phenolic content of the extract was determined in order to characterise the extracts. Aliquots of extracts were stored at -18 °C for HPLC analysis.

3.3.6. Gravimetric determination of extract yield

The soluble solids contents of all extract filtrates were determined gravimetrically in triplicate. An extract filtrate of 10 mL was transferred to pre-weighed nickel moisture dishes on a Merck Model 402 steam-bath and evaporated until visibly dry. Final drying occurred in a forced-air laboratory drying oven set at 100 °C for 60 min, whereafter the samples were cooled under desiccation, re-weighed and the resulting difference in mass calculated. The theoretical extract yield was calculated based on the total solvent volume and mass of plant material that was used, and expressed in terms of g SS.100 g⁻¹ plant material (%; m.m⁻¹).

3.3.7. HPLC analysis of extracts

Reversed-phase high performance liquid chromatography with diode array detection (RP-HPLC-DAD) was performed to quantify the major rooibos flavonoids of the extracts using the method described by Walters *et al.* (2017), but with the column temperature slightly reduced to 42.5 °C. An Agilent

1260 Affinity II HPLC system consisting of a quaternary pump, autosampler, in-line degasser, column oven and diode-array detector controlled by Chemstation software (Agilent Technologies, Waldbronn, Germany) was used. Separation was achieved on a Poroshell 120 SB-C₁₈ column (150 x 4.6 mm, 2.7 µm particle size) (Agilent Technologies), protected by an Acquity UPLC in-line filter (Waters; 0.2 µm) and a ZORBAX SB-C₁₈ analytical guard column (12.5 x 4.6 mm, 5 µm). Gradient elution was performed using 2% acetic acid (A) and acetonitrile (B) at 1 mL.min⁻¹ as follows: 10–14.8% B (0–28.5 min), 14.8–19.2% B (28.5–33 min), 19.2–100% B (33–33.5 min), 100% B isocratic (33.5–38 min), 100–10% B (38–39 min), 10% B isocratic (39–46 min). UV-Vis spectra were recorded for all samples from 200 to 700 nm with quantification of the dihydrochalcones at 288 nm, whilst the flavones were quantified at 350 nm. Stock solutions of authentic reference standards (ca. 1 mg.mL⁻¹) in dimethyl sulfoxide were used to prepare a standard calibration mixture. It was injected at different injection volumes to obtain 8-point calibration curves. The results were expressed as a percentage of the plant material (dry basis) or soluble solids content of the extracts (g.100 g⁻¹ = %; m.m⁻¹). Peaks were identified based on comparison at UV-Vis spectra and retention times with those of authentic standards. Prior to HPLC analysis, the diluted extracts with ascorbic acid added and the standard calibration mixtures were filtered using 0.22 µm pore-size Millex-HV hydrophilic polyvinylidene difluoride syringe filter devices (Merck Millipore) with 33 mm diameters.

3.3.8. CIELAB Colour and turbidity measurements of dust extracts

Objective colour measurements of all extracts (L*, a*, b*) were conducted in transmission mode with a CM-5 Konica Minolta Spectrophotometer (Konica Minolta Sensing Inc., Tokyo, Japan), using a 10 mm path length polystyrene cuvette. The C* and h* values were calculated from the L*, a* and b* values. A preliminary colour experiment was conducted to determine whether extract dilution would be necessary in order to obtain a linear relationship between the SS concentration of the dust extracts and CIELAB colour values (C*, a*, b*). Experimental results showed that there was no linear relationship between very concentrated extracts and the CIELAB colour values (C*, a*, b*) (Addendum A, Figures A3.1a-e). Therefore, all extracts were subsequently diluted 20x before colour measurements were conducted to ensure that the colour parameters were within the linear range. Triplicate measurements were performed on each extract.

Turbidity measurements of undiluted dust extracts were performed in triplicate, using a HACH 2100N turbidimeter (ISO Method 7027) (Hach, Loveland, USA) and sample cells provided by the manufacturer. The equipment were calibrated prior to turbidity measurements, using a StablCal® calibration set containing five sealed vials of 0 to 1000 NTU standards. The Formazin turbidity standards were supplied by the manufacturer.

3.3.9. Statistical analysis

Univariate analysis of variance (ANOVA) was carried out on all EE and OFAT experimental data to determine whether differences between treatment means were significant. The least significant difference (LSD) of the Student's t-test ($P = 0.05$) was calculated to compare treatment means where significant differences were found ($P \leq 0.05$). Levene's test was used to test for treatment homogeneity of variance. In instances where variances were not equal, a weighted analysis of variance was used for the combined analyses. The Shapiro-Wilk test was performed on the standardised residuals from the models to assess for normal distribution of the data (Shapiro & Wilk, 1965). All statistical analyses were performed, using SAS® software (Version 9.2, SAS Institute Inc., Cary, NC, USA).

Statistica 12.0 (Statsoft Southern Africa, Sandton, South Africa) was used to analyse all data generated by RSM. The statistical significance and suitability of the regression model, its factors and their interactions were determined at the 5% probability level ($P \leq 0.05$) using ANOVA. Standardised Pareto charts were used to illustrate the significant effects obtained for the different response values by ANOVA. The fitting efficiency of the data (extract yield) to the model was evaluated by calculating the correlation coefficient (R^2), the adjusted correlation coefficient (R^2_{adj}) and the significance of lack-of-fit (LOF). The regression equation that was generated for extract yield was illustrated as two-dimensional contour plots and three-dimensional response surface plots. The intra-class correlation coefficient (ICC) was used to assess the predictive ability of the model. Desirability profiling was performed to determine the optimum of the hot water extraction parameters.

3.4. Results and discussion

Rooibos extracts have been used in a number of consumer products in the nutraceuticals, food and cosmetics industries (Biénabe *et al.*, 2009; Joubert & De Beer, 2011). However, plant materials are naturally complex and the extraction of desired compounds is affected by processing factors such as solvent type, temperature, solubility of the target compounds, mechanical action (e.g. shaking or ultrasonication) and extraction time (Azmir *et al.*, 2013). Moreover, the positioning of the target compounds may vary between plant material batches (Takeuchi *et al.*, 2009). The application of enzymes on rooibos has been explored, but not widely. Previous studies on EE of rooibos made use of various commercial and fungal enzymes for potentially enhancing the quality of rooibos plant material. The results of the application of enzymes on rooibos dust and the optimisation of soluble matter extraction from rooibos dust using RSM are discussed below.

3.4.1. Enzyme-assisted extraction

The efficacy of three commercial food-grade enzymes to enhance extraction of soluble matter from rooibos dust was tested at six different concentrations while extraction time (20 min), temperature (50°C) and plant-material-to-water ratio (1:20 m.v⁻¹) were kept constant throughout each experiment. The extracts were also evaluated in terms of colour, turbidity and phenolic composition.

An increase in each enzyme concentration resulted in a gradual increase in extract yield. However, there was no significant difference ($P \leq 0.05$) in extract yield between the control dust extract and extracts of enzyme-treated dust at low concentrations ($\leq 0.2\%$ for Filtrase; $\leq 0.5\%$ for Rapidase and Validase) (Table 3.3). The increase in extract yield was most notable for enzyme concentrations of 5% and 10%, with a significant difference ($P \leq 0.05$) between the performances of each enzyme. Rapidase used at 10% produced the highest extract yield (25.7 g SS.100 g⁻¹ PM, %), with an 8.4% increase in SS in comparison to the control extract, and was thus regarded as the most efficient enzyme for the extraction of SS.

The Rapidase enzyme used is characterised by pectinase with arabinolytic and cellulolytic activity; and dried fermented rooibos plant material has been found to contain approximately 42% cellulose and 4.2% arabinose (Pengilly *et al.*, 2008). This finding therefore suggests that cellulases would be most effective for the hydrolysis of rooibos plant material for the release of SS, and further elucidates why Rapidase was able to achieve higher extract yields in comparison to Validase and Filtrase. The second most efficient enzyme for the extraction of maximum SS was Validase used at 10% (23.7% extract yield), followed by Filtrase used at 10% (22.4% extract yield). However, Filtrase used at high concentrations possibly did not reach its full extraction potential due to the fact that it was not used at its optimum operating temperature of 70-75 °C as the performance of each enzyme had to be compared at the same temperature.

The SS yield of the control dust extract (17.3%) was relatively high in comparison to other studies on EE where the SS yields of the control extract ranged from 7.7-14.01% (Coetzee *et al.*, 2014; Pengilly *et al.*, 2008 & Zwane 2014). Moreover, it was higher than the SS yield (15.2%) previously determined by Joubert & De Beer (2012) for rooibos extracted for 30 min at 93 °C at 1:10 ratio. This may possibly be due to the difference in plant material type and particle size used in this study and in the studies mentioned above. A small particle size would increase the rate of extraction due to decreased diffusion distances for soluble matter within the plant material. Similar results were achieved by Coetzee *et al.* (2014), where the treatment of fermented rooibos plant material with various commercial enzymes (40 °C, 2 h) increased the yield of SS by more than 10%. Moreover, fungal cocktails of hydrolysing enzymes (93 °C, 30 min) were also shown to improve the yield of extracted SS by 47% (Pengilly *et al.*, 2008).

The characteristic red-brown colour of rooibos infusions and extracts is a result of a “fermentation” process, and it is important for the application of rooibos extracts in the food and beverage industry. The effect of dilution on the CIELAB colour measurements is shown in Addendum A, Figures 3.1a-e. The L^* (lightness) and h (hue angle) values decreased with an increase in extract concentration. Furthermore, inversion occurred for the C^* , a^* and b^* values. Inversion of the C^* , a^* and b^* values occurred at 0.35, 0.5 and 0.3% SS concentration, respectively (Addendum A, Figures A3.1a-e). Dilution (20x) resulted in C^* , a^* and b^* values well within the linear range. The elimination of inversion could be achieved by decreasing the cell path length or by diluting the extracts (Joubert, 1995) in order to raise the luminosity level. The disadvantage of this, however, is that the colour of such extracts cannot be directly compared with tea infusions (in a cup), and extrapolations are unreliable (Joubert, 1995). Rooibos extracts display dichroism, i.e. the colour intensity is dependent on the degree of dilution or container size. Dilute rooibos extracts are light yellow instead of the characteristic red-brown of more concentrated extracts. This partly results in the yellowish ring often observed at the rim of a cup of rooibos tea (Joubert, 1995). This phenomenon was partially observed with the naked eye at the 20x dilution of the dust extracts. The dilution was performed in a relatively small 20 mL volumetric flask which may have hindered the full observation of dichroism. However according to Addendum A, Figure A3.1e, the dilution of dust extracts changed the hue of the dust extracts from red-brown to yellow as seen by the increase in h^* values with a decrease in SS concentration.

Enzyme treatment had little effect on the colour parameters of the dust extracts, except for some treatments, most notably the two highest concentrations of Rapidase (5% & 10%). Rapidase produced extracts that were slightly lighter, less yellow and less saturated in colour in comparison to the control extract due to higher L^* values, lower b^* values and lower C^* values, respectively (Table 3.3). Given that these treatments also delivered the significantly higher SS yields, it could be postulated that the extraction of non-coloured compounds such as sugars and ferulic acid contributed largely to increase in SS yields. One of the fungal enzymes used in a study conducted by Pengilly *et al.* (2008) lowered the L^* value of rooibos extracts, but increased the b^* value in comparison to the control extract.

Another parameter that indicated an increase in dust “solubilisation” by some enzyme treatments is turbidity. Turbidity refers to the haziness of a fluid caused by individual particles suspended in large number in solution, and is a relative measure of the clarity of a liquid. It is sometimes used as a quality measure of extracts, and noticeable turbidity may have a negative effect on the visual quality of infusions (Joubert, 1995). Clear instant iced teas in particular are preferred over cloudy beverages which typically form a “tea cream” at the bottom of the glass (Coetzee *et al.*, 2014). Overall, the turbidity of the undiluted dust extracts of enzyme-treated dust was relatively low (≤ 26). The NTU level of lager beer is ca. 13.3-17.3 (Wylter *et al.*, 2015). Moreover, the turbidity of extracts of Filtrase-

or Validase-treated dust in comparison to the control dust extract was not significantly different ($P \leq 0.05$) meaning that these two enzymes had no effect on the turbidity. At high concentrations (5% & 10%), however, the extracts of Rapidase-treated dust displayed a significant increase in turbidity ($P \leq 0.05$) relative to the control extract and that of the extracts of Filtrase- and Validase-treated dust (Table 3.3). The increase in turbidity at high Rapidase concentrations is possibly due to the hydrolysis of plant material which increased the amount of particles, perhaps various organic polymers, suspended in the liquid. Enzymes used by Coetzee *et al.* (2014) were able to enhance the clarity of extracts of enzyme-treated rooibos by ten-fold (1.19 NTU) in comparison to the control extract (11.70 NTU). This was attributed to breakdown of oligosaccharides. Although low, the turbidity values in the current study (Table 3.3) were relatively higher than those of Coetzee *et al.* (2014) as much finer plant material was used. Although the dust extracts were filtered and centrifuged, some particles which could possibly be tiny bits of plant material remained suspended in the liquid, unlike filtered normal rooibos extracts that have minimal particles suspended in the liquid.

When the effect of enzymes on the phenolic content of extracts of enzyme-treated dust was evaluated, neither Filtrase, Rapidase nor Validase had a significant effect ($P \leq 0.05$) on the nothofagin and orientin content of the dust extracts relative to the control extract. However, these three enzymes significantly increased ($P \leq 0.05$) the isoorientin content of the dust extracts at high concentrations (5% & 10%) (Table 3.4). In addition, no significant difference ($P \leq 0.05$) was seen in the aspalathin content of extracts of Validase-treated dust relative to the control extract. However, extracts of Filtrase- and Rapidase-treated dust at high concentrations (5% & 10%) demonstrated significant increases ($P \leq 0.05$) in aspalathin content relative to the control extract (Table 3.4). Rapidase at 10% was the most efficient enzyme for increasing the aspalathin content of the dust extracts (0.130 g aspalathin.100 g⁻¹ PM, %). A study conducted by Joubert & De Beer (2012) demonstrated that on average hot water extracts of unrefined rooibos contain 0.088% aspalathin, 0.011% nothofagin, 0.127% nothofagin and 0.120% orientin. The phenolic content values of the control dust extract were within the range of the above-mentioned phenolic content values of normal rooibos extracts (Table 3.4). Pengilly *et al.* (2008) also demonstrated an increase in the aspalathin, nothofagin, isoorientin and orientin content of extracts of enzyme-treated fermented rooibos. On the contrary, Coetzee *et al.* (2014) encountered a major loss of aspalathin, nothofagin and orientin accompanied by an increase in isoorientin in extracts of enzyme-treated fermented rooibos, where Zwane (2014) encountered a loss in aspalathin, nothofagin, isoorientin and orientin.

3.4.2. One-factor-at-a-time (OFAT) hot water extraction

A number of factors have an influence on the extraction of soluble matter from plant material. Each of these factors have an individual effect on the mass transfer kinetics of plant material, which makes the optimisation of extraction procedures necessary (Wijngaard *et al.*, 2012). A series of OFAT hot water extraction experiments was conducted to determine the individual effects of extraction time, extraction temperature and plant material-to-water ratios on the extract yield, as well as feasible treatment level ranges in which optimal responses would potentially be located.

3.4.2.1. Effect of extraction time on extraction yield

The effect of different extraction times (10, 15, 20, 25 and 30 min) on the extraction of soluble solids was investigated at a fixed extraction temperature (50 °C) and fixed plant material-to-water ratio of 1:20 (m.v⁻¹). A gradual increase in extract yield (g SS.100 g⁻¹ PM, %) was observed over time (Figure 3.1). Longer extraction times were also shown to increase the soluble solids yield from fermented rooibos (Joubert, 1990b). However, the major portion of the hot water soluble solids had already been extracted after just 10 min, considering that the extract yields at 10 and 30 min were 14.70% and 16.27%, respectively. A similar trend was observed when OFAT analysis was applied to green rooibos by Miller *et al.* (2017) where the extract yield did not differ significantly ($P \leq 0.05$) between the 10, 20 and 30 min extraction times. A study conducted by Joubert & Hansmann (1990) demonstrated that ca 50% of the hot water-soluble solids of fermented rooibos were extracted after 5 min when using a flow-through batch system at 90 °C.

3.4.2.2. Effect of extraction temperature on extraction efficiency

The second OFAT experiment investigated the effect of different extraction temperatures (30, 40, 50, 60 and 90 °C) on the extraction of soluble solids. The extraction time and plant material-to-water ratio were fixed at 20 min and 1:20 (m.v⁻¹), respectively, as these fixed points were regarded as centre points of their experimental ranges. Considering the boiling point of water and the experimental setup, a 90 °C temperature was used as an upper limit. The extract yield increased significantly ($P \leq 0.05$) with each increasing increment of extraction temperature tested. No plateau was seen within the temperature range tested (Figure 3.2). Using high temperatures for enhancing the extraction of soluble matter from plant material boosts plant compound solubility and increases the mass transfer of solutes (Azmir *et al.*, 2013)

A study by Joubert (1988) demonstrated the same trend whereby the yield of soluble solids increased linearly with an increase in extraction temperature. In addition, Miller *et al.* (2017) also

showed a significant ($P \leq 0.05$) increase in the extract yield of green rooibos with an increase in extraction temperature.

3.4.2.3. Effect of water-to-plant material ratio on extraction efficiency

The third OFAT experiment investigated the effect of different plant material-to-water ratios (1:10, 1:15, 1:20, 1:25 and 1:30; m.v⁻¹) on the extraction of soluble solids. The extraction time and temperature were fixed at 20 min and 50 °C, respectively. Soluble solids were optimally extracted when a 1:30 ratio was employed, but the extract yield was not significantly different ($P \leq 0.05$) from ratios of 1:20 and 1:25 (Figure 3.3). However, it was significantly higher ($P \leq 0.05$) than the extract yield obtained with 1:10 and 1:15 ratios. If the use of a higher water-to-plant material ratio did not result in a significant ($P \leq 0.05$) increase in the soluble solids yield, the use of a lower water-to-plant material ratio yielding similar results would be beneficial for water and energy saving purposes. Moreover, although the soluble solids yield is improved with the use of a higher water-to-plant material ratio, the increased solvent usage would influence the cost-efficiency of the extraction process. High water-to-tea mass ratios in fermented rooibos extraction processes have been shown to be associated with higher soluble yields (Joubert, 1998). In addition, mass transfer principles state that the concentration gradient of the solute between the solid and the bulk of the solvent is the driving force of the extraction process. When a higher solvent-to-solid ratio is used, the concentration gradient is steeper irrespective of the solvent used (Takeuchi *et al.*, 2009).

3.4.3. Application of response surface methodology (RSM)

The OFAT experimental results indicated the ranges of levels of independent variables within which the optimal extract yield would most likely be located. Other responses measured were CIELAB colour parameters (C^* , L^* , a^* , b^* and h) and turbidity (NTU) but they were not used for the purpose of optimisation. The extraction temperature, time and plant material-to-water ratio were selected for inclusion in the CCD. The CCD consisted of 16 experimental runs conducted in triplicate in randomised order. It was decided that the optimal parameters would be applied to different individual batches of rooibos dust ($n=20$), selected from various plantations, to determine the possible extract yield natural variation anticipated.

3.4.3.1. Analysis of RSM data

The data obtained from the RSM experiments are summarised in (Table 3.5). The following ranges of response values were obtained: extract yield, 13.68-22.7%; L^* , 79.83-91.47; a^* , 2.52-18.30; b^* , 50.68-

94.01; C*, 50.76-95.78; h, 80.98-86.96; extract turbidity (NTU), 8.60-64.90; aspalathin yield, 0.074-0.146%. Variation between triplicates of the same treatment was also evident. This is attributed to poor homogeneity of the batch used for extraction and/or experimental variation. For example contact time between the dust particles and water was difficult to control as the plant material-to-water ratio had an effect on the filtration rate.

The suitability of the generated model was determined by conducting regression analysis. The significance of the linear, quadratic and interaction effects of the independent variables on extract yield was evaluated using a standardised Pareto chart. Desirability profiling was used for the determination of the optimal extraction conditions for maximum response values. Thereafter, the predictive ability of the model was assessed by conducting an additional set of verification experiments.

The statistical significance of the effect of the independent variables and their interactions on extract yield, and the fit of the data model, was estimated by conducting ANOVA, which compares the variance between different combinations of independent variables (treatments) and the variance due to random errors. The response value for EY was fitted as a function of the three independent variables X_1 , X_2 and X_3 . The regression coefficient that was generated by the ANOVA was used to generate a quadratic regression equation with which the values of the responses could be predicted. The ANOVA results, with estimated linear, quadratic and interaction regression coefficients for the EY response, are presented in Table 3.6. For EY, extraction time showed a significant linear effect ($P \leq 0.05$), while temperature and plant material-to-water ratio showed both a significant linear and quadratic effect ($P \leq 0.05$).

The R^2 or lack-of-fit (LOF) can be used to evaluate suitability of the model. In addition the amount of variation around the mean is represented by R^2 . R^2_{adj} is adjusted to account for the number of terms in the model, allowing for direct comparison of models with different amounts of independent variables. Usually, R^2_{adj} which is lower in value than R^2 , should preferably be at least 0.8 for a model with a good fit (Guan & Yao, 2008). The variability between observations of different replications of the independent variables with the variability of the model residuals is compared by the LOF test. It makes use of the mean square (MS) pure error as the error term and it is regarded as a more sensitive test of model fit than R^2_{adj} . A model is considered to have a good fit to the data if there is significant regression and non-significant LOF. LOF of the prediction model for extract yield was not significant ($P = 0.144$) and $R^2_{adj} > 0.8$ (Table 3.6.), indicating good predictive ability for the model. Table 3.7 provides the full prediction equation for extract yield.

Further insight into the relative effect of the independent variables and their interaction is provided by a standardised Pareto chart (Figure 3.4). Linear, quadratic and interaction effects of the independent variables are graphically depicted as rectangular bars, with the length of each bar being proportional to the absolute scale of the standard estimated effects. The effect of a given parameter is significant if its bar crosses the red vertical line which represents the $P = 0.05$ confidence level.

The standardised Pareto chart for EY shows that the linear effects of extraction temperature, time and plant material-to-water ratio had a significant effect on this response (Figure 3.4), with extraction temperature having the greatest effect. In addition, the interaction effects of extraction time and plant material-to-water ratio (1L x 3L), and extraction temperature and plant material-to-water ratio (2L x 3L) had a significant effect on this response. The quadratic effects of temperature and plant material-to-water ratio also had a significant effect on this response. This is in agreement with the results of the OFAT extractions, demonstrating that extraction temperature has the greatest individual effect on the EY. This phenomenon is further illustrated by response surface plots for EY (Figures 3.5a-c). The extract yield increases linearly with increasing temperature up to 100 °C, and fixed extraction time, and an optimal response (considering the practical limitations of water as solvent) is located between 10 to 30 min and 80 to 100 °C. The small effect of extraction time on extract yield is evident from the less steep gradient of the response surface in the direction of increasing extraction time. The relatively small effect of the plant material-to-water ratio on extract yield is demonstrated by the response surfaces depicted in Figure 3.5b & Figure 3.5c, showing that changes in this variable are accompanied by minor changes in extract yield. The effect of the plant material-to-water ratio was greater at the shorter extraction times, as seen from the steeper gradient of the response surface at extraction time <24 min (Figure 3.5b).

3.4.3.2. Verification of prediction models

In order to assess how well the experimental results would agree with predicted values, the prediction models would need to be verified. Therefore, one additional replication of the central composite design was carried out as a verification experiment. The predicted and experimentally observed response values for extract yield, as well as the over/underestimation obtained for all 16 standard runs in the verification experiment are presented in Table 3.8. The intraclass correlation coefficient (ICC) value was used to assess how well the experimental data fitted the model. This value represents the reliability of quantitative measurements, where reliability refers to the reproducibility of randomly repeated measurements. ICC values range between 0 and 1, with values closer to 1 indicating a better model fit and a greater reliability of the model to predict a response value. The ICC (agreement) value is a subclass of the ICC value which accounts for any bias that may have occurred by incorporating the standard error of measurement (SEM), whereas the ICC (consistency) value excludes the SEM and is less sensitive as a result. Predicted response values and the observed results from the verification experiments are presented in a scatter plot to demonstrate the distribution of the data (Figure 3.6).

Extract yield displayed high ICC values with ICC (agreement) and ICC (consistency) values of 0.956 and 0.962, respectively. For large-scale cost and profit predictions, it is required that ICC values be as close to 1.0 as possible. For further evaluation of the predictive ability of the model, a Bland-

Altman plot (Table 3.7) was used to assess the agreement between the observed and predicted values, and to analyse for the presence of data bias. The x-axis contains the means of the observed and predicted values, whereas the y-axis contains the differences between the observed and predicted values. The experimental data should ideally be scattered closely around the mean and within the 95% limits of agreement. A small difference between the predicted and observed results would thus be represented. The mean lies at 0 on the y-axis with the absence of bias. The Bland-Altman plot for the extract yield shows, with the exception of two points, that the experimental points were scattered between the 95% limits of agreement above and below the mean. This indicates satisfactory predictive ability. The mean was slightly below 0, indicating slight biasness (Giavarina, 2015) due to random variation and consequent overestimation.

3.4.3.3. Desirability profiling and selection of practical optimum extraction parameters

The concept of desirability profiling in RSM refers to the identification of a desirability function for the given dependent variable(s) under investigation. Response values are then predicted using these functions and are assigned scores ranging from 0 (most undesirable) to 1 (most desirable) (Bezerra *et al.*, 2008). A series of graphs, profiling the desirability of the desired response is generated for each of the independent variables. Responses may be maximised, minimised or kept at constant values. When the desirability scores for a given parameter are plotted, the remaining parameters are fixed at constant values. The desirability profile which is finally obtained gives an indication of the levels of the independent variables that would produce the most desirable predicted response values.

Figure 3.8 shows the desirability profile for EY which was optimised for a maximum response value. The assessment of the reliability of the predicted responses is aided by the 95% confidence intervals (blue lines). The magnitude of the effect on the response value is reflected by the gradients of the various desirability curves (green). The desirability plot is a reflection of the Pareto chart as it clearly indicates that the extraction temperature had a strong positive effect on the extract yield, followed by the plant material-to-water ratio and extraction time.

Although RSM is a convenient method for establishing statistical models for the optimisation of extraction processes, optimum conditions that have been theoretically predicted by models may not always be economically or practically feasible. As seen in Figure 3.8, the effect of increasing the extraction temperature stayed constant up to 94 °C (maximum of temperature range tested) and did not reach an optimum. The desirability profiles for extraction time and plant material-to-water ratio remained fairly constant with each increasing increment as they approached the upper limits of their experimental ranges. Despite 30 min being indicated as the optimum extraction time, there was barely an increase in desirability from 10 min to 30 min. From a practical point of view, 30 min could not be justified as an optimum extraction time because it did not result in a significant extract yield increase

from 20 min. The same point was valid for the optimal plant material-to-water ratio of 1:30. A very small increase in desirability was seen between the plant material-to-water ratio of 1:10 and 1:30, and thus the use of a higher plant material-to-water ratio, which would translate to higher solvent use and energy consumption to remove the extract water, could also not be justified. The practical “optimal” extraction time and plant material-to-water ratio were therefore 20 min and 1:20 m.v⁻¹, respectively. The proposed optimal extraction temperature of 94°C was accepted. A similar trend was observed by Miller *et al.* (2017) for hot water extraction optimisation of green rooibos where the optimum independent extraction variables could not be justified from a practical point of view.

3.4.3.4. Validation of optimum hot water extraction process

By substituting the selected optimal extraction conditions into the polynomial model provided in Table 3.7, the optimal extract yield response was determined to be 22.73%. The extract yield was determined for other batches of rooibos dust (n = 20) by conducting additional extractions using the practical optimal extraction conditions. The extract yield varied (Table 3.9) between 16.42% and 27.90% due to natural plant material variation and variation introduced during “fermentation”. Even the lowest dust extract yield was higher than the average extract yield of 15.24% obtained by Joubert & De Beer (2012) for unrefined fermented rooibos tea. This is largely due to the difference in plant material used. According to Azmir *et al.* (2013), smaller plant material particle size would decrease mass transfer limitations and increase the rate of extraction as a result of shorter diffusion distances for solutes within the plant matrix. A variation in CIELAB colour parameters, extract turbidity and aspalathin yield was also encountered (Table 3.9). This was expected in view of the results for extract yield.

3.5. Conclusion

The EE results confirmed that commercial food-grade enzymes are able to increase the extract and phenolic content yield of rooibos plant material. However, in this case, increases were only observed at very high enzyme concentrations. Enzyme choice is usually driven by end product needs, i.e. phenolic enrichment (for pharmaceutical and nutraceutical needs) or clear extracts (for beverage use). Rapidase was the most suited for application on rooibos dust for the extraction of maximum soluble solids, followed by Validase then Filtrase. Rapidase at high concentrations, produced extracts that were turbid. On a large scale, however, the use of Rapidase for the enhancement of the dust extract yield and aspalathin content would not be economically feasible, owing to the large amount of enzyme required to perform functions. EE of rooibos dust was thus not as effective as expected and their use would not be economically viable in industry.

A variation in the composition of the rooibos dust harvested from various plantations was shown by the batch-to-batch variation in the extract yield, CIELAB colour parameters, turbidity and aspalathin yield. Preliminary, single factor experiments showed that extraction time, extraction temperature and plant material-to-water ratio affected the extraction efficiency of soluble solids from rooibos dust. Optimal hot water extraction conditions (94°C temperature, 1:20 plant material-to-water ratio and 20 min extraction time) were identified using RSM, taking into consideration cost-efficiency and industrial viability. The maximum dust extract yield obtained by Rapidase-treatment of rooibos dust (25.7%) was lower than the maximum dust extract yield obtained using the selected optimal extraction conditions (27.90%). Therefore, this result further motivates the fact that EE of rooibos dust would be a costly endeavor and thus not be cost effective. Rooibos dust extraction would rather be performed at the optimal extraction conditions using a higher temperature to prevent additional expenses, instead of extraction at a lower temperature using enzymes which would not result in a large extract yield increase.

3.6. References

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Table 3.1 Commercial food grade enzymes applied to rooibos dust.

Enzyme	Activity	Source	Optimal conditions
Validase TRL	Multi enzyme cellulase complex containing endo-glucanase, exo-glucosidase, endo-xylanase and endo-mannase	<i>Trichoderma longibrachiatum</i>	pH: 3.8-6.4 Temp.: 45-55°C Recommended dosage: 0.01%
Rapidase Fiber	Pectinase with arabinolytic and cellulolytic activity	<i>Aspergillus niger</i> & <i>Trichoderma longibrachiatum</i>	pH: 3.6-5.5 Temp.: 45-55 °C Recommended dosage: 0.01%
Filtrase NL Fast	Endo-glucanase activity	<i>Talamyces emersonii</i>	pH: 5.5 Temp.: 70-75 °C Recommended dosage: 0.01%

Table 3.2 Independent variables and their levels as applied in a central composite design (CCD) for the optimisation of rooibos dust extraction.

Factor	Symbol	Levels				
		- α (-1.68)	-1	0	+1	+ α (1.68)
Extraction time (min)	X_1	10	14	20	26	30
Extraction temperature (°C)	X_2	40	51	67	83	94
Water-to-plant material ratio (v.m ⁻¹)	X_3	10	14	20	26	30

Table 3.3 Extract yield, CIELAB colour parameters and turbidity (means \pm standard deviations) of dust extracts obtained during enzyme-assisted extraction using varying enzyme concentrations. Values in each column with the same letter are not significantly different.

Enzyme	Enzyme concentration ¹ (%)	Extract yield ² (%)	CIELAB colour parameters ³					Turbidity (NTU) ⁴
			L*	a*	b*	C*	h*	
Filtrase	0	17.3 \pm 0.1 gh	88.39 \pm 0.1 bc	5.15 \pm 0.10 abc	60.46 \pm 0.43 abc	60.68 \pm 0.44 abc	85.14 \pm 0.06 cd	19.53 \pm 1.20 bcde
	0.05	17.4 \pm 0.2 gh	88.35 \pm 0.18 c	5.16 \pm 0.18 abc	60.45 \pm 0.58 abc	60.68 \pm 0.59 abc	85.12 \pm 0.13 cd	20.86 \pm 2.92 bcde
	0.1	17.2 \pm 0.2 gh	88.43 \pm 0.13 bc	5.09 \pm 0.09 abc	60.22 \pm 0.27 abc	60.43 \pm 0.28 abc	85.18 \pm 0.07 cd	21.53 \pm 3.28 bc
	0.2	17.6 \pm 0.1 g	88.32 \pm 0.32 bc	5.24 \pm 0.34 ab	60.73 \pm 1.14 ab	60.97 \pm 1.12 ab	85.10 \pm 0.27 cd	20.14 \pm 1.83 bcde
	0.5	17.0 \pm 0.1 h	88.11 \pm 0.50 bc	5.14 \pm 0.07 abc	60.24 \pm 0.23 abc	60.46 \pm 0.23 abc	85.13 \pm 0.06 cd	18.44 \pm 0.86 de
	5	19.0 \pm 1.2 f	88.50 \pm 0.29 bc	5.03 \pm 0.33 abc	60.08 \pm 1.24 abc	60.29 \pm 1.27 abc	85.21 \pm 0.22 cd	18.74 \pm 1.96 cde
	10	22.4 \pm 0.1 c	88.33 \pm 0.24 bc	5.25 \pm 0.28 a	60.88 \pm 0.93 ab	60.61 \pm 0.40 abc	85.08 \pm 0.19 d	17.89 \pm 1.02 e
Rapidase	0	17.3 \pm 0.1 gh	88.39 \pm 0.1 bc	5.15 \pm 0.1 abc	60.46 \pm 0.43 abc	60.68 \pm 0.44 abc	85.14 \pm 0.06 cd	19.53 \pm 1.21 bcde
	0.05	17.4 \pm 0.4 gh	88.50 \pm 0.15 bc	5.01 \pm 0.23 abc	59.98 \pm 0.83 abc	60.19 \pm 0.85 abc	85.23 \pm 0.15 cd	19.20 \pm 1.81 bcde
	0.1	17.4 \pm 0.1 gh	88.55 \pm 0.03 bc	4.93 \pm 0.02 abc	59.83 \pm 0.03 bc	60.03 \pm 0.03 bc	85.29 \pm 0.01 cd	21.78 \pm 2.61 b
	0.2	17.7 \pm 0.2 g	88.41 \pm 0.08 bc	5.15 \pm 0.13 abc	60.58 \pm 0.41 abc	60.80 \pm 0.42 abc	85.1 \pm 0.09 cd	19.88 \pm 0.81 bcde
	0.5	17.8 \pm 0.2 g	88.56 \pm 0.12 bc	4.94 \pm 0.13 abc	60.07 \pm 0.45 abc	60.27 \pm 0.46 abc	85.30 \pm 0.09 cd	20.86 \pm 1.36 bcde
	5	21.8 \pm 0.2 d	89.22 \pm 0.11 a	4.07 \pm 0.14 d	58.03 \pm 0.62 d	58.17 \pm 0.63 d	85.99 \pm 0.10 b	25.64 \pm 2.51 a
	10	25.7 \pm 0.5 a	89.46 \pm 0.69 a	3.52 \pm 0.29 e	56.55 \pm 1.25 e	56.66 \pm 1.27 e	86.44 \pm 0.21 a	25.50 \pm 2.08 a
Validase	0	17.3 \pm 0.1 gh	88.39 \pm 0.1 bc	5.15 \pm 0.10 abc	60.46 \pm 0.43 abc	60.68 \pm 0.44 abc	85.14 \pm 0.06 cd	19.53 \pm 1.21 bcde
	0.05	17.3 \pm 0.3 gh	88.31 \pm 0.05 bc	5.17 \pm 0.07 abc	60.44 \pm 0.32 abc	60.66 \pm 0.33 abc	85.11 \pm 0.04 cd	21.21 \pm 1.02 bcd
	0.1	17.4 \pm 0.2 gh	88.32 \pm 0.23 bc	5.23 \pm 0.24 ab	60.72 \pm 1.01 abc	60.95 \pm 1.03 ab	85.08 \pm 0.15 d	21.39 \pm 2.50 bcd
	0.2	17.4 \pm 0.5 gh	88.38 \pm 0.29 bc	5.26 \pm 0.31 a	61.10 \pm 0.84 a	61.32 \pm 0.86 a	85.09 \pm 0.22 d	18.20 \pm 0.90 e
	0.5	17.5 \pm 0.2 gh	88.38 \pm 0.11 bc	5.22 \pm 0.10 ab	60.67 \pm 0.25 abc	60.89 \pm 0.25 ab	85.42 \pm 0.65 c	19.68 \pm 1.76 bcde
	5	20.3 \pm 0.1 e	88.63 \pm 0.27 b	4.07 \pm 0.33	59.48 \pm 1.20 c	59.68 \pm 1.22 c	85.33 \pm 0.22 cd	19.68 \pm 0.27 bcde
	10	23.7 \pm 0.5 b	88.17 \pm 0.57 bc	4.91 \pm 0.12	59.80 \pm 0.48 bc	60.00 \pm 0.49 bc	85.30 \pm 0.08 cd	21.98 \pm 1.28 b

¹mL enzyme solution “as is” provided by the manufacturer. 100 g⁻¹ PM; ²g SS. 100 g⁻¹ PM; ³CIELAB colour system values for extracts diluted 20x; ⁴Undiluted extract turbidity in Nephelometric Turbidity Units (NTU)

Table 3.4 Major rooibos phenolic compounds (means \pm standard deviations) of dust extracts obtained during enzyme-assisted extraction using varying enzyme concentrations. Values in each column with the same letter are not significantly different ($P \leq 0.05$).

