Cape Peninsula University of Technology

EFFECT OF ROOIBOS PREPARATION ON THE TOTAL POLYPHENOL CONTENT AND ANTIOXIDANT CAPACITY OF HERBAL TEA AND ITS CONSUMER CHARACTERISTICS

by

HANNELISE PIEK

Thesis submitted in fulfilment of the requirements for the degree Master of Technology: Consumer Science: Food and Nutrition in the Faculty of Applied Sciences at the Cape Peninsula University of Technology

Supervisor: A Prof I Venter

Co-supervisor: Prof JL Marnewick

Cape Town
August 2016

DECLARATION

I, **Hannelise Piek**, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

H. Piek

Signed

August 2016

Date

ACKNOWLEDGEMENTS

I wish to thank:

- My supervisor, Associate Professor Irma Venter, for her guidance and all her patience and encouragement throughout the process;
- My co-supervisor, Professor Jeanine Marnewick, for her guidance and assistance throughout the process;
- Mr Fanie Rautenbach for his assistance throughout the process, as well as the assays and statistical analysis undertaken for Phase 1 of the study;
- Professor Dirk van Schalkwyk and Dr Corrie Uys for the statistical analysis undertaken for Phase 2 of the study;
- Ms Natasha Danster-Christians, 2014 - 2015 research assistant of the programme Consumer Science: Food and Nutrition, for her assistance with some aspects of the research work;
- The Cape Peninsula University of Technology (CPUT), for the University Research Funding (URF) received towards this research. Opinions expressed in this thesis and the conclusions arrived at, are those of the author, and are not necessarily to be attributed to the CPUT;
- The respondents, for taking the time to complete the questionnaire;
- My family, for their continuous support; and
- My husband, Francois Piek, for his patience and invaluable support and for making it possible for me to further my academic study.

ABSTRACT

Background: The different types and forms of rooibos and the ways in which it is prepared and flavoured for consumption influences its total polyphenol content and total antioxidant capacity (TAC) and hence depends on its consumer practices.

Design: Phase 1 of the study entailed the selection and preparation of different rooibos types and forms; rooibos brewed for different times; and with different household and commercially added flavourings to determine the total polyphenol content, TAC, flavonol and flavanol content; and subsequent identification of the optimal cup of rooibos based on the first two biochemical parameters. For Phase 2 a questionnaire was used to obtain information on the profile of the adult rooibos herbal tea consumer, as well as of those consuming the optimal cup of rooibos.

Results: The following prepared rooibos samples delivered the higher biochemical parameter content: green / unfermented (type representative); green / unfermented leaves and powdered extract (form representatives); that brewed for 10 minutes or longer; and those with added honey. The optimal cup of rooibos was identified as the one brewed for 10 minutes or longer. The older respondents and those with a lower level of education consumed a higher daily amount of rooibos ($p < 0.05$) and those who brewed rooibos in a teapot consumed the optimal cup ($p < 0.05$). However, very few respondents consumed the advised number of cups per day (< 1%) and the identified optimal cup (15.9%).

Conclusions: Rooibos consumers in this study did not consume it in sufficient amounts and did not brew it for long enough to fully gain from its attributed health benefits.

Keywords: *rooibos herbal tea, rooibos types, rooibos forms, rooibos brewing time, rooibos flavourings, rooibos preparation, rooibos consumption, optimal rooibos cup, total polyphenol content, total antioxidant capacity*

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

A

AAPH	2,2'-azobis (2-amidinopropane) dihydrochloride
ABTS	2,2'-azinobis-3-ethylbenzthiazoline-6-silphonic acid
ACE	Angiotensin-converting enzyme
A.linearis	*Aspalathus linearis*
ANOVA	One-way analysis of variance

B

B-PE	B-phycoerythrin
BMI	Body mass index

C

CAD	Coronary artery disease
CAT	Catalase
CE	Catechin equivalents
CPUT	Cape Peninsula University of Technology
C. sinensis	*Camellia sinensis*
CVD	Cardiovascular disease

D

DEN	Diethylnitrosamine
DMACA	Dimethylaminocinnamaldehyde
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenylpicrylhydrazyl

E

EC	Epicatechin
ECG	Epicatechin-3-gallate
EGC	Epigallocatechin
EGCG	Epigallocatechin-3-gallate
EtOH	Ethanol

F

FB1	Fumonisin B1
FBDGs	Food-based dietary guidelines
FC	Folin-Ciocalteu
FDA	Food and Drug Administration
FL	Fluorescein
FR	Fermented rooibos
FRAP	Ferric reducing antioxidant power

G

g	Gram
GA	Gallic acid
GSH	Glutathione

H

HCl	Hydrochloric acid
HDL	High density lipoprotein

L

L	Litre
LDL	Low density lipoprotein

M

MBN	Methylbenzylnitrosamine
µL	Microlitre
mg	Milligram
mL	Millilitre
mmol / µmol	Millimole
MRC	Medical Research Council

N

n	Number
NADPH	Nicotinamide-adenine dinucleotide phosphate
ND	Not detected
NHANES	National Health and Nutrition Examination Survey
nm	Nanometer
NO	Nitric oxide

O

ORAC	Oxygen radical absorbance capacity
OSRC	Oxidative Stress Research Centre

P

%	Percentage

Q

QE	Quercetin equivalents

R

RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RTD	Ready-to-drink

S

SA	South Africa
SOD	Superoxide dismutase

T

TAC	Total antioxidant capacity
TEAC	Trolox equivalent antioxidant capacity
TRAP	Chain breaking antioxidant activity

U

UK	United Kingdom
UR	Unfermented rooibos
US	United States of America
USDA	United States Department of Agriculture
UV	Ultraviolet

V

vs	Versus

CLARIFICATION OF BASIC TERMS AND CONCEPTS

Antioxidants – Substances that are capable of slowing or preventing oxidation of other molecules (Flora, 2009:191) and in so doing protect the body cells and structures against the damaging effect of free radicals (Young & Woodside, 2001:176; Sharma *et al*., 2008:124).

Fermented rooibos – The production of this type of rooibos involves a fermentation process where the polyphenols present are oxidised and thereby contribute to the characteristic flavour and red-brown colour of traditional rooibos herbal tea (Joubert & Schulz, 2006:139).

Flavonoids – The flavonoid structure consists of a heterocyclic pyran or pyrone ring interlinked by two phenolic benzene rings with an attached oxygen atom. As a result of this specific structure, flavonoids form part of polyphenols (Sampson *et al*., 2002:1414) and constitute a subclass of the polyphenols (Beecher, 2003:3248S).

Optimal rooibos cup – For the purpose of this study it denotes a traditional / fermented cup of rooibos in tea bag form brewed for 10 minutes or longer to provide for the higher total polyphenol content and total antioxidant capacity (TAC).

Oxidative stress – This biological condition arises when antioxidants are not adequately provided to the body, or are ineffective due to an excess of free radicals present. When this happens enzymes manufactured by the body help to reduce the damage caused by free radicals. Increased amounts of free radicals that overwhelm the antioxidant defence system of the body are prone to cause damage to the cells (Halliwell, 1994:721; Sies, 1997:291; Flora, 2009:191).

Phenolic – An organic compound containing an unsaturated ring of carbon atoms with an attached hydroxyl group that is easily oxidised (Bennion & Scheule, 2004:487).

Polyphenol – The same chemical structure as phenolics, but more than one –OH group is attached to the unsaturated ring of carbon atoms. Some polyphenols may cause bitterness in food sources such as tea and coffee (Bennion & Scheule, 2004:781).

Reactive oxygen species (ROS) – A damaging by-product produced by the body; for instance, during the reaction leading to energy production through mitochondrial and microsomal electron-transport chains (Serafini, 2006:533). Although ROS are by-products of the life supporting aerobic metabolism they are, when in excess, the main cause of cellular damage and ageing (Yu, 1996:651; Radak *et al*., 2008:153; Baba *et al*., 2009:702).

Rooibos herbal tea – Rooibos (*Aspalathus (A.) linearis*) is an indigenous plant of South Africa (SA) (Joubert & de Beer, 2011:869) that produces a popular beverage, especially among health-conscious consumers. Rooibos has its own unique polyphenolic content (Joubert & de Beer, 2011:875).

Total antioxidant capacity – The concept originated from chemistry and has been applied to medicine and nutrition that examines the strategies of antioxidant defence, development of assay systems and comparison of applying *in vitro* dietary composition data to *in vivo* plasma and tissue status (Sies, 2007:1493).

Unfermented rooibos - The oxidative changes to rooibos during its processing are minimised by controlling the moisture content in order to retain the green colour (Joubert & Schulz, 2006:139). As a prepared beverage it has a mild 'green' taste similar to green tea, but without the astringency (Erickson, 2003:37).

CHAPTER 1
INTRODUCTION

1.1 Statement of the research problem

The different types and forms in which rooibos herbal tea is available and the ways in which rooibos herbal tea is prepared and flavoured for consumption will influence the total polyphenol content and the total antioxidant capacity (TAC) of this health beverage. Preparation methods and flavouring of rooibos herbal tea is done according to the specific taste of the consumer. Each consumer has their own preferred way in which they prepare and consume their rooibos herbal tea. The 'perfect cup' of rooibos herbal tea delivering the highest total polyphenol content and TAC might not necessarily be the most acceptable way for consumers to drink this beverage.

1.2 Background to the research problem

Aspalathus (A.) linearis (rooibos) (Brum.f) Dahlg. (Fabaceae), a shrub-like bush (Joubert *et al*., 2008:376), is indigenous to the Cederberg mountains of the Western Cape, South Africa (SA) (Erickson, 2003:34). The leaves and stems of the bush are used to manufacture rooibos herbal tea (Joubert *et al*., 2008:376). Both fermented (traditional) and unfermented (green) rooibos herbal teas are available on the market. Fermentation decreases the antioxidant capacity of rooibos (Marnewick *et al*., 2009:220) due to a 'chemical oxidation' of the flavonoid compounds, i.e. aspalathin and nothofagin, present (Joubert *et al*., 2004:133). Therefore higher antioxidant levels are evident in the unfermented / green rooibos than in the fermented / traditional rooibos (Joubert & Schulz, 2006:138).

The unique polyphenolic composition of rooibos herbal tea may be the key to its health properties (Joubert & Ferreira, 1996:79). Rooibos was marketed for the first time in 1904 as a brewed beverage. Since then rooibos has developed from a herbal tea to a potential phytopharmaceutical. The health potential and increased consumption worldwide of rooibos herbal tea along with it being sold in more than 37 countries (Joubert & de Beer, 2011:869) supports the need to determine the total polyphenol content and TAC of rooibos herbal tea beverage preparations, as regular rooibos consumption could make a notable contribution to the daily polyphenolic content and dietary TAC in addition to it providing several health benefits, such as having chemoprotective (Joubert *et al*., 2008:401; Marnewick *et al*., 2009:220) and

cardioprotective (Marnewick *et al*., 2011:46; Pantsi *et al*., 2011:1220; Persson *et al*., 2010:730) properties.

Different brewing temperatures and times influence the antioxidant capacity of rooibos prepared as a beverage for consumption (Von Gadow *et al*., 1997a:1370). It has additionally become popular to flavour rooibos herbal tea – both in the household and commercially. Consumers usually add a sweetener like sugar or honey, and / or milk to traditional fermented rooibos herbal tea. These are added to the brewed beverage in different amounts, according to taste. A slice of lemon is also sometimes added for a refreshing taste. Rooibos herbal tea is also commercially flavoured. Flavourings such as lemon, camomile and honey are added for variations in the flavour. Various fruit flavours, such as strawberry and apple, are also added to make rooibos herbal tea more appealing to children. Several green rooibos herbal teas are also available on the market. Even a number of flavoured iced teas containing rooibos extract are available.

It is important to investigate the influence of the different types, forms, preparation methods and flavouring additions to rooibos herbal tea as some studies have found that milk addition to black tea decreased the TAC (Ryan & Petit, 2010:14); while other studies (Van Het Hof *et al*., 1998:356; Sharma *et al*., 2008:124) found that milk addition does not decrease the TAC of black tea. Some consumers also add lemon to their tea serving which, according to Campanella *et al*. (2003:733) and Komes *et al*. (2010:175), increases the TAC. Limited research has been done on the preparation to obtain a ‘perfect’ cup of rooibos herbal tea to deliver a maximal polyphenolic content and dietary TAC.

It seems that beverage consumption in general makes a large contribution to the daily polyphenolic intake (Manach *et al*., 2004:747) and the dietary TAC (Louwrens *et al*., 2009:195). Rooibos herbal tea is an excellent source of the polyphenolic flavonoids (Baba *et al*., 2009:700), with aspalathin being the main polyphenol (Van Heerden *et al*., 2003:885). Consuming a cup of rooibos herbal tea brewed for optimal polyphenol extraction could make a notable contribution to the daily polyphenolic content and dietary TAC. An adequate dietary polyphenol (Manach *et al*., 2004:747) and TAC provision have been proposed as important dietary considerations as these dietary provisions support health, such as reducing the risk of coronary heart disease, several common types of cancer and other chronic diseases of lifestyle, through numerous antioxidant and other mechanisms (Dufresne & Farnworth, 2001:404; Serafini, 2006:533).

Populations of different countries prepare and consume their tea as a beverage in different ways. According to Sharma *et al*. (2008:124), in countries such as Canada, India, Ireland and the United Kingdom (UK) tea is consumed with the addition of a considerable amount of milk, whereas in Japan and China the beverage is mostly consumed without milk. No indications are provided in the publication as to the type of tea or milk consumed in each case, but it is assumed to be black tea for the former and green tea for the latter, as Tang *et al*. (2009:282) indicated that in Western societies most people drink black tea while almost all the tea drinkers in Japan and China drink green tea. The ways in which consumers usually consume and prepare their tea and other beverages also differ (De Godoy *et al*., 2013:802) according to factors such as their gender, race and age (Storey *et al*., 2006:1992).

1.3 Research questions

How do the different types, forms, preparation methods and flavourings influence the total polyphenol content and TAC of a cup of rooibos herbal tea?

Which prepared cup of traditional / fermented rooibos herbal tea provides the higher total polyphenol content and TAC?

How do adult consumers in the George area in the Western Cape usually prepare their rooibos herbal tea for consumption?

What percentage of these rooibos herbal tea consumers drink traditional / fermented rooibos herbal tea as a beverage prepared to provide the higher total polyphenol content and TAC?

Which of these rooibos herbal tea consumer demographic and lifestyle characteristics, and in addition to consumption and preparation characteristics, are associated with drinking rooibos herbal tea as a beverage, and as a beverage prepared to provide the higher total polyphenol content and TAC?

1.4 Objectives of the research

To determine the total polyphenol content and the TAC of rooibos herbal tea taking into consideration

- the different rooibos types to prepare it as a beverage;
- the different rooibos forms to prepare it as a beverage;

- the different preparation methods to consume it as a beverage; and
- the different flavouring additions and the amounts added to it as a beverage.

To determine how the different types, forms, preparation methods and flavourings influence the total polyphenol content and TAC of a cup of rooibos herbal tea.

To determine the ways in which adult consumers in the George area in the Western Cape usually drink rooibos herbal tea by considering the different types, forms, preparation methods and flavouring additions and amounts added to prepare it as a beverage.

To determine the demographic and lifestyle characteristics of these rooibos herbal tea consumers.

To determine the percentage of these rooibos herbal tea consumers and their demographic and lifestyle characteristics, and in addition their consumption and preparation characteristics, who consume traditional / fermented rooibos herbal tea as a beverage prepared to provide the higher total polyphenol content and TAC.

CHAPTER 2
LITERATURE REVIEW

Considering the beverages consumed around the world, the most popular beverage consumed among the functional beverages is tea (Pekal *et al*., 2011:681; Pan *et al*., 2013:12). Among the commonly consumed teas, black tea is consumed far more than green tea or oolong tea, across the world (Pan *et al*., 2013:12). In the past few years substantial progress has been made on determining the health benefits of tea consumption. This has contributed to an increased consumption of tea, and even coffee, as the intake of these beverages has been indicated as providing protective effects against diseases associated with oxidative stress, which is partly due to the antioxidant components they contain (Pellegrini *et al*., 2003:2812).

Amid all the dietary antioxidants, polyphenols are the most abundantly provided in our diets. A major source of dietary polyphenols is tea. For those regularly consuming tea, coffee or wine, these beverages will likely be their major dietary sources of polyphenols (Scalbert & Williamson, 2000:2076S). It has been established that the major provider of flavonoids (a common group of plant polyphenolics) from food and beverages in the United States (US) diet is tea (Song & Chun, 2008:1543S) and it is also conceivably the same in the South African diet (Venter *et al*., 2013:392). Studies have reported that the potential health benefits of tea may be increased by the method of preparation (Venditti *et al*., 2010:1597) as preparation methods can influence the flavonoid, and as a result, the polyphenol content present in the prepared beverage (Peterson *et al*., 2005:496).

This literature review briefly explores oxidative stress and dietary antioxidants, placing emphasis on flavonoids as important dietary antioxidants, and then considers the cultivation and production, consumption and suggested intake, uses, nutritional composition and health promoting effects of rooibos herbal tea and other teas and herbal teas, before it focuses on how the type, form, preparation method and added flavouring influence the total polyphenol content and antioxidant capacity of tea. The tea drinking behaviour of consumers is also addressed as this varies from person to person due to taste and individual preferences. The use of a questionnaire to assess the tea drinking behaviour of consumers is also briefly conversed. Assays have been developed to ascertain the total polyphenol content and TAC of a dietary sample. These different assays are briefly presented at the end of this chapter.

2.1 Oxidative stress

Molecules or atoms with one or more unpaired electron(s) are termed free radicals. These unpaired electrons enhance the chemical reactivity of the molecule. Environmental radiation and physiological processes that occur in the body cause free radicals to form (Radak *et al*., 2008:153). Free radical production as a result takes place constantly in all cells of the body as part of cell functioning (Young & Woodside, 2001:176). Endogenous sources of free radical formation include respiratory burst, auto-oxidation and enzyme reactions as well as mitochondrial leak. Environmental sources of free radicals are ultraviolet (UV) light, cigarette smoke, xenobiotics, pollutants and ionising radiation (Young & Woodside, 2001:176).

Free radicals include various reactive species such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Radak *et al*., 2008:153). Due to their chemical reactivity, free radicals react with other molecules in the body causing damage to cells or changes to important cellular components such as deoxyribonucleic acid (DNA). Antioxidants provide protection to the body against the damaging effects of free radicals. When antioxidants are not adequately provided to the body, or are ineffective due to an excess of free radicals present, enzymes manufactured by the body help to reduce the damage caused by free radicals. Increased amounts of free radicals that overwhelm the antioxidant defence system of the body are prone to cause damage to the cells. This manifestation is referred to as oxidative stress (Halliwell, 1994:721; Sies, 1997:291; Flora, 2009:191).

Some organisms use ROS for cell signalling and other functionary tasks (Radak *et al*., 2008:153). Although ROS are by-products of the life supporting aerobic metabolism they are, when in excess, the main cause of cellular damage and ageing (Yu, 1996:651; Radak *et al*., 2008:153; Baba *et al*., 2009:702). Oxidative stress has been found to be interconnected to the development of a number of diseases associated with metabolic or vascular disorders (Young & Woodside, 2001:176; Wiernsperger, 2003:579). Ischaemic heart disease, stroke, diarrhoea, lower respiratory infections and tuberculosis have all been associated with oxidative stress (Ferrari & Torres, 2003:251). Oxygen derivatives, particularly the hydroxyl radical and superoxide, are the primary free radicals involved in these disease states (Young & Woodside, 2001:176). The reason that overproduction of free radicals is a feature of numerous diseases originates from the fact that the oxidative metabolism is a vital part of every cell's metabolism (McCord, 2000:657).

While many studies have reported on the effects of oxidative stress in relation to pro-inflammatory signalling and macrophage activation in disease development (Cachofeiro *et al*., 2008:54; Ambade & Mandrekar, 2012:1), others have rather referred to oxidative stress as being a secondary complication in many disorders (Surh & Packer, 2005:22). A strong shared involvement by oxidative stress and inflammation has been shown in atherosclerosis development, leading to coronary artery disease (CAD) (Kotur-Stevuljevic *et al*., 2007:181), with some suggesting inflammation can lead to oxidative stress and *vice versa* (Ortiz *et al*., 2013:1). More studies are needed, using advanced –omics-based technologies, to gain further insight into the underlying mechanisms of disease development where oxidative stress and inflammation plays a role.

2.2 Dietary antioxidants

To maintain a healthy functioning biological system, it is crucial that antioxidation and oxidation is balanced (Bouayed & Bohn, 2010:228). Antioxidants protect the body cells and structures against the damaging effect of free radicals (Young & Woodside, 2001:176; Sharma *et al*., 2008:124). A substance that is capable of slowing or preventing oxidation of other molecules is referred to as an antioxidant. In general, antioxidants provide protection as they can trap free radicals and thereby protect the cell against metal toxicity thus stopping the chain reaction and / or prevent reaction with ROS by chelating metal ions or by chelating metals and preserving them in a redox form which impairs their ability to decrease molecular oxygen (Flora, 2009:191). The antioxidant defence system can be grouped into three categories: (i) antioxidant enzymes (see the next paragraph for examples) which accelerates the destruction of free radical species, routinely intracellular; (ii) chain breaking antioxidants (e.g. flavonoids, carotenoids, urate, ascorbate) which are strong electron donors that react with a free radical before damage is caused to vital target molecules; and (iii) transition metal binding proteins (e.g. ferritin, lactoferrin and transferrin) which ward off the interaction of transition metals (e.g. iron and copper) with superoxide and hydrogen peroxide which would have produced highly reactive hydroxyl radicals (Young & Woodside, 2001:178).

Sources of antioxidants are either exogenous or endogenous (Willcox *et al*., 2004:275). The exogenous (dietary source) and endogenous antioxidants work together for the protective effect (Serafini, 2006:533). Exogenous antioxidants are obtained from fruits, vegetables, grains and other plant products and include vitamins (vitamins C and E), trace elements (selenium and zinc), carotenoids (β-carotene and lycopene), phenolic acids (caffeic acid) and a range of

flavonoids (Bouayed & Bohn, 2010:229) which predominantly form the second category of the antioxidant defence system indicated above. The endogenous antioxidants include enzymatic and non-enzymatic antioxidants which respectively form the first and third categories of the antioxidant defence system indicated above. Superoxide dismutase (SOD), catalase (CAT), glucose-6-phosphate dehydrogenase, thioredoxin reductase and glutathione reductase are enzymatic antioxidants. Uric acid, glutathione (GSH), nicotinamide-adenine dinucleotide phosphate (NADPH), coenzyme Q, lipoic acid, albumin and bilirubin are part of the non-enzymatic antioxidants (Bouayed & Bohn, 2010:229).

Antioxidant levels in the human body are affected by environmental and lifestyle conditions such as dietary intake, smoking habits and physical activity, along with hormones, aging and stress (Serafini, 2006:535). Adequate antioxidant provision is therefore an important consideration for health protection and disease prevention, particularly when oxidative stress is involved, such as in cardiovascular disease (CVD) and cancer (Barber & Harris, 1994:26; Young & Woodside, 2001:181). Free radical production, their potency, the concentration of antioxidants present and gene expression is related to the efficiency of the antioxidant network in the human body (Serafini, 2006:535).

2.2.1 Types and sources of dietary antioxidants

The consumption of plant foods through the diet is indispensable to provide antioxidants from exogenous sources needed to assist the body's overall antioxidant defence system. Plant foods contain vital nutrient antioxidants (vitamins C and E) as well as non-nutrient antioxidants (carotenoids, polyphenols and flavonoids) in addition to important trace minerals to support the endogenous antioxidant system for enzyme functioning (Willcox *et al*., 2004:275).

The main dietary sources of the polyphenolic antioxidants are plant foods and beverages such as tea, red wine, coffee and fruit juices. Chocolate, vegetables, cereals, legumes (Scalbert *et al*., 2005:287), fruit and herbs (Tsuji *et al*., 2013:1014), are also rich sources of polyphenols and consequently contribute to its intake (Scalbert *et al*., 2005:287). Polyphenols can perform as either hydrogen donating antioxidants or as chelators of metal ions whereby they decrease the ability to produce radical species by preventing metal-catalysed initiation. Their function depends on their specific chemical structure (Salah *et al*., 1995:342; Scalbert & Williamson, 2000:2073S). The structure of polyphenols is made up of at least one hydroxyl group attached to one of its benzene rings (Hodgson & Croft, 2010:496).

Vitamins C and E are powerful antioxidants which respectively work in the aqueous and hydrophobic environments of the body. These two vitamins also work together to regenerate vitamin E (α-tocopherol) (Young & Woodside, 2001:183). A cross-sectional study was conducted with a Spanish sample group of healthy volunteers consisting of men and women aged between 29 and 69 years to assess the principal food sources of vitamins C and E in the diet. Foods that provided at least two-thirds of the vitamin C intake were fruits and fruiting vegetables, while vegetable oils, non-citrus fruits, nuts and seeds were the major contributors to the vitamin E intake (Garcia-Closas *et al*., 2004:1005).

Dietary antioxidants also include the carotenoids. These bioactive compounds include β-carotene, β-cryptoxanthin, α-carotene, lycopene, lutein and zeaxanthin (Fernández-Garcia *et al*., 2012:440). The main dietary sources of each of these carotenoid subgroups, along with that of the flavonoid subgroups and vitamins C and E, are indicated in Table 2.1.

Table 2.1: Types and sources of dietary antioxidants

Type	Subgroup	Main dietary sources	Reference
Carotenoids	α-carotene	Carrots, pumpkin	Fernández-Garcia *et al*., 2012:440
	β-carotene	Carrots, red bell peppers, oranges, potatoes, green vegetables	
	β-cryptoxanthin	Ripe red peppers, papayas	
	Lutein	Spinach, Brussel sprouts, broccoli, peas	
	Zeaxanthin	Egg yolk, corn	
	Lycopene	Tomatoes, tomato products, watermelon, pink grapefruit	
Flavonoids	Flavones	Leafy vegetables, celery, parsley	Day *et al*., 2004:61
	Flavonols	Red wine, tea, onions, cherries, apples, broccoli	Yao *et al*., 2004:115
	Flavonones	Citrus fruit & juice, cumin, peppermint	Day *et al*., 2004:61
	Flavanols	Apples, hops, tea, beer	Yao *et al*., 2004:115
	Anthocyanidins	Red wine, berry fruit	Day *et al*., 2004:61
	Isoflavones	Soy products	Day *et al*., 2004:61
Vitamins	Vitamin C	Oranges, tomato, sweet pepper	Garcia-Closas *et al*., 2004:1005
	Vitamin E	Sunflower oil, olive oil, nuts, seeds	Garcia-Closas *et al*., 2004:1005

Dragland *et al*. (2003:1286) reported on the total dietary intake of antioxidants considering the dietary inclusion of culinary and medicinal herbs. The findings from this study demonstrated that there is a considerable difference in the TAC of the different herbs. Sage, garden thyme, peppermint, oregano, lemon balm, allspice, cinnamon and cloves were the dried culinary herbs that presented high concentrations of dietary antioxidants through the TAC determinations [more than 75 millimole (mmol) / per 100 gram (g)]. Herbs are considered a rich source of dietary antioxidants compared to other food groups. Herb use can therefore contribute greatly to the total dietary intake of antioxidants in a person's diet (Dragland *et al*., 2003:1286).

Louwrens *et al*. (2009:195) through an exploratory study using secondary data, estimated the adult suggested dietary TAC considering the five-a-day concept and other dietary consumption guidelines, in a South African context. The largest contribution to the estimated suggested dietary TAC was provided by beverage (tea and coffee) consumption, in line with the consumption guidelines, followed by grain, fruit and vegetable consumption according to their respective consumption guidelines. Legume and nut consumption, in line with their consumption guideline, made a small contribution. The South African adult population's dietary TAC, as calculated from food consumption data obtained from published food consumption surveys done in SA, contributes about 50% of the estimated dietary TAC suggested by Louwrens *et al*. (2009:201). The dietary TAC provision of the beverage and vegetable groups calculated from the adult South African consumption is far below the estimated suggested TAC (Louwrens *et al*., 2009:201).

Tea, in general, is a major source of flavonoids (Dufresne & Farnworth, 2001:404; Hodgson & Croft, 2010:495) and polyphenols (Campanella *et al*., 2003:725). This literature review will therefore focus on these compounds as the dietary antioxidant sources rather than the other antioxidants with their different food sources.

2.2.2 Flavonoids as important dietary antioxidants

The phenolic phytochemicals are the largest phytochemical group. The flavonoids and the phenolic acids form the major phenolic groups (King & Young, 1999:213). The flavonoid structure consists of a heterocyclic pyran or pyrone ring interlinked by two phenolic benzene rings with an attached oxygen atom. Because of this specific structure, flavonoids are part of the polyphenols (Sampson *et al*., 2002:1414) forming a subclass of the polyphenols (Beecher, 2003:3248S).

In the human diet, polyphenols account for the largest provision of antioxidants (Scalbert & Williamson, 2000:2073S). More than 4000 flavonoid compounds have been identified and classified to date and more than 50 of these flavonoids are present in food (Packer & Colman, 1999:118). Flavonoids are said to contribute to more than 60% of the total polyphenol intake (Packer & Colman, 1999:118). Several studies have suggested that flavonoid intake, through consuming flavonoid rich sources which include vegetables, fruit, tea and herbs (Tsuji *et al*., 2013:1014), could have protective effects against age-related diseases such as CVD and cancer (Yao *et al*., 2004:113).

Based on the oxidation of the oxygen heterocyte, flavonoids can be grouped into a number of different subgroups or classes (Scalbert & Williamson, 2000:2074S; Liu, 2004:3481S). The six subgroups of flavonoids common to usually consumed foods and beverages with their components are the flavonols (quercetin, kaempferol, myricetin, isorhamnetin), flavanols [gallocatechin, catechin, epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC), epigallocatechin-3-gallate (EGCG)], flavones (luteolin, apigenin), flavonones (hesperidin, naringenin, eriodictyol), anthocyanidins (cyanidin, delphinidin, malvadin, pelargonidin, petunidin, peonidin) and isoflavones (genistein, daidzein) (Beecher, 2003:3249S; Liu, 2004:3481S). Flavonols are the subgroup of flavonoids which are most abundant in food and provide a pale yellow colour to food. Quercetin and kaempferol are the most well-known flavonols. Flavanols are colourless or yellow and have an astringent taste (Yao *et al*., 2004:115). Anthocyanins resemble the blue, red and violet colours in fruit and flowers (Day *et al*., 2004:61). Table 2.1 presents the main dietary sources for each of the six flavonoid subgroups.

Research about tea (*Camellia (C.) sinensis*) has mainly been brought about due to the presence of flavonoids in tea. Flavonoids make up 30% of the dry matter from tea solutions (King & Young, 1999:217; Hodgson & Croft, 2010:495). Black and green teas are both rich dietary sources of flavonoids, in particular the flavanol subgroup (Hodgson & Croft, 2010:495). Compared to other foods, flavanols (catechins) are abundantly provided in tea (Peterson *et al*., 2005:488; Hodgson & Croft, 2010:496) and deliver a specific flavanol composition that is unique to this beverage (Peterson *et al*., 2005:488). Flavonols are also one of the common subclasses of flavonoids in tea, especially quercetin (Hodgson & Croft, 2010:496). Tea is generally the main source of flavonol intake in populations (Hodgson & Croft, 2010:497). Hodgson and Croft (2010:497) reported that one cup of tea will provide 150 to 200 milligram (mg) of flavonoids. A daily tea consumption of two to three cups will supply most of the flavonoids consumed by an individual per day. The daily total flavonoid intake, considering the intake obtained from all

dietary sources, is normally below 1000 mg. Tea is usually the main contributor to the intake of flavonoids in tea drinking populations as it contributes to more than 50% of the total flavonoid intake in these populations (Hodgson & Croft, 2010:497). Geleijnse *et al*. (2002:885) determined the associations between flavonoid intake and tea consumption with incident myocardial infarction in the Dutch population. The study findings also indicated that more than 50% of the flavonoid intake in this population is through the consumption of tea.

On drinking tea, the flavonoids present are instantly absorbed into the blood circulation. Several studies have provided evidence that these bioavailable compounds continue to hold their antioxidant properties *in vivo* (Langley-Evans, 2000:310). Several studies have suggested that flavanols could have protective effects against diseases such as CVD and cancer (Yao *et al*., 2004:113). In relation to cancer, the modulation of the carcinogen action in the cell is a possible explanation of flavonoids' role in chemoprevention. The flavonoid interactions with cells alter the reactive carcinogenic metabolite levels at the cells likely to bind to the macromolecules of the cells, such as the DNA and protein. The resulting reactions are then inhibited / reduced which limits cell damage. It also affects a cell's oxidative status (Joubert *et al*., 2008:401). Differences in the flavonoid content of the different types of tea, which may influence the biological effects, could be caused by the blending of different teas related to area of production, type of tea included and cost factors that may have influenced the production process (Beecher, 2003:3251S).

2.3 Rooibos herbal tea

Rooibos (*A. linearis*) is an indigenous plant of SA and is enjoyed in more than 37 countries worldwide as a herbal tea (Joubert & de Beer, 2011:869) and is a popular beverage especially among health-conscious consumers. Rooibos has its own unique polyphenolic content (Joubert & Ferreira, 1996:79; von Gadow *et al*., 1997b:75; Joubert & de Beer, 2011:875). In the past it was the fact that rooibos herbal tea is free of caffeine and has low tannin levels in relation to *C. sinensis* tea variations that conveyed the 'health message' to consumers. Currently, it is the antioxidant capacity of this herbal tea, as well as its flavour that enjoys attention on the international market (Joubert *et al*., 2011:877). The beverage has a smooth, non-bitter flavour and is enjoyed hot or chilled (Erickson, 2003:37). The flavour of this herbal tea can be described as having a fruity character with overtones of mango, caramel and vanilla and is mildly sweet. Koch *et al*. (2012:217) developed a rooibos sensory wheel which reflects the variation in sensory profiles of harvested rooibos samples. The flavour of rooibos in this appraisal was described as

a combination of herbal-floral notes, honey and woody with a subtle astringency and slight sweet taste. The rooibos flavour continues to develop as it steeps and the beverage refrigerates and reheats well (Rubin, 2010:48). The manufacturing of rooibos powdered extract for use in beverages and functional foods by a South African company in 2000 expanded the rooibos market (Joubert & de Beer, 2011:873).

Numerous studies have investigated the biological attributes of South African herbal teas. Most of this research had been performed on the herbal tea, rooibos, with fermented rooibos mostly used for this purpose (Joubert *et al*., 2008:396).

2.3.1 Cultivation and production

It has been estimated that there are about 350 to 550 rooibos farmers in SA. Secondary processing of rooibos is performed by eight large processors responsible for the largest part (about 90%) of the market (South Africa. Department of Agriculture, Forestry and Fisheries, 2012:3). Fermented or traditional and unfermented or 'green' rooibos are the two types of rooibos produced during the manufacturing process (Joubert *et al*., 2008:376). Both herbal tea types are available as organic or conventionally grown, plain or flavoured, loose leaves or in tea bags (Erickson, 2003:37).

Rooibos has been cultivated in the Cederberg mountain region of the Western Cape province, SA, for centuries (Erickson, 2003:34; Lee & Jang, 2004:285). The small towns of Clanwilliam and Wupperthal, north of Cape Town in the Cederberg region, have a long history of rooibos cultivation. These towns are popular with tourists because of their beautiful rural scenery and their role in the rooibos industry (Erickson, 2003:36). Seedlings are used to plant and grow rooibos, leading to a large variation in the cultivated plants (Joubert *et al*., 2008:386). Considerable variability occurs in the plant size, leaf size, flowering time, density of branching and development of short shoots (Joubert & Schulz, 2006:138). Harvesting of rooibos takes place during the summer months. The bush is cut just above the topping height either mechanically or manually with sickles. Only the stems and leaves are used, as the flowers have an unpleasant flavour. After the harvest, the rooibos branches are bound together and transported to be processed (Joubert & Schulz, 2006:139).

A fermentation process is part of the production of traditional rooibos herbal tea. This is an important step as it supports the red colour and allows the sweet flavour to develop (Joubert & Schulz, 2006:139) and hence it is referred to as 'red bush' (Erickson, 2003:37). The fermentation process, which includes the oxidation of the polyphenols, develops the characteristic flavour and red-brown colour of traditional rooibos. Bruising of the tea shoots and the addition of water accelerates the fermentation process (Joubert & Schulz, 2006:139). The tea changes in colour during fermentation due to the change in the polyphenol composition (Joubert, 1996:403). Depending on the climate, the processing conditions and plant material composition, the fermentation process can be eight to 24 hours with the average being 12 to 14 hours. Once the sweet aroma and red-brown colour are fully developed, the tea leaves and stems are spread out to dry in the sun. The tea leaves and stems are sieved before packaging to ensure the required cut and pasteurised to ensure a microbiologically safe product (Joubert & Schulz, 2006:139).

Green rooibos is processed differently to traditional rooibos. The oxidative changes are minimised by controlling the moisture content in order to retain the green colour (Joubert & Schulz, 2006:139). The green leaves and stems are immediately dried to prevent oxidation. The unfermented variety has a mild 'green' taste similar to green tea, but without the astringency (Erickson, 2003:37). The production and quality of green rooibos remains a challenge as rapid drying of the tea is required since slow drying will result in the development of an undesirable colour and flavour (Joubert & de Beer, 2011:876). Flavour forms an integral component in the grading process of rooibos (Koch *et al*., 2013:704).

Only pasteurised tea is available commercially. Consequently it is important to know the effect of pasteurisation on the phenolic content of infusions obtained from such samples (Stanimirova *et al*., 2013:596). Steam pasteurisation was introduced to ensure good microbial quality after *Salmonella* contamination of rooibos was identified (Joubert & de Beer, 2011:876). Koch *et al*. (2013:704) determined the influence of steam pasteurisation on the phenolic content and sensory characteristics of infusions prepared from fermented rooibos leaves and stems. Steam pasteurisation reduced the soluble solids, total polyphenols and aspalathin content of the rooibos herbal tea. It also affected the colour of the tea. The prominent 'green' flavour of unpasteurised rooibos is changed to a 'hay-like' flavour after steam pasteurisation which is more favourable (Koch *et al*., 2013:704).

Stanimirova *et al*. (2013:590) evaluated the effect of the production season, grade quality as well as steam pasteurisation on the phenolic content of a normal infused cup of rooibos. The phenolic content of the rooibos herbal tea infusion was influenced by all the investigated factors. Infusions from higher quality plant material samples reflected a higher overall phenolic content compared to those from lower quality batches. Steam pasteurisation use on the tea material decreased the phenolic content of almost all the phenolic compounds in the tea infusion. Stanimirova *et al*. (2013:598) indicated that ferrulic acid may be a good indicator for determining the quality of rooibos herbal tea as a lower quality tea infusion delivered an increased ferrulic acid level.

2.3.2 Consumption and suggested intake

Nel and Steyn (2002:116,121,124) indicated in a report compiled for the South African Department of Health based on adult food consumption surveys done in SA, that a higher percentage of adults consume coffee or black tea than rooibos herbal tea (Nel & Steyn, 2002:115,121). Between coffee and tea intake as a beverage, the report further indicated that a higher percentage of adults in general consume tea rather than coffee (Nel & Steyn, 2002:62,63, 92,94,78,79). This was also supported in a more recent study where women in an informal settlement in the Vaal reported a mean daily intake of 288 g black tea, 274 g rooibos herbal tea and 255 g coffee per consumer (Oldewage-Theron & Kruger, 2011:420). Tydeman-Edwards (2012:173) used data from other studies conducted to list the 10 most frequently consumed food items for urban and rural adults aged between 25 and 64 years in the Free State province, SA. The data indicated that tea (all types) was second most frequently consumed by the urban women, third most frequently consumed by the urban men and the rural women, and fourth most frequently consumed by the rural men, and that men consumed coffee more frequently than women.

Louwrens *et al*. (2009:200), in an exploratory study, used secondary data to calculate the dietary TAC of the average adult South African. The results of this exploratory study indicated that the intake of rooibos herbal tea (at 3%) contributed the least to the dietary TAC compared to black tea (at 68%) and coffee (at 29%) in the beverage group. Consuming five to six cups of tea daily on a regular basis could prove to be beneficial for human health (Popkin *et al*., 2006:532). Marnewick *et al*. (2011:50) reported that a daily intake of six cups of rooibos can reduce the risk of CVD in adults. According to Joubert and de Beer (2012:48) six cups of rooibos herbal tea

must be consumed as a minimum daily for it to have a beneficial health effect. Rooibos herbal tea consumption appears to be free and safe from side effects (Erickson, 2003:45).

2.3.3 Uses

The use of rooibos herbal tea has grown extensively since the 1990's (Joubert & Schulz, 2006:138). 'Eleven O'Clock' was the first brand of rooibos herbal tea available on the South African market (Joubert *et al*., 2008:386). The modern day consumer mostly purchases rooibos in the form of tea bags rather than loose leaf tea. Rooibos herbal tea, in general, is prepared by using freshly boiled water and one tea bag (± 2 g) infused for two to five minutes. This infusion time allows for sufficient flavour and colour extraction. The beverage is nevertheless brewed and prepared according to taste, which is with or without a sweetening agent or milk, and served hot (Joubert *et al*., 2008:377).

Rooibos herbal tea flavoured with honey is popular in SA. Other flavourings added for aroma are vanilla and lemon. Herbs such as rosemary or ginger can also be added for health benefits and flavour (Joubert & Schulz, 2006:141). For a refreshing beverage, it is served cold with added sugar and / or lemon juice in summer. Mixtures of rooibos with herbs (e.g. buchu, honeybush, *Sutherlandia frutescens* or fennel) and chai rooibos herbal tea are also some tea bag products which are commercially available (Joubert *et al*., 2008:378). The development of green rooibos is due to the high antioxidant levels of unfermented rooibos (Joubert & Schulz, 2006:138). Green rooibos extract has been introduced for the functional food market. The standard for green rooibos extract is 15% aspalathin. The fermented rooibos extract is standardised in terms of its orientin content (Joubert & Schulz, 2006:140). Cosmetic products and ready-to-drink (RTD) iced teas contain these extracts (Joubert & Schulz, 2006:138). Rooibos extracts are also used as food ingredients in jam, drinking yogurt and yogurt (Joubert & de Beer, 2011:878).

In the cosmetic field rooibos is used in haircare, skincare and baby products. The antioxidant and free radical scavenging properties of the flavonoid components present make it a functional ingredient for mature skin and anti-ageing products. The anti-inflammatory properties, also related to the flavonoid components present, make it effective for products manufactured for skin prone to problems or sensitivity, baby products and after sun lotions (Tiedtke & Marks, 2002:18). The first non-beverage items marketed that incorporated rooibos extracts were skincare products. The fermented form of rooibos is used in these products in SA (Joubert *et al*., 2008:388). Annetjie Theron was the initiator of a range of skincare products containing rooibos

extract that became popular and well-established in the skincare industry of SA (Joubert *et al*., 2008:378).

2.3.4 Nutritional composition

Rooibos herbal tea contains flavonoids and phenolic acids which are potent antioxidants (Erickson, 2003:38). A unique characteristic of rooibos is the presence of aspalathin (Joubert & Schulz, 2006:140) and nothofagin (Breiter *et al*., 2011:338) which are the two main flavonoids in rooibos (Breiter *et al*., 2011:338; Sissing *et al*., 2011:609). These two flavonoids have stronger antioxidant activities than other flavonoids (Baba *et al*., 2009:700). Aspalathin and nothofagin, constitutes almost 15% of the soluble solids of rooibos (Sissing *et al*., 2011:609). Aspalathin is unique to rooibos and can serve as a quality marker of unfermented rooibos (Joubert *et al*., 2008:389). Nothofagin has a similar structure to aspalathin and may have similar antioxidant capabilities (Erickson, 2003:39). Other flavonoids present in rooibos include orientin, iso-orientin, vitexin, rutin and isoquercetin (Joubert & Ferreira, 1996:81; van Heerden *et al*., 2003:885). Unfermented or 'green' rooibos contains higher levels of these polyphenolic antioxidants (Joubert & Schulz, 2006:138).

According to Joubert and Schulz (2006:142), the fermented and unfermented rooibos forms have different aspalathin and nothofagin levels. The aspalathin and nothofagin levels in rooibos from the same plantation also vary because seeds are used for planting. Since fermentation takes place in the outside environment and under uncontrolled conditions, this process will additionally influence the aspalathin and nothofagin levels in fermented rooibos. Joubert and Schulz (2006:142) reported that the average aspalathin content of unfermented rooibos is 6.62 g/100 g compared to that of fermented rooibos which is 0.26 g/100 g. The average nothofagin content of unfermented (0.68 g/100 g) and fermented (0.12 g/100 g) rooibos does not differ as much as the aspalathin content.

During fermentation (an oxidative environment), when traditional rooibos is produced, the aspalathin content remaining is about seven percent of the initial content (Joubert, 1996:410). Joubert *et al*. (2004:133) explained that the fermentation process decreases the anti-radical capacity of the aqueous extracts and the crude phenolic contents of this herbal tea.

Breiter *et al*. (2011:341) quantified the main flavonoid compounds in a rooibos infusion prepared from 10 g of dried rooibos leaves (green rooibos) and 500 millilitre (mL) of boiled water. The

aspalathin (287 ± 01 mg/500 mL) and nothofagin (34.4 ± 0.0 mg/500 mL) contents were the highest with iso-orientin (26 ± 0.0 mg/500 mL) and orientin (17 ± 0.0 mg/500 mL) also contributing to the total flavonoid content. Powdered rooibos extracts, manufactured from waste material, contain low levels of isovitexin, orientin and iso-orientin (Joubert *et al*., 2008:392).

Marnewick *et al*. (2003:8116) reported on the quantification of the main flavonoids in aqueous extracts of herbal teas, specifically rooibos and honeybush fed to rats during fumonisin B1 (FB1) promotion. The main flavonoid consumed by the rats that were given unfermented rooibos herbal tea, was aspalathin. Far less of the flavone components, iso-orientin and orientin were consumed. The results indicated that the aqueous extract of the fermented rooibos had a lower content of nothofagin and aspalathin which resulted in a higher intake of vitexin, isovitexin, orientin and iso-orientin compared to nothofagin and aspalathin (Marnewick *et al*., 2003:8116).

Rooibos herbal tea also contains minerals such as iron, potassium (Erickson, 2003:44), sodium (Olivier *et al*., 2012:1), calcium, copper, zinc and magnesium (Erickson, 2003:44). It contains greatly lower levels of most minerals compared to black or green tea (Olivier *et al*., 2012:6), except for sodium (Olivier *et al*., 2012:4). Rooibos herbal tea is caffeine free and therefore does not need to go through a decaffeination process which will decrease the polyphenol content as occurs in green and black tea production. Furthermore, rooibos is preservative, colourant and additive free (South Africa. Department of Agriculture, Forestry and Fisheries, 2012:3) and has a lower tannin content in comparison to *C.sinensis* teas (Erickson, 2003:45).

2.3.5 Health promoting effects

Rooibos has numerous reputed health benefits that contribute to its popularity (Joubert & de Beer, 2011:869). Rooibos has been recommended for the following health conditions: allergies (asthma and hay fever), skin problems (eczema and acne), digestive disorder and stomach problems due to its antispasmodic properties, nervous conditions and age-related problems (Tiedtke & Marks, 2002:16). The unique phenolic metabolites of rooibos herbal tea, which among others, act as potent antioxidants, may be the link to its health promoting effects. Research has indicated antimutagenic, anticarcinogenic, anti-inflammatory and antiviral activities in rooibos herbal tea infusions (Joubert & Ferreira, 1996:82). Table 2.2 provides a summary of the experimental and human research studies that investigated the health promoting properties of rooibos.