Major rooibos phenolic compounds (%) ²					
Enzyme		Aspalathin	Nothofagin	Isoorientin	Orientin
Enzyme	concentration ¹ (%)				
Filtrase	0	0.105 \pm 0.000 efgh	0.007 \pm 0.000 bcdef	0.158 \pm 0.001 e	0.205 \pm 0.002 abcd
	0.05	0.102 \pm 0.003 fgh	0.006 \pm 0.000 h	0.130 \pm 0.002 h	0.198 \pm 0.002 cde
	0.1	0.101 \pm 0.001 fgh	0.007 \pm 0.000 bcdef	0.137 \pm 0.002 f	0.205 \pm 0.003 abcd
	0.2	0.103 \pm 0.003 fgh	0.007 \pm 0.000 bcdef	0.137 \pm 0.002 fg	0.205 \pm 0.003 abcd
	0.5	0.100 \pm 0.002 gh	0.006 \pm 0.000 cdefg	0.136 \pm 0.001 fgh	0.204 \pm 0.002 abcd
	5	0.111 \pm 0.006 cde	0.007 \pm 0.000 abc	0.202 \pm 0.006 ab	0.205 \pm 0.006 abcd
	10	0.112 \pm 0.004 c	0.007 \pm 0.000 ab	0.204 \pm 0.005 a	0.208 \pm 0.006 a
Rapidase	0	0.105 \pm 0.000 efgh	0.007 \pm 0.000 bcdef	0.158 \pm 0.001 e	0.205 \pm 0.002 abcd
	0.05	0.103 \pm 0.003 fgh	0.006 \pm 0.000 gh	0.130 \pm 0.004 gh	0.199 \pm 0.005 bcde
	0.1	0.104 \pm 0.005 fgh	0.007 \pm 0.000 abcd	0.139 \pm 0.003 f	0.208 \pm 0.005 a
	0.2	0.107 \pm 0.004 cdef	0.007 \pm 0.000 bcdef	0.138 \pm 0.005 f	0.206 \pm 0.006 ab
	0.5	0.111 \pm 0.002 cd	0.007 \pm 0.000 abcd	0.138 \pm 0.001 f	0.208 \pm 0.002 a
	5	0.121 \pm 0.004 b	0.007 \pm 0.000 abcd	0.186 \pm 0.006 d	0.190 \pm 0.006 f
	10	0.130 \pm 0.002 a	0.007 \pm 0.000 a	0.196 \pm 0.004 bc	0.202 \pm 0.005 abcd
Validase	0	0.105 \pm 0.000 efgh	0.007 \pm 0.000 bcdef	0.158 \pm 0.001 e	0.205 \pm 0.002 abcd
	0.05	0.102 \pm 0.005 fgh	0.006 \pm 0.000 efgh	0.134 \pm 0.03 fgh	0.204 \pm 0.003 abcd
	0.1	0.099 \pm 0.006 h	0.006 \pm 0.000 defgh	0.136 \pm 0.007 fgh	0.203 \pm 0.010 abcd
	0.2	0.106 \pm 0.002 defg	0.006 \pm 0.000 cdefg	0.139 \pm 0.001 f	0.209 \pm 0.001 a
	0.5	0.104 \pm 0.002 fgh	0.007 \pm 0.000 bcde	0.137 \pm 0.002 d	0.206 \pm 0.003 abc
	5	0.101 \pm 0.003 fgh	0.006 \pm 0.000 fgh	0.189 \pm 0.001 d	0.193 \pm 0.004 ef
	10	0.105 \pm 0.006 fgh	0.006 \pm 0.000 cdef	0.190 \pm 0.009 cd	0.198 \pm 0.010 def

¹mL enzyme solution “as is” provided by the manufacturer. 100 g⁻¹ PM; ²g compound. 100 g⁻¹ PM

Table 3.5 Layout and response values of the central composite design (CCD), performed in triplicate, for the optimisation of hot water extraction of rooibos dust.

Treatment combination	CCD Run No. ¹	X ₁ Time (min)	X ₂ Temperature (°C)	X ₃ PM ² :Water (m.v ⁻¹)	Extract yield (%) ³	Extract concentration (g.100 mL ⁻¹) ⁴	CIELAB Colour parameters ⁵					Turbidity (NTU) ⁶	Asp yield (%) ⁷
							L*	a*	b*	C*	h		
1	6 (F)	26 (+1)	51 (-1)	1:26 (+1)	16.43	0.632	90.65	2.89	51.21	51.29	86.77	14.03	0.114
	22(F)	26 (+1)	51 (-1)	1:26 (+1)	16.11	0.620	90.70	2.87	51.12	51.20	86.78	12.23	0.108
	38(F)	26 (+1)	51 (-1)	1:26 (+1)	15.67	0.603	90.78	2.69	50.68	50.76	86.96	13.30	0.108
2	13 (A)	20 (0)	67 (0)	1:10 (-α)	18.00	1.800	79.83	18.30	94.01	95.78	78.98	32.95	0.095
	29 (A)	20 (0)	67 (0)	1:10 (-α)	17.78	1.778	80.28	17.59	92.90	94.55	79.28	32.47	0.093
	45 (A)	20 (0)	67 (0)	1:10 (-α)	17.67	1.767	80.15	17.78	93.03	94.72	79.18	31.97	0.093
3	1 (F)	14 (-1)	51 (-1)	1:14 (-1)	15.08	1.077	86.12	9.08	75.20	75.75	83.12	23.37	0.089
	17 (F)	14 (-1)	51 (-1)	1:14 (-1)	15.02	1.073	85.95	9.29	75.74	76.30	83.01	23.43	0.074
	33 (F)	14 (-1)	51 (-1)	1:14 (-1)	15.03	1.073	85.63	9.73	77.05	77.66	82.80	22.43	0.091
4	10 (A)	30 (+α)	67 (0)	1:20 (0)	19.15	0.957	87.29	6.53	64.00	64.33	84.18	30.17	0.101
	26 (A)	30 (+α)	67 (0)	1:20 (0)	19.30	0.965	87.00	6.85	65.04	65.06	83.99	30.20	0.110
	42 (A)	30 (+α)	67 (0)	1:20 (0)	19.19	0.960	87.12	6.67	64.70	64.72	84.08	35.23	0.104
5	11 (A)	20 (0)	40 (-α)	1:20 (0)	14.17	0.709	90.07	3.64	55.56	55.98	86.27	14.07	0.081
	27 (A)	20 (0)	40 (-α)	1:20 (0)	13.68	0.684	90.24	3.38	61.35	54.79	86.46	15.83	0.082
	43 (A)	20 (0)	40 (-α)	1:20 (0)	14.03	0.701	90.10	3.65	55.90	56.02	86.27	14.20	0.080

Treatment combination	CCD Run No. ¹	X ₁ Time (min)	X ₂ Temperature (°C)	X ₃ PM ² :Water (m.v ⁻¹)	Extract yield (%) ³	Extract concentration (g.100 mL ⁻¹) ⁴	CIELAB Colour parameters ⁵					Turbidity (NTU) ⁶	Asp yield (%) ⁷
							L*	a*	b*	C*	h		
6	8 (F)	26 (+1)	83 (+1)	1:26 (+1)	21.66	0.833	89.10	3.97	51.04	51.20	85.55	41.53	0.138
	24 (F)	26 (+1)	83 (+1)	1:26 (+1)	19.76	0.835	88.96	4.14	51.54	51.71	85.40	40.17	0.146
	40 (F)	26 (+1)	83 (+1)	1:26 (+1)	21.30	0.819	89.06	4.04	51.38	51.54	85.50	39.53	0.137
7	4 (F)	14 (-1)	83 (+1)	1:26 (+1)	21.68	0.834	88.83	4.55	54.50	54.69	85.23	32.47	0.138
	20 (F)	14 (-1)	83 (+1)	1:26 (+1)	21.42	0.824	88.53	4.69	55.20	55.40	85.14	32.53	0.136
	36 (F)	14 (-1)	83 (+1)	1:26 (+1)	21.29	0.819	88.46	4.77	55.65	55.85	85.10	30.23	0.134
8	7 (F)	26 (+1)	83 (+1)	1:14 (-1)	21.19	0.513	83.11	11.43	73.25	74.13	81.13	61.50	0.104
	23 (F)	26 (+1)	83 (+1)	1:14 (-1)	21.12	1.508	82.91	11.74	73.67	74.93	80.99	64.90	0.112
	39 (F)	26 (+1)	83 (+1)	1:14 (-1)	20.66	1.476	83.06	11.52	73.51	74.41	81.09	59.20	0.107
9	14 (F)	20 (0)	67 (0)	1:30 (+α)	19.15	0.638	90.77	2.70	48.55	48.62	86.61	23.13	0.107
	30 (F)	20 (0)	67 (0)	1:30 (+α)	18.76	0.625	90.78	2.71	48.58	48.66	86.81	22.47	0.104
	46 (F)	20 (0)	67 (0)	1:30 (+α)	18.54	0.618	90.90	2.64	48.45	48.52	86.88	19.47	0.105
10	9 (A)	10 (-α)	67 (0)	1:20 (0)	17.87	0.893	87.75	6.41	64.81	65.12	84.35	16.67	0.106
	25 (A)	10 (-α)	67 (0)	1:20 (0)	17.14	0.857	87.77	6.10	63.73	64.02	84.53	17.80	0.102
	41 (A)	10 (-α)	67 (0)	1:20 (0)	18.07	0.904	87.85	6.21	64.24	64.54	84.48	16.57	0.102
11	5 (F)	26 (+1)	51 (-1)	1:14 (-1)	15.91	1.136	85.65	9.86	77.15	77.78	82.72	17.73	0.093
	21 (F)	26 (+1)	51 (-1)	1:14 (-1)	15.85	1.132	85.36	10.13	77.83	78.48	85.59	20.13	0.091

Treatment combination	CCD Run No. ¹	X ₁ Time (min)	X ₂ Temperature (°C)	X ₃ PM ² :Water (m.v ⁻¹)	Extract yield (%) ³	Extract concentration (g.100 mL ⁻¹) ⁴	CIELAB Colour parameters ⁵					Turbidity (NTU) ⁶	Asp yield (%) ⁷
							L*	a*	b*	C*	h		
	37 (F)	26 (+1)	51 (-1)	1:14 (-1)	15.56	1.112	85.49	10.11	77.85	78.50	82.60	18.17	0.085
12	12 (A)	20 (0)	94 (+α)	1:20 (0)	22.61	1.131	86.83	6.41	56.54	56.9	83.53	55.60	0.120
	28 (A)	20 (0)	94 (+α)	1:20 (0)	22.69	1.135	86.83	6.41	56.54	56.90	83.53	56.20	0.123
	44 (A)	20 (0)	94 (+α)	1:20 (0)	22.70	1.135	86.43	6.75	57.62	58.02	83.32	56.07	0.122
13	2 (F)	14 (-1)	51 (-1)	1:26 (+1)	16.22	0.624	91.01	2.95	51.64	51.72	86.72	11.53	0.116
	18 (F)	14 (-1)	51 (-1)	1:26 (+1)	15.71	0.604	91.29	2.66	50.39	50.46	86.97	10.93	0.111
	34 (F)	14 (-1)	51 (-1)	1:26 (+1)	15.41	0.593	91.47	2.52	49.83	49.89	87.10	8.60	0.102
14	3 (F)	14 (-1)	83 (+1)	1:14 (-1)	20.19	1.442	82.86	12.31	77.47	78.45	80.98	62.13	0.110
	19 (F)	14 (-1)	83 (+1)	1:14 (-1)	19.76	1.412	83.20	12.11	77.61	78.55	81.15	57.43	0.104
	35 (F)	14 (-1)	83 (+1)	1:14 (-1)	20.10	1.436	82.95	12.15	77.11	78.07	81.04	63.70	0.105
15	15(C)	20 (0)	67 (0)	1:20 (0)	19.01	0.950	87.29	6.77	65.08	65.43	84.06	20.33	0.101
	16(C)	20 (0)	67 (0)	1:20 (0)	18.80	0.940	87.39	6.58	64.76	65.10	84.20	28.67	0.121
	31(C)	20 (0)	67 (0)	1:20 (0)	18.48	0.924	87.47	6.52	64.73	64.72	84.24	27.00	0.101
16	32(C)	20 (0)	67 (0)	1:20 (0)	18.55	0.927	87.43	6.61	64.65	64.99	84.17	20.10	0.114
	47(C)	20 (0)	67 (0)	1:20 (0)	18.68	0.934	87.44	6.60	65.65	64.98	84.17	20.83	0.114
	48(C)	20 (0)	67 (0)	1:20 (0)	18.58	0.929	87.41	6.63	64.92	65.26	84.17	27.90	0.103

¹(F) = factorial point; (A) = axial point; (C) = central point; ²PM = plant material; ³g SS.100 g⁻¹ PM; ⁴g SS.100 mL⁻¹; ⁵CIELAB colour system values for extracts diluted 20x; ⁶Turbidity of undiluted extract in Nephelometric Turbidity Units (NTU); ⁷Aspalathin yield = g.100 g⁻¹ PM

Table 3.6 ANOVA of experimental results for the polynomial regression equation for dust extract yield (EY; %).

Parameter ¹	Regr. Coeff. ²	SS ³	DF ⁴	MS ⁵	F	P
Intercept	0.622077					
(1) Time (min.) (L)	0.237516	4.717	1	4.717	70.613	0.000
Time (min.) (Q)	-0.002316	0.188	1	0.188	2.820	0.103
(2) Temperature (°C) (L)	0.206249	284.669	1	284.669	4261.508	0.000
Temperature (°C) (Q)	-0.000510	0.482	1	0.482	7.208	0.011
(3) PM:Water (m ⁻¹ .v) (L)	0.211907	4.910	1	4.910	73.497	0.000
PM:Water (m ⁻¹ .v) (Q)	-0.003683	0.476	1	0.476	7.130	0.012
1L x 2L	0.000056	0.001	1	0.001	0.011	0.919
1L x 3L	-0.004595	0.657	1	0.657	9.831	0.004
2L x 3L	0.001272	0.358	1	0.358	5.355	0.027
Lack of fit		0.594	5	0.112	1.779	0.144
Pure error		2.204	33	0.067		
Total SS		298.786	47			
R ²						0.991
R ² _{adj}						0.988

¹L = linear coefficient; Q = quadratic coefficient; L x L = interaction coefficient; PM = Plant material; ²Regression coefficients; ³Sum of squares; ⁴Degrees of freedom; ⁵Mean square

Table 3.7 Polynomial prediction equation for rooibos dust extract yield.

Dependent variable	Prediction equation
Extract yield	$\hat{Y} = 0.622077 + 0.237516X_1 + 0.206249X_2 + 0.211907X_3 - 0.002316X_1^2 - 0.000510X_2^2 - 0.003683X_3^2 - 0.000056X_1X_2 - 0.004595X_1X_3 + 0.001272X_2X_3$

¹X₁ = extraction time (min); X₂ = extraction temperature (°C); X₃ = water-to-plant material ratio (m.v⁻¹)

Table 3.8 Verification of prediction models for extract yield of rooibos dust.

Std. Run	X1 Time (min)	X2 Temp (°C)	X3 PM ¹ : Water (m.v ⁻¹)	Extract yield (%) ²		
				Obs ³	Pred ⁴	%Δ ⁵
1	20	40	20	14.09	13.78	2.25
2	26	51	26	16.08	15.95	0.81
3	14	51	14	14.98	14.90	0.55
4	26	51	14	15.14	15.83	-4.36
5	14	51	26	15.01	15,68	-4.27
6	20	67	10	17.99	17.58	2.31
7	20	67	20	17.77	18.53	-4.11
8	20	67	30	17.87	18.74	-4.66
9	10	67	20	16.77	17.81	-5.83
10	20	67	20	18.53	18.53	-0.01
11	30	67	20	18.79	18.79	-0.01
12	26	83	26	21.68	21.37	1.43
13	14	83	26	19.40	21.13	-8.17
14	26	83	14	20.36	20.77	-1.96
15	14	83	14	20.31	19.86	2.29
16	20	94	20	23.88	22.54	5.94

¹PM = plant material; ² g.100 g⁻¹ PM; ³Observed; ⁴Predicted; ⁵Δ = (Observed – Predicted)

Table 3.9 Extract yield, extract concentration, CIELAB colour parameters, turbidity and aspalathin yield of optimised hot water extracts of rooibos dust (n=20). Bold italic script indicates minimum and maximum values for the each parameter.

Batch	Extract yield (%) ¹	Extract concentration (g.100 mL ⁻¹) ²	CIELAB Colour parameters ³					Turbidity (NTU) ⁴	Asp yield (%) ⁵
			L*	a*	b*	C*	h		
1	21.71	1.086	86.40	7.24	58.63	59.07	82.97	61.60	0.085
2	16.42	0.822	90.13	3.11	46.34	46.44	86.17	43.85	0.097
3	26.86	1.151	86.08	7.34	58.53	58.88	82.84	65.75	0.184
4	22.35	1.118	86.11	8.06	68.54	69.01	83.29	42.17	0.098
5	20.44	1.022	88.49	4.76	49.76	49.99	84.54	45.58	0.110
6	18.03	0.902	90.15	3.01	44.12	44.19	86.09	41.65	0.083
7	19.41	0.970	88.94	4.11	49.09	49.26	85.21	43.00	0.089
8	19.46	0.973	89.90	3.49	45.08	45.22	85.57	54.87	0.159
9	27.42	1.137	85.52	8.03	61.11	61.64	82.52	156.67	0.189
10	23.66	1.183	87.31	5.70	55.56	55.85	84.14	69.17	0.108
11	23.15	1.158	86.67	6.46	57.63	57.99	83.60	71.27	0.105
12	23.36	1.168	87.13	5.85	56.23	56.53	84.06	67.00	0.124
13	21.97	1.099	87.14	5.43	54.95	55.21	79.36	59.95	0.089
14	23.83	1.189	87.22	5.73	54.38	54.68	83.98	63.80	0.137
15	23.75	1.188	87.01	5.80	55.64	55.94	84.05	67.40	0.109
16	22.56	1.128	87.61	5.32	54.27	54.53	84.40	60.92	0.103
17	27.90	1.392	85.42	7.94	60.67	61.18	82.59	196.67	0.202
18	22.30	1.115	87.05	6.09	58.20	58.51	84.03	54.60	0.091
19	21.40	1.070	87.99	4.91	52.84	53.07	84.73	54.20	0.096
20	23.17	1.158	87.45	5.42	55.16	55.44	84.39	57.38	0.101

¹g.100 g⁻¹ PM; ²g soluble solids.100 mL⁻¹; ³ CIELAB colour system values for extracts diluted 20x; ⁴ Undiluted extract turbidity in Nephelometric Turbidity Units (NTU); ⁵Aspalathin yield = g.100 g⁻¹ PM

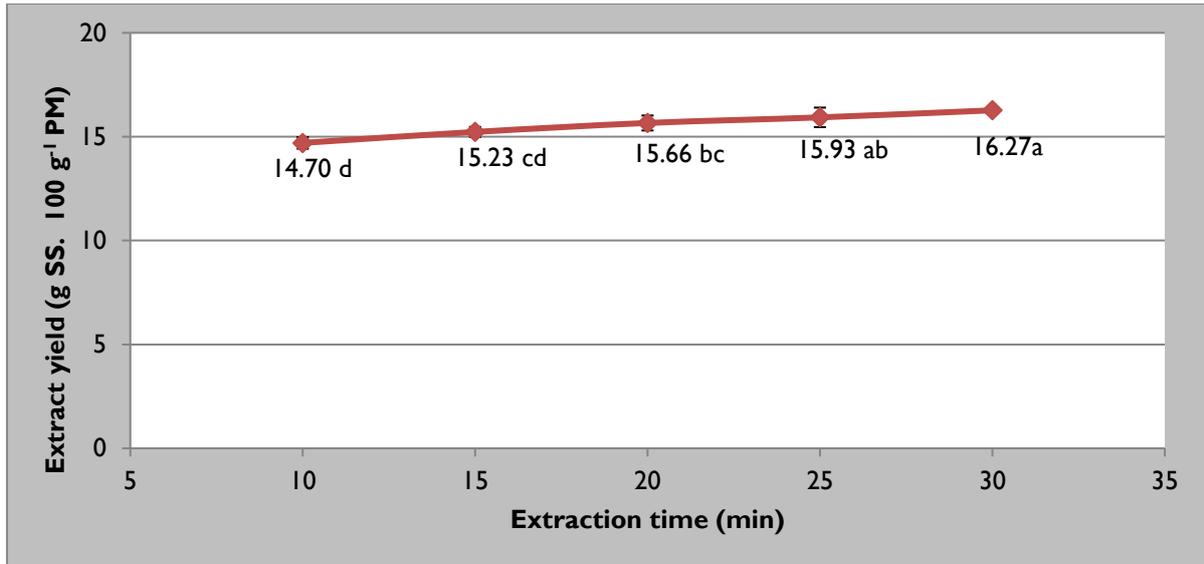


Figure 3.1 Effect of extraction time on extract yield of rooibos dust (extraction temperature = 50 °C; plant material-to-water ratio = 1:20; m.v⁻¹). Values (means with standard deviation as error bars) with the same letter are not significantly different ($P \leq 0.05$). SS, soluble solids; PM, plant material.

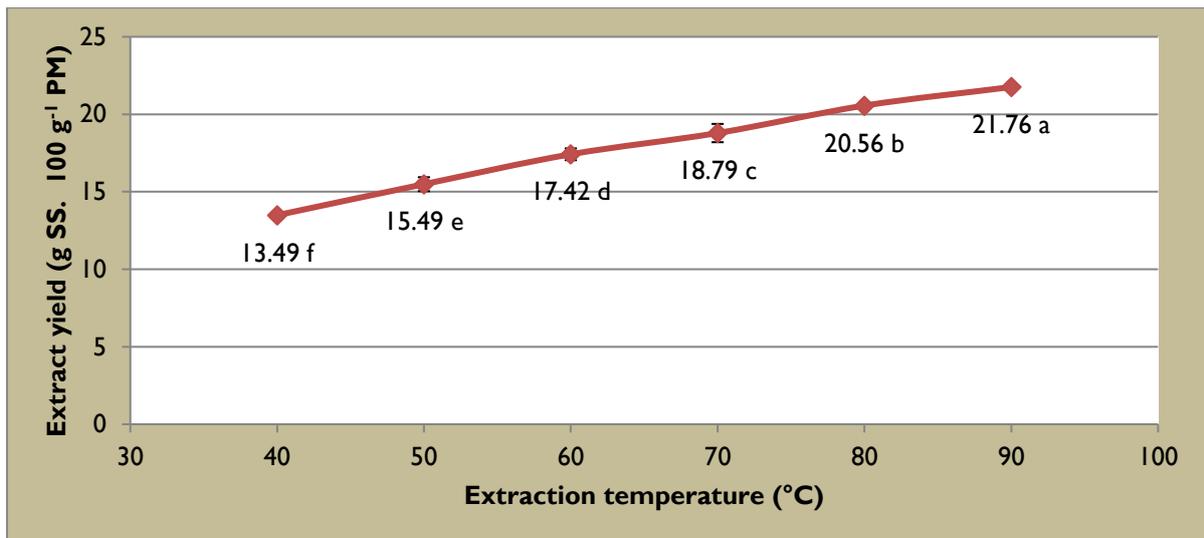


Figure 3.2 Effect of extraction temperature on extract yield of dust (extraction time = 20 min; plant material-to-water ratio = 1:20; m.v⁻¹). Values (means with standard deviation as error bars) with the same letter are not significantly different ($P \leq 0.05$). SS, soluble solids; PM, plant material.

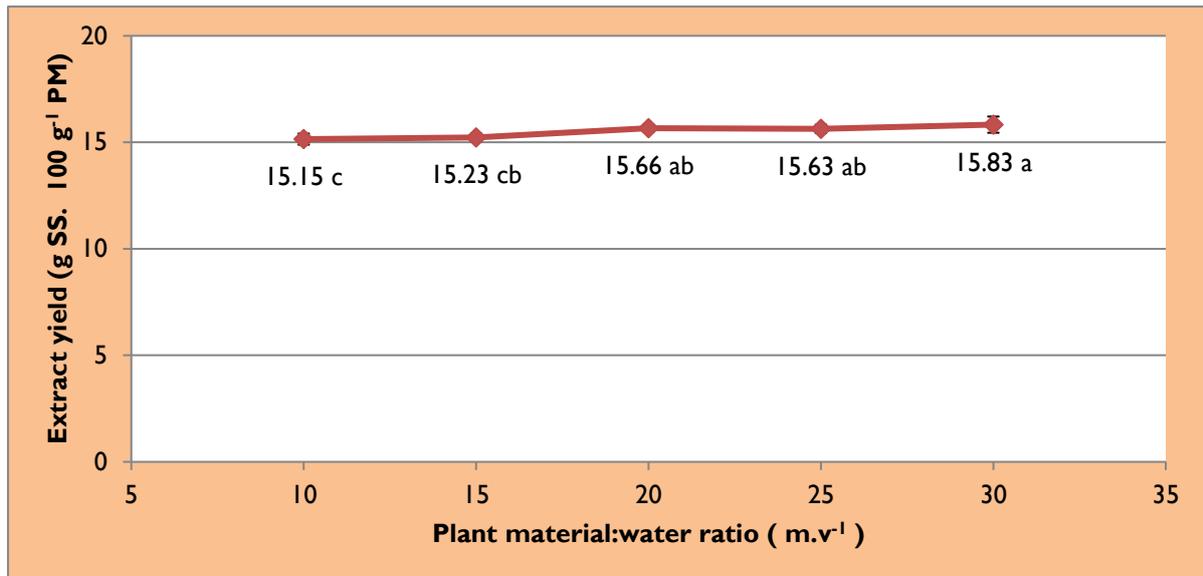


Figure 3.3 Effect of water-to-plant material ratio on dust extract yield of dust (extraction time = 20 min; extraction temperature = 50 °C). Values (means with standard deviation as error bars) with the same letter are not significantly different ($P \leq 0.05$). SS, soluble solids; PM, plant material.

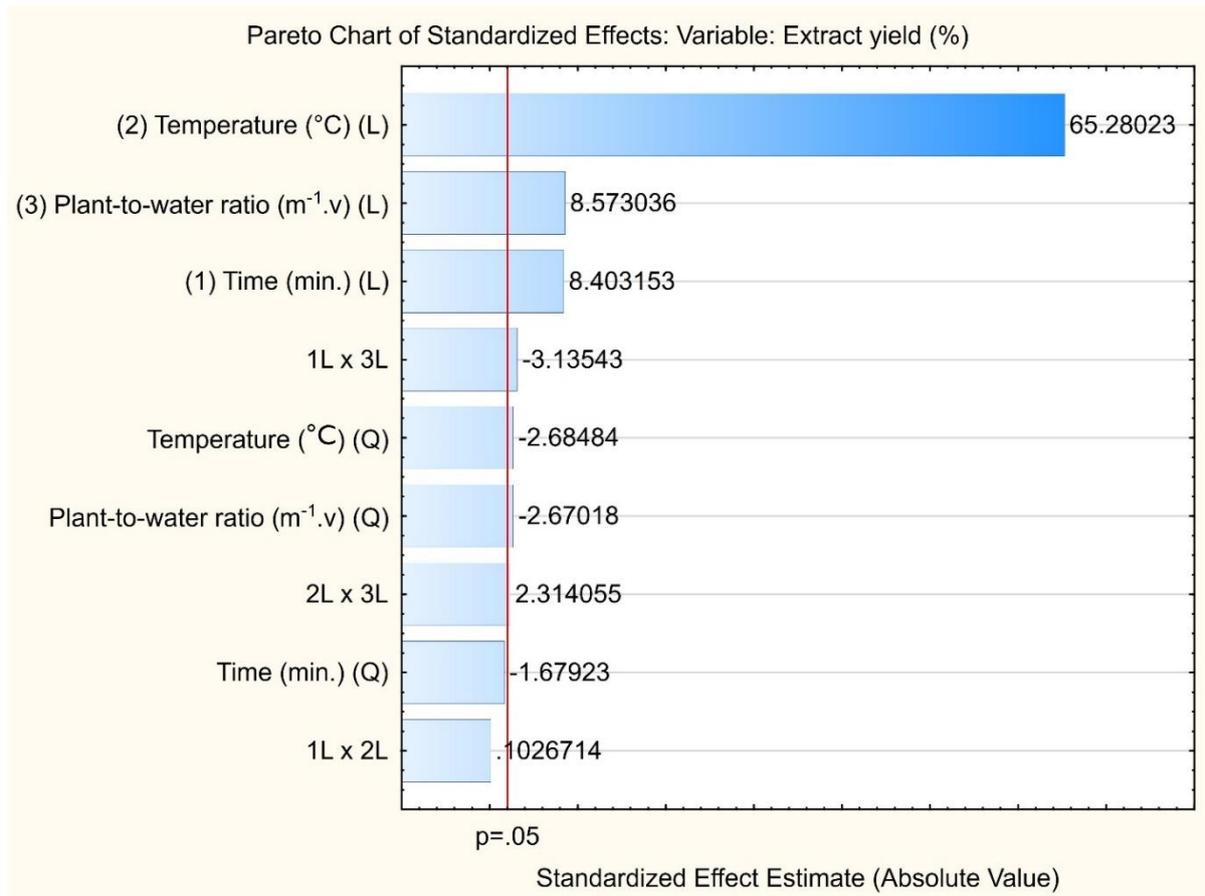
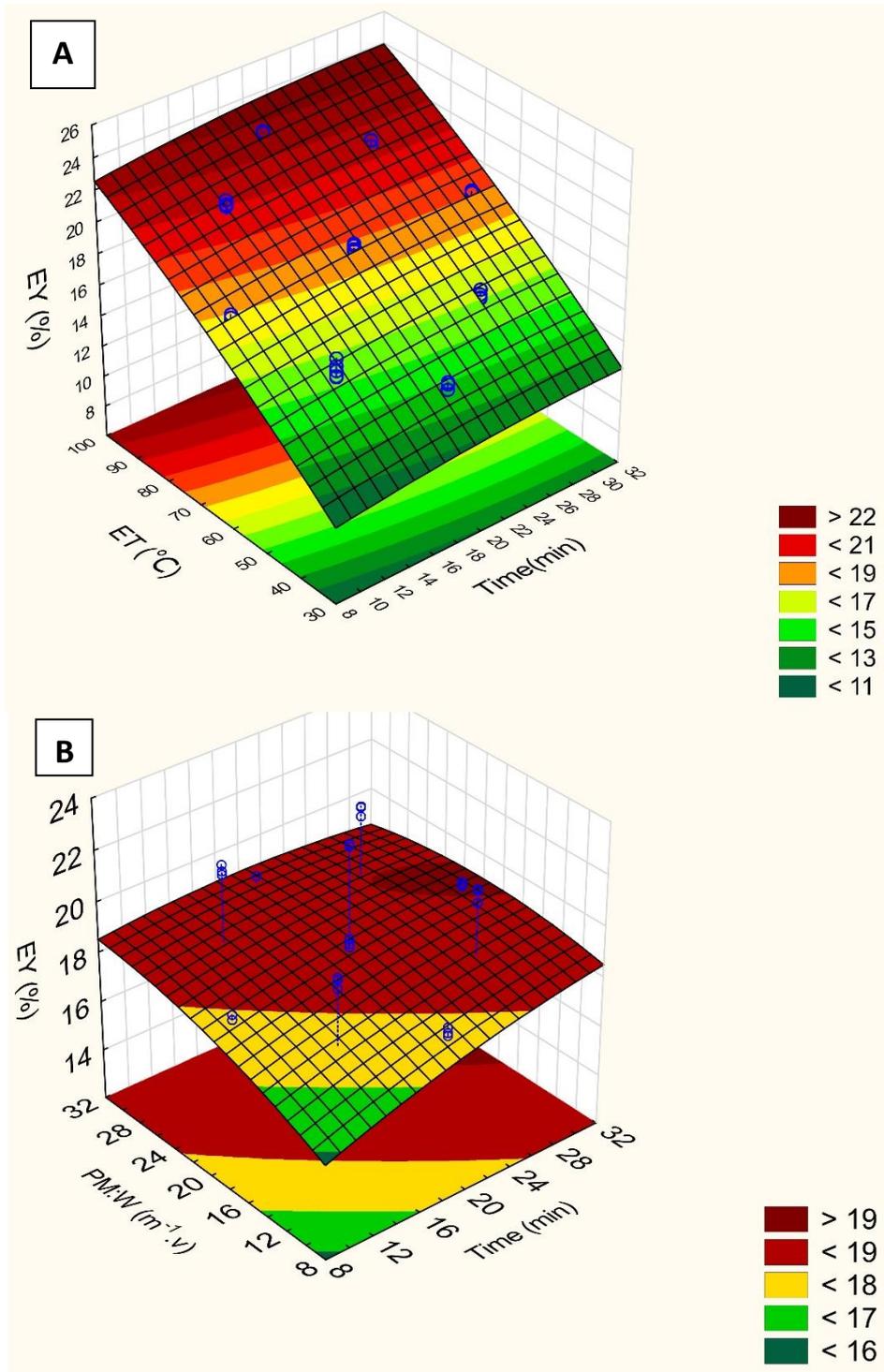


Figure 3.4 Standardised Pareto chart showing linear, quadratic and interaction effects for dust extract yield (EY: g.100 g⁻¹ plant material, %). L = linear effect; Q = quadratic effect; LxL = interaction effect.



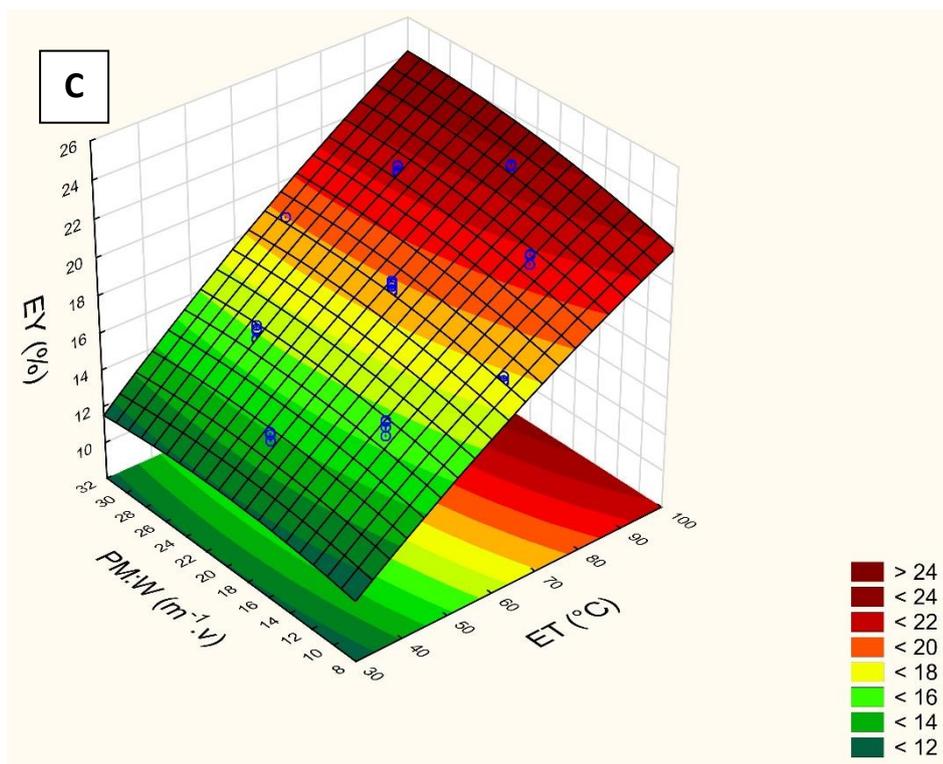


Figure 3.5 Response surface plots for dust extract yield (EY; g.100 g⁻¹ PM, %), showing effects (a) of extraction temperature (ET; °C) and time (min) at fixed plant material-to-water ratio (PM:W) of 1:20 (m:v-l); (b) of PM:W and time (min) at fixed ET of 50°C; (c) of PM:W and ET (°C) at fixed time (20 min).

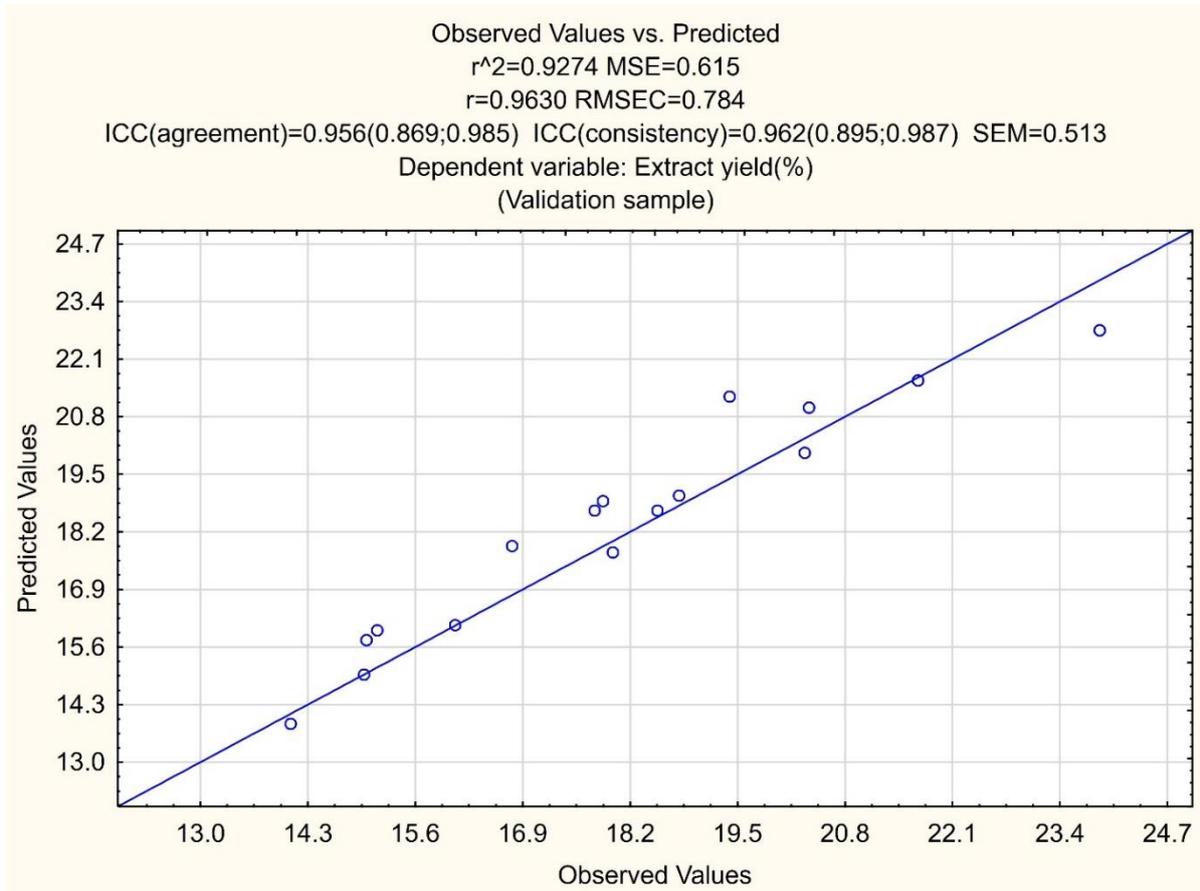


Figure 3.6 Correlation of predicted and observed values for dust extract yield (EY) model verification.