Table 2.2: Summary of reported research on the health promoting effects of rooibos herbal tea

Bioactivity	Form(s) of rooibos	Objective(s) of study in relation to consumption	Outcome(s) on consumption	Reference
Cardiacprotection	Aqueous extract UR[1] and FR[2]	Effect of rooibos supplementation on ischaemia / reperfusion injury in the isolated perfused rat heart	Decreased pro-apoptotic proteins; Improved aortic output recovery	Pantsi *et al*., 2011:1220-1228
	FR consumed as beverage	Effect of rooibos on biochemical and oxidative stress parameters in adults at risk for CVD[3]	Increased plasma total polyphenol content; Decreased lipid peroxidation, serum LDL[4]-cholesterol and triacylglycerols; Increased HDL[5]-cholesterol	Marnewick *et al*., 2011:46-52
	Aqueous extract rooibos herbal tea	Effects of green, black and rooibos herbal tea on ACE[6] and NO[7] of healthy volunteers	Inhibited ACE activity; No significant effect on NO concentration	Persson *et al*., 2010:730-737
Oxidative stress reduction	Aqueous extract UR and FR	Antioxidant status as reflected by redox state of glutathione and the ORAC[8] in the liver of rats exposed to the various tea preparations and modulation of drug metabolising enzymes in the liver of rats by rooibos and honeybush	UR increased the activity of the microsomal UDP-glucoronosyl transferase; Reduced oxidised glutathione levels; Increased reduced glutathione	Marnewick *et al*., 2003:8113-8119
	Ready-to-drink UR and FR formulated with rooibos extract powder	Effect of rooibos on TAC[9], lipid triacylglycerols, cholesterol and glycaemia plasma levels in humans	Increased plasma antioxidant capacity; No changes in triacylglycerols, cholesterol and glycaemia plasma levels	Villano *et al*., 2010:679-683
Chemoprotection	Aqueous extract UR and FR	Modulating properties of herbal teas, black and green tea against oxidative parameters and cancer promoting activity induced by FB1 in rat liver	FR decreased lipid peri-oxidation; UR decreased number of foci	Marnewick *et al*., 2009:220-229
	Aqueous extract UR and FR	Effects of rooibos and honeybush herbal teas on the development of esophageal papillomas in Fischer rats	UR reduced mean total papilloma size	Sissing *et al*., 2011:600-610
Anti-inflammatory effect	Aqueous extract UR	Effects of rooibos herbal tea on prevention of inflammation and dextran sodium sulphate induced rat colitis	Increased SOD[10] levels; Decreased 8-hydroxy-2'deoxyguanosine	Baba *et al*., 2009:700-704

[1] UR: Unfermented rooibos
[2] FR: Fermented rooibos
[3] CVD: Cardiovascular disease
[4] LDL: Low-density lipoprotein
[5] HDL: High-density lipoprotein
[6] ACE: Angiotensin-converting enzyme
[7] NO: Nitric oxide
[8] ORAC: Oxygen radical absorbance capacity
[9] TAC: Total antioxidant capacity
[10] SOD: Superoxide dismutase

In relation to its cardioprotective effects, Marnewick *et al*. (2011:46) investigated the effect of regular rooibos herbal tea consumption on oxidative stress and biochemical indicators in subjects at risk for CVD. The study was the first to report the influence rooibos has on oxidative stress and the lipid profile of adults at risk for the onset of CVD, which were found to be improved on the daily consumption of six cups (1 200 mL) per day for six weeks (Marnewick *et al*., 2011:50). Villano *et al*. (2010:682) and Nikolova *et al*. (2007:120) also confirmed the lipid profile modulation and oxidative stress reduction properties of unfermented and fermented rooibos herbal teas in healthy humans and that these rooibos herbal teas boost plasma antioxidant capacity. Pantsi *et al*. (2011:1224) also reported on the cardioprotective properties of rooibos. In this study male Wistar rats consumed fermented and unfermented rooibos for seven weeks whereafter the rat hearts were exercised and placed on working cardio perfusion equipment. The rats that received the fermented rooibos (67.7 ± 8.12 mg daily) ingested significantly ($p < 0.05$) lower total polyphenols in comparison to those that received unfermented rooibos (74.62 ± 3.41 mg daily) and green tea (95.58 ± 7.16 mg daily). The flavonol intake of those that received green tea (3.81 ± 0.56 mg daily) was significantly ($p < 0.05$) lower in comparison to that of the rats that received the unfermented (13.61 ± 1.07 mg daily) and fermented (20.65 ± 3.41 mg daily) rooibos (Pantsi *et al*., 2011:1222). The rats receiving the unfermented and fermented rooibos indicated an improved aortic output recovery due to the higher intake of flavonols (Pantsi *et al*., 2011:1225).

Marnewick *et al*. (2009:220) investigated the chemoprotective attributes of fermented and unfermented honeybush and rooibos herbal teas and black and green teas against FB1 elevation in rat liver utilising diethylnitrosamine (DEN) as cancer originator. The protective effects of the herbal teas under study were decreased by the fermentation process as the unfermented honeybush and rooibos significantly ($p < 0.05$) to marginally ($p < 0.1$) decreased the total amount of foci respectively, while the relative amount of larger foci was reduced with all the teas studied (Marnewick *et al*., 2009:220). The effect that flavonoids have in chemoprotection is possibly as a result of their inflection on the action of the carcinogen in the cell. A flavonoid cell interaction affects the cell's level of xenobiotic metabolising enzymes and its oxidative profile, which inhibits and / or reduces the binding of reactive carcinogenic metabolites to the cell macromolecules, such as the protein and DNA (Joubert *et al*., 2008:401). The two rooibos flavonoids, quercetin and luteolin are furthermore known to initiate death of cancer cells (Rubin, 2010:48).

Baba *et al.* (2009:700) used an experimental rat study to investigate the anti-inflammatory properties of rooibos. Seven-week-old Wistar rats were grouped with one group receiving rooibos herbal tea and the other group water. The 8-hydroxy-2'-deoxyguanosine levels in the urine decreased significantly ($p < 0.05$) while the SOD levels in the serum increased significantly ($p < 0.05$) in the group consuming rooibos in comparison to the control group which received water. The findings from the study indicated that rooibos herbal tea may prevent inflammation and damage to DNA *in vivo* due to its antioxidant activity (Baba *et al*., 2009:700).

2.4 Other teas and herbal teas

Wide and comprehensive research has been done on tea consumption (Mukhtar & Ahmad, 1999:111S). Along with the green and black tea varieties, oolong tea forms the three main types of manufactured tea (Shimada *et al*., 2004:227). The origin of all three these teas are from the same plant, *C.sinensis* (Hilal & Engelhardt, 2007:414). Green tea is the unfermented form of *C. sinensis*, while oolong tea is the partially fermented form, and black tea is the fermented form of *C. sinensis* (Pekal *et al*., 2011:681). Green tea has mainly been the focus of the research completed on tea. Therefore, not as much is known about black tea (Mukhtar & Ahmad, 1999:111S). As green tea is non-toxic and not expensive it is a popular beverage (Mukhtar & Ahmad, 1999:115S). In Japan and China, over 90% of tea consumed is green tea, whereas in Western societies mostly black tea is consumed (Tang *et al*., 2009:282). Another traditional South African herbal tea is honeybush (*Cyclopia spp.*). During the past few years, sales of this herbal tea have expanded to 25 countries (Joubert *et al*., 2011:887). A dark red-brown colour and a sweet flavour and aroma are the characteristic sensory features of honeybush (Joubert *et al*., 2011:901).

2.4.1 Cultivation and production

Green tea is produced to limit the oxidation of the polyphenols in the green tea leaves (Graham, 1992:334). The leaves of *C. sinensis* are processed and rapidly heated or steamed so that the polyphenol oxidase enzyme present can be inactivated (Komes *et al*., 2010:169). In black tea production, oxidation is actually encouraged so that oxidation of most of these substances can occur (Graham, 1992:334). The more extensive processing applied during black tea production converts most of the green tea catechins into complex and condensed flavonoids such as theaflavins and thearubigins (Hodgson & Croft, 2010:497; King & Young, 1999:217; van Het Hof

et al., 1998:356). Considering all the dried tea manufactured, oolong tea forms less than two percent and green tea one fifth (20%) of the production (Graham, 1992:334).

In localised areas from the southern part of the Western Cape province (Overberg) to the Eastern Cape province (Langkloof) of SA, over 200 hectares of honeybush are cultivated (Joubert *et al*., 2008:388; Joubert *et al*., 2011:888). The plant grows well in the soil and climatic conditions of these specific areas and most species flower in spring (September to October) (Joubert *et al*., 2011:888). During summer to late autumn the plant material is harvested (Joubert *et al*., 2008:388). The name honeybush comes from the honey-like, sweet aroma of the plant when it is in full bloom (Joubert *et al*., 2011:888). The preparation of the tea includes a fermentation and drying process of the flowers and leaves (Joubert *et al*., 2011:888). Honeybush, like rooibos, goes through fermentation. This process occurs either at a temperature of 70^0C for a period of 60 hours or at a temperature of 80 to 85^0C for a period of 18 hours. The drying of the herbal tea takes place on drying racks in the sun or in a controlled environment by using a rotary drier (Joubert *et al*., 2008:388). The unique sweet flavour and aroma specific to honeybush, as well as the leaves' dark red-brown colour, develop during the processing of honeybush herbal tea (Joubert *et al*., 2011:901). To acquire a green honeybush product, the browning and oxidation or fermentation of the honeybush plant material is not undertaken (Joubert *et al*., 2011:902). Each manufacturer has their own set of quality parameters for the cut size, colour and flavour (Joubert *et al*., 2008:388).

2.4.2 Consumption and suggested intake

The Department of Health report summarising South African food consumption studies conducted in the period 1983 to 2000 indicate the average consumption of black tea within rural and urban areas of SA for different age groups. The findings for rural areas indicate 224.7 g/ consumer/day for children five years of age; 264.5 g/consumer/day for children six to nine years of age and for the age group ten years and older, 468.8 g/consumer/day. The average black tea consumption determined within the urban areas is 224.2 g/consumer/day for children one to five years; 250 g/consumer/day for six to nine year olds and 441.4 g/consumer/day for persons older than 10 years (Nel & Steyn, 2002:75-92).

Louwrens *et al.* (2009:201) concluded from an exploratory study that beverages, such as black tea, contribute approximately 50% of the suggested daily dietary TAC determined in the study. An increase in the intake of beverages, especially tea, along with fruit and vegetables can increase the dietary TAC and promote health (Louwrens *et al*., 2009:195). Tea contains high amounts of the polyphenolic flavonoids which may provide up to 45% of the daily consumption of antioxidants (Langley-Evans, 2001:75). Song and Chun (2008:1543S) reported on tea flavonoids, the major flavonoid sources in the US diet and the sociodemographic characteristics of tea consumers. The results from this study indicated that the tea non-consumers' total daily flavonoid intake was more than 20 times less than that of the tea consumers' (32.6 mg/day versus (vs) 697.9 mg/day). The daily total flavonoid intake per capita from tea was 157 mg. For both the tea consumer and non-consumer groups, further flavonoid food sources included citrus juice, wine and citrus fruits.

The Beverage Guidance System, which has been developed in the US, focuses on obtaining a fluid intake on a daily basis from beverages which have a lower energy content and a higher nutrient content (Popkin *et al*., 2006:529). Due to tea containing various flavonoids and antioxidants at increased levels, it has been taken up in the Beverage Guidance System which supports the regular consumption of tea in an amount of five to six cups daily (Popkin *et al*., 2006:532). If green tea is regularly consumed in amounts of at least 0.6 to 1.5 litre (L) per day, this beverage may increase the TAC, reduce lipid / protein peroxidation and may, in healthy subjects, protect against DNA damage (Ellinger *et al*., 2011:913). The US Food and Drug Administration (FDA) states that the intake of green tea may reduce the risk of prostate and breast cancers, but commented that the scientific evidence to support this claim is inadequate (US Food and Drug Association, 2013:1).

2.4.3 Uses

Various cosmetic and health care products, in addition to beverages and ice creams, fortified with green tea are available as consumer products (Mukhtar & Ahmad, 1999:112S). Green tea polyphenols are added to foodstuffs with a higher water content or added to processed food to replace controversial synthetic antioxidants with natural antioxidants. Green tea polyphenols are resistant to heat and are water soluble, in addition to being potent antioxidants, which makes them greatly suitable for this purpose (Dufresne & Farnworth, 2001:416).

Mixtures of rooibos and honeybush, with only a small percentage of honeybush added to the rooibos, are other herbal tea products that can be bought commercially (Joubert *et al*., 2008:378; Joubert *et al*., 2011:891). The market for honeybush in a RTD form as iced tea, as well as powdered honeybush extract which has similar applications as rooibos, are not yet as advanced as that of rooibos. The market for cosmetic and toiletry products containing honeybush as ingredient is at this stage also far behind such products containing rooibos as ingredient (Joubert *et al*., 2008:378). Similar to rooibos herbal tea, honeybush is also available in the fermented and unfermented form for use as infusions (Joubert *et al*., 2008:388; Joubert *et al*., 2011:902). In the year 2000 the industry recognised green honeybush as an alternative herbal tea. Small amounts of unfermented (green) honeybush are available and sold commercially (Joubert *et al*., 2011:891).

2.4.4 Nutritional composition

The chemical composition of teas, regarding black and green teas, includes mainly polyphenols (especially catechins), in addition to caffeine and minerals (Wu & Wei, 2002:443). Tea is the main dietary source of catechins (Dufresne & Farnworth, 2001:408). Catechins are a very powerful group of flavonoids that has formed the basis of numerous research studies (Dufresne & Farnworth, 2001:404). Other polyphenols present in tea include quercetin, myricetin and kaempherol (Dufresne & Farnworth, 2001:408). Antioxidant nutrients such as carotenoids, tocopherols and vitamin C are also found in tea (Wu & Wei, 2002:443).

Far higher amounts of catechins are present in green tea compared to black tea (Dufresne & Farnworth, 2001:405). The catechin content in green tea is 30 to 42% of its dry weight, whereas in black tea it is only 10 to 12% of the dry weight (Dufresne & Farnworth, 2001:405). Green tea also has a higher TAC than rooibos herbal tea (both fermented and unfermented forms) as well as a higher total polyphenol content. Unfermented rooibos herbal tea has half the TAC of green tea (Bramati *et al*., 2003:7473,7474). A cup of green tea contains about 400 mg of polyphenolic antioxidants and half of these antioxidants are EGCG (Mukhtar & Ahmad, 1999:112S). It is accepted that the EGCG present in green tea is responsible for its chemoprotective properties (Mukhtar & Ahmad, 1999:111S).

The main flavonoids identified and analysed to date in all the species of honeybush are mangiferin, xanthones and isomangiferin, as well as hesperidin, which is a flavanone. Other flavonoid compounds identified in honeybush include the flavones, isoflavones and flavonols (Joubert *et al*., 2011:900). In a study by Marnewick *et al*. (2003:8116) which quantified the major flavonoid compounds in aqueous extracts of honeybush and rooibos herbal tea fed to rats, the monomeric polyphenols were xanthonemangiferin and hesperidin, in both fermented and unfermented honeybush.

The caffeine and tannin content, as well as the polyphenolic compounds of black, green, rooibos and honeybush, differ. Honeybush, like rooibos herbal tea, contains less tannin and no caffeine which is different to black and green teas which contain high levels of tannin and caffeine. The polyphenolic content also differs from that of black and green tea and also differ from one another (Sissing *et al*., 2011:601). Chemical analysis indicates that black tea, in addition to theaflavins, also contains high amounts of green tea catechins. Therefore, both catechins and theaflavins contribute to the bioactivity of black tea either by additive and / or synergistic effects (Pan *et al*., 2013:12).

Olivier *et al*. (2012:1) reported on traditional and herbal teas' mineral composition covering green and black tea, as well as rooibos and honeybush herbal tea. All samples were infused for six minutes. All the teas tested had its own unique mineral profile, although in very small amounts compared to the daily mineral requirements. Green and black tea has a higher mineral content, especially of potassium, copper and sulphur, compared to rooibos and honeybush herbal tea. Higher aluminium levels were also identified in green and black tea samples. Black tea provided the highest content of all the minerals assessed in relation to the individual mineral levels, while rooibos and honeybush contained lower levels of almost all the assessed minerals compared to the other teas studied (Olivier *et al*., 2012:6).

2.4.5 Health promoting effects

Polyphenolic antioxidants, which are abundantly present in teas, may perform a vital function in the control and prevention of cancer. The functions of tea polyphenols include acting as antioxidants, targeting and repairing damaged DNA and inhibiting the activity of specific enzymes (Dufresne & Farnworth, 2001:409). The biological actions of tea and the polyphenols present in tea on reducing the development of lung, skin, digestive and other cancers (Dufresne & Farnworth, 2001:410) and CVD (Dufresne & Farnworth, 2001:410; Hodgson & Croft,

2010:497) have been studied extensively. Population studies have suggested that increased tea consumption is associated with a lowered risk of CVD, with the degree of this benefit from black and green tea seemingly similar (Hodgson & Croft, 2010:497). Tea consumption can also positively impact diabetes, skin and eye function, and has renal effects, antihistaminic and anti-arthritis effects, antibacterial and antiviral effects, neurological and psychological effects, as well as effects on dental caries (Dufresne & Farnworth, 2001:414). Only green tea has been awarded a qualified health claim by the US FDA. The claim states that green tea is linked to a lower cancer risk and that it refers to green tea containing dietary supplements and conventional foods, as well as green tea itself (US Food and Drug Association, 2009:1).

Numerous studies have presented the biological actions of black tea and black tea polyphenols which include antioxidant, anti-tumor and anti-inflammatory effects, as well as metabolic regulation. Tea polyphenols such as theaflavins and catechins are considered to be multifunctional. These compounds could be effective in the prevention and treatment of chronic inflammation, metabolic syndrome, various cancers, CVD, obesity and several neurodegenerative diseases (Pan *et al*., 2013:12).

Langley-Evans (2000:309) performed a study which indicated that higher habitual consumption of black tea (as six or more measures daily) increased the TAC *in vivo*. The TAC was assessed using the ferric reducing antioxidant power (FRAP) methodology implicating that black tea may have resulting antioxidant effects *in vivo*.

In a prospective study, Geleijnse *et al*. (1999:2170) investigated the association of aortic atherosclerosis, which is a strong indicator of CVD, and black tea consumption. The study population consisted of men and women aged 55 years and older. In this prospective study, tea consumption, adjusted for gender and age, was inversely correlated with smoking, intake of coffee and alcohol and body mass index (BMI) in addition to aortic sclerosis with a stronger inverse association for women with regard to the latter.

Hakim *et al*. (2003:64) assessed the association between black tea consumption and CVD. Cross-sectional data from 1993 to 1998 were collected from the National Epidemiological Survey for Coronary Artery Disease in Saudi Arabia. The participants were adult women and men aged 30 to 70 years with symptoms of CVD. The results indicated that those consuming black tea daily in an amount of more than six cups had a significant ($p < 0.001$) decreased risk of CVD compared to those not consuming black tea (Hakim *et al*., 2003:68).

Mukamal *et al*. (2002:2476) performed a prospective cohort study comprising 1 900 hospitalised patients who suffered an acute myocardial infarction with a median follow-up of three years and eight months. The study methodology included chart reviews and face-to-face interviews. The participant weekly tea consumption was categorised as average (drinking less than 14 servings of tea weekly) or above average (drinking more than 14 servings of tea weekly) (Mukamal *et al*., 2002:2477). Cardiovascular deaths were more prevalent among the non-consumers (14%) than among the average or moderate tea consumers (11%) and those consuming tea above average (10%) (Mukamal *et al*., 2002:2479).

Bahorun *et al*. (2012:98S) performed a prospective randomised controlled clinical trial. The objective of this trial was to determine the influence of black tea intake on antioxidant status, lipid profiles and fasting blood plasma levels of glucose within a normal population. Three 200 mL black tea infusions were consumed per day by the group under study for three months, whereafter a three week wash-out period followed. The tea was consumed with no additions. The study results indicated a significant reduction in the fasting levels of triglycerides (35.8%; $p < 0.01$) and serum glucose (18.4%; $p < 0.001$) in addition to a significant reduction in the low density lipoprotein (LDL) / high density lipoprotein (HDL) plasma cholesterol ratio (16.6%; $p < 0.05$), a low increase in the HDL plasma cholesterol levels (20.3%) and a significant increase in the plasma TAC (FRAP: 41.8%; $p < 0.001$). Table 2.3 provides a summary of the research reported above on the health promoting effects of consuming black tea.

Table 2.3: Summary of reported research on the health promoting effects of green, black and oolong tea as well as honeybush herbal tea

Type of tea / herbal tea	Health property	Objective(s) of study in relation to tea consumption	Outcome(s) of study on tea consumption	Reference
Black tea	Antioxidant effects	Contribution of broader family of black tea flavonoids to circulating antioxidant potential	FRAP[1] increased by 65 to 75% after a few hours of consumption	Langley-Evans, 2000:309-315
	Cardioprotective properties	Association between aortic atherosclerosis and consumption	Significant, inverse association of tea intake with advanced aortic atherosclerosis; Stronger association in women than in men	Geleijnse *et al*., 1999:2170-2174
		Tested hypothesis that tea consumers have better long term survival after acute myocardial infarction	Self-reported tea consumption during the year before infarction associated with lower subsequent mortality after infarction	Mukamal *et al*., 2002:2476-2481
		Effects of black tea consumption on fasting serum glucose, total cholesterol, triglycerides, HDL[2], LDL[3] and antioxidant status in a normal population	Highly significant decrease of fasting serum glucose and triglyceride levels, significant decrease in LDL / HDL plasma cholesterol ratio and non-significant increase in HDL plasma cholesterol levels, while highly significant rise in plasma antioxidant propensity	Bahorun *et al*., 2012:98S-102S
Green tea	Chemoprotection	Association between consumption and risk of cancers of the colon, rectum and pancreas	Regular green tea drinking associated with a slight overall reduction in risk of rectum and pancreas cancers, not colon cancer in men and overall reduction in risk of all three cancers in women	Ji *et al*., 1997:255-258
		Meta-analysis to summarise results from prospective and case control studies on role in lung cancer	Increased intake of two cups per day associated with an 18% decreased risk of developing lung cancer	Tang *et al.*, 2009:274-283
	Cardioprotective properties	Effect of green tea drinking on lipid profile	Decrease in cholesterol, LDL cholesterol, apolipoprotein B, and ratio of cholesterol / HDL-C, but an increase in HDL-C and apolipoprotein A-I, observed	Coimbra *et al*., 2006:604-607

Table 2.3: Summary of reported research on the health promoting effects of green, black and oolong tea as well as honeybush herbal tea (cont.)

Type of tea / herbal tea	Health property	Objective(s) of study in relation to tea consumption	Outcome(s) of study on tea consumption	Reference
Green tea	Cardioprotective properties	Long term effects of consumption on risk of newly diagnosed hypertension subjects	Habitual moderate strength consumption, 120 mL[4] per day or more for one year, significantly reduces risk of developing hypertension	Yang *et al.*, 2004:1534-1540
Oolong tea	Cardioprotective properties	Relationship between plasma adiponectin levels, LDL-particle size and long term intake in patients with CAD[5]	Elevated plasma adiponectin; Increased LDL-particle size; Significant difference in plasma adiponectin levels before and after one month intake	Shimada *et al*., 2004:227-234
	Antioxidant effects	Effect of acute intake (500 mL) on antioxidant status in healthy volunteers	Increased plasma antioxidant status	Villano *et al*., 2012:2102-2106
Honey-bush herbal tea	Chemoprotection	Ability of fermented and unfermented honeybush herbal tea to modulate MBN[6]-induced esophageal carcinogenesis in rats	Reduced mean total papilloma size	Sissing *et al*., 2011:600-610
	Reduced oxidative status	Antioxidant status as reflected by redox state of glutathione and the ORAC[7] in the liver of rats exposed to various tea preparations and modulation of drug metabolising enzymes in the liver of rats by rooibos and honeybush	Enhanced activity of cytosolic glutathione S-transferase alpha; Increased activity of microsomal UDP-glucuronosyl transferase	Marnewick *et al*., 2003:8113-8119

[1] FRAP: Ferric reducing / antioxidant power
[2] HDL: High density lipoprotein
[3] LDL: Low density lipoprotein
[4] mL: Millilitres
[5] CAD: Coronary artery disease
[6] MBN: Methylbenzylnitrosamine
[7] ORAC: Oxygen radical absorbance capacity

In Shanghai, China, Ji *et al*. (1997:255) performed a large case-control study within the population to assess the consumption of green tea on cancers of the colon, rectum and pancreas. Subjects were between 30 and 74 years of age and newly diagnosed with one of the considered cancers. The incidence of each cancer was inversely associated with a higher intake of green tea, with the inverse association the strongest for pancreatic and rectal cancer. The results from this study suggested that the risk for pancreatic, colonic and rectum cancer may be reduced with green tea consumption. Studies on tea have also proposed that green tea polyphenols may be protective against skin cancer, as well as inflammatory responses (Mukhtar & Ahmad, 1999:115S). Tang *et al*. (2009:274) performed a meta-analysis of the association of black tea and green tea consumption with cancer of the lungs. The results of 22 case control

and prospective studies were summarised for the analysis. The results indicated a profound association between a lowered risk of lung cancer and a higher consumption of green tea. An increased intake of green tea of two cups daily was associated with a lowered risk of 18% in developing lung cancer. No statistically significant association was observed for a decreased risk of lung cancer with black tea consumption.

Epidemiological research has indicated that green tea consumption may provide protection against several cancers as well as CVD. This is primarily due to the antioxidant properties of the flavanols present in green tea (Ellinger *et al*., 2011:903). Research has proposed that, due to their strong antioxidant power, polyphenols, specifically flavonoids, can safeguard cells against the negative effects of ROS (Dufresne & Farnworth, 2001:408). If a minimum daily amount of 0.6 to 1.5 L is consumed on a regular basis, green tea may increase the TAC, decrease lipid peroxidation, particularly LDL oxidation, and provide protection against DNA damage in healthy subjects. The beneficial health effects related to green tea consumption appear to be more probable in subjects exposed to increased oxidative stress (Ellinger *et al*., 2011:913).

Coimbra *et al*. (2006:604) evaluated the relation between lipid risk factors and the outcome of green tea consumption on CVD. Twenty-nine healthy subjects aged 22 to 63 years, participated in the study. The subjects consumed one L of water per day for three weeks and for four weeks one L of green tea per day. No milk was added to the prepared tea. The same infusion time, concentration and temperature were applied throughout with the daily preparation of the tea (Coimbra *et al*., 2006:604). The results from this study indicated that green tea consumption has a favourable effect on blood lipid levels, thereby decreasing the risk of CVD through improving the blood lipid levels (Coimbra *et al*., 2006:604).

Yang *et al*. (2004:1534) studied the outcome of regular moderate strength oolong or green tea intake on newly diagnosed hypertensive subjects. In comparison to non-habitual tea consumers, the risk of developing hypertension decreased by 46% for those who consume a daily amount of at least 120 mL and by 65% for those who consume a daily amount of 600 mL or more. The study findings concluded that in the Chinese population, an intake of oolong or green tea of 120 mL or more per day for a year significantly reduces the development risk of hypertension. The research on the health supporting properties of green tea consumption reported above, in addition to the reported research on black tea consumption, is summarised in Table 2.3.

The effect of oolong tea on obesity and CVD has also been researched (Shimada *et al*., 2004:227). In a cross-over study by Shimada *et al*. (2004:231), 22 subjects consumed one L oolong tea or water for one month. The oolong tea was prepared with two tea bags (total weight of 6 g of tea leaves) which were infused in one L of hot water every morning for 10 minutes as a particular batch. The tea was then left to cool at room temperature. The subjects were free to consume the tea as and when they liked during the day until they went to sleep (Shimada *et al*., 2004:229). An increased adiponectin level resulted from the long term oolong tea consumption (Shimada *et al*., 2004:229) (see Table 2.3). Increased plasma adiponectin levels may provide protection against vascular damage and may be deregulated in conditions prone to atherosclerotic vascular disease (Okamoto *et al*., 2002:2767). The results from the study also indicated that the LDL particle size increased (see Table 2.3). Patients with CVD have a significant lower plasma LDL particle size (Shimada *et al*., 2004:228). Hodgson and Croft (2010:500) states in their report that the ability of tea flavonoids to improve the nitric oxide (NO) status and to enhance endothelial function may partly explain the protective effects of tea consumption on the risk of CVD.

Villano *et al*. (2012:2102) studied the effect of a RTD oolong beverage to modify plasma antioxidant levels in healthy humans compared to a control drink with added ascorbic acid but containing no oolong tea extract. The research findings indicated that the intake of 500 mL of a RTD oolong tea elevates plasma antioxidant levels in healthy humans in comparison to the control drink (Villano *et al*., 2012:2105) (see Table 2.3).

Joubert *et al*. (2008:398) reported that honeybush, in general, compared to rooibos herbal tea, green tea and black tea has the weakest TAC assessed via antioxidant assays applied *in vitro*. Marnewick *et al*. (2003:8115) also reported that the total phenolic content of green rooibos herbal tea's soluble solids was meaningfully higher than that of the corresponding type honeybush. Potential applications of honeybush herbal tea are for relief of menopausal symptoms, the prevention of skin cancer and decreasing blood glucose levels (Joubert *et al*., 2011:905). Two animal studies (see Table 2.3) respectively provide support for a reduced oxidative status (Marnewick *et al*., 2003:8113) and esophageal papilloma size (Sissing *et al*., 2011:600) on exposure to honeybush.

2.5 Effects of type, form, preparation method and additions on the total phenolic content and the total antioxidant capacity of tea

Several factors determine a cup of brewed tea's flavonoid content. The variety of the tea and the weight of the tea bag are the two major factors that determine the flavonoid contents of brewed tea. A further influencing factor is the brewing technique which includes the brewing temperature and brewing time (Hakim *et al*., 2000:1720; Peterson *et al*., 2004:404; Peterson *et al*., 2005:496; Sharpe *et al*., 2016:385). The brewing characteristics of tea include the ease of the compound extraction and the strength of the infusion. The weight of the tea bags affects the amount of compounds available and the size of the particles affects the available surface area provided for extraction. Extraction conditions such as the time and temperature have an influence because the infusion strength is determined by the amount of water used. With an extended infusing time of more than four minutes, the effect of the tea-to-water ratio reduces (Peterson *et al*., 2005:497).

2.5.1 Type of tea

Pantsi *et al*. (2011:1225) quantified the major polyphenols in unfermented and fermented rooibos. Unfermented rooibos provided higher levels of aspalathin, orientin, iso-orientin, vitexin, isovitexin and rutin compared to fermented rooibos. The flavonol content of both the unfermented and fermented rooibos were higher in comparison to the flavonol content of green tea. Green tea though had a higher flavanol content than both unfermented and fermented rooibos (Pantsi *et al*., 2011:1225).

Bramati *et al*. (2003:7472) also quantified the major flavonoids in unfermented rooibos herbal tea. The main compounds determined were also aspalathin, iso-orientin, orientin and rutin. The total flavonoid content detected in the unfermented rooibos herbal tea was 59.08 ± 0.59 mg/g compared to that of the fermented type which was 5.521 ± 0.055 mg/g. The level of aspalathin in unfermented rooibos was found to be 50 times higher than that of the fermented rooibos (49.92 ± 0.8 mg/g and 1.234 ± 0.01 mg/g respectively) and the TAC of the unfermented infusion double that of the fermented infusion (Bramati *et al*., 2003:7473).

The flavonoid and total polyphenol content in rooibos and honeybush herbal tea (fermented and unfermented) were recorded by Marnewick *et al*. (2000:160). On fermentation the flavonoid and total polyphenol content of both these herbal teas decreased significantly ($p < 0.001$). The total flavonoid values for unfermented rooibos (28.06 ± 0.25 g/100 g) and unfermented honeybush (27.1 ± 0.2 g/100 g) were almost the same, while the difference for the total polyphenols was higher (41.15 ± 0.25 g/100 g and 35.52 ± 0.03 g/100 g respectively). The flavonoid values for fermented honeybush were almost half (9.86 ± 0.18 g/100 g) that of fermented rooibos (18.8 ± 0.35 g/100 g). The fermented rooibos also had a higher total polyphenol content (29.74 ± 0.36 g/100 g) in comparison to the fermented honeybush (19.8 ± 0.26 g/100 g) (Marnewick *et al*., 2000:160).

Joubert (1996:403) confirmed that processing of rooibos decreased the aspalathin and nothofagin content with the decrease depending on the extent of the oxidation of the tea material. Most of the aspalathin and nothofagin is oxidised in the period prior to the fermentation process. The method of drying, as controlled drying vs sun-drying, had no effect on the rooibos apalathin, nothofagin or total polyphenol content (Joubert, 1996:411).

Von Gadow *et al*. (1997b:75) assessed rooibos herbal tea's antioxidant activity against that of black, green and oolong tea. Semi-fermented and fermented rooibos had a lower percentage of respectively total polyphenols and flavonoids than unfermented rooibos, expressed as percentage of the total water-soluble solids. This is a further indication that fermented rooibos herbal tea has a lower antioxidant activity. Green tea had the highest flavonoid content and fermented rooibos herbal tea the lowest (Von Gadow *et al*., 1997b:75).

Villano *et al*. (2010:679) investigated the influence of the rooibos herbal tea on the plasma TAC of healthy human volunteers. This study indicated that both fermented and unfermented rooibos herbal teas increased the plasma TAC. The unfermented rooibos herbal tea produced a 28% higher *in vitro* antioxidant capacity than fermented rooibos herbal tea, measured as the chain breaking antioxidant activity (TRAP) (Villano *et al*., 2010:680). Both the unfermented and fermented form of rooibos herbal tea produced a lower plasma TAC than black tea or green tea in this study (Villano *et al*., 2010:681). The lower antioxidant capacity is linked to the fermentation process where a decline in the polyphenol content of rooibos occurs (Villano *et al*., 2010:682). Joubert and Schulz (2006:142), in describing rooibos herbal tea's quality aspects, confirmed that unfermented rooibos herbal tea contains more aspalathin than fermented rooibos herbal tea.

In an investigative study, unfermented and fermented honeybush and rooibos herbal teas were tested for their antimutagenic properties. The *Salmonella typhimurium* mutagenicity assay was used. The study concluded that the unfermented form of the rooibos extract displayed greater antimutagenic properties compared to the fermented form of rooibos with these protective effects not observed with honeybush herbal tea (Marnewick *et al*., 2000:162). The fermentation process seems to decrease the protective effects of the herbal teas studied (Marnewick *et al*., 2009:220).

The ability of unfermented and fermented honeybush and rooibos herbal teas to regulate methylbenzylnitrosamine (MBN)-induced oesophageal cancer in experimental rats was evaluated by Sissing *et al*. (2011:601). It was reported that aspalathin was the major flavonoid in unfermented rooibos, whereas in the fermented form, orientin was the major flavonoid (Sissing *et al*., 2011:603). The aspalathin content of fermented rooibos herbal tea (0.48% of soluble solids) was much lower than that of the unfermented type (14.73% of soluble solids). In the unfermented form of honeybush the mangiferin content was 4.59% of the soluble solids, whereas in the fermented form it was 0.42% of the soluble solids (Sissing *et al*., 2011:605). The researchers concluded that fermentation of herbal teas does indeed decrease the protective effects on papilloma progression which is associated with the reduced polyphenolic constituent content (Sissing *et al*., 2011:608).

Literature has also indicated that after the fermentation process of green tea to black tea, the percentage of catechins present decreases to less than 10% (Dreosti, 2000:692). These changes that take place during tea processing can be ascribed to oxidative changes (Peterson *et al*., 2004:397). This possibly explains why there is a qualified health claim for green and not for black tea as the catechins are a group of very active flavonoids having biological properties that may be responsible for the health promoting effects (Hodgson & Croft, 2010:497). The flavanol content of blended and unblended teas also differ to some extent (Peterson *et al*., 2004:397) as blended teas are lower in catechins than unblended teas (Peterson *et al*., 2004:400).

2.5.2 Tea form

Ryan and Petit (2010:16) undertook an experimental study to determine and evaluate the antioxidant capacity of black tea brewed from black tea bag and tea leaf brands (n = 5). The results from the effect of the tea bag experiment indicated a significant difference ($p < 0.05$) between those tea preparations from the tea bags and those from the tea leaves. The tea infused using tea leaves delivered a higher TAC compared to the tea infusions prepared from the tea bags. A possible explanation for this could be that when the tea is enclosed in a bag, the flow resistance, caused by the packed leaves and by the bag material, results in a slower transfer of the soluble components from inside the bag into the water (Astill *et al*., 2001:5345).

Komes *et al*. (2010:169) investigated the effect on the phenolic compounds and the antioxidant capacity of different green tea extraction conditions. While the bag-prepared green tea provided the highest flavanol (especially EGCG) and methylxanthine contents, the powdered green tea provided the highest phenolic acid content (Komes *et al*., 2010:173). Cheong *et al*. (2005:747) determined the types and amounts of flavonoids in Korean green tea with the tea samples prepared at different temperatures and brewing times. The flavonoid extraction rate of green tea in powder form was found to be faster than that of green tea leaves.

Costa *et al*. (2012:324) analysed the 2,2-diphenylpicrylhydrazyl (DPPH) scavenging activity, total phenolics, total flavonoid content and the ascorbic acid of fruit juices, various green teas and other herbal infusions along with dietary supplements. The following samples were prepared to evaluate the bioactive compounds and the TAC of the teas: green tea from a bag (2 g) infused in 200 mL hot water (75^0C) for five minutes; green tea leaves (1.62 g) infused in 200 mL of hot water (75^0C) for three minutes and rooibos herbal tea prepared from a bag (1.5 g) infused in 200 mL boiling water for four minutes (Costa *et al*., 2012:325). The total phenolics for the green tea prepared from leaves were 22.9 mg gallic acid (GA)/100 mL and from the bag 29.1 mg GA/100mL. There was no significant difference in the total flavonoids between these two forms of green tea. The total phenolics of rooibos infused from tea bags were 21.9 ± 0.1 mg GA/100mL and the counterpart green tea 29.1 ± 0.5 mg GA/100 mL. The total flavonoids were higher in rooibos brewed from bags than green tea brewed from bags (11.6 mg EC/100 mL and 8.4 ± 0.1 mg EC/100 mL respectively) (Costa *et al*., 2012:326).

The total polyphenol content of rooibos herbal tea prepared from tea powder (300 mg / 150 mL) was found to be 82 mg compared to the 88 mg of that prepared from a tea bag (2.5 g tea bag / 150 mL infused for 10 minutes) and the aspalathin content 1.05 mg compared to 3.09 mg, respectively. The aspalathin, orientin, iso-orientin, vitexin and isovitexin content of the rooibos herbal tea prepared from the powder was less than the rooibos herbal tea prepared from the tea bag (Joubert & Schulz, 2006:142). The processes involved in the production of instant and RTD tea lowers the flavanol level or levels of both the flavanols and thearubigins (Beecher, 2003:3251S). Joubert *et al*. (2008:392) confirmed low levels of isovitexin, orientin and iso-orientin in powdered rooibos extract produced from waste materials.

Joubert and de Beer (2012:49) determined the phenolic content and antioxidant activity of rooibos food ingredient extracts. 'Instant rooibos' is another form of rooibos sold commercially. This is a convenient way to consume rooibos as it is made up of extract powder mixed with sugar. The results indicated that the extract with added hot water, converted to a cup of tea infusion (a single cup serving based on the soluble solid content) would deliver a lower flavone content, but approximately the same flavonoid and total polyphenol content as a freshly brewed cup of rooibos herbal tea (Joubert & de Beer, 2012:49). Findings from experimental studies indicate that iced teas produced from rooibos extract can contribute an appreciable amount of aspalathin if the formulation and processing of iced teas is done with caution during the manufacturing process. The aspalathin level can decrease in stored iced teas, which is linked to the stability of aspalathin during the storage period. The aspalathin stability depends on the type of the extract and the product formulation. Milk-based rooibos products do not retain aspalathin due to the relative high pH (Joubert & de Beer, 2011:880). A lower pH enhances aspalathin stability (De Beer *et al*., 2011:274).

2.5.3 Preparation method

Peterson *et al*. (2004:401) determined the influence of the tea variety and brewing techniques on the catechin, theaflavin and thearubigin contents of black tea and reported that brewing techniques influence the flavonol content. The flavonoid content of prepared tea was found to be higher if the brewing time is four minutes or longer, as opposed to shorter brewing times (two minutes). The amount of water used affects the strength of the infusion but it does not affect the total amount of flavanols extracted from the dry tea. The weight of the tea used, the tea variety used and the length of the brewing time applied primarily determines the total flavanol content (Peterson *et al*., 2004:403). Peterson *et al*. (2004:401) additionally confirmed that consumers do

not infuse their tea bag for long enough for a more complete compound withdrawal and as a result overestimate their flavonoid intake.

Sharpe *et al*. (2016:380) investigated the effects of brewing conditions on the antioxidant capacity of several green tea varieties. According to the study results, a brewing time of five to 10 minutes at a temperature of 80 to 100^0C will result in an infusion with a higher TAC than for infusions brewed for a shorter period and at a lower temperature (Sharpe *et al*., 2016:385). The experimental study of Ryan and Petit (2010:16) also indicated that the antioxidant potential increased with the infusion time. All the tea brands they investigated followed the same trend of a gradual increase. A maximal value was obtained after ten minutes; however, more than 50% of the maximum value was obtained after only one minute of brewing. More than 95% of the antioxidant capacity was available after five minutes and more than 90% available after four minutes for all of the teas.

Campanella *et al*. (2003:731) studied the effect of different brewing times on hot tea infusions. Hot infusions with distilled water, but with various different infusion times (one, three, five and 10 minutes), for black and green tea were prepared. The analysis performed on the regular tea bag infusions showed that a hot tea infusion brewed for five minutes produced the highest antioxidant capacity. The antioxidant compounds increased at the onset of the infusion and continued to increase but after five minutes, the antioxidant capacity decreased. The researchers provided the following explanation for the results. The hot water's ability to extract the antioxidants present in the tea increases with an increased infusion time from the onset. However, numerous antioxidant constituents probably begin to swift out or begin to create miscelles in solution after five minutes, which occurs due to the steady cooling. Another explanation, although less likely, is the partial thermal degradation of certain antioxidant substances as a result of the high temperature, which leads to the entire mixture's antioxidant capacity to decrease after an infusion time of more than five minutes (Campanella *et al*., 2003:732).

Komes *et al*. (2010:173) indicated that a shorter infusion time with a higher water temperature and a longer infusion time with a lower water temperature are the combinations allowing for the optimal withdrawal efficiency of bioactive compounds of green tea. Venditti *et al*. (2010:1599) tested several teas (black, oolong, green, white and lyons), prepared in either hot or cold water, for delivering the higher total polyphenol content. With both black and green tea the total polyphenol content was higher with the beverages prepared from hot water. According to Arab

and Il'yasova (2003:3317S) the health promoting compounds present in tea seem to loose its efficiency and the amount reduced when the beverage has cooled. This occurs because these compounds precipitate.

2.5.4 Tea additions

The processes involved in the production of black tea results in a tea with a bitter taste, which is often the reason for black tea being consumed with milk (Langley-Evans, 2001:75) and sugar (Sharma *et al*., 2008:124). A study investigated the effect of added sugar and milk on the antioxidant status of black tea. Black tea with only sugar added, black tea with only milk added, black tea with milk and sugar added and plain black tea were prepared for the analysis. The results indicated that the antioxidant activity of black tea is enhanced and stabilised with sugar or milk added (Sharma *et al*., 2008:124). A different study conducted by van Het Hof *et al*. (1998:356) assessed blood catechin levels after black or green tea consumption and the influence of adding skim milk to black tea. The study findings indicated that black and green tea catechins are quickly absorbed and that milk does not weaken the bioavailability of these flavonoid compounds.

Hollman *et al*. (2001:297) performed a study that evaluated the influence of milk addition to tea on flavonol absorption from tea in 18 healthy subjects. Each subject consumed only two of the following four beverages for a period of three days: black tea without additions (plain black tea), black tea with added milk, plain green tea and water. One cup of the beverage was consumed every two hours resulting in an intake of eight cups daily. The black tea (135 mL) itself was consumed with 15 mL milk to represent the black tea with added milk sample. The findings indicated that the flavonols from tea are absorbed and that milk addition does not influence the bioavailability. Studies done by van Het Hof *et al*. (1998:356), Leenen *et al*. (2000:87) and Catterall *et al*. (2003:3863) additionally confirmed that milk addition to tea does not reduce the bioavailability of the antioxidants present in tea.

Ryan and Petit (2010:14) also evaluated the influence of added skim, semi-skim and whole milk in various amounts on the antioxidant capacity of prepared black tea samples from five brands. The results found were in contrast to that of the above studies with regards to milk addition to tea. The TAC of all the black tea brands evaluated decreased when 10 mL, 15 mL and 20 mL of bovine milk (skim, semi-skim and whole milk) were added to a 200 mL tea infusion. For all the brands of black teas that were compared, the addition of the skim milk significantly ($p < 0.05$)

reduced the TAC in comparison to the tea with the same volume of water added (Ryan & Petit, 2010:14). The study concluded that the effect of milk on the *in vitro* TAC may be related to the fat content of the milk. A number of fat-soluble antioxidants are present in milk (tocopherols, retinols and carotenoids). Therefore, the antioxidant potential of milk is decreased when its fat content is decreased which is due to the reduction in the fat soluble antioxidant components present (Ryan & Petit, 2010:17). A study was also undertaken by Ryan and Sutherland (2011:3115) to determine whether the addition of different types of soya milk or semi-skim bovine milk decreased the TAC of black tea. Soya milk addition to black tea was found to be a valuable substitute to using the semi-skim form of bovine milk for persons who wish to uphold the total antioxidant potential of a cup of tea.

In a study performed by Dubeau *et al*. (2010:539), the influence of milk addition on the antioxidant capacity of green, Darjeerling and English breakfast tea was investigated. The mean antioxidant capacity of the teas with milk addition was significantly ($p < 0.05$) lower than those with no milk (the plain teas). The researchers stated that milk decreased the electron donation ability of tea polyphenols at the electrode surface. The different teas were not equally affected by the addition of milk. It was suggested that some of the polyphenol compounds present in the teas might interact more readily with milk than others (Dubeau *et al*., 2010:542). Savage *et al*. (2003:415) also confirmed the antioxidant reducing effect associated with milk addition to tea. The study confirmed that regular tea consumption with added milk will result in the absorption of only a small amount of oxalate from the tea.

Langley-Evans (2000:309) performed a study with nine healthy adults for three days. The objective of the research was to evaluate the contribution of black tea flavonoids to the plasma TAC using the FRAP assay. On the first day of the study the participants consumed no tea. On the second and third day they consumed either black tea with or without milk at hourly interims from 09:00 to 14:00. Subjects who consumed no tea showed an unchanged plasma FRAP through the evaluation period. Those consuming black tea with milk showed no significant changes in the plasma FRAP between 09:00 and 12:00 and a 50% higher plasma FRAP between 12:00 and 15:00 which was not statistically significant. Considering those participants who consumed plain black tea, the plasma FRAP was significantly higher (65%; $p = 0.02$) between 09:00 and 12:00 and at 15:00 it was still higher than at 09:00 (76%; $p = 0.002$). This research also established that milk addition to black tea negatively influenced the antioxidant content.

Honey is also sometimes added to tea as a natural sweetener. Substances present in honey, such as vitamins, minerals, proteins and antioxidants are of nutritional value (Ajibola *et al*., 2012:61). Toydemir *et al*. (2015:127) determined the effects of honey addition on the antioxidant properties of different herbal teas. Two types of honey (flower and pine honey) were added to nine different herbal teas (melissa, ginger, fennel, green tea, rosehip, linden, daily, sage and echinacea) at different temperatures. The total phenolic content and the TAC of the tea samples with added honey were higher (up to 57% for both the honey types) than the samples without honey addition (Toydemir *et al*., 2015:127).

Campanella *et al*. (2003:734) conducted tests to determine the TAC of tea with different additions, for example a normal cup of tea, with or without lemon or herbal flavours added or milk added. Tea flavoured with camomile had the lowest TAC, while green tea and tea with lemon added had the highest TAC. Bottled tea with lemon added had a higher TAC than bottled tea with peach added (Campanella *et al*., 2003:734). Hot tea with added lemon or milk respectively resulted in an increased and a decreased antioxidant capacity, in comparison to a normal tea infusion. The increased antioxidant capacity of the lemon infused tea was said to be due to the ascorbic acid present in lemon (Campanella *et al*., 2003:733). Majchrzak *et al*. (2004:447) through *in vitro* experimentation compared the antioxidant activity of several types of tea and evaluated the effect of the addition of lemon juice on the TAC. The researchers found that the tea samples with added lemon or lemon juice had a higher TAC than those without lemon juice added (Majchrzak *et al*., 2004:450). The same results were reported by Komes *et al*. (2010:175). These researchers found that the addition of lemon juice resulted in an increased antioxidant capacity of all the green teas studied. Commercially flavoured teas are popular in European countries because of its flavour and therapeutic properties. Dried fruit aromas or natural aromas are added to these teas as a last step before the packaging (Pekal *et al*., 2011:681).

2.6 Consumer tea drinking behaviour

The methods of how tea is prepared and consumed vary worldwide (Astill *et al*., 2001:5340; Venditti *et al*., 2010:1597). Consumer tea and overall beverage consumption differ and is dependent on consumer gender, ethnicity / race and age (Storey *et al*., 2006:1992). Factors such as the local temperature, cultural habits and income also influence the different ways in which tea is consumed (De Godoy *et al*., 2013:802).

2.6.1 Profile of tea consumers

Hakim *et al*. (2000:1715) reported on the consumption patterns, composition and preparation of tea-based beverages in Arizona. A detailed tea questionnaire was compiled to obtain the information. The results concluded that more women (35%) than men (29%) indicated drinking tea (non-herbal) at least once a week, with more men (38%) consuming no tea in the previous year than women (27%). More women (34%) preferred brewed iced tea than men (16%). From the population under study, 66.4% of the participants indicated consuming tea during the previous year (Hakim *et al*., 2000:1722).

Geleijnse *et al*. (1999:2172; 2002:884) commented that in Western populations, tea consumption is mostly linked with a healthier diet and lifestyle. From the results of the study that investigated the link between tea consumption and aortic atherosclerosis, the researchers reported that tea consumption was generally associated with leaner, higher educated people and those that take vitamin antioxidants. Consumers that drink larger amounts of tea also have a lower intake of total fat, alcohol and coffee and do not smoke as much as those that consume less tea. The number of tea consumers was high in both men (84%) and women (91%). Female tea consumers on average consumed more tea than male tea consumers (438 vs 375 mL) (Geleijnse *et al*., 1999:2170).

The results from the Saudi Arabia National Study indicated that most men (91.8%) and women (87.2%) had a daily intake of black tea. Women consumed almost three and a half (3.4) cups of black tea and men five and a half (5.6) cups of black tea, with a cup equalling 80 mL tea. The daily consumption of six or more cups of black tea was indicated by 11.9% of women and 26.4% of men (Hakim *et al*., 2003:66). Compared to those that consume less tea (one to three cups and four to six cups daily), the heavy tea consumers (more than six cups daily) were more likely to be younger, male, current smokers, coffee consumers and having a lower dietary fat intake

and a lower treatment rate for blood pressure and diabetes mellitus (all $p < 0.01$) (Hakim *et al*., 2003:67).

Mukamal *et al*. (2002:2476) indicated from their prospective cohort study of 1 900 hospitalised patients with acute myocardial infarction that 1 019 patients did not consume tea, while 615 patients consumed less than 14 cups of tea weekly and only 266 consumed more than 14 cups of tea weekly. The researchers reported that the heavy tea consumers (> 14 cups weekly) in the study were more likely to be women, older and to have a lower BMI than those patients drinking less and no tea weekly, but with similar education and household income levels. The incidence of hypertension and smoking status were also the same in these two patients groups (Mukamal *et al*., 2002:2477). A higher percentage of heavy tea consumers also consumed no alcohol and less coffee than those patients drinking less and no tea weekly (Mukamal *et al*., 2002:2478).