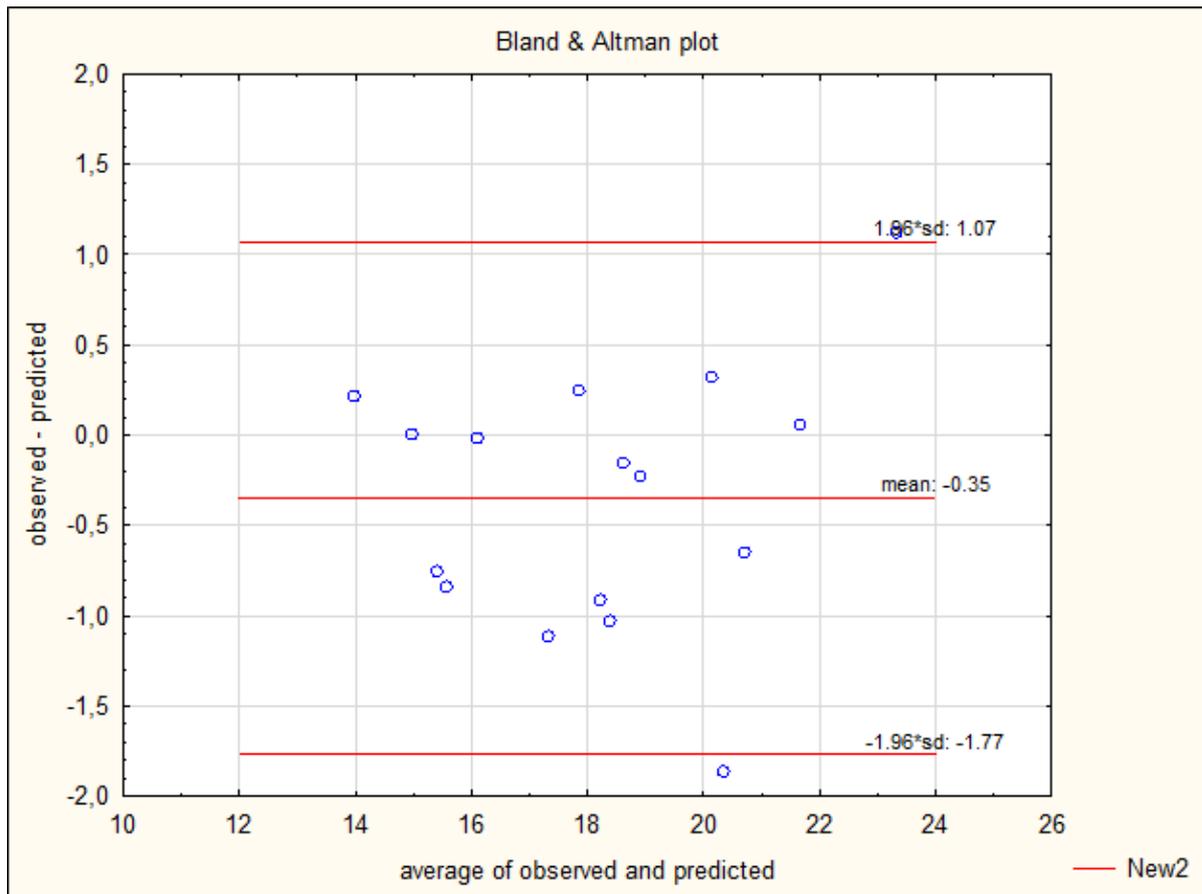


Figure 3.7 Bland-Altman plot for extract yield of rooibos dust model verification.

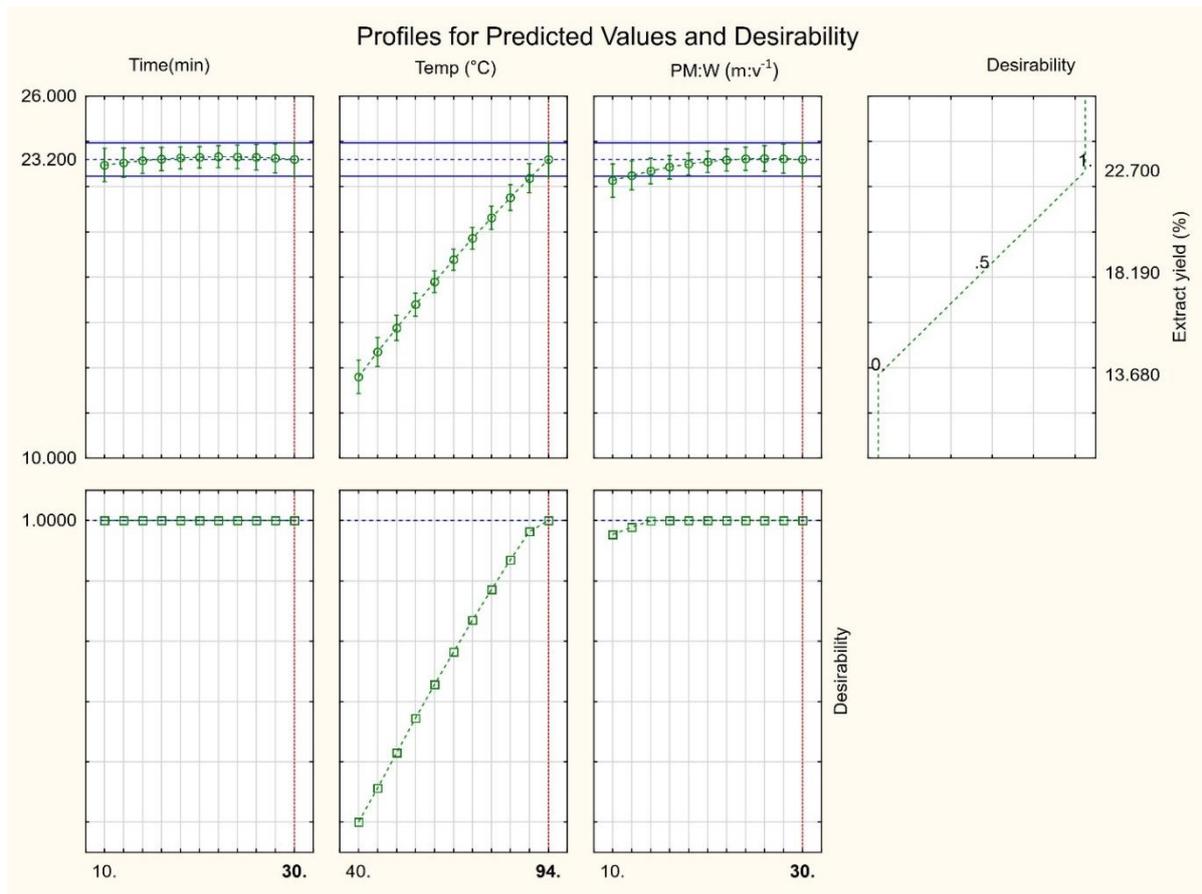


Figure 3.8 Desirability profiles for dust extract yield. Optimal values for each independent variable are indicated in bold at the bottom.

4. Sensory profiling of rooibos waste plant material

4.1. Abstract

A substantial amount of waste ($\pm 10\%$ per production batch) in the form of coarse stems and fine dust is generated during rooibos processing. Current shortages of rooibos merited investigation of the viability of converting rooibos waste material into a good quality rooibos product. Conversion of this waste material into valuable tea products offers not only a waste reduction opportunity, but also an option to stretch annual production. A total of 40 waste material samples, consisting of 20 fine rooibos dust and 20 coarse rooibos stems batches, were collected from one area (Niewoudtville, Northern Cape) and season (2016). The samples were analysed individually and in combinations thereafter using descriptive sensory analysis (DSA) to evaluate 41 aroma, flavour, taste and mouthfeel attributes. Diluted dust extracts, 50/50 ratio diluted dust extract and stem infusion combinations, and 75/25 ratio diluted dust extract and stem infusion combinations at “cup-of-tea” strength produced infusions of similar sensory quality as normal rooibos tea. The diluted dust extracts in particular were of good sensory quality and thus have significant value for the potential production of good quality rooibos beverages. Stem infusions made at “cup-of-tea” strength on the other hand produced weak infusions, suggesting that stem plant material cannot be used alone in teabags, as it would negatively affect the quality of rooibos infusions. In addition, non-typical “planky/pencil shavings”, “raisin” and “almond” aroma attributes were perceived in the stem infusions. The “planky/pencil shavings” aroma note in particular, which was unpleasant and thus regarded as a taint, was carried through into the 50/50 and 75/25 ratio dust extract and stem infusion combinations. Reducing the stem plant material content in the latter combination at “cup-of-tea” strength to 25% was not enough to significantly reduce and mask the undesirable “planky/pencil shavings” aroma. Therefore, careful analysis and understanding of the sensory attributes of the waste plant material is imperative for its potential reutilisation. Good quality rooibos beverages could potentially be produced from waste plant material should the “planky/pencil shavings” aroma note be eliminated satisfactorily. Overall, the results suggest that rooibos waste material could be of significant market value to benefit the rooibos industry.

4.2. Introduction

Rooibos (*Aspalathus linearis*) is an indigenous herbal tea with a global footprint and is enjoyed by consumers worldwide. Unfortunately, persisting droughts and demand that exceeds production, have put rooibos production under pressure. Production yields and export volumes have been affected negatively and have resulted in product shortages. Therefore, the increasing demand for rooibos in conjunction with the current shortages provided motivation for the present investigation into the utilisation of fermented rooibos waste plant material in the form of fine dust and coarse stems. In Chapter 3, soluble matter was successfully extracted from the fine rooibos dust. Moreover, it was

established that extracts could be prepared from rooibos dust for food ingredient use or for the “tea-value” enhancement of rooibos stems. However, rooibos dust plant material would not be suitable for addition in teabags as it is extremely fine and would seep through and result in a turbid infusion. In contrast, rooibos stems have been shown to produce weak infusions and are thus regarded as a poor quality product with low tea-value (Joubert, 1984). However, processed stems can be blended at certain percentages with normal fermented rooibos tea leaves to make good quality infusions in order to stretch annual production. Because rooibos stems cannot completely replace fermented rooibos tea leaves to make good quality infusions, rooibos dust and stems could potentially be combined to produce a rooibos product, where the stems could act as a carrier of soluble dust matter. The soluble dust matter would thus enhance the quality of the stems. Alternative uses for rooibos dust and stems are, therefore, required for the conversion of plant material waste into a good quality rooibos product.

Market research based on consumer liking of healthy products demonstrated that flavour and taste are the main drivers of consumers’ decisions to purchase such products. However, no sensory data are available on infusions made from rooibos tea waste as no sensory research has been conducted on the waste plant material to date. In addition, no data are available on properties such as colour, turbidity and phenolic composition of infusions made from individual rooibos waste products or combinations of rooibos dust and stems when reconstituted to “cup-of-tea” strength. Whether the sensory attributes of waste plant material will be similar or different to those of normal rooibos tea, when used individually or in combination, is unknown. The latter, therefore, needs to be established as it is critical for the future use of rooibos waste plant material. Descriptive sensory analysis (DSA), using a sensory lexicon and wheel as reference, is an ideal method for profiling rooibos waste plant material. This method enables the complete sensory description of products compiled by a panel of well-trained judges through the differentiation and description of qualitative (attributes) and quantitative (intensity) sensory characteristics (Meilgaard *et al.*, 1999, Lawless & Heymann, 2010).

Research by Koch *et al.* (2012) and Jolley *et al.* (2017) described the characteristic sensory profile of rooibos as “honey”, “rooibos-woody” and “fynbos-floral” notes coupled with a slightly sweet taste and astringent mouthfeel. In addition to this primary characteristic aroma profile, “fruity-sweet”, “caramel”, “apricot” and “hay/dried grass” aromas were considered to be part of the secondary characteristic aroma profile of rooibos. Therefore, profiling the rooibos waste plant material will provide valuable information regarding sensory attribute similarities and differences in comparison to the primary and secondary characteristic profiles of rooibos infusions.

In view of the above-mentioned shortcomings, this investigation was conducted to characterise and quantify sensory attributes (aroma, flavour, taste and mouthfeel) associated with diluted dust extracts and stem infusions individually, and dust extract and stem infusion combinations at “cup-of-tea” strength. The individual diluted dust extracts, stem infusions, and dust extract and

stem infusion combinations at “cup-of-tea” strength were also characterised in terms of colour, turbidity and phenolic content. This was done in order to determine the potential commercial viability of producing a valuable rooibos product with the same sensory quality parameters as a cup of rooibos tea produced from fermented leaves.

4.3. Materials and methods

The sensory profiling of rooibos waste plant material individually and in combinations was conducted in the following order: diluted dust extracts, stem infusions, diluted dust extract and stem infusion combinations (50/50 ratio), and lastly diluted dust extract and stem infusion combinations (75/25 ratio). The manner in which all samples for each experiment were prepared is described below.

4.3.1. Rooibos and rooibos waste plant material samples

Twenty (n=20) batches of fine dust and coarse stems each, drawn from various production batches (Batches 1 to 20; ≈2kg per batch), were sourced from Bokkeveld Rooibos (Nieuwoudtville, South Africa) in 2016. A rooibos reference sample was made up by blending six batches of B-grade rooibos samples sourced from Rooibos Ltd, Clanwilliam, South Africa in 2012. This rooibos reference sample was used to make control infusions for use during both the DSA training and testing phases of the diluted dust extracts and stem infusions. For the DSA training and testing phases of the 50/50 ratio diluted dust extract and stem infusion combinations, 20 batches of good quality rooibos tea of the 2017 production season were sourced from Rooibos Ltd and used as reference samples. For the DSA training phase of the 75/25 diluted dust extract and stem infusion combinations, six samples from the latter 20 batches of good quality rooibos tea were selected and blended to make a reference sample for use as a control. All reference samples used served as fixed points to which all other experimental samples could be compared, thus permitting panel members to calibrate their sensory perception at the beginning of all training and testing sessions.

4.3.2. Sample preparation

Control samples To ascertain that no sample differences arose from variations in the preparation of the infusions it was necessary to ensure that a standardised tea preparation protocol was followed. Normal rooibos infusions were prepared as described by Jolley *et al.* (2017) by pouring 1000 g of freshly boiled distilled water onto 19.3 g of pasteurised rooibos leaves. After the infusion was stirred for 5 s, it was covered with foil and left to infuse for 5 min. Thereafter it was strained through a fine mesh tea strainer into a pre-warmed thermos flask (1000 mL). Approximately 100 mL of infusion was

then poured into each of the white porcelain mugs, which were covered with plastic lids to ensure that no evaporation or loss of volatiles took place. Thereafter, approximately 50 mL of each infusion was filtered using Whatman No.4 filter paper for turbidity measurements. A portion of the filtered infusions was transferred into several 2 mL microfuge tubes which were stored in a freezer at -18°C until required for further high-performance liquid chromatography (HPLC) analyses (Section 4.3.5). Another 50 mL of each infusion was used unfiltered for the determination of soluble solids (SS) (Section 4.3.6) and CIELAB colour measurements (Section 4.3.7). Each rooibos sample was prepared in triplicate. The layout of the sample preparation and analysis protocol is shown in Figure 4.1. One control sample, replicated over 4 testing sessions, was used during the diluted dust extract and stem infusion experiments, and 20 control samples were used during the 50/50 ratio diluted dust extract and stem infusion combination experiments (Figure 4.2).

During preparation of the rooibos infusions it was essential that the temperature of the infusions was kept as constant as possible at all times. Similar to Jolley *et al.* (2017), various actions were taken to ensure that the temperature of the infusions was at no point compromised. Stainless steel thermos flasks, used to aid in the maintenance of the constant temperature of the infusions, were pre-heated prior to the addition of the infusion. The white porcelain mugs were also pre-heated in an oven (Hobart industrial oven, USA, temperature setting = 70°C) prior to the addition of the infusions. A consistent infusion temperature was not only essential during the preparation stage but also during the DSA training and testing phases so as not to compromise the sensory quality and attributes intensities of the infusions. This was achieved by placing the infusion filled mugs in scientific water-baths (SMC, Cape Town, South Africa, temperature setting = 65°C) where they were kept throughout the analysis period.

Diluted dust extracts The 20 batches of fine rooibos dust were used to firstly produce 20 dust extracts using the optimised extraction conditions indicated in Chapter 3 (Section 3.4.3.4). As the SS content of the dust extracts were very high, the dust extracts were diluted to 1000 mL with hot distilled water to simulate “cup-of-tea” strength, i.e. according to the average SS content of hot water rooibos infusions ($0.224\text{ g SS}\cdot 100\text{ mL}^{-1}$) (Figure 4.2) as previously determined by Koch (2011). Therefore, a specific amount of freshly boiled distilled water was poured onto a pre-determined amount of concentrated dust extract (Addendum B; Table B4.1), where after the mixture was stirred for 5 s and then poured directly into a pre-warmed thermos flask (1000 mL). The rest of the preparation process was the same as that of the control samples as described above.

Stem infusions The 20 batches of coarse stem were used to prepare 20 stem infusions at “cup-of-tea” strength in the exact same manner the control samples were prepared (Figure 4.2). Freshly boiled

distilled water (1000 g) was poured onto 19.3 g rooibos stems. Thereafter, the rest of the control sample preparation process was followed.

Diluted dust extract and stem infusion combinations The 20 batches of fine dust waste plant material were used to first make 20 dust extracts as described above. The 20 dust extracts were randomly paired with the 20 batches of stems to make combination infusions at “cup-of-tea” strength in 50/50 and 75/25 ratios (Table 4.1). However, only six batches of dust extracts and stems pairs used for the 50/50 ratio combinations sensory analysis were selected and paired for the 75/25 ratio combinations sensory analysis. The respective ratios represented the SS content of the “cup-of-tea” strength, i.e. the 50/50 combination infusions contained 50% SS (± 0.112 g SS.100 mL⁻¹) from the concentrated dust extract and 50% SS (± 0.112 g SS.100 mL⁻¹) from the stem plant material, where the 75/25 combination infusions contained 25% (± 0.056 g SS.100 mL⁻¹) from the stem plant material and 75% (± 0.168 g SS.100 mL⁻¹) from the concentrated dust extract (Figure 4.2). Therefore, for each combination experiment, a pre-determined amount of concentrated dust extract (in mL) was poured onto a pre-determined amount of coarse stems (in g) depending on the desired SS ratio (Addendum B, Table B4.3). Thereafter, a specific amount of freshly boiled distilled water was poured onto the concentrated dust extract and stem plant material mixture, and the mixture was topped up to 1000 g. The infusion was stirred for 5s, covered with foil and left to infuse for 5 min. The rest of the control sample preparation process was followed.

4.3.3. Descriptive sensory analysis

4.3.3.1. Panel training

The panel members were selected according to availability and sensory analysis experience. The majority of the panel members took part in previous rooibos studies by Koch *et al.* (2012) and Jolley *et al.* (2017). A total of 12 female assessors participated in this study. Panel training was done in accordance with the consensus method set out by Lawless and Heymann (2010), and Koch *et al.* (2012). At the beginning of the training phase, the assessors were informed of the objectives and outline of the current study, and were re-familiarised with the training methods and protocol involved in descriptive sensory analysis (DSA). When analysing a sample, the panel was instructed to remove the sample from the water-bath, remove the plastic lid and swirl the contents of the mug several times before analysing the aroma (orthonasal aroma). Flavour (retronasal aroma), as well as taste and mouthfeel attributes were analysed by directing the assessors to suck up a mouthful of the infusion off a rounded tablespoon whilst breathing in, as opposed to sipping the infusion from the mug. This action draws the infusion aroma up to the olfactory nerve located in the nose, allowing assessors to identify

the aromas, i.e. specific or a combination of volatile compounds present in the infusions. Non-volatile rooibos compounds such as quercetin-3-O-glucoside, iso-orientin and aspalathin give rise to the basic taste modalities (sweet, sour and bitter taste) and the mouthfeel attribute astringency, and are all perceived by the mouth (Owour, 2003). The panel was directed to swallow, i.e. not expectorate the cup contents, and to cleanse their palates between samples with water biscuits (Woolworths, Cape Town, South Africa) and distilled water.

Control samples were used to calibrate the sensory perception of the panel at the start of each training session, as well as during the DSA testing sessions. The control samples embodied rooibos tea infusions with the perfect balance between positive and negative attributes and had to represent a “characteristic” cup of rooibos tea. Other reference standards were also used during the training phase, primarily to familiarise assessors with specific sensory attributes, such as “rooibos-woody” and “planky/pencil shavings” aromas. The latter reference samples were actual rooibos samples exhibiting a high intensity of the specific attribute in question.

During the training phase the definitions for each of the attributes were adjusted, where necessary. These changes ensured that the definitions used were both clear and concise. Any attributes found not to be important to the rooibos profile of the samples, or not frequently present in the samples, were removed from the initial, standard list of attributes. The final lists of attributes used in the DSA training and testing periods are summarised in Table 4.2 and Table 4.3.

4.3.3.2. Analysis of rooibos waste plant material samples using DSA

Once the training of the panel was completed, the panel members proceeded to the testing phase of DSA, which entailed scoring the intensities of the attributes of each sample. This was done using the Compusense® *five* program (Compusense, Guelph, Canada). The assessors rated the intensities of 19 aroma attributes, 18 flavour attributes, 3 taste attributes and 1 mouthfeel attribute of the samples being tested (Table 4.2 and Table 4.3). The intensity rating of each attribute was scored on an unstructured line-scale, ranging from 0 (not detectable) to 100 (extremely high intensity). The testing took place over a total of 21 days, with samples being tested in triplicate daily. Between each testing session, the panel was required to take a 10-min break; this allowed the panel to rest and limit panel fatigue. The samples, labelled with 3-digit codes for blind testing, were presented to each of the assessors in a randomised order. The control samples used during the training and testing phases of the diluted dust extracts, stem infusions and 50/50 ratio diluted dust extract and stem infusion combinations were tested blind in order to see how similar or different the waste material infusions were when compared to the control. However, the control sample used during the 75/25 ratio diluted dust extract and stem infusion combinations was indicated as such and was thus not scored.

4.3.4. Chemicals

Authentic reference standards (purity $\geq 95\%$) were obtained from Extrasynthese (Genay, France; iso-orientin, orientin, isovitexin, hyperoside), Karl Roth (Karlsruhe, Germany; vitexin), Sigma-Aldrich (St. Louis, MO, USA; isoquercitrin), Transmit (Gießen, Germany; rutin) and the South African Medical Research Council (PROMEC Division, Bellville, South Africa; aspalathin and nothofagin). Z-2-(β -D-glucopyranosyloxy)-3-phenylpropenoic acid (PPAG) was obtained from the compound library of the Post-Harvest & Agro-Processing Technologies Division of the Agricultural Research Council (Infruitec-Nietvoorbij) of South Africa. Glacial acetic acid ($\geq 98-100\%$) and HPLC gradient grade acetonitrile were purchased from Merck Millipore (Darmstadt, Germany), and ascorbic acid was purchased from Sigma-Aldrich. Deionised water prepared using an Elix Advantage 5 water purification system (Merck Millipore) was purified further to obtain high performance liquid chromatography (HPLC) grade water using a Milli-Q Reference A⁺ water purification system (Merck Millipore). Deionised water was used to make all infusions.

4.3.5. Determination of SS content

The SS content of all control infusions, diluted dust extracts, stem infusions, and diluted dust extract and stem infusion combinations was determined as described in Chapter 3 (section 3.3.6). Results were expressed as g SS.100 mL⁻¹ infusion (%; m.v⁻¹).

4.3.6. HPLC analysis

HPLC analysis of all control infusions, diluted dust extracts and stem infusions was conducted as described in Chapter 3 (section 3.3.7).

4.3.7. CIELAB colour and turbidity measurements

The objective colour and turbidity measurements of all control infusions, diluted dust extracts, stem infusions, and diluted dust extract and stem infusion combinations were determined as described in Chapter 3 (section 3.3.8).

4.4. Statistical analysis

A complete block design was used and the data were analysed using various appropriate statistical methods. Panel performance was tested using PanelCheck software (Version 4.1.0, Nofima, Norway).

Reliability of the panel was determined using test-retest analysis of variance (ANOVA), using SAS® software version 9.2 (SAS Institute, Cary, NC, USA). The normality of the residuals was determined using the Shapiro-Wilk test (Shapiro & Wilk, 1965). Where necessary, outliers were identified and removed until the data were normally distributed. Least significant difference (LSD; $P = 0.05$) was calculated to determine if there were significant differences between the attributes of the control infusions and experimental samples. XLSTAT (Version 2014.01.02, Addinsoft, France) was used to create principal component analysis (PCA) plots and histograms.

4.5. Results

DSA was used to profile the control and rooibos waste plant material samples individually and in combinations thereof. In addition, the SS content, CIELAB colour parameters, turbidity and phenolic content of the control samples, diluted dust extracts, stem infusions, diluted dust extract and stem infusion combinations (50/50 ratio), and diluted dust extract and stem infusion combinations (75/25 ratio) were determined. The results are given below.

4.5.1. Determination of similarities and differences between the control infusion and diluted dust extracts

One control sample, replicated over four testing sessions, was used during the sensory profiling of diluted dust extract samples ($n=20$). The control infusion was used as a reference point for profiling the diluted dust extracts. Analysis of variance (ANOVA) was conducted to determine whether the control infusion differed significantly ($P \leq 0.05$) from the diluted dust extracts in terms of the sensory attributes (Table 4.4). The PCA bi-plot (Figure 4.3) illustrates the association between the control infusion, diluted dust extracts and rooibos aroma, taste and mouthfeel attributes, with PC 1 (Factor 1) explaining 45.4% of the variance and PC 2 (Factor 2) 21.7% of the variance. According to Figure 4.3 there is a definite split between the samples, with the control infusion samples grouping on the right of PC 1 and diluted dust extract samples grouping on the left of PC 1.

The control infusion and the diluted dust extracts both illustrated high intensities of “fynbos-floral” and “honey” and furthermore did not differ significantly ($P > 0.05$) in terms of these two, typical positive aroma attributes (Table 4.4). This tendency is also illustrated in the PCA bi-plot with both aroma attributes being situated in the centre of the PCA bi-plot (Figure 4.3), thus associating equally with the control sample and diluted dust extracts. In contrast, other typical positive rooibos aroma attributes such as “rooibos-woody”, “apricot”, “fruity-sweet” and “caramel”, including “sweet spice”, differed significantly ($P \leq 0.05$) between the control infusion and the diluted dust extracts (Table 4.4). With the exception of “caramel” and “sweet spice” aroma, these attributes were more associated

with the control infusion than with the diluted dust extracts on the right of the PCA bi-plot (Figure 4.3). “Apricot” was particularly low in the control infusion (mean intensity = 3.8) and “sweet spice” (mean intensity = 1.1) in the diluted dust extracts. Therefore, both attributes illustrated mean intensities that were barely perceptible. On the other hand, “hay/dried grass” which is a typical negative rooibos aroma attribute, differed significantly ($P \leq 0.05$) between the control infusion and the diluted dust extracts. According to Figure 4.3, the “hay/dried grass” aroma associated more with the control infusion. In addition, other negative rooibos aroma attributes such as “green grass”, “seaweed” and “medicinal/rubber”, which differed significantly ($P \leq 0.05$) between the control infusion and the diluted dust extracts ($P \leq 0.05$), were associated more with the control infusion, but they were all perceived at extremely low intensities (<5). Furthermore, although negative rooibos aroma attributes “rotting plant water”, “burnt caramel”, “dusty” and “musty/mouldy” seemed to associate more with the control infusion than the diluted dust extracts (Figure 4.3), no significant differences were observed ($P > 0.05$) in these attributes that were all perceived at extremely low intensities (Table 4.4).

In terms of the taste and mouthfeel attributes, sweet, sour and bitter taste and astringency, differed significantly ($P \leq 0.05$) between the control infusion and diluted dust extracts (Table 4.4). With the exception of the sweet taste, which is a typical positive rooibos taste attribute, the typical rooibos mouthfeel attribute, astringency, and the negative taste attributes were associated more with the control infusion (Figure 4.3). The sour and bitter tastes in particular were perceived at extremely low intensities in the control infusion (mean intensities = 1.83 and 6.05, respectively).

Similar trends were noted for the flavour attributes, which were less prominent and mostly perceived at lower intensities than the corresponding aroma attributes (Table 4.4). A PCA bi-plot including all aroma, flavour, taste and mouthfeel attributes is supplied in the addendum (Addendum B; Figure B4.1).

To further characterise the diluted dust extracts, their SS content, turbidity, CIELAB colour parameters and phenolic content were determined for comparison with the control infusions. The results are summarised in Table 4.5 and Table 4.6. The mean SS content and all CIELAB colour parameters of the diluted dust extracts were within the same range as those of the control infusions. However, the mean turbidity of the diluted dust extracts (10.06 NTU) was much lower than that of the control infusions (28.08 NTU). According to Table 4.6, the major phenolic compounds in the diluted dust extracts were aspalathin, orientin, isoorientin and phenylpyruvic acid glucosidase (PPAG) ($>10 \text{ mg.L}^{-1}$), followed by vitexin and isovitexin ($>5 \text{ mg.L}^{-1}$). The mean aspalathin content of the diluted dust extracts, however, was approximately half the mean aspalathin content of the control infusion. Orientin had the highest mean concentration in the diluted dust extracts and in the control infusion. In addition, the mean orientin, isoorientin, vitexin, isovitexin and PPAG content of the diluted dust extracts was lower than that of the control infusion. Nothofagin, isoquercitrin, hyperoside and rutin

in the control infusion, grade B rooibos infusions and diluted dust extracts ranged from not being detected to being detected in small amounts.

4.5.2. Determination of similarities and differences between the control infusion and stem infusions

The same control sample used for profiling the diluted dust extracts, replicated over 4 testing sessions, was used during the sensory profiling of stem infusion samples (n=20). The control infusion served as a reference point for profiling the stem infusions. Table 4.7 summarises the overall means of the sensory attributes of the control infusion and stem infusions. The association between the control infusion, stem infusions and rooibos aroma, taste and mouthfeel attributes is illustrated in Figure 4.4. The PCA bi-plot portrays the variation of the samples as they are plotted in relation to each other based on their sensory profiles, with PC 1 (Factor 1) explaining 48.3% of the variance and PC 2 (Factor 2) 22.3% of the variance. Similar to the diluted dust extract samples (Figure 4.3), there is a split between the samples, with the control infusion samples grouping on the right side of PC 1 and a large majority of the stem infusion samples grouping more to the left of PC 1.

All aroma, taste and mouthfeel attributes differed significantly ($P \leq 0.05$) between the control infusion and stem infusions, except for the “caramel/vanilla”, “musty/mouldy” and “rotting plant water” aroma notes (Table 4.7). The three typical positive rooibos aroma attributes, “fynbos-floral”, “rooibos-woody” and “honey”, illustrated high intensities and were more associated with the control infusion, while “apricot”, “fruity-sweet” and “sweet spice”, which were all perceived at extremely low intensities (≤ 5), were associated more with the stem infusions (Figure 4.4). “Hay/dried grass”, a typical negative rooibos aroma attribute, along with other negative rooibos aroma attributes (“green grass”, “rotting plant water”, “seaweed” and “burnt caramel”) were perceived at extremely low intensities (< 5) and associated more with the control infusion (Figure 4.4).

Interestingly, with the analysis of the stem infusions, specific aroma attributes emerged, i.e. “planky/pencil shavings”, “raisin” and “almond” (Figure 4.4). These attributes which are not typical of rooibos were not perceived during the analysis of the control infusion and diluted dust extracts at notable intensities (Figure 4.3). “Planky/pencil shavings” in particular was regarded as a negative attribute and was perceived at a low, but perceptible intensity (> 5 , Table 4.7).

With the exception of the sweet taste, which is a typical positive rooibos taste, astringency and sour and bitter tastes, associated more with the control infusion (Table 4.7, Figure 4.4).

Again, similar trends were noted with the flavour attributes that were less prominent and mostly perceived at lower intensities than the corresponding aroma attributes (Table 4.7). Similar to the diluted dust extracts, “fynbos-floral”, “rooibos-woody” and “hay/dried grass”, which are all typical rooibos flavour attributes, were the most prominent flavours in the control infusion and stem infusions. The “planky/pencil shavings” flavour, which is regarded as negative and not typical of rooibos,

was perceived at low intensities in the stem infusions (mean intensity = 5.55, Table 4.7). A PCA biplot including all sensory attributes, including the flavour attributes, is supplied in the addendum (Addendum B; Figure B4.2).

Table 4.5 and Table 4.6 summarises the SS content, turbidity, CIELAB colour parameters and phenolic content of the stem infusions. The SS content of the stem infusions had a large range and its mean was almost half the mean SS content of the control infusions, although the stem infusions were produced at “cup-of-tea” strength i.e. using the same mass of plant material as for the control infusions. Similar to the SS content, the turbidity of the stem infusions also had a large range and its mean (18.91) was lower than the mean turbidity of the control infusions (28.08). On average, the stem infusions had a higher L* value, and a lower a* and C* value in comparison to the control infusions. This means that on average the colour of the stem infusions was lighter, less red and less saturated. The major phenolic compounds in the stem infusions were orientin (>10 mg.L⁻¹), followed by aspalathin, isoorientin, PPAG and quercetin-3-O-robinobioside (>5 mg.L⁻¹). Similar to diluted dust extracts, orientin had the highest concentration in the stem infusions. The mean isoorientin content of the stem infusions, however, was much lower than the mean isoorientin content of the control infusion, grade B rooibos infusions and diluted dust extracts. The difference in aspalathin content between the stem infusions, diluted dust extracts and grade B rooibos infusions was not large. Nothofagin, vitexin, isovitexin isoquercitrin, hyperoside and rutin in the stem infusions ranged from not being detected to detection in small amounts.

4.5.3. Determination of similarities and differences between the control infusions, and diluted dust extract and stem infusion combinations

The sensory profiles of the diluted dust extract and stem infusion combinations at “cup-of-tea” strength were investigated, firstly a 50/50 ratio diluted dust extract and stem infusion combination, and thereafter a 75/25 ratio diluted dust extract and stem infusion combination. In the latter experiment, the 50/50 ratio diluted dust extract and stem infusion combinations were repeated and compared to that of the 75/25 ratio diluted dust extract and stem infusion combinations in terms of sensory profile, SS content, CIELAB colour parameters and turbidity. In this chapter and henceforth the former will be referred to as the “50/50 ratio combinations” and the latter as the “75/25 ratio combinations”.

4.5.3.1. 50/50 ratio diluted dust extract and stem infusion combinations

A summary of the overall means of the sensory attributes of the control infusions (n=20) and 50/50 ratio combinations (n=20) is displayed in Table 4.8. The association of the control infusions, 50/50 ratio combinations and the rooibos aroma, taste and mouthfeel attributes is illustrated in Figure 4.5. This PCA bi-plot portrays the variation of the samples as they are plotted in relation to each other, based on their sensory profiles, with PC 1 (Factor 1) explaining 34.1% of the variance and PC 2 (Factor 2) 19.5% of the variance. Similar to Figure 4.2 and Figure 4.3, there is a definite split between the samples, with the control infusion samples grouped on the left of PC 1 and 50/50 ratio combinations on the right of PC 1 with the exception of one sample (Combo 2).

The typical positive rooibos aroma attributes, “fynbos-floral”, “rooibos-woody”, “honey” and “caramel/vanilla”, differed significantly ($P \leq 0.05$) between the control infusions and 50/50 ratio combinations, and were all associated more with the control infusions, except for “caramel/vanilla” which associated significantly ($P \leq 0.05$) more with the 50/50 ratio combinations (Table 4.8, Figure 4.5). However, although significant ($P \leq 0.05$), the differences in the intensities of these attributes in the 50/50 ratio combinations and control infusions were not large. The histograms illustrated in Figure 4.6 visualise the distribution of the three above-mentioned typical positive rooibos aroma attribute intensities over a 100-point scale. The “fynbos-floral”, “rooibos-woody” and “honey” histograms of the 50/50 ratio combinations and control infusions had similar shapes indicating that these aroma notes were perceived at similar intensities in the 50/50 ratio combinations and control infusions. “Apricot” and “fruity-sweet” which are also regarded as typical positive rooibos aroma attributes did not differ significantly ($P > 0.05$) between the control infusions, and dust extract and stem combination infusions although they seem to be associated more with the control infusions on PC 1 (Figure 4.5).

Positive rooibos aroma attributes, “apple” and “sweet spice”, perceived at extremely low intensities (<2), differed significantly ($P \leq 0.05$) between the control infusions and 50/50 ratio combinations. These two attributes associated more with the 50/50 ratio combinations. The three non-typical rooibos aroma attributes, “planky/pencil shavings”, “raisin” and “almond” which were previously perceived in the stem infusions, were present in the 50/50 ratio combinations. “Planky/pencil shavings” and “almond” illustrated significantly higher intensities in the 50/50 ratio combinations ($P \leq 0.05$), however, this was not the case for “raisin” aroma (Table 4.8). The histograms in Figure 4.7 visualise the distribution of these three aroma attribute intensities over a 100-point scale. The large majority (91.7%) of the 50/50 ratio combinations had a “planky/pencil shavings” aroma intensity between 0 and 15, where all the control infusion samples had a “planky/pencil shavings” aroma intensity between 0 and 10. From the shape of the density curve it is clear that the “planky/pencil shavings” aroma intensity of the 50/50 ratio combinations had a larger standard deviation, indicating substantial sample variation. The perceived “raisin” aroma intensities of both the control infusions and

50/50 ratio combinations ranged between 17 and 27, and the shape of their density curves was similar. This demonstrates that their “raisin” aroma intensity standard deviations were also similar and the perceived “raisin” aroma did not differ significantly between treatments ($P \leq 0.05$) (Table 4.8). Most (85%) of the 50/50 ratio combinations had an “almond” aroma intensity between 0 and 5, where all the control infusions had an “almond” aroma intensity between 0 and 5. Although low, the “almond” aroma intensity in the 50/50 ratio combinations was more perceptible and had a larger standard deviation in comparison to the control infusions, judging from the shape of the density curves. Furthermore, in comparison to the stem infusions, the “planky/pencil shavings” and “almond” aroma attributes were more perceptible in the 50/50 ratio combinations (mean intensities = 10.98 and 3.53, respectively).

“Hay/dried grass”, which is a typical rooibos aroma, differed significantly ($P \leq 0.05$) between the control infusions and the 50/50 ratio combinations. Although it associated more with the control infusions, on average, it did not differ largely from the 50/50 ratio combinations. In addition, negative rooibos aromas “green grass”, “rotting plant water”, “seaweed”, “burnt caramel” and “musty/mouldy” associated more with the control infusions, however, in both treatments the intensities of these negative aroma attributes were barely perceptible (<4). “Dusty”, on the other hand, associated more with the 50/50 ratio combinations, but was also perceived at very low intensities (<4).

All taste and mouthfeel attributes, with the exception of astringency differed significantly ($P \leq 0.05$) between the control infusions and 50/50 ratio combinations. The typical rooibos mouthfeel attribute, astringency, and the bitter taste associated more with the control infusions, whereas the typical sweet taste and negative sour taste associated more with the 50/50 ratio combinations. Similarly to the diluted dust extracts and the stem infusions, both negative sour and bitter taste were perceived at extremely low intensities (<4 ; Table 4.8).

As with the diluted dust extracts and stem infusions, the flavour attributes of the 50/50 ratio combinations were less prominent than that of the corresponding aroma attributes. The non-typical “planky/pencil shavings” flavour perceived in the stem infusions was also perceived at low, but perceptible intensities in the 50/50 ratio combinations (>10). However, the “raisin” and “almond” flavour attributes were not perceptible in the 50/50 ratio combinations (<0.17 ; Table 4.8). A PCA bi-plot including all sensory attributes is supplied in the addendum (Addendum B; Figure B4.3).

The SS content, turbidity and CIELAB colour parameters of the 50/50 ratio combinations are summarised in Table 4.5. Although the SS content of the 50/50 ratio combinations had a large range, the mean SS content of the 50/50 ratio combinations was within the SS content range of the control infusions. The turbidity of the 50/50 ratio combinations and control infusions both had a large range, where the mean turbidity of the 50/50 ratio combinations did not differ largely from the mean turbidity of the control infusions. Similar to the diluted dust extracts, all mean CIELAB colour parameters of the 50/50 ratio combinations were within the range of CIELAB colour parameters of the control

infusions and therefore differed in small amounts. However, the mean C^* value of the 50/50 ratio combinations was slightly lower than that of the control infusions, meaning that the colour of the 50/50 ratio combinations was slightly less saturated.

4.5.3.2. 75/25 ratio diluted dust extract and stem infusion combination infusions

Figure 4.8 illustrates the association of the diluted dust extract infusions, stem infusions, 50/50 ratio combinations, 75/25 ratio combinations and rooibos aroma, taste and mouthfeel attributes. The PCA bi-plot portrays the variation of the samples as they are plotted in relation to each other based on their sensory profiles, with PC 1 (Factor 1) explaining 40.57% of the variance and PC 2 (Factor 2) 15.35% of the variance. Unlike Figures 4.3, 4.4 and 4.5, there is no definite split between the samples as all individual diluted dust extract, stem infusion, 50/50 ratio combination and 75/25 ratio combination samples are scattered across PC 1. This indicates that no specific sensory attribute is driving the separation of samples on PC 1.

Table 4.9 summarises the overall means of the sensory attributes of the diluted dust extracts, stem infusions, 50/50 ratio combinations and 75/25 ratio combinations. Interestingly, most aroma, taste and mouthfeel attributes did not differ significantly ($P \leq 0.05$) between the diluted dust extract, stem infusion, 50/50 ratio combination and 75/25 ratio combination samples. In view of this, only aroma attributes associating with the stem infusions will be discussed, i.e. “planky/pencil shavings”, “raisin” and “almond”, particularly to ascertain whether the 75/25 ratio had a diluting effect on these three attributes when comparing the results of the 50/50 and 75/25 combination ratios. The “almond” aroma which was perceived in the stem infusions and 50/50 ratio combinations was also perceived in the 75/25 ratio combinations, however, at significantly lower intensities ($P \leq 0.05$) in the 75/25 ratio combination than the stem infusions. Although this attribute was perceived at extremely low intensities (<2), there seems to be some diluting effect when the ratio of the stem infusion is decreased. There were no significant ($P > 0.05$) differences in terms of “raisin” aroma when comparing all treatments. Therefore, increasing the ratio of diluted dust extract will not result in a more prominent “raisin” aroma. Similar to previous stem infusion results (Table 4.7), the “planky/pencil shavings” aroma was high in the stem infusions (>11) of this experiment. Although not significant ($P > 0.05$), the mean “planky/pencil shavings” aroma intensity of the stem infusion samples decreased from 11.3 to 8.57 for the 50/50 ratio combinations samples and finally to 6.26 for the 75/25 ratio combinations. This indicates that the inclusion of less stem plant material could potentially decrease the intensity of the “planky/pencil shavings” aroma in diluted dust extract and stems infusion combinations.

As already indicated, the flavour attributes of the samples were less prominent and mostly perceived at very low intensities when compared to that of the aroma intensities (Table 4.9). A PCA bi-plot including all sensory attributes is supplied in the addendum (Addendum; Figure B4.4).

The SS content and CIELAB colour parameters of the 75/25 ratio combinations are summarised in Table 4.5. Similarly to the 50/50 ratio combinations, the SS content of the 75/25 ratio combinations had a large range, where the mean SS content of the 75/25 ratio combinations was within the SS content range of the control infusions. In addition, the difference between the mean SS content of the 75/25 ratio combinations (0.240 g SS.100 mL⁻¹) and the mean SS content of rooibos infusions at “cup-of-tea” strength (0.224 g SS.100 mL⁻¹) was not large. All mean CIELAB colour parameters of the 75/25 ratio combinations were within the range of those of the control infusions and therefore only differed in small amounts.

4.6. Discussion

The positive sensory characteristics of rooibos have been established and include the primary aroma attributes “rooibos-woody”, “fynbos-floral” and “honey”, as well as the secondary aroma attributes “fruity-sweet”, “caramel” and “apricot” (Jolley *et al.*, 2017). A number of negative sensory attributes such as “hay/dried grass”, “green grass” and bitter taste also form part of the full sensory profile of fermented rooibos. When setting up a sample set to evaluate the sensory quality of fermented rooibos, it is important that all potential positive and negative sensory attributes are included in the sample set. For this reason rooibos waste plant material samples were collected randomly from a large number of different production batches to include sufficient product variation (Næs *et al.*, 2010). Reference samples, representing the typical sensory profile of rooibos, i.e. having a mixture of honey, woody and floral notes were used as control samples in the current study. The control samples served as a “fixed” point during DSA, allowing the panellists to calibrate their sensory perception at the start of each testing session (Koch *et al.*, 2012).

Several factors such as infusion time, plant material-to-water ratio, water type used and water temperature influence and determine the extraction of rooibos soluble solids (Joubert, 1988; Joubert, 1990; Joubert & Hansmann, 1990; Dos *et al.*, 2005). For this reason, the latter factors were kept as constant as possible. In addition, the dilution of dust extracts as well as that of the diluted dust extract and stem infusions combinations was calculated mathematically in order to obtain infusions at “cup-of-tea” strength, thus similar to that of a normal cup of tea (Figure 4.2; Addendum B, Table B4.2 and Table B4.3). Distilled water, instead of tap water, was used to prepare all infusions as the type of water used may have an effect on the clarity and acceptability of tea (Dos *et al.*, 2005).

4.6.1. Sensory profile of diluted dust extracts, stem infusions and combinations thereof

Firstly, the sensory profile of twenty **diluted dust extracts** was compared to that of a control sample to ascertain the typicality of the sensory profile of the diluted dust extracts. Overall, the sensory

profile of the diluted dust extracts associated well with the positive rooibos aroma attributes, in particular with the primary sensory attributes, i.e. “fynbos-floral” and “honey”, as well as the secondary sensory attribute, “caramel”. Although the control sample illustrated significantly higher intensities ($P \leq 0.5$) of “rooibos-woody”, “apricot” and “fruity-sweet”, the diluted dust extracts also had notable intensities of these three important aroma notes, suggesting that the diluted dust extracts are of good sensory quality with noteworthy value. The control sample had perceptible intensities of two of the negative aroma attributes, i.e. “hay/dried grass” (>24) and “green grass” (>7), significantly more so than that of the diluted dust extracts. This means that the control sample used in this experiment, which was a blend of six good quality 2012 B-grade rooibos samples used in previous research (Jolley *et al.*, 2017), could no longer be regarded as optimum quality rooibos (Koch, 2011) and thus not as an ideal point of reference.

The sensory profile of twenty **stem infusions** was compared to that of the control sample used in the above-mentioned experiment on diluted dust extracts. A number of dissimilarities between the aroma attributes of the control sample (representing the characteristic profile of fermented rooibos) and that of the stem infusions were observed. Firstly, the stem infusions indicated much lower intensities of all positive aroma attributes typically associated with fermented rooibos (Jolley *et al.*, 2017), except for “caramel/vanilla” aroma. The stem infusions were also significantly lower in the two negative attributes, “hay/dried grass” and “green grass”. These significant ($P \leq 0.05$) trends are most probably the result of a much lower soluble solids content of the stem infusions as observed by Joubert (1984). Some of the stem infusions elicited “caramel”, as well as “vanilla” aromas which elucidates why “caramel” was subsequently referred to as “caramel/vanilla” in the sensory lexicon adapted for the stem infusions (Table 4.3). “Caramel/vanilla” has been perceived in the floral-like herbal tea *Cyclopia intermedia* (Bergh *et al.*, 2017). These two attributes are extremely typical of wooded wines (Fernández de Simon *et al.*, 2014), however, more research needs to be conducted to establish the origin of these two aroma notes in rooibos stem infusions. The stem infusions also illustrated non-typical aroma notes, namely “planky/pencil shavings”, “raisin” and “almond”. In previous research on the full sensory profile of rooibos (Koch *et al.*, 2012; Jolley *et al.*, 2017), these three aroma attributes have never been perceived in fermented rooibos infusions at perceptible intensities (>5), however, the attributes “raisin” and “almond” are quite typical of fermented honeybush, in particular *C. intermedia* (Bergh *et al.*, 2017). In contrast, the “planky/pencil shavings” aroma note could be regarded as an unfamiliar attribute as it is not similar to “rooibos-woody”, an aroma characteristic of fermented rooibos (Jolley *et al.*, 2017). In the analysis of red wines, the sensory descriptor “pencil shavings” is not foreign to barrel fermented wines (Schmidtke *et al.*, 2010). Both the descriptors “wooded” and “pencil shavings” usually form part of wine sensory lexicons and thus also of the “positive” aroma profile of wines (Oberholster *et al.*, 2015). For the purpose of the current rooibos research, it was decided that both “planky/pencil shavings” and “rooibos-woody” should form

part of the rooibos sensory lexicon (Table 4.3). Food-based reference standards illustrating the latter attributes were introduced during DSA training of the current research, primarily to enable the panelists to clearly distinguish between these two woody aroma notes in stem infusions (Drake & Civille, 2002). The question is, however, whether the attribute “planky/pencil shavings” should be regarded as a taint or as part of the broader aroma woody profile of fermented rooibos. During this research project it was decided to view the aroma attribute “planky/pencil shavings” as “foreign” in terms of what is regarded as the characteristic sensory profile of fermented rooibos, i.e. based on previous research using large samples sets of fermented rooibos (Koch *et al.*, 2012; Jolley *et al.*, 2017). Furthermore, other researchers have also stated that rooibos stems result in tea of low quality (Joubert, 1984).

The initial aim of this research project was to assess the sensory profile of diluted dust extracts and stem infusions, first as separate entities and thereafter in combination. The formulation of the diluted dust extract and stem infusion combinations was based on soluble solids content (SS), primarily to try to achieve a characteristic rooibos sensory profile at “cup-of-tea” strength. The mean SS content of the stem infusions was approximately 50% of that of the control infusions and diluted dust extracts, however, the SS range of the stem infusions was quite large, ranging from 0.0072 to 0.234 g SS.100 mL⁻¹. This wide range is a direct result of the natural variability of the plant material of the respective batches of stems. Some of the batches of stems separated satisfactorily from other rooibos plant material and consisted of coarse stems only, while in some batches, the stems were mixed with rooibos leaves and fine dust. Therefore, the stem batches that contained fractions of leaves and fine dust produced infusions with an average SS content.

Two diluted dust extract and stem infusion combinations, at 50/50 and 75/25 ratios, were tested. The ratio of 75/25 was included to offset the negative effect of “planky/pencil shavings”. In the first diluted dust extract and stem infusion combination, i.e. the **50/50 ratio combination**, 20 diluted dust extracts and 20 stem infusions were randomly combined in 50/50 ratios at “cup-of-tea” strength. The latter combinations were compared to 20 new control samples, i.e. infusions of good quality commercial rooibos tea, in terms of sensory attributes. This was done in order to determine the practicality of the 50/50 ratio for industry application, but also to ascertain the effect of the latter combination on the attribute that could be regarded as a potential taint, i.e. “planky/pencil shavings”. Similar to the results of the stem infusions, the 50/50 ratio combination resulted in significantly ($P \leq 0.05$) lower intensities for the primary rooibos aroma attributes (“fynbos-floral”, “rooibos-woody” and “honey”), however, the aroma attribute “planky/pencil shavings” was still perceptible in the majority of the 50/50 combinations with a mean intensity of >10 .

Because of the above-mentioned results, a subset of six samples were selected (Figure 4.2) to prepare the **75/25 ratio combinations** at “cup-of-tea” strength. This was done to ascertain if a higher amount of diluted dust extract would effectively mask the “planky/pencil shavings” aroma

associated with stem infusions as well as with the 50/50 ratio combinations, thereby producing a product of good and acceptable rooibos quality from waste material. To choose a subset of samples illustrating optimum product variability, the sensory attributes of each of the 75/25 ratio combinations were compared to that of the individual dust extract and stem infusion samples used to produce the 75/25 ratio combinations. The sensory attributes of each of the 75/25 ratio combinations were also compared to those of the 50/50 ratio combinations made from the exact same dust extract and stem infusion samples. Overall, both combination ratios did not result in a significantly ($P \leq 0.05$) lower intensity of “planky/pencil shavings” when compared to that of the stem infusions, however, there was a slight trend that increased amounts of diluted extract could potentially mask this so-called taint. This result is promising because it means that the 75/25 ratio combinations have the potential to be used for the production of good quality rooibos beverages if the “planky/pencil shavings” aroma can be masked. According to Figure 4.8, it was observed that the specific dust extract and stem infusions samples used to make specific ratio combinations tended to associate with each other on the PCA bi-plot. For example, the “combo 19 (75/25)” and “combo 19 (50/50)” samples were made up of the “dust 19” and “stem 27” dust extract and stem infusion samples. According to the PCA bi-plot (Figure 4.8), “combo 19 (75/25)”, “combo 19 (50/50)” and “stem 27” associated with “planky/pencil shavings” aroma, probably as a result of the fact that “stem 27” was one of the samples with a higher “planky/pencil shavings” intensity (mean intensity = 15.77; Addendum B, Table B4.13). On the opposite end of PC I, the “combo 10 (75/25)” and “combo 10 (50/50)” samples were made up of the “dust 10” and “stem 14” dust extract and stem infusion samples. The “combo 10 (75/25)”, “combo 10 (50/50)”, “dust 10” and “stem 14” samples associated less with the aroma attribute “planky/pencil shavings” on PC I, and more with “sweet” taste since “dust 10” was one of the dust extract samples with a higher “sweet” taste intensity (Mean intensity = 21.11; Addendum, Table B4.14).

4.6.2. Instrumental and chemical profile of diluted dust extracts and stem infusions and combinations thereof

The mean SS content, turbidity and CIELAB colour parameters of twenty diluted dust extracts, stem infusions, 50/50 ratio combinations and six 75/25 ratio combinations were compared to 20 control infusions. The range in SS content, turbidity and CIELAB colour parameters of the diluted dust extracts, stem infusions, 50/50 ratio combinations and 75/25 ratio combinations and control infusions was possibly attributable to natural variation of plant material used, which is ultimately unavoidable. The large range in SS content of the stem infusions was most likely result of the variation of the size plant material in the stem plant material batches. Some batches were separated satisfactorily from other rooibos plant material and consisted of coarse stems only, while some batches were slightly mixed with rooibos leaves and fine dust. The mean SS content of the control infusions (0.222 g SS.100

mL⁻¹), diluted dust extracts (0.232 g SS.100 mL⁻¹), 50/50 ratio combinations (0.233 g SS.100 mL⁻¹) and 75/25 ratio combinations (0.240 g SS.100 mL⁻¹) was also close to the mean SS content of rooibos infusions at “cup-of-tea” strength (0.224 g SS.100 mL⁻¹) determined by Koch (2011). However, mean SS content of the stem infusions (0.134 g SS.100 mL⁻¹) at “cup-of-tea” strength was almost half the mean SS content of the control infusions and of rooibos infusions at “cup-of-tea” strength determined by Koch (2011). The stem infusions were thus weak due to their low mean SS content. Joubert (1984) who, in addition, stated that rooibos stems are of low tea value also observed this. This occurrence may also be attributable to the variation in plant material size of the stem batches. The stem batches that contained fractions of leaves and fine dust produced infusions with an average SS content close to the SS content of infusions at “cup-of-tea” strength (0.224 g SS.100 mL⁻¹).

The diluted dust extracts were on average less turbid than the control infusions, stem infusions and 50/50 ratio combinations. This is because after the dust extracts were produced, they had to be centrifuged in order to remove the very small dust particles. This processing step therefore contributed to the improvement of the quality of the diluted dust extracts. The infusions on the other hand were not centrifuged, but were filtered with a fine mesh tea strainer to remove plant material. Therefore, small dust particles were possibly suspended in the control and stem infusions, hence the higher turbidity. In addition, stem batches with coarse stems only produced infusions with low turbidity values, where stem batches containing more leaves and dust produced infusions with higher turbidity values. The turbidity of the 75/25 ratio combinations was not determined as the focus of the experiment was the attempt to significantly reduce the undesirable “planky/pencil shavings” aroma. In addition, the turbidity of the ratio combinations would be a less important factor to consider, in comparison to the sensory attributes, for the viability of the proposed waste reutilisation endeavor. The desired “tea leaf” end product would essentially be a dried combination of dust extract and stem plant material for reconstitution by the consumer.

No large differences between the mean CIELAB colour parameters of the control infusions, diluted dust extracts, 50/50 ratio combinations and 75/25 ratio combinations were observed. Their a* values in particular, which are important for rooibos quality, were all similar which is a noteworthy result. On average, the colour of the stem infusions was lighter, less red and less saturated in comparison to the control infusions, diluted dust extracts, 50/50 ratio combinations and 75/25 ratio combinations. The stem infusions did not possess the characteristic red-brown colour of rooibos infusions, which is a result of a “fermentation” process. Since rooibos stems are usually discarded after separation from rooibos leaves and do not undergo the “fermentation” process, it was expected that the colour of the stem infusions would not be as red-brown and saturated as that of normal, good quality rooibos infusions. Joubert (1995) stated that the red colour (a* value) of rooibos infusions is important and plays a pivotal role during visual evaluation of rooibos quality. Therefore, the fact that the stem infusion’s mean a* value was much lower than that of the control infusions, diluted dust

extracts, 50/50 ratio combinations and 75/25 ratio combinations extracts further suggests that rooibos stems cannot be used in isolation to make rooibos infusions. The use of rooibos stems only in tea bags in order to meet the ever-growing demand for rooibos tea would not thus be a fruitful endeavour as the quality of rooibos infusions would be decreased drastically.

One control sample, replicated over four testing sessions, was used in the diluted dust extract and stem infusion experiments where natural variation was not factored into the control infusion. As a result of this, phenolic content values were compared to published literature values for grade B rooibos infusions from Joubert *et al.* (2012). The mean phenolic compounds in the control infusion were generally higher than those of the diluted dust extracts, stem infusions and grade B rooibos infusions. The major phenolic compounds of the diluted dust extracts further suggest that the diluted dust extracts were of good quality and could potentially be used solely for the production of rooibos beverages. The mean aspalathin content of the stem infusions did not differ largely from the mean aspalathin content of the diluted dust extracts and grade B rooibos infusions. This result suggests that the phenolic quality of the stem plant material is good.

4.7. Conclusions

With the rooibos demand currently exceeding production, it was crucial to determine the sensory profile of fermented rooibos waste plant material, i.e. dust and stems, individually and in combinations, primarily for the future potential reutilisation of rooibos waste plant material for production of rooibos products to make up for the current shortages experienced by the industry.

The results indicated that dust extracts diluted to “cup-of-tea” strength associated with the positive aroma attributes, along with the “sweet” taste of normal rooibos tea. Due to the noteworthy sensory profile of the diluted dust extracts, dust extracts on their own could potentially be used for the production of rooibos beverages, including iced tea. On the other hand, the stem plant material used produced reasonably weak infusions at “cup-of-tea” strength and these infusions did not possess the characteristic red-brown colour of rooibos infusions. Moreover, a non-typical rooibos aroma note, namely “planky/pencil shavings” was perceived in the stem infusions. These results indicated that rooibos stems cannot be used in isolation to make rooibos infusions as the quality of rooibos infusions would be affected negatively. However, when the stem plant material was combined with diluted dust extracts in a ratio of 75/25, this negative aroma attribute decreased and resulted in infusions of reasonably acceptable sensory quality. The results open up the potential for combining these two waste material fractions for the rooibos beverage industry. However, it is vitally important that a low percentage of stem plant material ($\leq 25\%$) is used in the diluted dust extract and stem infusion combinations to mask the non-typical “planky/pencil shavings” aroma note. Another alternative is blending dust extract and stem combinations further with good quality rooibos tea. This would ensure

that the quality of rooibos beverages would not be compromised. Nonetheless, these rooibos two rooibos tea agro-processing waste materials could have significant market value.

4.8. References

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Table 4.1 Dust extract and stem sample pairs used to make combination infusions at "cup-of-tea" strength in 50/50 and 75/25 ratios. Bold, italic, red text indicates samples selected for the 75/25 ratio combinations.

Dust extract sample	Stem sample	Combination sample
D1	S1	C1
D2	S3	C2
D3	S15	C3
D4	S7	C4
<i>D5</i>	<i>S6</i>	<i>C5</i>
D6	S9	C6
D7	S8	C7
<i>D8</i>	<i>S10</i>	<i>C8</i>
<i>D9</i>	<i>S13</i>	<i>C9</i>
<i>D10</i>	<i>S14</i>	<i>C10</i>
<i>D11</i>	<i>S18</i>	<i>C11</i>
D12	S19	C12
D13	S21	C13
D14	S22	C14
D15	S23	C15
D16	S24	C16
D17	S25	C17
D18	S26	C18
<i>D19</i>	<i>S27</i>	<i>C19</i>
D20	S28	C20

Table 4.2 List of sensory attributes used during descriptive sensory analysis (DSA) of diluted dust extracts.

Classification of attributes	Primary attributes	Descriptive attributes	Description of attributes
Positive attributes	Floral	Fynbos-floral	The unique, somewhat sweet aromatics associated with fynbos ^a vegetation
	Woody	Rooibos-woody	Aromatics associated with dry bushes, stems and twigs of the rooibos vegetation
	Fruity	Apricot	Aromatics associated with apricot jam or dried apricot
		Apple	Sweet aromatics associated with cooked apples or apple pie
		Citrus	The sweet aroma associated with ripe oranges
	Sweet	Fruity-sweet	Aromatics associated with the sweet/sour smell of non-specific fruit
		Honey	Aromatics associated with the sweet fragrance of fynbos honey or Alyssum blossoms
		Caramel	Sweet aromatics characteristic of caramelised sugar
	Spicy	Sweet spice	Aromatics associated with sweet spice mainly cinnamon
Negative attributes	Vegetative	Hay/Dried grass	Slightly sweet aromatics associated with dried grass or hay
		Green grass	Aromatics associated with freshly cut grass/stale cut grass
		Rotting plant water	Aromatics associated with the rotting aroma of old flower water
		Seaweed	Aromatics associated with seaweed
	General	Burnt caramel	Aromatics associated with burnt sugar, burnt caramel or burnt caramelised vegetables
		Medicinal/Rubber	Aromatics associated with Band-Aid®
		Dusty	Earthy aromatics associated with dust from a gravel road or ground
		Musty/Mouldy	Mouldy aromatics associated with mildew or damp cellars

^aFynbos is natural shrub land vegetation occurring in the Western Cape, South Africa

Table 4.3 List of sensory attributes used during descriptive sensory analysis (DSA) of stems infusions, 50/50 ratio combinations and 75/25 ratio combinations.

Classification of attributes	Primary attributes	Descriptive attributes	Description of attributes
Positive attributes	Floral	Fynbos-floral	The unique, somewhat sweet aromatics associated with fynbos ^a vegetation
	Woody	Rooibos-woody	Aromatics associated with dry bushes, stems and twigs of the rooibos vegetation
	Fruity	Apricot	Aromatics associated with apricot jam or dried apricot
		Apple	Sweet aromatics associated with cooked apples or apple pie
		Citrus	The sweet aroma associated with ripe oranges
	Sweet	Fruity-sweet	Aromatics associated with the sweet/sour smell of non-specific fruit
		Honey	Aromatics associated with the sweet fragrance of fynbos honey or Alyssum blossoms
		Caramel	Sweet aromatics characteristic of caramelised sugar
Spicy	Sweet spice	Aromatics associated with sweet spice mainly cinnamon	
Negative attributes	Vegetative	Hay/Dried grass	Slightly sweet aromatics associated with dried grass or hay
		Green grass	Aromatics associated with freshly cut grass/stale cut grass
		Rotting plant water	Aromatics associated with the rotting aroma of old flower water
		Seaweed	Aromatics associated with seaweed
	General	Burnt caramel	Aromatics associated with burnt sugar, burnt caramel or burnt caramelised vegetables
		Medicinal/Rubber	Aromatics associated with Band-Aid®
		Dusty	Earthy aromatics associated with dust from a gravel road or ground
		Musty/Mouldy	Mouldy aromatics associated with mildew or damp cellars

^aFynbos is natural shrub land vegetation occurring in the Western Cape, South Africa

Table 4.4 Sensory attribute means \pm standard deviations of the control infusion and the diluted dust extracts. Values in each row with the same letter are not significantly different ($P \leq 0.05$).

Attribute classes	Rooibos attributes	Control infusion (n=1)	Diluted dust extract (n=20)
Aroma attributes	Fynbos-floral	31.89 \pm 2.20 a	32.25 \pm 1.72 a
	Rooibos-woody	40.99 \pm 1.83 a	37.49 \pm 2.38 b
	Apricot	3.81 \pm 0.61 a	1.18 \pm 0.64 b
	Fruity-sweet	6.31 \pm 0.85 a	4.63 \pm 1.21 b
	Honey	18.17 \pm 2.50 a	18.17 \pm 2.54 a
	Caramel	18.13 \pm 1.56 b	21.66 \pm 2.53 a
	Sweet spice	0.16 \pm 0.21 b	1.11 \pm 0.60 a
	Hay/Dried grass	24.91 \pm 1.89 a	20.21 \pm 1.96 b
	Green grass	7.55 \pm 1.01 a	0.36 \pm 0.33 b
	Rotting plant water	1.55 \pm 0.45 a	0.41 \pm 0.37 a
	Seaweed	2.06 \pm 1.35 a	0 \pm 0 b
	Burnt caramel	4.13 \pm 1.16 a	3.47 \pm 1.14 a
	Medicinal/Rubber	1.14 \pm 1.08 a	0.18 \pm 0.22 b
	Dusty	0.89 \pm 0.66 a	0.45 \pm 0.43 a
	Musty/Mouldy	0.79 \pm 0.89 a	0.43 \pm 0.31 a
Taste and mouthfeel attributes	Sweet	19.15 \pm 0.32 b	21.91 \pm 0.69 a
	Astringent	28.23 \pm 0.49 a	25.91 \pm 0.59 b
	Sour	1.83 \pm 0.58 a	0.53 \pm 0.37 b
	Bitter	6.05 \pm 0.95 a	2.39 \pm 0.67 b
Flavour attributes	Fynbos-floral	27.65 \pm 2.98 a	27.61 \pm 1.36 a
	Rooibos-woody	38.63 \pm 1.47 a	35.96 \pm 1.83 b
	Apricot	0 \pm 0 a	0 \pm 0 a
	Fruity-sweet	0.11 \pm 0.12 a	0.18 \pm 0.35 a
	Honey	1.40 \pm 0.35 a	1.93 \pm 0.79 a
	Caramel	2.04 \pm 0.87 a	2.4 \pm 1.08 a
	Sweet spice	0 \pm 0 a	0 \pm 0 a
	Hay/Dried grass	24.95 \pm 1.49 a	22.13 \pm 1.59 b
	Green grass	4.32 \pm 0.90 a	0.87 \pm 0.48 b
	Rotting plant water	2.29 \pm 0.96 a	0.47 \pm 0.27 b
	Seaweed	1.93 \pm 1.24 a	0 \pm 0 b
	Burnt caramel	3.67 \pm 0.78 a	1.73 \pm 0.60 b
	Medicinal/Rubber	0.13 \pm 0.26 a	0.08 \pm 0.25 a
	Dusty	0.39 \pm 0.43 a	0.48 \pm 0.40 a
	Musty/Mouldy	0.06 \pm 0.13 a	0.13 \pm 0.17 a

Table 4.5 Mean values \pm standard deviations and range in brackets for SS content, turbidity and CIELAB colour parameters of diluted dust extracts, stem infusions, 50/50 ratio combinations and 75/25 ratio combinations.

	Control infusions (n=20)	Diluted dust extract (n=20)	Stem infusions (n=20)	50/50 ratio combinations (n=20)	25/75 ratio combinations (n=6)
Soluble solids content (g SS.100 mL⁻¹)	0.222 \pm 0.014 (0.198 – 0.250)	0.232 \pm 0.019 (0.203 – 0.282)	0.134 \pm 0.040 (0.072 – 0.234)	0.233 \pm 0.050 (0.161 – 0.351)	0.240 \pm 0.037 (0.193 – 0.290)
Turbidity (NTU)	28.08 \pm 7.14 (19.70 – 43.7)	10.06 \pm 1.85 (7.06 – 16.60)	18.91 \pm 11.31 (6.58 - 57.3)	26.42 \pm 11.11 (11.77 – 52.95)	na ^a
L*	64.50 \pm 1.48 (52.42 - 73.03)	66.40 \pm 2.32 (63.13 – 70.64)	78.65 \pm 6.40 (65.20 – 88.21)	66.17 \pm 4.65 (61.51 – 66.60)	64.32 \pm 3.41 (59.54 – 69.08)
a*	33.38 \pm 1.60 (31.00 – 35.66)	32.40 \pm 2.32 (27.99 – 36.97)	17.19 \pm 8.68 (4.96 – 35.01)	29.57 \pm 4.50 (22.41 – 42.01)	33.58 \pm 3.96 (28.37 – 39.76)
b*	100.81 \pm 1.15 (98.34 – 102.74)	99.24 \pm 1.53 (97.18 – 103.09)	84.26 \pm 13.95 (54.83 – 105.46)	96.78 \pm 3.10 (90.84 – 101.14)	98.60 \pm 1.48 (96.86 – 100.80)
C*	106.20 \pm 1.04 (102.92 - 107.84)	104.59 \pm 1.79 (100.66 – 109.06)	86.12 \pm 15.35 (54.99 – 109.64)	101.47 \pm 3.48 (93.56 – 105.51)	104.23 \pm 2.41 (101.00 – 107.13)
h	71.68 \pm 0.90 (68.76 – 73.29)	71.97 \pm 1.04 (69.76 – 73.55)	79.26 \pm 43.81 (71.29 – 85.63)	73.18 \pm 2.48 (64.35 – 77.82)	71.24 \pm 1.93 (68.08 – 73.73)

^a not analysed

Table 4.6 Mean values \pm standard deviations for phenolic of the control infusion, diluted dust extracts and stem infusions compared to baseline values^e for rooibos infusions.

Phenolic compounds	Control infusion (n=1)	Diluted dust extracts (n=20)	Stem infusions (n=20)	Rooibos infusions - baseline data ^b (n=30)
Aspalathin	20.82 \pm 0.25	10.84 \pm 1.02	7.30 \pm 1.86	9.02 \pm 4.37
Nothofagin	1.56 \pm 0.02	1.09 \pm 0.07	0.62 \pm 0.17	1.57 \pm 0.80
Orientin	25.53 \pm 0.18	22.52 \pm 0.42	13.14 \pm 2.10	17.22 \pm 1.99
Isorientin	17.98 \pm 0.19	15.41 \pm 0.47	8.44 \pm 1.52	24.02 \pm 2.99
Vitexin	5.55 \pm 0.08	5.21 \pm 0.15	3.11 \pm 0.50	3.74 \pm 0.40
Isovitexin	9.16 \pm 0.13	7.76 \pm 0.45	4.32 \pm 0.95	3.84 \pm 0.49
Isoquercitrin	nd ^c	nd	nd	1.51 \pm 1.00
Hyperoside	4.66 \pm 0.08	2.68 \pm 0.22	1.52 \pm 0.43	3.18 \pm 1.04
Rutin	2.40 \pm 1.17	0.92 \pm 0.16	1.34 \pm 0.43	2.43 \pm 1.36
PPAG ^d	15.45 \pm 0.03	11.32 \pm 0.30	8.71 \pm 0.75	11.41 \pm 3.06
Quercetin-3-O-robinobioside	11.34 \pm 5.52	4.14 \pm 0.30	6.22 \pm 1.53	12.61 \pm 4.73

^amg.L⁻¹; ^bGrade B rooibos infusions from Joubert *et al.* (2012); ^cnot detected; ^dZ-2-(β -D-glucopyranosyloxy)-3-phenylpropenoic acid; ^evalues from Joubert *et al.* (2012) were adjusted from 12.5 g PM.1000 g⁻¹ H₂O to 19.3 g PM.1000 g⁻¹ H₂O due to stronger infusions prepared for sensory analysis of rooibos waste infusions.

Table 4.7 Sensory attribute means \pm standard deviations of the control infusion and stem infusions. Values in each row with the same letter are not significantly different ($P \leq 0.05$).

Attribute classes	Rooibos attributes	Control infusion (n=1)	Stem infusions (n=20)
Aroma attributes	Fynbos-floral	36.48 \pm 0.79 a	28.82 \pm 2.90 b
	Rooibos-woody	45.54 \pm 0.64 a	34.44 \pm 3.29 b
	Planky/Pencil shavings	1.95 \pm 0.6 b	5.27 \pm 2.55 a
	Apricot	3.03 \pm 0.34 b	5.09 \pm 1.22 a
	Raisin	6.45 \pm 1.82 b	11.72 \pm 1.46 a
	Almond	0.32 \pm 0.17 b	2.30 \pm 0.95 a
	Fruity-sweet	2.69 \pm 0.46 b	4.63 \pm 1.28 a
	Honey	27.35 \pm 0.41 a	20.84 \pm 2.47 b
	Caramel/Vanilla	26.40 \pm 1.05 a	26.03 \pm 2.25 a
	Sweet spice	1.09 \pm 0.66 b	1.91 \pm 0.68 a
	Hay/Dried grass	26.59 \pm 1.42 a	22.15 \pm 1.44 b
	Green grass	4.87 \pm 0.91 a	0.82 \pm 0.62 b
	Rotting plant water	0.54 \pm 0.37 a	0.34 \pm 0.28 a
	Seaweed	3.39 \pm 0.4 a	0.37 \pm 0.30 b
	Burnt caramel	5.73 \pm 1.53 a	1.88 \pm 0.62 b
	Medicinal/Rubber	0.20 \pm 0.40 a	0.0 \pm 0.0 b
	Dusty	0.20 \pm 0.18 b	1.28 \pm 0.95 a
	Musty/Mouldy	0.27 \pm 0.28 a	0.54 \pm 1.14 a
	Taste and mouthfeel attributes	Sweet	21.91 \pm 0.71 b
Astringent		28.44 \pm 0.70 a	26.64 \pm 0.89 b
Sour		0.47 \pm 0.39 a	0.26 \pm 0.22 a
Bitter		5.33 \pm 0.88 a	2.02 \pm 1.05 b
Flavour attributes	Fynbos-floral	30.63 \pm 0.83 a	23.97 \pm 2.40 b
	Rooibos-woody	40.04 \pm 0.42 a	31.95 \pm 2.71 b
	Planky/Pencil shavings	1.29 \pm 0.64 a	5.55 \pm 1.98 b
	Apricot	0 \pm 0 a	0 \pm 0 a
	Raisin	0.0 \pm 0.0 a	0.21 \pm 0.31 a
	Almond	0.0 \pm 0.0 a	0.25 \pm 0.31 a
	Fruity-sweet	0.11 \pm 0.13a	0.16 \pm 0.26 a
	Honey	0.44 \pm 0.34 a	0.05 \pm 0.15 b
	Caramel/Vanilla	0.55 \pm 0.69 a	0.56 \pm 0.44 a
	Sweet Spice	0.07 \pm 0.13 a	0.0 \pm 0.0 b
	Hay/Dried grass	27.72 \pm 0.46 a	22.87 \pm 1.90 b
	Green grass	3.91 \pm 0.28 a	0.93 \pm 0.53 b
	Rotting plant water	1.16 \pm 0.32 a	0.52 \pm 0.33 b
	Seaweed	3.42 \pm 0.24 a	0.34 \pm 0.42 b
	Burnt caramel	3.54 \pm 1.04 a	1.03 \pm 0.58 b
	Medicinal/Rubber	0.0 \pm 0.0 a	0.03 \pm 0.11 a
	Dusty	0.13 \pm 0.26 a	0.94 \pm 0.91 a
Musty/Mouldy	0.0 \pm 0.0 a	0.33 \pm 0.82 a	

Table 4.8 Sensory attribute means \pm standard deviations of the control infusions and 50/50 ratio combinations. Values in each row with the same letter are not significantly different ($P \leq 0.05$).

Attribute classes	Rooibos attributes	Control infusions (n=20)	50/50 ratio combinations (n=20)
Aroma attributes	Fynbos-floral	32.47 \pm 1.52 a	31.22 \pm 1.90 b
	Rooibos-woody	39.55 \pm 1.60 a	37.75 \pm 1.62 b
	Planky/Pencil shavings	2.92 \pm 1.44 b	10.98 \pm 4.45 a
	Apricot	17.26 \pm 1.51 a	16.16 \pm 2.34 a
	Apple	0.57 \pm 0.36 b	1.67 \pm 0.54 a
	Raisin	20.26 \pm 1.07 a	20.87 \pm 1.70 a
	Almond	1.22 \pm 0.57 b	3.53 \pm 1.23 a
	Fruity-sweet	21.21 \pm 1.18 a	21.04 \pm 1.83 a
	Honey	24.11 \pm 1.56 a	22.81 \pm 1.78 b
	Caramel/Vanilla	25.75 \pm 0.86 b	27.19 \pm 1.48 a
	Sweet spice	0.17 \pm 0.28 b	0.54 \pm 0.48 a
	Hay/Dried grass	24.15 \pm 0.96 a	22.73 \pm 1.49 b
	Green grass	3.21 \pm 1.49 a	0.96 \pm 0.51 b
	Rotting Plant water	0.46 \pm 0.41 a	0.14 \pm 0.27 b
	Seaweed	1.11 \pm 0.41 a	0.29 \pm 0.37 b
	Burnt caramel	1.57 \pm 0.94 a	0.50 \pm 0.47 b
	Medicinal/Rubber	0.0 \pm 0.0 a	0.0 \pm 0.0 a
	Dusty	1.04 \pm 0.67 b	3.28 \pm 1.45 a
	Musty/Mouldy	1.49 \pm 0.55 a	1.04 \pm 0.55 b
Taste and mouthfeel attributes	Sweet	22.25 \pm 0.56 b	22.75 \pm 0.85 a
	Astringent	28.33 \pm 0.53 a	27.97 \pm 0.74 a
	Sour	1.71 \pm 0.66 b	2.28 \pm 0.53 a
	Bitter	2.58 \pm 1.25 b	3.50 \pm 0.64 a
Flavour attributes	Fynbos-floral	27.14 \pm 1.34 a	26.99 \pm 1.49 a
	Rooibos-woody	35.24 \pm 1.48 b	36.89 \pm 1.34 a
	Planky/Pencil shavings	3.34 \pm 1.19 b	10.3 \pm 3.71 a
	Apricot	0.0 \pm 0.0 a	0.0 \pm 0.0 a
	Raisin	0.0 \pm 0.0 a \pm a	0.03 \pm 0.11 a
	Almond	0.0 \pm 0.0 a \pm b	0.16 \pm 0.30 a
	Fruity-sweet	0.0 \pm 0.0 a \pm a	0.0 \pm 0.0 a
	Honey	0.0 \pm 0.0 a \pm a	0.0 \pm 0.0 a
	Caramel/Vanilla	0.0 \pm 0.0 a \pm b	0.23 \pm 0.34 a
	Sweet spice	0.0 \pm 0.0 a \pm a	0.13 \pm 0.06 a
	Hay/Dried grass	25.31 \pm 0.86 a	23.41 \pm 1.36 b
	Green Grass	2.54 \pm 0.78 a	0.91 \pm 0.65 b
	Rotting plant water	0.28 \pm 0.28 a	0.14 \pm 0.26 a
	Seaweed	0.03 \pm 0.13 b	0.34 \pm 0.44 a
	Burnt caramel	0.77 \pm 0.58 a	0.23 \pm 0.34 b
	Medicinal/Rubber	0.0 \pm 0.0 a	0.03 \pm 0.13 a
	Dusty	1.14 \pm 0.45 b	2.86 \pm 0.88 a
Musty/Mouldy	0.10 \pm 0.24 a	0.10 \pm 0.21 a	

Table 4.9 Sensory attribute means \pm standard deviations of the control infusions and 75/25 ratio diluted dust extract and stem infusion combination infusions. Values in each row with the same letter are not significantly different ($P \leq 0.05$).