From their research on the association between black tea consumption and risk of total stroke and stroke types in a prospective study, Larsson *et al*. (2013:158) also commented on the sociodemographic characteristics of the study population. Compared with men and women who never consumed tea, those who consumed four or more cups per day were more likely to have a post secondary education and a lower BMI. These heavy tea consumers were also less likely to be smokers or to have a family history of hypertension. In addition to these characteristics, these heavy tea consumers moreover tended to eat more fruits and vegetables but drink less alcohol and coffee than those who did not consume tea.

Storey *et al*. (2006:1992) assessed the consumption of beverages across factors such as gender, ethnicity / race and age in the US population. The researchers used publicly available dietary survey data from the National Health and Nutrition Examination Survey (NHANES) 1999 - 2002. The results indicated that young children had a higher average intake of tea compared to coffee, but which was still low compared to the amounts of the other beverages consumed. The results further indicated that white children had a higher intake of tea and coffee with sugar than African-American children. Considering the adolescents, the average tea intake was higher among white than African-American teenagers. The researchers indicated that the highest tea intake was among white adults, aged 20 to 39 years, 40 to 59 years and 60 years and older. The Mexican-American and African-American adults, in contrast consumed small amounts of tea. In this study for all the race and age groups, boys / men had a higher tea intake than girls / women, except for the men and women aged 40 to 59 years where women had a slightly higher average tea intake (245.7 g) compared to the men (203.8 g). Song and Chun (2008:1543S) used the

same survey data referred to in the above paragraph (NHANES 1999 – 2002) to examine, among other factors, the sociodemographic characteristics of tea consumers in the US population. The study reported that older persons, females and those with a higher income are likely to be the tea consumers ($p < 0.001$). Walcott (2012:359) reported that previous research indicated that the largest US tea consumer category consisted of households with no children and with an income in the lower-middle and highest socioeconomic class groups. Tea consumption was also higher in the north-western part of the US with the consumption on average higher in the rural parts than in the urban areas.

2.6.2 Consumer tea drinking preferences

Worldwide, black tea is more popular than green tea (Tang *et al*., 2009:274; Hodgson & Croft, 2010:496; Venditti *et al*., 2010:1597; Pan *et al*., 2013:12). Tang *et al*. (2009:274) reported that worldwide 78% of the population consumes black tea compared to 20% that consumes green tea. The results from the Arizona-study indicated that green tea was the least preferred (8.7%), with most participants consuming iced black tea (51.8%) and hot black tea (30.7%) (Hakim *et al*., 2000:1717). Most (96.3%) tea consumers in the Chinese population prefer green or oolong tea (Yang *et al*., 2004:1534). Many Americans prefer iced tea prepared from hot tea and cooled with ice (Venditti *et al*., 2010:1597). Only nine percent of the participants from the Arizona-study indicated preparing iced tea with cold water-soluble instant tea (Hakim *et al*., 2000:1722).

Tea drinking is popular in Saudi Arabia where the tea preparation normally involves brewing it in a teapot and consuming it in small teacups (80 mL each) (Hakim *et al*., 2003:65). The Japanese prefer their tea in a more diluted form than South African tea consumers (Joubert *et al*., 2008:377). They use green tea leaves that are steeped in hot water for about two minutes. They reuse these leaves for two to three infusions. The Chinese population, on the other hand, steep tea leaves in hot water for only 20 to 40 seconds. These leaves are often repeatedly used for up to seven times (Venditti *et al*., 2010:1597). Most of the tea consumers from the Arizona-study consumed black tea of medium strength – 68.5% brewing their tea for two to three minutes and only 19.4% brewing their tea for more than three minutes (Hakim *et al*., 2000:1717). Hot (90.4%) or warm (9.6%) tea was mostly preferred by the participants (Hakim *et al*., 2000:1719). Most of the participants also prepared their hot black tea by infusing a tea bag (2.26 g) in 240 mL hot water (Hakim *et al*., 2000:1722).

De Godoy *et al*. (2013:801) used a questionnaire to assess consumers' taste preferences, consumption, behaviour and beliefs on Brazilian mate tea [a tea prepared using the leaves of the locally known plant, yerba mate (*Ilex paraguariensis*)]. On the question regarding the preferred type of mate tea consumed, 95% of the participants indicated using tea bags and 48% iced teas, with only five percent who indicated using the loose tea leaf form and two percent instant tea. With regards to flavoured tea bags, most (69%) of the participants preferred the natural flavour of mate tea with about a third (35%) that preferred mate tea flavoured with lemon. Iced teas flavoured with peach and lemon were also preferred (25% and 24% respectively), with 29% still preferring the natural taste. The temperature of the mate tea was preferred by 61% of the participants to be hot. Most (90%) participants indicated consuming tea before bedtime. Mate tea was also consumed either at breakfast (36%) or between meals (37%) and most (90%) of the consumers consumed their tea at home (De Godoy *et al*., 2013:803).

In a study by Bryan *et al*. (2012:342) which assessed the relationship between beverage consumption on mood and work performance, participants indicated consuming a range of none to seven and a half cups of tea per day, with the mean intake one cup per day. Milk alone was added most to all the beverages consumed (tea, coffee, other caffeinated and decaffeinated beverages) and second most was milk and sugar. Sugar alone added to the beverages was the additive that was added by the lowest number of participants.

In Canada, the UK and Ireland tea is enjoyed with a considerable amount of milk added to it in addition to sugar also sometimes being added. The Japanese mostly consume their diluted tea without milk (Sharma *et al*., 2008:124). The effect of habitual tea intake on hypertension in the Chinese population was assessed by Yang *et al*. (2004:1534). The researchers found that of the 600 habitual green or oolong tea consumers, less than five percent (4.8%) added milk to their tea.

2.7 Questionnaire compilation for assessing consumer tea and herbal tea drinking behaviour

Consumer research commonly involves the completion of a questionnaire. The compilation of the questionnaire is an important part of a study (Kemp *et al*., 2009:121) and will have an important influence on both the quality of information received and the response rate (Murray, 1999:148). A questionnaire not only permits the collection of the required data from the respondent but also offers a structure and reliable format for the gathering of the question responses (Kemp *et al*., 2009:121).

2.7.1 Questionnaire construction

The compilation of a questionnaire is a complex process and is time consuming. A sufficient input of time and effort is needed to ensure that the tool developed limits errors of completion and comprehension on both the researcher's and respondent's part (Murray, 1999:148). Before starting with the questionnaire, questions such as 'what is the purpose of the research?', 'what is the research question to be answered?' and 'is the questionnaire the best method of acquiring this information?' must be asked and thought through (Murray, 1999:149).

Question wording formulation should cover aspects relating to literacy, comprehension and cultural background, as well as the age of the target population (Murray, 1999:149). Questions on the same subject should be grouped together for unity, and questions should have a logical flow from one topic to another. Important questions should be asked first (Murray, 1999:151).

Questions can either be open or closed. With open questions, the respondent must formulate his / her own answer, in his / her own words. These questions are time consuming to answer and could lower the response rate. Handwriting could be a problem with this type of question. Factors such as the size of the handwriting, the depth of the required response and the subject under investigation should be considered when allocating the provided space for answering. Too many open questions in a questionnaire can have a negative effect, while having too few could indicate that individual comprehension is not questioned (Murray, 1999:150).

Different forms of closed questions can be used. Dichotomous questions, providing a *Yes* or *No* answer should be limited in order to reduce successful guessing (Murray, 1999:150). Statements with tick box categories, such as *Yes, No, Don't know*, measures general attitude, is easily understood and quick to complete. These statements generate data suitable for non-parametric statistical analysis (Boynton & Greenhalgh, 2004:1313). A fixed or multiple choice question is where the respondent can choose from a list of alternative answers as the response. Checklists, where the respondent is requested to tick all the boxes appropriate to him or her, can also be used. Caution must be taken to make sure that the list of alternatives is complete, although the inclusion of an open question, such as 'other ... please specify' is a suitable possibility to ensure that a respondent can indicate his / her response where not included on the provided list. In a ranking question respondents are asked to state their order of preference from a pre-arranged response list. The drawbacks with this type of question are that offering too many response options could result in confusion and respondents may have equal preference for two response items (Murray, 1999:150).

The following are some guidelines on questionnaire compilation necessary for consideration:

- questions should be short (Kemp *et al*., 2009:122), 12 words or less (Marshall, 2005:132);
- questions should be simple (Kemp *et al*., 2009:122);
- question wording choice should be relevant to the respondent vocabulary (Hein *et al*., 2008:657);
- questions should be kept within the respondent capability;
- two questions should not be asked in one;
- appropriate instructions should be provided on how to complete the questionnaire;
- it should be decided beforehand how each response will be entered into spreadsheets for the analysis of the data (Kemp *et al*., 2009:122). Scale responses to a statement can be scored by assigning it a specific value, depending on the degree of agreement or disagreement with the statement and a total sum for the scale calculated for each respondent. This total sum is then interpreted by the researcher during data analysis (Murray, 1999:150); and
- the questionnaire should be pre-tested with a representative group of the individuals envisaged for participation (Kemp *et al*., 2009:122).

2.7.2 Questionnaire pilot testing

After the questionnaire has been compiled, it must be pilot tested. This will identify any difficulties with the questionnaire in terms of language, clarity and comprehension before the questionnaire is sent out to the required population (Murray, 1999:152). This phase of the study can enhance the validity and reliability of the questionnaire (Marshall, 2005:135) although it does not mean that the questionnaire is valid and reliable (Boynton & Greenhalgh, 2004:1313).

Adequate time and resources should be allocated for the pilot test to allow for necessary modifications and should be repeated until the questionnaire is correct and user friendly. The questionnaire should be tested on a part of the target population (Murray, 1999:152; Rattray & Jones, 2007:237). There is no fixed rule which refers to the sample size necessary for the pilot testing. The correct sample size depends on the purpose of the study and the nature of the population being investigated (Cohen *et al*., 2000:101). It is also advised that the questionnaire must be proof read by an objective outsider. The questionnaire can then be sent out with instructions on how to complete it and when it is to be returned to the researcher (Murray, 1999:152).

2.7.3 Questionnaire validity

A valid questionnaire measures what it says it measures (Boynton & Greenhalgh, 2004:1313; Rattray & Jones, 2007:238; Bannigan & Watson, 2009:3238). Validity contains the concepts of concurrent, construct, face, discriminant, predictive, content, factorial, criterion and convergent validity (Bannigan & Watson, 2009:3238). Face validity and content validity are two narrowly linked forms of validity and are a minimum requirement of acceptance of a measurement tool. Face validity is the fastest method of determining validity. It is an assessment of whether a measurement tool looks sensible and practical. Face validity is tested by experts or by the researcher(s) involved. An assessment of face validity is essential because suitability of a measurement tool is important to its value (Bannigan & Watson, 2009:3240).

Content validity refers to expert opinion concerning whether the items represent the proposed fields or ideas the questionnaire is intended to measure (Rattray & Jones, 2007:238; Bannigan & Watson, 2009:3240). Content validity is therefore performed to attain authenticity - to confirm that all concepts relevant to the construct of interest are included in the instrument. Content

validity is normally assessed via a critical review by an expert panel for clarity and completeness or by comparing it with the literature or by using both (Bannigan & Watson, 2009:3240).

2.8 Total polyphenol, flavanol, flavonol and total antioxidant capacity assay methodology

In order to establish the total polyphenol, flavanol and flavonol content and TAC of food or beverage samples, several methods have been designed and used in the industry.

2.8.1 Assay for testing the total polyphenol content

The chemistry behind this method relies on the movement of electrons in a phase from reducing species and phenolic compounds to molybdenum, resulting in the formation of blue complexes. This can be spectrophotometrically monitored at 750 to 765 nanometer (nm) (Magalhaes *et al*., 2008:15). According to Singleton and Rossi (1965:144) this assay is grounded on the reaction between the Folin-Ciocalteu (FC) reagent and the aromatic group in polyphenols. The reaction yields a blue-coloured product.

The FC reducing capacity assay is useful for assessing the TAC of food samples. The assay is not complicated, it is reproducible and convenient because the reagent can be purchased commercially, it follows a standardised procedure, and the absorption of the specific sample at a long-wavelength minimises the influence from the matrix of a sample. Disadvantages include the fact that the assay involves a long process (two hours) which will make routine analysis difficult. It is furthermore carried out in water, and can therefore not be used for lipophilic compounds (Magalhaes *et al*., 2008:15). The FC method does not reflect total phenolics, although it measures the reducing capacity of a sample (Karadag *et al*., 2009:48).

2.8.2 Assay for testing the flavanol content

An assay using hydrochloric acid (HCl)-acidified 4-dimethylaminocinnamaldehyde (DMACA) is used to test for the flavanol content (Li *et al*., 1996:89). The product to be tested must be freeze-dried and grounded in order to pass through a 0.6 mm mesh. The flavanol contents are expressed as mg catechin equivalents (CE) per L (mg CE/L) (Li *et al*., 1996:89).

2.8.3 Assay for testing the flavonol content

The method of Mazza *et al*. (1999:4010) applies to the reaction of the HCl reacting with the flavonols. Quercetin is used as standard. This method consists of filling a test tube with the standard or sample, 4.55 mL of 2% HCl and 0.1% HCl in 95% ethanol. The solution is then mixed and left to stand for about 15 minutes before taking the reading of absorbance at 280 nm with a spectrophotometer. Results for the flavonol content are expressed as mg quercetin equivalents (QE) per L of beverage (mg QE/L) (Mazza *et al*., 1999:4010).

2.8.4 Assays for testing the total antioxidant capacity

Several steps are involved in determining the TAC of a food or beverage sample. These are: preparing the sample to be evaluated, extracting the antioxidants, measuring the antioxidant capacity and consequently reporting the results (Perez-Jiménez *et al*., 2008:283). The most widely used procedures to measure the TAC are the oxygen radical absorbance capacity (ORAC), 2,2'-azinobis-3-ethylbenzthiazoline-6-silphonic acid (ABTS), DPPH and FRAP (Pérez-Jiménez *et al*., 2008:274). The ABTS or Trolox equivalent antioxidant capacity (TEAC), DPPH and ORAC assays measure a sample's free radical scavenging ability, while the FRAP assay measures the metal reducing ability of a sample (Pérez-Jiménez *et al*., 2008:280).

2.8.4.1 ORAC assay

The improved ORAC assay uses fluorescein (FL) as fluorescent probe instead of B-phycoerythrin (B-PE). This improved method measures the hydrophilic chain breaking antioxidant ability against the peroxyl radical directly (Ou *et al*., 2001:4619). The distinctiveness of this assay is that the TAC of a sample is determined on completion of the oxidation reaction (Cao *et al*., 1993:303). Broadly, the controls, the samples and the standard are mixed with a FL solution and incubated at a constant temperature of 37^0C before the addition of 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) which starts the reaction (MacDonald-Wicks *et al*., 2006:2050). The ORAC assay is done for extended periods in order to obtain accurate results and not underestimate the antioxidant capacity as well as to consider the effects of secondary antioxidant compounds (Karadag *et al*., 2009:45).

Advantages of this assay include: it uses applicable biological free radicals, the assay is standardised which allows for inter-laboratory comparisons and it assimilates both the time of the antioxidant reaction and degree of action. A further advantage is the capability of using different oxidants which is an important factor for the measured antioxidant capacity of a sample. This application is important as the measured antioxidant capacity of a sample depends on which oxidant is used in the method (Karadag *et al*., 2009:45). Disadvantages are that it normally requires the use of costly equipment, the differences in data can be large across the equipment used, the method is sensitive to pH and it requires quite a long time to quantify results (Zulueta *et al*., 2009:311). The outcomes from validation studies clearly demonstrate that the ORAC method is antioxidant specific and that it is precise, sensitive and strong within accepted criteria (Ou *et al*., 2001:4624).

2.8.4.2 ABTS assay or TEAC assay

The ABTS or TEAC assay is a decolourisation assay which is suitable for both hydrophilic and lipophilic antioxidants (Re *et al*., 1999:1231; Floegel *et al*., 2011:1047), including flavonoids. It is also applicable for investigating pure food extract and compounds (Re *et al*., 1999:1232; Karadag *et al*., 2009:48). A more appropriate format of this assay has been developed in that the radical is created straight into a steady form before the reaction with a supposed antioxidant (Re *et al*., 1999:1231). The ABTS reaction is created by the oxidation of ABTS with the compound potassium persulfate, and is condensed in the presence of hydrogen donating antioxidants. Factors such as the effect of the extent and concentration of the antioxidant reaction on the inhibition of the radical cation absorption are considered when measuring the antioxidant activity (Re *et al*., 1999:1231). Floegel *et al*. (2011:1046) proposed that the ABTS method gives a better reflection of the antioxidant content in various foods compared to the DPPH.

Advantages of this assay include: economical and easy to use, stable to pH (Zulueta *et al*., 2009:311) in the sense that it allows study over a varied range of pH (Karadag *et al*., 2009:48) (hence it can be used to determine the effect of pH on activity) and it has a fast reaction. Disadvantages include: an extra step is necessary to create free radicals from ABTS salts necessary for the assay, the created free radical is unstable and the assay is not standardised, therefore it cannot be used to compare values across laboratories (Zulueta *et al*., 2009:311).

2.8.4.3 DPPH assay

The DDPH has a rich purple colour and can be referred to as an organic nitrogen radical. The purple chromogen radical is changed to a corresponding lighter yellow hydrazine by antioxidant compounds. The ability of an antioxidant to reduce towards DPPH can be determined by monitoring the decreased absorbance at 515 to 528 nm until it stabilises, or by the resonance of the electron spin (Karadag *et al*., 2009:49).

This is a quick and technically easy assay. A UV-visible spectrophotometer is the only instrument that is needed to perform the assay (Karadag *et al*., 2009:49). Drawbacks associated with this method are that another compound can absorb at 515 nm; there could be asteric interference for the molecules with an increased molecular weight; and *in vivo,* the free radical that was used is not present and it is relatively stable, unlike radicals present in living organisms (Pérez-Jiménez *et al*., 2008:281). Karadag *et al*. (2009:49) reported on a limitation in the sense that the DDPH cannot be dissolved in aqueous media, only in organic media.

2.8.4.4 FRAP assay

The FRAP assay is grounded on the effect of phenolics which can alter the complex yellow ferric tripyridyltriazine to a blue ferrous complex. This is done by the electron donating antioxidant actions (Benzie *et al*., 1999:147). The only difference between the FRAP and TEAC assays is that the TEAC assay is performed at a more neutral pH compared to the FRAP assay, which performs in a more acidic environment to ensure iron solubility (Karadag *et al*., 2009:50).

Advantages of the FRAP assay is that it is rapid, robust, not expensive, easy to perform and does not need special or expensive equipment (Karadag *et al*., 2009:50). Disadvantages of using this method include: absorption of other compounds may occur at 595 nm; a compound having a redox status less than 0.77 volt, although not necessarily an antioxidant *in vivo,* may decrease iron and it is conducted at a pH which is non-physical (Pérez-Jiménez *et al*., 2008:281). Compounds acting by radical quenching can also not be detected by the FRAP assay (Karadag *et al*., 2009:50).

2.9 Summary

Oxidative stress, brought about by internal and external bodily factors, leads to the development of various non-communicable diseases which can be controlled by following a healthy lifestyle incorporating an adequate diet supplying sufficient dietary antioxidants. Antioxidants are predominantly found in fruit, vegetables and beverages, particularly tea, coffee, fruit juice and red wine in addition to herbs (Dragland *et al*., 2003:1286). Ample research has been undertaken which indicates that tea, in general, is indeed a healthy beverage to consume due to its antioxidant provision. This might be a contributory reason as to why tea is a popular beverage consumed worldwide (Yang *et al*., 2004:1534). Several studies have indicated that if tea is consumed in sufficient amounts, it can have positive risk reduction effects on diseases such as cancer and CVD (Dufresne & Farnworth, 2001:404). Green and black teas have been studied widely. These teas, together with oolong tea and honeybush (another South African herbal tea) contain valuable antioxidants (especially the flavonoid subgroup catechins) (Peterson *et al*., 2005:488) in variable amounts.

Rooibos, as a herbal tea beverage and dietary antioxidant source, is the focus of this research. Flavonoids are the group of antioxidants prominent in teas and herbal teas, such as rooibos. Flavonoids are important polyphenols which have gained popularity and a lot of attention, especially in health research related to tea consumption. Several animal and human studies have been performed utilising the different types of tea which have confirmed beneficial effects of tea consumption in relation to various health aspects, particularly in relation to green tea consumption, with it receiving a qualified health claim in relation to cancer risk reduction.

Rooibos herbal tea is rich in antioxidants and has an unique flavonoid content. It contains aspalathin, a flavonoid only found in rooibos herbal tea (Erickson, 2003:39). This herbal tea, which is produced in SA, has gained popularity over the last few years due to its antioxidant content and health properties. Most studies performed on rooibos herbal tea has been performed with the fermented form of rooibos. Rooibos is nowadays available in different types, forms and flavours as herbal tea and also incorporated in cosmetic products and dietary supplements. Rooibos herbal tea is normally flavoured in the household with milk, sugar or honey, as well as commercially utilising herb and fruit flavours. Iced teas are becoming a popular alternative for soft drinks, with rooibos iced teas also available on the market.

There are several factors which influence a cup of tea's polyphenol content and antioxidant capacity. Factors such as the type, form, added flavouring and preparation method might influence the polyphenol content and antioxidant potential either positive or negative. From the available literature it seems that the unfermented form of rooibos herbal tea yields a higher total polyphenol content and TAC. It also seems that using tea leaves or tea powder for the infusion results in a cup of tea with a higher polyphenol content and TAC and that a longer brewing time also increases the polyphenol content and TAC. Teas are flavoured in the household according to taste and also commercially with different herbs or flavourants. Studies performed on the flavouring of tea have provided conflicting results with respect to the antioxidant capacity, especially regarding the addition of milk and sugar. This research will broaden these results but specifically pertaining to the effects of the different types, forms, preparation methods and flavourings coupled to rooibos herbal tea. By consuming an optimal cup of rooibos herbal tea, it might uphold further health benefits by providing an increased total polyphenol intake and dietary TAC. The total polyphenol content and TAC of a food or beverage can be analysed with various assays available to determine the TAC, each having its advantages and disadvantages.

Studies that investigated the association between tea consumption and the incidence of various diseases reported that tea consumers tended to be older, female and those with a healthier lifestyle and higher level of education. The method to prepare and consume tea differs worldwide. The type of tea selected to drink, the brewing method applied and the flavourings added by consumers are directed by their individual tastes.

CHAPTER 3
RESEARCH DESIGN AND METHODOLOGY

The methodology of this study consisted of two phases: Phase 1 dealt with the selection and preparation of rooibos herbal tea samples which included different rooibos herbal tea types, forms, brewing times and flavourings for the analysis of the total polyphenol, flavanol and flavonol content and the TAC that provided the composition information for a database to screen the rooibos herbal tea samples for identification of those with the highest polyphenol content and TAC; and Phase 2 obtained information (using a questionnaire as research tool) on how consumers drink their rooibos herbal tea (bearing in mind the identified rooibos beverage with the highest total polyphenol content and TAC in Phase 1), as well as identifying the demographic and lifestyle information of rooibos herbal tea consumers, and in particular those who consume rooibos as a prepared beverage providing the highest total polyphenol content and TAC.

3.1 Study design

For the first phase of the study different rooibos herbal tea types, forms, brewing times and flavourings (added household and commercially flavoured) were considered as factors which influence the total polyphenol content and the TAC of rooibos prepared as a beverage. It incorporated a rooibos sample selection and preparation of these samples as beverages and analysis of the total polyphenol content and the TAC of these samples in triplicate (n = 3). An experimental study of a comparative design was followed for this first phase. The study design allowed for consideration of the factors (independent variables) which may cause or influence a particular condition or situation (dependent variable) (Leedy & Ormrod, 2010:223) which in this study related to the different rooibos herbal tea types, forms, brewing times and flavourings as factors which influence the total polyphenol content and the TAC as the condition of rooibos prepared as a beverage.

The second phase of this study followed a quantitative research design conducted as a descriptive survey. In quantitative research the aim is to determine the relationship between one characteristic (an independent variable) and another (a dependent or outcome variable) in a population (Hopkins, 2000:1). This phase was performed to assess the ways in which consumers drink rooibos herbal tea and to determine the rooibos consumer demographic, lifestyle and health profile and, in particular, of those who consume an optimal cup of rooibos herbal tea in the provision of the total polyphenol content and the TAC. A detailed questionnaire

was compiled as the research tool to obtain the required information. Figure 3.1 provides a schematic flow diagram of the study design, presenting the major methodological steps for the two study phases.

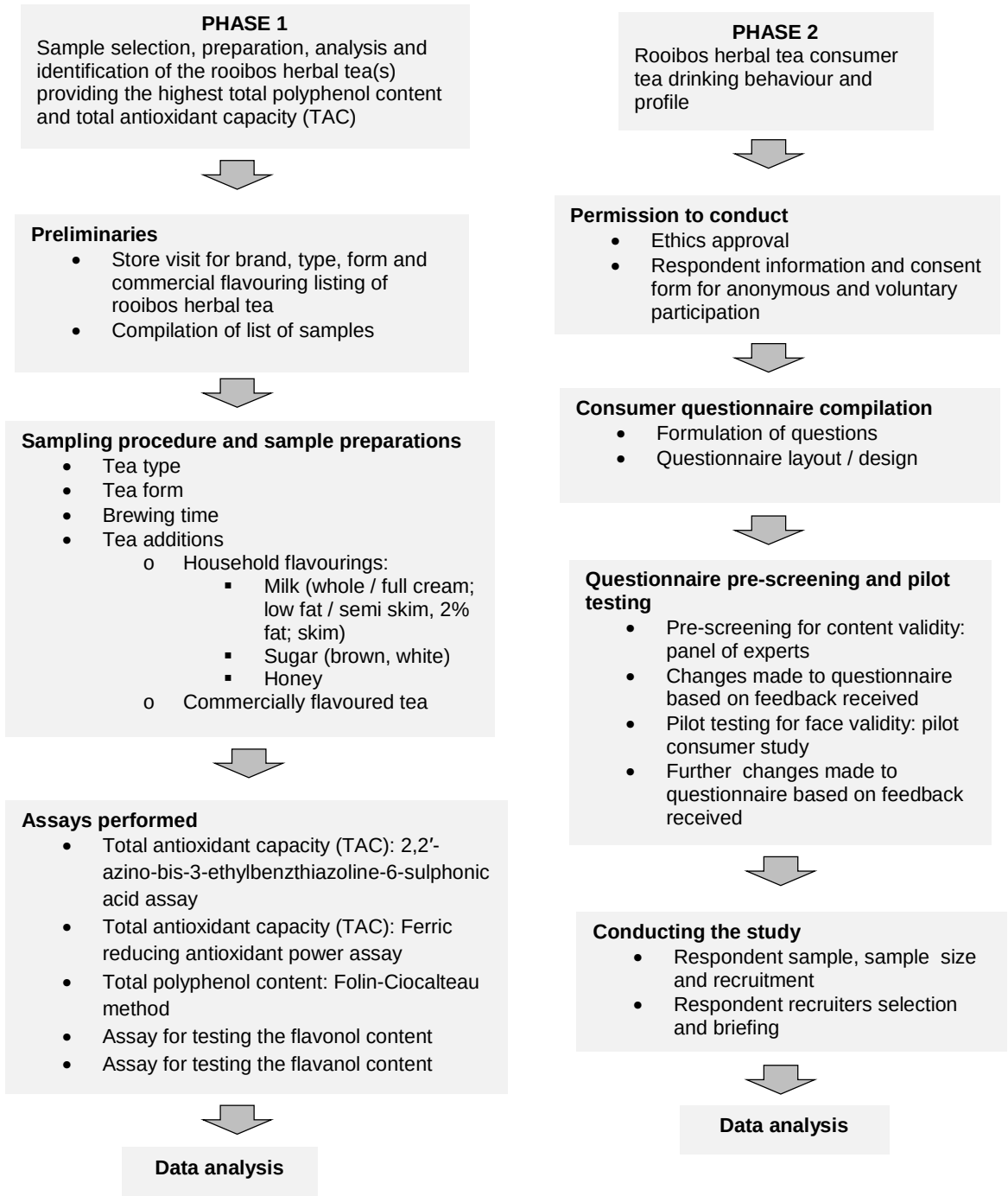


Figure 3.1: Schematic flow diagram of study design presenting the major methodological steps for the two study phases

3.2 Phase 1: Sample selection, preparation, analysis and identification of the rooibos herbal tea(s) providing the highest total polyphenol content and total antioxidant capacity

A total of 667 rooibos samples were prepared and analysed to represent different rooibos beverage options; with the researcher responsible for the preparation of the samples, the sample extractions and dilutions at the Oxidative Stress Research Centre (OSRC), Cape Peninsula University of Technology (CPUT) Bellville campus; and the OSRC responsible for the assays. The large number of samples prepared addressed the wide spectrum of rooibos variations available which consumers drink. Contained within the 667 samples were the different sample brands (n = 2 - 3) bought from two different retail chain supermarkets, providing for duplicate samples and prepared to represent each of the various factors investigated in this study [being, rooibos types, forms, brewing times and flavourings (household and commercially flavoured)]. The prepared samples analysed provided a database for the total polyphenol content and the TAC of prepared rooibos herbal teas.

3.2.1 Preliminaries

Several tasks had to be performed prior to the study in order to gain sufficient information to conduct Phase 1 of the study. In order to determine the total polyphenol content and the TAC of the different rooibos herbal tea formulations, the following information was obtained regarding the rooibos herbal teas available in the local retail market for the typical consumer to purchase and consume: the different available rooibos herbal tea types, rooibos herbal tea forms, flavoured rooibos herbal teas as well as brands of each to cover and obtain the available spectrum of the different types, forms and commercial flavourings. Two large and differently branded retail chain supermarkets were visited to obtain the information on the rooibos herbal tea available to the consumer to purchase. A letter (see Addendum A) was provided to the store managers in order to acquire permission for the researcher to obtain the required information on the rooibos herbal teas availablein their stores.

The information as obtained for the different brands and forms, types and commercial flavourings within each brand is included as Addendum B. This list represents the envisaged number of brands per sample (n = 2 - 3) that were sourced from the two local retail chain supermarkets that a typical consumer frequents, providing for duplicate purchases and samples. Three brands were mostly sourced in order to allow for consideration of the production factors which may influence the total polyphenol content or TAC of each brand.

A limitation found in the brand listing of the rooibos herbal teas was that most brands were only available in the tea bag form. The availability of green / unfermented rooibos brands was limited, and rooibos herbal tea in leaf and powder form not available or having limited availability on the supermarket shelves. Consequently it was not possible to obtain the envisaged number of brand samples (n = 2 - 3) for each type and form of rooibos. Green (unfermented) rooibos leaves, rooibos leaves (fermented / traditional), rooibos powder and bagged organic green rooibos were not available at the supermarkets that were visited. These forms of rooibos were consequently supplied by Rooibos Ltd (Clanwilliam, South Africa). For the purpose of confidentiality, brand names and suppliers were replaced with Brand A, Brand B, etc. or as Supplier A, etc. in Addendum B and the thesis text.

3.2.2 Sampling procedure and sample preparations

Random samples of brand-specific rooibos and flavoured rooibos herbal tea types and forms (n = 2 - 3 brands in each case) were sourced from two local retail chain supermarkets (providing for duplicate purchases and samples). A retail chain supermarket located in the central and in the northern regional sectors of the City of Cape Town was used for this purpose as these stores offer a wider product range.

The controls for the different brewing times and the different flavouring additions and flavouring amounts added individually and in combination, were a standard cup of traditional / fermented rooibos herbal tea prepared using tea bags from the different selected brands and prepared according to the specifications of the SA Rooibos Council without any additions. The controls were taken from the same batches of tea prepared for the experimental samples. A batch for each brand of traditional / fermented rooibos from each supermarket was made up. Seven tea bags from each brand were steeped in 1.26 L of boiling water, stirred for 30 seconds and steeped for three minutes. The time for stirring and steeping was checked against a countdown timer. The tea bags were removed with tweezers and caution was taken to remove the least amount of liquid with the tea bags. Samples from these batches were also used for the samples with added household flavourings. For all the samples, except for the analysis of the form and type of rooibos herbal tea, traditional / fermented rooibos herbal tea bags were used as this form is the most widely available in supermarkets, representing the most commonly available rooibos herbal tea form to the typical consumer. The standard procedure was adapted to represent different brewing times as independent variables.

Prepared samples (each sample being 18 mL) were stored in a test tube and marked with a code for identification purposes with a permanent marker. The extracted and diluted samples were stored at 4^{o}C until analysed.

3.2.2.1 Tea type sample preparations

The different brands of traditional / fermented (n = 5), green / unfermented (n = 1) and organic traditional / fermented (n = 4) rooibos herbal tea purchased from the two retail chain supermarkets (see Addendum B) were prepared using tea bags. The time for stirring and steeping were checked against a countdown timer. The tea bags were removed with tweezers and caution was taken to remove the least amount of liquid with the tea bags.

3.2.2.2 Tea form sample preparations

In addition to the tea bag sample preparations presented above, samples were also prepared from traditional / fermented (n = 1 and n = 1, respectively), green / unfermented (n = 1 and n = 0, respectively) and organic (n = 1 and n = 0, respectively) rooibos herbal tea leaves and powder (see Addendum B). The amount of tea leaves (2.6 g) was weighed on a *Sartorius 2006 MP* scale and 180 mL boiling water was added, stirred for 30 seconds and steeped for three minutes (checked against the timer). The samples were strained through a sieve to separate the tea leaves from the tea sample.

Only one brand of rooibos herbal tea powder (see Addendum B) could be prepared (done in triplicate) as this form of tea is not yet readily available on supermarket shelves. Green / unfermented rooibos herbal tea powder is not yet available. A small amount of powder (50 mg) was weighed on a *Sartorius 2006 MP* scale and dissolved in 25 mL boiling water in ratio as per the product preparation instructions. The sample was stirred for 30 seconds and left for three minutes (checked against the timer) before the sampling took place.

RTD, unflavoured iced teas with traditional / fermented rooibos herbal tea (n = 3) (see Addendum B) were sampled directly from the containers. The iced teas in powder form (n = 6) (see Addendum B) were prepared according to the instructions on the sachet or container. These iced tea powders were properly mixed and dissolved in the sample preparation before the sampling took place.

3.2.2.3 Brewing time sample preparations

Tea samples (n = 24) utilising different brewing times were prepared from traditional / fermented rooibos herbal tea bags. The standard brewing time according to the instructions of the SA Rooibos Council, is three minutes. The three different times that were used to evaluate the different preparation methods were chosen to represent a quick brewing time (one minute), an average (five minutes) and a long (10 minutes) brewing time (Campanella *et al*., 2003:732). Samples with a brewing time of 20 and 30 minutes respectively were also prepared to determine the effect of a prolonged brewing time on the total polyphenol content and TAC. Each sample from the different brands were prepared with 180 mL boiling water added to one tea bag, stirred for 30 seconds and then steeped for the various indicated times checked against the timer.

3.2.2.4 Tea addition sample preparations

Various rooibos herbal tea samples with household flavourings / additions and commercially flavoured rooibos herbal tea samples (see Addendum B) were prepared using the batches of rooibos herbal tea as described in 3.2.2, then flavoured and sampled.

3.2.2.4.1 Household flavourings

The different amounts of household flavourings added to each sample had to be predetermined. The small, medium and large quantities of added milk (10 mL, 20 mL and 30 mL respectively of skim, low fat and whole milk) and added sugar (4 g, 6 g and 10 g respectively of white and brown granulated sugar) to a cup of tea [180 mL as indicated by Langenhoven *et al*. (1991:148)] were obtained from the Medical Research Council (MRC) Food Quantities Manual (Langenhoven *et al*., 1991:9,10,140). The small, medium and large amounts of honey added were determined by the weight of half a teaspoon (5 g), a level teaspoon (9 g) and a heaped teaspoon (15 g) of honey (Langenhoven *et al*., 1991:139).

Due to logistical reasons, long life milk cartons were purchased. One batch of milk (of skim, low fat and whole milk), sugar (of white and brown granulated sugar) and honey were used for the different samples in order to eliminate any interference through nuisance variables. Each added amount of sugar and honey were weighed on a calibrated *Sartorius 2006 MP* scale beforehand. Each added amount of the different types of milk was measured with calibrated *Eppendorf* pipettes. The tea samples (n = 594) were taken from the prepared tea batches described in

3.2.2.1. All the combinations of added household flavourings undertaken are presented in Addendum B.

Rooibos herbal tea with added sweetener (tablet or powder form) was not sampled due to the wide variety of sweeteners available on the supermarket shelves and the different amounts in which each consumer adds this sweetening agent. There was also no rooibos sample prepared with added lemon juice as using lemon juice as a household flavouring was added to the consumer questionnaire (Phase 2 of the study) only after the preparation and analysis of the samples was completed.

3.2.2.4.2 Commercially flavoured rooibos herbal tea

Camomile, lemon and honey flavoured rooibos herbal tea bag samples (n = 10) were prepared. Boiling water (180 mL) was added to these commercially flavoured rooibos herbal tea bags and steeped for three minutes (checked against the timer). The tea bags were removed from the infused water with tweezers with caution taken to remove the least amount of liquid. No additional flavourings were added.

Flavoured RTD iced teas were sampled directly from the container. These included iced-teas (n = 11 samples) flavoured with peach, berry and lemon. The flavoured iced-teas in powder form were prepared according to the instructions on the sachet, whereafter the sample was taken. Samples (n = 6) prepared from flavoured powdered iced-tea included the peach, apple, lemon and fruit punch flavours.

3.2.3 Assays performed

The assays to determine the total polyphenol content, the flavonol and flavanol contents and the TAC were performed by the OSRC (with the researcher involved in the preparation and dilution of the samples). All chemicals were bought from *Sigma Aldrich* and calibrated *Eppendorf* pipettes were used. Each 100 microlitre (µL) sample was diluted with 900 µL of distilled water before the assays were performed. Each assay performed on the 667 samples prepared was done in triplicate for precision – resulting in three values per assay for each sample. All the sample values for a specific factor (e.g. one minute brewing time) across the different brand and supermarket purchases were used to calculate an average for that factor.

3.2.3.1 ABTS assay

This decolourisation assay used to evaluate the TAC, applicable to both lipophilic and hydrophilic antioxidants, and being more accurate than the original TEAC assay (Re *et al*., 1999:1231), was used. Floegel *et al*. (2011:1045) commented that the ABTS assay is more strongly correlated with the ORAC and better reflects the antioxidant contents in a variety of foods than the DPPH assay. The ABTS assay estimates the antioxidant capacity of particularly fruits, vegetables and beverages better than the DPPH assay (Floegel *et al*., 2011:1048).

Twenty four hours before the assay was performed, the ABTS mix was prepared. In a 15 mL screw cap tube, 88 µL potassium-peroxodisulphate solution was added to 5 mL of the ABTS solution and mixed. This ABTS mix was left for 24 hours in a dark place at room temperature. Six tubes were marked A to F and the amount of standard (Trolox) stock solution and diluents added to each tube. In a clear well plate, 25 µL of standard was added to tubes A1 to B6 and 25 µl of the sample added to the rest of the wells (C1 to H12) to provide for triplicate measurements. The ABTS mix solution was diluted with ethanol to read an absorbance of approximately two. The ABTS mix (300 µL) was added to each well using a multichannel pipette. The plate was left at room temperature for 30 minutes before a reading was taken. The plate was inserted into the plate reader (*Thermo Electron Multiscan Spectrum*) and read at 734 nm at a temperature of 25^0C. The concentration (x) of the samples was calculated using the equation y = mx + c of the line on the standard curve where m = slope, c = y-intercept, y = absorbance of sample.

3.2.3.2 FRAP assay

The FRAP assay was also selected to evaluate the antioxidant activities of the rooibos herbal tea samples for the following reasons. Firstly, the FRAP assay treats the antioxidants in the samples as reductants in a redox-linked colorimetric reaction. Secondly, the procedure of the FRAP assay is relatively simple and easy to be standardised (Guo *et al*., 2003:1724).

The FRAP reagent was prepared before the assay was performed. Six tubes were marked A to F and the fixed amount of standard (ascorbic acid) stock solution and diluents added to each tube. In a clear well plate, 10 µL of standard was added to tubes A1 to B6 and 10 µL of sample added in triplicate to the rest of the wells (C1 to H12). A volume of 300 µL of the FRAP reagent was added to each well with a multichannel pipette. The plate was incubated for 30 minutes at

37^0C after which it was read (*Thermo Electron Multiscan Spectrum* plate reader) at 593 nm. The concentration (x) of the samples was calculated using the equation y = mx + c of the line on the standard curve where m = slope, c = y-intercept, y = absorbance of sample.

3.2.3.3 Folin-Ciocalteau method

The Folin-Ciocalteau method (Singleton & Rossi, 1965:144) was followed to measure the total polyphenol content of the samples. Six tubes were marked A to F and a fixed amount of standard (gallic acid) stock solution and diluents added to each tube. An amount of 25 µL of standard was added to each well in the designated wells (A1 to B6) in a clear well plate and 25 µL of sample added in triplicate to the rest of the wells (C1 to H12). With a multichannel pipette and a solution basis, 125 µL Folin reagent was added to each well. After five minutes, 100 µL Na_2CO_3 was added to each well with a multichannel pipette. The plate was left for two hours at room temperature whereafter it was read (*Thermo Electron Multiscan Spectrum* plate reader) at 665 nm. The concentration (x) of the samples was calculated using the equation y = mx + c of the line on the standard curve where m = slope, c = y-intercept, y = absorbance of sample.

3.2.3.4 Assay for testing the flavonol content

The flavonol content was measured using the method of Mazza *et al*. (1999:4010). Six tubes were marked A to F and a fixed amount of standard (quercetin) stock solution and diluents added to each tube. A volume of 12.5 µL of standard was added to each well in the designated wells (A1 to B6) in a clear well UV plate and 12.5 µL of sample added in triplicate to the rest of the wells (C1 to H12). To each well, 12.5 µL of 0.1% HCl in 95% ethanol (EtOH) and 225 µL 2% HCl was added. The plate was incubated for 30 minutes at room temperature whereafter it was read (*Thermo Electron Multiscan Spectrum* plate reader) at 360 nm and 25^0C. The concentration (x) of the samples was calculated using the equation y = mx + c of the line on the standard curve where m = slope, c = y-intercept, y = absorbance of sample.

3.2.3.5 Assay for testing the flavanol / proanthocyanidin content

The method of Nagel and Glories (1991:364) was used to determine the flavanol / proanthocyanidin content. The calibrators were prepared by marking six tubes (A to F) and adding a fixed amount of standard (catechin) solution and methanol to each. The standard (50 µL) was added to wells A1 to B6 and 50 µL of sample to the designated wells. The reactions were initiated by adding 250 µL of DMACA to all the wells using a multichannel pipette. The plate was incubated at room temperature for 30 minutes whereafter it was read (*Thermo Electron Multiscan Spectrum* plate reader) at 640 nm. The concentration (x) of the samples was calculated using the equation $y = mx + c$ of the line on the standard curve where m = slope, c = y-intercept, y = absorbance of sample.

3.2.4 Data analysis

Statistical analyses were individually performed on the rooibos herbal tea samples representing the tea type, tea form, tea prepared with different brewing times, tea with added household flavourings and the commercial flavourings. Statistical analyses for the household flavourings (being the addition of the different milk types, the addition of the different milk amounts added and the addition of white sugar, brown sugar or honey and in their differing added amounts) were determined separately and on the combinations of the household flavourings (milk and sugar or honey added).

One-way analysis of variance (ANOVA) was performed to test for differences between the rooibos herbal tea samples, i.e. the samples representing the rooibos types, the rooibos forms, the brewing times, the rooibos household flavouring additions and amounts and the commercially flavoured rooibos herbal teas. The Levene's Test for equality of variances and the Student-Newman-Keuls Test were applied. The Levene's Test for equality of variances was done as it is an inferential statistic used to assess the equality of variances for a variable calculated for two or more groups, and the Student-Newman-Keuls Test was done as a post-hoc test if a positive ANOVA result ($p < 0.05$) was obtained. The Student-Newman-Keuls Test through multiple pairwise comparisons determined where the significant ($p < 0.05$) differences in the sample means transpired. The Kruskal-Wallis one-way ANOVA by ranks was performed to determine whether the rooibos samples, where the sample data did not represent a normal distribution, originated from the same population. A positive ANOVA result ($p < 0.05$) obtained was followed by a post-hoc analysis through multiple pairwise sample comparisons to determine

where the significance ($p < 0.05$) transpired. This is a non-parametric alternative to the ANOVA. The statistics were done on all the assays executed (total polyphenol assay, FRAP, ABTS, flavanol and flavonol assay). The program used for the statistics was *MedCalc Statistical software, version 12.1.0.0.*

3.3 Phase 2: Rooibos herbal tea consumer tea drinking behaviour and profile

This phase aimed to determine how consumers usually drink rooibos herbal tea considering the different types, forms, brewing times and flavouring additions and amounts added to prepare it as a beverage. Phase 2 also aimed to determine the demographic, lifestyle and health characteristics of rooibos herbal tea consumers. This phase of the study also determined the percentage of rooibos herbal tea consumers who consume the optimal cup of traditional / fermented rooibos herbal tea as a beverage prepared to provide the higher total polyphenol content and TAC, and also the demographic-, lifestyle- and health-related characteristics of these consumers.

3.3.1 Permission to conduct the consumer study

Ethical approval (Ref. 12 / 2013) was obtained from the Faculty of Applied Sciences Research Ethics Committee (CPUT) for this phase of the study (Addendum C). All the respondents who participated voluntary in the study were fully informed about the study details and were required to sign the consent form (Addendum D) before completing the questionnaire (Addendum E). Each respondent received a copy of the signed consent form. A participant code was allocated to each respondent to facilitate anonymous participation.

3.3.2 Consumer questionnaire compilation

The questionnaire cover page informed the respondents of what the questionnaire entailed and indicated the instructions for its completion, as advised by Marshall (2005:134). The approximate time that it would take to complete the questionnaire was also stated on this cover page. A block for the respondent code and date was added for office use and for data capture.

The consumer questionnaire (Addendum E) comprised two sections. Section A included questions related to the respondent beverage, tea and herbal tea consumption, and rooibos herbal tea consumption (n = 12 questions). Willet (1998:95), as the expert resource on the

consumption categories incorporated within food frequency questionnaires, was consulted for the beverage consumption frequency response options for questions A4 ("*How often do you usually consume tea / herbal tea?"*) and A5 ("*How often do you usually consume rooibos herbal tea?"*). Nine frequency response categories were as a result provided, ranging from '*Almost never / Seldom'* to '*More than 6 cups per day*'. As many possible response options were provided in the included questions, especially for the consumed beverages, teas / herbal teas and flavoured teas questions (questions A1, A2 and A7 respectively), with each of these questions ending in the added response option '*Other… (please indicate)*' where the respondent could add whatever consumed option was not listed. Murray (1999:150) advises that one should ensure that the list of alternatives is complete, although the inclusion of an open response (and question), such as *'Other ... please specify'* is a suitable addition. This section also included a table, as question A12, which questioned the flavouring(s) usually added and the amount(s) in which these were usually added to the respondent's cup of rooibos herbal tea. The amounts of sweetening agent and lemon juice added to rooibos were illustrated with spoon(s) to incorporate the household amounts added as small, medium and large amounts. The amounts of the different types of milk added to rooibos were illustrated by a tea cup filled with 180 mL liquid (rooibos herbal tea) and the added milk as a layer added on top of the tea to illustrate small, medium and large amounts of added milk. Clear instructions for the completion of this table were provided. To determine whether a respondent consumed an optimal cup of rooibos herbal tea (with regards to the TAC and total polyphenols), the time of infusion was considered. If the respondent chose 'Strong (brewed for longer than 10 minutes)' within question A9 it was considered to be an optimal cup of rooibos herbal tea. This was based on the statistical analysis of the total polyphenol content and TAC of the samples considering the analysis of the different brewing times. A block where the researcher indicated whether the respondent consumed an optimal cup of rooibos herbal tea (with 'yes' coded '1') or not (with 'no' coded '2') formed the last part of Section A, indicated within A13 in the 'Office use' section of the questionnaire.

Section B consisted of respondent demographic, lifestyle- and health related questions (n = 12 questions). These questions formed the last part of the questionnaire to engage participants and prevent boredom (Rattray & Jones, 2007:237). The list of age categories was taken from the age listings on the Census 2011 (Western Cape) report (Lehohla, 2012:46). The age category listing started at 25 years of age due to the finding that tea consumers are mostly older persons (Storey *et al*., 2006:1992; Song & Chun, 2008:1543S). Age was as a result considered an inclusion / exclusion criterion for the study participation. The response options for questions B7

("*Are you currently active?"*) and B9 ("*What is your smoking status*?") were in line with those defined by Yusuf *et al*. (2004:939) respectively.

All the questions included were multiple-choice with the number of responses varying between the questions where the respondent could choose from a list of alternative answers as responses (Marshall, 2005:132). The following criteria were applied in the wording of each test item: questions were short and simple (Kemp *et al*., 2009:122) and questions on the same subject were grouped together and had a logical flow from one topic to another (Marshall, 2005:134). The questionnaire was ended by thanking the respondent for their participation as advised by Murray (1999:152). The layout of the questionnaire was carefully considered as a good design and layout improves survey response rates (Boynton & Greenhalgh, 2004:1315; Marshall, 2005:132).

3.3.3 Questionnaire pre-screening and pilot testing

Face validity and content validity are two closely related forms of validity and are the minimum requirement of acceptance of a measure (Bannigan & Watson, 2009:3240).