Attribute classes	Rooibos attribute	Diluted dust extracts (n=6)	Stem infusions (n=6)	50/50 ratio combinations (n=6)	75/25 ratio combinations (n=6)
Aroma attributes	Fynbos-floral	32.00 \pm 2.07 a	30.12 \pm 3.70 a	32.13 \pm 2.27 a	31.96 \pm 1.29 a
	Rooibos-woody	36.85 \pm 2.20 a	36.22 \pm 2.79 a	37.13 \pm 1.40 a	37.12 \pm 1.00 a
	Planky/Pencil shavings	4.20 \pm 2.07 b	11.23 \pm 7.33 a	8.57 \pm 3.48 ab	6.26 \pm 1.13 ab
	Apricot	6.11 \pm 1.57 a	4.74 \pm 1.71 a	5.41 \pm 1.13 a	4.88 \pm 1.13 a
	Apple	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
	Raisin	17.22 \pm 1.11 a	17.09 \pm 2.70 a	18.71 \pm 1.06 a	18.15 \pm 1.11 a
	Almond	0.44 \pm 0.40 b	1.44 \pm 0.95 a	0.90 \pm 0.86 ab	0.34 \pm 0.38 b
	Fruity-sweet	18.43 \pm 1.47 a	18.02 \pm 1.65 a	18.72 \pm 1.64 a	18.72 \pm 1.55 a
	Honey	22.56 \pm 1.58 a	21.79 \pm 3.49 a	21.92 \pm 1.53 a	21.88 \pm 1.12 a
	Caramel/Vanilla	25.24 \pm 0.74 a	23.15 \pm 2.59 b	23.49 \pm 1.77 ab	24.59 \pm 1.10 ab
	Sweet Spice	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
	Hay/Dried grass	16.28 \pm 1.68 a	18.00 \pm 1.58 a	18.08 \pm 1.43 a	17.04 \pm 0.83 ab
	Green grass	0.21 \pm 0.33 a	0.11 \pm 0.27 a	0.11 \pm 0.27 a	0.00 \pm 0.00 a
	Rotting plant water	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
	Seaweed	0.11 \pm 0.27 a	0.13 \pm 0.32 a	0.21 \pm 0.51 a	0.00 \pm 0.00 a
	Burnt caramel	2.94 \pm 1.26 a	1.48 \pm 0.62 b	2.06 \pm 0.51 ab	2.20 \pm 0.37 ab
	Medicinal/Rubber	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
	Dusty	3.43 \pm 1.40 b	5.50 \pm 2.30 a	4.40 \pm 1.78 ab	3.93 \pm 0.49 ab
	Musty/Mouldy	1.48 \pm 0.63 a	1.35 \pm 0.37 a	1.75 \pm 1.19 a	1.64 \pm 0.76 a
	Taste and mouthfeel attributes	Sweet	20.45 \pm 0.46 a	19.64 \pm 0.65 a	19.62 \pm 0.89 a
Astringent		29.51 \pm 0.57 a	30.19 \pm 0.58 a	30.39 \pm 0.98 a	30.36 \pm 0.81 a
Sour		2.32 \pm 0.75 b	3.97 \pm 0.60 a	3.54 \pm 1.36 a	3.03 \pm 0.80 ab
Bitter		4.82 \pm 0.86 a	3.15 \pm 0.68 b	4.35 \pm 0.83 a	3.99 \pm 0.94 ab
Flavour attributes	Fynbos-floral	29.32 \pm 0.95 a	26.04 \pm 2.17 b	27.07 \pm 1.42 b	26.81 \pm 0.71 b
	Rooibos-woody	36.22 \pm 0.75 a	34.94 \pm 2.86 a	36.53 \pm 1.05 a	35.98 \pm 1.12 a
	Planky/Pencil shavings	3.51 \pm 0.60 c	10.71 \pm 4.23 a	8.26 \pm 2.79 ab	7.05 \pm 2.03 b
	Apricot	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
	Raisin	0.00 \pm 0.00 a	0.10 \pm 0.25 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
	Almond	0.00 \pm 0.00 a	0.09 \pm 0.22 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
	Fruity-sweet	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
	Honey	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
	Caramel/Vanilla	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
	Sweet spice	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
	Hay/Dried grass	17.69 \pm 2.10 b	19.47 \pm 0.81 a	19.21 \pm 1.07 a	18.86 \pm 0.39 ab
	Green grass	0.31 \pm 0.34 a	0.00 \pm 0.00 b	0.22 \pm 0.34 ab	0.00 \pm 0.00 b
	Rotting plant water	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
	Seaweed	0.04 \pm 0.10 a	0.00 \pm 0.00 a	0.15 \pm 0.36 a	0.00 \pm 0.00 a
	Burnt caramel	2.00 \pm 0.87 a	0.58 \pm 0.67 b	0.77 \pm 0.59 b	0.87 \pm 0.65 b
	Medicinal/Rubber	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
	Dusty	3.11 \pm 1.16 a	3.75 \pm 1.58 a	3.91 \pm 1.28 a	3.79 \pm 1.11 a
Musty/Mouldy	0.78 \pm 0.78 a	0.41 \pm 0.51 a	0.69 \pm 0.89 a	0.20 \pm 0.36 a	

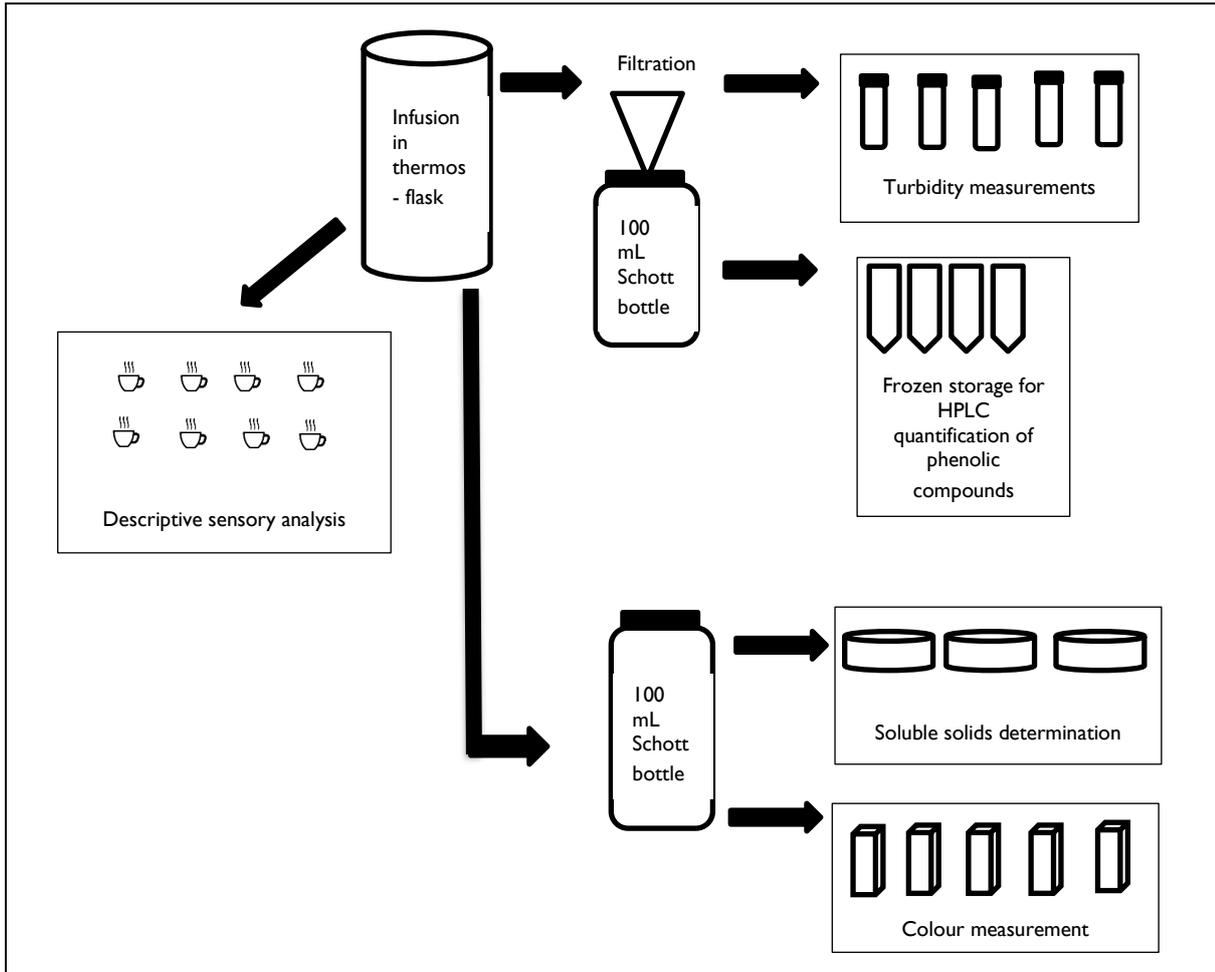


Figure 4.1 Layout of sample preparation and analyses

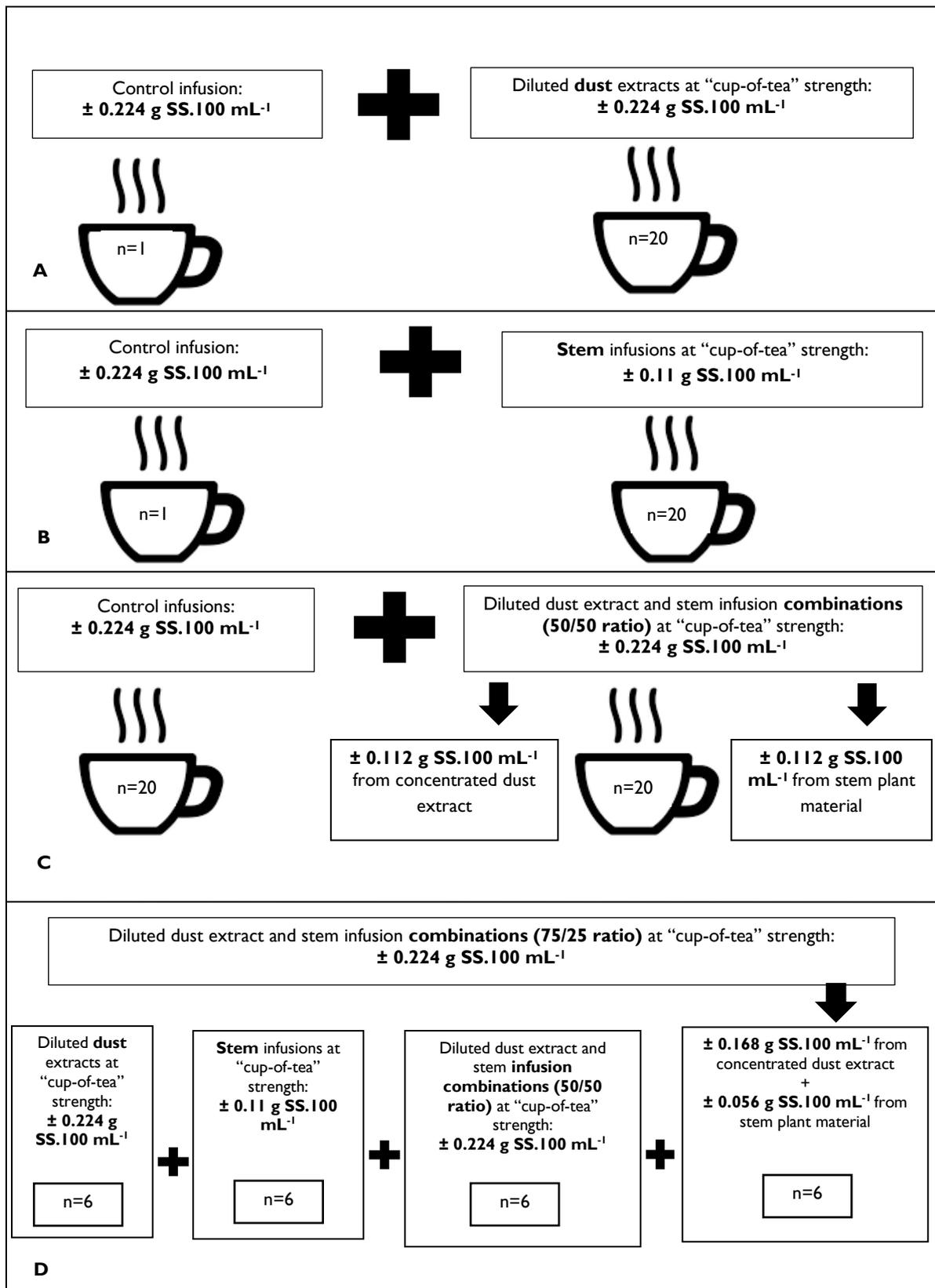


Figure 4.2 Schematic representation of the layout and SS content at “cup-of-tea” strength of (a) control infusion and diluted dust extracts, (b) control and stem infusions, (c) control infusions and 50/50 ratio diluted dust extract and stem infusion combinations and (d) 75/25 ratio diluted dust extract and stem infusion combinations.

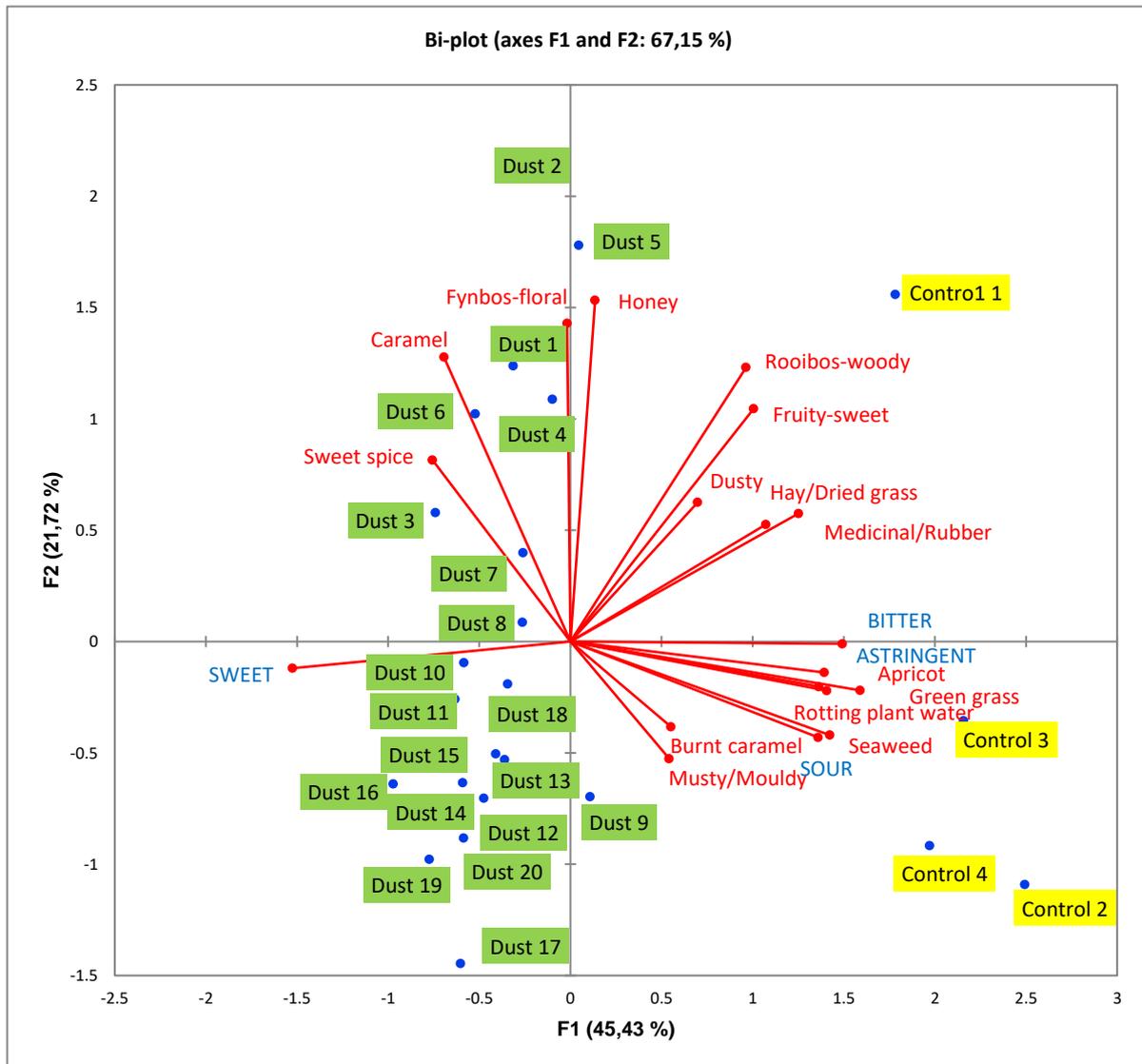


Figure 4.3 Principal component analysis (PCA) bi-plot illustrating aroma, taste and mouthfeel attributes of the control rooibos infusion and diluted dust extracts.

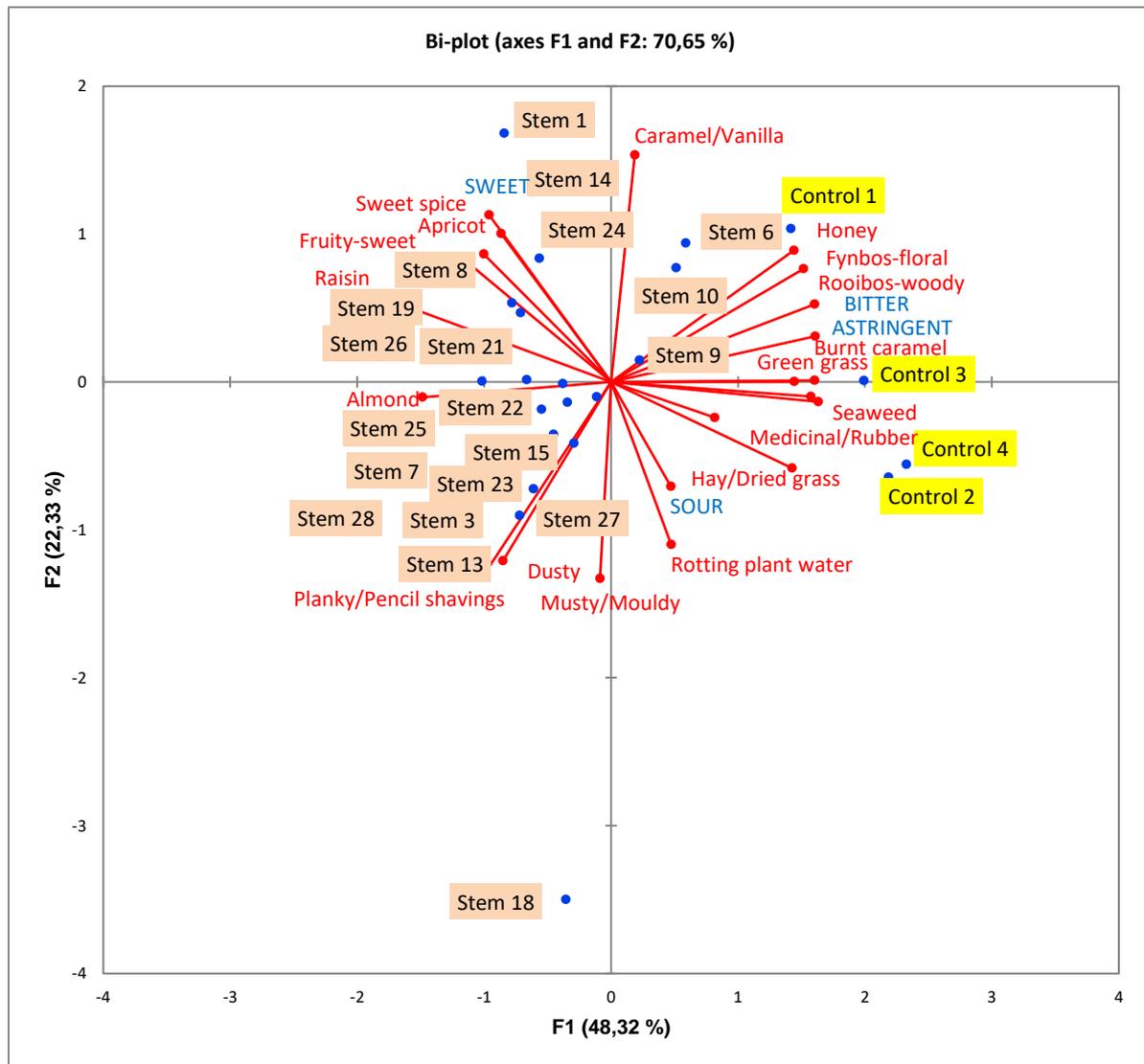


Figure 4.4 Principal component analysis (PCA) bi-plot illustrating aroma, taste and mouthfeel attributes of the control rooibos infusion and stem infusions.

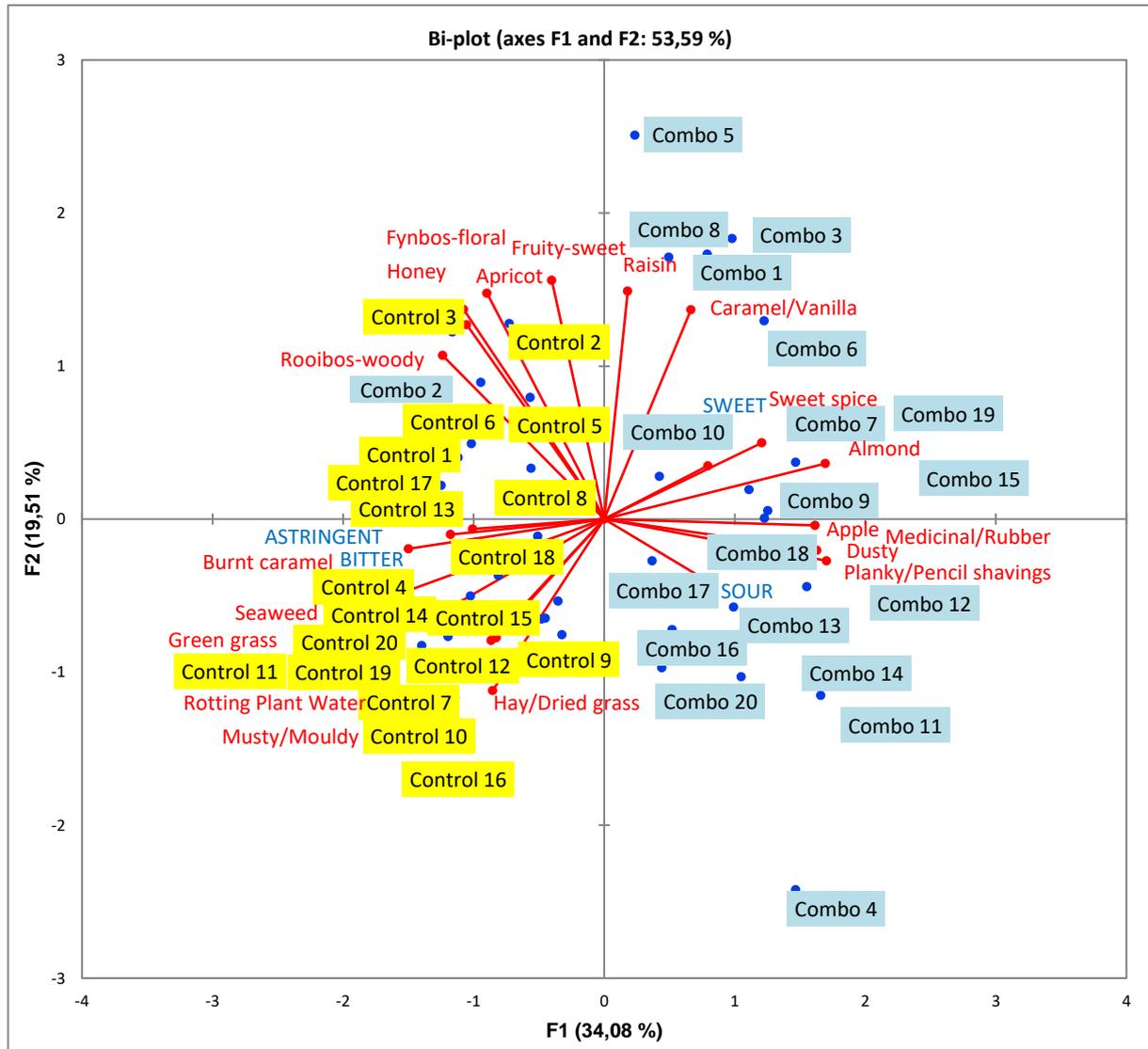


Figure 4.5 Principal component analysis (PCA) bi-plot illustrating aroma, taste and mouthfeel attributes of the control rooibos infusions and the 50/50 ratio combinations. The word “Combo” before each sample number refers to the diluted dust extract and stem combinations.

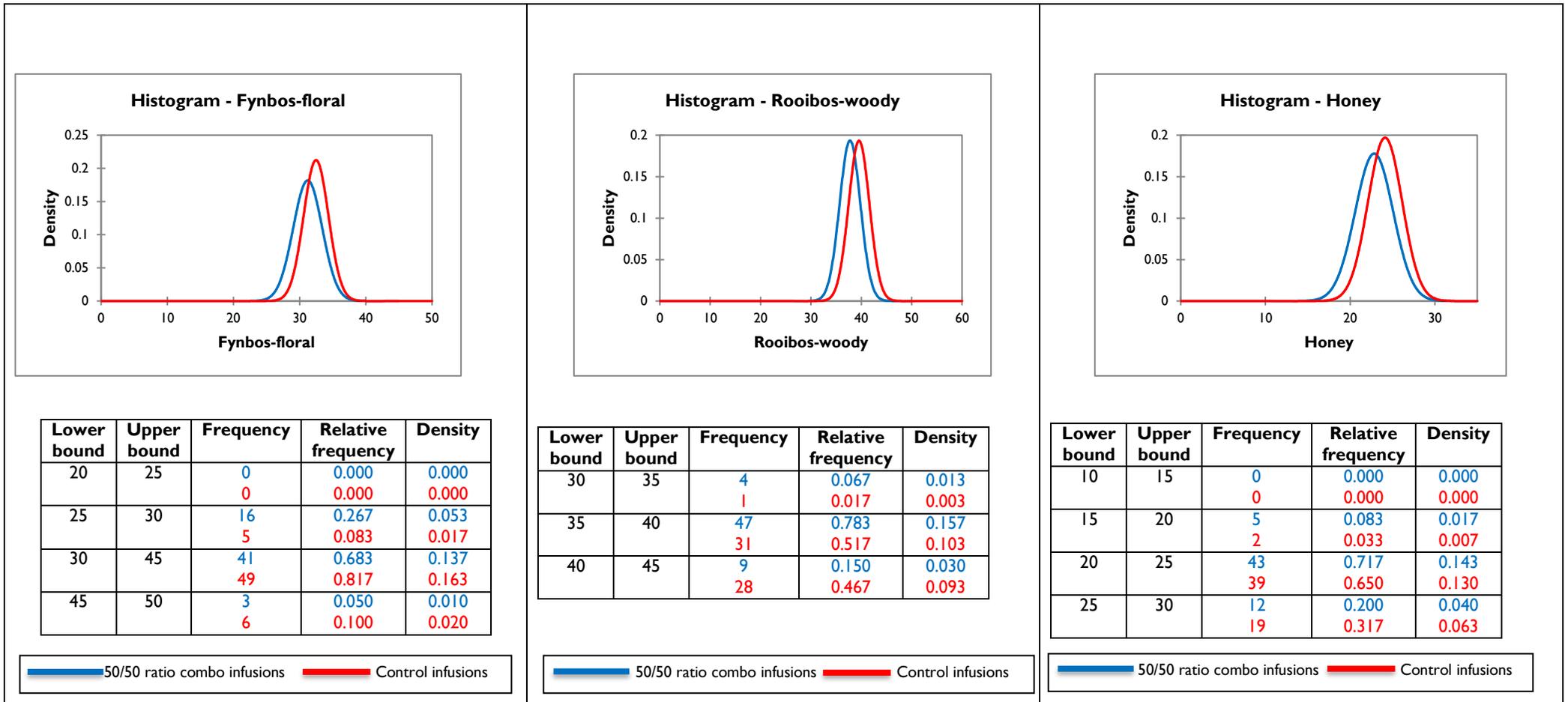


Figure 4.6 Histograms of typical rooibos aroma attributes perceived in the 50/50 ratio diluted dust extract and stem infusion combinations. “Combo” refers to diluted dust extract and stem infusion combination samples.

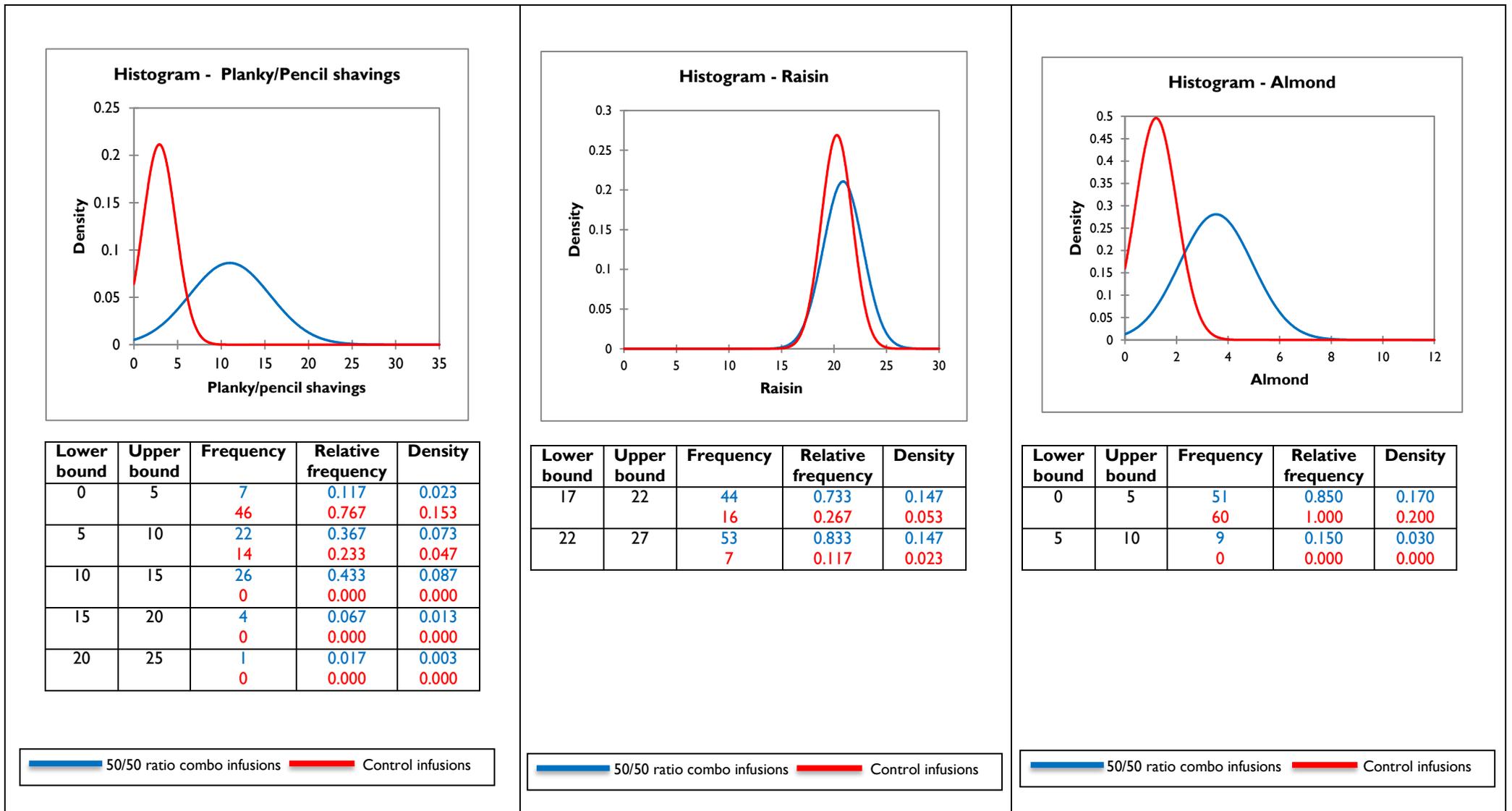


Figure 4.7 Histograms of non-typical rooibos aroma attributes perceived in the 50/50 ratio combinations. “Combo” refers to diluted dust extract and stem infusion combination samples.

5. General discussion, recommendations and conclusions

In view of the burgeoning demand for rooibos coupled with the current product shortages due to persisting droughts, the utilisation of rooibos waste plant material could be a meaningful pursuit to extend annual production. The bulk of rooibos production is comprised of the fermented plant material (Joubert & De Beer, 2011). However, the rooibos production process generates a significant amount of waste ($\pm 10\%$ per production batch), in the form of fine dust and coarse stems. Currently, rooibos waste is used for compost production or the coarse stems are cut into smaller pieces and mixed with tea leaves to produce a cheaper product (E. Joubert, Agricultural Research Council, Stellenbosch, South Africa, 2016, personal communication). Although this processing action is now applied frequently, it results in a lower quality tea with a less distinctive rooibos aroma. Annual production could potentially be increased by converting rooibos waste plant material into rooibos products of good and acceptable sensory quality.

Rooibos (*Aspalathus linearis*) is a fynbos species endemic to South Africa, which grows mostly in the Western and Northern Cape regions. To date rooibos is produced for consumption as a herbal tea and increasingly processed as a food ingredient and nutraceutical extract. Its popularity and demand has risen gradually as a result of its caffeine-free status and relatively low tannin levels in combination with health-promoting properties, specifically antioxidant activity. (Joubert & De Beer, 2011). Representing 10% of the global herbal tea market, the rooibos tea market is worth approximately R550 million annually (Anon., 2015). The South African rooibos industry recently obtained geographical indication (GI) certification; this important achievement permits better control of rooibos sales both on local and international scales. In addition, the improvement of livelihoods and development are some of the socioeconomic benefits that are a direct result of the GI status of rooibos (Anon., 2014). Therefore, because of GI certification of rooibos, the rooibos market is bound to expand and continue growing within the rooibos regions (WIPO, 2014).

The prosperity of any food or beverage product is highly dependent on its overall quality and consistency, which are two aspects that are vital when considering consumer expectations and consumer satisfaction. Since a number of herbal teas and tea blends with health benefit claims are continuously emerging on the tea market, it is vital that rooibos tea should be differentiated from its competitors. This could be accomplished through marketing and careful monitoring of its unique sensory profile apart from its characteristic red-brown colour. Although the demand for rooibos has been rising steadily locally and internationally, it is of utmost importance that the quality of this GI-certified South African product be maintained consistently. This ultimately guarantees not only industry growth, but also customer loyalty.

The main aim of this study was, therefore, to investigate the utilisation of fermented rooibos dust and stems for the eventual production of a valuable, commercially viable rooibos product for the herbal tea market, i.e. a product with the same sensory quality parameters as a cup of rooibos tea produced from the “tea bag” fraction of fermented leaves. To achieve this, the extraction of soluble

solids from rooibos dust using commercial enzymes was firstly explored and thereafter the hot water extraction (HWE) conditions were optimised using response surface methodology (RSM). The sensory attributes (aroma, flavour, taste and mouthfeel) associated with diluted dust extracts and stem infusions individually, and diluted dust extract and stem infusion combinations, at “cup-of-tea” strength were characterised thereafter.

Since significant amounts of waste material (dust and stems) are left over after fermented rooibos tea processing, rooibos dust was used as a plant material source for extraction optimisation. The “tea value” of rooibos stems could then be enhanced by application of extracts prepared from rooibos dust. It was therefore imperative to maximise the extraction of soluble matter from rooibos dust. Enzyme-assisted extraction (EE) was chosen as an alternative extraction method to the conventional HWE process since previous research (Pengilly *et al.*, 2008; Coetzee *et al.*, 2014; Zwane, 2014) showed positive results although the topic has not been studied extensively. Previous studies on EE of rooibos reported that enzymes resulted in significant increases in extract yields when applied to green and fermented rooibos, enhanced clarity of fermented rooibos extracts and reduced the aspalathin content in green and fermented rooibos extracts (Pengilly *et al.*, 2008; Coetzee *et al.*, 2014; Zwane, 2014). In the current study, rooibos dust was treated with three food-grade enzymes (Validase, Rapidase and Filtrase) each at varying concentrations (0.05, 0.1, 1, 2, 5 & 10%), while extraction time (2 hr), temperature (50 °C) and plant-material-to-water ratio (1:20 m.v⁻¹) were kept constant throughout each experiment. Although the commercial enzymes used had slightly different optimum extraction temperatures, their performance was evaluated at the same temperature (50 °C) for comparative purposes. Enzyme treatment of rooibos dust resulted only in a slight improvement in the soluble solids (SS) yield unlike in other studies by Pengilly *et al.* (2008), Coetzee *et al.* (2014) and Zwane (2014). At the highest dose of 10% (1000 times the dosage recommended by the supplier), Rapidase was the most efficient enzyme, delivering an increase of 8.4% SS. EE of rooibos dust was thus not as effective as expected possibly due to the fact that rooibos plant material composition is not fully understood. Selecting an appropriate enzyme for maximum extraction of SS from rooibos dust was therefore not straightforward. The Rapidase enzyme used is characterised by pectinase with arabinolytic and cellulolytic activity. According to Pengilly *et al.* (2008), dried fermented rooibos plant material has been found to contain approximately 42% cellulose and 4.2% arabinose. This finding suggests that cellulases would be most effective for the hydrolysis of rooibos plant material for the release of SS, and further elucidates why Rapidase was able to achieve highest extract yields. Given that Rapidase delivered significantly higher extract yields, it could be postulated that the extraction of non-coloured compounds such as sugars and ferulic acid contributed largely to this increase. In addition, Rapidase-treated dust produced significantly lighter and more turbid extracts than the control extract. The increase in turbidity at high Rapidase concentrations is possibly due to the hydrolysis of plant material, which increased the amount of particles, perhaps various organic polymers, suspended

in the liquid. However, the turbidity of the Rapidase-treated dust was still fairly low (<26 NTU) and would not have a large negative effect on the visual quality of extracts produced from enzyme-treated dust. In future, the use of other enzymes characterised by cellulase activity specifically, or a mixture of commercial enzymes, could be applied to rooibos dust for the optimisation of SS extraction. These enzymes could also be applied at different concentrations, temperatures and times to evaluate the effect on the extraction of SS. Alternatively, RSM could be applied to EE of rooibos dust where time and money could be saved in the process by obtaining useful information with a minimal number of possible experiments. Therefore, upon assessing the results of EE of rooibos dust for the extraction of maximum SS, the need to use a high enzyme concentration would ultimately increase the extract production costs. Optimisation of the HWE process was thus considered more feasible.

Preliminary “one-factor-at-a-time” (OFAT) experiments showed that the extraction time, extraction temperature and plant material-to-water ratio all had significant effects ($P \leq 0.05$) on the rooibos dust extract yield. These three variables were optimised by employing a central composite design (CCD) and RSM. Desirability profiling was used to identify optimal extraction conditions: extraction temperature (94 °C), extraction time (30 min) and plant material-to-water ratio (1:20 m.v⁻¹). In spite of 30 min and 1:30 m.v⁻¹ being indicated as the optimal extraction time and plant material-to-water ratio, minor increases in desirability were observed from 10 to 30 min and 1:10 to 1:30 m.v⁻¹. These optimal extraction conditions were thus unjustifiable as they would lead to the use of more energy and solvent. Therefore, the practical “optimal” extraction conditions were 20 min and 1:20 m.v⁻¹, where 94°C was acceptable as an optimal extraction temperature. The prediction model displayed satisfactory predictive ability for extract yield ($R^2_{adj} = 0.988$) with verification results further showing the suitability of the prediction model.

Application of the practically optimal extraction conditions (94 °C, 20 min and 1:20 plant material-to-water ratio (m.v⁻¹)) to 20 batches of rooibos dust led to extract yields varying between 16.4% and 27.9% compared to the yield of 22.7% predicted by the polynomial prediction model. This demonstrates that predicted values were not always achievable when producing extracts with batches of plant material different from that which was used to generate data for the model. The most probable cause for this was the inherent natural variation between the respective batches. Despite some extract yields being lower than the predicted value from the model, all extract yields from the 20 batches of dust were higher than the lowest extract yield (12.5%) obtained previously for 74 batches of unrefined fermented rooibos tea (Joubert & De Beer, 2012). This is largely due to the differences in particles size of plant material used. The maximum dust extract yield obtained by Rapidase-treatment of rooibos dust (25.7%) was lower than the maximum dust extract yield obtained using the selected optimal extraction conditions (27.9%). Therefore, this result further motivates the fact that EE of rooibos dust would not be cost effective. Analysis of the 20 dust extracts also demonstrated a considerable amount of variation in the colour, turbidity and phenolic compound content. The aspalathin yield from the 20

dust extracts varied from 0.083 to 0.202%. These values compared well with those of hot water extracts of unrefined rooibos containing 0.088% aspalathin (calculated from average extract yield and aspalathin content of 15.24% and 0.581%, respectively) (Joubert & De Beer, 2012). In addition, Schulz *et al.* (2003) reported the aspalathin content of fermented rooibos dust of 0.120% aspalathin. In comparison to this finding, more than half of the 20 dust extracts had a lower aspalathin content. Therefore, from the above-mentioned results it was established that extracts could be prepared from rooibos dust for food ingredient use or for the “tea-value” enhancement of rooibos stems due to the successful extraction of soluble matter. However, rooibos dust plant material would not be suitable for addition in teabags and would seep through and result in turbid infusions due to its extremely fine texture.

An interesting observation during this study was dichroism of the dust extracts. Extract colour parameters (C^* , a^* and b^*) did not have a linear relationship with SS concentration, and the inversion of the C^* , a^* and b^* values occurred at 0.35, 0.5 and 0.3% SS concentration, respectively. This result necessitated dilution of dust extract (20x) to enable comparison of objective colour parameters. Rooibos extracts are known to display dichroism, i.e. the degree of dilution or container size determines the colour intensity. Rooibos extracts that have been diluted are light yellow instead of the characteristic red-brown of more concentrated extracts, which results in the yellowish ring often seen at the rim of a cup of rooibos tea (Joubert, 1995). This phenomenon was somewhat observed with the naked eye at the 20x dilution of the dust extracts. The full observation of dichroism may have been limited by the dilution of the dust extracts, which was performed in a relatively small 20 mL volumetric flask.

The primary characteristic sensory profile of rooibos has been described as “honey”, “rooibos-woody” and “fynbos-floral” notes coupled with a slightly sweet taste and astringent mouthfeel, with “fruity-sweet”, “caramel”, “apricot” and “hay/dried grass” aromas forming part of the secondary characteristic profile of rooibos (Koch *et al.*, 2012; Jolley *et al.*, 2017). The sensory profile of diluted dust extracts and stem infusions individually and in combinations was analysed using descriptive sensory analysis (DSA) to quantify 41 aroma, flavour, taste and mouthfeel attributes associated with the positive and negative sensory profile of rooibos. Diluted dust extracts, 50/50 ratio diluted dust extract and stem infusion combinations, and 75/25 ratio diluted dust extract and stem infusion combinations, at “cup-of-tea” strength produced infusions of similar sensory quality as that of normal rooibos tea, although some significant differences were observed. Therefore, the sensory profiling of rooibos waste plant material provided valuable information regarding sensory attribute similarities and differences when compared to that of standard rooibos tea.

The dust extracts diluted to “cup-of-tea” strength, in particular, were of good sensory quality and could thus have significant value for the potential production of good quality rooibos beverages. For this reason, rooibos dust (without extraction) could potentially be included in rooibos espresso

plant material, since plant material is milled finely to produce this product. In contrast, stem infusions made at “cup-of-tea” strength produced weak infusions, suggesting that stem plant material cannot be used alone in teabags, as it would negatively affect the quality of rooibos infusions. Joubert (1984) also observed this and stated that rooibos stems are of low tea value. In addition, non-typical “planky/pencil shavings”, “raisin” and “almond” aroma attributes were perceived in the stem infusions. The “planky/pencil shavings” aroma note, in particular, was perceived as unpleasant and thus regarded as a taint. This taint was also observed in the 50/50 and 75/25 ratio dust extract and stem infusion combinations, although to a lesser extent. A “caramel” note often coupled with a “vanilla” aroma note was also perceived in the stem infusions at moderate intensities. “Caramel/vanilla”, however, has been perceived in the floral-like herbal tea *Cyclopia intermedia* (Bergh *et al.*, 2017) and these two attributes are very typical of wooded wines (Fernández de Simon *et al.*, 2014). It is known that vanilla originates from vanillin in oak fermented wine (Campbell, 2006). However, more research needs to be conducted to establish the origin of these two aroma notes in the rooibos stem infusions. Interestingly, in the analysis of red wines, the sensory descriptor “pencil shavings”, originating from nonanal, is not foreign to barrel fermented wines (Schmidtke *et al.*, 2010). The question is, however, whether the attribute “planky/pencil shavings” aroma in rooibos stems should be regarded as a taint or as part of the broader aroma woody profile of fermented rooibos. It will also be of value if the origin of this attribute in rooibos stems could be established. Perhaps a correlation between a particular volatile compound and the “planky/pencil shavings” aroma note exists, however, that would have to be ascertained.

Aspalathin, orientin, isoorientin and *Z*-2-(β -D-glucopyranosyloxy)-3-phenylpropenoic acid (PPAG) (>10 mg.L⁻¹), followed by vitexin and isovitexin (>5 mg.L⁻¹), were the major phenolic compounds in the diluted dust extracts, where orientin (>10 mg.L⁻¹), followed by aspalathin, isoorientin, PPAG and quercetin-3-*O*-robinobioside (>5 mg.L⁻¹), were the major phenolic compounds in the stem infusions. Fermented grade B rooibos infusions analysed by Joubert *et al.* (2012) contained the same major phenolic compounds. In addition, the aspalathin content of the diluted dust extracts, stem infusions and grade B rooibos infusions did not differ largely, although they were much lower than the control used for the sensory experiments. Therefore, the phenolic quality of the diluted dust extracts and stem infusions were good as suggested by these results. In addition, these results suggest that the diluted dust extracts could potentially be used for the production of rooibos beverages.

Reducing the stem plant material content at “cup-of-tea” strength to 25% was not enough to significantly reduce and mask the undesirable “planky/pencil shavings” aroma note. However, there was a slight indication that a greater portion of diluted dust extract could possibly mask the supposed taint was observed. Therefore, in order for rooibos waste plant material to be potentially re-used, its sensory attributes must be analysed precisely and understood fully. Good quality rooibos could potentially be produced from waste plant material should the “planky/pencil shavings” aroma note be eliminated satisfactorily by blending the waste material with good quality rooibos tea. However, it is

vitaly important that a low percentage of stem plant material is used. Therefore, the effect of lower percentages of stem plant material in the diluted dust extract and stem infusion combinations, as well as blending with good quality rooibos tea, should be evaluated in future.

The exact percentage of rooibos dust and stems generated during rooibos processing is also important for the future reutilisation of the waste plant material. As demonstrated, using 25% of stem plant material in the dust extract and stem infusion combinations was not sufficient for the significant reduction and masking of the undesirable “planky/pencil shavings” aroma. Therefore, if more stems are generated than dust, dust extract and stem infusion combination production will be severely limited as the stem plant material content cannot exceed 25%. Bearing in mind the end dry product of dust extract and stem infusion combinations where the stems would act as a carrier of the rooibos dust, getting the dust extract to be carried by the stems will be a challenge if the stem percentage is reduced below 25%.

In summary, the primary objectives of this study were realised, pointing the way towards extending annual fermented rooibos production to make up for the current shortages experienced by the industry. These two rooibos tea agro-processing waste materials could have significant market value in combination or dust on its own to benefit the rooibos industry.

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6. Addendum A: Chapter 3 supplementary data

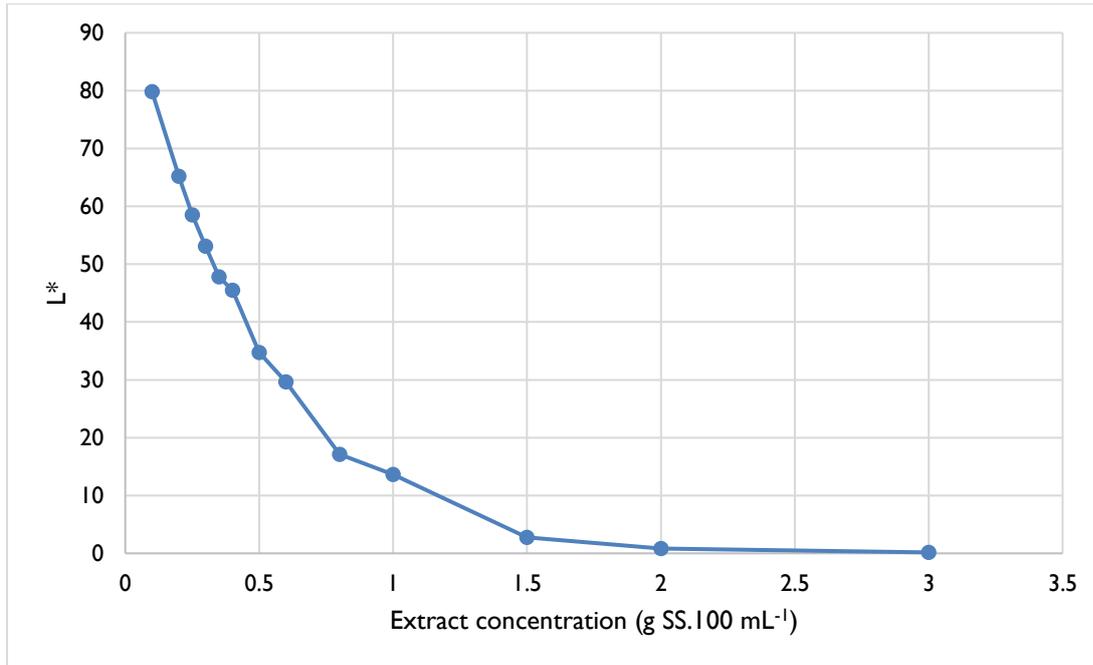


Figure A3.1a Effect of dilution on the L* value of dust extracts.

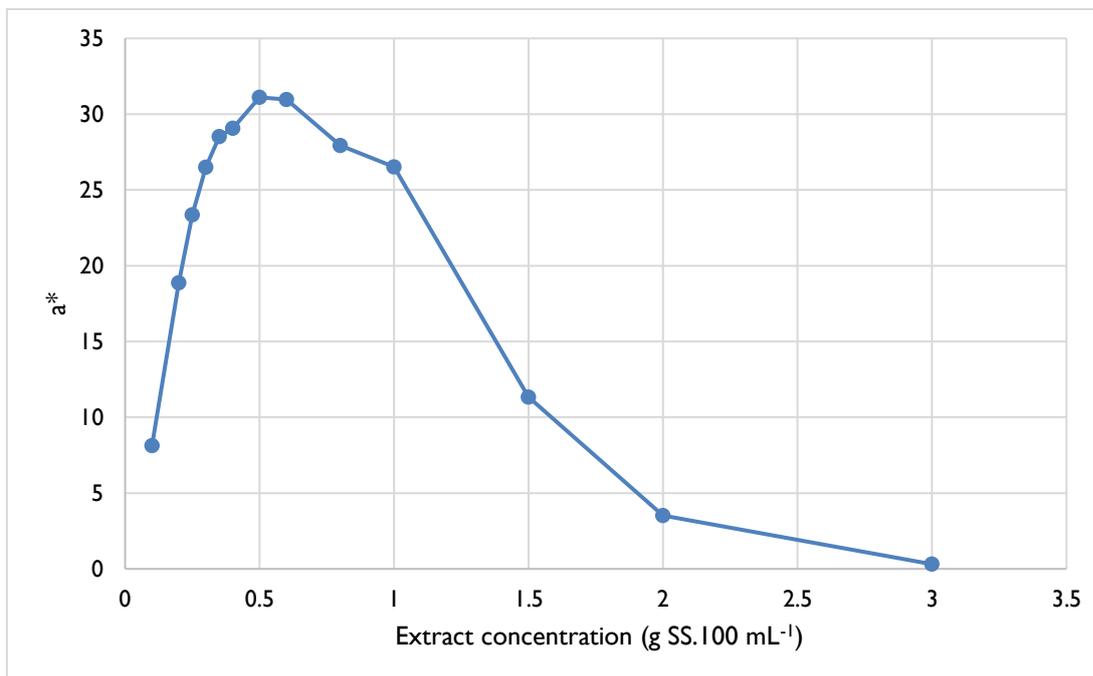


Figure A3.1b Effect of dilution on the a* value of dust extracts.

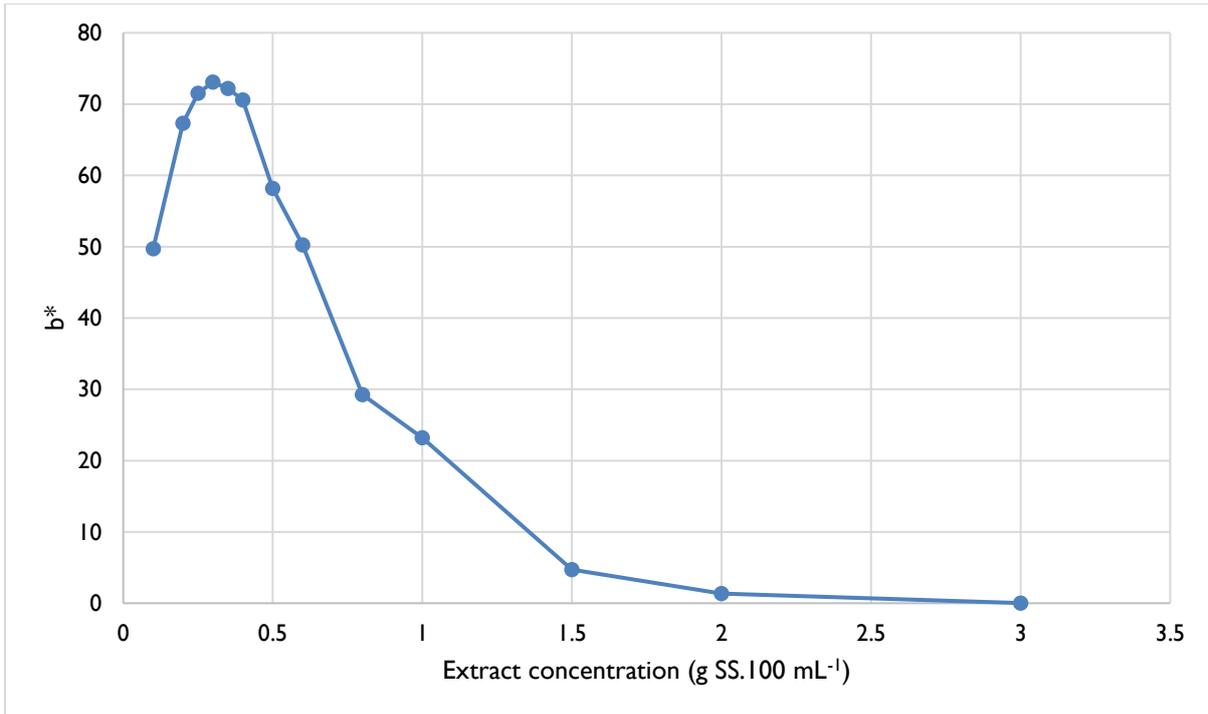


Figure A3.1c Effect of dilution on the b^* value of dust extracts.

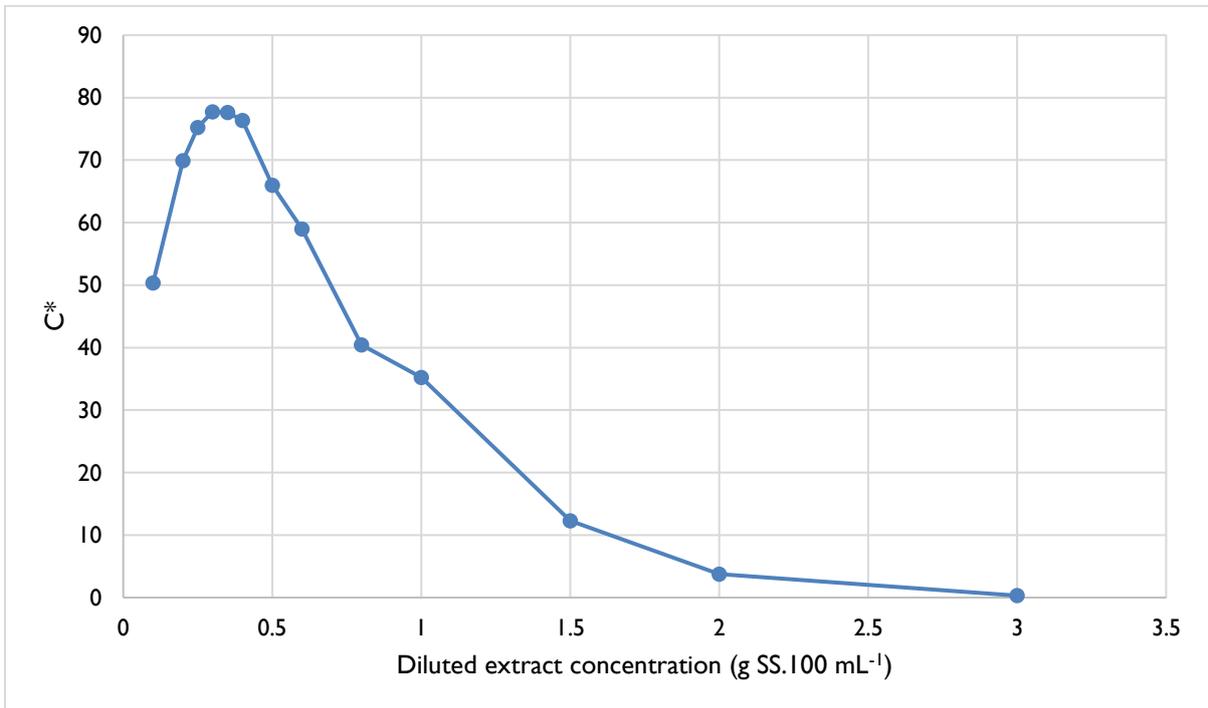


Figure A3.1d Effect of dilution on the C^* value of dust extracts.

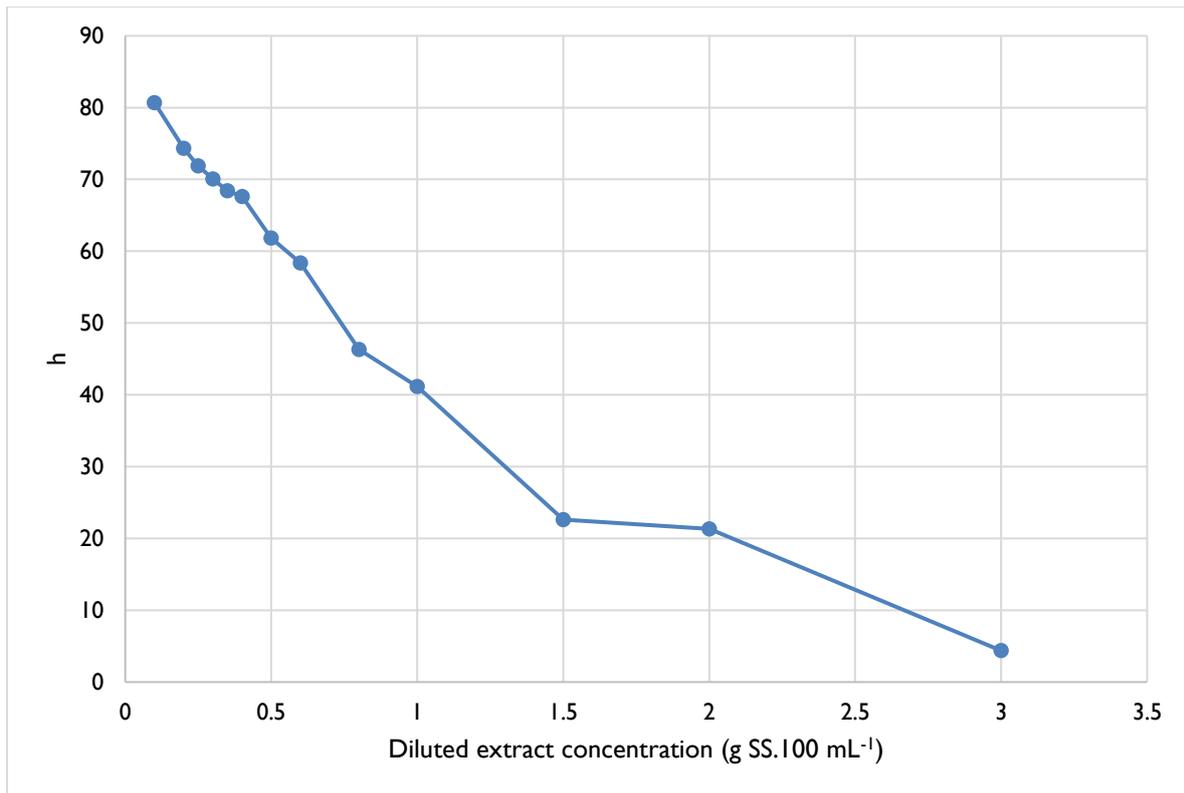


Figure A3.1e Effect of dilution on the h value of dust extracts.

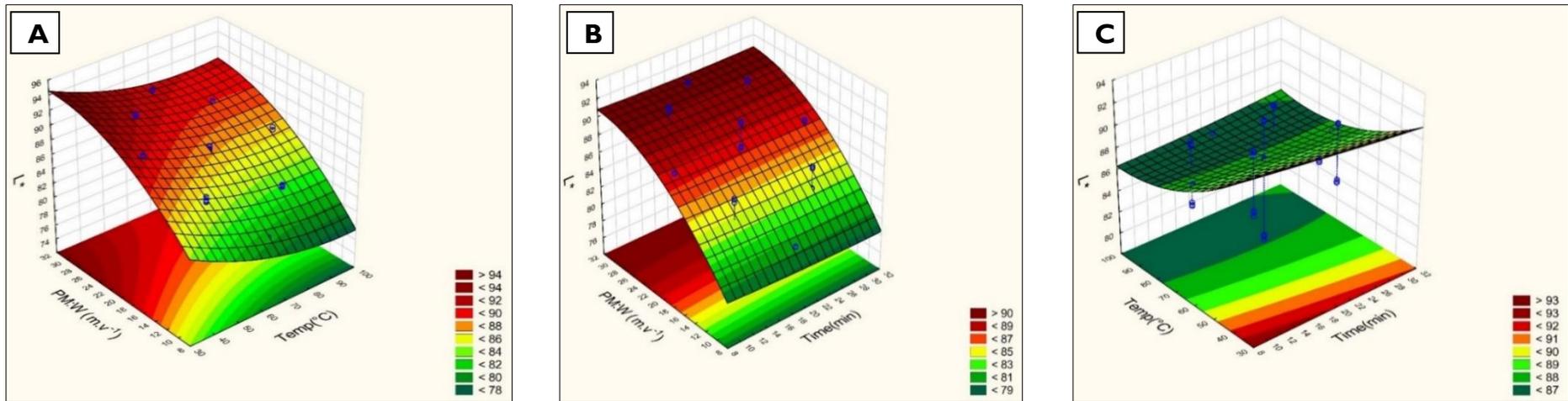


Figure A3.2a Response surface plots for L* value showing effects of (a) PM:W and ET at fixed extraction time (20 min); (b) PM:W and extraction time at fixed ET (50°C); (c) ET and extraction time (min) at fixed PM:W (1:20).

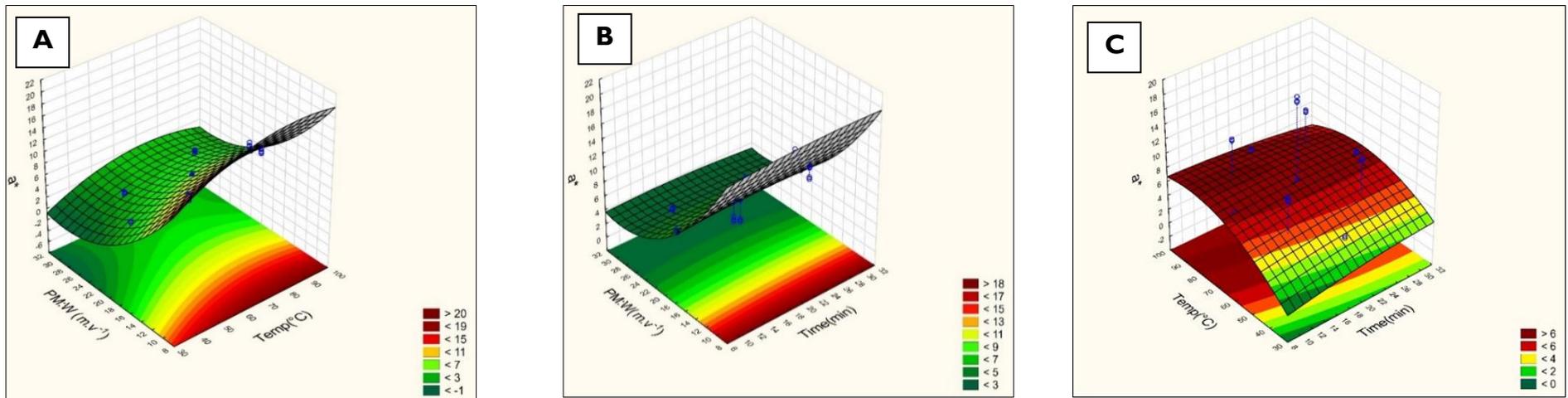


Figure A3.2b Response surface plots for a* the value showing effects of (a) PM:W and ET at fixed extraction time (20 min), (b) PM:W and extraction time at fixed ET (50°C), (c) ET and extraction time (min) at fixed PM:W (1:20).

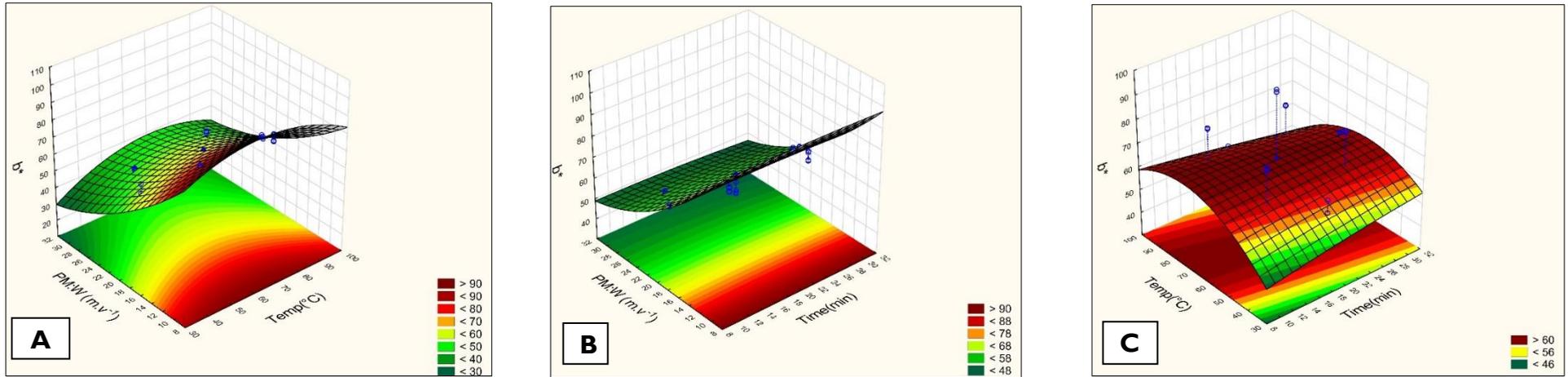


Figure A3.2c Response surface plots for b^* the value showing effects of (a) PM:W and ET at fixed extraction time (20 min), (b) PM:W and extraction time at fixed ET (50°C), (c) ET and extraction time (min) at fixed PM:W (1:20).

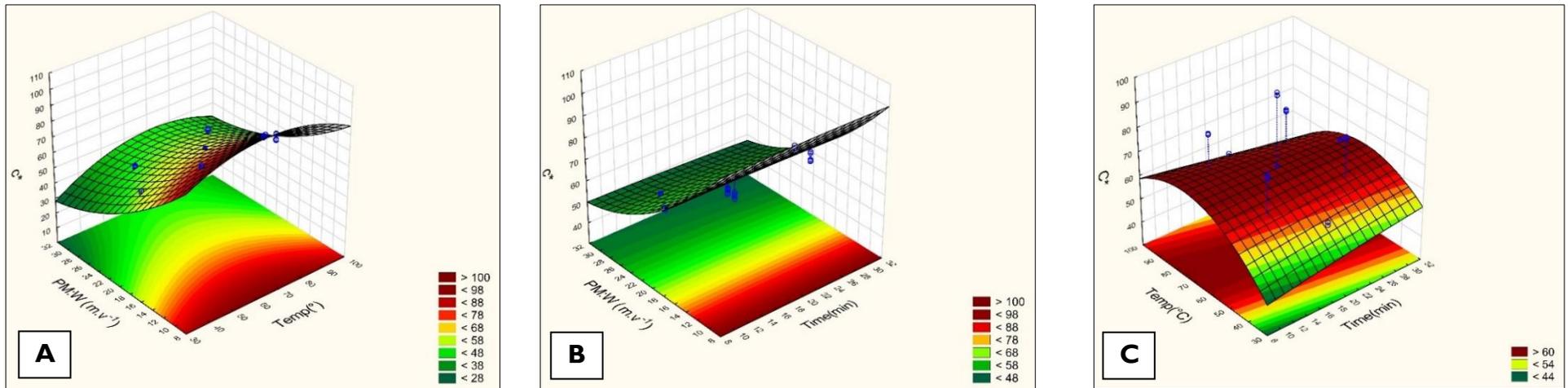


Figure A3.2d Response surface plots for C^* the value showing effects of (a) PM:W ET at fixed extraction time (20 min), (b) PM:W and extraction time at fixed ET (50°C), (c) ET and extraction time (min) at fixed PM:W (1:20).

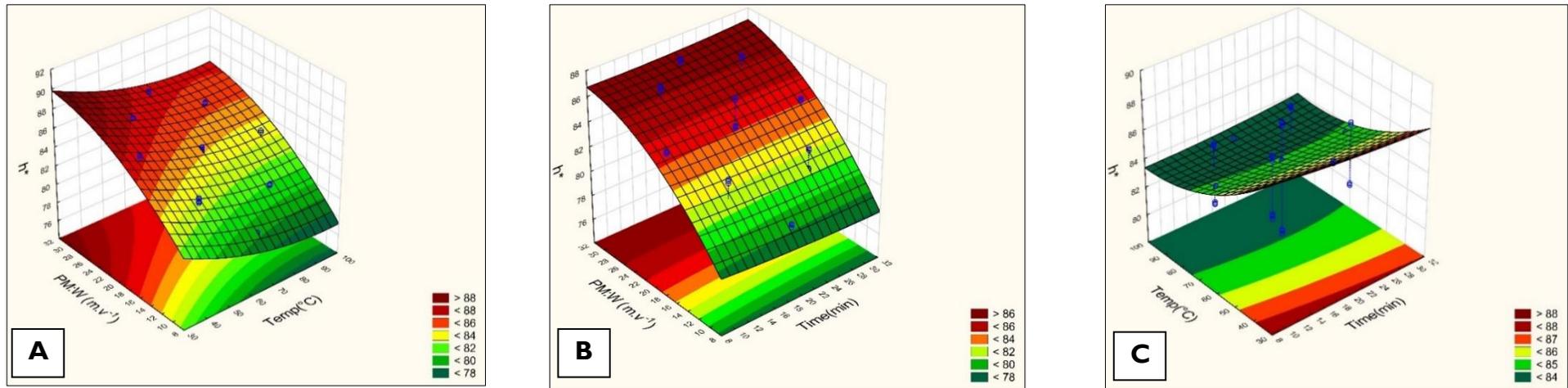


Figure A3.2e Response surface plots for the h^* value showing effects of (a) PM:W and ET at fixed extraction time (20 min), (b) PM:W and extraction time at fixed ET (50°C), (c) ET and extraction time (min) at fixed PM:W (1:20).

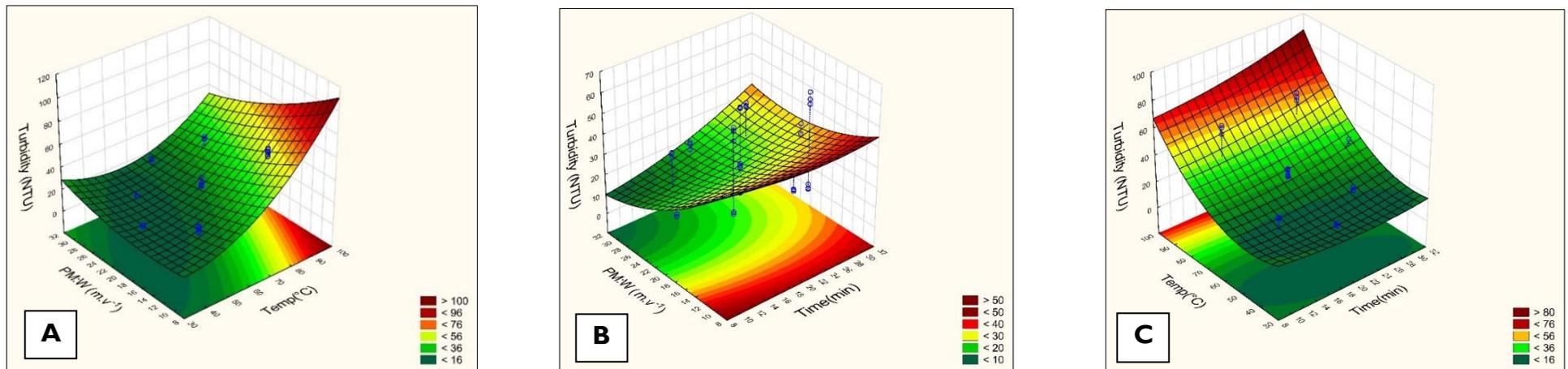
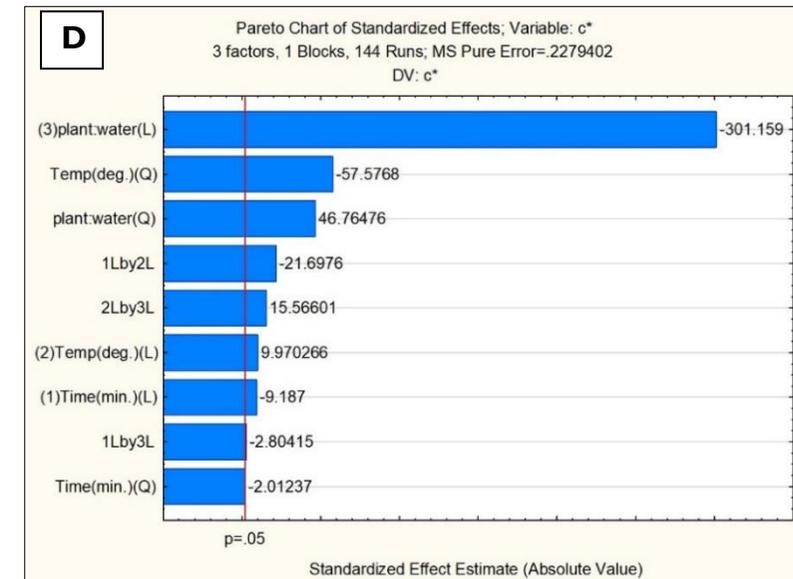
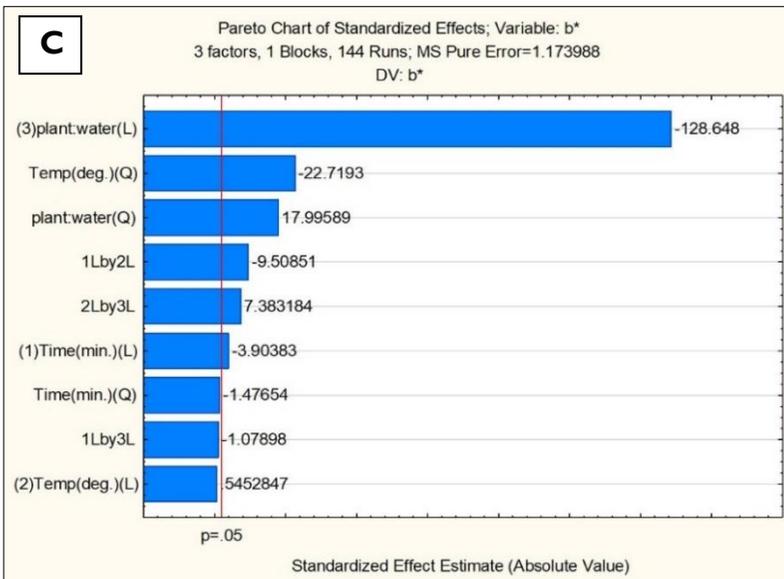
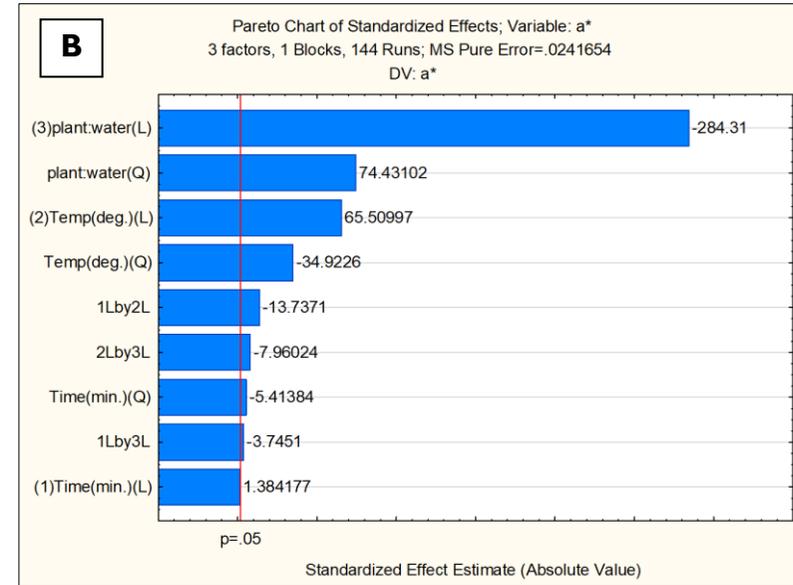
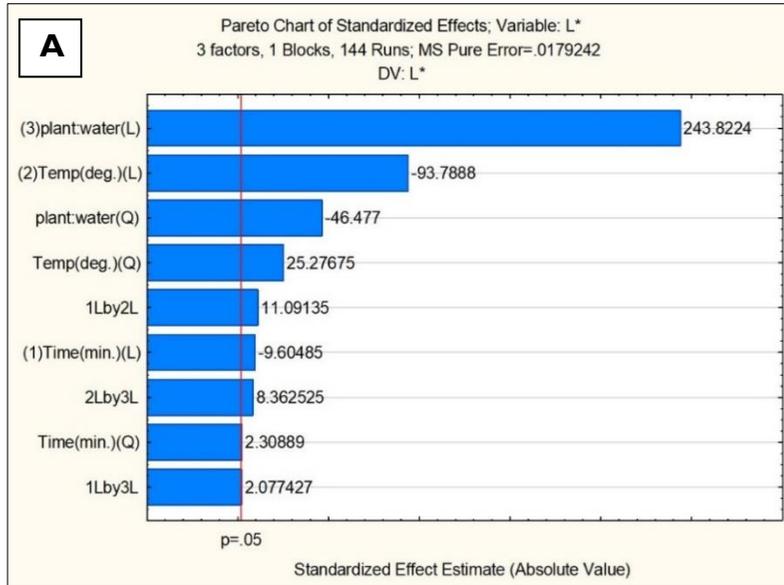


Figure A3.2f Response surface plots for the turbidity (NTU) value showing effects of (a) PM:W and ET at fixed extraction time (20 min), (b) PM:W and extraction time at fixed (50°C), (c) ET and extraction time (min) at fixed PM:W (1:20).