3.3.3.1 Content validity evaluation

The compiled questionnaire was pre-screened for content-related evidence of validity by a panel of experts. Food and food science (n = 3) as well as biochemical (n = 2) experts were asked to undertake the content validity evaluation to ensure the content domain reflected relevant information on tea and herbal tea consumption and demographic-, lifestyle-and health-related information of tea consumers. These experts also considered the consumer understanding of the questionnaire (face validity) in their evaluation of the questions.

The following is a summary of the changes made to the consumer questionnaire after being screened for content validity:

- the wording 'herbal tea' replaced 'tea' as a response option for preferred non-alcoholic beverages in question A1;
- a brief description of what 'squash' and 'fruit juice concentrate' is was added to question A1 for clarity;
- the words 'fermented' and 'unfermented' were removed from the rooibos herbal tea indication in question A2 as a consumer might not be familiar with these terms;

- a question (as A3) regarding the reason for the consumption of tea / herbal tea was added as "*Why do you mostly drink this tea / herbal tea*?";
- a question (as A6) regarding the time of day that rooibos herbal tea is usually consumed was added as "*What time of the day do you usually consume rooibos herbal tea*?";
- the size of a cup or small mug was quantified as 180 mL in questions A4 and A5;
- sweeteners, as powder or tablet form, were added as a flavouring option in question A12. The amount column was blocked out from this flavouring option as there are many amount options for this type of sweetening agent added and the provided amount information was not essential for this study;
- the fact that only one type of sugar and milk should be marked in the flavouring table (question A12) was added to the instructions on how to complete the table;
- a question (A11) regarding the temperature of rooibos herbal tea consumption was added as *"How 'warm' do you usually consume your rooibos herbal tea?"*;
- the questions regarding rooibos herbal tea consumption and preparation were rearranged for a more logical flow;
- more flavour examples were added in question A7 to the commercially flavoured rooibos herbal tea options available on the supermarket shelves to limit writing for the respondents; and
- the wording of questions B11 and B12 were respectively changed from "*Do you know if you have any of the following chronic diseases of lifestyle?*" and "*Do you know if you have a family history of any of the following chronic diseases of lifestyle?*" to "*Do you have any of the following chronic diseases of lifestyle?*" and "*Do you have a family history of any of the following chronic diseases of lifestyle?*" respectively.

3.3.3.2 Face validity evaluation

The questionnaire was pilot tested on approximately 10% (n = 38) of the envisaged sample size with the respondents representative of the study sample. These respondents met the age grouping and rooibos herbal tea consumer inclusion criteria. The objective of the pilot study was to determine the participant understanding of the questions within the questionnaire, the ease of the question answering as well as whether the time period of 10 minutes allocated to the participant to complete the questionnaire could be adhered to.

The following presents a summary of the changes made to the consumer questionnaire after being tested for face validity:

- the time to complete the questionnaire was changed on the cover page of the questionnaire to five to 10 minutes;
- the time to complete the questionnaire was changed in the information and consent form to five to 10 minutes;
- a further response option ('*Throughout the day*') was added to question A6 ("*What time of the day do you usually consume rooibos herbal tea?"*);
- question A12 (flavouring table) was divided into two steps for ease of understanding and completion;
- the pictures and the accompanying labels in the flavouring table (question A12) were made clearer;
- the instructions for completion of the flavouring table were rewritten and important words highlighted in bold;
- the words 'rooibos herbal tea' in the questions related to this herbal tea were made bold and underlined to highlight the type of tea referred to;
- question B3 was rephrased from "*With which population group do you associate yourself*?" to "*Under which population group do you fall?*"; and
- a further health related question (as B10) with four response options was added to Section B to ascertain the respondents' perception of their own body weight status as '*How would you describe your body weight status?*'.

3.3.4 Recruitment of respondents

A convenient and purposive sample of adult respondents aged 25 years and older who consume rooibos herbal tea and residing in the George area, Western Cape, SA was recruited. Individuals who consume rooibos herbal tea or 'rooibos', even if they consume it almost never or seldom, were unsystematically invited to complete the questionnaire. The distribution of the population by age for the George Municipality from the *Census 2011* (Western Cape) report (Lehohla, 2012:46) was used to determine the total number of respondents to be used, as well as the number in each age group) representing the formation of a non-probability quota sample (that parallels a stratified sample in probability sampling) (see Table 3.1). The sample size was determined by using a margin of error (or precision) of 0.06, a confidence interval of 95% under the normal distribution (i.e. the distribution of when *n* is sufficiently large), and a prior proportion

of 0.5. The prior proportion follows a binomial distribution when the observations are independent. This distribution has a maximum variance when the proportion is 0.5 [since var (X) = np (1-p)], which is why it was considered prudent to choose this as the prior proportion.

A minimum sample size of 267 individuals was calculated, but considering the possibility of non-response bias across the age stratus representing the quota sample, the age strata was multiplied with 1.5 to make sure that the required sample size within each stratus was obtained but having the same ratio representation as the age strata of the population. Table 3.1 provides the envisaged respondent numbers calculated to representatively answer the questionnaire within the total population, considering age as an inclusion criterion.

Table 3.1: Envisaged sample size calculated using the George Municipality Census 2011 Western Cape report

Age category (years)	Population (n)	Percentage (%) contribution per age strata	Sample size			
			Minimum number (n) and %[a]		Aim number (n) and %[b]	
			n	%	n	%
25 - 29	17 419	16.0	43	15.8	66	15.8
30 - 34	14 423	13.2	36	13.2	55	13.2
35 - 39	14 269	13.1	35	12.9	54	12.9
40 - 44	13 728	12.6	34	12.5	52	12.4
45 - 49	12 186	11.2	30	11.0	46	11.0
50 - 54	10 010	9.2	25	9.2	38	9.1
55 - 59	7 915	7.3	20	7.4	31	7.4
60 - 64	6 679	6.1	17	6.3	26	6.2
65 - 69	4 493	4.1	12	4.4	19	4.5
70 - 74	3 412	3.1	9	3.3	14	3.3
75 - 79	4 466	4.1	11	4.0	17	4.1
Total:	**109 000**	**100**	**272**	**100**	**418**	**100**

[a] Minimum calculated sample size of 267 but considering the percentage contribution per age stratum within the total population a minimum sample size of 272 of the total population was reached.
[b] Considering the possibility of non-response bias across the age stratus representing the stratified sample, the age strata was multiplied with 1.5 to make sure that the required minimum sample size within each stratus was obtained but providing the same ratio representation as the age strata of the population.

Recruiters (n = 12) from different age and cultural groups were identified and approached to assist in obtaining the required respondent sample size. These recruiters were also asked to check whether the questionnaires were fully answered. The recruiters assisted by distributing questionnaires to persons known to them (i.e. family, friends and colleagues) that met the inclusion criteria. A briefing was provided to the recruiters on how to uniformly address potential respondents for participation in the research. The researcher discussed the aims of the study, the questionnaire instructions and each question in order for the recruiter to have a clear understanding of the research and the questionnaire and to be able to answer questions of the respondents. The participation in this study was voluntary and anonymous as no identification of the respondents was required for the completion of the consumer questionnaire.

3.3.5 Data analysis

All information obtained during the research study (kept in a locked cabinet by the researcher) was handled in a confidential manner. The researcher captured the data (using a password protected computer) and the statistician undertook the analysis of the data (based on the allocated respondent study numbers and using a password protected computer). The SPSS 22 Statistics Data Editor program was used.

The data obtained from the questionnaire was used to describe the respondent sample of those who participated in the study, as well as their rooibos herbal tea consumption and the profile of the optimal cup rooibos herbal tea consumer. Frequencies as descriptive statistics of the questionnaire question responses, and associations (Pearson's chi-squared statistic) between the respondent rooibos herbal tea consumption, considering the respondent consumption of the optimal cup of rooibos herbal tea or not, and the respondent profile responses at a significance level of five percent ($p < 0.05$) were used for this data analysis. To account for empty cells and low cell numbers of response options to questions in the questionnaire, some response options had to be combined to reduce the occurrence of empty cells and low cell counts in the statistical analysis.

CHAPTER 4
RESULTS

The data of Phase 1 and Phase 2 of the study are presented in this chapter. The first phase delivered data on the analysis of the TAC, total polyphenol, flavonol and flavanol contents after the selection and preparation of rooibos samples from different rooibos types, forms and with different brewing times and added flavourings. The FRAP and TEAC assays were performed to determine the TAC.

Only one sample of green / unfermented rooibos (tea bag), organic green / unfermented rooibos (tea bag), traditional / fermented rooibos powder, green / unfermented rooibos leaves and organic traditional / fermented rooibos leaves were prepared due to the limited / unavailability of these rooibos product types and forms in the local supermarkets. Rooibos herbal tea samples without any additions, except for when flavourings were investigated as an influencing factor, were prepared for analysis. Also, unless stated otherwise, tea bags were used to prepare the samples.

During Phase 2, by using a questionnaire, data was obtained on how rooibos herbal tea consumers drink their rooibos herbal tea, bearing in mind the results from Phase 1, as well as identifying the demographic and lifestyle information of rooibos herbal tea consumers, and in particular those who consume rooibos as a prepared beverage providing the highest TAC and total polyphenol content.

4.1 Phase 1: Sample selection, preparation, analysis and identification of the rooibos sample(s) providing the highest total polyphenol content and total antioxidant capacity

4.1.1 Tea type

The differences in and the TAC, total polyphenol, flavonol and flavanol contents of the various rooibos types analysed in the study are presented in Table 4.1. A significant difference was found in the TAC (both the FRAP and TEAC assays) ($p < 0.05$) and the flavonol ($p < 0.05$) and flavanol ($p< 0.05$) contents of the different rooibos types. The green / unfermented rooibos had a significantly ($p < 0.05$) higher FRAP (3 219 ± 82 µmol/L) and significantly ($p < 0.05$) higher flavanol content (29.40 ± 1.55 mg/L) than each of the other rooibos types. The TEAC of the organic green / unfermented rooibos (866.7 ± 57.2 µmol/L) was significantly ($p < 0.05$) lower

than that of the organic traditional / fermented rooibos (1 068 ± 110 µmol/L) and the traditional / fermented rooibos (1 140 ± 143 µmol/L) respectively. Traditional / fermented rooibos had a significantly ($p < 0.05$) higher flavonol content (34.47 ± 18.88 mg/L) compared to the green / unfermented (15.39 ± 2.08 mg/L) and organic green / unfermented (16.57 ± 5.70 mg/L) rooibos types. The flavanol content of the organic green / unfermented rooibos (22.95 ± 1.69 mg/L) was also significantly ($p < 0.05$) higher than that of the organic traditional / fermented (9.71 ± 4.33 mg/L) and the traditional / fermented (9.09 ± 3.02 mg/L) rooibos types (see Table 4.1).

Table 4.1: Comparison of the total antioxidant capacity and the total polyphenol, flavonol and flavanol contents of the different rooibos types analysed in the study

Different rooibos types[a]	Number of samples	Total antioxidant capacity and -content				
		FRAP (µmol/L)[b]	TEAC (µmol/L)[c]	Total polyphenols (mg/L)[d]	Flavonols (mg/L)[d]	Flavanols (mg/L)[d]
Green / unfermented (1)	1	3 219±82[e]	963.6±46.7	457.7±40.9	15.39±2.08	29.40±1.55
Organic green / unfermented (2)	1	2 494±20	866.7±57.2	431.5±13.1	16.57±5.70	22.95±1 .69
Organic traditional / fermented (3)	3	2 546±351	1 068±110	400.0±109.0	24.72±5.99	9.71±4 .33
Traditional / fermented (4)	6	2 303±361	1 140±143	377.2±66.0	34.47±18.88	9.09±3 .02
Significant difference		(1)-(2)(3)(4)[f]	(2)-(3)(4)[f]		(4) – (1)(2)[g]	(1)-(2)(3)(4)[f] (2)-(3)(4)[f]

[a] Rooibos samples prepared as bags with freshly boiled water, stirred for 30 seconds and steeped for three minutes (checked against a laboratory timer).
[b] FRAP: Ferric reducing antioxidant power assay presented as micromole (µmol) per litre (L).
[c] TEAC: Trolox equivalent antioxidant capacity assay presented as micromole (µmol) per litre (L).
[d] Total polyphenols, flavonol content and flavanol content presented as milligram (mg) per litre (L).
[e] Values: Mean ± standard deviation.
[f] Overall significant ($p<0.05$) difference in the Levene's Test for equality of variances of the samples with the significant ($p < 0.05$) differences transpiring in the pairwise multiple comparisons of the Student-Newman-Keuls Test indicated as a rooibos type (number presented in brackets [e.g. green / unfermented rooibos as 1] significantly different (-) to another rooibos type with each type presented as a number in brackets [e.g. organic green / unfermented rooibos as 2]).
[g] Overall significant ($p<0.05$) difference in the Kruskal-Wallis Test one-way analysis of variance by ranks of the samples with the significant ($p<0.05$) contrasts transpiring on the post-hoc pairwise multiple comparisons presented as in f above.

4.1.2 Tea form

Differences in the TAC, total polyphenol, flavonol and flavanol contents of the various rooibos forms are presented in Table 4.2. A significant ($p < 0.05$) difference was only found in the flavanol content of the different rooibos forms. The rooibos prepared from the green / unfermented leaves and the rooibos powdered extract (traditional / fermented) both had a significantly ($p < 0.05$) higher flavanol content (32.70 ± 1.55 mg/L and 34.21 ± 1.41 mg/L respectively) than the other rooibos forms (see Table 4.2).

Table 4.2: Total antioxidant capacity, total polyphenol, flavonol and flavanol contents of the various rooibos forms and the differences between the forms

Different rooibos forms	Number of samples[a]	Total antioxidant capacity and -content				
		FRAP (µmol/L)[b]	TEAC (µmol/L)[c]	Total polyphenols (mg/L)[d]	Flavonols (mg/L)[d]	Flavanols (mg/L)[d]
Leaves (green / unfermented) (1)	1	3 743±144[e]	972.7±44.8	579.2±23.0	20.12±3.78	32.70±1.55
Iced tea (with rooibos extract – traditional / fermented) (2)	3	2 840±1130	1 701±565	440.5±209.3	22.09±18.27	4.21 ±6.80
Leaves (organic traditional / fermented) (3)	1	2 189±48	931.8±28.4	372.3±7.3	23.28±3.81	9.21±1.1 4
Rooibos powdered extract (traditional / fermented) (4)	1	4 004±395	1 170±53	604.6±39.0	21.30±4.58	34.21±1. 41
Tea bag (traditional / fermented) (5)	6	2 303±361	1 140±143	377.2±66.0	34.47±18.88	9.09±3 .02
Leaves (rooibos superior – traditional / fermented) (6)	1	2 863±57	1 033±57	498.5±31.0	28.80±2.82	12.78±0.8 6
Significant difference						(1)-(2)(3)(5)(6)[f] (4)-(2)(3)(5)(6)[f]

[a] Leave and bag rooibos samples prepared with freshly boiled water, stirred for 30 seconds and steeped for three minutes (checked against a laboratory timer). RTD, unflavoured iced teas with traditional / fermented rooibos were sampled directly from the containers. The unflavoured iced teas in powder form were prepared according to the instructions on the sachet or container.
[b] FRAP: Ferric reducing antioxidant power assay presented as micromole (µmol) per litre (L).
[c] TEAC: Trolox equivalent antioxidant capacity assay presented as micromole (µmol) per litre (L).
[d] Total polyphenols, flavonol content and flavanol content presented as milligram (mg) per litre (L).
[e] Values: Mean ± standard deviation.
[f] Overall significant ($p<0.05$) difference in the Levene's Test for equality of variances of the samples with the significant ($p < 0.05$) differences transpiring in the pairwise multiple comparisons of the Student-Newman-Keuls Test indicated as a rooibos form (number presented in brackets [e.g. leaves (green / unfermented) as 1] significantly different (-) to another rooibos form with each form presented as a number in brackets [e.g. iced tea (with rooibos extract – traditional / fermented) as 2]).

4.1.3 Brewing time

As can be construed from Table 4.3 which presents the TAC, total polyphenol, flavonol and flavanol contents of fermented rooibos tea bags across different brewing times, significant differences were found in the all of the parameters measured across the different brewing times. The TAC (measured by the FRAP and TEAC assays), total polyphenol content, flavonol content and flavanol content of the sample brewed for one minute (1 943 ± 284 µmol/L, 912.88 ± 58.52 µmol/L, 309.4 ± 57.6 mg/L, 22.03 ± 4.45 mg/L and 7.12 ± 2.46 mg/L respectively) were significantly ($p < 0.05$) lower than that of the samples brewed for five, ten, twenty and thirty minutes respectively. The TAC (as measured by the FRAP assay) and the flavanol content of the sample brewed for five minutes (2 547 ± 317 µmol/L and 10.74 ± 3.32 mg/L respectively) were also respectively significantly ($p < 0.05$) lower than that of the sample brewed for ten minutes (2 857 ± 417 µmol/L and 13.49 ± 4.01 mg/L respectively). The TAC (as measured by the TEAC assay) of the sample brewed for five minutes (978.0 ± 50.2 µmol/L) was also significantly ($p < 0.05$) lower than that of each of the other longer brewed samples. The total polyphenol content of the rooibos sample with a brewing time of five minutes (414.0 ± 71.7 mg/L) was significantly ($p < 0.05$) lower than that of the samples brewed for ten and thirty minutes respectively (491.7 ± 76.9 mg/L and 543.2 ± 125.7 mg/L respectively) (see Table 4.3).

Table 4.3: Influence of the different brewing times on the total antioxidant capacity, total polyphenol, flavonol and flavanol contents in freshly brewed fermented rooibos herbal tea samples

Different brewing times	Number of samples[a]	Total antioxidant capacity and -content				
		FRAP (µmol/L)[b]	TEAC (µmol/L)[c]	Total polyphenols (mg/L)[d]	Flavonols (mg/L)[d]	Flavanols (mg/L)[d]
1 minute (1)	6	1 943±284[e]	912.9±58.5	309.4±57.6	22.03±4.45	7.12±2.46
5 minutes (2)	6	2 547±317	978.0±50.2	414.0±71.7	28.60±6.13	10.74± 3.32
10 minutes (3)	6	2 857±417	1 069±120	491.7±76.9	32.09±6.63	13.49±4 .01
20 minutes (4)	3	2 934±719	1 162±217	520.8±116.8	34.39±12.20	14.06 ±5.99
30 minutes (5)	3	3 107±731	1 212±270	543.2±125.7	36.53±12.84	15.14 ±6.23
Significant difference		(1)-(2)(3)(4)(5)[f] (2)-(3)[f]	(1)-(2)(3)(4)(5)[g] (2)-(3)(4)(5)[g]	(1)-(2)(3)(4)(5)[f] (2)-(3)(5)[f]	(1)-(2)(3)(4)(5)[f]	(1)-(2)(3)(4)(5)[f] (2)-(3)[f]

[a] Samples of all brewing times prepared as traditional / fermented rooibos bags with freshly boiled water, stirred for 30 seconds and steeped for three minutes (checked against a laboratory timer).
[b] FRAP: Ferric reducing antioxidant power assay presented as micromole (µmol) per litre (L).
[c] TEAC: Trolox equivalent antioxidant capacity assay presented as micromole (µmol) per litre (L).
[d] Total polyphenols, flavonol content and flavanol content presented as milligram (mg) per litre (L).
[e] Values: Mean ± standard deviation.
[f] Overall significant ($p<0.05$) difference in the Levene's Test for equality of variances of the samples with the significant ($p < 0.05$) differences transpiring in the pairwise multiple comparisons of the Student-Newman-Keuls Test indicated as a brewing time (number presented in brackets [e.g. one minute as 1] significantly different (-) to another brewing time with each brewing time presented as a number in brackets [e.g. five minutes as 2]).
[g] Overall significant ($p<0.05$) difference in the Kruskal-Wallis Test one-way analysis of variance by ranks of the samples with the significant ($p<0.05$) contrasts transpiring on the post-hoc pairwise multiple comparisons presented as in f above.

4.1.4 Household flavourings

White sugar, brown sugar and honey represented the household sweetening agents which were singly added in small, medium and large amounts to the rooibos samples with the base sample consistently prepared as traditional / fermented rooibos herbal tea using tea bags. Different types of milk (skim, low fat and whole milk) were also singly added in small, medium and large amounts as well as a combination of each type of milk and a sweetening agent in the different added amounts to represent the household flavoured rooibos that a consumer could prepare as a beverage for consumption.

4.1.4.1 Sweetening agent and different amount additions

To differentiate between the TAC and antioxidant contents of the rooibos samples with the addition of the different sweetening agents, the sweetening agents were compared as per amount that would be added to rooibos herbal tea by consumers; i.e. as small, medium and large amount additions. This allowed for comparison of the different sweetening agents across a

comparable added amount of sweetening agent of which the data is presented in Table 4.4. A significant difference was found in the TEAC ($p < 0.05$) and flavonol content ($p < 0.05$) of the rooibos samples with the different types of sweetening agents added in turn.

Table 4.4: The total antioxidant capacity and the total polyphenol, flavonol and flavanol contents of rooibos herbal tea samples with added white sugar, brown sugar and honey to present small, medium and large amount additions

Type of sweetening agent added in small, medium and large gram (g) amounts	Number of samples[a]	Total antioxidant capacity and -content				
		FRAP (µmol/L)[b]	TEAC (µmol/L)[c]	Total polyphenols (mg/L)[d]	Flavonols (mg/L)[d]	Flavanols (mg/L)[d]
Sweetening agent added as small amount						
White sugar: 4 g (1)	6	2 223±415[e]	1 130±108	374.0±65.2	34.59±18.76	8.72±3.13
Brown sugar: 4 g (2)	6	2 399±296	1 179±154	371.0±56.3	27.80±12.24	9.30±3 .24
Honey: 5 g (3)	6	2 489±437	1 868±172	407.2±71.8	48.55±27.71	8.40±3 .25
Significant difference			(3)-(1)(2)[f]		(2)-(3)[f]	
Sweetening agent added as medium amount						
White sugar: 6 g (1)	6	2 284±432	1 165±143	376.9±60.8	33.01±18.96	8.65±2 .97
Brown sugar: 6 g (2)	6	2 308±317	1 188±130	374.1±62.5	28.95±11.83	9.25±3 .16
Honey: 9 g (3)	6	2 351±275	1 801±192	387.3±64.7	46.80±25.85	8.29±3 .27
Significant difference			(3)-(1)(2)[f]		(3)-(1)(2)[f]	
Sweetening agent added as large amount						
White sugar:10 g (1)	6	2 381±374	1 138±115	373.5±62.9	32.41±18.88	8.59±2 .83
Brown sugar: 10 g (2)	6	2 380±396	1 155±86	375.1±63.0	26.55±15.59	9.07±3. 15
Honey:15 g (3)	6	2 220±288	1 768±97	413.0±72.7	46.73±27.62	8.84±3. 62
Significant difference			(3)-(1)(2)[f]		(2)-(3)[f]	

[a] Samples with different sweetening agents added in the same amounts (small, medium and large amounts) prepared as traditional / fermented rooibos bags with freshly boiled water, stirred for 30 seconds and steeped for three minutes (checked against a laboratory timer).
[b] FRAP:Ferric reducing antioxidant power assay presented as micromole (µmol) per litre (L).
[c] TEAC: Trolox equivalent antioxidant capacity assaypresented as micromole (µmol) per litre (L).
[d] Total polyphenols, flavonol content and flavanol content presented as milligram (mg) per litre (L).
[e] Values: Mean ± standard deviation.
[f] Overall significant ($p<0.05$) difference in the Levene's Test for equality of variances of the samples with the significant ($p < 0.05$) differences transpiring in the pairwise multiple comparisons of the Student-Newman-Keuls Test indicated as a type of sweetening agent (number presented in brackets [e.g. white sugar as 1] significantly different (-) to another type of sweetening agent with each sweetening agent presented as a number in brackets [e.g. brown sugar as 2]).

The rooibos sample with honey added as a small, medium and large amount had a significantly ($p < 0.05$) higher TEAC (1 868 ± 172 µmol/L, 1 801 ± 192 µmol/L and 1 768 ± 97 µmol/L, respectively) than rooibos with either white or brown sugar added at the corresponding amount additions. The addition of honey to the rooibos sample in a small (5 g) and a large (15 g) amount respectively resulted in a significantly ($p < 0.05$) higher flavonol content (48.55 ± 27.71 mg/L and 46.73 ± 27.62 mg/L respectively) than the sample with the added brown sugar in these amount (4 g and 6 g respectively) additions (27.80 ± 12.24 mg/L and 26.55 ± 15.59 mg/L respectively), while the addition of honey to the rooibos sample in a medium (9 g) amount had a significantly ($p < 0.05$) higher flavonol content (46.80 ± 25.85 mg/L) than the respective samples with white and brown sugar added as medium (6 g) amounts (33.01 ± 18.96 mg/L and 28.95 ± 11.83 mg/L respectively) (see Table 4.4). When considering the different amount (i.e. small, medium and large) additions per sweetening agents no significant ($p > 0.05$) differences were respectively found in the TAC (as measured by both the FRAP and TEAC assays) and the total polyphenol, flavonol and flavanol contents of the rooibos herbal tea samples with the different amounts of white sugar, brown sugar or honey added.

4.1.4.2 Milk type addition

The antioxidant characteristics of the rooibos herbal tea samples on the addition of different types of milk, were compared as per the amount of milk usually added by the consumer (i.e., small, medium and large amount additions) and are presented in Table 4.5. Significant ($p < 0.05$) differences were respectively found across most of the analysed antioxidant parameters when considering the different types of milk added.

Table 4.5: Total antioxidant capacity and -contents of rooibos samples with skim, low fat and whole milk added to present small, medium and large amount additions

Type of milk added in small, medium and large amounts	Number of samples[a]	Total antioxidant capacity and -content				
		FRAP (µmol/L)[b]	TEAC (µmol/L)[c]	Total polyphenols (mg/L)[d]	Flavonols (mg/L)[d]	Flavanols (mg/L)[d]
Small amount addition (10 mL)						
Skim milk (1)	6	1 828±133[e]	1 552±216	385.6±57.3	51.15±14.16	8.31±2.98
Low fat milk (2)	6	1 988±296	1 501±169	411.7±60.0	69.29±18.49	11.81±3.51
Whole milk (3)	6	2 037±271	1 435±94	431.2±66.3	81.32±20.84	14.51±3.69
Significant difference		(1)-(2)(3)[f]			(1)-(2)(3)[g]	(1)-(2)(3)[g]
Medium amount addition (20 mL)						
Skim milk (1)	6	1 794±240	2 210±259	421.9±53.9	56.29±17.40	8.52±2.44
Low fat milk (2)	6	1 967±208	2 348±239	454.9±48.2	95.83±19.44	15.04±2.91
Whole milk (3)	6	2 002±263	1 792±169	472.6±74.7	115.3±23.3	20.54±5.24
Significant difference		(1)-(2)(3)[f]	(3)-(1)(2)[g]		(1)-(2)(3)[g] (2)-(3)[g]	(1)-(2)(3)[g] (2)-(3)[g]
Large amount addition (30 mL)						
Skim milk (1)	6	1 800±246	3 025±325	444.2±44.5	60.97±18.29	8.56±2.65
Low fat milk (2)	6	1 938±298	2 926±311	497.4±55.9	124.4±31.6	18.04±3.69
Whole milk (3)	6	2 100±453	2 289±391	519.1±82.7	145.8±37.6	23.67±7.38
Significant difference		(1)-(3)[f]	(3)-(1)(2)[g]	(1)-(3)[g]	(1)-(2)(3)[g]	(1)-(2)(3)[g]

[a] Samples with different milk types added in the same amounts (small, medium and large millilitre (mL) amounts) prepared as traditional / fermented rooibos bags with freshly boiled water, stirred for 30 seconds and steeped for three minutes (checked against a laboratory timer).
[b] FRAP: Ferric reducing antioxidant power assay presented as micromole (µmol) per litre (L).
[c] TEAC: Trolox equivalent antioxidant capacity assay presented as micromole (µmol) per litre (L).
[d] Total polyphenols, flavonol content and flavanol content presented as milligram (mg) per litre (L).
[e] Values: Mean ± standard deviation.
[f] Overall significant ($p<0.05$) difference in the Kruskal-Wallis Test one-way analysis of variance by ranks of the samples with the significant ($p<0.05$) contrasts transpiring on the post-hoc pairwise multiple comparisons indicated as a type of milk (number presented in brackets [e.g. skim milk as 1] significantly different (-) to another type of milk with each milk type presented as a number in brackets [e.g. low fat milk as 2]).
[g] Overall significant ($p<0.05$) difference in the Levene's Test for equality of variances of the samples with the significant ($p < 0.05$) differences transpiring in the pairwise multiple comparisons of the Student-Newman-Keuls Test presented as in f above.

The rooibos samples with the added small and medium amounts of skim milk had a significantly ($p < 0.05$) lower FRAP (1 828 ± 133 µmol/L and 1 794 ± 240 µmol/L respectively) than those with low fat or whole milk added in small and medium amounts to the rooibos samples. The rooibos herbal tea sample with a large amount of skim milk added also had a significantly ($p < 0.05$) lower FRAP than the rooibos sample with the same amount of whole milk added (1 800 ±

246 µmol/L vs 2 100 ± 453 µmol/L). The TEAC of the rooibos sample with added whole milk as a medium and as a large amount (1 792 ± 169 µmol/L and 2 289 ± 391 µmol/L respectively) was significantly ($p < 0.05$) lower than that of the rooibos samples with the other types of milk added in these amounts (see Table 4.5).

The total polyphenol content of the rooibos sample with skim milk added as a large amount (444.2 ± 44.5 mg/L) was significantly ($p < 0.05$) lower than that of the rooibos sample with added whole milk in a large amount (519.1 ± 82.7 mg/L). The flavonol and flavanol contents of the rooibos samples with added skim milk in small (51.15 ± 14.16 mg/L and 8.31 ± 2.98 mg/L respectively), medium (56.29 ± 17.40 mg/L and 8.52 ± 2.44 mg/L respectively) and large (60.97 ± 18.29 mg/L and 8.56 ± 2.65 mg/L respectively) amounts were significantly ($p < 0.05$) lower than that of the rooibos samples with low fat and whole milk added in these amounts, respectively. The flavonol and flavanol contents of the rooibos sample with low fat milk added in a medium amount (95.83 ± 19.44 mg/L and 15.04 ± 2.91 mg/L respectively) were also significantly ($p < 0.05$) lower than that on the addition of whole milk in a medium amount (115.3 ± 23.3 mg/L and 20.54 ± 5.24 mg/L respectively) (see Table 4.5).

4.1.4.3 Milk amount addition

The antioxidant characteristics of the rooibos samples with different milk amount additions (i.e., small, medium and large amounts) per milk type as representation of the amounts added by consumers and the differences in the characteristics of the samples are presented in Table 4.6. Significant ($p < 0.05$) differences were found for most of the analysed biochemical contents of the rooibos samples as prepared with different amounts of milk added per type of milk.

Table 4.6: Antioxidant characteristics of rooibos samples with skim, low fat and whole milks added in different amounts

Amount of milk added (millilitres)	Number of samples[a]	Antioxidant capacity and -content				
		FRAP (µmol/L)[b]	TEAC (µmol/L)[c]	Total polyphenols (mg/L)[d]	Flavonols (mg/L)[d]	Flavanols (mg/L)[d]
Skim milk						
Small amount: 10 mL (1)	6	1 828±133[e]	1 552±216	385.6±57.3	51.15±14.16	8.31±2.98
Medium amount: 20 mL (2)	6	1 794±240	2 210±259	411.7±53.9	56.29±17.40	8.52±2 .44
Large amount: 30 mL (3)	6	1 800±246	3 025±325	431.2±44.5	60.97±18.29	8.56±2 .65
Significant difference		(1)-(2)(3)[f]	(1)-(2)(3)[g] (2)-(3)[g]			
Low fat milk						
Small amount: 10 mL (1)	6	1 988±296	1 501±169	421.9±60.0	69.29±18.49	11.81± 3.51
Medium amount: 20 mL (2)	6	1 967±208	2 348±239	454.9±48.2	95.83±19.44	15.04± 2.91
Large amount: 30 mL (3)	6	1 938±298	2 926±311	497.4±55.9	124.4±31.6	18.04±3 .69
Significant difference		(1)-(2)(3)[f]	(1)-(2)(3)[g] (2)-(3)[g]		(1)-(2)(3)[g] (2)-(3)[g]	(1)-(2)(3)[g]
Whole milk						
Small amount: 10 mL (1)	6	2 037±271	1 435±94	444.2±66.3	81.32±20.84	14.51±3 .69
Medium amount: 20 mL (2)	6	2 002±263	1 792±169	472.6±74.7	115.3±23.3	20.54±5 .24
Large amount: 30 mL (3)	6	2 100±453	2 289±391	519.1±82.7	145.8±37.6	23.67±7 .38
Significant difference		(1)-(3)[f]	(1)-(2)(3)[g] (2)-(3)[g]	(1)-(3)[g]	(1)-(2)(3)[g] (2)-(3)[g]	(1)-(2)(3)[g]

[a] Samples with different milk types added in small, medium and large millilitre (mL) amounts prepared as traditional / fermented rooibos herbal tea bags with freshly boiled water, stirred for 30 seconds and steeped for three minutes (checked against a laboratory timer).
[b] FRAP: Ferric reducing antioxidant power assay presented as micromole (µmol) per litre (L).
[c] TEAC: Trolox equivalent antioxidant capacity assay presented as micromole (µmol) per litre (L).
[d] Total polyphenols, flavonol content and flavanol content presented as milligram (mg) per litre (L).
[e] Values: Mean ± standard deviation.
[f] Overall significant ($p<0.05$) difference in the Kruskal-Wallis Test one-way analysis of variance by ranks of the samples with the significant ($p<0.05$) contrasts transpiring on the post-hoc pairwise multiple comparisons presented as an amount of milk (number presented in brackets [e.g. small amount: 10 mL as 1] significantly different (-) to another amount of milk with each amount presented as a number in brackets [e.g. medium amount: 20 mL as 2]).
[g] Overall significant ($p<0.05$) difference in the Levene's Test for equality of variances of the samples with the significant ($p < 0.05$) differences transpiring in the pairwise multiple comparisons of the Student-Newman-Keuls Test presented as f above.

The FRAP of the rooibos samples with an added small amount (10 mL) of skim and low fat milks (1 828 ± 133 µmol/L and 1 988 ± 296 µmol/L respectively) was significantly ($p < 0.05$) higher than that of the rooibos samples with medium (20 mL) and large (30 mL) amounts of these milks added respectively. The FRAP of the rooibos sample with a small amount of whole milk added (2 037 ± 271 µmol/L) was significantly ($p < 0.05$) lower than that of rooibos sample with a large amount of whole milk added (2 100 ± 453 µmol/L) (see Table 4.6).

The TEAC of the rooibos samples with the medium and large amounts of skim milk (2 210 ± 259 µmol/L and 3 025 ± 325 µmol/L respectively), low fat milk (2 348 ± 239 µmol/L and 2 926 ± 311 µmol/L respectively) and whole milk (1 792 ± 169 µmol/L and 2 289 ± 391 µmol/L respectively) added was significantly ($p < 0.05$) higher than the TEAC of the rooibos samples with small added amounts for each of the milk types (1 552 ± 216 µmol/L, 1 501 ± 169 µmol/L and 1 435 ± 94 µmol/L respectively for skim, low fat and whole milks). The rooibos samples with large amount additions of the different types of milk added had in addition, a significantly ($p < 0.05$) higher TEAC than the rooibos samples with the medium amount additions (see Table 4.6).

The total polyphenol content of the rooibos sample to which a small amount of whole milk was added (444.2 ± 66.3 mg/L), was significantly ($p < 0.05$) lower than that of the addition of a large amount of whole milk (519.1 ± 82.7 mg/L). The rooibos samples with an added small amount of low fat and whole milks had a significantly ($p < 0.05$) lower flavonol (69.29 ± 18.49 mg/L and 81.32 ± 20.84 mg/L respectively) and flavanol (11.81 ± 3.51 mg/L and 14.51 ± 3.69 mg/L respectively) contents compared to when medium and large amounts of these respective milk types were added. The flavonol content of the rooibos samples to which a medium amount of low fat milk and whole milk was added (95.83 ± 19.44 mg/L and 115.3 ± 23.3 mg/L respectively) was also significantly ($p < 0.05$) lower than that found on the addition of a large amount of low fat milk and whole milk respectively (124.4 ± 31.6 mg/L and 145.8 ± 37.6 mg/L respectively) (see Table 4.6).

4.1.4.4 Combined household flavourings

The TAC, as determined by the FRAP and TEAC assay, and the total polyphenol, flavonol and flavanol contents of rooibos samples with different amount additions per milk type and sweetening agent as added to rooibos by consumers (i.e. small, medium and large amount additions) and the antioxidant charateristics differences between these are presented in the next few tables (n = 9) per amount and type of milk addition (as 10 mL skim milk, 10 mL low fat milk, etc.) and accompanied by the sweetening agent additions.

i Small skim milk amount addition combined with sweetening agents

The TEAC of the rooibos samples prepared with skim milk as a small amount addition of 10 mL and with the various sweetening agent additions differed significantly ($p < 0.05$) (see Table 4.7). The TAC, as measured by the TEAC assay, of the rooibos samples prepared with 10 mL skim milk and to which a small (5 g), medium (9 g) and large (15 g) amount of honey was added, respectively delivered the samples with the highest TEAC (3 337 ± 119 µmol/L, 3 369 ± 134 µmol/L and 3 385 ± 103 µmol/L respectively) in comparison to the rooibos samples with the other sweetening agents added in the corresponding amount additions. The TEAC of the rooibos samples to which honey was added in either a small, medium or large amount, was significantly ($p < 0.05$) higher than that of the other rooibos samples prepared with 10 mL skim milk and the similar amount addition of either white or brown sugar, i.e. as small (4 g), medium (6 g) and large (10 g) amounts added, respectively. In addition, the TEAC of the rooibos sample with 10 mL skim milk added along with a large amount (10 g) of brown sugar (at 1 514 ± 78 µmol/L) was significantly ($p < 0.05$) lower than that of the rooibos samples with 10 mL skim milk added along with a small (4 g), medium (6g) and large (10g) amount of white sugar respectively (at 1 599 ± 189 µmol/L, 1 644 ± 171 µmol/L and 1 629 ± 262 µmol/L respectively). The TEAC of the rooibos sample with 10 mL added skim milk along with a small amount (4 g) of brown sugar (1 536 ± 87 µmol/L) was significantly ($p < 0.05$) lower than that of the rooibos sample with 10 mL added skim milk and a medium amount (6 g) of white sugar (1 644 ± 171 µmol/L) (see Table 4.7).

Table 4.7: The antioxidant characteristics of rooibos samples with 10 mL skim milk and sweetening agents added in small, medium and large amounts and the differences between these rooibos samples

Flavourings	Number of samples[a]	Antioxidant capacity and -content				
		FRAP (µmol/L)[b]	TEAC (µmol/L)[c]	Total polyphenols (mg/L)[d]	Flavonols (mg/L)[d]	Flavanols (mg/L)[d]
10 mL skim milk & 4 g white sugar (1)	6	1 917±213[e]	1 599±189	387.6±56.1	50.90±15.17	8.15±2.97
10 mL skim milk & 6 g white sugar (2)	6	1 841±243	1 644±171	384.5±57.8	50.02±15.34	7.97±2 .69
10 mL skim milk & 10 g white sugar (3)	6	1 897±279	1 629±262	391.2±59.0	49.55±15.38	8.04±2 .84
10 mL skim milk & 4 g brown sugar (4)	6	1 901±398	1 536±87	386.2±51.7	45.64±8.44	7.92±2.8 4
10 mL skim milk & 6 g brown sugar (5)	6	1 846±304	1 544±91	392.8±65.6	44.92±7.72	8.56±3.1 1
10 mL skim milk & 10 g brown sugar (6)	6	2 005±364	1 514±78	386.2±57.2	43.93±8.81	7.83±2.3 0
10 mL skim milk & 5 g honey (7)	6	1 876±264	3 337±119	401.3±54.3	63.43±30.07	7.44±2 .58
10 mL skim milk & 9 g honey (8)	6	1 926±299	3 369±134	402.2±51.9	59.87±28.92	7.28±2 .41
10 mL skim milk & 15 g honey (9)	6	1 911±226	3 385±103	419.7±59.3	60.24±29.21	6.69±2 .40
Significant difference			(4)-(2)[f] (6)-(1)(2)(3)[f] (7)-(1)(2)(3)(4)(5)(6)[f] (8)-(1)(2)(3)(4)(5)(6)[f] (9)-(1)(2)(3)(4)(5)(6)[f]			

[a] Samples with 10 millilitre (mL) skim milk and different sweetening agents added in small, medium and large gram (g) amounts prepared as traditional / fermented rooibos bags with freshly boiled water, stirred for 30 seconds and steeped for three minutes (checked against a laboratory timer).
[b] FRAP: Ferric reducing antioxidant power assay presented as micromole (µmol) per litre (L).
[c] TEAC: Trolox equivalent antioxidant capacity assay presented as micromole (µmol) per litre (L).
[d] Total polyphenols, flavonol content and flavanol content presented as milligram (mg) per litre (L).
[e] Values: Mean ± standard deviation.
[f] Overall significant ($p<0.05$) difference in the Kruskal-Wallis Test one-way analysis of variance by ranks of the samples with the significant ($p<0.05$) contrasts transpiring on the post-hoc pairwise multiple comparisons indicated as a combined flavouring (number presented in brackets [e.g. 10 mL skim milk & 4 g white sugar as 1] significantly different (-) to another combined flavouring with each flavouring presented as a number in brackets [e.g. 10 mL skim milk & 6 g white sugar as 2]).

ii Small low fat milk amount addition combined with sweetening agents

As with the TEAC of the rooibos samples prepared with skim milk as a small amount addition of 10 mL and with the various sweetening agent additions, the TEAC of the rooibos samples prepared with low fat milk at a small amount addition of 10 mL with the various sweetening agent additions, also differed significantly ($p < 0.05$). In addition, the flavanol content also differed significantly ($p < 0.05$) between these rooibos samples (see Table 4.8).

Table 4.8: Total antioxidant capacity and the total polyphenol, flavonol and flavanol contents of rooibos samples with 10 mL low fat milk and sweetening agents added in small, medium and large amounts and the differences between these rooibos samples

Flavourings	Number of samples[a]	Antioxidant capacity and -content				
		FRAP (µmol/L)[b]	TEAC (µmol/L)[c]	Total polyphenols (mg/L)[d]	Flavonols (mg/L)[d]	Flavanols (mg/L)[d]
10 mL low fat milk & 4 g white sugar (1)	6	1 851±192[e]	1 571±156	398.5±51.2	67.08±18.82	11.01±2.52
10 mL low fat milk & 6 g white sugar (2)	6	1 878±287	1 582±127	408.7±57.1	72.01±18.98	11.22± 2.24
10 mL low fat milk & 10 g white sugar (3)	6	1 914±245	1 618±139	403.3±61.2	70.23±22.25	11.77± 2.93
10 mL low fat milk & 4 g brown sugar (4)	6	2 027±329	1 677±175	436.8±78.9	62.09±11.96	12.59± 3.44
10 mL low fat milk & 6 g brown sugar (5)	6	1 950±230	1 763±495	407.2±59.6	62.34±9.58	11.20±2 .91
10 mL low fat milk & 10 g brown sugar (6)	6	1 728±353	1 582±176	367.6±98.6	57.70±17.93	9.41±3 .32
10 mL low fat milk & 5 g honey (7)	6	1 905±295	3 288±257	419.2±66.5	75.73±31.37	8.79±4 .08
10 mL low fat milk & 9 g honey (8)	6	1 836±232	3 312±229	420.6±60.1	76.28±30.84	10.14± 2.96
10 mL low fat milk & 15 g honey (9)	6	1 939±221	3 389±183	439.5±67.9	74.61±29.51	11.22± 2.61
Significant difference			(6)-(4)[f] (7)-(1)(2)(3)(4)(5)(6)[f] (8)-(1)(2)(3)(4)(5)(6)[f] (9)-(1)(2)(3)(4)(5)(6)[f]			(4)-(6)(7)[g]

[a] Samples with 10 millilitre (mL) low fat milk and different sweetening agents added in small, medium and large gram (g) amounts prepared as traditional / fermented bags with freshly boiled water, stirred for 30 seconds and steeped for three minutes (checked against a laboratory timer).
[b] FRAP: Ferric reducing antioxidant power assay presented as micromole (µmol) per litre (L).
[c] TEAC: Trolox equivalent antioxidant capacity assay presented as micromole (µmol) per litre (L).
[d] Total polyphenols, flavonol content and flavanol content presented as milligram (mg) per litre (L).
[e] Values: Mean ± standard deviation.
[f] Overall significant ($p<0.05$) difference in the Kruskal-Wallis Test one-way analysis of variance by ranks of the samples with the significant ($p<0.05$) contrasts transpiring on the post-hoc pairwise multiple comparisons indicated as a combined flavouring (number presented in brackets [e.g. 10 mL low fat milk & 4 g white sugar as 1] significantly different (-) to another combined flavouring with each flavouring presented as a number in brackets [e.g. 10 mL low fat milk & 6 g white sugar as 2]).
[g] Overall significant ($p<0.05$) difference in the Levene's Test for equality of variances of the samples with the significant ($p < 0.05$) differences transpiring in the pairwise multiple comparisons of the Student-Newman-Keuls Test indicated as a combined flavouring (number presented in brackets [e.g. 10 mL low fat milk & 4 g white sugar as 1] significantly different (-) to another combined flavouring with each flavouring presented as a number in brackets [e.g. 10 mL low fat milk & 6 g white sugar as 2]).

The rooibos samples prepared with 10 mL low fat milk and to which a small (5 g), medium (9 g) and large (15 g) amount of honey was added respectively again delivered the rooibos samples with the highest TEAC (3 288 ± 257 µmol/L, 3 312 ± 229 µmol/L and 3 389 ± 183 µmol/L respectively). The TEAC of each of these rooibos samples to which honey was added in a different amount was also significantly ($p < 0.05$) higher than that of the other rooibos samples prepared with 10 mL low fat milk added and the corresponding amount addition of either white and brown sugar, i.e. as a small (4 g), medium (6 g) or large (10 g) amount, respectively. The TEAC of the rooibos sample with added 10 mL low fat milk and a large amount (10 g) of brown sugar (1 582 ± 176 µmol/L) was in addition significantly ($p < 0.05$) lower than that of the rooibos sample with 10 mL low fat milk and a small amount (4 g) of brown sugar added (1 677 ± 175 µmol/L). The flavanol content of the rooibos sample with 10 mL low fat milk and a small amount (4 g) of brown sugar added (12.59 ± 3.44 mg/L) was significantly ($p < 0.05$) higher than that of the rooibos samples with 10 mL low fat milk and a large amount (10 g) of brown sugar (9.41 ± 3.32 mg/L) and a small amount (5 g) of honey added (8.79 ± 4.08 mg/L) respectively (see Table 4.8).

iii Small whole milk amount addition combined with sweetening agents

The TEAC of the rooibos samples prepared with whole milk at a small amount addition of 10 mL with the various sweetening agent additions, also differed significantly ($p < 0.05$) as with the TEAC of the rooibos samples respectively prepared with skim milk and low fat milk as a small amount addition of 10 mL and with the various sweetening agent additions (see Table 4.9). The rooibos samples prepared with 10 mL whole milk and an added small (5 g), medium (9 g) and large (15 g) amount of honey (3 124 ± 131 µmol/L, 3 232 ± 126 µmol/L and 3 262 ± 197 µmol/L respectively) was, as before, significantly ($p <0.05$) higher than that of the other rooibos samples prepared with 10 mL whole milk added and small (4 g), medium (6 g) and large (10 g) amounts of either white and brown sugar added respectively. These three rooibos samples with the

differing added honey amounts also again delivered the samples with the highest TEAC. The TEAC of the rooibos sample with the 10 mL whole milk and a medium amount (6 g) of brown sugar added (1 909 ± 826 µmol/L) was in addition significantly ($p < 0.05$) higher than that of the rooibos samples with the 10 mL whole milk and a small amount (4 g) and a medium amount (6 g) of white sugar added respectively (1 470 ± 119 µmol/L and 1 483 ± 103 µmol/L respectively). The rooibos sample with the 10 mL whole milk and a large amount (10 g) of brown sugar added (2 256 ± 1 013 µmol/L) furthermore had a significantly ($p < 0.05$) higher TEAC than the rooibos samples with the 10 mL whole milk and either small (4 g), medium (6 g) and large (10 g) amounts of white sugar added (1 470 ± 119 µmol/L, 1 483 ± 103 µmol/L and 1 519 ± 119 µmol/L respectively), and a small amount (4 g) of brown sugar added (1 609 ± 293 µmol/L) respectively (see Table 4.9).