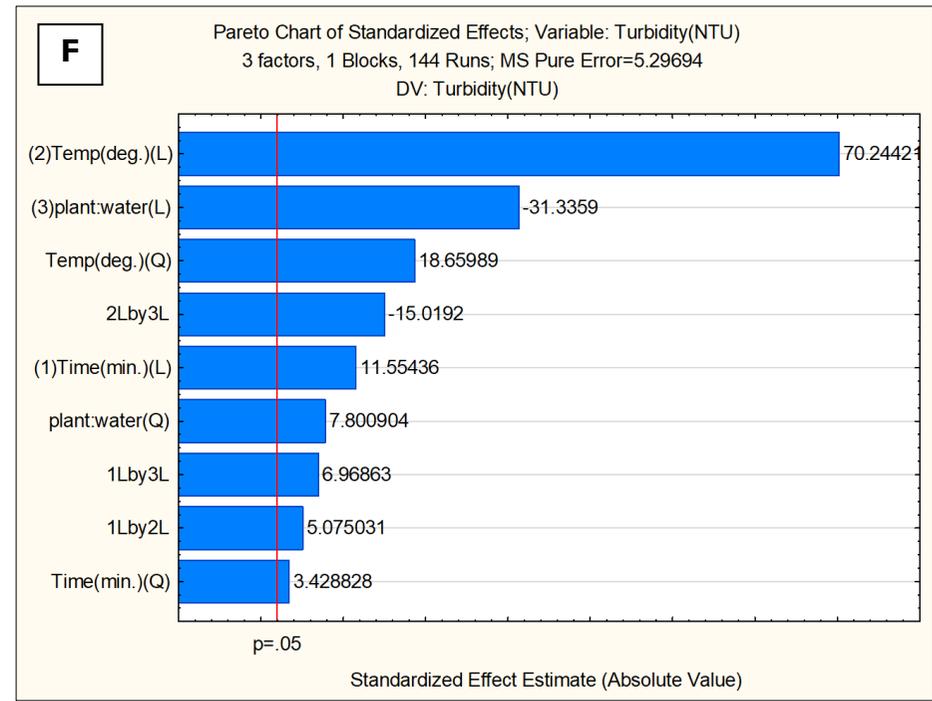
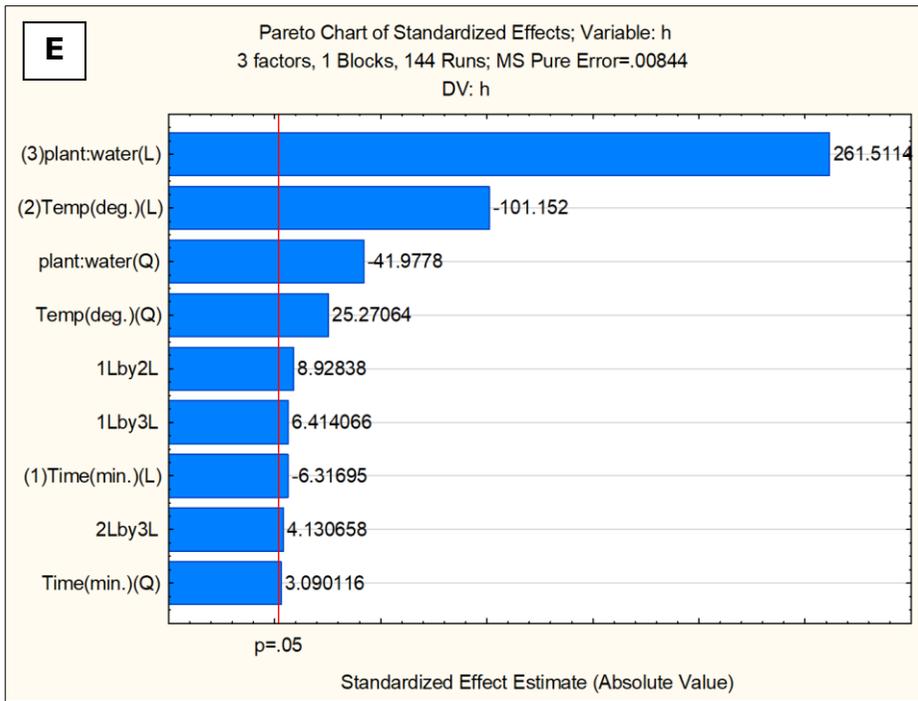


Figure A3.3a-f Standardised Pareto charts showing linear, quadratic and interaction effects for the L*, a*, b*, C*, h* and turbidity (NTU) values. L = linear effect; Q = quadratic effect; LxL = interaction effect.

7. Addendum B: Chapter 4 supplementary data

Table B4.1 Concentrated rooibos dust extract SS content (in triplicate) used to determine the dilution of dust extracts in order to make diluted dust extracts at “cup-of-tea” strength.

Dust Sample	g SS.100 mL ⁻¹	Concentrated extract (mL)	Freshly boiled distilled water (mL) needed for dilution to 1000 mL	Total Volume in flask (mL)
D1 A	1.098	204	796	1000
D1 B	1.113	201	799	1000
D1 C	1.108	202	798	1000
D2 A	0.925	242	758	1000
D2 B	0.875	256	744	1000
D2 C	0.885	253	747	1000
D3 A	1.323	169	831	1000
D3 B	1.339	167	833	1000
D3 C	1.329	169	831	1000
D4 A	1.124	199	801	1000
D4 B	1.152	194	806	1000
D4 C	1.090	206	794	1000
D5 A	1.015	221	779	1000
D5 B	1.014	221	779	1000
D5 C	1,011	222	778	1000
D6 A	0.911	246	754	1000
D6 B	0.916	244	756	1000
D6 C	0.903	248	752	1000
D7 A	0.989	227	773	1000
D7 B	0.985	227	773	1000
D7 C	1.022	219	781	1000
D8 A	1.079	208	792	1000
D8 B	0.960	233	767	1000
D8 C	0.976	230	770	1000
D9 A	1.369	164	836	1000
D9 B	1.275	176	824	1000
D9 C	1.354	165	835	1000
D10 A	1.205	186	814	1000
D10 B	1.243	180	820	1000
D10 C	1.230	182	818	1000
D11 A	1.217	184	816	1000
D11 B	1.155	194	806	1000
D11 C	1.183	189	811	1000
D12 A	1.119	200	800	1000
D12 B	1.131	198	802	1000
D12 C	1.136	197	803	1000
D13 A	1.155	194	806	1000
D13 B	1.172	191	809	1000
D13 C	1.129	198	802	1000

D14 A	1.245	180	820	1000
D14 B	1.251	179	821	1000
D14 C	1.225	183	817	1000
D15 A	1.218	184	816	1000
D15 B	1.189	188	812	1000
D15 C	1.199	187	813	1000
D16 A	1.195	187	813	1000
D16 B	1.182	190	810	1000
D16 C	1.181	190	810	1000
D17 A	1.397	160	840	1000
D17 B	1.373	163	837	1000
D17 C	1.407	159	841	1000
D18 A	1.193	188	812	1000
D18 B	1.197	187	813	1000
D18 C	1.162	193	807	1000
D19 A	1.049	214	786	1000
D19 B	1.038	216	784	1000
D19 C	1.012	221	779	1000
D20 A	1.162	193	807	1000
D20 B	1.447	155	845	1000
D20 C	1.337	168	832	1000

Table B4.2 Concentrated rooibos dust extract SS content (in triplicate) used to determine the dilution of dust extracts for the 50/50 dust extract and stem infusion combinations at “cup-of-tea” strength.

Combination sample	Stem plant material sample	Dust Sample	g SS.100 mL ⁻¹	Concentrated extract (mL) needed for ± 0.112 g SS.100 mL ⁻¹	Freshly boiled distilled water (mL) needed for dilution to 1000 mL	Total Volume in flask (mL)	Stem plant material needed for ± 0.112 g SS.100 mL ⁻¹ (g)
C1 A	S1 A	D1 A	1.118	89	911	1000	19.3
C1 B	S1 B	D1 B	1.095	91	909	1000	19.3
C1 C	S1 C	D1 C	1.104	91	909	1000	19.3
C2 C	S3 A	D2 A	0.926	108	892	1000	19.3
C2 B	S3 B	D2 B	0.873	115	885	1000	19.3
C2 C	S3 C	D2 C	0.882	113	887	1000	19.3
C3 A	S15 A	D3 A	1.330	75	925	1000	19.3
C3 B	S15 B	D3 B	1.320	76	924	1000	19.3
C3 C	S15 C	D3 C	1.304	77	923	1000	19.3
C4 A	S7 A	D4 A	1.264	79	921	1000	19.3
C4 B	S7 B	D4 B	1.036	97	903	1000	19.3
C4 C	S7 C	D4 C	1.068	94	906	1000	19.3
C5 A	S6 A	D5 A	1.016	98	902	1000	19.3
C5 B	S6 B	D5 B	1.021	98	902	1000	19.3
C5 C	S6 C	D5 C	1.013	99	901	1000	19.3
C6 A	S9 A	D6 A	0.879	114	886	1000	19.3
C6 B	S9 B	D6 B	0.871	115	885	1000	19.3
C6 C	S9 C	D6 C	0.878	114	886	1000	19.3
C7 A	S8 A	D7 A	1.173	85	915	1000	19.3
C7 B	S8 B	D7 B	1.123	89	911	1000	19.3
C7 C	S8 C	D7 C	1.140	88	912	1000	19.3
C8 A	S10 A	D8 A	1.031	97	903	1000	19.3
C8 B	S10 B	D8 B	1.002	100	900	1000	19.3
C8 C	S10 C	D8 C	1.084	92	908	1000	19.3
C9 A	S13 A	D9 A	1.351	74	926	1000	19.3
C9 B	S13 B	D9 B	1.388	72	928	1000	19.3
C9 C	S13 C	D9 C	1.344	74	926	1000	19.3
C10 A	S14 A	D10 A	1.228	81	919	1000	19.3
C10 B	S14 B	D10 B	1.179	85	915	1000	19.3
C10 C	S14 C	D10 C	1.193	84	916	1000	19.3
C11 A	S18 A	D11 A	1.169	86	914	1000	19.3
C11 B	S18 B	D11 B	1.152	87	913	1000	19.3
C11 C	S18 C	D11 C	1.140	88	912	1000	19.3
C12 A	S19 A	D12 A	1.122	89	911	1000	19.3
C12 B	S19 B	D12 B	1.119	89	911	1000	19.3
C12 C	S19 C	D12 C	1.075	93	907	1000	19.3
C13 A	S21 A	D13 A	1.094	91	909	1000	19.3
C13 B	S21 B	D13 B	1.104	91	909	1000	19.3
C13 C	S21 C	D13 C	1.158	86	914	1000	19.3
C14 A	S22 A	D14 A	1.179	85	915	1000	19.3
C14 B	S22 B	D14 B	1.165	86	914	1000	19.3
C14 C	S22 C	D14 C	1.163	86	914	1000	19.3
C15 A	S23 A	D15 A	1.191	84	916	1000	19.3
C15 B	S23 B	D15 B	1.200	83	917	1000	19.3
C15 C	S23 C	D15 C	1.164	86	914	1000	19.3
C16 A	S24 A	D16 A	1.111	90	910	1000	19.3
C16 B	S24 B	D16 B	1.098	91	909	1000	19.3
C16 C	S24 C	D16 C	1.105	90	910	1000	19.3
C17 A	S25 A	D17 A	1.367	73	927	1000	19.3
C17 B	S25 B	D17 B	1.385	72	928	1000	19.3
C17 C	S25 C	D17 C	1.354	74	926	1000	19.3
C18 A	S26 A	D18 A	1.115	90	910	1000	19.3
C18 B	S26 B	D18 B	1.142	88	912	1000	19.3
C18 C	S26 C	D18 C	1.106	90	910	1000	19.3
C19 A	S27 A	D19 A	0.971	103	897	1000	19.3

C19 B	S27 B	D19 B	0.981	102	898	1000	19.3
C19 C	S27 C	D19 C	1.006	99	901	1000	19.3
C20 A	S28 A	D20 A	1.220	82	918	1000	19.3
C20 B	S28 B	D20 B	1.209	83	917	1000	19.3
C20 C	S28 C	D20 C	1.690	59	941	1000	19.3

Table B4.3 Concentrated dust extract SS content (in triplicate) used to determine the dilution of dust extracts in order to make 75/25 diluted dust extract and stem infusion combination at “cup-of-tea” strength.

Diluted dust extracts							
Combination sample	Stem plant material sample	Dust Sample	g SS.100 mL ⁻¹	Concentrated extract (mL) needed for ± 0.224 SS.100 mL ⁻¹	Freshly boiled distilled water (mL) needed for dilution to 1000 mL	Total Volume in flask (mL)	Stem plant material (g)
n/a	n/a	D11 A	1.234	181	819	1000	n/a
n/a	n/a	D11 B	1.248	179	821	1000	n/a
n/a	n/a	D11 C	1.235	181	819	1000	n/a
n/a	n/a	D9 A	1.426	157	843	1000	n/a
n/a	n/a	D9 B	1.400	160	840	1000	n/a
n/a	n/a	D9 C	1.426	157	843	1000	n/a
n/a	n/a	D19 A	1.009	222	778	1000	n/a
n/a	n/a	D19 B	1.049	214	786	1000	n/a
n/a	n/a	D19 C	0.995	225	775	1000	n/a
n/a	n/a	C10 A	1.297	173	827	1000	n/a
n/a	n/a	C10 B	1.251	179	821	1000	n/a
n/a	n/a	C10 C	1.051	213	787	1000	n/a
n/a	n/a	D5 A	1.069	210	790	1000	n/a
n/a	n/a	D5 B	1.088	206	794	1000	n/a
n/a	n/a	D5 C	1.053	213	787	1000	n/a
n/a	n/a	D8 A	1.126	199	801	1000	n/a
n/a	n/a	D8 A	1.083	207	793	1000	n/a
n/a	n/a	D8 C	1.056	212	788	1000	n/a
50/50 dust extract and stem infusion combinations							
Combination sample	Stem plant material sample	Dust Sample	g SS.100 mL ⁻¹	Concentrated extract (mL) needed for ± 0.112 g SS.100 mL ⁻¹	Freshly boiled distilled water (mL) needed for dilution to 1000 mL	Total Volume in flask (mL)	Stem plant material needed for ± 0.112 g SS.100 mL ⁻¹ (g)
C11 A	S18 A	D11 A	1.166	86	914	1000	19.3
C11 B	S18 B	D11 B	1.150	87	913	1000	19.3
C11 c	S18 C	D11 C	1.154	87	913	1000	19.3
C9 A	S13 A	D9 A	1.318	76	924	1000	19.3
C9 B	S13 B	D9 B	1.369	73	927	1000	19.3
C9 C	S13 C	D9 C	1.363	73	927	1000	19.3
C19 A	S27 A	D19 A	0.952	105	895	1000	19.3
C19 B	S27 B	D19 B	0.976	102	898	1000	19.3
C19 C	S27 C	D19 C	0.946	106	894	1000	19.3
C10 A	S14 A	C10 A	1.190	84	916	1000	19.3
C10 B	S14 B	C10 B	1.205	83	917	1000	19.3
C10 C	S14 C	C10 C	1.209	83	917	1000	19.3
C5 A	S6 A	D5 A	0.988	101	899	1000	19.3
C5 B	S6 B	D5 B	0.987	101	899	1000	19.3
C5 C	S6 B	D5 C	0.929	108	892	1000	19.3
C8 A	S10 A	D8 A	0.919	109	891	1000	19.3
C8 C	S10 B	D8 A	1.000	100	900	1000	19.3
C8 C	S10 C	D8 C	0.949	105	895	1000	19.3

25/75 dust extract and stem infusion combination							
Combination sample	Stem plant material sample	Dust Sample	g SS.100 mL⁻¹	Concentrated extract (mL) needed for ± 0.168 g SS.100 mL⁻¹	Freshly boiled distilled water (mL) needed for dilution to 1000 mL	Total Volume in flask (mL)	Stem plant material needed for ± 0.056 g SS.100 mL⁻¹ (g)
C11 A	S18 A	D11 A	1.161	145	855	1000	9.65
C11 B	S18 B	D11 B	1.193	141	859	1000	9.65
C11 C	S18 C	D11 C	1.184	142	858	1000	9.65
C9 A	S13 A	D9 A	1.397	120	880	1000	9.65
C9 B	S13 B	D9 B	1.417	119	881	1000	9.65
C9 C	S13 C	D9 C	1.417	119	881	1000	9.65
C19 A	S27 A	D19 A	1.020	165	835	1000	9.65
C19 B	S27 B	D19 B	0.981	171	829	1000	9.65
C19 C	S27 C	D19 C	0.988	170	830	1000	9.65
C10 A	S14 A	C10 A	1.240	136	864	1000	9.65
C10 B	S14 B	C10 B	1.225	137	863	1000	9.65
C10 C	S14 C	C10 C	1.274	132	868	1000	9.65
C5 A	S6 A	D5 A	1.078	156	844	1000	9.65
C5 B	S6 B	D5 B	1.023	164	836	1000	9.65
C5 C	S6 B	D5 C	1.050	160	840	1000	9.65
C8 A	S10 A	D8 A	1.039	162	838	1000	9.65
C8 B	S10 B	D8 B	1.028	163	837	1000	9.65
C8 C	S10 C	D8 C	0.999	168	832	1000	9.65

Table B4.4 Aroma attributes of the control rooibos infusion and diluted dust extracts. The letters “C” and “D” refer to the control infusion and the diluted dust extracts, respectively.

AROMA ATTRIBUTES															
Sample	Fynbos-floral	Rooibos-woody	Apricot	Fruity-sweet	Honey	Caramel / Vanilla	Sweet spice	Hay/Dried grass	Green grass	Rotting plant water	Seaweed	Burnt caramel	Medicinal/Rubber	Dusty	Musty/Mouldy
C1	35.13	35.13	3.53	7.57	21.88	20.21	0.44	27.22	7.02	0.93	0.87	2.55	1.58	1.37	0.51
C2	30.24	38.95	3.10	5.86	16.82	16.99	0.19	25.55	9.09	1.68	3.84	3.96	0.56	1.54	2.10
C3	31.09	40.99	4.46	5.69	17.36	18.47	0.00	23.97	7.05	1.56	1.16	4.90	2.42	0.38	0.26
C4	31.09	40.62	4.14	6.14	16.62	16.87	0.00	22.88	7.05	1.98	2.27	5.04	0.00	0.27	0.27
D1	34.18	41.45	1.62	5.08	23.25	24.35	0.65	21.29	0.96	0.25	0.00	3.18	0.26	0.14	0.28
D2	36.59	41.07	1.72	6.73	23.17	25.22	1.85	22.95	0.00	0.79	0.00	4.21	0.65	0.97	0.27
D3	32.55	37.22	1.02	5.19	21.01	24.70	0.44	20.43	0.41	0.00	0.00	1.65	0.00	0.14	0.28
D4	32.09	40.55	0.79	5.53	19.93	25.93	2.02	20.53	0.56	0.26	0.00	5.06	0.50	1.31	0.27
D5	34.26	41.24	0.44	7.04	21.77	25.79	1.98	23.16	0.15	0.98	0.00	2.51	0.56	0.59	0.26
D6	33.47	40.00	0.85	4.78	19.84	22.29	1.50	19.88	0.24	0.22	0.00	1.89	0.51	1.01	0.00
D7	34.03	38.96	1.09	5.83	18.06	19.80	1.22	21.77	0.00	0.00	0.00	3.45	0.00	0.73	0.81
D8	33.08	38.58	1.79	4.13	17.23	21.14	1.47	20.88	0.49	0.00	0.00	4.33	0.00	0.44	0.31
D9	31.06	35.45	2.02	5.58	17.71	18.44	0.53	21.73	0.83	1.10	0.00	5.18	0.00	0.40	0.63
D10	30.71	36.60	1.05	3.35	15.74	22.39	2.15	20.83	0.28	0.51	0.00	5.06	0.00	1.09	0.29
D11	31.77	37.08	0.58	4.90	17.97	21.67	1.03	20.04	0.50	0.42	0.00	2.29	0.00	0.13	0.59
D12	32.33	36.68	0.83	3.50	16.32	18.46	0.51	21.28	0.26	0.77	0.00	4.03	0.00	0.00	0.42
D13	30.48	37.37	0.83	4.51	16.62	21.54	0.79	18.27	0.21	0.65	0.00	4.82	0.20	0.38	0.00
D14	30.88	34.96	0.56	4.05	16.30	19.24	0.77	21.69	0.59	0.19	0.00	2.09	0.22	0.47	0.71
D15	30.66	36.83	1.0	3.22	17.14	21.09	0.92	21.55	0.00	0.00	0.00	2.87	0.27	0.24	0.54
D16	31.32	35.35	0.86	3.09	17.79	20.76	1.31	17.15	0.00	0.00	0.00	2.67	0.00	0.00	0.97
D17	29.14	33.93	0.96	4.27	14.04	17.01	1.18	19.96	1.04	0.19	0.00	4.17	0.00	0.00	0.23
D18	32.59	36.99	3.19	5.29	18.02	21.81	1.28	16.97	0.26	1.03	0.00	4.22	0.19	0.00	0.53
D19	32.71	33.91	0.88	2.81	15.83	21.5	0.53	16.69	0.26	0.51	0.00	2.20	0.00	1.00	1.12
D20	31.31	35.87	1.41	3.38	15.67	20.04	0.0	17.22	0.00	0.23	0.00	3.42	0.17	0.00	0.00

Table B4.5 Taste and mouthfeel attributes of the control rooibos infusion and diluted dust extracts. The letters “C” and “D” refer to the control infusion and the diluted dust extracts, respectively.

TASTE AND MOUTHFEEL ATTRIBUTES				
Sample	Sweet	Astringent	Sour	Bitter
C1	19.51	28.06	1.24	4.66
C2	18.74	28.55	1.95	6.51
C3	19.26	27.61	2.58	6.44
C4	19.09	28.68	1.50	6.59
D1	22.29	25.13	0.36	3.26
D2	21.77	25.69	0.00	3.40
D3	22.43	25.70	0.00	2.18
D4	21.37	26.25	0.96	3.37
D5	20.62	27.01	0.69	2.27
D6	21.49	24.81	0.28	2.77
D7	21.63	26.28	0.73	1.64
D8	20.58	26.43	0.49	2.60
D9	20.95	25.93	0.83	2.41
D10	21.95	25.40	0.49	1.37
D11	22.50	26.13	0.24	2.83
D12	21.78	25.71	0.23	1.95
D13	21.79	25.17	1.28	3.05
D14	21.95	25.60	0.22	2.19
D15	21.65	26.21	0.77	3.24
D16	22.40	25.17	0.13	2.70
D17	22.53	26.23	0.71	1.64
D18	22.74	26.13	0.77	1.47
D19	22.38	26.80	1.01	1.48
D20	23.32	26.43	0.25	1.93

Table B4.6 Flavour attributes of the control rooibos infusion and diluted dust extracts. The letters “C” and “D” refer to the control infusion and the diluted dust extracts, respectively.

FLAVOUR ATTRIBUTES															
Sample	Fynbos-floral	Rooibos-woody	Apricot	Fruit-sweet	Honey	Caramel/Vanilla	Sweet spice	Hay/dried grass	Green grass	Rotting plant water	Seaweed	Burnt caramel	Medicinal/Rubber	Dusty	Musty/Mouldy
C1	31.17	39.88	0.00	0.22	1.88	2.43	0.00	26.47	4.37	0.23	2.72	0.00	0.00	1.00	0.00
C2	23.92	36.59	0.00	0.00	1.12	0.91	0.00	24.38	5.58	3.19	3.27	0.00	0.00	0.27	0.26
C3	27.78	38.65	0.00	0.21	1.17	2.92	0.00	25.81	3.97	1.91	4.18	0.51	0.51	0.00	0.00
C4	27.62	39.35	0.00	0.00	1.42	1.88	0.00	23.14	3.39	2.32	4.41	0.00	0.00	0.29	0.00
D1	29.65	38.06	0.00	0.00	2.19	3.88	0.00	23.40	1.15	0.00	2.08	0.00	0.99	0.49	0.26
D2	29.91	38.54	0.00	0.00	2.68	4.56	0.00	24.97	1.46	0.00	2.26	0.98	0.00	0.88	0.26
D3	29.88	37.03	0.00	0.00	3.05	3.19	0.00	21.74	0.76	0.00	0.80	0.00	0.00	1.19	0.00
D4	26.55	36.91	0.00	0.00	1.84	2.97	0.00	22.83	1.18	0.00	1.49	0.00	0.53	1.36	0.00
D5	28.74	38.41	0.00	0.00	2.01	3.44	0.00	23.39	1.26	0.00	1.68	0.53	0.00	1.22	0.00
D6	28.15	39.68	0.00	0.00	1.00	1.17	0.00	20.75	0.00	0.00	0.82	0.00	0.00	0.31	0.00
D7	29.58	36.54	0.00	0.22	0.79	1.40	0.00	23.24	0.00	0.00	2.48	0.00	0.00	0.49	0.28
D8	26.86	37.20	0.00	0.00	0.50	0.91	0.00	20.98	1.13	0.00	1.74	0.00	0.00	0.37	0.26
D9	25.47	35.41	0.00	0.00	0.37	0.41	0.00	24.82	1.86	0.00	2.93	0.00	0.00	0.51	0.51
D10	27.46	35.62	0.00	0.26	1.17	1.04	0.00	21.97	0.47	0.00	1.85	0.00	0.00	0.66	0.00
D11	27.52	36.03	0.00	0.14	2.91	3.04	0.00	22.41	0.71	0.00	2.15	0.00	0.00	0.26	0.00
D12	26.68	34.62	0.00	1.28	2.81	2.22	0.00	22.30	1.03	0.00	1.86	0.00	0.00	0.45	0.47
D13	26.40	34.39	0.00	0.00	1.82	3.14	0.00	20.93	0.51	0.00	1.70	0.00	0.00	0.67	0.22
D14	25.91	35.18	0.00	0.00	2.03	3.06	0.00	23.91	1.42	0.00	0.86	0.00	0.00	0.22	0.00
D15	26.54	34.94	0.00	0.51	2.58	2.58	0.00	22.75	0.92	0.00	2.17	0.00	0.00	0.23	0.26
D16	27.72	35.11	0.00	0.00	2.32	2.36	0.00	19.78	0.67	0.00	1.32	0.00	0.00	0.27	0.00
D17	26.58	33.79	0.00	0.91	2.18	2.27	0.00	21.85	1.03	0.00	1.71	0.00	0.00	0.00	0.00
D18	28.03	34.79	0.00	0.00	1.92	1.41	0.00	20.97	0.90	0.00	2.25	0.00	0.00	0.00	0.00
D19	26.78	32.13	0.00	0.24	2.01	2.58	0.00	19.82	0.23	0.00	1.00	0.00	0.00	0.14	0.00
D20	27.78	34.63	0.00	0.00	2.46	2.23	0.00	19.45	0.64	0.00	1.14	0.00	0.00	0.00	0.00

Table B4.7 Aroma attributes of the control rooibos infusion and stem infusions. The letters “C” and “S” refer to the control infusion and the stem infusions, respectively.

AROMA ATTRIBUTES																		
Sample	Fynbos-floral	Rooibos-woody	Planky/Pencil shavings	Apricot	Raisin	Almond	Fruity-sweet	Honey	Caramel/Vanilla	Sweet spice	Hay/Dried grass	Green grass	Rotting plant water	Seaweed	Burnt caramel	Medicinal/Rubber	Dusty	Musty/Mouldy
C1	37.64	45.01	1.21	3.26	9.03	0.57	3.17	27.64	27.82	2.08	25.21	4.50	0.00	3.03	4.10	0.00	0.26	0.00
C2	36.10	46.45	2.01	3.28	4.76	0.23	2.05	26.76	25.64	0.67	28.08	3.84	0.64	3.07	6.19	0.81	0.13	0.66
C3	36.29	45.53	1.91	3.03	5.93	0.21	2.79	27.36	26.59	0.74	25.55	5.19	0.77	3.80	5.03	0.00	0.41	0.28
C4	35.90	45.17	2.67	2.54	6.06	0.28	2.74	27.63	25.55	0.87	27.51	5.95	0.77	3.67	7.64	0.00	0.00	0.14
S1	30.23	37.14	2.37	7.50	13.22	3.83	7.93	24.15	30.71	2.85	21.86	0.40	0.14	0.00	2.59	0.00	1.13	0.00
S21	26.44	31.54	6.27	5.47	10.69	3.15	3.18	19.07	27.09	1.62	22.79	1.26	0.28	0.57	1.76	0.00	1.49	0.00
S3	27.32	32.46	6.58	4.75	13.37	2.05	6.08	20.65	25.93	2.06	23.34	1.69	0.26	0.55	1.74	0.00	1.35	1.49
S22	27.30	32.47	6.90	5.67	13.41	1.85	3.96	21.85	27.95	1.66	22.81	1.08	0.26	0.72	1.69	0.00	0.76	0.80
S6	34.14	41.41	2.09	4.80	10.76	0.89	3.66	26.49	27.76	2.14	23.63	1.69	0.00	0.82	2.31	0.00	0.68	0.00
S7	28.32	33.64	5.23	5.18	10.89	2.47	4.77	20.47	26.31	1.63	23.51	0.49	0.53	0.00	2.43	0.00	1.26	0.00
S23	27.85	32.85	4.65	5.27	11.86	2.03	3.68	20.10	25.50	1.82	23.32	1.88	0.64	0.14	2.13	0.00	1.19	0.79
S8	28.64	34.36	5.58	6.62	13.78	2.26	6.08	19.55	26.13	2.31	21.67	1.64	0.00	0.00	1.32	0.00	0.78	0.86
S24	29.92	33.13	4.20	5.10	14.14	2.29	5.62	21.96	27.86	1.46	21.64	0.96	0.00	0.16	2.32	0.00	0.46	0.00
S9	33.19	37.45	4.79	5.57	11.07	1.12	3.68	21.89	26.12	0.99	24.38	0.58	0.00	0.21	3.14	0.00	0.86	0.50
S10	34.22	40.91	1.06	3.55	10.45	1.16	3.05	24.41	28.74	2.05	21.28	1.17	0.54	0.64	2.29	0.00	0.27	0.83
S25	28.24	33.91	5.57	4.07	10.14	1.91	5.47	18.54	23.72	2.05	21.22	0.00	0.54	0.55	2.06	0.00	0.70	0.00
S13	26.33	33.21	9.29	6.13	12.79	2.00	4.74	19.05	23.23	1.51	21.20	0.13	0.66	0.00	2.33	0.00	1.90	0.55
S26	26.59	30.19	6.24	3.53	12.35	4.58	5.28	20.27	26.46	2.46	19.78	0.14	0.00	0.00	0.92	0.00	1.62	0.00
S14	32.91	39.67	1.54	6.46	10.12	1.41	5.53	23.51	27.04	2.64	20.56	0.78	0.00	0.47	2.08	0.00	1.00	0.00
S15	29.86	34.26	4.58	6.06	10.47	1.29	4.96	20.31	25.23	1.74	20.91	0.49	0.77	0.77	1.82	0.00	1.04	0.00
S27	25.15	30.78	6.09	3.76	10.76	3.41	3.83	20.26	23.09	0.51	21.99	0.54	0.53	0.24	1.01	0.00	0.96	0.00
S18	24.80	31.69	12.14	2.36	9.01	2.65	2.58	15.41	20.50	0.89	25.34	1.45	0.77	0.76	1.50	0.00	4.88	5.04
S28	25.79	31.74	4.79	5.25	12.33	2.83	3.97	18.51	24.82	2.45	20.32	0.00	0.53	0.54	0.55	0.00	1.61	0.00
S19	29.12	36.05	5.42	4.74	12.67	2.82	4.56	20.30	26.41	3.31	21.50	0.14	0.53	0.27	1.51	0.00	1.74	0.00

Table B4.8 Taste and mouthfeel attributes of the control rooibos infusion and stem infusions. The letters “C” and “S” refer to the control infusion and the stem infusions, respectively.

TASTE AND MOUTHFEEL ATTRIBUTES				
Sample	Sweet	Astringent	Sour	Bitter
C1	22.82	28.37	4.85	0.19
C2	21.38	27.61	4.34	0.58
C3	22.12	29.30	5.93	0.14
C4	21.30	28.51	6.21	0.97
S1	23.03	24.99	1.70	0.00
S21	23.30	25.78	1.05	0.00
S3	22.08	26.46	2.00	0.53
S22	22.51	27.05	1.17	0.50
S6	22.12	28.29	3.97	0.00
S7	22.36	26.39	2.05	0.29
S23	22.16	26.32	1.49	0.24
S8	22.84	26.36	1.24	0.14
S24	23.00	26.05	1.24	0.00
S9	22.51	27.77	2.97	0.38
S10	22.71	28.09	4.05	0.00
S25	22.82	25.84	0.96	0.26
S13	22.49	26.23	1.46	0.71
S26	22.57	25.78	1.38	0.62
S14	23.12	27.41	4.36	0.20
S15	22.29	27.99	2.78	0.44
S27	23.14	25.83	1.37	0.39
S18	21.09	27.12	1.72	0.19
S28	22.54	26.58	1.43	0.27
S19	23.42	26.51	1.91	0.00

Table B4.9 Flavour attributes of the control rooibos infusion and stem infusions. The letters “C” and “S” refer to the control infusion and the stem infusions, respectively.

	FLAVOUR ATTRIBUTES																	
	Fynbos-floral	Rooibos-woody	Planky/Pencil shavings	Apricot	Raisin	Almond	Fruity-sweet	Honey	Caramel/Vanilla	Sweet spice	Hay/Dried grass	Green grass	Rotting plant water	Seaweed	Burnt caramel	Medicinal/Rubber	Dusty	Musty/Mouldy
C1	31.60	40.23	1.44	0.00	0.00	0.00	0.00	0.34	1.53	0.26	27.53	3.73	1.21	3.29	2.64	0.00	0.00	0.00
C2	29.58	40.22	0.43	0.00	0.00	0.00	0.22	0.00	0.54	0.00	28.32	4.32	0.77	3.35	2.77	0.00	0.51	0.00
C3	30.76	39.41	1.97	0.00	0.00	0.00	0.23	0.71	0.00	0.00	27.81	3.73	1.55	3.24	3.89	0.00	0.00	0.00
C4	30.59	40.29	1.33	0.00	0.00	0.00	0.00	0.71	0.13	0.00	27.22	3.86	1.09	3.78	4.84	0.00	0.00	0.00
S1	24.32	31.62	4.68	0.00	0.50	0.50	0.00	0.00	1.36	0.00	21.86	0.54	0.39	0.26	0.26	0.00	0.13	0.00
S21	22.60	30.13	6.85	0.00	0.54	0.00	0.00	0.00	0.72	0.00	23.86	0.53	0.64	0.28	0.76	0.00	1.50	0.00
S3	22.82	31.26	7.01	0.00	0.00	0.00	0.00	0.50	0.54	0.00	23.49	0.54	0.39	0.00	1.26	0.00	0.55	0.66
S22	20.14	31.05	8.07	0.00	0.00	0.22	0.53	0.00	0.59	0.00	23.53	0.86	0.26	0.00	0.26	0.00	0.70	0.00
S6	28.87	38.26	1.82	0.00	0.00	0.00	0.00	0.49	0.16	0.00	27.32	1.77	0.66	0.39	2.10	0.00	0.26	0.45
S7	23.68	32.01	6.53	0.00	0.00	0.45	0.00	0.00	0.45	0.00	22.20	0.62	0.66	0.00	1.00	0.00	1.29	0.25
S23	23.37	30.95	5.76	0.00	0.00	0.00	0.00	0.00	0.00	0.00	21.92	1.25	0.26	0.27	1.26	0.50	0.67	0.54
S8	23.78	30.64	6.80	0.00	0.50	0.00	0.00	0.00	0.46	0.00	20.04	0.41	0.13	0.00	0.51	0.00	0.62	0.00
S24	24.17	32.50	4.58	0.00	0.54	0.55	0.54	0.00	0.76	0.00	21.37	0.87	0.13	0.62	1.14	0.00	0.66	0.00
S9	25.28	35.47	5.70	0.00	0.00	0.00	0.54	0.00	0.00	0.00	25.61	1.08	0.00	0.21	1.40	0.00	0.51	0.00
S10	28.96	36.46	3.44	0.00	0.00	0.00	0.00	0.00	0.49	0.00	24.64	2.64	1.34	1.11	1.85	0.00	1.08	0.00
S25	23.10	29.63	5.56	0.00	0.00	0.49	0.00	0.00	0.51	0.00	22.72	1.04	0.53	0.00	1.43	0.00	0.36	0.53
S13	22.64	30.91	7.22	0.00	0.00	0.00	0.00	0.00	0.23	0.00	21.93	0.85	0.53	0.00	0.45	0.00	0.59	0.59
S26	22.83	29.21	6.08	0.00	0.50	0.76	0.00	0.00	1.35	0.00	20.46	0.85	0.00	0.00	0.67	0.00	1.32	0.00
S14	28.53	36.92	2.60	0.00	0.00	0.00	0.76	0.00	0.74	0.00	24.59	1.42	0.79	1.58	2.11	0.00	0.76	0.00
S15	23.67	31.36	6.12	0.00	0.00	0.00	0.50	0.00	0.24	0.00	23.96	0.72	0.81	0.74	1.60	0.00	0.00	0.00
S27	22.58	28.69	5.03	0.00	0.86	0.77	0.00	0.00	1.35	0.00	21.26	0.45	0.53	0.00	0.47	0.00	0.97	0.00
S18	20.77	30.19	10.17	0.00	0.00	0.00	0.00	0.00	0.17	0.00	24.26	0.81	0.77	0.58	0.79	0.00	4.24	3.67
S28	22.35	29.33	3.63	0.00	0.83	0.77	0.00	0.00	1.05	0.00	19.99	0.51	0.77	0.36	0.58	0.00	0.69	0.00
S19	24.88	32.33	3.42	0.00	0.00	0.47	0.38	0.00	0.00	0.00	22.49	0.96	0.77	0.45	0.78	0.00	1.87	0.00

Table B4.10 Aroma attributes of the control rooibos infusions and 50/50 ratio combinations. The letters “C” and “CB” before the sample number refer to the control infusion and combination samples, respectively.

AROMA ATTRIBUTES																			
	Fynbos-floral	Rooibos-woody	Planky/Pencil shavings	Apricot	Apple	Raisin	Almond	Fruity-sweet	Honey	Caramel/Vanilla	Sweet spice	Hay/Dried grass	Green grass	Rotting plant water	Seaweed	Burnt caramel	Medicinal/Rubber	Dusty	Musty/Mouldy
C1	32.74	37.71	6.41	17.99	0.36	22.47	2.09	23.08	26.11	27.53	0.00	24.82	6.01	0.00	1.47	2.23	0.00	1.81	1.58
C2	31.01	39.37	1.94	16.91	1.23	19.71	1.62	20.22	22.42	25.88	0.00	24.39	3.51	0.62	1.41	1.97	0.00	0.00	2.18
C3	32.30	39.64	1.04	16.92	0.91	18.71	0.00	20.71	23.80	26.64	0.00	25.09	6.33	0.19	2.13	2.99	0.00	1.08	1.35
C4	30.79	40.56	4.18	14.76	0.79	19.77	0.83	19.68	22.71	24.39	0.00	23.76	2.42	0.00	1.06	0.57	0.00	0.79	0.53
C5	33.54	41.38	3.66	18.01	0.68	19.53	2.26	21.32	25.05	25.92	0.00	23.22	3.67	0.65	1.56	2.18	0.00	0.26	1.62
C6	33.03	38.87	0.70	15.76	0.00	20.50	0.97	20.01	23.70	27.03	0.46	24.72	3.03	1.13	0.67	2.73	0.00	0.79	2.46
C7	30.66	36.77	1.94	15.95	0.76	20.81	0.94	21.45	21.96	25.11	0.89	23.80	3.79	0.00	0.66	1.40	0.00	1.18	1.57
C8	32.45	39.50	2.22	15.77	0.89	20.10	1.04	19.71	22.92	25.09	0.00	26.39	5.78	0.54	1.34	2.43	0.00	0.54	2.21
C9	34.64	41.62	3.13	17.25	0.00	20.04	1.54	21.32	25.83	26.60	0.00	23.60	4.00	0.96	0.68	3.10	0.00	0.78	1.81
C10	32.26	39.18	3.96	15.09	0.51	19.13	1.69	20.54	24.54	26.09	0.00	23.45	1.85	0.00	0.79	0.74	0.00	1.18	0.79
C11	31.29	39.46	2.99	16.36	0.83	18.39	0.53	20.62	25.34	25.07	0.00	22.63	3.11	1.31	1.54	2.83	0.00	0.67	1.64
C12	34.71	41.40	2.64	19.32	0.81	22.01	1.35	23.13	25.86	25.37	0.51	24.35	1.56	0.26	1.08	0.51	0.00	0.53	1.01
C13	31.38	38.74	2.96	17.30	0.68	19.05	1.55	20.97	24.93	25.04	0.00	25.57	2.97	0.00	1.43	1.55	0.00	0.53	1.80
C14	35.94	42.32	5.38	19.20	0.00	20.61	0.53	22.08	26.04	26.94	0.00	23.42	0.58	0.17	0.93	1.38	0.00	2.42	1.05
C15	31.19	37.03	2.33	18.07	0.26	20.36	0.53	23.70	23.23	24.61	0.00	24.69	3.91	0.69	0.83	1.24	0.00	1.69	1.08
C16	33.03	40.67	0.64	19.07	0.80	21.63	1.82	22.17	23.47	25.88	0.50	24.24	2.47	0.00	1.09	0.35	0.00	0.83	0.81
C17	32.96	41.10	2.64	20.58	0.00	21.59	1.21	22.24	24.47	25.39	0.00	24.24	2.07	0.58	0.58	0.26	0.00	1.83	2.43
C18	30.26	37.93	3.26	17.18	0.58	20.45	1.54	20.61	22.42	25.46	0.57	24.70	2.80	0.76	1.36	0.54	0.00	2.42	1.32
C19	33.79	39.99	3.84	16.35	0.54	20.29	1.26	20.16	26.29	26.09	0.54	22.46	2.04	0.74	0.76	0.96	0.00	1.00	1.56
C20	31.47	37.67	2.55	17.45	0.77	20.14	1.03	20.38	21.05	24.88	0.00	23.50	2.38	0.59	0.72	1.41	0.00	0.55	1.05
CB 1	34.14	39.29	8.64	18.10	1.57	24.37	4.99	23.45	23.20	27.64	0.66	22.18	0.53	0.00	0.13	0.80	0.00	3.84	0.77
CB 2	30.75	38.36	7.84	16.26	2.00	20.59	2.47	21.55	23.82	28.66	0.00	22.95	1.11	0.24	0.72	0.25	0.00	1.51	0.81

CB 3	27.14	35.37	19.29	12.88	2.00	20.21	4.08	20.33	22.79	25.77	0.72	24.03	1.33	0.00	0.13	0.27	0.00	5.19	0.77
CB 4	30.86	35.96	13.34	15.04	2.29	20.14	5.41	19.03	22.33	27.22	0.97	23.45	1.32	0.00	0.23	0.43	0.00	4.72	1.03
CB 5	29.46	37.32	8.75	15.07	1.42	18.51	4.57	20.42	21.73	25.96	0.00	22.83	1.18	0.00	0.00	0.55	0.00	2.26	0.79
CB 6	30.96	37.81	6.21	14.20	1.42	19.18	1.57	18.99	21.15	24.68	0.00	22.93	0.53	0.00	0.19	0.00	0.00	1.95	1.50
CB 7	31.78	38.43	9.45	13.59	1.15	19.93	3.61	19.69	21.71	26.78	1.32	21.74	0.54	0.00	0.00	0.47	0.00	2.95	0.77
CB 8	31.27	37.42	8.92	13.65	1.07	19.58	2.34	18.80	23.22	27.23	0.51	24.50	2.10	0.49	0.86	0.53	0.00	2.32	1.03
CB 9	31.61	38.08	9.51	14.96	1.73	20.59	1.41	19.99	23.46	25.67	0.00	23.89	0.78	0.00	1.09	0.28	0.00	3.00	0.77
CB 10	31.60	37.92	11.78	16.00	1.38	18.77	3.24	20.19	21.73	26.91	0.57	22.54	1.13	0.00	0.00	0.13	0.00	3.16	0.77
CB 11	31.44	38.38	12.53	15.79	1.56	19.88	3.89	21.01	22.08	27.61	0.54	21.76	0.79	0.14	0.59	0.19	0.00	2.68	0.77
CB 12	32.38	37.43	8.64	20.53	0.26	23.31	2.49	23.89	24.17	28.51	0.00	23.09	1.54	0.20	0.88	1.93	0.00	2.50	1.78
CB 13	30.92	37.47	12.66	14.25	2.15	19.51	2.53	18.13	20.29	25.82	0.00	21.92	0.26	0.54	0.79	1.01	0.00	3.86	0.77
CB 14	31.76	38.62	14.24	19.33	1.54	23.38	4.06	24.08	26.27	29.33	1.30	23.00	1.26	0.00	0.00	0.79	0.00	4.31	1.05
CB 15	27.86	33.82	21.87	13.92	2.46	20.42	5.19	19.54	19.03	24.67	0.86	26.71	1.70	1.05	0.00	0.00	0.00	6.84	3.04
CB 16	34.72	41.26	4.27	20.41	1.78	23.50	3.16	23.81	24.70	29.72	0.58	21.15	0.77	0.00	0.24	1.19	0.00	1.05	0.77
CB 17	31.17	37.74	12.09	19.09	1.70	22.74	4.97	22.62	24.43	28.21	1.35	23.17	0.54	0.00	0.00	0.13	0.00	3.57	0.79
CB 18	30.10	36.76	12.68	16.72	1.36	20.60	5.00	21.74	22.00	29.26	0.00	21.00	0.27	0.00	0.00	0.26	0.00	4.79	1.28
CB 19	34.59	40.39	3.62	18.05	2.76	21.35	3.04	22.04	25.86	27.54	0.57	19.49	0.27	0.19	0.00	0.51	0.00	1.21	0.82
CB 20	30.03	37.08	13.36	15.42	1.73	20.75	2.66	21.44	22.18	26.65	0.92	22.36	1.22	0.00	0.00	0.26	0.00	3.89	0.79

Table B4.11 Taste and mouthfeel attributes of the control rooibos infusions and 50/50 ratio combinations. The letters “C” and “CB” before the sample number refer to the control infusions and combination samples, respectively.

TASTE AND MOUTHFEEL ATTRIBUTES					
Sample	Sweet	Astringent	Sour	Bitter	
C1	21.27	28.92	1.82	3.49	
C2	22.31	27.75	2.72	5.04	
C3	21.58	28.59	2.95	3.09	
C4	21.97	28.34	1.73	4.22	
C5	22.18	28.64	2.32	3.28	
C6	22.74	27.97	1.33	3.10	
C7	22.50	28.95	1.87	3.84	
C8	22.93	28.05	2.12	3.14	
C9	22.81	28.46	2.58	2.57	
C10	21.92	28.60	0.94	4.06	
C11	22.86	28.46	1.63	3.45	
C12	22.86	27.42	0.66	2.70	
C13	21.38	29.18	1.54	3.99	
C14	21.20	28.54	0.75	4.39	
C15	22.11	28.63	1.96	2.82	
C16	22.62	28.18	0.89	3.04	
C17	22.62	28.75	1.61	4.00	
C18	21.87	28.09	2.06	2.84	
C19	22.67	26.97	0.92	3.58	
C20	22.50	28.10	1.87	3.41	
CB 1	23.00	27.97	1.87	2.59	
CB 2	22.84	27.93	2.93	4.01	
CB 3	21.83	28.04	3.15	2.41	
CB 4	22.88	28.24	2.72	1.96	
CB 5	23.12	27.07	1.86	2.22	
CB 6	22.54	28.18	1.86	2.83	
CB 7	23.35	27.18	1.70	1.81	
CB 8	24.01	27.93	2.03	1.55	
CB 9	23.20	27.81	1.08	2.38	
CB 10	22.74	28.21	2.41	2.22	
CB 11	23.32	27.79	2.76	1.37	
CB 12	20.07	30.60	2.78	6.84	
CB 13	23.01	27.83	2.45	1.18	
CB 14	22.68	28.10	2.15	1.44	
CB 15	21.38	28.12	2.14	4.01	
CB 16	23.60	27.51	1.85	2.82	
CB 17	22.54	27.29	2.12	2.68	

CB 18	22.78	27.25	3.19	2.36
CB 19	23.32	28.61	2.42	2.50
CB 20	22.73	27.71	2.17	2.38

Table B4.12 Flavour attributes of the control rooibos infusions and 50/50 ratio combinations. The letters “C” and “CB” before the sample number refer to the control infusions and combination samples, respectively.

FLAVOUR ATTRIBUTES																		
	Fynbos-floral	Rooibos-wood	Planky/Pencil shaving	Apricot	Raisin	Almond	Fruit-sweet	Honey	Caramel/Vanilla	Sweet spice	Hay/Dried grass	Green grass	Rotting plant water	Seaweed	Burnt caramel	Medicinal/Rubber	Dusty	Musty/Mouldy
C1	28.13	34.91	9.65	0.00	0.00	0.50	0.00	0.00	0.00	0.27	22.53	0.88	0.00	0.00	0.24	0.00	3.67	0.00
C2	29.16	37.01	6.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.13	0.84	0.13	0.00	0.00	0.00	1.76	0.00
C3	25.89	35.38	14.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	24.04	1.49	0.00	0.00	0.00	0.00	2.74	0.00
C4	27.18	33.75	12.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	23.96	0.88	0.00	0.00	0.21	0.00	3.46	0.00
C5	26.18	33.97	6.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	24.06	0.53	0.00	0.00	0.50	0.00	2.32	0.00
C6	28.85	35.14	5.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	23.17	0.76	0.00	0.00	0.00	0.00	1.82	0.00
C7	29.01	36.03	7.87	0.00	0.00	0.00	0.00	0.00	0.51	0.00	21.32	0.53	0.09	0.00	0.00	0.00	2.28	0.00
C8	26.24	35.18	7.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	24.83	1.53	0.00	0.00	0.00	0.00	2.35	0.00
C9	25.70	36.13	9.73	0.00	0.00	0.74	0.00	0.00	0.47	0.00	24.36	0.79	0.00	0.00	0.00	0.00	2.59	0.00
C10	26.95	35.76	13.32	0.00	0.00	0.51	0.00	0.00	0.55	0.00	24.53	0.97	0.00	0.00	0.00	0.00	3.49	0.00
C11	27.34	35.16	12.16	0.00	0.00	0.76	0.00	0.00	0.71	0.00	20.55	0.27	0.41	0.59	0.53	0.00	1.99	0.00
C12	27.11	36.83	9.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.79	3.16	0.67	0.00	1.34	0.57	3.05	0.63
C13	27.03	34.09	10.27	0.00	0.51	0.76	0.00	0.00	0.79	0.00	22.22	0.96	0.37	0.00	0.49	0.00	2.84	0.00
C14	26.03	34.25	12.91	0.00	0.00	0.00	0.00	0.00	0.00	0.00	24.59	1.18	0.28	0.00	0.00	0.00	4.07	0.49
C15	23.50	33.28	18.78	0.00	0.00	0.00	0.00	0.00	0.00	0.00	23.36	1.28	0.95	0.00	0.16	0.00	3.88	0.47
C16	29.63	38.11	6.39	0.00	0.00	0.00	0.00	0.00	0.80	0.00	23.70	0.38	0.00	0.00	0.17	0.00	2.08	0.00
C17	26.15	34.93	13.84	0.00	0.00	0.00	0.00	0.00	0.00	0.00	21.80	0.51	0.00	0.00	0.00	0.00	3.43	0.00
C18	25.63	32.92	12.03	0.00	0.00	0.00	0.00	0.00	0.51	0.00	22.49	0.26	0.00	0.00	0.66	0.00	3.42	0.00
C19	28.07	38.14	3.92	0.00	0.00	0.00	0.00	0.00	0.82	0.00	23.51	0.67	0.00	0.00	0.00	0.00	1.30	0.00
C20	26.09	33.80	13.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	22.19	0.26	0.00	0.00	0.23	0.00	4.59	0.49
CB I	27.01	36.07	6.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	26.36	2.74	0.00	0.00	0.00	0.00	2.24	0.00

CB 2	27.64	37.74	2.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	24.36	1.73	0.13	0.46	1.49	0.00	0.51	0.00
CB 3	26.96	36.26	1.81	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	26.03	4.11	0.00	0.00	0.56	0.00	1.12	0.72
CB 4	27.94	38.49	3.73	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	24.12	1.54	0.00	0.01	0.16	0.00	1.33	0.00
CB 5	28.91	36.86	4.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.09	2.58	0.00	0.51	1.42	0.00	0.74	0.00
CB 6	26.62	35.69	3.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.18	2.91	0.54	0.53	1.51	0.00	0.81	0.68
CB 7	26.51	35.78	2.32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	26.63	2.58	0.15	0.13	0.54	0.00	0.62	0.00
CB 8	27.34	37.96	2.72	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.49	3.75	0.50	1.57	0.56	0.00	1.34	0.00
CB 9	27.78	38.41	4.62	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	24.99	2.93	0.46	0.00	1.84	0.00	0.82	0.00
CB 10	25.49	36.96	3.68	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	26.95	2.49	0.00	0.00	0.79	0.00	1.55	0.00
CB 11	28.01	37.31	3.47	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.63	3.36	0.89	0.63	1.20	0.00	1.38	0.00
CB 12	29.50	37.24	2.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	24.42	1.21	0.55	0.00	0.00	0.00	0.79	0.00
CB 13	25.96	36.47	2.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	26.50	3.21	0.33	1.14	1.59	0.00	1.31	0.00
CB 14	30.00	40.41	5.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.74	1.41	0.00	0.00	0.61	0.00	0.97	0.53
CB 15	24.90	34.88	2.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	24.87	3.08	0.70	0.68	0.51	0.00	1.27	0.00
CB 16	27.10	37.51	1.86	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	24.32	3.00	0.00	0.63	0.14	0.00	0.59	0.00

Table B4.13 Aroma attributes of the control rooibos infusions and 75/25 ratio combinations. The letters “D”, “S” and “CB” before the sample number refer to the diluted dust extract, stem infusion and combination samples, respectively.

Sample	AROMA ATTRIBUTES																		
	Fynbos-floral	Rooibos-wood y	Plank y/Pencil shavings	Apricot	Apple	Raisin	Almond	Fruity-sweet	Honey	Caramel/Vanilla	Sweet spice	Hay/Dr ied grass	Green grass	Rotting plant water	Seaweed	Burnt caramel	Medicinal/Rubber	Dusty	Musty/Mouldy
D10	30.84	34.13	4.10	3.49	0.00	15.24	0.94	17.46	22.30	25.99	0.00	14.34	0.00	0.00	0.00	0.99	0.00	2.88	1.16
D11	34.61	38.73	3.18	5.99	0.00	18.06	0.31	18.53	22.74	26.21	0.00	14.91	0.00	0.00	0.67	4.25	0.00	3.50	1.49
D19	33.06	37.58	2.47	5.42	0.00	17.42	0.59	17.12	21.86	24.97	0.00	15.48	0.00	0.00	0.00	2.76	0.00	2.22	1.27
D5	32.52	37.19	2.95	7.03	0.00	16.78	0.00	21.06	24.67	25.15	0.00	18.64	0.68	0.00	0.00	4.10	0.00	3.67	2.34
D8	28.58	34.21	8.18	8.05	0.00	18.33	0.81	17.48	20.09	24.90	0.00	17.77	0.00	0.00	0.00	2.09	0.00	6.00	0.59
D9	32.40	39.25	4.32	6.70	0.00	17.47	0.00	18.91	23.68	24.21	0.00	16.55	0.59	0.00	0.00	3.45	0.00	2.29	2.03
S6	31.17	38.06	4.70	6.11	0.00	19.36	1.24	19.37	24.73	24.72	0.00	17.06	0.00	0.00	0.00	0.97	0.00	2.73	1.00
S10	33.58	37.88	6.16	7.16	0.00	17.76	0.02	17.84	24.92	24.12	0.00	18.84	0.00	0.00	0.00	2.47	0.00	4.11	1.68
S13	27.56	34.83	16.45	4.09	0.00	15.28	2.20	17.85	20.82	22.50	0.00	18.50	0.00	0.00	0.00	1.23	0.00	6.42	1.38
S14	34.65	38.97	3.47	5.07	0.00	19.63	1.00	20.08	24.06	25.73	0.00	16.04	0.00	0.00	0.00	1.23	0.00	3.81	1.31
S18	28.79	36.17	20.84	2.63	0.00	17.94	2.71	17.65	20.22	23.44	0.00	17.07	0.00	0.00	0.00	2.04	0.00	8.64	1.86
S27	24.97	31.39	15.77	3.38	0.00	12.56	1.48	15.32	15.97	18.36	0.00	20.45	0.00	0.00	0.00	0.97	0.00	7.32	0.91
CB10 (50/50)	35.36	39.25	4.12	4.53	0.00	20.53	0.97	20.67	23.74	24.04	0.00	15.89	0.00	0.00	0.00	2.37	0.00	3.04	0.74
CB11 (50/50)	34.06	36.94	13.71	5.93	0.00	18.94	2.10	19.46	23.44	25.80	0.00	19.47	0.00	0.00	0.00	2.00	0.00	7.69	3.47
CB19 (50/50)	29.25	34.92	9.74	4.67	0.00	17.39	0.69	16.08	19.55	21.05	0.00	18.92	0.00	0.00	0.00	1.53	0.00	4.20	2.73
CB 5 (50/50)	32.41	37.50	6.26	4.24	0.00	18.23	1.66	18.35	21.64	21.75	0.00	17.42	0.66	0.00	1.25	1.69	0.00	2.75	0.71
CB 8 (50/50)	31.02	36.65	6.84	7.21	0.00	18.94	0.00	17.92	21.36	24.05	0.00	17.33	0.00	0.00	0.00	2.92	0.00	3.95	2.05
CB 9 (50/50)	30.72	37.48	10.77	5.89	0.00	18.23	0.00	19.86	21.80	24.28	0.00	19.42	0.00	0.00	0.00	1.84	0.00	4.77	0.79
CB10 (75/25)	33.22	37.99	5.26	2.94	0.00	18.31	0.54	19.39	23.07	23.29	0.00	17.47	0.00	0.00	0.00	2.06	0.00	4.12	1.13
CB11 (75/25)	32.93	37.47	7.59	5.23	0.00	20.10	0.81	20.43	23.17	24.81	0.00	16.67	0.00	0.00	0.00	2.87	0.00	4.51	0.91

CB19 (75/25)	30.97	36.47	5.41	5.27	0.00	16.73	0.00	16.85	20.53	24.59	0.00	17.23	0.00	0.00	0.00	2.20	0.00	4.20	1.73
CB 5 (75/25)	32.44	37.31	5.62	6.09	0.00	18.23	0.67	20.14	21.82	26.45	0.00	15.89	0.00	0.00	0.00	1.75	0.00	3.98	1.94
CB 8 (75/25)	29.85	35.44	5.89	5.53	0.00	17.81	0.00	18.58	20.69	23.71	0.00	16.68	0.00	0.00	0.00	2.25	0.00	3.13	1.16
CB 9 (75/25)	32.38	38.05	7.81	4.23	0.00	17.70	0.00	16.95	22.00	24.70	0.00	18.33	0.00	0.00	0.00	2.06	0.00	3.62	2.98

Table B4.14 Taste and mouthfeel attributes of the control rooibos infusions and 75/25 ratio combinations. The letters “D”, “S” and “CB” before the sample number refer to the diluted dust extract, stem infusion and combination infusions, respectively.

TASTE AND MOUTHFEEL ATTRIBUTES				
Sample	Sweet	Astringent	Sour	Bitter
D10	21.11	29.61	1.70	3.50
D11	20.36	29.43	1.57	5.72
D19	20.18	29.87	1.65	4.83
D5	20.61	30.36	2.82	4.09
D8	19.77	28.86	3.03	5.38
D9	20.70	28.92	3.14	5.39
S6	19.39	29.59	3.98	3.16
S10	20.16	29.50	3.61	3.17
S13	19.21	30.08	4.25	3.28
S14	20.60	30.99	2.96	4.29
S18	19.67	30.47	4.46	2.21
S27	18.83	30.52	4.55	2.82
CB10 (50/50)	20.87	29.39	2.00	3.50
CB11 (50/50)	19.43	30.67	4.68	4.49
CB19 (50/50)	19.13	29.84	2.34	3.94
CB 5 (50/50)	20.55	29.65	3.48	3.59
CB 8 (50/50)	18.59	32.05	5.53	5.61
CB 9 (50/50)	19.15	30.74	3.20	4.98
CB10 (75/25)	20.39	30.56	3.18	3.41
CB11 (75/25)	19.47	30.40	4.11	3.90
CB19 (75/25)	19.38	30.70	3.14	3.68
CB 5 (75/25)	21.02	28.86	1.65	2.86
CB 8 (75/25)	19.42	30.34	3.30	4.59

(75/25)
CB 9
(75/25)

18.89

31.32

2.82

5.52

Table B4.15 Flavour attributes of the control rooibos infusions and 75/25 ratio combinations. The letters “D”, “S” and “CB” before the sample number refer to the diluted dust extract, stem infusion and combination samples, respectively.

AROMA ATTRIBUTES																			
Sample	Fynbos -floral	Rooib os- wood y	Plank y/Pen cil shavi ngs	Apric ot	App le	Raisi n	Almo nd	Fruit y- sweet	Honey	Caram el/ Vanilla	Sweet spice	Hay/Dr ied grass	Gree n grass	Rotting plant water	Seaweed	Burnt cara mel	Medicina l/Rubber	Dusty	Musty/ Mouldy
D10	28.29	35.28	3.89	0.00	0.00	0.00	0.94	0.00	0.00	0.00	0.00	14.47	0.00	0.00	0.00	0.73	0.00	3.14	0.00
D11	28.97	36.08	3.29	0.00	0.00	0.00	0.31	0.00	0.00	0.00	0.00	17.03	0.00	0.00	0.23	2.46	0.00	4.34	0.29
D19	29.80	36.16	2.44	0.00	0.00	0.00	0.59	0.00	0.00	0.00	0.00	17.55	0.64	0.00	0.00	2.22	0.00	2.86	1.08
D5	30.42	37.36	3.78	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20.95	0.62	0.00	0.00	3.23	0.00	4.52	2.11
D8	28.23	35.64	4.15	0.00	0.00	0.00	0.81	0.00	0.00	0.00	0.00	18.42	0.00	0.00	0.00	1.33	0.00	2.25	0.97
D9	30.20	36.77	3.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17.69	0.61	0.00	0.00	2.02	0.00	1.55	0.26
S6	28.64	37.71	6.55	0.00	0.00	0.00	1.24	0.00	0.00	0.00	0.00	19.91	0.00	0.00	0.00	0.77	0.00	2.74	0.00
S10	27.74	37.23	7.77	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	18.36	0.00	0.00	0.00	1.78	0.00	2.45	0.00
S13	23.66	33.70	13.11	0.00	0.00	0.62	2.20	0.00	0.00	0.00	0.00	19.45	0.00	0.00	0.00	0.29	0.00	3.89	1.27
S14	27.59	37.43	7.31	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	18.74	0.00	0.00	0.00	0.00	0.00	3.24	0.00
S18	24.34	31.29	17.37	0.00	0.00	0.00	2.71	0.00	0.00	0.00	0.00	20.60	0.00	0.00	0.00	0.00	0.00	6.80	0.54
S27	24.30	32.30	12.14	0.00	0.00	0.00	1.48	0.00	0.00	0.00	0.00	19.75	0.00	0.00	0.00	0.63	0.00	3.39	0.67
CB10 (50/50)	29.50	38.29	4.47	0.00	0.00	0.00	0.97	0.00	0.00	0.00	0.00	17.96	0.00	0.00	0.00	0.75	0.00	2.90	0.00
CB11 (50/50)	26.42	35.73	12.72	0.00	0.00	0.00	2.10	0.00	0.00	0.00	0.00	19.35	0.00	0.00	0.00	0.93	0.00	6.41	2.35
CB19 (50/50)	27.26	35.29	7.48	0.00	0.00	0.00	0.69	0.00	0.00	0.00	0.00	18.12	0.70	0.00	0.00	0.00	0.00	3.94	0.00
CB 5	27.67	36.41	7.11	0.00	0.00	0.00	1.66	0.00	0.00	0.00	0.00	20.05	0.00	0.00	0.00	0.48	0.00	3.56	0.26

(50/50)																				
CB 8																				
(50/50)	25.53	36.45	7.92	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	19.06	0.59	0.00	0.89	1.78	0.00	3.08	0.67
CB 9																				
(50/50)	26.06	37.03	9.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20.73	0.00	0.00	0.00	0.66	0.00	3.58	0.88
CB10																				
(75/25)	27.65	37.76	7.99	0.00	0.00	0.00	0.54	0.00	0.00	0.00	0.00	0.00	18.76	0.00	0.00	0.00	0.00	0.00	4.94	0.32
CB11																				
(75/25)	25.92	35.49	10.31	0.00	0.00	0.00	0.81	0.00	0.00	0.00	0.00	0.00	18.88	0.00	0.00	0.00	0.57	0.00	5.10	0.00
CB19																				
(75/25)	26.64	35.11	4.44	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	18.56	0.00	0.00	0.00	1.95	0.00	3.19	0.88
CB 5																				
(75/25)	27.70	35.66	5.55	0.00	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00	18.84	0.00	0.00	0.00	0.70	0.00	3.70	0.00
CB 8																				
(75/25)	26.53	34.94	7.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	19.60	0.00	0.00	0.00	1.06	0.00	2.12	0.00
CB 9																				
(75/25)	26.41	36.94	6.91	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	18.52	0.00	0.00	0.00	0.95	0.00	3.69	0.00

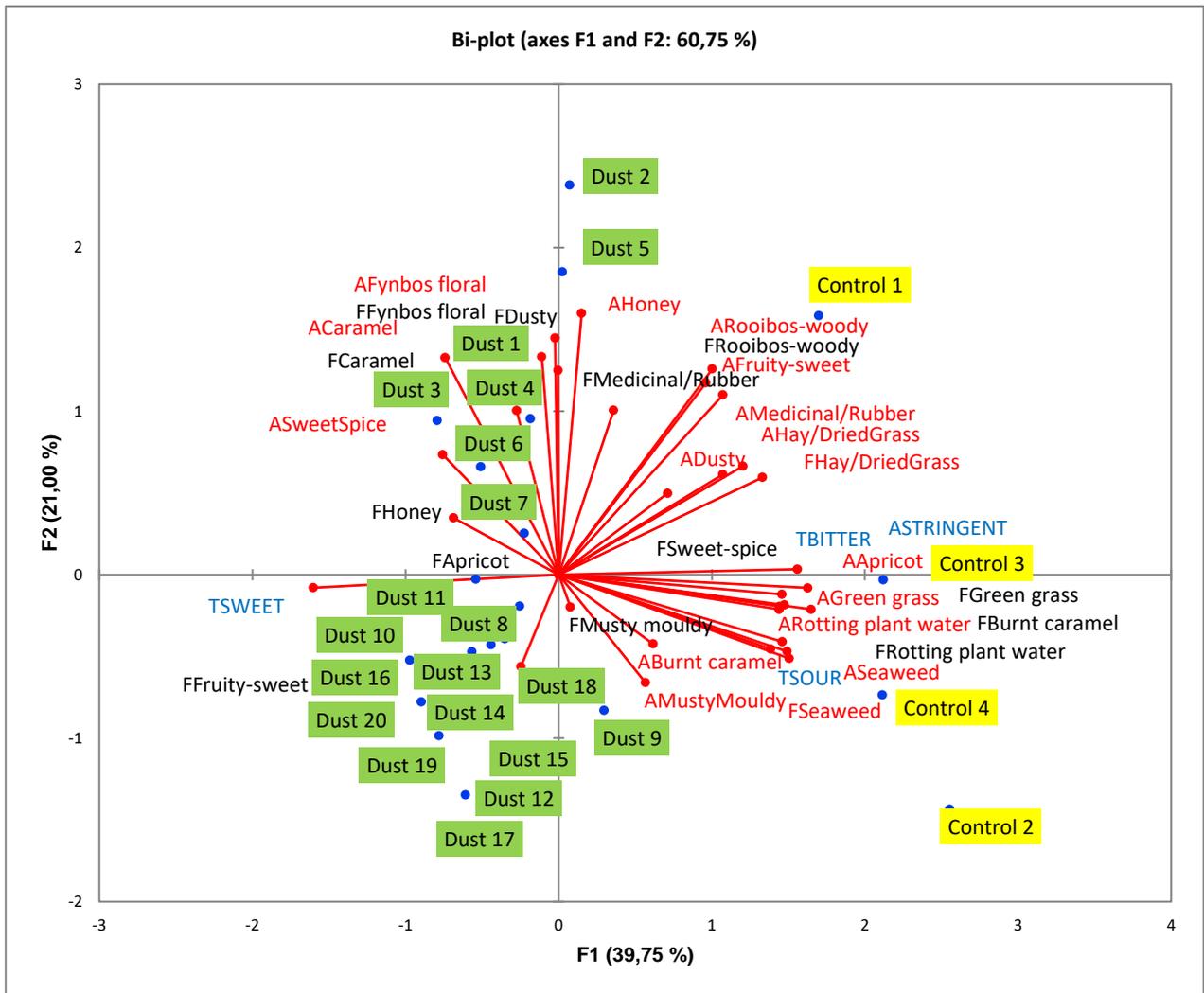


Figure B4.1 Principal component analysis (PCA) bi-plot illustrating flavor, aroma, taste and mouthfeel attributes of the diluted rooibos dust extracts and control infusions. Except for astringency, the letters “A”, “F” and “T” in front of the attribute name refer to aroma, flavour and taste attributes, respectively.

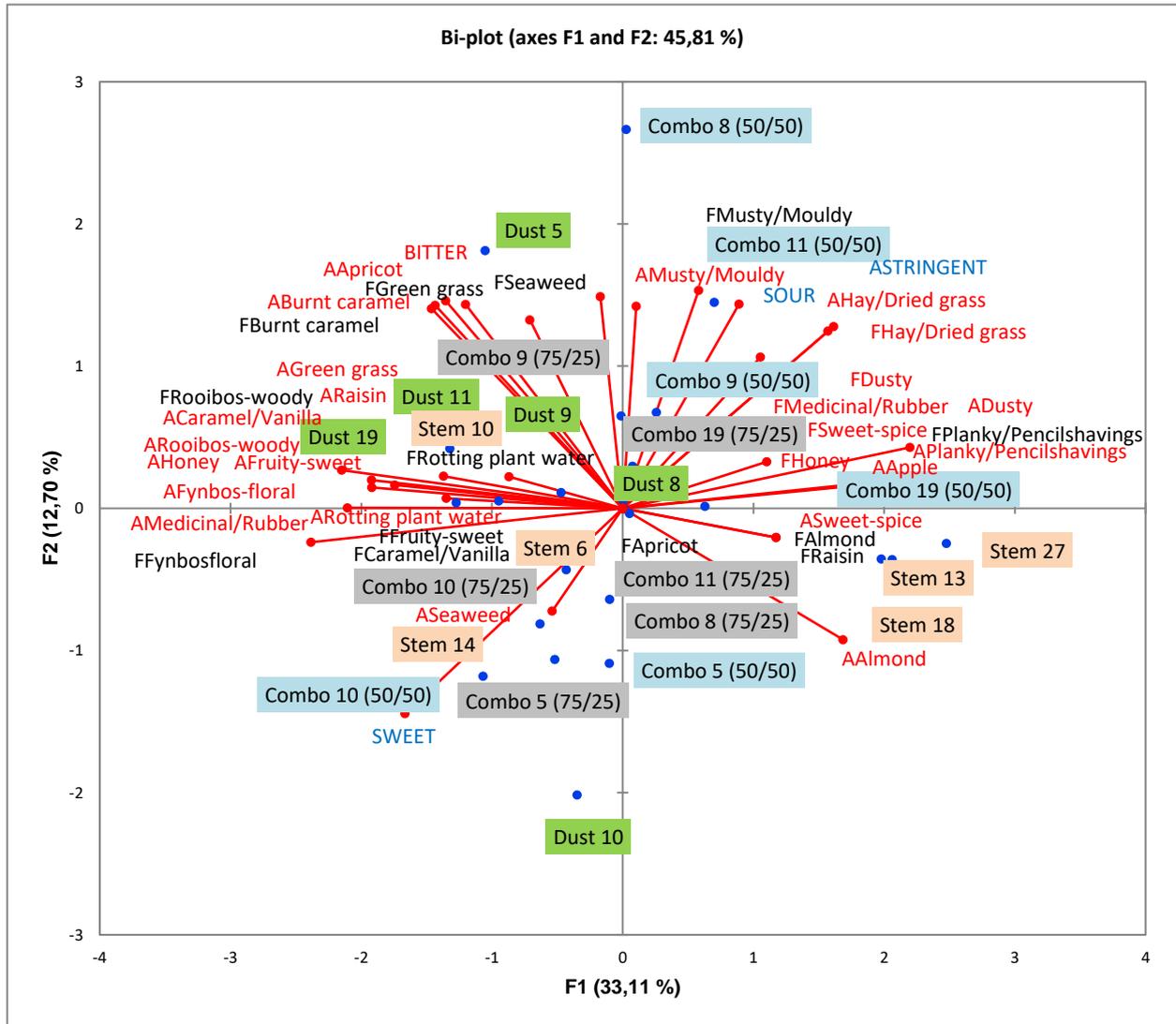


Figure B4.4 Principal component analysis (PCA) bi-plot illustrating flavor, aroma, taste and mouthfeel attributes of the control rooibos infusions and 75/25 ratio combinations. Except for astringency, the letters “A”, “F” and “T” in front of the attribute name refer to aroma, flavour and taste attributes, respectively. The word “Combo” before each sample number refers to the dust extract and stem combination infusions.