Table 4.9: Antioxidant characteristics of the rooibos herbal tea samples with 10 mL whole milk and various sweetening agents added

Flavourings	Number of samples[a]	Antioxidant capacity and –content				
		FRAP (µmol/L)[b]	TEAC (µmol/L)[c]	Total polyphenols (mg/L)[d]	Flavonols (mg/L)[d]	Flavanols (mg/L)[d]
10 mL whole milk & 4 g white sugar (1)	6	1 861±275[e]	1 470±119	393.3±72.2	78.37±24.69	13.05±3.17
10 mL whole milk & 6 g white sugar (2)	6	1 959±278	1 483±103	415.5±65.4	80.76±19.55	14.29± 3.17
10 mL whole milk & 10 g white sugar (3)	6	2 024±433	1 519±119	421.2±64.7	86.10±22.04	14.63± 3.14
10 mL whole milk & 4 g brown sugar (4)	6	1 997±293	1 609±293	420.0±61.7	77.41±12.20	13.99± 2.86
10 mL whole milk & 6 g brown sugar (5)	6	1 972±399	1 909±826	423.3±64.3	77.26±11.17	13.99± 2.63
10 mL whole milk & 10 g brown sugar (6)	6	1 950±377	2 256±1 013	417.3±58.8	73.00±11.64	13.7 8±2.71
10 mL whole milk & 5 g honey (7)	6	1 857±335	3 124±131	416.5±65.3	80.80±23.63	12.43± 4.10
10 mL whole milk & 9 g honey (8)	6	1 993±305	3 232±126	436.5±57.8	77.52±24.59	12.73± 3.09
10 mL whole milk & 15 g honey (9)	6	1 955±205	3 262±197	449.6±70.8	81.83±27.73	12.73± 4.19
Significant difference			(5)-(1)(2)[f] (6)-(1)(2)(3)(4)[f] (7)-(1)(2)(3)(4)(5)(6)[f] (8)-(1)(2)(3)(4)(5)(6)[f] (9)-(1)(2)(3)(4)(5)(6)[f]			

[a] Samples with 10 millilitre (mL) whole milk and different sweetening agents added in small, medium and large gram (g) amounts prepared as traditional / fermented rooibos bags with freshly boiled water, stirred for 30 seconds and steeped for three minutes (checked against a laboratory timer).
[b] FRAP: Ferric reducing antioxidant power assay presented as micromole (µmol) per litre (L).
[c] TEAC: Trolox equivalent antioxidant capacity assay presented as micromole (µmol) per litre (L).
[d] Total polyphenols, flavonol content and flavanol content presented as milligram (mg) per litre (L).
[e] Values: Mean ± standard deviation.
[f] Overall significant ($p<0.05$) difference in the Kruskal-Wallis Test one-way analysis of variance by ranks of the samples with the significant ($p<0.05$) contrasts transpiring on the post-hoc pairwise multiple comparisons indicated as a combined flavouring (number presented in brackets [e.g. 10 mL whole milk & 4 g white sugar as 1] significantly different (-) to another combined flavouring with each flavouring presented as a number in brackets [e.g. 10 mL whole milk & 6 g white sugar as 2]).

iv Medium skim milk amount addition combined with sweetening agents

The TEAC of the rooibos samples prepared with 20 mL skim milk as a medium amount addition and the various sweetening agent additions also differed significantly ($p < 0.05$) (see Table 4.10) as was found with the TEAC of the rooibos samples prepared with small amount additions of each of the milk types with the various sweetening agent additions. The rooibos samples prepared with 20 mL skim milk and to which a small (5 g), medium (9 g) and large (15 g) amount of honey was added again respectively delivered the rooibos samples with the highest TEAC (3 549 ± 142 µmol/L, 3 619 ± 106 µmol/L and 3 636 ± 92 µmol/L respectively) which is also, as before, significantly ($p < 0.05$) higher than that of the other rooibos samples prepared with 20 mL skim milk added and small (4 g), medium (6 g) and large (10 g) amount additions of either white and brown sugar respectively. The rooibos samples with the 20 mL skim milk and the different amounts of white and brown sugar added had a TEAC which ranged from the lowest being for a small amount (4 g) of added brown sugar (at 2 138 ± 163 µmol/L) to the highest being for a medium amount (6 g) of added brown sugar (at 2 249 ± 213 µmol/L) (see Table 4.10).

Table 4.10: Antioxidant characteristics of rooibos herbal tea samples with 20 mL skim milk and various sweetening agents added in small, medium and large amounts

Flavourings	Number of samples[a]	Antioxidant capacity and -content				
		FRAP (µmol/L)[b]	TEAC (µmol/L)[c]	Total polyphenols (mg/L)[d]	Flavonols (mg/L)[d]	Flavanols (mg/L)[d]
20 mL skim milk & 4 g white sugar (1)	6	1 826±285[e]	2 178±247	415.9±61.6	55.23±15.14	8.29±1.92
20 mL skim milk & 6 g white sugar (2)	6	1 845±290	2 223±224	411.8±53.8	53.00±14.52	8.15±2 .34
20 mL skim milk & 10 g white sugar (3)	6	1 802±334	2 207±278	416.0±55.6	54.79±12.78	8.54±2 .09
20 mL skim milk & 4 g brown sugar (4)	6	1 893±423	2 138±163	415.9±56.7	49.33±8.10	8.31±2. 56
20 mL skim milk & 6 g brown sugar (5)	6	1 889±308	2 249±213	415.5±54.2	49.61±7.42	8.38±2. 45
20 mL skim milk & 10 g brown sugar (6)	6	1 817±296	2 186±171	408.1±57.1	48.16±11.81	7.74±2 .62
20 mL skim milk & 5 g honey (7)	6	1 930±274	3 549±142	434.8±56.0	69.06±27.14	8.68±3 .91
20 mL skim milk & 9 g honey (8)	6	1 948±284	3 619±106	448.6±53.2	63.44±17.85	7.62±2 .99
20 mL skim milk & 15 g honey (9)	6	1 924±249	3 636±92	458.1±60.6	67.47±22.02	7.81±3. 08
Significant difference			(7)-(1)(2)(3)(4)(5)(6)[f] (8)-(1)(2)(3)(4)(5)(6)[f] (9)-(1)(2)(3)(4)(5)(6)[f]			

[a] Samples with 20 millilitre (mL) skim milk and different sweetening agents added in small, medium and large gram (g) amounts prepared as traditional / fermented rooibos bags with freshly boiled water, stirred for 30 seconds and steeped for three minutes (checked against a laboratory timer).
[b] FRAP: Ferric reducing antioxidant power assay presented as micromole (µmol) per litre (L).
[c] TEAC: Trolox equivalent antioxidant capacity assay presented as micromole (µmol) per litre (L).
[d] Total polyphenols, flavonol content and flavanol content presented as milligram (mg) per litre (L).
[e] Values: Mean ± standard deviation.
[f] Overall significant ($p<0.05$) difference in the Kruskal-Wallis Test one-way analysis of variance by ranks of the samples with the significant ($p<0.05$) contrasts transpiring on the post-hoc pairwise multiple comparisons indicated as a combined flavouring (number presented in brackets [e.g. 20 mL skim milk & 4 g white sugar as 1] significantly different (-) to another combined flavouring with each flavouring presented as a number in brackets [e.g. 20 mL skim milk & 6 g white sugar as 2]).

v Medium low fat milk amount addition combined with sweetening agents

The TEAC of the rooibos samples prepared with 20 mL low fat milk added as medium amount and the various sweetening agent additions also differed significantly ($p < 0.05$) (see Table 4.11). The rooibos samples prepared with 20 mL low fat milk and to which a small (5 g), medium (9 g) and large (15 g) amount of honey was added as before presented a significantly ($p < 0.05$) higher TEAC (3 608 ± 151 µmol/L, 3 577 ± 129 µmol/L and 3 627 ± 179 µmol/L respectively) than that of the other rooibos samples prepared with 20 mL low fat milk added and small (4 g), medium (6 g) and large (10 g) amount additions of either white and brown sugar, respectively. These three rooibos samples with the differing amounts of honey added also delivered the highest TEAC. The TEAC of the rooibos sample with the 20 mL low fat milk and a medium amount (6 g) of white sugar added (2 201 ± 193 µmol/L) was furthermore significantly ($p < 0.05$) lower than that of the rooibos samples with the 20 mL low fat milk and a small amount (4 g) of white sugar and a large amount (10 g) of white and brown sugar added respectively (2 402 ± 358 µmol/L, 2 290 ± 159 µmol/L and 2 580 ± 454 µmol/L respectively). In addition, the rooibos sample with the 20 mL low fat milk and brown sugar added in a large amount (10 g) had a significantly ($p < 0.05$) higher TEAC than the rooibos sample with the 20 mL low fat milk and brown sugar added in a medium amount (6 g) (2 580 ± 454 µmol/L vs 2 240 ± 107 µmol/L) (see Table 4.11).

Table 4.11: Antioxidant characteristics of rooibos herbal tea samples prepared with 20 mL low fat milk and various sweetening agents added in small, medium and large amounts

Flavourings	Number of samples[a]	Antioxidant capacity and –content				
		FRAP (µmol/L)[b]	TEAC (µmol/L)[c]	Total polyphenols (mg/L)[d]	Flavonols (mg/L)[d]	Flavanols (mg/L)[d]
20 mL low fat milk & 4 g white sugar (1)	6	1 809 ±280[e]	2 402±358	454.2±64.1	95.44±18.30	15.45±1.31
20 mL low fat milk & 6 g white sugar (2)	6	1 876 ±252	2 201±193	444.2±62.7	94.94±21.33	14.88 ±2.42
20 mL low fat milk & 10 g white sugar (3)	6	1 905±289	2 290±159	454.5±58.6	93.91±20.33	15.27± 1.89
20 mL low fat milk & 4 g brown sugar (4)	6	1 965±279	2 285±149	452.3±56.8	80.62±12.37	14.47± 3.12

Table 4.11: Antioxidant characteristics of rooibos herbal tea samples prepared with 20 mL low fat milk and various sweetening agents added in small, medium and large amounts (cont.)

20 mL low fat milk & 6 g brown sugar (5)	6	1 825±251	2 240±107	439.7±58.8	79.12±12.70	13.69± 2.82
20 mL low fat milk & 10 g brown sugar (6)	6	1 964±280	2 580±454	446.5±66.7	81.58±12.58	14.06± 3.56
20 mL low fat milk & 5 g honey (7)	6	1 935±311	3 608±151	454.6±61.9	95.18±30.48	12.52± 4.56
20 mL low fat milk & 9 g honey (8)	6	1 930±196	3 577±129	465.4±60.3	91.39±25.88	13.78± 5.83
20 mL low fat milk & 15 g honey (9)	6	1 928±124	3 627±179	479.5±59.7	93.07±28.38	13.32± 5.35
Significant difference			(2)-(1)(3)(6)[f] (6)-(5)[f] (7)-(1)(2)(3)(4)(5)(6)[f] (8)-(1)(2)(3)(4)(5)(6)[f] (9)-(1)(2)(3)(4)(5)(6)[f]			

[a] Samples with 20 millilitre (mL) low fat milk and different sweetening agents added in small, medium and large gram (g) amounts prepared as traditional / fermented rooibos herbal tea bags with freshly boiled water, stirred for 30 seconds and steeped for three minutes (checked against a laboratory timer).
[b] FRAP: Ferric reducing antioxidant power assay presented as micromole (µmol) per litre (L).
[c] TEAC: Trolox equivalent antioxidant capacity assay presented as micromole (µmol) per litre (L).
[d] Total polyphenols, flavonol content and flavanol content presented as milligram (mg) per litre (L).
[e] Values: Mean ± standard deviation.
[f] Overall significant ($p<0.05$) difference in the Kruskal-Wallis Test one-way analysis of variance by ranks of the samples with the significant ($p<0.05$) contrasts transpiring on the post-hoc pairwise multiple comparisons indicated as a combined flavouring (number presented in brackets [e.g. 20 mL low fat milk & 4 g white sugar as 1] significantly different (-) to another combined flavouring with each flavouring presented as a number in brackets [e.g. 20 mL low fat milk & 6 g white sugar as 2]).

vi Medium whole milk amount addition combined with sweetening agents

Similar to the results described for the TEAC of the rooibos samples prepared with skim milk and low fat milk (20 mL) and with the various sweetening agent additions, the TEAC of the rooibos samples prepared with whole milk at a medium amount addition of 20 mL with the various sweetening agent additions, also differed significantly ($p < 0.05$) (see Table 4.12). The rooibos samples prepared with 20 mL whole milk and to which a small (5 g), medium (9 g) and large (15 g) amount of honey was added, also again respectively delivered the rooibos samples with the highest TEAC (3 492 ± 163 µmol/L, 3 557 ± 151 µmol/L and 3 540 ± 289 µmol/L respectively) and were significantly ($p < 0.05$) higher than that of the rooibos samples prepared with 20 mL whole milk added and small (4 g), medium (6 g) and large (10 g) amounts of either white and brown sugar added respectively. The TEAC of the rooibos sample with the 20 mL whole milk

and a medium amount (6 g) of white sugar added (2 392 ± 658 µmol/L) was in addition significantly ($p < 0.05$) higher than that of the rooibos sample with the 20 mL whole milk and a small amount (4 g) of white sugar added (2 035 ± 225 µmol/L) (see Table 4.12).

Table 4.12: Antioxidant characteristics of rooibos herbal tea samples prepared with 20 mL whole milk and various sweetening agents added in small, medium and large amounts

Flavourings	Number of samples[a]	Antioxidant capacity and -content				
		FRAP (µmol/L)[b]	TEAC (µmol/L)[c]	Total polyphenols (mg/L)[d]	Flavonols (mg/L)[d]	Flavanols (mg/L)[d]
20 mL whole milk & 4 g white sugar (1)	6	1 989±229[e]	2 035±225	466.9±58.6	128.4±32.6	20.99±3.48
20 mL whole milk & 6 g white sugar (2)	6	2 008±322	2 392±658	479.1±60.3	124.0±26.6	20.40±2 .32
20 mL whole milk & 10 g white sugar (3)	6	2 035±301	2 087±194	479.6±68.9	131.0±30.0	21.91±3 .91
20 mL whole milk & 4 g brown sugar (4)	6	2 094±305	2 443±803	474.2±61.6	109.2±22.1	20.42±3 .28
20 mL whole milk & 6 g brown sugar (5)	6	2 256±403	2 300±808	521.2±94.1	117.4±21.7	22.14±3 .85
20 mL whole milk & 10 g brown sugar (6)	6	2 092±317	2 206±379	475.3±63.2	112.9±20.9	20.61±4 .21
20 mL whole milk & 5 g honey (7)	6	2 046±267	3 492±163	497.3±76.3	116.2±30.2	23.01±4 .03
20 mL whole milk & 9 g honey (8)	6	2 004±261	3 557±151	493.5±57.9	121.1±27.4	18.91±5 .91
20 mL whole milk & 15 g honey (9)	6	2 019±266	3 540±289	496.8±63.0	122.4±34.1	18.48±6 .98
Significant difference			(2)-(1)[f] (7)-(1)(2)(3)(4)(5)(6)[f] (8)-(1)(2)(3)(4)(5)(6)[f] (9)-(1)(2)(3)(4)(5)(6)[f]			

[a] Samples with 20 millilitre (mL) whole milk and different sweetening agents added in small, medium and large gram (g) amounts prepared as traditional / fermented rooibos herbal tea bags with freshly boiled water, stirring for 30 seconds and steeping for three minutes (checked against a laboratory timer).
[b] FRAP: Ferric reducing antioxidant power assay presented as micromole (µmol) per litre (L).
[c] TEAC: Trolox equivalent antioxidant capacity assay presented as micromole (µmol) per litre (L).
[d] Total polyphenols, flavonol content and flavanol content presented as milligram (mg) per litre (L).
[e] Values: Mean ± standard deviation.
[f] Overall significant ($p<0.05$) difference in the Kruskal-Wallis Test one-way analysis of variance by ranks of the samples with the significant ($p<0.05$) contrasts transpiring on the post-hoc pairwise multiple comparisons indicated as a combined flavouring (number presented in brackets [e.g. 20 mL whole milk & 4 g white sugar as 1] significantly different (-) to another combined flavouring with each flavouring presented as a number in brackets [e.g. 20 mL whole milk & 6 g white sugar as 2]).

vii Large skim milk amount addition combined with sweetening agents

The TEAC of the rooibos samples prepared with skim milk as a large amount addition of 30 mL with the various sweetening agent additions, also differed significantly ($p < 0.05$), as found before and in addition to the flavonol content of these rooibos samples (see Table 4.13). The rooibos samples with the added skim milk in a large amount (30 mL) and honey added in small (5 g), medium (9 g) and large (15 g) amounts had a significantly ($p < 0.05$) higher TEAC (3 773 ± 146 µmol/L, 3 762 ± 211 µmol/L and 3 771 ± 182 µmol/L respectively) than the other rooibos samples prepared with 30 mL skim milk added and small (4 g), medium (6 g) and large (10 g) amount additions of either white and brown sugar respectively. The rooibos samples with the large amount addition of 30 mL skim milk and the honey additions in small, medium and large amounts also delivered the samples with the highest TEAC (see Table 4.13).

Table 4.13: Antioxidant characteristics of the rooibos herbal tea samples with 30 mL skim milk and various sweetening agents added in small, medium and large amounts

Flavourings	Number of samples[a]	Antioxidant capacity and -content				
		FRAP (µmol/L)[b]	TEAC (µmol/L)[c]	Total polyphenols (mg/L)[d]	Flavonols (mg/L)[d]	Flavanols (mg/L)[d]
30 mL skim milk & 4 g white sugar (1)	6	1 906±155[e]	2 949±346	470.0±41.2	62.61±17.21	10.42±1.91
30 mL skim milk & 6 g white sugar (2)	6	1 840±292	2 853±359	442.3±57.9	60.40±10.01	9.16±3 .04
30 mL skim milk & 10 g white sugar (3)	6	1 772±243	2 913±443	428.6±55.0	60.92±12.36	8.20±2 .00
30 mL skim milk & 4 g brown sugar (4)	6	1 802±272	2 849±126	435.5±56.0	58.20±15.89	8.95±2 .11

Table 4.13: Antioxidant characteristics of the rooibos herbal tea samples with 30 mL skim milk and various sweetening agents added in small, medium and large amounts (cont.)

Flavourings	Number of samples[a]	Antioxidant capacity and -content				
		FRAP (µmol/L)[b]	TEAC (µmol/L)[c]	Total polyphenols (mg/L)[d]	Flavonols (mg/L)[d]	Flavanols (mg/L)[d]
30 mL skim milk & 6 g brown sugar (5)	6	1 782±224	2 857±103	449.6±55.6	54.31±10.17	8.97±2 .21
30 mL skim milk & 10 g brown sugar (6)	6	1 817±260	2 960±201	449.4±56.0	55.59±11.05	8.91±2 .50
30 mL skim milk & 5 g honey (7)	6	1 735±370	3 773±146	451.5±70.7	77.56±19.26	8.36±3 .57
30 mL skim milk & 9 g honey (8)	6	1 844±236	3 762±211	471.5±62.7	71.25±19.59	6.80±4 .45
30 mL skim milk & 15 g honey (9)	6	1 885±376	3 771±182	486.3±59.9	72.89±19.68	9.73±4 .47
Significant difference			(7)-(1)(2)(3)(4)(5)(6)[f] (8)-(1)(2)(3)(4)(5)(6)[f] (9)-(1)(2)(3)(4)(5)(6)[f]		(7)-(1)(2)(3)(4)(5)(6)[f] (8)-(4)(5)(6)[f] (9)-(3)(4)(5)(6)[f]	

[a] Samples with 30 millilitre (mL) skim milk and different sweetening agents added in small, medium and large gram (g) amounts prepared as traditional / fermented rooibos bags with freshly boiled water, stirred for 30 seconds and steeped for three minutes (checked against a laboratory timer).
[b] FRAP: Ferric reducing antioxidant power assay presented as micromole (µmol) per litre (L).
[c] TEAC: Trolox equivalent antioxidant capacity assay presented as micromole (µmol) per litre (L).
[d] Total polyphenols, flavonol content and flavanol content presented as milligram (mg) per litre (L).
[e] Values: Mean ± standard deviation.
[f] Overall significant ($p<0.05$) difference in the Kruskal-Wallis Test one-way analysis of variance by ranks of the samples with the significant ($p<0.05$) contrasts transpiring on the post-hoc pairwise multiple comparisons indicated as a combined flavouring (number presented in brackets [e.g. 30 mL skim milk & 4 g white sugar as 1] significantly different (-) to another combined flavouring with each flavouring presented as a number in brackets [e.g. 30 mL skim milk & 6 g white sugar as 2]).

The rooibos sample with 30 mL skim milk and a small (5 g) amount of honey added delivered the highest flavonol content (77.56 ± 19.26 mg/L) and a significantly ($p < 0.05$) higher flavonol content than the rooibos samples with 30 mL skim milk and the different amount additions of either white and brown sugar respectively [ranging from the lowest flavonol content at 54.31 ± 10.17 mg/L for the rooibos sample with a medium (6 g) amount of brown sugar added and the highest flavonol content at 62.61 ± 17.21 mg/L for the rooibos sample with a small (4 g) amount of white sugar added]. The rooibos sample with 30 mL skim milk and a medium (9 g) amount of honey added) had a significantly ($p < 0.05$) higher flavonol content (71.25 ± 19.59 mg/L) than the rooibos samples with 30 mL skim milk and brown sugar additions in small (4 g), medium (6 g) and large (10 g) amounts respectively (58.20 ± 15.89 mg/L, 54.31 ± 10.17 mg/L and 55.59 ± 11.05 mg/L respectively). The flavonol content of the rooibos sample with the 30 mL skim milk and a large (15 g) amount of honey added (72.89 ± 19.68 mg/L) was significantly ($p < 0.05$)

higher than the rooibos sample with the 30 mL skim milk and a large (10 g) amount of white sugar added (60.92 ± 12.36 mg/L) and the rooibos samples with the 30 mL skim milk and brown sugar additions in small (4 g), medium (6 g) and large (10 g) amounts respectively (58.20 ± 15.89 mg/L, 54.31 ± 10.17 mg/L and 55.59 ± 11.05 mg/L respectively) (see Table 4.13).

viii Large low fat milk amount addition combined with sweetening agents

The rooibos samples with 30 mL low fat milk and to which a small (5 g), medium (9 g) and large (15 g) amount of honey was added respectively delivered the highest TEAC (3 765 ± 151 µmol/L, 3 742 ± 214 µmol/L and 3 652 ± 270 µmol/L respectively) as well as a significantly ($p < 0.05$) higher TEAC than the other rooibos samples prepared with 30 mL low fat milk and small (4 g), medium (6 g) and large (10 g) amount additions of either white and brown sugar, respectively (ranging from the lowest TEAC at 3 009 ± 204 µmol/L for the rooibos sample with a medium (6 g) amount addition of white sugar and the highest TEAC at 3 206 ± 329 µmol/L for the rooibos sample with a small (4 g) amount addition of brown sugar). In general, as previously encountered, no other significant ($p >0.05$) differences were found in the antioxidant characteristics of these combined household flavourings (see Table 4.14).

Table 4.14: Antioxidant characteristics of rooibos herbal tea samples prepared with 30 mL low fat milk and various sweetening agents added in small, medium and large amounts

Flavourings	Number of samples[a]	Antioxidant capacity and -content				
		FRAP (µmol/L)[b]	TEAC (µmol/L)[c]	Total polyphenols (mg/L)[d]	Flavonols (mg/L)[d]	Flavanols (mg/L)[d]
30 mL low fat milk & 4 g white sugar(1)	6	1 871±258[e]	3 160±454	490.1±57.9	115.4±25.2	19.39±2.17
30 mL low fat milk & 6 g white sugar (2)	6	1 881±247	3 009±204	480.9±50.4	114.6±26.9	18.93±1 .98
30 mL low fat milk & 10 g white sugar (3)	6	1 984±281	3 076±338	488.7±52.8	112.6±25.7	18.70±1 .44
30 mL low fat milk & 4 g brown sugar (4)	6	1 927±299	3 206±329	497.3±59.3	101.0±17.7	18.32±3 .66

Table 4.14: Antioxidant characteristics of rooibos herbal tea samples prepared with 30 mL low fat milk and various sweetening agents added in small, medium and large amounts (cont.)

Flavourings	Number of samples[a]	Antioxidant capacity and -content				
		FRAP (µmol/L)[b]	TEAC (µmol/L)[c]	Total polyphenols (mg/L)[d]	Flavonols (mg/L)[d]	Flavanols (mg/L)[d]
30 mL low fat milk & 6 g brown sugar (5)	6	1 967±225	3 016±159	491.8±66.5	103.1±14.8	18.22±3 .63
30 mL low fat milk & 10 g brown sugar (6)	6	1 949±311	3 067±405	484.6±55.4	105.3±12.5	17.79±3 .81
30 mL low fat milk & 5 g honey (7)	6	1 934±251	3 765±151	505.4±52.8	110.6±29.6	16.62±6 .88
30 mL low fat milk & 9 g honey (8)	6	1 936±258	3 742±214	500.4±47.4	113.2±33.5	16.78±6 .49
30 mL low fat milk & 15 g honey (9)	6	1 889±210	3 652±270	500.4±54.7	112.8±32.3	17.01±6 .16
Significant difference			(7)-(1)(2)(3)(4)(5)(6)[f] (8)-(1)(2)(3)(4)(5)(6)[f] (9)-(1)(2)(3)(4)(5)(6)[f]			

[a] Samples with 30 millilitre (mL) low fat milk and different sweetening agents added in small, medium and large gram (g) amounts prepared as traditional / fermented rooibos herbal tea bags with freshly boiled water, stirred for 30 seconds and steeped for three minutes (checked against a laboratory timer).
[b] FRAP: Ferric reducing antioxidant power assay presented as micromole (µmol) per litre (L).
[c] TEAC: Trolox equivalent antioxidant capacity assay presented as micromole (µmol) per litre (L).
[d] Total polyphenols, flavonol content and flavanol content presented as milligram (mg) per litre (L).
[e] Values: Mean ± standard deviation.
[f] Overall significant ($p<0.05$) difference in the Kruskal-Wallis Test one-way analysis of variance by ranks of the samples with the significant ($p<0.05$) contrasts transpiring on the post-hoc pairwise multiple comparisons indicated as a combined flavouring (number presented in brackets [e.g. 30 mL low fat milk & 4 g white sugar as 1] significantly different (-) to another combined flavouring with each flavouring presented as a number in brackets [e.g. 30 mL low fat milk & 6 g white sugar as 2]).

ix Large whole milk amount addition combined with sweetening agents

Such as with the TEAC of the rooibos samples prepared with skim and low fat milks as large amount additions of 30 mL and with the various sweetening agent additions respectively, the TEAC of the rooibos samples prepared with whole milk at a large amount addition of 30 mL with the various sweetening agent additions, also differed significantly ($p < 0.05$) (see Table 4.15). The rooibos samples prepared with 30 mL whole milk and to which a small (5 g), medium (9 g) and large (15 g) amount of honey was added again respectively delivered the rooibos samples with the highest TEAC (3 674 ± 217 µmol/L, 3 654 ± 249 µmol/L and 3 706 ± 230 µmol/L respectively) and followed by that of the 30 mL whole milk rooibos samples with brown sugar added at a medium (6 g) and a large (10 g) amount (3 236 ± 585 µmol/L and 3 119 ± 567 µmol/L respectively) (see Table 4.15).

Table 4.15: Antioxidant characteristics of the rooibos herbal tea samples prepared with 30 mL whole milk and various sweetening agents added in small, medium and large amounts

Flavourings	Number of samples[a]	Antioxidant capacity and –content				
		FRAP (µmol/L)[b]	TEAC (µmol/L)[c]	Total polyphenols (mg/L)[d]	Flavonols (mg/L)[d]	Flavanols (mg/L)[d]
30 mL whole milk & 4 g white sugar (1)	6	2 161±330[e]	2 892±556	513.9±57.6	158.8±41.9	26.92±3.35
30 mL whole milk & 6 g white sugar (2)	6	2 061±187	2 735±620	514.9±49.5	158.9±37.3	24.63±7 .14
30 mL whole milk & 10 g white sugar (3)	6	2 114±423	2 857±569	507.8±59.2	155.1±29.3	24.57±4 .19
30 mL whole milk & 4 g brown sugar (4)	6	2 016±249	2 786±335	514.5±55.2	137.2±34.0	25.69±4 .49
30 mL whole milk & 6 g brown sugar (5)	6	2 274±525	3 236±585	517.2±53.0	136.8±31.2	26.33±4 .67
30 mL whole milk & 10 g brown sugar (6)	6	2 310±609	3 119±567	511.9±59.3	142.5±25.3	25.30±4 .61
30 mL whole milk & 5 g honey (7)	6	2 095±242	3 674±217	535.4±56.1	143.1±30.2	28.66±3 .66
30 mL whole milk & 9 g honey (8)	6	2 104±345	3 654±249	538.2±63.5	143.6±33.3	27.89±5 .37
30 mL whole milk & 15 g honey (9)	6	2 117±323	3 706±230	545.1±65.71	141.8±29.6	27.75± 9.07
Significant difference			(5)-(1)(2)(3)(4)[f] (6)-(2)[f] (7)-(1)(2)(3)(4)(5)(6)[f] (8)-(1)(2)(3)(4)(5)(6)[f] (9)-(1)(2)(3)(4)(5)(6)[f]			

[a] Samples with 30 millilitre (mL) whole milk and different sweetening agents added in small, medium and large gram (g) amounts prepared as traditional / fermented rooibos bags with freshly boiled water, stirred for 30 seconds and steeped for three minutes (checked against a laboratory timer).
[b] FRAP: Ferric reducing antioxidant power assay presented as micromole (µmol) per litre (L).
[c] TEAC: Trolox equivalent antioxidant capacity assay presented as micromole (µmol) per litre (L).
[d] Total polyphenols, flavonol content and flavanol content presented as milligram (mg) per litre (L).
[e] Values: Mean ± standard deviation.
[f] Overall significant ($p<0.05$) difference in the Kruskal-Wallis Test one-way analysis of variance by ranks of the samples with the significant ($p<0.05$) contrasts transpiring on the post-hoc pairwise multiple comparisons indicated as a combined flavouring (number presented in brackets [e.g. 30 mL whole milk & 4 g white sugar as 1] significantly different (-) to another combined flavouring with each flavouring presented as a number in brackets [e.g. 30 mL whole milk & 6 g white sugar as 2]).

The TEAC of the rooibos samples to which honey was added in amounts representing small, medium and large additions was, as before, significantly ($p < 0.05$) higher than that of the other rooibos samples prepared with 30 mL whole milk added and small (4 g), medium (6 g) and large (10 g) amounts of either white and brown sugar added respectively. The TEAC of the rooibos sample with the 30 mL whole milk and a medium amount (6 g) of brown sugar added (3 236 ± 585 µmol/L) was in addition significantly ($p < 0.05$) higher than the rooibos samples with the 30 mL whole milk and either small (4 g), medium (6 g) and large (10 g) amounts of white sugar added (2 896 ± 556 µmol/L, 2 735 ± 620 µmol/L and 2 857 ± 569 µmol/L respectively) and a small (4 g) amount of brown sugar added (2 786 ± 335 µmol/L) respectively. The TEAC of the rooibos sample prepared with 30 mL whole milk to which a large (10 g) amount of brown sugar was added (3 119 ± 567 µmol/L) was significantly ($p < 0.05$) higher than the rooibos sample with 30 mL whole milk and a medium (6 g) amount of white sugar added (2 735 ± 620 µmol/L) (see Table 4.15).

4.1.5 Commercially flavoured rooibos

The TAC, total polyphenol, flavonol and flavanol contents of the commercially flavoured rooibos samples and their respective antioxidant characteristics are presented in Table 4.16. Significant ($p < 0.05$) differences were found within each of the antioxidant parameters measured. The FRAP of the camomile flavoured rooibos (traditional / fermented) (1 311 ± 185 µmol/L) and the berry flavoured iced tea (RTD) (724 ± 325 µmol/L) was the lowest of all the samples with the FRAP of these two samples also significantly ($p < 0.05$) lower than that of each of the other commercially flavoured rooibos samples respectively. The FRAP of the honey flavoured rooibos (traditional / fermented) (1 971.99 ± 425.66 µmol/L), the lemon flavoured iced tea (RTD) (3 008 ± 2 537 µmol/L) and the peach flavoured iced tea (RTD) (2 001 ± 1 459 µmol/L) were each also significantly ($p < 0.05$) lower than that of the lemon flavoured rooibos (traditional / fermented) (3 632 ± 1424 µmol/L), the lemon flavoured iced tea (powder) (2 734 ± 269 µmol/L), the instant apple fruit iced tea (powder) (4 750 ± 79 µmol/L) and the instant fruit punch and lemon flavoured iced tea (powder) (3 255 ± 314 µmol) respectively (see Table 4.16).

Table 4.16: Antioxidant characteristics of the commercially flavoured rooibos products

Flavour	Number of samples	Antioxidant capacity and -content				
		FRAP (µmol/L)[a]	TEAC (µmol/L)[b]	Total polyphenols (mg/L)[c]	Flavonols (mg/L)[c]	Flavanols (mg/L)[c]
Camomile, (traditional / fermented)[d] (1)	4	1 311±185[e]	1 299±108	176.5±46.2	16.96±4.07	ND[f]
Honey (traditional / fermented)[d] (2)	3	1 972±426	1 247±104	331.0±83.2	21.04±5.41	7.56±3. 22
Lemon (traditional / fermented)[d] (3)	3	3 632±1424	1 741±864	626.7±293.0	26.30±4.85	65.26 ±79.95
Iced tea – lemon (powder)[g] (4)	2	2 734±269	1 113±91	300.4±42.1	10.26±3.63	ND[f]
Iced tea – peach (powder)[g] (5)	1	2 395±147	1 655±154	298.5±16.5	8.68±4.00	ND[f]
Instant apple fruit iced tea (powder)[g] (6)	1	4 750±79	987.9±95.8	629.2±47.0	5.13±1.04	ND[f]
Instant fruit punch iced tea (powder)[g] (7)	1	2 276±172	1 200±86	373.1±30.4	3.55±4.72	ND[f]
Instant fruit punch & lemon iced tea (powder)[g] (8)	1	3 255±314	843.9±63.8	625.4±39.3	3.55±3.99	ND[f]
Iced tea – berry (RTD)[h] (9)	2	723.9±325.0	1 498±76	41.9±79.4	7.10±4.47	ND[f]
Iced tea – lemon (RTD)[h] (10)	4	3 008.34±2537	1 347±112	371.5±264.8	15.09±9.70	ND[f]
Iced tea – peach (RTD)[h] (11)	6	2 001±1459	1 378±120	323.7±225.3	10.92±6.68	ND[f]
Significant difference		(1)-(2)(3)(4)(5)(6)(7)(8)(10)(11)[i] (2)-(3)(4)(6)(8)[i] (9)-(2)(3)(4)(5)(6)(7)(8)(10)(11)[i] (10)-(3)(4)(6)(8)[i] (11)- (3)(4)(6)(8)[i]	(1)-(4)(8)(9)[i] (3)-(4)(6)(7)(8)(9)[i] (5)-(1)(2)(3)(4)(6)(7)(8)(10)(11)[i] (6)-(1)(9)(10)(11)[i] (8)-(2)[i] (9)-(2)(4)(7)(8)(10)(11)[i] (10)-(4)(8)(9)[i] (11)-(2)(4)(7)(8)[i]	(1)-(3)(4)(6)(7)(8)[i] (2)-(1)(3)(6)(8)[i] (5)-(3)(6)[i] (9)-(2)(3)(4)(5)(6)(7)(8)(10)(11)[i] (10)-(1)(3)(6)(8)(11)[i] (11)-(2)(3)(4)(6)(7)(8)[i]	(1)-(2)(4)(7)(10) (3)-(1)(2)(5)(10)(11) (6)-(1)(2)(5)(10)(11) (8)-(1)(2)(10)(11) (9)-(2)(3)(4)(5)(6)(7)(8)(10)(11) (11)-(2)(4)(7)(10)	

[a] FRAP: Ferric reducing antioxidant power assay presented as micromole (µmol) per litre (L).
[b] TEAC: Trolox equivalent antioxidant capacity assay presented as micromole (µmol) per litre (L).
[c] Total polyphenols, flavonol content and flavanol content presented as milligram (mg) per litre (L).
[d] Samples with different flavourings prepared as rooibos bags with freshly boiled water, stirring for 30 seconds and steeping for three minutes (checked against a laboratory timer).
[e] Values: Mean ± standard deviation.
[f] Flavanol content not detected (ND) in all samples providing for a negligible mean excluded in the data analysis.
[g] Commercially flavoured samples in powder form were prepared according to the instructions on the container.
[h] Ready-to-drink (RTD) samples were sampled directly from the container.
[i] Overall significant ($p<0.05$) difference in the Kruskal-Wallis Test one-way analysis of variance by ranks of the samples with the significant ($p<0.05$) contrasts transpiring on the post-hoc pairwise multiple comparisons indicated as a combined flavouring (number presented in brackets [e.g. camomile, (traditional / fermented) as 1] significantly different (-) to another commercially flavoured sample with each flavour presented as a number in brackets [e.g.honey (traditional / fermented) as 2]).

The TEAC of the commercially flavoured rooibos samples was the highest for the lemon flavoured rooibos (traditional / fermented) and the peach flavoured iced tea (powder). The TEAC of the lemon flavoured rooibos (traditional / fermented) (1 741 ± 864 µmol/L) was significantly ($p < 0.05$) higher than that of the lemon flavoured iced tea (powder) (1 113 ± 91 µmol/L), the instant apple fruit flavoured iced tea (powder) (987.9 ± 95.8 µmol), the instant fruit punch flavoured iced tea (powder) (1 200 ± 86 µmol/L), the instant fruit punch and lemon flavoured iced tea (powder) (843.9 ± 63.8 µmol/L) and the berry flavoured iced tea (RTD) (1 498 ± 76 µmol/L) respectively. The TEAC of the peach flavoured iced tea (powder) (1 655 ± 154 µmol/L) in contrast was significantly ($p < 0.05$) higher than that of each of the other commercially flavoured rooibos samples respectively, besides for that of the berry flavoured iced tea (RTD) (1 498 ± 76 µmol/L) (see Table 4.16).

The TEAC of the commercially flavoured rooibos samples was the lowest for the instant apple flavoured fruit iced tea (powder) and the instant fruit punch and lemon flavoured iced tea (powder). The TEAC of the instant apple flavoured fruit iced tea (powder) (987.9 ± 95.8 µmol/L) (see Table 4.16) was significantly ($p < 0.05$) lower than that of camomile flavoured rooibos (traditional / fermented) (1 299 ± 108 µmol/L), the berry flavoured iced tea (RTD) (1 498 ± 76 µmol/L), the lemon flavoured iced tea (RTD) (1 347 ± 112 µmol/L) and the peach flavoured iced tea (RTD) (1 378 ± 120 µmol/L) in addition to the two commercially flavoured rooibos samples having the highest TEAC. The TEAC of the instant fruit punch and lemon flavoured iced tea (powder) (843.9 ± 63.8 µmol/L) (see Table 4.16) was significantly ($p < 0.05$) lower than that of the honey flavoured rooibos (traditional / fermented) (1 247 ± 104 µmol/L) in addition to the two commercially flavoured rooibos samples having the highest TEAC, the camomile flavoured rooibos (traditional / fermented) (1 299 ± 108 µmol/L) and all the three RTD flavoured iced tea samples (results elaborated below).

The TEAC of the camomile flavoured rooibos (traditional / fermented) (1 299 ± 108 µmol/L) and the lemon flavoured iced tea (RTD) (1 347 ± 112 µmol/L) were both significantly ($p < 0.05$) higher than that of the lemon flavoured iced tea (powder) (1 113 ± 91 µmol/L) and the instant fruit punch and lemon flavoured iced tea (powder) (843.9 ± 63.8 µmol/L) respectively and significantly ($p <0.05$) lower than that of the berry flavoured iced tea (RTD) (1 498 ± 76 µmol/L). In addition, the TEAC of both the berry flavoured iced tea (RTD) (1 498 ± 76 µmol/L) and the peach flavoured iced tea (RTD) (1 378 ± 120 µmol/L) was significantly ($p <0.05$) higher than that of the honey flavoured rooibos (traditional / fermented) (1 247 ± 104 µmol/L), the lemon flavoured iced tea (powder) (1 113 ± 91 µmol/L), the instant fruit punch flavoured iced tea

(powder) (1 200 ± 86 µmol/L) and the instant fruit punch and lemon flavoured iced tea (powder) (843.9 ± 63.8 µmol/L), respectively. The TEAC of the berry flavoured iced tea (RTD) (1 498 ± 76 µmol/L) was in addition significantly ($p < 0.05$) higher than that of the lemon flavoured iced tea (RTD) (1 347 ± 112 µmol/L) and the peach flavoured iced tea (RTD) (1 378 ± 120 µmol/L) (see Table 4.16).

The total polyphenol content of the commercially flavoured rooibos samples was the highest for the instant apple fruit flavoured iced tea (powder) (629.2 ± 47.0 mg/L), the lemon flavoured rooibos (traditional / fermented) (626.7 ± 293.0 mg/L) and the instant fruit punch and lemon flavoured iced tea (powder) (625.4 ± 39.3 mg/L) and lowest for the berry flavoured iced tea (RTD) (41.92 ± 79.37 mg/L). The total polyphenol content of the instant apple fruit flavoured iced tea (powder) and the lemon flavoured rooibos (traditional / fermented) was significantly ($p < 0.05$) higher than that of the berry flavoured iced tea (RTD) and in addition to that of the camomile flavoured rooibos (traditional / fermented) (176.5 ± 46.2 mg/L), the honey flavoured rooibos (traditional / fermented) (331.0 ± 83.2 mg/L), the peach flavoured iced tea (powder) (298.5 ± 16.5 mg/L), the lemon flavoured iced tea (RTD) (371.5 ± 264.8 mg/L) and the peach flavoured iced tea (RTD) (323.7 ± 225.3 mg/L) respectively, while the total polyphenol content of the instant fruit punch and lemon flavoured iced tea (powder) was also significantly higher than that of all these samples respectively besides for that of the peach flavoured iced tea (powder). The berry flavoured iced tea (RTD) had a significantly ($p < 0.05$) lower total polyphenol content (41.92 ± 79.37 mg/L) than that of all the commercially flavoured rooibos samples respectively, except for the camomile flavoured rooibos (traditional / fermented) (176.5 ± 46.2 mg/L). The total polyphenol content of the camomile flavoured rooibos (traditional / fermented) (176.5 ± 46.2 mg/L) and the peach flavoured iced tea (RTD) (323.7 ± 225.3 mg/L) were in addition also significantly ($p < 0.05$) lower than that of the honey flavoured rooibos (traditional / fermented) (331.0 ± 83.2 mg/L), the instant fruit punch flavoured iced tea (powder) (373.1 ± 30.4 mg/L) and the lemon flavoured iced tea (RTD) (371.5 ± 264.8 mg/L) respectively, while the total polyphenol content of the camomile flavoured rooibos (traditional / fermented) was significantly ($p < 0.05$) lower than that of the lemon flavoured iced tea (powder) (300.4 ± 42.1 mg/L) and that of the peach flavoured iced tea significantly ($p < 0.05$) higher than that of the lemon flavoured iced tea (powder) (see Table 4.16).

The commercially flavoured rooibos samples in tea bag form, i.e. camomile (traditional / fermented), honey (traditional / fermented) and lemon (traditional / fermented), delivered the highest flavonol content while instant fruit punch flavoured iced tea (powder) and instant fruit punch and lemon flavoured iced tea (powder) delivered the lowest flavonol content. The flavonol content of these commercially flavoured rooibos samples in tea bag form (fermented / traditional) (camomile, honey and lemon at 16.93 ± 4.07 mg/L, 21.04 ± 5.41 mg/L and 26.30 ± 4.85 mg/L respectively) were significantly ($p < 0.05$) higher than that of the other commercially flavoured rooibos samples respectively, except for the flavonol content of camomile flavoured rooibos (traditional / fermented) not being significantly ($p > 0.05$) higher than that of the lemon flavoured iced tea (RTD) (15.09 ± 9.70 mg/L). The lemon flavoured rooibos (traditional / fermented) in addition also yielded a significantly ($p < 0.05$) higher flavonol content than the camomile flavoured rooibos (traditional / fermented). The flavonol content of the lemon flavoured iced tea (RTD) (15.09 ± 9.70 mg/L) and the peach flavoured iced tea (RTD) (10.92 ± 6.68 mg/L) was significantly ($p < 0.05$) higher than that of the instant fruit punch flavoured iced tea (powder) (3.55 ± 4.72 mg/L) and the instant fruit punch and lemon flavoured iced tea (powder) (3.55 ± 3.99 mg/L). The lemon flavoured iced tea (RTD) had in addition a significantly ($p < 0.05$) higher flavonol content (15.09 ± 9.70 mg/L) than the instant apple fruit flavoured iced tea (powder) (5.13 ± 1.04 mg/L) and the berry flavoured iced tea (RTD) (7.10 ± 4.47 mg/L) respectively (see Table 4.16).

Only two commercially flavoured rooibos samples delivered a flavanol content reading. The lemon flavoured rooibos (traditional / fermented) provided the higher flavanol content (at 65.26 ± 79.95 mg/L) and the honey flavoured rooibos (traditional / fermented) the lower (at 7.56 ± 3.22 mg/L) (see Table 4.16).

4.1.6 Rooibos sample(s) identification providing the highest total polyphenol content and total antioxidant capacity

The rooibos preparation method providing the highest total polyphenol content and TAC are presented in Table 4.17 taking cognisance of the rooibos types and forms available to the consumer and the different brewing times and added flavourings that could be used. Considering the different rooibos types investigated, the green / unfermented rooibos provided the highest total polyphenol content as well as the highest FRAP, while the traditional / fermented rooibos the highest TEAC. Rooibos in the form of a powdered extract (traditional / fermented) yielded the highest total polyphenol content and FRAP and iced tea (with rooibos

extract – fermented / traditional) the highest TEAC considering the different available rooibos forms. A brewing time of 30 minutes resulted in the highest total polyphenol content and TAC compared to the other brewing times. The addition of honey as sweetening agent resulted in the highest total polyphenol content and TAC. When considering the amounts of sweetening agent added, the addition of a large amount of honey resulted in the highest total polyphenol content whereas the addition of a small amount of honey resulted in the highest TAC. The addition of whole milk in a large amount resulted in the highest total polyphenol content and FRAP, while the addition of a large amount of skim milk resulted in the highest TEAC. The combination of 30 mL (large amount) whole milk and 15 g (large amount) honey, 30 mL whole milk and 10 g (large amount) brown sugar and 30 mL skim milk and 5 g (small amount) honey added to rooibos samples resulted in the highest total polyphenol content, FRAP and TEAC respectively. Instant apple fruit flavoured iced tea (powder) yielded the highest total polyphenol content and FRAP, while the lemon flavoured rooibos sample prepared from a tea bag the highest TEAC (see Table 4.17).

Table 4.17 Rooibos preparation methods providing the highest total polyphenol content and total antioxidant capacity

Rooibos preparation method	**Data extracted from table**	**Biochemical contents[a]**			**Comment**
		Total polyphenols[b] (mg/L)	**FRAP[c] (µmol/L)**	**TEAC[d] (µmol/L)**	
Type					
Green, unfermented (1)	4.1	457.7±40.9[e]	3 219±82[f]		Availability to consumer is limited
Traditional, fermented (2)				1 140±143[e]	
Form					
Powdered extract (traditional / fermented) (3)	4.2	604.6±39.0[e]	4 004±395[e]		Availability to consumer is limited
Iced tea (with rooibos extract – traditional / fermented) (4)				1 701±565[e]	More costly to the consumer, although convenient
Brewing time					
30 minutes (5)	4.3	543.2±125.7[f]	3 107±731[f]	1 212±270[f]	
Household flavourings addition type and amounts					
Type and amount of sweetening agent added					
Honey (6)	4.4 & 4.5	413.0±72.7[f] (large amount)	2 489±437[e] (small amount)	1 868±172[f] (small amount)	Health implications of a large amount of honey (fructose) questionable

Table 4.17 Rooibos preparation methods providing the highest total polyphenol content and total antioxidant capacity (cont.)

Rooibos preparation method	Data extracted from table	Biochemical contents[a]			Comment
		Total polyphenols[b] (mg/L)	FRAP[c] (µmol/L)	TEAC[d] (µmol/L)	
Type of milk added[g]					
Whole milk	4.6 & 4.7	519.1±82.7[e] (large amount)	2 100±453[e] (large amount)		High polyphenol content probably due to interference with the absorbance of the assay by some of the milk constituents
Skim milk				3 025±325[e] (large amount)	
Combined household flavourings[g]					
30 mL whole milk & 15 g honey	4.8 - 4.16	545.1±65.71[e]			High polyphenol content probably due to interference with the absorbance of the assay by some of the milk constituents
30 mL whole milk & 10 g brown sugar			2 310±609[e]		High polyphenol content probably due to interference with the absorbance of the assay by some of the milk constituents
30 mL skim milk & 5 g honey				3 773±146[f]	
Commercially flavoured rooibos[g]					
Instant apple fruit iced tea (powder)	4.17	629.2±47.0[f]	1 741±864[f]		More costly to the consumer, although convenient
Lemon (traditional / fermented) (tea bag)				4 750±79[f]	More costly to the consumer, although convenient
Significant difference		(6)-(3)(5)[h]	(3)-(1)[h] (6)-(1)(3)(5)[h]	(2)-(4)(6)[i] (5)-(4)(6)[i]	

[a] Biochemical content values: Mean ± standard deviation.
[b] Total polyphenol content presented as milligram (mg) per litre (L).
[c] FRAP: Ferric reducing antioxidant power assay presented as micromole (µmol) per litre (L).
[d] TEAC: Trolox equivalent antioxidant capacity assaypresented as micromole (µmol) per litre (L).
[e] Highest in preparation method grouping.
[f] Significantly highest in preparation method grouping.
[g] The rooibos samples representing the type of milk added and the combined household flavourings, in addition to the commercially flavoured rooibos samples, were not included in the statistics carried out due to the interference with the polyphenol content analysis of the former and the nature of the latter.
[h] Overall significant($p<0.05$) difference in the Levene'sTestfor equality of variances of the samples with the significant ($p < 0.05$) differences transpiring in the pairwise multiple comparisons of the Student-Newman-Keuls Test indicated as a rooibos sample providing a high total polyphenol and total antioxidant capacity (number presented in brackets [e.g. green, unfermentedas1] significantly different (-) to another rooibos sampleprovidng a high total polyphenol content and total antioxidant capacity with each sample presented as a number in brackets[e.g. traditional, fermented as 2]).
[i] Overall significant ($p<0.05$) difference in the Kruskal-Wallis Test one-way analysis of variance by ranks of the samples with the significant ($p<0.05$) contrasts transpiring on the post-hoc pairwise multiple comparisons indicated as a rooibos sample providing a high total polyphenol content and total antioxidant capacity (number presented in brackets [e.g. green, unfermented as 1] significantly different (-) to another rooibos sample providing ahigh total polyphenol content and total antioxidant capacity with each sample presented as a number in brackets [e.g. traditional, fermented as 2]).

Only the rooibos type, form, brewing time and the type and amount of honey as the sweetening agent were included for this determination as the household preparation of rooibos was the foremost consideration. The findings of the commercially flavoured rooibos were not included in the determination in addition to the type and amount of milk added (see the comments column in Table 4.17 for type of milk added and the combined household flavourings). The total polyphenol content of the rooibos sample with a brewing time of 30 minutes (543.2 ± 125.7 mg/L) was significantly ($p < 0.05$) higher than that of the rooibos sample with a large (15 g) amount addition of honey (413.0 ± 72.7 mg/L), while the rooibos sample prepared with rooibos in powder form had a significantly ($p < 0.05$) higher total polyphenol content than the rooibos sample with a large (15 g) amount addition of honey (604.6 ± 39.0 mg/L vs 413.0 ± 72.7 mg/L) (see Table 4.17).

The FRAP of the rooibos sample with a small (5 g) amount addition of honey (2 489 ± 437 µmol/L) was significantly ($p < 0.05$) lower than that of the rooibos sample prepared with green, unfermented rooibos (3 219 ± 82 µmol/L), the sample prepared with rooibos in powder (traditional / fermented) form (4 004 ± 395 µmol/L) and the rooibos sample brewed for 30 minutes (3 107 ± 731 µmol/L), respectively. The sample prepared with rooibos powder (traditional / fermented) had a significantly ($p < 0.05$) higher FRAP (4 004 ± 395 µmol/L) than the sample prepared with green, unfermented rooibos (3 219 ± 82 µmol/L). The TEAC of the rooibos sample prepared with traditional / fermented rooibos (1 140 ± 143 µmol/L) and the rooibos sample brewed for 30 minutes (1 212 ± 270 µmol/L) were both significantly ($p < 0.05$) lower than that of the rooibos sample with the small (5 g) amount addition of honey (1 868 ± 172 µmol/L) and that of the rooibos iced tea sample (1 701 ± 565 µmol/L) (see Table 4.17).

Based on this phase of the study, a longer brewing time was identified to deliver a higher TAC and total polyphenol content. This meant a more optimal cup of rooibos when considering the different brewing times and methods used by consumers to prepare rooibos. Factors such as the market availability and cost of the different types and forms of rooibos to the consumer restricted the obtainability of rooibos to traditional / fermented rooibos in bag form (see Table 4.17). The addition of honey as added flavouring was considered on the completion of Phase 2 of the study by taking into account the number of respondents who indicated that they added it to their prepared rooibos.

4.2 Phase 2: Respondent profile and tea drinking behaviour

4.2.1 Respondent sample size, representation and response rate

Respondents from both genders and all population groups were invited to complete the questionnaire considering the envisaged respondent inclusion coverage of the different age categories and them being rooibos consumers who resided in the George area, Western Cape. A total of 344 respondents who met the inclusion criteria were invited to participate, of whom 36 declined, resulting in a response rate of 89.5%. The sample comprised 308 respondents of whom all completed the questionnaire. In all the age categories more than the minimum number of respondents completed the questionnaire, except for the age categories 45 to 49 years, 50 to 54 years and 70 to 74 years where one less respondent than the minimum envisaged number participated (see Table 4.18).

Table 4.18: Age category representation of the respondent sample

Age category	Envisaged respondent number (range) per age category[a]	Respondent number per age category	Number of individuals who declined participation per age category
25-29 years	43-66	62	0
30-34 years	36-55	49	0
35-39 years	35-54	35	3
40-44 years	34-52	35	2
45-49 years	30-46	29	4
50-54 years	25-38	24	3
55-59 years	20-31	20	4
60-64 years	17-26	18	3
65-69 years	12-19	15	4
70-74 years	9-14	8	6
75 years and older	11-17	13	7
Total	**272-418**	**308**	**36**

[a] The total number of respondents to be used, as well as the number in each age category, were determined by the distribution of the population by age for the George Municipality from the *Census 2011* (Western Cape) report (Lehohla, 2012:46).

4.2.2 Respondent sample profile

One of the objectives of Phase 2 of the research was to determine the demographic, lifestyle and health characteristics of rooibos consumers. These characteristics of the rooibos consumers who participated in Phase 2 of the study are presented below.

4.2.2.1 Demographic characteristics

The sample had a high representation of female (68.2%) and white (70.1%) respondents along with a high representation of respondents who had obtained a post-school qualification (i.e. a certificate, diploma, degree or postgraduate qualification) (70.1%). The sample furthermore had a near equal representation of respondents aged 25 to 39 years (47.4%) and those aged 40 years and older (52.6%) (see Table 4.19), which represented the envisaged sample age distribution coverage (see Table 4.18), and a near equal representation of respondents married and / or living together with children (34.7%) and respondents being single and living without children (30.8%) (see Table 4.19).

Table 4.19: Demographic characteristics of the respondent sample

Demographic characteristics		**Total sample (n = 308)**	
		Number	**%**
Gender	Male	98	31.8
	Female	210	68.2
Age category (years)	25 - 29	62	20.1
	30 – 34	49	15.9
	35 – 39	35	11.4
	40 – 44	35	11.4
	45 – 49	29	9.4
	50 – 54	24	7.8
	55 - 59	20	6.5
	60 – 64	18	5.8
	65 – 69	15	4.9
	70 - 74	8	2.6
	75 and older	13	4.2
Population groups	Black	20	6.5
	Coloured	69	22.4
	Indian	3	1.0
	White	216	70.1

Table 4.19: Demographic characteristics of the respondent sample (cont.)

Demographic characteristics		Total sample (n = 308)	
		Number	%
Highest level of education	Standard 9/Grade 11 and lower	25	8.1
	Standard 10/Grade 12	67	21.8
	Grade 12 + certificate	54	17.5
	Grade 12 + diploma	64	20.8
	Grade 12 + degree	76	24.7
	Postgraduate (Masters/Doctorate)	22	7.1
Marital status	Married/living together with children	107	34.7
	Married/living together without children	73	23.7
	Single and living with children	33	10.7
	Single and living without children	95	30.8

4.2.2.2 Lifestyle and health characteristics

The lifestyle and health characteristics of the respondent sample are presented in Table 4.20. About two-thirds (62.3%) of the respondent sample indicated that they consumed foods / beverages popular with and consumed by most adults of the same age and not necessarily healthier food / beverage choices and just over half (51.3%) indicated that they were physically active. Just less than a fifth (18.5%) of the respondents indicated that they used dietary supplements regularly, while just over a third (35.1%) indicated that they never used dietary supplements. The majority (73.4%) of the respondents indicated that they were non-smokers and just more than half (56.8%) indicated that their body weight status was optimal / normal. A few respondents indicated that they were underweight (3.2% or 10 respondents) or obese (4.5% or 14 respondents) (see Table 4.20).

Table 4.20: Lifestyle and health characteristics of the respondent sample

Lifestyle and health characteristics		Total sample (n = 308)	
		Number	%
Food and beverage intake	Consume foods/beverages popular with and consumed by most adults of same age (similar food and beverage intakes as most friends, family and/or colleagues)	192	62.3
	Consume foods/beverages considered healthier choices than those consumed by most adults of same age (as most friends, family and/or colleagues)	116	37.7
Physically active	Yes[a]	158	51.3
	No	150	48.7
Dietary supplement[b] use	Never	108	35.1
	Seldom	81	26.3
	When remembered	27	8.8
	Fairly regularly	35	11.4
	Regularly	57	18.5
Smoking status[c]	Non-smoker	226	73.4
	Current smoker	48	15.6
	Former smoker	34	11.0
Body weight status	Underweight	10	3.2
	Optimal/normal body weight	175	56.8
	Slightly overweight/overweight	109	35.4
	Obese	14	4.5
Cardiovascular disease diagnosis	Yes	14	4.5
	No	284	92.2
	Don't know/unsure	10	3.2
Diabetes mellitus type 2 diagnosis	Yes	7	2.3
	No	292	94.8
	Don't know/unsure	9	2.9
Inflammatory condition (e.g. arthritis) diagnosis	Yes	35	11.4
	No	262	85.1
	Don't know/unsure	11	3.6
Cancer (skin, lung, breast, liver or prostate) diagnosis	Yes	4	1.3
	No	301	97.7
	Don't know/unsure	3	1.0
Family history of cardiovascular disease	Yes	101	32.8
	No	196	63.6
	Don't know/unsure	11	3.6
Family history of diabetes mellitus type 2	Yes	86	27.9
	No	213	69.2
	Don't know/unsure	9	2.9

Table 4.20: Lifestyle and health characteristics of the respondent sample (cont.)

Lifestyle and health characteristics		Total sample (n = 308)	
		Number	%
Family history of inflammatory conditions (e.g. arthritis)	Yes	95	30.8
	No	206	66.9
	Don't know/unsure	7	2.3
Family history of cancer (skin, lung, breast, liver or prostate)	Yes	115	37.3
	No	183	59.4
	Don't know/unsure	10	3.2

[a] Regular moderate exercise (e.g. walking or cycling) or strenuous exercise (e.g. jogging, football and vigorous swimming) for four hours or more per week (Yusuf *et al*., 2004:939).
[b] A vitamin, mineral, herbal, plant extract, amino acid, metabolite, constitute, or extract, or a combination of any of these substances (Marriott, 2000:1731S).
[c] Non-smoker: has never smoked; current smoker: smoked in the last 12 months or quit in the past year; and former smoker: quit smoking more than a year ago (Yusuf *et al*., 2004:939).

The majority of respondents indicated that they had not been diagnosed with cardiovascular disease (92.2%), diabetes mellitus type 2 (94.8%), inflammatory conditions (85.1%) or cancer (97.7%), with the highest number (11.4% or 35 respondents) indicating an inflammatory disease diagnosis. About a third of the respondents indicated to have a family history of cardiovascular disease (32.8%), inflammatory conditions (30.8%) and cancer (37.3%), while about a quarter (27.9%) indicated to have a family history of diabetes mellitus type 2 (see Table 4.20).

4.2.3 Respondent tea and herbal tea consumption

This phase of the research also aimed to determine the respondent tea preferences and how the respondents usually drink their tea / herbal tea by considering the different types, forms, brewing times and flavouring additions and amounts added to prepare it as a beverage, and in particular reference to rooibos herbal tea.

4.2.3.1 Tea and herbal tea

The respondents' preferred beverage choice and their tea / herbal tea consumption are presented in Table 4.21. Coffee was indicated as the preferred non-alcoholic beverage choice by most (36%) of the respondents, closely followed by tea / herbal tea (28.6%) and then water (12.7%) and fruit juice (as pure, nectars and concentrates) (11.7%). Very few (10 and less) of the respondents indicated that the other beverage options provided were their beverage of choice (which included soda drinks, milk and milk beverages and milk-based hot drinks, respectively).

On questioning the respondents on their tea and / or herbal tea consumption, almost half (47.4%) of the respondent sample indicated that they mostly consumed traditional rooibos herbal tea (plain), while about a fifth (18.8%) indicated that they mostly consumed black tea (plain). Somewhat less than a fifth (15.9%) of the respondents indicated that they mostly consumed the commercially flavoured versions of traditional rooibos herbal tea. The reasons indicated by the respondents for drinking the tea / herbal tea mostly consumed was foremost taste indicated by about half (49.4%) of the respondents followed by health reasons indicated by about a quarter (24.7%) of the respondents. Almost a third (32.1%) of the respondents indicated that they consumed two to three cups of tea / herbal tea per day and just over a tenth indicated that they consumed only one cup per day (14.3%), two to three cups per week (13.6%) and almost never / seldom (11.4%). Only six respondents (1.9%) indicated that they consumed more than six cups of tea / herbal tea per day (see Table 4.21).

Table 4.21: Respondent beverage preference and tea and herbal tea consumption

Beverage preference and tea / herbal tea consumption		**Total sample (n = 308)**	
		Number	**%**
Preferred non-alcoholic beverage choice	Coffee	111	36.0
	Cold drinks / squash	12	3.9
	Fruit juice (pure, nectars)	31	10.1
	Fruit juice concentrates	5	1.6
	Milk and milk beverages	6	1.9
	Milk based hot beverages	6	1.9
	Soda drinks	10	3.2
	Tea / herbal tea	88	28.6
	Water	39	12.7

Table 4.21: Respondent beverage preference and tea and herbal tea consumption (cont.)

Beverage preference and tea / herbal tea consumption		Total sample (n = 308)	
		Number	%
Tea / herbal tea mostly consumed	Black tea, commercially flavoured	10	3.2
	Black tea, plain	58	18.8
	Green tea, commercially flavoured	5	1.6
	Green tea, plain	5	1.6
	Honeybush herbal tea, commercially flavoured	2	0.6
	Honeybush herbal tea, plain	4	1.3
	Iced tea, flavoured[a]	17	5.5
	Iced tea, plain[a]	3	1.0
	Traditional rooibos herbal tea, commercially flavoured	49	15.9
	Traditional rooibos herbal tea, plain	146	47.4
	Green rooibos herbal tea, plain	9	2.9
Reason for drinking mostly consumed tea / herbal tea	Availability	30	9.7
	Habit	50	16.2
	Health reasons	76	24.7
	Preferred taste	152	49.4
Tea / herbal tea consumption frequency	Almost never / seldom	35	11.4
	1-3 cups per month	24	7.8
	1 cup per week	21	6.8
	2-3 cups per week	42	13.6
	4-6 cups per week	20	6.5
	1 cup per day	44	14.3
	2-3 cups per day	99	32.1
	4-6 cups per day	17	5.5
	More than 6 cups per day	6	1.9

[a] Includes the respondents who purchase iced teas (n = 9) and who prepare iced tea at home from tea bags (n = 11), either flavoured or plain (n = 20).

4.2.3.2 Rooibos herbal tea

Different aspects in relation to the respondent consumption of specifically rooibos herbal tea were investigated in the study, which is presented in Table 4.22. A fifth (20.8%) of the respondent sample indicated that they consumed two to three cups of this herbal tea per day, while just less than a fifth indicated that they consumed this amount per week (17.5%) and almost the same number indicated that they almost never / seldom consumed rooibos (16.9%). Only two respondents (0.6%) indicated that they consumed more than six cups of rooibos per day. About a third (35.4%) of the respondent sample indicated that they mostly consumed

rooibos throughout the day, followed by a quarter (25.3%) of the respondents who indicated that they consumed it during the morning and just less than a fifth (16.6%) who indicated that they consumed it in the evening. Very few respondents indicated that they consumed it early morning (6.5%), at lunchtime (4.9%) and early and late afternoon (4.5% and 6.8% respectively). While almost half (47.1%) of the respondent sample indicated that they did not consume commercially flavoured rooibos, the rest of the respondent sample indicated to consume a range of commercially flavoured rooibos herbal teas with peach flavoured rooibos iced tea (14.9%) and lemon flavoured rooibos prepared from tea bags (13.3%) the most popular commercially flavoured rooibos options (see Table 4.22).

Table 4.22: Rooibos consumption and the herbal tea preparation by the respondent sample

Rooibos consumption and the herbal tea preparation		Total sample (n = 308)	
		Number	%
Consumption frequency	Almost never / seldom	52	16.9
	1-3 cups per month	44	14.3
	1 cup per week	18	5.8
	2-3 cups per week	54	17.5
	4-6 cups per week	25	8.1
	1 cup per day	37	12.0
	2-3 cups per day	64	20.8
	4-6 cups per day	12	3.9
	More than 6 cups per day	2	0.6
Time of day mostly consumed	Early morning	20	6.5
	During the morning	78	25.3
	Lunchtime	15	4.9
	Early afternoon	14	4.5
	Late afternoon	21	6.8
	In the evening	51	16.6
	Throughout the day	109	35.4
Commercially flavoured rooibos usually consumed	Do not consume	145	47.1
	Lemon flavoured tea bags	41	13.3
	Honey flavoured tea bags	15	4.9
	Camomile flavoured tea bags	19	6.2
	Ginger flavoured tea bags	6	1.9
	Lemon flavoured iced tea	20	6.5
	Peach flavoured iced tea	46	14.9
	Pomegranate flavoured iced tea	1	0.3
	Apple and ginger flavoured iced tea	2	0.6
	Berry flavoured iced tea	11	3.6
	Fruit punch flavoured iced tea	2	0.6

Table 4.22: Rooibos consumption and the herbal tea preparation by the respondent sample (cont.)

Rooibos consumption and the herbal tea preparation		Total sample (n = 308)	
		Number	%
Form usually consumed	Tea bags	286	92.9
	Loose tea leaves	2	0.6
	Iced tea – ready to drink	7	2.3
	Iced tea – powder form	2	0.6
	Iced tea – tea bag form	11	3.6
Strength usually consumed	Very weak (brewed for less than 1 min)	11	3.6
	Weak (brewed for 1 min)	27	8.8
	Weak to medium (brewed between 1-5 min)	83	26.9
	Medium (brewed for 5 min)	77	25.0
	Medium to strong (brewed between 5-10 min)	61	19.8
	Somewhat strong (brewed for 10 min)	23	7.5
	Strong (brewed for longer than 10 min)	26	8.4
Preparation method[a]	Tea bag brewed in a cup or mug	271	88.0
	Tea bag(s) brewed in a teapot (teapot not kept warm)	25	8.1
	Tea bag(s) brewed in a teapot placed on the stove or warmer to keep warm	10	3.2
	Loose tea leaves brewed in cup or mug	0	0.0
	Loose tea leaves brewed in a teapot (teapot not kept warm)	2	0.6
	Loose tea leaves brewed in a teapot placed on the stove or warmer to keep warm	0	0.0
Temperature at which usually consumed[a]	Hot / near boiling	107	34.7
	Warm / still heated	184	59.7
	Cooled / near cold	17	5.5

[a] The representation also includes the rooibos preparation and consumption of the respondents who usually consumed rooibos herbal iced tea.

The majority (92.9%) of the respondents indicated that they usually used rooibos in the form of tea bags to prepare the herbal tea. Rooibos usually consumed in the form of iced tea was indicated by a few of the respondents (6.5% or 20), of whom 11 (3.6%) indicated that they consumed iced tea prepared with tea bags while nine respondents (2.9%) indicated that they consumed the RTD and powder forms of iced tea. Only two respondents (0.6%) indicated that they usually consumed rooibos in the form of loose tea leaves.

Just over a quarter (26.9%) of the respondents indicated that they consumed their rooibos at weak to medium strength (brewed between one and five minutes), while a quarter (25%) indicated that they consumed it at medium strength (brewed for five minutes). Less than a fifth (15.9%) of the respondents indicated that they usually consumed rooibos that had been brewed for ten minutes (7.5% or 23 respondents) or longer than ten minutes (8.4% or 26 respondents).

The majority (88%) of the respondent sample indicated that they prepared rooibos by brewing a tea bag in a cup or mug, while only two respondents (0.6%) indicated that they brewed loose tea leaves in a teapot without keeping it warm. More than half (59.7%) of the respondents indicated that they consumed rooibos warm or still heated, while about a third (34.7%) indicated that they consumed it hot or near boiling (see Table 4.22).

Numerous flavourings and in different amounts (see Table 4.23) were usually added by the respondent sample to their prepared rooibos. Just over a tenth (14.9%) of the respondents indicated that they usually did not add flavouring. Just more than a fifth (23.7%) of the respondents indicated that they usually added white sugar and whole milk, which was followed by about a tenth (11.4%) of the respondents who indicated that they usually added white sugar and low fat milk. Among those respondents who indicated that they usually only added a sweetening agent to their prepared rooibos, more indicated that they usually added white sugar (8.4%) than any of the other types of sweetening agents (brown sugar at 4.2%, honey at 3.2% and sweetener at 3.2%). Of those who indicated that they usually only added milk to their prepared rooibos, slightly more respondents indicated that they added whole milk (1.9% or 6 respondents) compared to those who indicated that they only added low fat or skim milk (1.6% and 0.6% or 5 and 2 respondents respectively). Only six respondents (1.9%) indicated that they added lemon juice to their prepared rooibos (see Table 4.23).

Table 4.23: Flavouring types and amounts usually added to rooibos by the respondent sample

Flavouring types and amounts added to rooibos		**Total sample (n = 308)**	
		Number	**%**
Flavouring usually added (n = 308)	Do not add flavouring(s)	46	14.9
	Sweetener only	10	3.2
	Brown sugar only	13	4.2
	White sugar only	26	8.4
	Honey only	10	3.2
	Milk, whole only	6	1.9
	Milk, low fat only	5	1.6
	Milk, skim only	2	0.6
	Lemon juice only	6	1.9
	Sweetener and whole milk	5	1.6
	Sweetener and low fat milk	18	5.8
	Sweetener and skim milk	1	0.3
	Brown sugar and whole milk	22	7.1
	Brown sugar and low fat milk	19	6.2

Table 4.23: Flavouring types and amounts usually added to rooibos by the respondent sample (cont.)

Flavouring types and amounts added to rooibos		Total sample (n = 308)	
		Number	%
Flavouring usually added (n = 308)	Brown sugar and skim milk	0	0.0
	White sugar and whole milk	73	23.7
	White sugar and low fat milk	35	11.4
	White sugar and skim milk	3	1.0
	Honey and whole milk	6	1.9
	Honey and low fat milk	2	0.6
	Honey and skim milk	0	0.0
Amount of brown sugar[a] (n = 54)	Add less than small amount (less than level teaspoon / less than 4 g)	2	3.7
	Small amount (level teaspoon / 4 g)	20	37.0
	Medium amount (heaped teaspoon / 6 g)	14	25.9
	Large amount (level teaspoon plus a heaped teaspoon / 10 g)	17	31.5
	Add more than large amount (more than one level plus one heaped teaspoon / more than 10 g)	1	1.9
Amount of white sugar[a] (n = 137)	Add less than small amount (less than level teaspoon / less than 4 g)	8	5.8
	Small amount (level teaspoon / 4 g)	27	19.7
	Medium amount (heaped teaspoon / 6 g)	49	35.8
	Large amount (level teaspoon plus a heaped teaspoon / 10 g)	43	31.4
	Add more than large amount (more than one level plus one heaped teaspoon / more than 10 g)	10	7.3
Amount of honey[a] (n = 18)	Add less than small amount (less than ½ teaspoon / less than 5 g)	1	5.6
	Small amount (½ teaspoon / 5 g)	3	16.7
	Medium amount (level teaspoon / 9 g)	13	72.2
	Large amount (heaped teaspoon / 15 g)	0	0.0
	Add more than large amount (more than heaped teaspoon / more than 15 g)	1	5.6
Amount of milk, whole[a] (n = 112)	Less than small amount (less than 10 mL / less than 10 g)	5	4.5
	Small amount (10 mL / 10 g)	39	34.8
	Medium amount (20 mL / 20 g)	59	52.7
	Large amount (30 mL / 30 g)	8	7.1
	More than large amount (more than 30 mL / more than 30 g)	1	0.9
Amount of milk, low fat[a] (n = 79)	Less than small amount (less than 10 mL / less than 10 g)	5	6.3
	Small amount (10 mL / 10 g)	24	30.4
	Medium amount (20 mL / 20 g)	45	57.0
	Large amount (30 mL / 30 g)	5	6.3
	More than large amount (more than 30 mL / more than 30 g)	0	0.0

Table 4.23: Flavouring types and amounts usually added to rooibos by the respondent sample (cont.)

Flavouring types and amounts added to rooibos		Total sample (n = 308)	
		Number	%
Amount of milk, skim[a] (n = 6)	Less than small amount (less than 10 mL / less than 10 g)	0	0.0
	Small amount (10 mL / 10 g)	1	16.7
	Medium amount (20 mL / 20 g)	3	50.0
	Large amount (30 mL / 30 g)	2	33.3
	More than large amount (more than 30 mL / more than 30 g)	0	0.0
Amount of lemon juice[a] (n = 6)	Less than small amount (less than level teaspoon`)	1	16.7
	Small amount (level teaspoon)	3	50.0
	Medium amount (2 x level teaspoons)	1	16.7
	Large amount (level tablespoon)	1	16.7
	More than large amount (more than level tablespoon)	0	0.0

[a] Amounts in questionnaire illustrated in household measurements only as obtained from Langenhoven *et al*. (1991:9,10,100,139,140).

The amount of brown sugar added to rooibos by the respondents who indicated that they usually added brown sugar, either adding it alone or in combination with milk (17.5% or 54 respondents), was indicated by just more than a third (37%) to be a small amount (level teaspoon), by almost a third (31.5%) to be a large amount (level plus a heaped teaspoon) and a quarter (25.9%) to be a medium amount (heaped teaspoon). The respondents who indicated that they added white sugar (44.5% or 137 respondents), either added it alone (19%) or in combination with milk (81%). Just over a third of the respondents indicated that they usually added white sugar as a medium amount (heaped teaspoon) (35.8%) or a large amount (level teaspoon plus a heaped teaspoon) (31.4%). About a fifth (19.7%) indicated that they usually added a small amount (level teaspoon) of white sugar. The majority (72.2%) of the respondents who indicated that they usually added honey, indicated that they added a medium amount (level teaspoon), while nearly all the other respondents (4 of the 5 respondents) indicated that they usually added smaller amounts (½ teaspoon and less) (see Table 4.23).

The respondents who indicated that they usually added whole milk (36.4% or 112 respondents) to their prepared rooibos, either added it alone (1.9% or 6 respondents) or in combination with a sweetening agent (34.4%). Just more than half (52.7%) of these respondents added whole milk in a medium amount (20 mL), while just more than a third (34.8%) of these respondents added whole milk in a small amount (10 mL). More than half (57%) of the respondents who indicated that they usually added low fat milk, either alone or in combination with a sweetening agent (25.6% or 79 respondents), added it in a medium amount (20 mL) and just less than a third

(30.4%) indicated that they added a small amount (10 mL) of low fat milk. Half of the respondents (50% or 3 respondents) who indicated that they usually added skim milk (6 respondents), added a medium amount (20 mL), while only one respondent indicated that he / she added a small amount (10 mL) of skim milk. Half of the respondents (50% or 3 respondents) who indicated that they usually added lemon juice (1.9% or 6 respondents), added a small amount (level teaspoon) (see Table 4.23).

4.2.3.3 Characteristics of respondents with different rooibos consumption frequencies

The characteristics of the respondents regarding their different consumption frequencies of rooibos are presented in Table 4.24. The respondent rooibos consumption frequency did not differ significantly ($p > 0.05$) across the various respondent characteristics (gender, racial group, marital status, food and beverage intake, dietary supplement use, smoking status or body weight status) (see Table 4.24).

Table 4.24: Characteristics of respondents with different rooibos consumption frequencies

Characteristic		Rooibos herbal tea consumption frequency								Significance ($p < 0.05$)[a]
		Less than 3 cups/month (n=96)		1-6 cups/week (n=97)		1 cup/day (n=37)		More than 1 cup/day (n=78)		
		n	%	n	%	n	%	n	%	
Gender	Male	40	41.7	25	25.8	13	35.1	20	25.6	0.058
	Female	56	58.3	72	74.2	24	64.9	58	74.4	
Age[b]	Young adults	54	56.3	56	57.7	9	24.3	27	34.6	0.000
	Middle aged adults	35	36.5	36	37.1	21	56.8	34	43.6	
	Older adults	7	7.3	5	5.2	7	18.9	17	21.8	
Racial group	Black/Coloured/Indian[c]	32	33.3	30	30.9	7	18.9	23	29.5	0.436
	White	64	66.7	67	69.1	30	81.1	55	70.5	
Highest level of education	Grade 12 and lower	24	25.0	23	23.7	11	29.7	34	43.6	0.001
	Grade 12 & certificate	25	26.0	11	11.3	7	18.9	11	14.1	
	Grade 12 & diploma	25	26.0	26	26.8	3	8.1	10	12.8	
	Grade 12 & degree / post- graduate qualification (Masters/Doctorate)	22	22.9	37	38.1	16	43.2	23	29.5	
Marital status	Married/living together with children	28	29.2	40	41.2	15	40.5	24	30.8	0.465
	Married/living together without children	22	22.9	20	20.6	10	27.0	21	26.9	
	Single and living with or without children	46	47.9	37	38.1	12	32.4	33	42.3	

Table 4.24: Characteristics of respondents with different rooibos consumption frequencies (cont.)

Characteristic		Rooibos herbal tea consumption frequency								Significance ($p < 0.05$)[a]
		Less than 3 cups/month (n=96)		1-6 cups/week (n=97)		1 cup/day (n=37)		More than 1 cup/day (n=78)		
		n	%	n	%	n	%	n	%	
Food and beverage intake	Consume foods/beverages popular with and consumed by most adults of same age	65	67.7	62	63.9	19	51.4	46	59.0	0.313
	Consume foods/beverages considered healthier choices than consumed by most adults of same age	31	32.3	35	36.1	18	48.6	32	41.0	
Physically active	Yes[d]	38	39.6	52	53.6	26	70.3	42	53.8	0.012
	No	58	60.4	45	46.4	11	29.7	36	46.2	
Dietary supplement[e] use	Never	37	38.5	30	30.9	11	29.7	30	38.5	0.145
	Seldom or when remembered	35	36.5	42	43.3	10	27.0	21	26.9	
	Fairly regularly or regularly[c]	24	25.0	25	25.8	16	43.2	27	34.6	
Smokingstatus[f]	Non-smoker	65	67.7	74	76.3	29	78.4	58	74.4	0.473
	Current or former smoker[c]	31	32.3	23	23.7	8	21.6	20	25.6	
Body weight status	Underweight or optimal/normal body weight[c]	57	59.4	60	61.9	25	67.6	43	55.1	0.613
	Slightly overweight/overweight or obese[c]	39	40.6	37	38.1	12	32.4	35	44.9	

[a] Pearson's chi-square
[b] Young adults as the age group 25 to 39 years; middle aged adults as the age group 40 to 64 years; and older adults as the age group 65 years and older (Hamarat *et al*., 2001:181).
[c] Response options grouped together due to low cell counts.
[d] Regular moderate exercise (e.g. walking or cycling) or strenuous exercise (e.g. jogging, football and vigorous swimming) for four hours or more per week (Yusuf *et al*., 2004:939).
[e] A vitamin, mineral, herbal, plant extract, amino acid, metabolite, constitute, or extract, or a combination of any of these substances (Marriott, 2000:1731S).
[f] Non-smoker: has never smoked; current smoker: smoked in the last 12 months or quit in the past year; and former smoker: quit smoking more than a year ago (Yusuf *et al*., 2004:939).

A significant ($p < 0.001$) difference was identified within the respondent consumption frequency of rooibos and their age groups. The young adult respondent age group formed most of the respondents who indicated that they consumed less than three cups of rooibos per month (56.3%) and one to six cup(s) of rooibos per week (57.7%), while the middle aged adult respondent age group formed most of the respondents who indicated that they consumed one cup of rooibos per day (56.8%) and more than one cup of rooibos per day (43.6%). In line with this finding that more respondents in the middle aged adult age group compared to those in the

young adult age group consumed rooibos at a higher frequency rate, the older adult age group also consumed rooibos at a higher rather than a lower consumption frequency rate (seven respondents at a frequency of one cup per day and 17 respondents at a frequency of more than one cup per day vs seven respondents at a frequency of less than three cups per month and five respondents at a frequency of one to six cup(s) per week) (see Table 4.24).

The rooibos consumption frequency also differed significantly ($p = 0.001$) considering the respondents' highest level of education. Most of the respondents who indicated a consumption frequency of one to six cup(s) per week have grade 12 and a degree / postgraduate qualification (38.1%) followed by grade 12 and a diploma (26.8%) as their highest level of education. Most of those respondents who indicated a consumption frequency of one cup per day also have grade 12 and a degree / postgraduate qualification (43.2%), but followed by grade 12 and lower (29.7%), as their highest level of education. In contrast most of the respondents who indicated a consumption frequency of more than one cup per day have grade 12 and lower (43.6%) followed by grade 12 and a degree / postgraduate qualification (29.5%) as their highest level of education (see Table 4.24).

A significant ($p < 0.05$) difference was additionally identified for the respondent consumption frequency of rooibos and them being physically active or not. Most of the respondents who indicated that they consumed less than three cups of rooibos per month indicated that they were not active (60.4%), while most of the respondents who indicated that they consumed one to six cup(s) per week, one cup per day and more than one cup per day indicated that they were physically active (53.6%, 70.3% and 53.8% respectively) (see Table 4.24).

4.2.4 Characteristics of the respondents consuming the optimal cup of rooibos

An objective of the second phase of the study was to determine the rooibos herbal tea consumers who consumed the optimal cup of traditional / fermented rooibos herbal tea as a beverage prepared to provide the higher total polyphenol content and TAC, and the demographic-, lifestyle- and health-related characteristics of these consumers. Based on the first phase of this study, a longer brewing time was identified to deliver a higher TAC and total polyphenol extraction, and therefore a more optimal cup of rooibos, allowing for the household preparation of rooibos as the foremost consideration. Along with being a practical consumer determinant in achieving a more optimal beverage, the commercial factors (type, form etc.) greatly restricted the consumer availability of rooibos to traditional / fermented rooibos in bag

form (see 4.1.6). Where added flavourings were concerned, honey was not considered as very few respondents (n = 18 or 5.8%, see Table 4.23) used it as flavouring. The biochemical results from the first phase of the study indicated that the extraction of polyphenols is greatly completed at 10 minutes with no significant differences ($p > 0.05$) found between the biochemical parameters measured on the brewing times of 10, 20 and 30 minutes (see Table 4.3). The respondent sample was divided into two groups to obtain the results of this phase of the study – those who consumed an optimal cup of rooibos and those who did not. The respondents who indicated that they brewed their rooibos for 10 minutes (somewhat strong) or longer (strong) were considered to consume an optimal cup of rooibos. Those who indicated that they applied medium to strong brewing times (brewing time between 5 and 10 minutes) or shorter brewing times were considered to not consume an optimal cup of rooibos. Based on the above, a total of 49 respondents (15.9%) were identified as consuming an optimal cup of rooibos, and 259 respondents (84.1%) as not consuming an optimal cup of rooibos.

4.2.4.1 Respondent tea and herbal tea consumption

The preferred non-alcoholic beverage choice, tea and herbal tea consumption of the respondents who consumed an optimal cup of rooibos and those who did not are presented in Table 4.25. The indicated preferred non-alcoholic beverage choice, the tea / herbal tea mostly consumed, the reason for consuming this tea / herbal tea and the consumption frequency thereof did not differ significantly ($p > 0.05$) between the respondents who consumed an optimal cup of rooibos and those who did not (see Table 4.25).

Table 4.25: Relation between the respondent optimal cup of rooibos consumption and the respondent beverage preference and tea / herbal tea consumption

Respondent beverage preference and tea/herbal tea consumption (n = 308)		Optimal cup of rooibos consumption				Significance ($p < 0.05$)[a]
		Yes (n = 49)		No (n = 259)		
		n	%	n	%	
Preferred non-alcoholic beverage choice	Coffee	24	49.0	87	33.6	0.080
	Cold drinks (squash, soda drinks)	3	6.1	19	7.3	
	Fruit juice (pure, nectars, concentrates)	1	2.0	35	13.5	
	Milk beverages (milk, hot and cold milk based beverages)	0	0	12	4.6	
	Tea/herbal tea	14	28.6	74	28.6	
	Water (still, sparkling, flavoured)	7	14.3	32	12.4	
Tea/herbal tea mostly consumed	Black tea/green tea (commercially flavoured and plain)	14	28.6	64	24.7	0.850
	Herbal tea (commercially flavoured and plain)	32	65.3	178	68.7	
	Iced tea (commercially flavoured and plain)	3	6.1	17	6.6	
Reason for consuming tea/herbal tea mostly consumed	Availability	5	10.2	25	9.7	0.259
	Habit	10	20.4	40	15.4	
	Health reasons	16	32.7	60	23.2	
	Preferred taste	18	36.7	134	51.7	
Consumption frequency of tea/herbal tea	Seldom (almost never, 1-3 cups per month)[b]	13	26.5	46	17.8	0.121
	Weekly (1-6 cups per week)[b]	7	14.3	76	29.3	
	One cup per day	9	18.4	35	13.5	
	More than one cup per day[b]	20	40.8	102	39.4	

[a] Pearson's chi-square

4.2.4.2 Respondent rooibos consumption

The rooibos herbal tea consumption of the respondents who consumed an optimal cup of rooibos and those who did not are presented in Table 4.26. The preparation method used for their rooibos consumption differed significantly ($p = 0.001$) between the respondents who consumed an optimal cup and those who did not (see Table 4.26). Among all these respondents the majority indicated that they brewed a tea bag in a cup or mug and not in a teapot (74.5% and 91.1% respectively). However, a far higher proportion of the respondents among those who consumed an optimal cup of rooibos, brewed their rooibos in a teapot compared to among those respondents who did not consume the optimal cup (25.5% vs 8.9%) (see Table 4.26). No

significant ($p > 0.05$) differences were found in the respondent rooibos consumption frequency, the time of day rooibos is usually consumed, the commercially flavoured rooibos usually consumed, the form of rooibos usually consumed and the temperature at which rooibos is usually consumed between those who consumed the optimal cup of rooibos and those who did not (see Table 4.26).

Table 4.26: Relation between the respondent optimal cup of rooibos consumption and the respondent rooibos consumption and preparation

Respondent rooibos consumption and preparation (n = 308)		**Optimal cup of rooibos consumption**				**Significance** ($p < 0.05$)[a]
		Yes (n = 49)		**No** (n = 259)		
		n	**%**	**n**	**%**	
Rooibos consumption frequency	Seldom (almost never, 1-3 cups per month)[b]	16	32.7	80	30.9	0.968
	Weekly (1-6 cups per week)[b]	16	32.7	81	31.3	
	One cup per day	6	12.2	31	12.0	
	More than one cup per day[b]	11	22.4	67	25.9	
Time of day rooibos is usually consumed	Morning (early, during morning)[b]	15	30.6	83	32.0	0.979
	Afternoon (lunchtime, early afternoon, late afternoon)[b]	8	16.3	42	16.3	
	In the evening/throughout the day[b]	26	53.1	134	51.7	
Commercially flavoured rooibos usually consumed	Do not consume	21	42.9	124	47.9	0.811
	Tea bags (lemon, honey, camomile flavoured tea bags)[b]	14	28.6	67	25.9	
	Iced tea (lemon, peach, berry, pomegranate, apple & ginger, fruit punch)[b]	14	28.6	68	26.2	
Form of rooibos usually consumed	Tea bags	45	91.8	241	93.0	0.762
	Loose tea leaves/iced tea (ready to drink, powder form)[b]	4	8.2	18	6.9	
Usual preparation method	Tea bag brewed in cup or mug	35[d]	74.5	236	91.1	0.001
	Tea bags brewed in teapot (kept warm, not kept warm)[b]	12[d]	25.5	23	8.9	
Temperature at which rooibos is usually consumed	Hot/near boiling	19	38.8	88	34.0	0.485
	Warm/still heated	26	53.1	158	61.0	
	Cooled/near cold	4	8.2	13	5.0	

[a] Pearson's chi-square
[b] Response options grouped together due to low cell counts.
[c] Fisher's exact test (done for 2 x 2 tables)
[d] n = 47; tea leaves as response option omitted due to low respondent usage (n = 2)

The flavouring types and amounts added to rooibos by the respondents, i.e. sweetening agents, milk and sweetening agents added together with milk in small, medium and large amounts, did not differ significantly ($p > 0.05$) between the respondents who consumed an optimal cup of rooibos and those who did not (see Table 4.27). Honey, skim milk and lemon juice, with their different amounts usually added to rooibos, were omitted in this analysis due to low usage of the flavouring agents by the respondents (18, 6 and 6 respondents respectively) (see Table 4.23).

Table 4.27: Relation between the respondent optimal cup of rooibos consumption and the flavouring types and amounts added to rooibos by the respondents

Flavouring types and amounts added to rooibos by respondents (n = 308)		**Optimal cup of rooibos consumption**				**Significance** ($p < 0.05$)[a]
		Yes		**No**		
		n	**%**	**n**	**%**	
Flavouring usually added to rooibos	Do not add flavouring(s)/lemon juice only[b]	13	26.5	39	15.1	0.228
	Sweetening agent	9	18.4	50	19.3	
	Milk only	1	2.0	12	4.6	
	Sweetening agent and milk	26	53.1	158	61.0	
	Total:	49		259		
Brown sugar and its added amount (n = 54)	Small amount or less (level teaspoon or less)[b]	3	33.3	19	42.2	0.723[c]
	Medium amount, large amount and more (heaped-/level + heaped teaspoon or more)[b]	6	66.7	26	57.8	
	Total:	9		45		
White sugar and its added amount (n = 137)	Small amount or less (level teaspoon or less)[b]	3	16.7	32	26.9	0.169
	Medium amount (heaped teaspoon)	10	55.6	39	32.8	
	Large amount or more (level + heaped teaspoon or more)[b]	5	27.8	48	40.3	
	Total:	18		119		
Milk, whole and its added amount (n = 112)	Small amount or less (10 mL or less)[b]	8	50.0	36	37.5	0.343
	Medium amount, large amount or more (20 mL, 30 mL or more)[b]	8	50.0	60	62.5	
	Total:	16		96		
Milk, low fatand its added amount (n = 79)	Small amount or less (10 mL or less)[b]	5	50.0	24	34.8	0.484[c]
	Medium amount, large amount or more (20 mL, 30 mL or more)[b]	5	50.0	45	65.2	
	Total:	10		69		

[a] Pearson's chi-square
[b] Response options grouped together due to low cell counts.
[c] Fisher's exact test (done for 2 x 2 tables)

4.2.4.3 Respondent demographic characteristics

The gender, age grouping, race representation, highest level of education achieved and marital status of the respondents who consumed an optimal cup of rooibos did not differ significantly ($p > 0.05$ for each) from those who did not consume an optimal cup of rooibos (see Table 4.28).

Table 4.28: Relation between the respondent optimal cup of rooibos consumption and the respondent demographic characteristics

Respondent demographic characteristics (n = 308)		Optimal cup of rooibos consumption				Significance ($p < 0.05$)[a]
		Yes (n = 49)		No (n = 259)		
		n	%	n	%	
Gender	Males	17	34.7	81	31.3	0.620[b]
	Females	32	65.3	178	68.7	
Age group[c]	Young adults	24	49.0	122	47.1	0.735
	Middle aged adults	18	36.7	108	41.7	
	Older adults	7	14.3	29	11.2	
Racial group	Black/Coloured/Indian[d]	15	30.6	77	29.7	0.901
	White	34	69.4	182	70.3	
Highest level of education	Standard 10/Grade 12 or lower	12	24.5	80	30.9	0.383
	Grade 12 + certificate	10	20.4	44	17.0	
	Grade 12 + diploma	14	28.6	50	19.3	
	Grade 12 + degree/Postgraduate qualification (Masters/Doctorate)	13	26.5	85	32.8	
Marital status	Married/living together with children	16	32.7	91	35.1	0.870
	Married/living together without children	13	26.5	60	23.2	
	Single and living with/without children[d]	20	40.8	108	41.7	

[a] Pearson's chi-square
[b] Fisher's exact test (done for 2 x 2 tables)
[c] Young adults as the age group 25 to 39 years; middle aged adults as the age group 40 to 64 years; and older adults as the age group 65 years and older (Hamarat *et al*., 2001:181).
[d] Response options grouped together due to low cell counts.

4.2.4.4 Respondent lifestyle and health characteristics

The respondent lifestyle characteristics, i.e. food and beverage intake, physical activity, intake of dietary supplements, smoking and body weight status, between those who consumed an optimal cup of rooibos and those who did not consume an optimal cup of rooibos did not differ significantly ($p > 0.05$) (see Table 4.29). The respondent indication of cardiovascular disease, diabetes mellitus type 2, inflammatory conditions and cancer were omitted from the analysis due

to a low incidence of individual diagnoses (14, 7, 35 and 4 respondents respectively), as well as their family history of these health conditions, as a number of respondents were not aware of or unsure of having a family history of these diseases (11, 9, 7 and 10 respondents respectively) (see Table 4.20).

Table 4.29: Relation between the respondent optimal cup of rooibos consumption and the respondent lifestyle and health characteristics

Respondent lifestyle and health characteristics (n = 308)		**Optimal cup of rooibos consumption**				**Significance** ($p < 0.05$)[a]
		Yes (n = 49)		**No** (n = 259)		
		n	**%**	**n**	**%**	
Food and beverage intake	Consumed foods/beverages popular with and consumed by most adults of same age	34	69.4	158	61.0	0.334[b]
	Consumed foods/beverages considered healthier choices	15	30.6	101	39.0	
Physically active	Yes[c]	23	46.9	135	52.1	
	No	26	53.1	124	47.9	0.535[b]
Dietary supplement[d] use	Never	19	38.8	89	34.3	
	Seldom	13	26.5	95	36.7	0.388
	Irregularly (when remembered, fairly regularly), regularly[e]	17	34.7	75	29.0	
Smoking status[f]	Non-smoker	35	71.4	191	73.7	
	Current smoker/former smoker[e]	14	28.6	68	26.3	0.727[b]
Body weight status	Underweight/optimal/normal body weight[e]	28	57.1	157	60.6	0.649
	Slightly overweight/overweight/obese[e]	21	42.9	102	39.4	

[a] Pearson's chi-square
[b] Fisher's exact test (done for 2 x 2 tables)
[c] Regular moderate exercise (e.g. walking or cycling) or strenuous exercise (jogging, football and vigorous swimming) for four hours or more per week (Yusuf *et al*., 2004:939).
[d] A vitamin, mineral, herbal, plant extract, amino acid, metabolite, constitute, or extract, or a combination of any of these substances (Marriott, 2000:1731S).
[e] Response options grouped together due to low cell counts.
[f] Non-smoker have never smoked; current smoker smoked in the last 12 months or quit in the past year and former smoker quit smoking more than a year ago (Yusuf *et al*., 2004:939).

CHAPTER 5
SUMMARY AND DISCUSSION OF RESULTS

The findings of the study are summarised and discussed in two parts. The first part relates to the biochemical analysis and identification of the obtained rooibos samples, considering the different types, forms, brewing times and flavourings (as commercial and household additions), providing the highest TAC and total polyphenol content. The second part relates to the rooibos herbal tea consumer profile and tea drinking behaviour with specific reference to that of respondents consuming the more optimal cup of rooibos based on providing the higher TAC and total polyphenol content. The strengths and limitations of the study are also addressed.

5.1 Rooibos type, form, brewing time and flavourings providing the highest total antioxidant capacity and total polyphenol content

5.1.1 Type

Green / unfermented rooibos provided a significantly higher TAC (measured by the FRAP assay) compared to the other types of rooibos investigated. Other studies had confirmed this finding. Bramati *et al*. (2003:7472) indicated that the TAC of an unfermented rooibos infusion was double that of the fermented rooibos prepared as a similar infusion. Von Gadow *et al*. (1997b:75) aimed to determine whether fermentation affects the antioxidant capacity of rooibos herbal tea. The antioxidant activity was assessed by the DPPH radical scavenging method. The study likewise confirmed that fermented rooibos herbal tea provided a lower antioxidant activity in comparison to unfermented or semi-fermented rooibos.

Despite the organic green / unfermented rooibos having a significantly lower TEAC, it had a significantly higher flavanol content compared to the organic traditional / fermented and the traditional / fermented rooibos. Green / unfermented rooibos had the highest flavanol content which was also significantly higher than that of the other types of rooibos samples prepared including that of the organic green / unfermented rooibos. It had been reported that catechins, which is a flavanol structure (Zhang *et al*., 2013:1786), are present in lower concentrations in fermented rooibos (Snijman *et al*., 2007:111) as catechins decrease with fermentation (Peterson *et al*., 2005:495). The traditional / fermented rooibos sample though had a significantly higher flavonol content compared to the green / unfermented and organic green / unfermented forms of rooibos. Bramati *et al*. (2003:7473) quantified the flavonoids in fermented and unfermented

rooibos. The fermented rooibos had a higher quercetin (flavonol compound) content compared to the unfermented rooibos. During the fermentation of rooibos the main flavonol-glycoside rutin is partly converted to the aglycone quercetin, as evidenced by its increased level in fermented rooibos (Bramati *et al*., 2003:7474).

Marnewick *et al*. (2000:160) recorded the flavonoid and total polyphenol content of fermented and unfermented rooibos herbal tea and confirmed that on fermentation the total polyphenol and flavonoid content decreased significantly as the fermentation process includes the oxidation of polyphenols (Joubert & Schulz, 2006:139). During the production of green rooibos the oxidative changes are minimised through the control of the moisture content in order to retain the green colour. This also minimises the oxidation of the polyphenols (Joubert & Schulz, 2006:139). In this study the total polyphenol content, however, did not differ significantly between the prepared rooibos samples, although the content of the green / unfermented and organic green / unfermented samples were higher than that of the organic traditional / fermented and traditional / fermented samples.

5.1.2 Form

The rooibos samples prepared with green / unfermented leaves and the rooibos powdered extract (traditional / fermented) had a significantly higher flavanol content compared to the other forms of rooibos investigated, which included the bag form. According to Cheong *et al*. (2005:750) the extraction rate of green tea powder is faster due to a wider surface area than that of green tea leaves for example, although no significant difference was found in the biochemical content of the rooibos samples prepared with the rooibos powdered extract (traditional / fermented) and the green / unfermented leaves in this study. Astill *et al.* (2001:5344) indicated that a loose leaf infusion extracts tea solids into the water more efficiently than an infusion from a tea bag. During loose leaf infusion, soluble components are transported from the tea leaves directly into the water. Whereas when preparing tea from a tea bag, the flow resistance, caused by the packed bed of leaves and by the bag material, results in a slower transfer of the soluble components from inside the bag into the water. The catechin and flavonol contents in tea infusions furthermore increase in a linear way relative to the amount of tea leaves used for brewing (Bhagwat *et al*., 2014:3). When considering green tea (*C. sinensis*) Komes *et al*. (2010:173) indicated that the bagged infusion provided a higher flavanol content than the powdered or loose leaf green tea infusion. The infusions prepared from the different tea forms in

that green tea study were not completely comparable as they were prepared at different water temperatures, brewing and storage times.

Having reported on the above mentioned parameters, it is important to keep in mind when comparing results of various studies that a number of factors such as differences in the biochemical assay protocols, different sources of plant material, composition of plant material, harvest date and the manufacturing processes all influence the phenolic composition and quantity in the final product (Joubert *et al*., 2011:874).

5.1.3 Brewing time

Brewing time has a major influence on the flavonoid and polyphenol content of tea infusions (Hakim *et al*., 2000:1720). Peterson *et al*. (2004:401) reported the influence of brewing techniques and brewing times on the flavonoid content of black tea. The flavonoid content of prepared black tea was found to be higher if the brewing time was four minutes, as opposed to two minutes (Peterson *et al*., 2004:403). The experimental study of Ryan and Petit (2010:16) also indicated that the antioxidant potential of black tea increased with an increased infusion time. The researchers recorded that the antioxidant potential increased gradually according to the infusion time, with a maximum value obtained after 10 minutes, while more than 50% of the maximum value was already obtained after one minute of infusion. More than 95% of the antioxidant capacity was available after five minutes of infusion and more than 90% available after four minutes. The findings from this current rooibos study indicated that a longer brewing time imply an increased TAC, total polyphenol, flavonol and flavanol content. The rooibos infusion brewed for one minute had a significantly lower phenolic content compared to longer brewing times for all of the investigated parameters. The rooibos infusion brewed for five minutes also had a significantly lower biochemical content compared to a 10 minute brewing time for nearly all the investigated biochemical parameters. The sample brewed for five minutes had a significantly lower TEAC compared to the samples brewed for 20 and 30 minutes, as well as a significantly lower total polyphenol content compared to the sample brewed for 30 minutes. The rooibos infusions brewed for 10, 20 and 30 minutes, however, did not differ significantly from each other for any of the investigated biochemical parameters, although an increase was found in each of the investigated biochemical parameters for these brewing times. Bhagwat *et al*. (2014:3) indicated that the majority of the flavonoids present in tea are extracted into the infusion within the early stage of infusion and do not seem to increase markedly with prolonged brewing times, compared to short brewing times based on the findings of the studies of Hertog *et*

al. (1993:1242) and Arts *et al*. (2000:1752). This finding is supported by the brewing time results of this rooibos study.

While the results provided above imply that the major extraction of the phenolic contents of tea / herbal tea occurs after a short brewing time and that the contents seemingly increase on continued brewing for a noticeable period of time, some study results suggest a decrease in the phenolic contents with prolonged brewing. Campanella *et al*. (2003:731) found that a hot tea infusion brewed for five minutes produced the highest antioxidant capacity and that the antioxidant compounds increased at the onset of the infusion and continued to increase, but that after five minutes the antioxidant capacity decreased (Campanella *et al*., 2003:732). Cheong *et al*. (2005:754) confirmed that a traditional brewing time of 10 minutes is sufficient for contributing to the flavonoid intake but that prolonged extraction of green tea leaves is not favourable as the flavonoids decompose over time. Schwalfenberg *et al*. (2013:1) however warns against the exposure to heavy metals, such as lead and aluminium, when brewing black, green, white and oolong teas for longer than three minutes. No information in relation to this was obtained for rooibos.

5.1.4 Flavourings

The TAC (measured by the TEAC assay) of the rooibos samples with added honey in small, medium and large amounts were significantly higher than the TAC (measured by the TEAC assay) of the rooibos samples with small, medium and large amounts of white or brown sugar added. The samples with honey added in these differing amounts also had a significantly higher flavonol content compared to the samples with brown sugar added in these different amounts. The flavonol content of the sample with honey added in a medium amount was also significantly higher than the sample with white sugar added in a medium amount. Honey consists of the two natural sugars, glucose and fructose (Erejuwa *et al*., 2012:1900), with fructose being the most abundant (Ajibola *et al*., 2012:2). It also contains phenolic compounds, proteins, vitamins and minerals, amino acids and organic acids, enzymes and volatile compounds (da Silva *et al*., 2016:309). The main functional components of honey are flavonoids, with the flavonol quercetin being the most abundant, which contribute to the total antioxidant activity of honey, resulting in beneficial effects for human health (Alvarez-Suarez *et al*., 2012:1515). Whole grains, as a further example, are richer in antioxidants than refined grains as antioxidant components, such as flavonoids, decrease with refining. Refining decreases the antioxidant content of not only whole grains, but that of sugars as well (Phillips *et al*., 2009:64) and may explain the higher phenolic

contents found in the prepared rooibos with the addition of honey, compared to brown, and in particular, white sugar.

The rooibos herbal tea samples with whole milk added in small, medium and large amounts, had a significantly higher TAC (measured by the FRAP assay) than the samples with added skim milk in small, medium and large amounts. The FRAP of the samples with low fat milk added in small and medium amounts were also significantly higher than that of the samples with small and medium amounts of added skim milk. The TAC, measured by the TEAC assay, of the samples with added whole milk in medium and large amounts were significantly lower than that of the samples with added skim or low fat milk in medium and large amounts. Ryan and Petit (2010:17) confirmed that a number of fat-soluble antioxidants are present in milk such as tocopherols, retinols and carotenoids. As a result the antioxidant potential of milk is increased when its fat content is higher which may explain the significantly higher TAC found of the rooibos samples with whole milk added in comparison to skim and low fat milk added in the same amounts. The fat content of whole milk is required to be 3.3 to 4.5%, that of low fat milk 0.5 to 1.5% and that of skim milk not more than 0.5% (South Africa. Department of Agriculture, Forestry and Fisheries, 2015:7).

The addition of whole milk in a large amount resulted in a significantly higher total polyphenol content compared to the sample with added skim milk in a large amount. For both the flavonols and flavanols, the rooibos samples with added whole milk or low fat milk in small, medium and large amounts were also significantly higher than the samples with skim milk added in small, medium and large amounts. Whole milk added in a medium amount furthermore had a significantly higher value for these two biochemical parameters than the sample with low fat milk added in a medium amount. Although significant differences were greatly noted in these biochemical parameters for the whole milk and low fat milk additions compared to the skim milk addition, it most probably occurred because of the interference with the absorbance of the assay by some of the milk constituents, and in particular the milk protein, and not as a result of a reflection of a true increase in the phenolic compounds (Prior *et al*., 2005:4297).

Considering the amounts of the different types of milk added, the samples with a small amount of skim and low fat milk added had a significantly higher FRAP than the samples with added skim and low fat milk in medium and large amounts. The FRAP of the sample with a small amount of whole milk added was significantly lower than that of the sample with a large amount of whole milk added. The TAC (measured by the TEAC assay) of the rooibos samples with the

added large amount of skim, low fat and whole milk were significantly higher than the samples with the added small and medium amounts of skim, low fat or whole milk. This finding was also noted with the flavonol content of the samples with the differing amounts of added low fat milk and whole milk. The flavanol content of the samples with low fat and whole milks added in small amounts was further significantly lower than those samples of these milk types when added in medium and large amounts. This is due to the protein and fat present in the milk which causes turbidity in the assays. The plate reader reads the turbidity as antioxidants or flavonols which results in a false higher reading for both the assays (Rautenbach, 2016). Considering the addition of whole milk, a large amount addition resulted in a significantly higher total polyphenol content compared to the addition of a small amount of whole milk and again may be ascribed to the protein and fat present in milk interfering with the assay reading (Rautenbach, 2016).

Considering the samples with the added household flavourings combined, the TEAC of the samples with the different types and amounts of milk added, along with brown or white sugar added in different amounts, were all significantly lower than the samples with the different amounts of honey added to the different types and amounts of added milk. This could be attributed to the antioxidant compounds present in honey (Alvarez-Suarez *et al*., 2012:1515).

Commercially flavoured teas are popular due to their taste as well as added antioxidant properties through the flavourant addition (Pekal *et al*., 2012:742). Rooibos as a cold water soluble extract is excellent for use in iced tea / RTD applications, especially in an environment that is not very acidic and where the application does not require 100% clarity. The extract consists of the cold water soluble fraction of rooibos and contains a minimum of 24% polyphenols. It has a very attractive chestnut reddish brown rooibos colour when dissolved. The dosage solution for the extract is 2 g per L (Rooibos Ltd, 2011). The phenolic quality of commercial South African fermented rooibos iced teas in terms of aspalathin, iso-orientin, and orientin contents in comparison to a standard cup of rooibos herbal tea has been shown to be lower. The role of the different manufacturing stages of powdered extract used in iced tea formulations and, more specifically, the impact of pasteurisation and sterilisation on the colour and phenolic content of the beverage, were assessed as potential causes of its inferior phenolic quality (Joubert *et al*., 2009:4204).

With the commercially flavoured rooibos samples, camomile flavoured rooibos had a significantly lower TAC (measured by the FRAP assay) compared to the other samples, except for berry flavoured iced tea (RTD). Berry flavoured iced tea (RTD) had a significantly lower FRAP

compared to the rest of the samples, but not significantly lower compared to that of the camomile flavoured rooibos. Honey flavoured rooibos and peach flavoured iced tea (RTD) had a significantly lower FRAP compared to lemon flavoured rooibos, lemon flavoured iced tea (powder), instant apple fruit flavoured iced tea (powder) and instant fruit punch and lemon flavoured iced tea (powder). Lemon flavoured iced tea (RTD) also had a significantly lower FRAP compared to lemon flavoured rooibos, instant apple fruit flavoured iced tea (powder) and instant fruit punch and lemon flavoured iced tea (powder), but a significantly higher FRAP compared to the powder form of the lemon flavoured iced tea. The lemon flavoured rooibos (traditional / fermented) had the highest TEAC, which was significantly higher than that of the lemon flavoured iced tea (powder), instant apple fruit iced tea (powder), instant fruit punch flavoured iced tea (powder), instant fruit punch and lemon flavoured iced tea (powder) and the berry flavoured iced tea (RTD). The flavonoid (quercetin) content, according to the United States Department of Agriculture (USDA) Database for the Flavonoid Content of Selected Foods (Release 3.2), for lemon is 1.14 mg per 100 g, for peach 0.66 mg per 100 g and for honey 0.31 mg per 100 g (Bhagwat & Haytowitz, 2015:46,50,116) which may explain some of the differences found in the investigated phenolic parameters of the commercially flavoured rooibos samples. Campanella *et al*. (2003:734) reported that bottled tea with lemon added had a higher TAC than bottled tea with peach added. This was in line with the findings of the current study, although the TAC of the two samples was not found to differ significantly.

The total polyphenol content of berry flavoured iced tea (RTD) was the lowest, and was also significantly lower than that of all the other commercially flavoured rooibos samples, except for camomile flavoured rooibos (traditional / fermented). Lemon flavoured rooibos (traditional / fermented) had the highest flavonol content with the flavonol content significantly higher than that of camomile flavoured rooibos (traditional / fermented), honey flavoured rooibos (traditional / fermented), peach flavoured iced tea (powder), lemon flavoured iced tea (RTD) and peach flavoured iced tea (RTD). Lemon-flavoured black tea has been described as a mixture of two powerful antioxidant matrixes consisting of that of the black tea and that of the lemon (Pereira *et al*., 2013:233) which contribute to its polyphenol and flavonoid provision. The health benefits of lemon and its peels and juices have been attributed to the presence of bioactive compounds, such as phenolic compounds and vitamin C which are powerful antioxidants with radical-scavenging activity (Pereira *et al*., 2013:233).

5.1.5 Highest total polyphenol content and total antioxidant capacity identification

The rooibos samples with the highest total polyphenol content and TAC (for both the FRAP and TEAC assays) for the different types, forms, brewing times and household flavourings added were recorded and considered in order to conclude on the optimal cup of rooibos based on the above mentioned biochemical parameters. The total polyphenol content of the rooibos sample with honey added in a large amount was significantly lower compared to that of the sample prepared with powdered rooibos extract (traditional / fermented) and the sample brewed for 30 minutes. The powdered rooibos extract (traditional / fermented) had a significantly higher FRAP compared to that of the sample prepared with green unfermented rooibos and the sample with a large amount of honey added. The FRAP of the sample prepared with green unfermented rooibos and the sample brewed for 30 minutes were both significantly higher than that of the sample with honey added in a large amount. The sample with a large amount of honey added and the iced tea (with rooibos extract – traditional / fermented) sample had the highest TAC (measured by the TEAC) with the TAC of these samples also significantly higher than that of the sample prepared with traditional / fermented rooibos and the sample brewed for 30 minutes. Due to limited availability and cost implications to the consumer, the rooibos type, as green unfermented rooibos, and the rooibos form, as powdered rooibos extract (traditional / fermented) and iced tea (with rooibos extract – traditional / fermented) were not considered to conclude an optimal cup.

The sample with the addition of honey in a large amount was also not considered due to the possible health implications of the addition of a large amount fructose contained in honey. Striving for the consumption of at least four (Yang & Hong, 2013:161) to six (Popkin *et al*., 2006:532; Marnewick *et al*., 2011:50) cups of tea per day is advised to obtain the health benefits of tea, while a large amount of honey added to each cup may be ill-advised. Several studies (Chepulis, 2007:224S; Nemoseck *et al*., 2011:55; Ajibola *et al*., 2012:12) though have indicated that honey has more health promoting properties than sucrose. Nemoseck *et al*. (2011:55) compared the influences of feeding male rats a honey-based diet vs a sucrose-based diet for 33 days, on weight regulation, adiposity and related biomarkers and lipid metabolism. The results suggested that there may be numerous promising health benefits, such as improved weight regulation and reduced triglyceride levels when sucrose is substituted with honey in the diet. Honey is a natural substance with many medicinal properties, including antibacterial, hepatoprotective, hypoglycaemic, antioxidant and antihypertensive effects (Erejuwa *et al*., 2012:1900). The fructose content of honey might contribute to the hypoglycaemic effect of this

form of sweetener (Erejuwa *et al*., 2012:1900). Grobler *et al*. (1994:147) also reported that honey does not have an effect on erosion of the tooth enamel over a period of 30 minutes. This could be due to the calcium, fluoride and phosphorus levels in honey.

Though there is reason to believe that moderate fructose ingestion could be beneficial for public health, an excess intake would be a risk to health (Livesey, 2009:1250S). Kretowicz *et al*. (2011:3) reported that fructose, consumed in excessive amounts, might contribute to diabetes and cardio-renal disease as well as obesity. Fructose can induce insulin resistance in humans. Fructose does not acutely stimulate leptin or insulin release and hence may not trigger normal satiety responses. Fructose intake from added sugars is furthermore also associated with elevated blood pressure in humans. The acute ingestion of fructose (60 g) can increase systolic blood pressure in humans, and this is not seen in subjects given the same dose of glucose (Brown *et al*., 2008:R730). Fructose and sucrose are also known to induce renal hypertrophy and tubulointerstitial disease in rats (Nakayama *et al*., 2010:F712). Clinical studies have also linked the intake of excessive fructose with the development of nonalcoholic fatty liver disease in humans (Ouyang *et al*., 2008:993; Abdelmalek *et al*., 2010:1961).

Therefore permitting for the household preparation of rooibos and taking cognisance of the consumer availability and cost implications of the rooibos type and form, the traditional / fermented in bag form rooibos sample brewed for 30 minutes was considered as the preparation method that provided for the highest total polyphenol content and TAC. Astill *et al*. (2001:5340) confirmed that the preparation method of tea, such as the brewing time utilised, is a major determinant of the component concentrations in tea and Sharpe *et al*. (2016:380) stated that brewing time significantly affects the tea antioxidant capacity. Yet, as the biochemical parameters of the samples brewed for 10, 20 and 30 minutes did not differ significantly, traditional / fermented rooibos in bag form brewed for 10 minutes and longer was considered to be the optimal rooibos consumption representation to provide for the highest total polyphenol content and TAC. Ryan and Petit (2010:16) indicated that the antioxidant potential of black tea increased gradually according to the infusion time, with a maximum value obtained on a 10 minute brewing time, while Cheong *et al*. (2005:754) confirmed from a 10 minute brewing time was sufficient for contributing to flavonoid intake.

5.2 Rooibos herbal tea consumer profile and tea drinking behaviour

Most of the respondents indicated that they consumed food / beverages popular with and consumed by most adults of the same age (similar food and beverage intake to most friends, family and / or colleagues), were non-smokers, had an optimal / normal body weight and did not use or seldom used dietary supplements, while slightly more respondents indicated to be physically active rather than not. In the Caerphilly study undertaken in South Wales, the UK (Hertog *et al*., 1997:1489), tea consumption was generally positively linked to a less healthy lifestyle with regards to smoking and a higher fat intake and also to a lower social class, whereas in most other studies (seemingly coming forward in this study), tea consumption was associated with a healthier lifestyle, with tea intake higher in lean, educated people who smoked less and also consumed a relatively healthy diet (Geleijnse *et al*., 2002:880; Hakim *et al*., 2003:64) and occasionally higher social class (Geleijnse *et al*., 2002:880). The majority of the respondents furthermore indicated that they were not diagnosed with CVD, diabetes mellitus type 2, inflammatory conditions (e.g. arthritis) or cancer (skin, lung, breast, liver or prostate) and most indicated that they did not have a family history of these dieases.

Although all the respondents were rooibos consumers, most of the respondents indicated that coffee is their preferred non-alcoholic beverage, which was closely followed by tea / herbal tea. Oldewage-Theron and Kruger (2011:420) reported that women in an informal settlement in the Vaal region (SA) have a mean daily intake of 288 g black tea, 274 g rooibos herbal tea and 255 g coffee per consumer. Tydeman-Edwards (2012:173) listed the 10 most frequently consumed food items of adults aged 25 to 64 years in the Free State province, SA. Tea was second most frequently consumed item by urban women; third most frequently consumed by the urban men and rural women; and fourth most frequently consumed by rural men in the study. Men consumed coffee more frequently (5th urban and 6th rural positions) than women (10th position in both rural and urban areas).

Almost half of the respondent sample indicated that traditional rooibos (plain) is the tea / herbal tea mostly consumed with the foremost reason for this indicated to be the preferred taste. Brunso *et al*. (2002:12) indicated taste to be one of the primary determining factors for food choices. The taste of fermented rooibos has been described as sweet and caramel-like (Joubert & Schulz, 2006:141), as sweet and fruity (Erickson, 2003:37) or as a combined taste of honey, woody and herbal-floral notes accompanied by a sweet taste as described by Koch *et al*. (2012:217; 2013:704). Reed *et al*. (2006:215) acknowledged the universality of the ‘goodness’ of

the sweet taste and ‘badness’ of the bitter taste within the basic taste qualities. This acknowledgement, along with the sweet sensory attribute ascribed to rooibos, provides support for taste being provided by the respondent sample as the foremost reason for their rooibos consumption.

Two to three cups of tea / herbal tea were indicated by almost a third of the respondent sample as the daily consumption frequency of tea / herbal tea, while a very small number of the respondents indicated a consumption frequency of four to six cups and more than six cups per day. This was a rather disappointing finding. Hakim *et al*. (2003:66) reported a consumption of six or more cups of tea per day for 26.4% of the men and 11.9% of the women who participated in their study. Popkin *et al*. (2006:532) advised the consumption of five to six cups of tea and Yang and Hong (2013:161) four to six cups on a daily basis to be beneficial for human health. Studies conducted in the US, continental Europe and Asia reported a significant reduction in the incidence of stroke of 12% per three cups of consumed tea per day. Hakim *et al*. (2003:64) also reported that those who drink more than six cups of tea per day had a significantly lower prevalence of coronary heart disease than the non-tea drinkers.

A rooibos herbal tea consumption frequency of two to three cups per day was indicated by a fifth of the respondents, while only a few of the respondents indicated a consumption frequency of four to six cups per day, with not even one percent of the respondent sample having a consumption frequency of more than six cups of rooibos per day. This was a further rather disappointing finding as the respondent group comprised individuals who consume rooibos herbal tea. Marnewick *et al*. (2011:50) reported a consumption of six cups of rooibos daily to reduce the risk for CVD in adults. According to Joubert and de Beer (2012:48) six cups of rooibos herbal tea must be consumed as a minimum daily for it to have a beneficial health effect. The consumption of various plant foods and beverages like tea which are rich sources of antioxidants, and in particular flavonoids, have been found to reduce the risk of chronic diseases (Yao *et al*., 2004:113; Tsuji *et al*., 2013:1014) with tea consumption greatly contributing to the dietary intake of polyphenols (Scalbert & Williamson, 2000:2076S) and flavonoids (Song & Chun, 2008:1543S).

Considering the characteristics of the respondents having different rooibos consumption frequencies, there was a significant difference between the respondent rooibos consumption frequencies and the age of the respondents as well as their highest level of education. The respondents having the higher consumption frequencies were mostly older and had a lower level

of education, with the latter also established in the Caerphilly study conducted in South Wales, the UK (Hertoge*t al*., 1997:1489). Other studies (Geleijnse *et al*., 2002:883; Mukamal *et al*., 2002:2477) have indicated that tea consumption is associated with a higher level of education. Hakim *et al*. (2000:1717) reported that more females consume tea than males. In their study on the flavonoid intake in the US diet, Song and Chun (2008:1543S) also reported that tea consumers are more likely to be female and older and also white and to have a higher income than those who do not consume tea. Mukamal *et al*. (2002:2477) also reported from their study that heavy tea drinkers were more likely to be female and older. The respondents from the study were mostly married / living together with children or single and living without children.

Most of the respondents indicated that they mostly consumed rooibos throughout the day or during the morning. Almost half of the respondent sample indicated that they did not consume commercially flavoured rooibos. The respondents, who did indicate to consume commercially flavoured rooibos, mostly consumed either peach flavoured iced tea or lemon flavoured tea bags and lemon flavoured iced tea. De Godoy *et al*. (2013:807) also reported the same – that iced teas flavoured with peach and lemon was preferred by their study participants.

The preparation methods of tea vary in different countries and with different people (Bhagwat *et al*., 2014:3). Tea bags were indicated to be the form of rooibos used by the respondents to prepare it as a beverage which can probably be ascribed to the wide market availability of rooibos in the bag form. This was also encountered in the studies conducted by Hakim *et al*. (2000:1722) and de Godoy *et al*. (2013:801). The majority of the respondents indicated that they consumed a weak to medium to strong rooibos infusion (brewed for one to between five to 10 minutes) and not a rooibos infusion brewed for 10 minutes or longer (somewhat strong or strong infusion). In the Arizona-study, most of the tea consumers also brewed their tea for two to three minutes and only about a fifth brewed their tea for more than three minutes (Hakim *et al*., 2000:1717). A significant difference was identified in this study between the usual preparation method of those consuming an optimal cup of rooibos and those who did not. Although the majority of the respondents indicated that they brewed their tea bag in a cup or mug, a quarter of the respondents indicated that they usually prepared their tea bags brewed in a teapot. It was these latter respondents that were inclined to consume an optimal cup of rooibos for the higher total polyphenol content and TAC provision on considering the brewing time. More than half of the respondents indicated that they consumed tea when warm or still heated, while other studies (Hakim *et al*., 2000:1719; de Godoy *et al*., 2013:801) reported their participants preferred their tea hot or warm. To consume an optimal cup of rooibos for the higher polyphenol content and

TAC provision taking into account the brewing time, the respondent who likes to consume tea / herbal tea warm or still heated will normally brew the tea in a cup or mug. It must however, be brewed for 10 minutes or longer and therefore may undergo more cooling when compared to being brewed in a teapot (with the teapot also permitting tea / herbal tea to be kept warm easier than in a cup or mug).

Less than a fifth of the respondents indicated that they did not add a flavouring to their prepared rooibos. Most of the respondents indicated that they added milk together with a sweetening agent to their rooibos, using primarily whole milk and white sugar, followed by low fat milk and white sugar. Among those respondents who indicated that they added whole milk, low fat milk or skim milk to their prepared rooibos, half of them in each case added a medium amount. Brown sugar was indicated by the respondents who added it to their rooibos to be added in a small amount, followed by a large amount. White sugar was indicated to be added in a medium amount, followed by large amount by the respondents who indicated that they added it to their prepared rooibos. In a study by Bryan *et al*. (2012:342), which assessed the relationship between beverage consumption and mood and work performance, milk alone was mostly added to all the beverages consumed (tea, coffee, other caffeinated and decaffeinated beverages) and second most was milk and sugar, while most of the respondents in this study indicated that they added milk together with a sweetening agent to their rooibos. Sugar added alone was the flavouring that was added by the lowest number of respondents to the investigated beverages in the study by Bryan *et al*. (2012:342). Most of the respondents who indicated that they added honey to their prepared rooibos, added it in a medium amount. Only a few of the respondents indicated that they added lemon juice to their prepared rooibos.

5.3 Strengths and limitations of the study

5.3.1 Strengths

A total of 667 samples representing different rooibos types and forms, brewing times, with different added household flavourings and commercially added flavourings from different rooibos brands were prepared, sampled and analysed for a number of biochemical parameters. The data collected on the content of these analysed samples provides the foundation for a possible rooibos composition database for these biochemical parameters. The total polyphenol content and the TAC for an optimal cup of rooibos could as a result be obtained, with the testing of

different methods in which consumers prepare their rooibos which provided valuable information that can be used to advise consumers on the consumption of rooibos.

A questionnaire was designed to gather the information required on the respondent sample profile and respondent tea and herbal tea consumption. The questionnaire did not only permit the collection of data pertinent to the study, but also offered a structure of reliable format for the gathering of the required information through the question responses as promoted by Kemp *et al*. (2009:121). The questionnaire was pilot tested on the target population as advised by Murray (1999:152) and Rattray and Jones (2007:237), and evaluated by several experts in the food and nutrition field for face and content validity as recommended by Bannigan and Watson (2009:3240). As a result the respondents only had to complete a short developed questionnaire that consisted of compiled multiple choice questions, which were time-saving for the respondents to complete and made it easy for the data capturing process and the accuracy thereof.

The respondents who participated in this study were rooibos herbal tea consumers. Their tea drinking habits and in particular information on rooibos consumption behaviour obtained through the questionnaire, provided valuable information as to the tea / rooibos herbal tea drinking behaviour of South Africans. Taking into account the findings of this study, the aforementioned could be considered inadequate based on the consumption frequency and utilised brewing time. The respondents were from different adult age, gender and population groups, which made it more representative of the larger community.

5.3.2 Limitations

The interference of the non-phenolic components present in milk in the assays resulted in a false total polyphenol content, hence not providing an accurate value for the samples with milk added as flavouring. The market unavailability of some of the types and forms of rooibos does not make the biochemical parameter findings related to these samples of pertinent value to the consumer.

As the respondent selection for the quota sample was non-random, sample bias is an almost certain limitation of Phase 2 of the study. The sample would also have been more representative if more individuals from the non-white population groups and the older age categories completed the questionnaire. Language barriers limited the recruitment and participation of these

respondents in answering the questionnaire, which was only available in English, while cognitive disabilities limited the recruitment and participation of the elderly in answering the questionnaire. The questionnaire was only available in English as there was no need identified for its availablility in other languages after the questionnaire pilot testing. Only consumers of rooibos tea were recruited. This limited the interpretation of the results on demographic and lifestyle characteristics of rooibos herbal tea consumers. It is therefore not known whether demographic and lifestyle characteristics differ between consumers and non-consumers of rooibos in this specific study population.

The few research studies completed that have a likeness to that of Phase 1, while those similar to Phase 2 of this study had mostly been done on other types of tea and in other countries. This restricted the discussion of the results of this study.

CHAPTER 6
CONCLUSIONS

Based on the research questions and objectives of the study, the following can be concluded from Phase 1 which investigated how the different types, forms, preparation methods and added flavourings influenced the total polyphenol content and TAC of a cup of rooibos herbal tea, and also determined which prepared cup of rooibos herbal tea provided the higher total polyphenol content and TAC. The different rooibos types, forms, preparation methods and flavouring additions all had an influence on the total polyphenol content and TAC of rooibos. In terms of the rooibos types, green / unfermented rooibos had a significantly higher TAC (measured by the FRAP assay) and flavanol content as well as the highest total polyphenol content, although not significantly higher than that of the other types. In terms of the rooibos forms, the rooibos powdered extract had the highest total polyphenol content and TAC (measured by the FRAP assay) although not significantly higher than that of the other forms. This form and green / unfermented leaves both had a significantly higher flavanol content than the other rooibos forms. Green / unfermented rooibos as the type and rooibos powdered extract and green / unfermented rooibos leaves as rooibos forms have limited consumer market availability and are currently not of pertinent value to the consumer; although they could have, based on their determined biochemical parameter content and them having market availability at a competitive price range. Allowing for the brewing time as representative of the rooibos preparation method, it was determined that a longer brewing time resulted in a higher total polyphenol content and TAC. The rooibos samples that brewed for one minute had a significantly lower TAC, total polyphenol, flavonol and flavanol content than the samples brewed for longer as five, 10 and 20 minutes respectively. The sample brewed for 10 minutes also had a higher TAC, total polyphenol and flavanol content than the sample brewed for five minutes.

Considering the addition of household flavourings to rooibos, the addition of honey to traditional / fermented rooibos in amounts that represented a small, medium and large amount positively influenced the biochemical parameters tested due to the phenolic compounds present. Although the addition of whole milk also resulted in a higher total polyphenol content and TAC, these findings should be interpreted with caution due to assay interferences. Rooibos commercially flavoured with lemon, although more costly than the unflavoured traditional / fermented option, had a higher total polyphenol content and TAC compared to the other investigated commercial rooibos with added flavourings. Phase 1 of the study based on the rooibos types, forms, brewing times and added flavourings, whether household or commercially, also denoted the more

optimal cup of rooibos, in terms of the total polyphenol content and TAC. This was determined to be the one brewed for a longer time, alluding to a brewing time of 10 minutes or longer, taking cognisance of relevance to the consumer in terms of rooibos market availability (lack of green / unfermented rooibos and powdered rooibos), expenditure (higher pricing range of commercially flavoured rooibos and the higher cost of honey as an added sweetener) and health implications (fructose consumption through honey added to each cup of rooibos consumed during the day).

Based on the research questions and objectives of this study, the following can be concluded from Phase 2 concerning the ways in which consumers, represented by this non-probability sample of rooibos herbal tea drinkers, usually consume rooibos considering the different types, forms, preparation methods and flavouring additions and amounts added to prepare it as a beverage. Plain traditional rooibos herbal tea was indicated to be the preferred tea / herbal tea by almost half of the respondents, with the clear reason provided being its taste. The majority of the respondents indicated that the form usually used is rooibos in tea bag form which is also the form most readily available on the market. The majority of the respondents also indicated that they usually prepared their tea by brewing the tea bag in a cup or mug, and most that they consumed it warm or still heated. It can moreover be concluded that the respondents, although they were consumers of rooibos herbal tea, did not brew their prepared rooibos long enough to be considered an optimal cup for providing the higher total polyphenol content and TAC. Most of the respondents indicated that they brewed their rooibos between one to five minutes or for five minutes, being of weak to medium or medium strength respectively. Most of the respondents indicated that they usually added milk and a type of sweetener to rooibos – mostly whole milk and white sugar. Milk was indicated to be added in a medium amount. Most of those who indicated that they added white sugar either added it in a medium or a large amount, while most of those who indicated that they added brown sugar either added it in a small or a large amount. The addition of sugar as a flavouring practise occurred despite the taste of fermented rooibos being described as sweet. Only a few of the respondents indicated that they added honey to their rooibos, of which most added a medium amount which additionally supports not having considered the addition of honey to rooibos as an option for provision of the optimal cup.

Tea / herbal tea, and in addition, rooibos herbal tea were not merely consumed enough of by the respondents as only a few indicated that they consumed four or more cups of it per day. For rooibos itself, only a fifth of the respondents indicated that they even consumed two to three cups of rooibos per day. Most of the respondents furthermore indicated that they consumed rooibos throughout the day and about half that they did not consume commercially flavoured

rooibos, which additionally provides support for the decision not to have considered commercially flavoured rooibos for optimal rooibos consumption in addition toits higher pricing range.

Concerning the demographic and lifestyle characteristics of rooibos herbal tea consumers, as a further research question and objective of Phase 2 of the study, it can be concluded that rooibos consumers as the respondents of this study consumed food and beverages popular with and consumed by most other adults of the same age. They were generally healthy in the sense that most indicated that they were physically active, had an optimal / normal body weight and that the majority indicated that they were non-smokers and had not been diagnosed, nor had a family history of CVD, diabetes mellitus (type 2), inflammatory conditions (e.g. arthritis) or cancer (skin, lung, breast, liver or prostate). It can also be concluded that it was the older adults and those with a lower level of education that consumed a significantly higher amount of rooibos as the number of cups per day.

In relation to the last objective of the study, which was to determine the percentage of rooibos herbal tea consumers and their demographic and lifestyle characteristics that consumed traditional / fermented rooibos herbal tea as a beverage prepared to provide the higher total polyphenol content and TAC, it could be concluded that a mere 15.9% of the respondents consumed an optimal cup of rooibos; based on a brewing time of 10 minutes and longer. There was no significant difference between the respondents consuming an optimal cup of rooibos and those who did not, where their demographic and lifestyle characteristics were concerned. The only significant difference found was related to the preparation method of rooibos. Although the respondents generally brewed their tea bag in a cup or mug, more of those respondents who brewed it in a teapot consumed the optimal cup of rooibos considering the brewing time of 10 minutes and longer. This finding should be considered in relation to the finding that those respondents who consumed rooibos also did not consume it in an amount (as a minimum of four to six cups per day) which would fully provide for obtaining its health benefits.

CHAPTER 7
RECOMMENDATIONS

The type, form, preparation method (represented by the brewing time) and added flavouring all had an influence on the total polyphenol content and TAC of rooibos herbal tea prepared as beverage. Considering the rooibos type and form, it can be recommended that the rooibos industry should strive to make green / unfermented rooibos as well as the rooibos powdered extract more readily available to the consumer and in addition provide for more affordable products as both green / unfermented rooibos (as a rooibos type) and the powdered extract (as a rooibos form), provided the higher total polyphenol content and TAC among the different rooibos types and forms respectively investigated.

In terms of the brewing time, the rooibos industry should strive to inform rooibos consumers that a longer brewing time provides for the more optimal cup of rooibos in terms of the total polyphenol content and the TAC. A brewing time reaching 10 minutes or longer was found to be the optimal for the provision of the higher total polyphenol content and the TAC, whereas a brewing time of one minute or shorter was found not to be sufficient for this provision. This guidance on the optimal brewing time could possibly be placed on the packaging of rooibos or communicated via consumer health campaigns in relation to rooibos consumption. However, rooibos brewed for 10 minutes or longer will taste different than rooibos brewed for a shorter period. Consumer acceptance should therefore be considered when making recommendations on the brewing time. As the majority of the rooibos consumers seemed to enjoy their rooibos warm / still heated or hot / near boiling, cognisance should be taken of the temperature of the prepared beverage on its consumption in the provided brewing time guidance. Consumers could, for example, be advised to brew their rooibos in a teapot to provide a warmer beverage for consumption, as this study did find that those respondents who brewed their rooibos in a teapot represented those who consumed the more optimal cup of rooibos in terms of the brewing time and the resultant higher total polyphenol content and TAC. While no information was found in relation to rooibos, Schwalfenberg *et al*. (2013:1) warned against consumer exposure to heavy metals, such as lead and aluminium, when brewing black, green, white, and oolong teas for longer than three minutes, which may propose an investigation should be undertaken to determine the presence of heavy metals in rooibos and possible heavy metal accumulation brewed for a longer period of time. Malik *et al*. (2008:520) determined the presence of certain metallic elements, such as aluminium, copper, iron, manganese, nickel and zinc in the plant stimulants and their infusions they investigated. The results, however, indicated that the amounts of all these elements in the rooibos infusion were low. The aluminium and

nickel content found in the rooibos infusion, as representative of the presence of metallic contaminants, was in particular significantly lower than that found in the other tea infusions which included mate, honeybush and camomile tea as well as a coffee infusion. The tea infusions were prepared with tea leaves and infused for 15 minutes (Malik *et al*., 2008:524) which suggests that consumer exposure to heavy metals over a longer brewing time may not be an aspect of concern with regards to rooibos consumption as a beverage on infusion.

The addition of honey as a flavourant, rather than brown or white sugar, could be advised due to its polyphenol content contribution made to rooibos as a beverage. Consideration should however be given to the amount of honey added, as the addition of honey to the suggested number of cups of tea to be consumed per day is debateable from a consumer health perspective. Consumers should also consider preparing homemade iced tea as a cost-effective replacer for commercial iced tea. Adding flavourings such as lemon should be considered as it would increase the phytochemical content and even nutritional value of the herbal tea as a beverage.

The consumption of the advised servings of tea should be encouraged as Phase 2 of the study found that tea / herbal tea, and specifically rooibos consumers did not consume the advised number of cups of tea / herbal tea (four to six cups per day) (Yang & Hong, 2013:161) and specifically rooibos (a minimum of six cups per day) (Marnewick *et al*., 2011:50; Joubert & de Beer, 2012:48). Consumers will only optimally benefit from the attributed health benefits of tea consumption when the suggested number of tea servings is consumed on a daily basis (Yang & Hong, 2013:161). In addition to the inadequate daily intake of rooibos, and also tea in general, the study found that rooibos consumers generally did not consume the optimal cup of rooibos as they did not brew rooibos for long enough to reach the optimal brewing period. By addressing the daily amount of rooibos consumed and applying a longer brewing time, the consumption of tea / herbal tea, and specifically rooibos, can make a valuable contribution to the daily dietary TAC. A diet rich in antioxidants is associated with a reduced risk of several diseases, including CHD and several common types of cancer and other chronic diseases (Nalsén *et al*., 2006:64), while consumption of various plant foods and beverages which are rich sources of antioxidants, and in particular flavonoids, have also been found to reduce the risk of these chronic diseases (Yao *et al*., 2004:113; Tsuji *et al*., 2013:1014). Health professionals should in their communications with consumers and patients assist them to better understand the health benefits of dietary antioxidants and encourage them to increase their intake of antioxidant-rich foods as well as beverages like tea / herbal tea.

Van Graan *et al*. (2013:77S) described the importance of consuming lots of clean, safe water as part of the South African food-based dietary guidelines (FBDGs). The general recommendation for total daily water intake is between 2 and 3.7 L for adult women and men (Van Graan *et al*., 2013:77S). Water is an important liquid nutrient as it is involved in important functions of the body and it is also the key dietary source of fluoride. The dietary sources of fluid include drinking water and other beverages and food that contain water (Van Graan *et al*., 2013:80S). It is normally assumed that the contribution of beverage consumption to the total water intake is 70 to 80% (Jéquier & Constant, 2010:117). The intake of tea can make an important contribution to the water / fluid intake as well as fluoride intake of an individual and should be encouraged. Rooibos herbal tea as a beverage is mostly made up of water and additionally provides protective effects against disease occurrence and particularly of those associated with oxidative stress (Baba *et al*., 2009:700), which is ascribed to the phenolic constituents they contain (Joubert & Ferreira, 1996:82; van Heerden *et al*., 2003:885).

The second phase of the study concluded that the older adult respondents consumed a significantly higher amount of rooibos as the number of cups per day compared to younger adult respondents. It can therefore be recommended that tea, and in particular rooibos, should be encouraged as the beverage of choice for consumption among younger adults for a more health conscious dietary approach. Those respondents with a lower level of education also consumed a significantly higher amount of rooibos as the number of cups per day, but these consumers as all rooibos consumers, should be advised on how to prepare it as a beverage to provide for the more optimal cup of rooibos.

The biochemical data collected of all the prepared rooibos samples can be used in a rooibos polyphenol and TAC database and for future rooibos research, including acute and chronic clinical intervention trials, as a database of this nature has not yet been compiled. The research tool, as a selected response questioning multiple choice questionnaire, used in the second phase of the study, may be used in further research to advance the available information on rooibos consumption. Further investigations on rooibos consumption are needed for more descriptive consumption information and comparison to a study of this nature, as well as to exclude the sampling bias believed to exist in this study. Future studies could for instance include rooibos herbal tea non-consumers to determine if the demographic and lifestyle characteristics of rooibos herbal tea non-consumers differ from that of rooibos herbal tea consumers.

REFERENCES

Abdelmalek, M.F., Suzuki, A., Guy, C., Unalp-Arida, A., Colvin, R., Johnson, R.J. & Diehl, A.M. 2010. Increased fructose consumption is associated with fibrosis severity in patients with nonalcoholic fatty liver disease. *Hepatology*, 56(6):1961-1971.

Ajibola, A., Chamunorwa, J.P. & Erlwanger, K.H. 2012. Nutraceutical values of natural honey and its contribution to human health and wealth. *Nutrition and Metabolism,* 9:1-12.

Alvarez-Suarez, J.M., Giampieri, F., Gonzalez-Paramas, A.M., Damiani, E., Astolfi, P., Martinez-Sanchez, G., Bompadre, S., Quiles, J.L., Santos-Buelga, C. & Battino, M. 2012. Phenolics from monofloral honeys protect human erythrocyte membranes against oxidative damage*. Food and Chemical Toxicology,* 50:1508-1516.

Ambade, A. & Mandrekar, P. 2012. Oxidative Stress and inflammation: essential partners in alcoholic liver disease. *International Journal of Hepatology*, 2012:1-9.

Arab, L. & Il'yasova, D. 2003. The epidemiology of tea consumption and colorectal cancer incidence. *Journal of Nutrition*, 133:3310S-3318S.

Arts, I.C.W., van de Putte, B. & Hollman, P.C.H. 2000. Catechin contents of foods commonly consumed in the Netherlands. Tea, wine, fruit juices, and chocolate milk. *Journal of Agricultural and Food Chemistry*, *48*(5):1752-1757.

Astill, C., Birch, M.R., Dacombe, C., Humphrey, P.G. & Martin, P.T. 2001. Factors affecting the caffeine and polyphenol contents of black and green tea infusions. *Journal of Agricultural and Food Chemistry,* 49:5340-5347.

Baba, H., Ohtsuka, Y., Haruna, H., Lee, T., Nagata, S., Maeda, M., Yamashiro, Y. & Shimizu, T. 2009. Studies of anti-inflammatory effect of rooibos tea in rats. *Pediatrics International*, 51:700-704.

Bahorun, T., Luximon-Ramma, A., Neergheen-Bhujun, V.S., Gunness, T.K., Googoolye, K., Auger, C., Crozier, A. & Aruoma, O.I. 2012. The effect of black tea on risk factors of cardiovascular disease in a normal population. *Preventive Medicine*, 54:98S-102S.

Bannigan, K. & Watson, R. 2009. Reliability and validity in a nutshell. *Journal of Clinical Nursing*, 18:3237-3243.

Barber, D.A. & Harris, S.R. 1994. Oxygen free radicals and antioxidants: a review. *American Pharmacy*, 34(9):26-35.

Beecher, G.R. 2003. Overview of dietary flavonoids: Nomenclature, occurrence and intake. *Journal of Nutrition*, 133:3248S-3254S.

Bennion, M. & Scheule, B. 2004. *Introductory foods*. 12th ed. New Jersey: Pearson Prentice Hall.

Benzie, I.F.F., Chung, W.Y. & Strain, J.J. 1999. "Antioxidant" (reducing) efficiency of ascorbate in plasma is not affected by concentration. *Journal of Nutritional Biochemistry*, 10:146-150.

Bhagwat, S. & Haytowitz, D.B. 2015. *USDA database for the flavonoid content of selected foods, Release 3.2*. U.S. Department of Agriculture, Agricultural Research Service. Nutrient Data Laboratory.

Bhagwat, S., Haytowitz, D.B. & Holden, J.M. 2014. *USDA database for the flavonoid content of selected foods, Release 3.1*. U.S. Department of Agriculture, Agricultural Research Service. Nutrient Data Laboratory.

Bouayed, J. & Bohn, T. 2010. Exogenous antioxidants - double-edged swords in cellular redox state. *Oxidative Medicine and Cellular Longevity*, 3(4):228-237.

Boynton, P.M. & Greenhalgh, T. 2004. Selecting, designing and developing your questionnaire. *British Medical Journal,* 328:1312-1315.

Bramati, L., Aquilano, F. & Pietta, P. 2003. Unfermented rooibos tea: quantitative characterization of flavonoids by HPLC−UV and determination of the total antioxidant activity. *Journal of Agricultural and Food Chemistry*, 51(25):7472-7474.

Breiter, T., Laue, C., Kressel, G., Gröll, S., Engelhardt, U.H. & Hahn, A. 2011. Bioavailability and antioxidant potential of rooibos flavonoids in humans following the consumption of different rooibos formulations. *Food Chemistry*, 128:338-347.

Brown, C.M., Dulloo, A.G., Yepuri, G. & Montani, J. 2008. Fructose ingestion acutely elevates blood pressure in healthy young humans. *American Journal of Physiology*, 294(3):R730-R737.

Brunso, K., Fjord, T.A. & Grunert, K.G. 2002. *Consumers' food choice and quality perception*. https://www.researchgate.net/profile/Klaus_G_Grunert/publication/5092936_Consumer's_food_choice_and_quality_perception/links/00b7d5256e24280d1e000000.pdf [14 Janaury 2016].

Bryan, J., Tuckey, M., Einöther, S.J.L., Garczarek, U., Garrick, A. & de Bruin, E.A. 2012. Relationships between tea and other beverage consumption to work performance and mood. *Appetite,* 58:339-346.

Cachofeiro, V., Goicochea, M., de Vinuesa, S.G., Oubiña, P., Lahera, V. & Luño, J. 2008. Oxidative stress and inflammation, a link between chronic kidney disease and cardiovascular disease. *Kidney International*, 74(111):4S-9S.

Campanella, L., Bonanni, A. & Tomasetti, M. 2003. Determination of the antioxidant capacity of samples of different types of tea, or of beverages based on tea or other herbal products, using a superoxide dismutase biosensor. *Journal of Pharmaceutical and Biomedical Analysis*, 32:725-736.

Cao, G., Alessio, H.M. & Cutler, R.G. 1993. Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biology and Medicine*, 14(3):303-311.

Catterall, F., Kassimi, A.I., Cliffort, M.N. & Ioannides, C. 2003. Influence of milk on the antimutagenic potential of green and black teas. *Anticancer Research*, 23(5A):3863-3867.

Cheong, W.J., Park, M.H., Kang, G.W., Ko, J.H. & Seo, Y.J. 2005. Determination of catechin compounds in Korean green tea infusions under various extraction conditions by high performance liquid chromatography. *Bulletin of the Korean Chemical Society*, 26(5):747-754.

Chepulis, L.M. 2007. The effect of honey compared to sucrose, mixed sugars, and a sugar-free diet on weight gain in young rats. *Journal of Food Science*, 27(3):224S-229S.

Cohen, L., Manion, L. & Morrison, K. 2000. *Research methods in education.* 5th ed. London: Routledge Falmer.

Coimbra, S., Santos-Silva, A., Rocha-Pereira, P., Rocha, S. & Castro, E. 2006. Green tea consumption improves plasma lipid profiles in adults. *Nutrition Research*, 26:604-607.

Costa, A.S.G., Nunes, M.A., Almeida, I.M.C., Carvalho, M.R., Barroso, M.F. & Alves, R.C. 2012. Teas, dietary supplements and fruit juices: a comparative study regarding antioxidant activity and bioactive compounds. *Food Science and Technology*, 49:324-328.

Da Silva, P.M., Gauche, C., Gonzaga, L.V., Costa, A.C.O. & Fett, R. 2016. Honey: chemical composition, stability and authenticity. *Food Chemistry*, 196:309-323.

Day, A.J., Rothwell, J.A. & Morgan, R.A. 2004. Characterization of polyphenol metabolites. In Bao, Y. & Fenwick, R. (eds). *Phytochemicals in health and disease*. New York: Marcel Dekker.

De Beer, D., Joubert, E., Viljoen, M. & Manley, M. 2011. Enhancing aspalathin stability in rooibos (*Aspalathus linearis*) ready-to-drink iced teas during storage: the role of nano-emulsification and beverage ingredients, citric and ascorbic acids. *Journal of the Science of Food and Agriculture*, 92(2):274-282.

De Godoy, R.C.B., Deliza, R., Gheno, L.B., Licodiedoff, S., Frizon, C.N.T., Ribani, R.H. & dos Santos, G.G. 2013. Consumer perceptions, attitudes and acceptance of new and traditional mate tea products. *Food Research International*, 53:801-807.

Dragland, S., Senoo, H., Wake, K., Holte, K. & Blomhoff, R. 2003. Several culinary and medicinal herbs are important sources of dietary antioxidants. *Journal of Nutrition*, 133:1286-1290.

Dreosti, I.E. 2000. Antioxidant polyphenols in tea, cocoa, and wine. *Nutrition*, 16(7/8):692-694.

Dubeau, S., Samson, G. & Tajmir-Riahi, H. 2010. Dual effect of milk on the antioxidant capacity of green, Darjeeling, and English breakfast teas. *Food Chemistry*, 122:539-545.

Dufresne, C.J. & Farnworth, E.R. 2001. A review of latest research findings on the health promotion properties of tea. *Journal of Nutritional Biochemistry*, 12:404-421.

Ellinger, S., Müllera, N., Stehlea, P. & Ulrich-Merzenichb, F. 2011. Consumption of green tea or green tea products: is there an evidence for antioxidant effects from controlled interventional studies? *Phytomedicine*, 18:903-915.

Erejuwa, O.O., Sulaiman, S.A. & Wahab, M.S.A. 2012. Fructose might contribute to the hypoglycemic effect of honey. *Molecules,* 17:1900-1915.

Erickson, L. 2003. Rooibos tea: research into antioxidant and antimutagenic properties. *Herbal Gram*, 59:34-45.

Fernández-Garcia, E., Carvajal-Lérida, I., Jarén-Galán, M., Garrido-Fernández, J., Pérez-Gálvez, A. & Hornero-Méndez, D. 2012. Carotenoids bioavailability from foods: from plant pigments to efficient biological activities. *Food Research International*, 46:438-450.

Ferrari, C.K.B. & Torres, E.A.F.S. 2003. Biochemical pharmacology of functional foods and prevention of chronic diseases of aging. *Biomedicine & Pharmacotherapy*, 57:251-260.

Floegel, A., Kim, D., Chung, S., Koo, S.I. & Chun, O.K. 2011. Comparison of ABTS / DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *Journal of Food Composition and Analysis*, 24(7):1043-1048.

Flora, S.J.S. 2009. Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure. *Oxidative Medicine and Cellular Longevity*, 2(4):191-206.

Garcia-Closas, R., Berenguer, A., Tormo, M.J., Sanchez, M.J., Quiros, J.R., Navarro, C., Arnaud, R., Dorronsoro, M., Chirlaque, M.D., Barricarte, A., Ardanaz, E., Amiano, P., Martinez, C., Agudo, A. & Gonzalez, C.A. 2004. Dietary sources of vitamin C, vitamin E and specific carotenoids in Spain. *British Journal of Nutrition*, 91:1005-1011.

Geleijnse, J.M., Launer, L.J., Hofman, A. & Pols, H.A.P. 1999. Tea flavonoids may protect against atherosclerosis – the Rotterdam study. *Archives of International Medicine*, 159:2170-2174.

Geleijnse, J.M., Launer, L.J., van der Kuip, D.A.M., Hofman, A. & Witteman, J.C.M. 2002. Inverse association of tea and flavonoid intakes with incident myocardial infarction: the Rotterdam Study. *American Journal of Clinical Nutrition*, 75:880-886.

Graham, H.N. 1992. Green tea composition, consumption and polyphenol chemistry. *Preventive Medicine*, 21(3):334-350.

Grobler, S.R., Du Toit, I.J. & Basson, N.J. 1994. The effect of honey on human tooth enamel *in vitro* observed by electron microscopy and micro hardness measurements. *Archives of Oral Biology*, 39(2):147-153.

Guo, C., Yang, J., Wei, J., Li, Y., Xu, J. & Jiang, Y. 2003. Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. *Nutrition Research*, 23:1719-1726.

Hakim, I.A., Alsaif, M.A., Alduwaihy, M., Al-Rubeaan, K., Al-Nuaim, A.R. & Al-Attas, O.S. 2003. Tea consumption and the prevalence of coronary heart disease in Saudi adults: results from a Saudi national study. *Preventative Medicine*, 36(1):64-70.

Hakim, I.A., Weisgerber, U.M., Harris, R.B., Balentine, D., van-Mierlo, C.A.J. & Paetau-Robinson, I. 2000. Preparation, composition and consumption patterns of tea-based beverages in Arizona. *Nutrition Research*, 20(12):1715-1724.

Halliwell, B. 1994. Free radicals, antioxidants, and human disease curiosity, cause, or consequence? *Lancet*, 344:721-724.

Hamarat, E., Thompson, D., Zabrucky, K.M., Steele, D., Matheny, K.B. & Aysan, F. 2001. Perceived stress and coping resource availability as predictors of life satisfaction in young, middle-aged, and older adults. *Experimental Aging Research*, 27(2):181-196.

Hein, K.A., Jaeger, S.R., Carr, B.T. & Delahunty, C.M. 2008. Comparison of five common acceptance and preference methods. *Food Quality and Preference*, 19:651-661.

Hertog, M.G.L., Hollman, P.C.H. & van de Putte, B. 1993. Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. *Journal of Agricultural and Food Chemistry*, 41(8):1242-1246.

Hertog, M.G., Sweetnam, P.M., Fehily, A.M., Elwood, P.C. & Kromhout, D. 1997. Antioxidant flavonols and ischemic heart disease in a Welsh population of men: the Caerphilly Study. *The American Journal of Clinical Nutrition*, 65(5):1489-1494.

Hilal, Y. & Engelhardt, U. 2007. Characterisation of white tea – comparison to green and black tea. *Journal of Consumer Protection and Food Safety*, 2:414-421.

Hodgson, J.M. & Croft, K.D. 2010. Tea flavonoids and cardiovascular health. *Molecular Aspects of Medicine*, 31:495-502.

Hollman, P.C., van Het Hof, K.H., Tijburg, L.B. & Katan, M.B. 2001. Addition of milk does not affect the absorption of flavonols from tea in man. *Free Radical Research*, 34(3):297-300.

Hopkins, W.G. 2000. Quanititative research designs. *Sportscience*, 4(1):1-10.

Jéquier, E. & Constant, F. 2010. Water as an essential nutrient: the physiological basis of hydration. *European Journal of Clinical Nutrition*, 64:115-123.

Ji, B., Chow, W., Hsing, A.W., McLaughlin, J.K., Dai, Q., Gao, Y., Blot, W.J. & Fraumeni, J.F. 1997. Green tea consumption and the risk of pancreatic and colorectal cancers. *International Journal of Cancer*, 70:255-258.

Joubert, E. 1996. HPLC quantification of the dihydrochalcones, aspalathin and nothofagin in rooibos tea (*Aspalathus linearis*) as affected by processing. *Food Chemistry*, 55(4):403-411.

Joubert, E. & de Beer, D. 2011. Rooibos (*Aspalathus linearis*) beyond the farm gate: from herbal tea to potential phytochemical. *South African Journal of Botany*, 77:869-886.

Joubert, E. & de Beer, D. 2012. Phenolic content and antioxidant activity of rooibos food ingredient extracts. *Journal of Food Composition and Analysis*, 27:45-51.

Joubert, E. & Ferreira, D. 1996. Antioxidants of rooibos tea – a possible explanation for its health promoting properties? *South African Journal of Food Science and Nutrition*, 8(3):79-83.

Joubert, E., Gelderblom, W.C.A., Louw, A. & de Beer, D. 2008. South African herbal teas: *Aspalathus linearis, Cyclopia spp.* and *Athrixiaphylicoides* - a review. *Journal of Ethnopharmacology*, 119(3): 376-412.

Joubert, E., Joubert, M.E., Bester, C., de Beer, D. & de Lange, J.H. 2011. Honeybush (*Cyclopia spp.*): from local cottage industry to global markets – the catalytic and supporting role of research. *South African Journal of Botany*, 77:887-907.

Joubert, E. & Schulz, H. 2006. Production and quality aspects of rooibos tea and related products - a review. *Journal of Applied Botany and Food Quality*, 80:138-144.

Joubert, E., Viljoen, M., de Beer, D. & Manley, M. 2009. Effect of heat on aspalathin, iso-orientin, and orientin contents and color of fermented rooibos (*Aspalathus linearis*) iced tea. *Journal of Agricultural Food Chemistry, 57*(10):4204-4211.

Joubert, E., Winterton, P., Britz, T.J. & Ferreira, D. 2004. Superoxide anion and an a-diphenyl-b-picrylhydrazyl radical scavenging capacity of rooibos (*Aspalathus linearis*) aqueous extracts, crude phenolic fractions, tannin and flavonoids. *Food Research International*, 37:133-138.

Karadag, A., Ozcelik, B. & Saner, S. 2009. Review of methods to determine antioxidant capacities. *Food Analytical Methods*, 2:41-60.

Kemp, S.E., Hollowood, T. & Hort, J. 2009. *Sensory evaluation: a practical handbook*. 1st ed. Chichester: Wiley-Blackwell.

King, A. & Young, G. 1999. Characteristics and occurance of phenolic phytochemicals. *Journal of the American Dietetic Association*, 99:213-218.

Koch, I.S., Muller, N., de Beer, D., Naes, T. & Joubert, E. 2013. Impact of steam pasteurization on the sensory profile and phenolic composition of rooibos (*Aspalathus linearis*) herbal tea infusions. *Food Research International*, 53:704-712.

Koch, I.S., Muller, M., Joubert, E., van der Rijst, M. & Naes, T. 2012. Sensory characterization of rooibos tea and the development of a rooibos sensory wheel and lexicon. *Food Research International*, 46:217-228.

Komes, D., Horzic D., Belscak, A., Ganic, K.K. & Vulic, I. 2010. Green tea preparation and its influence on the content of bioactive compounds. *Food Research International*, 43:167-176.

Kotur-Stevuljevic, J., Memon, L., Stefanovic, A., Spasic, S., Spasojevic-Kalimanovska, V., Bogavac-Stanojevic, N., Kalimanovska-Ostric, D., Jelic-Ivanovic, Z. & Zunic, G. 2007. Correlation of oxidative stress parameters and inflammatory markers in coronary artery disease patients. *Clinical Biochemistry*, 40(3-4):181-187.

Kretowicz, M., Johnson, R.J., Ishimoto, T., Nakagawa, T. & Manitius, J. 2011. The impact of fructose on renal function and blood pressure. *International Journal of Nephrology*, 2011:1-5.

Langenhoven, M.L., Conradie, P.J., Wolmarans, P. & Faber, M. 1991*. MRC food quantities manual.* 2nd ed. Parow: Medical Research Council, National Research Programme for Nutritional Intervention.

Langley-Evans, S. 2000. Consumption of black tea elicits an increase in plasma antioxidant potential in humans. *International Journal of Food Sciences and Nutrition*, 51(5):309-315.

Langley-Evans, S. 2001. Tea as a protective agent in cardiovascular health. *Nutrition and Food Science*, 31(2):75-78.

Larsson, S.J., Virtamo, J. & Wolk, A. 2013. Black tea consumption and risk of stroke in women and men. *Annals of Epidemiology*, 23:157-160.

Lee, E. & Jang, H. 2004. Antioxidant activity and protective effect on DNA strand scission of Rooibos tea (*Aspalathus linearis*). *BioFactors*, 21:285-292.

Leedy, P.D. & Ormrod, J.E. 2010. *Practical research: planning and design.* New Jersey: Pearson Education.

Leenen, R., Roodenberg, A.J., Tijburg, L.B. & Wiseman, S.A. 2000. A single dose of tea with or without milk increases plasma antioxidant activity in humans. *European Journal of Clinical Nutrition*, 54(1):87-92.

Lehola, P. 2012. *Census 2011 Municipal report, Western Cape*. Pretoria: Statistics South Africa.

Li, Y., Tanner, G. & Larkin, P. 1996. The DMACA-HCl protocol and the threshold procanthocyanidin content for bloat safety in forage legumes. *Journal of the Science of Food and Agriculture*, 70:89-101.

Liu, R.H. 2004. Potential synergy of phytochemicals in cancer prevention: mechanism of action. *Journal of Nutrition*, 134(12):3479S-3485S.

Livesey, G. 2009. Fructose ingestion: dose-dependent responses in health research. *Journal of Nutrition*, 139:1246S-1252S.

Louwrens, H., Rautenbach, F. & Venter, I. 2009. South African dietary total antioxidant capacity based on secondary intake data. *South African Journal of Clinical Nutrition*, 22(4):195-202.

MacDonald-Wicks, L.K., Wood, L.G. & Garg, M.L. 2006. Methodology for the determination of biological antioxidant capacity *in vitro*: a review. *Journal of the Science of Food and Agriculture,* 86:2046-2056.

Magalhaes, L.M., Segundo, M.A., Reis, S. & Lima, J.L.F.C. 2008. Methodological aspects about *in vitro* evaluation of antioxidant properties. *Analytica Chimica Acta,* 613:1-19.

Majchrzak, D., Mitter, S. & Elmadfa, I. 2004. The effect of ascorbic acid on total antioxidant activity of black and green teas. *Food Chemistry*, 88:447-451.

Malik, J., Szakova, J., Drabek, O., Balik, J. & Koskoska, L. 2008. Determination of certain micro and macro elements in plant stimulants and their infusions. *Food Chemistry*, 111(2):520-525.

Manach, C., Scalbert, A., Morand, C., Rémésy, C. & Jimenez, L. 2004. Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition*, 79:727-747.

Marnewick, J.L., Gelderblom, W.C.A. & Joubert, E. 2000. An investigation on the antimutagenic properties of South African herbal teas. *Mutation Research*, 471:157-166.

Marnewick, J.L., Joubert, E., Swart, P., van der Westhuizen, F. & Gelderblom, W.C. 2003. Modulation of hepatic drug metabolizing enzymes and oxidative stress by rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia intermedia*), green and black (*Camellia sinensis*) teas in rats. *Journal of Agricultural and Food Chemistry*, 51:8113-8119.

Marnewick, J.L., Rautenbach, F., Venter, I., Neethling, H., Blackwurst, D.M., Wolmarans, P. & Macharia, M. 2011. Effects of rooibos (*Aspalathus linearis*) on oxidative stress and biochemical parameters in adults at risk for cardiovascular disease. *Journal of Ethnopharmacology*, 133:46-52.

Marnewick, J.L., van der Westhuizen, F.H., Joubert, E., Swanevelder, S., Swart, P. & Gelderblom, W.C.A. 2009. Chemoprotective properties of rooibos (*Aspalathus linearis*), honeybush (*Cyclopia intermedia*) herbal and green and black (*Camellia sinensis*) teas against cancer promotion induced by fumonisin B1 in rat liver. *Food and Chemical Toxicology*, 47:220-229.

Marriot, B.M. 2000. Functional foods: an ecologic perspective. *American Journal of Clinical Nutrition*, 71:1728S-1734S.

Marshall, G. 2005. The purpose, design and administration of a questionnaire for data collection. *Radiography*, 11:131-136.

Mazza, G., Fukumoto, L., Delaquis, P., Girard, B. & Ewert, B. 1999. Anthocyanins, phenolics and colour in cabernet franc, merlot and pino noir wines from British Columbia. *Journal of Agricultural and Food Chemistry*, 47:4009-4017.

McCord, J.M. 2000.The evolution of free radicals and oxidative stress. *American Journal of Medicine*, 108:652-659.

Mukamal, K.J., Malcolm Maclure, M., Muller, J.E., Sherwood, J.B. & Mittleman, M.A. 2002. Tea consumption and mortality after acute myocardial infarction. *Circulation*, 105:2476-2481.

Mukhtar, H. & Ahmad, N. 1999. Green tea in chemoprevention of cancer. *Toxicology Sciences*, 52:111S-117S.

Murray, P. 1999. Fundamental issues in questionnaire design. *Accident and Emergency Nursing*, 7:148-153.

Nagel, C.W. & Glories, Y. 1991. Use of a modified dimethylaminocinnamaldehyde reagent for analysis of flavanols. *American Journal of Enology and Viticulture,* 42:364-366.

Nakayama, T., Kosugi, T., Gersch, M., Connor, T., Sanchez-Lozada, L.G., Lanaspa, M.A., Roncal, C., Perez-Pozo, S.E., Johnson, R.J. & Nakagawa, T. 2010. Dietary fructose causes tubulointerstitial injury in the normal rat kidney. *American Journal of Physiology*, 298(3):F712-F720.

Nalsén, C., Basu, S., Wolk, A. & Vessby, B. The importance of dietary antioxidants on plasma antioxidant capacity and lipid peroxidation in vivo in middle-aged men. *Scandinavian Journal of Food and Nutrition,* 50(2):64-70.

Nel, J.H. & Steyn, N.P. 2002. *Report on South African food consumption studies undertaken amongst different population groups (1983-2000): Average intakes of foods most commonly consumed*. Pretoria: Department of Health.

Nemoseck, T.M., Carmody, E.G., Furchner-Evanson, A., Gleason, M., Li, A., Potter, H., Rezende, L.M., Lane, K.J. & Mark, K. 2011. Honey promotes lower weight gain, adiposity, and triglycerides than sucrose in rats. *Nutrition Research*, 31(1):55-60.

Nikolova, V., Petrova, S., Petkova, V., Pavlova, A. & Georgieva, T. 2007. Antioxidant effects of rooibos tea on workers occupationally exposed to lead. *Toxicology Letters*, 172:120S-121S.

Okamoto, Y., Kihara, S., Ouchi, N., Nishida, M., Arita, Y., Kumada, M., Ohashi, K., Sakai, N., Shimomura, I., Kobayashi, H., Terasaka, N., Inaba, T., Funahashi, T. & Matsuzawa, Y. 2002. Adiponectin reduces atherosclerosis in apolipoprotein e-deficient mice. *Circulation*, 106:2767-2770.

Olderwage-Theron, W. & Kruger, R. 2011. Dietary diversity and adequacy of women caregivers in a peri-urban informal settlement in South Africa. *Nutrition*, 27(4):420-427.

Olivier, J., Symington, E.A., Jonker, C.Z., Rampedi, I.T. & van Eeden, T.S. 2012. Comparison of the mineral composition of leaves and infusions of traditional and herbal teas. *South African Journal of Science*, 108(1/2):1-7.

Ortiz, G.G., Pacheco-Moisés, F.P., Bitzer-Quintero, O.K., Ramírez-Anguiano, A.C., Flores-Alvarado, L.J., Ramírez-Ramírez, V., Macias-Islas, M.A. & Torres-Sánchez, E.D. 2013. Immunology and oxidative stress in multiple sclerosis: clinical and basic approach. *Clinical and Developmental Immunology*, 2013:1-14.

Ou, B., Hampsch-Woodmill, M. & Prior, R.L. 2001. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent. *Journal of Agricultural and Food Chemistry*, 49:4619-4626.

Ouyang, X., Cirillo, P., Sautin, Y., McCall, S., Bruchette, J.L., Diehl, A.M., Johnson, R.J. & Abdelmalek, M.F. 2008. Fructose consumption as a risk factor for non-alcoholic fatty liver disease. *Journal of Hepatology*, 48(6):993-999.

Packer, L. & Colman, C. 1999. *The antioxidant miracle*. New York: John Wiley.

Pan, M., Lai, C., Wang, H., Lo, C., Ho, C. & Li, S. 2013. Black tea in chemo-prevention of cancer and other human diseases*. Food Science and Human Wellness,* 2:12-21.

Pantsi, W.G., Marnewick, J.L., Esterhuyse, A.J., Rautenbach, F. & van Rooyen, J. 2011. Rooibos (*Aspalathus linearis*) offers cardiac protection against ischaemia / reperfusion in the isolated rat heart. *Phytomedicine*, 18:1220-1228.

Pekal, A., Dro'zdz, P., Biesaga, M. & Pyrzynska, K. 2011. Evaluation of the antioxidant properties of fruit and flavoured black teas. *European Journal of Nutrition*, 50:681-688.

Pekal, A., Dro'zdz, P., Biesaga, M. & Pyrzynska, K. 2012. Polyphenolic content and comparative antioxidant capacity of flavoured black teas*. International Journal of Food Sciences and Nutrition*, 63(6):742-748.

Pellegrini, N., Serafini, M., Colombi, B., Del Rio, D., Salvatore, S., Bianchi, M. & Brighenti, F. 2003. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. *Journal of Nutrition*, 133(9):2812-2819.

Pereira, C., Barros, L., Vilas-Boas, M. & Ferreira, I.C.F.R. 2013. Potentiating effects of honey on antioxidant properties of lemon-flavoured black tea. *International Journal of Food Sciences and Nutrition*, 64(2):230-234.

Pérez-Jiménez, J., Arranz, S., Tabernero, M., Diaz-Rubio, M., Serrano, J., Goni, I. & Saura-Calixto, F. 2008. Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: extraction, measurement and expression of results. *Food Research International*, 41(3):274-285.

Persson, I.A.L., Persson, K., Hägg, S. & Anderson R.G.G. 2010. Effects of green tea, black tea and rooibos tea on angiotensin-converting enzyme and nitric oxide in healthy volunteers. *Public Health Nutrition*, 13(5):730-737.

Peterson, J., Dwyer, J., Bhagwat, S., Haytowitz, D., Holden, J., Eldridge, A.L., Beecher, G. & Aladdesanmi, J. 2005. Major flavonoids in dry tea. *Journal of Food Composition and Analysis*, 18:487-501.

Peterson, J., Dwyer, J., Jacques, P., Rand, W., Prior, R. & Chui, K. 2004. Tea variety and brewing techniques influence flavonoid content of black tea. *Journal of Food Composition and Analysis*, 17:397-405.

Phillips, K.M., Carlsen, M.H. & Blomhoff, R. 2009. Total antioxidant content of alternatives to refined sugar. *Journal of the American Dietetic Association*, 109(1):64-71.

Popkin, B.M., Armstrong, L.E., Bray, G.M., Caballero, B., Frei, B. & Willett, W.C. 2006. A new proposed guidance system for beverage consumption in the United States. *American Journal of Clinical Nutrition*, 83:529-542.

Prior, R.L., Wu, X. & Schaich, K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, 53(10):4290-4302.

Radak, Z., Chung, H.Y. & Goto, S. 2008. Systematic adaptation to oxidative challenge induced by regular exercise. *Free Radical Biology & Medicine*, 44:153-159.

Rattray, J. & Jones, M.C. 2007. Essential elements of questionnaire design and development. *Journal of Clinical Nursing*, 16:234-243.

Rautenbach, F. 2016. Personal communication, 10 March.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. & Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26(9/10):1231-1237.

Reed, D.R., Tanaka, T. & McDaniel, A.H. 2006. Diverse tastes: genetics of sweet and bitter perception. *Physiology & Behavior*, 215-226.

Rooibos Ltd. 2011. *Rooibos extracts*. http://www.rooibosltd.co.za/rooibos-extracts.php [15 January 2016].

Rubin, T. 2010. Make a splash with rooibos tea. *Better Nutrition*: February, 48-49.

Ryan, L. & Petit, S. 2010. Addition of whole, semi-skimmed, and skimmed bovine milk reduces the total antioxidant capacity of black tea. *Nutrition Research*, 30:14-20.

Ryan, L. & Sutherland, S. 2011. Comparison of the effect of different types of soya milk on the total antioxidant capacity of black tea infusions. *Food Research International*, 44(9):3115-3117.

Salah, N., Miller, N.J., Paganga, G., Tijburg, L., Bolwell, G.P. & Rice-Evans, C. 1995. Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. *Archives of Biochemistry and Biophysics*, 322(2):339-346.

Sampson, L., Rimm, E., Hollman, P.C.H., de Vries, J.H.M. & Katan, M.B. 2002. Flavonol and flavone intakes in US health professionals. *Journal of the American Dietetic Association*, 102(10):1414-1420.

Savage, G.P., Charrier, M.J. & Vanhanen, L. 2003. Bioavailability of soluble oxalate from tea and the effect of consuming milk with the tea. *European Journal of Clinical Nutrition*, 57(3):415-419.

Scalbert, A., Manach, C., Morand, C., Rémésy, C. & Jiménez, L. 2005. Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Science and Nutrition*, 45(4):287-306.

Scalbert, A. & Williamson, G. 2000. Dietary intake and bioavailability of polyphenols. *Journal of Nutrition*, 130:2073S-2085S.

Schwalfenberg, G., Genuis, S.J. & Rodushkin, I. 2013. The benefits and risks of consuming brewed tea: beware of toxic element contamination. *Journal of Toxicology*, 2013:1-8.

Serafini, M. 2006. The role of antioxidants in disease prevention. *Medicine*, 34(12):33-535.

Sharma, V., Kumar, H.V. & Rao, L.J.M. 2008. Influence of milk and sugar on antioxidant potential of black tea. *Food Research International*, 41:124-129.

Sharpe, E., Hua, F., Schuckers, S., Andreescu, S. & Bradley, R. 2016. Effects of brewing conditions on the antioxidant capacity of twenty-four commercial green tea varieties. *Food Chemistry*, 192:380-387.

Shimada, K., Kawarabayashi, T., Tanaka, A., Fukuda, D., Nakamura, Y., Yoshiyama, M., Takeuchi, K., Sawaki, T., Hosoda, K. & Yoshikawa, J. 2004. Oolong tea increases plasma adiponectin levels and low-density lipoprotein particle size in patients with coronary artery disease. *Diabetes Research and Clinical Practice*, 65:227-234.

Sies. H. 2007. Total antioxidant capacity: appraisal of a concept. *Journal of Nutrition*, 137(6):1493-1495.

Sies, H. 1997. Oxidative stress: oxidants and antioxidants. *Experimental Physiology*, 82:291-295.

Singleton, V.L. & Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16:144-158.

Sissing, L., Marnewick, J., de Kock, M., Swanevelder, S., Joubert, E. & Gelderblom, W. 2011. Modulating effects of rooibos and honeybush herbal teas on the developing of esophageal papillomas in rats. *Nutrition and Cancer*, 63(4):600-610.

Snijman, P.W., Swanevelder, S., Joubert, E., Green, I.R. & Gelderblom, W.C.A. 2007. The antimutagenic activity of the major flavonoids of rooibos (*Aspalathus linearis*): some dose–response effects on mutagen activation–flavonoid interactions. *Mutation Research / Genetic Toxicology and Environmental Mutagenesis*, 631(2):111-123.

Song, W.O. & Chun, O.K. 2008. Tea is the major source of flavan-3-ol and flavonol in the US diet. *Journal of Nutrition*, 138:1543S-1547S.

South Africa. Department of Agriculture, Forestry and Fisheries. 2012. *A profile of the South African rooibos tea market value chain*. Pretoria: Government Printer.

South Africa. Department of Agriculture, Forestry and Fisheries. 2015. *Regulations relating to the classification, packing and marking of dairy products and imitation dairy products intended for sale in the Republic of South Africa.* Pretoria: Government Printer.

Stanimirova, E., Kazura, M., de Beer, D., Joubert, E., Schulze, A.E., Beelders, T., de Villiers, A. & Walczak, B. 2013. High-dimensional nested analysis of variance to assess the effect of

production, season, quality grade and steam pasteurization on the phenolic composition of fermented rooibos herbal tea. *Talanta*, 115:590-599.

Storey, M.L., Forshee, R.A. & Anderson, P.A. 2006. Beverage consumption in the US population. *Journal of the American Dietetic Association*, 106:1992-2000.

Surh, Y. & Packer, L. 2005. *Oxidative stress, inflammation and health*. Florida: Taylor & Francis.

Tang, N., Wu, Y., Zhou, B., Wang, B. & Yu, R. 2009. Green tea, black tea consumption and risk of lung cancer: a meta-analysis. *Lung Cancer*, 65:274-283.

Tiedtke, J. & Marks, O. 2002. Rooibos – the new 'white tea' for hair and skin care. *Euro Cosmetics*, 6:16-19.

Toydemir, G., Capanoglu, E., Kamiloglu, S., Firatligil-Durmus, E., Sunay, A.E., Samanci, T. & Boyacioglu, D. 2015. Effects of honey addition on antioxidative properties of different herbal teas. *Polish Journal of Food and Nutrition Sciences*, 65(2):127-135.

Tsuji, P.A., Stephenson, K.K., Wade, K.L., Liu, H. & Fahey, J.W. 2013. Structure-activity analysis of flavonoids: direct and indirect antioxidant, and anti-inflammatory potencies and toxicities. *Nutrition and Cancer*, 65(7):1014-1025.

Tydeman-Edwards, R. 2012. *Obesity, undernutrition and the double burden of disease in the Free State*. Unpublished Magister Scientiae Dietetics dissertation, University of the Free State, Bloemfontein.

United States of America. Food and Drug Association. 2009. *Guidance for industry: A food labeling guide*.http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/LabelingNutrition/ucm064923.html [3 June 2013].

United States of America. Food and Drug Association. 2013. *Guidance for Industry: A Food Labeling Guide* (12. Appendix D: Qualified Health Claims). http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/LabelingNutrition/ucm064923.html [3 June 2013].

Van Graan, A.E., Bopape, M., Phooko, D., Bourne, L. & Wright, H.H. 2013. "Drink lots of clean, safe water": a food-based dietary guideline for South Africa. *South African Journal of Clinical Nutrition*, 26(3):77S-86S.

Van Heerden, F.R., van Wyk, B.E., Viljoen, A.M. & Steenkamp, P.A. 2003. Phenolic variation in wild populations of *Aspalathus linearis* (rooibos tea). *Biochemical Systematics and Ecology*, 31:885-895.

Van Het Hof, K.A., Kivits, G.A.A., Weststrate, J.A. & Tijburg, L.B.M. 1998. Bioavailability of catechins from green tea: the effect of milk. *European Journal of Clinical Nutrition*, 52:356-359.

Venditti, E., Bacchetti, T., Tiano, L., Carloni, P., Greci, L. & Damiani, E. 2010. Hot vs. cold water steeping of different teas: do they affect antioxidant acitivity? *Food Chemistry*, 119:1597-1604.

Venter, I., Nel, D., Herselman, M. & Marnewick, J.L. 2013. *Development and evaluation of a food frequency questionnaire to assess daily total flavonoid intake using a rooibos intervention study model*. Unpublished PhD dissertation, Stellenbosch University, Stellenbosch.

Villano, D., Lettieri-Barbato, D., Guadagni, F., Schmid, M. & Serafini, M. 2012. Effect of acute consumption of oolong tea on antioxidant parameters in healthy individuals. *Food Chemistry*, 132:2102-2106.

Villano, D., Pecorari, M., Testa, M.F., Raguzzini, A., Stalmach, A., Crozier, A., Tubili, C. & Serafini, M. 2010. Unfermented and fermented rooibos tea (*Aspalathus linearis*) increase plasma total antioxidant capacity in healthy humans. *Food Chemistry*, 123:679-683.

Von Gadow, A., Joubert, E. & Hansmann, C.F. 1997a. Effect of extraction time and additional heating on the antioxidant activity of rooibos tea (*Aspalathus linearis*) extracts. *Journal of Agricultural and Food Chemistry*, 45(4):1370-1374.

Von Gadow, A., Joubert, E. & Hansmann, C.F. 1997b. Comparison of the antioxidant acitivity of rooibos tea (*Aspalathus linearis*) with green, oolong and black tea. *Food Chemistry*, 60(1):73-77.

Walcott, S.M. 2012. Brewing a new American tea industry. *Geographical Review,* 102(3):350-363.

Wiernsperger, N.F. 2003. Oxidative stress as a therapeutic target in diabetes: revisiting the controversy. *Diabetes & Metabolism*, 29(6):579-585.

Willcox, J.K., Ash, S.L. & Catignani, G.L. 2004. Antioxidants and prevention of chronic disease. *Critical Reviews in Food Science and Nutrition*, 44:275-295.

Willet, W. 1998. *Nutritional epidemiology*. 2nd ed. New York: Oxford University Press.

Wu, C.D. & Wei, G. 2002. Tea as a functional food for oral health. *Nutrition*, 18:443-444.

Yang, C.S. & Hong, J. 2013. Prevention of chronic diseases by tea: possible mechanisms and human relevance. *Annual Review of Nutrition,* 33:161-181.

Yang, Y., Lu, F., Wu, J., Wu, C. & Chang, C. 2004. The protective effect of habitual tea consumption on hypertension. *Archives of Internal Medicine*, 164:1534-1540.

Yao, L.H., Jiang, Y.M., Shi, J., Thomás-Barberán, F.A., Datta, N., Singanusong, R. & Chen, S.S. 2004. Flavonoids in food and their health benefits. *Plant Foods for Human Nutrition*, 59:113-122.

Young, I.S. & Woodside, J.V. 2001. Antioxidants in health and disease. *Journal of Clinical Pathology*, 54:176-186.

Yu, B.P. 1996. Aging and oxidative stress: modulation by dietary restriction. *Free Radical Biology and Medicine*, 21(5):651-668.

Yusuf, S., Hawken, S., Ôunpuu, S., Dans, T., Avezum, A., Lanas, F., McQueen, M., Budaj, A., Pais, P., Varigos, J. & Lisheng, L., on behalf of the INTERHEART Study Investigators. 2004. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet*, 364:937-952.

Zhang, D., Liu, Y., Chu, L., Wei, Y., Wang, D., Cai, S., Zhou, F. & Ji, B. 2013. Relationship between the structures of flavonoids and oxygen radical absorbance capacity values: a quantum chemical analysis. *Journal of Physical Chemistry*, 117:1784-1794.

Zulueta, A., Esteven, M.J. & Frigola, A. 2009. ORAC and TEAC assays comparison to measure the antioxidant capacity of food products. *Food Chemistry*, 114:310-316.

Addendum A

Letter to store manager

Cape Peninsula
University of Technology

MEMORANDUM

To:	The Store Manager
From:	Dr I Venter – Supervisor and Senior Lecturer: Department of Agricultural and Food Sciences, Faculty of Applied Sciences, CPUT, Cape Town campus
Date:	25 June 2013
Subject:	Types, forms, flavours and brands of rooibos herbal tea available in supermarkets

I confirm that Mrs Hannelise Piek (CPUT student number 204163633) is a MTech Consumer Science: Food and Nutrition student at the Cape Peninsula University of Technology (CPUT). As part of the requirements to obtain the degree Hannelise needs to conduct a research study. To initiate her research titled *'Effect of rooibos type, form, preparation method and added flavouring on the total polyphenol content and antioxidant capacity of the herbal tea'* she first needs to record the different types, forms, flavours and brands of rooibos herbal tea retail stores has on offer. The project is aimed at determining the effect of the type (traditional or fermented and green or unfermented), form (loose leaved, bagged or powdered), preparation method (brewing time) and flavouring (herbs, milk, sugar, etc.) used on the total polyphenol content and antioxidant capacity of rooibos herbal tea. Based on the recorded availability information of roobos herbal teas, different rooibos herbal tea types, forms, flavours and brands will be purchased across supermarkets for the execution of the project to represent the commonly available types, forms, flavours and brands.

Your assistance in providing her the opportunity to record the required information of the rooibos herbal tea products available in your store will be highly appreciated. The information will only be used for the purpose of this research project. Hannelise will confirm the time points to record the required information considering that best suited to the store activities.

Kind regards

Dr I Venter
Supervisor
Senior lecturer: Programme Consumer Science: Food and Nutrition
Faculty of Applied Sciences: Cape Town campus

Dr S Crafford
Head of Department: Agricultural and Food Sciences
Faculty of Applied Sciences: Cape Town campus

PO Box 652, Cape Town, 8000
Tennant Street, Cape Town

Addendum B

Envisaged brands per sample sourced from two local retail chain supermarkets

The following samples of rooibos herbal teas (n = 2-3 brands depending on the availability of the type / form / flavours) will be randomly sourced from local supermarkets that the average consumer frequents (n = 2 providing for duplicate purchases and samples):

1. Samples to assess different rooibos herbal tea types

1.1 Samples[1] with **traditional / fermented** rooibos herbal tea

Sample 1: Brand A
Sample 2: Brand B
Sample 3: Brand C

[1]Samples will be prepared using the specific brand traditional/fermented rooibos herbal tea bag and prepared according to the specifications of the SA Rooibos Council with 180ml boiling water, stirred for 30 seconds and steeped for three minutes without any additions.

1.2 Samples[1] with **green / unfermented** rooibos herbal tea

Sample 1: Brand A
Sample 2: Supplier A

[1]Samples will be prepared using the specific brand green/unfermented rooibos herbal tea bag and prepared according to the specifications of the SA Rooibos Council with180ml boiling water, stirred for 30 seconds and steeped for three minutes without any additions.

1.3 Samples[1] with **organic traditional / fermented** rooibos herbal tea

Sample 1: Brand D
Control samples from 1.1 Samples 1, 2 and 3 (Brands A, B and C)

[1]Samples will be prepared using the specific brand traditional/fermented rooibos herbal tea bag and prepared according to the specifications of the SA Rooibos Council with 180ml boiling water, stirred for 30 seconds and steeped for three minutes without any additions.

2. Samples to assess different rooibos herbal tea forms

2.1 Samples with traditional / fermented rooibos herbal tea

2.1.1Samples[1] with**traditional / fermented** rooibos herbal tea **bags**

Sample 1: Brand A
Sample 2: Brand B
Sample 3: Brand C

[1]Samples will be prepared using the specific brand traditional/fermented rooibos herbal tea bag and prepared according to the specifications of the SA Rooibos Council with 180ml boiling water, stirred for 30 seconds and steeped for three minutes without any additions.

2.1.2 Samples[1] with **traditional / fermented**rooibos herbal tea **leaves**

Sample 1: Supplier A

[1]Samples will be prepared using the specific brand traditional/fermented rooibos herbal tea leaves and prepared according to the specifications of the SA Rooibos Council with 180ml boiling water, stirred for 30 seconds and steeped for three minutes without any additions.

2.1.3 **Iced teas**[1] with **traditional / fermented**rooibos herbal tea extracts

Sample 1: Brand B
Sample 2: Brand E

[1]Samples will be prepared according to the instructions on the product packaging.

2.1.4 Samples[1] with **traditional / fermented hot water soluble / powdered** rooibos herbal tea

Sample 1: Supplier A

[1]Sample will be prepared using the specific brand traditional/fermented rooibos herbal tea hot water soluble/powdered formper product packaging amount and prepared according to the specifications of the SA Rooibos Council with 180ml boiling water, stirred for 30 seconds and steeped for three minutes without any additions.

2.2 Samples with green / unfermented rooibos herbal tea

2.2.1 Samples[1] with **green / unfermented** rooibos herbal tea **bags**

Sample 1: Brand A
Sample 2: Supplier A

[1]Samples will be prepared using the specific brand green/unfermented rooibos herbal tea bag and prepared according to the specifications of the SA Rooibos Council with 180ml boiling water, stirred for 30 seconds and steeped for three minutes without any additions.

2.2.2 Samples[1] with **green / unfermented** rooibos herbal tea **leaves**

Sample 1: Supplier A

[1]Samples will be prepared using the specific brand green/unfermented rooibos herbal tea leaves and prepared according to the specifications of the SA Rooibos Council with 180ml boiling water, stirred for 30 seconds and steeped for three minutes without any additions.

2.2.3 **Iced teas**[1]with **green/unfermented**rooibos herbal tea extracts

Sample 1: No product currently available in selected supermarkets – will continue to search in other outlets

[1]Samples will be prepared according to the instructions on the product packaging.

2.2.4 Samples[1] with **green / unfermentedhot water soluble / powdered** rooibos herbal tea

Sample 1: Supplier A

[1]Sample will be prepared using the specific brand green/unfermented rooibos herbal tea hot water soluble/powdered formper product packaging amount and prepared according to the specifications of the SA Rooibos Council with 180ml boiling water, stirred for 30 seconds and steeped for three minutes without any additions.

3. Samples[1] to assess <u>different brewing times</u> to consume rooibos herbal tea as beverage

Sample 1: Brand C steeped for 1minutes
Sample 2: Brand C steeped for 5 minutes
Sample 3: Brand C steeped for 10 minutes
Sample 4: Brand F steeped for 1minutes
Sample 5: Brand Fsteeped for 5 minutes
Sample 6: Brand Fsteeped for 10 minutes
Sample 7: Brand G steeped for 1minutes
Sample 8: Brand Gsteeped for 5 minutes
Sample 9: Brand G steeped for 10 minutes
Control samples: Plain traditional/fermented rooibos herbal tea bags [from 1.1 Samples 1, 2 and 3 (Brands A, B and C)].

[1]Samples will be prepared with the specific brand rooibos herbal tea and 180ml boiling water, stirred for 30 seconds and steeped for three minutes the specific additions added after the steeping period.

4. Samples to assess the <u>different flavouring additions and the amounts added</u> to rooibos herbal tea

4.1 <u>Household flavouring</u>of rooibos herbal tea samples[1] in different amounts[2]

Sample 1: Brand C with whole, fresh milk - 10ml
Sample 2: Brand C with whole, fresh milk- 20ml
Sample 3: Brand Cwith whole, fresh milk - 30ml
Sample 4: Brand C with low fat/semi-skimmed/2% fat milk - 10ml
Sample 5: Brand C with low fat/semi-skimmed/2% fat milk - 20ml
Sample 6: Brand C with low fat/semi-skimmed/2% fat milk - 30ml
Sample 7: Brand C with skim, fresh milk - 10ml
Sample 8: Brand C with skim, fresh milk - 20ml
Sample 9: Brand Cwith skim, fresh milk - 30ml
Sample 10: Brand Cwith white sugar – 2g
Sample 11: Brand C with white sugar – 4g
Sample 12: Brand C with white sugar – 6g
Sample 13: Brand C with brown sugar - 2g

Sample 14: Brand C with brown sugar - 4g
Sample 15: Brand Cwith brown sugar - 6g
Sample 16: Brand C with honey – 5g[3]
Sample 17: Brand Cwith honey - 9g[3]
Sample 18: Brand C with honey - 15g[3]
Sample 19: Brand Fwith whole, fresh milk - 10ml
Sample 20: Brand Fwith whole, fresh milk - 20ml
Sample 21: Brand Fwith whole, fresh milk - 30ml
Sample 22: Brand F with low fat/semi-skimmed/2% fat milk - 10ml
Sample 23: Brand Fwith low fat/semi skimmed/2% fat milk – 20ml
Sample 24: Brand F with low fat/semi-skimmed/2% fat milk - 30ml
Sample 25: Brand Fwith skim, fresh milk - 10ml
Sample 26: Brand F with skim, fresh milk - 20ml
Sample 27: Brand F with skim, fresh milk - 30ml
Sample 28: Brand Fwith white sugar– 2g
Sample 29: Brand F with white sugar– 4g
Sample 30: Brand F with white sugar– 6g
Sample 31: Brand F with brown sugar - 2g
Sample 32: Brand F with brown sugar - 4g
Sample 33: Brand F with brown sugar - 6g
Sample 34: Brand F with honey – 5g[3]
Sample 35: Brand Fwith honey - 9g[3]
Sample 36: Brand F with honey - 15g[3]
Sample 37: Brand G with whole, fresh milk - 10ml
Sample 38: Brand G with whole, fresh milk - 20ml
Sample 39: Brand G with whole, fresh milk - 30ml
Sample 40: Brand G with low fat/ semi-skimmed/2% fat milk - 10ml
Sample 41: Brand G with low fat/ semi-skimmed/2% fat milk – 20ml
Sample 42: Brand G with low fat/ semi-skimmed/2% fat milk - 30ml
Sample 43: Brand G with skim, fresh milk - 10ml
Sample 44: Brand G with skim, fresh milk - 20ml
Sample 45: Brand Gwith skim, fresh milk - 30ml
Sample 46: Brand G with white sugar – 2g
Sample 47: Brand Gwith white sugar – 4g
Sample 48: Brand G with white sugar – 6g
Sample 49: Brand G with brown sugar - 2g
Sample 50: Brand G with brown sugar - 4g
Sample 51: Brand Gwith brown sugar - 6g
Sample 52: Brand Gwith honey – 5g[3]
Sample 53: Brand G with honey - 9g[3]
Sample 54: Brand G with honey - 15g[3]
Control samples: Plain traditional/fermented rooibos herbal tea bags [from 1.1 Samples 1, 2 and 3 (Brands A, B and C)].

[1]Samples will be prepared using the specific brand traditional/fermented rooibos herbal tea bag and prepared according to the specifications of the SA Rooibos Council with 180ml boiling water, stirred for 30 seconds and steeped for three minutes with the specific additions added after the three minute steeping period.
[2]Added milk and sugar amounts obtained fromLangenhoven*et al*. (1991:9;10;140)

[3]Added average honey amounts obtained from Langenhoven *et al*. (1991:139) and the USDA Agricultural Research Service.

4.2 Combinations of household flavourants to rooibos herbal tea[1] added in different amounts[2]

Sample 1: Brand C with 10ml whole, fresh milk and 2g white sugar
Sample 2: Brand Cwith 10ml whole, fresh milk and 4g white sugar
Sample 3: Brand C with 10ml whole, fresh milk and 6g white sugar
Sample 4: Brand C with 20ml whole, fresh milk and 2g white sugar
Sample 5: Brand C with 20ml whole, fresh milk and 4g white sugar
Sample 6: Brand Cwith 20ml whole, fresh milk and 6g white sugar
Sample 7: Brand Cwith 30ml whole, fresh milk and 2g white sugar
Sample 8: Brand C with 30ml whole, fresh milk and 4g white sugar
Sample 9: Brand Cwith 30ml whole, fresh milk and 6g white sugar
Sample 10: Brand Cwith 10ml low fat/semi-skimmed/2% fat milk and 2g white sugar
Sample 11: Brand C with 10ml low fat/semi-skimmed/2% fat milk and 4g white sugar
Sample 12: Brand Cwith 10ml low fat/semi-skimmed/2% fatmilk and 6g white sugar
Sample 13: Brand C with 20ml low fat/semi-skimmed/2% fat milk and 2g white sugar
Sample 14: Brand C with 20ml low fat/semi-skimmed/2% fat milk and 4g white sugar
Sample 15: Brand C with 20ml low fat/semi-skimmed/2% fat milk and 6g white sugar
Sample 16: Brand Cwith 30ml low fat/semi-skimmed/2% fat milk and 2g white sugar
Sample 17: Brand C with 30ml low fat/semi-skimmed/2% fat milk and 4g white sugar
Sample 18: Brand C with 30ml low fat/semi-skimmed/2% fat milk and 6g white sugar
Sample 19: Brand C with 10ml skim, fresh milk and 2g white sugar
Sample 20: Brand C with 10ml skim, fresh milk and 4g white sugar
Sample 21: Brand C with 10ml skim, fresh milk and 6g white sugar
Sample 22: Brand C with 20ml skim, fresh milk and 2g white sugar
Sample 23: Brand Cwith 20ml skim, fresh milk and 4g white sugar
Sample 24: Brand C with 20ml skim, fresh milk and 6g white sugar
Sample 25: Brand C with 30ml skim, fresh milk and 2g white sugar
Sample 26: Brand C with 30ml skim, fresh milk and 4g white sugar
Sample 27: Brand C with 30ml skim, fresh milk and 6g white sugar
Sample 28: Brand C with 10ml whole, fresh milk and 2g brown sugar
Sample 29: Brand C with 10ml whole, fresh milk and 4g brown sugar
Sample 30: Brand C with 10ml whole, fresh milk and 6g brown sugar
Sample 31: Brand C with 20ml whole, fresh milk and 2g brown sugar
Sample 32: Brand C with 20ml whole, fresh milk and 4g brown sugar
Sample 33: Brand C with 20ml whole, fresh milk and 6g brown sugar
Sample 34: Brand C with 30ml whole, fresh milk and 2g brown sugar
Sample 35: Brand C with 30ml whole, fresh milk and 4g brown sugar
Sample 36: Brand C with 30ml whole, fresh milk and 6g brown sugar
Sample 37: Brand C with 10ml low fat/semi-skimmed/2% fat milk and 2g brown sugar
Sample 38: Brand C with 10ml low fat/semi-skimmed/2% fat milk and 4g brown sugar
Sample 39: Brand C with 10ml low fat/semi-skimmed/2% fat milk and 6g brown sugar
Sample 40: Brand C with 20ml low fat/semi-skimmed/2% fat milk and 2g brown sugar
Sample 41: Brand C with 20ml low fat/semi-skimmed/2% fat milk and 4g brown sugar
Sample 42: Brand C with 20ml low fat/semi-skimmed/2% fat milk and 6g brown sugar
Sample 43: Brand C with 30 ml low fat/semi-skimmed/2% fat milk and 2g brown sugar

Sample 44: Brand C with 30ml low fat/semi-skimmed/2% fat milk and 4g brown sugar
Sample 45: Brand Cwith 30ml low fat/semi-skimmed/2% fat milk and 6g brown sugar
Sample 46: Brand C with 10ml skim, fresh milk and 2g brown sugar
Sample 47: Brand C with 10ml skim, fresh milk and 4g brown sugar
Sample 48: Brand C with 10ml skim, fresh milk and 6g brown sugar
Sample 49: Brand C with 20ml skim, fresh milk and 2g brown sugar
Sample 50: Brand C with 20ml skim, fresh milk and 4g brown sugar
Sample 51: Brand C with 20ml skim, fresh milk and 6g brown sugar
Sample 52: Brand Cwith 30ml skim, fresh milk and 2g brown sugar
Sample 53: Brand Cwith 30ml skim, fresh milk and 4g brown sugar
Sample 54: Brand Cwith 30ml skim, fresh milk and 6g brown sugar
Sample 55: Brand C with 10ml whole, fresh milk and 5g honey[3]
Sample 56: Brand Cwith 10ml whole, fresh milk and 9g honey[3]
Sample 57: Brand Cwith 10ml whole, fresh milk and 15g honey[3]
Sample 58: Brand C with 20ml whole, fresh milk and 5g honey[3]
Sample 59: Brand Cwith 20ml whole, fresh milk and 9g honey[3]
Sample 60: Brand C with 20ml whole, fresh milk and 15g honey[3]
Sample 61: Brand C with 30ml whole, fresh milk and 5g honey[3]
Sample 62: Brand Cwith 30ml whole, fresh milk and 9g honey[3]
Sample 63: Brand Cwith 30ml whole, fresh milk and 15g honey[3]
Sample 64: Brand C with 10ml low fat/semi-skimmed/2% fatmilk and 5g honey[3]
Sample 65: Brand Cwith 10ml low fat/semi-skimmed/2% fat milk and 9g honey[3]
Sample 66: Brand C with 10ml low fat/semi-skimmed/2% fat milk and 15g honey[3]
Sample 67: Brand C with 20ml low fat/semi-skimmed/2% fat milk and 5g honey[3]
Sample 68: Brand C with 20ml low fat/semi-skimmed/2% fat milk and 9g honey[3]
Sample 69: Brand C with 20ml low fat/semi-skimmed/2% fat milk and 15g honey[3]
Sample 70: Brand C with 30ml low fat/semi-skimmed/2% fat milk and 5g honey[3]
Sample 71: Brand C with 30ml low fat/semi-skimmed/2% fat milk and 9g honey[3]
Sample 72: Brand C with 30ml low fat/semi-skimmed/2% fat milk and 15g honey[3]
Sample 73: Brand Cwith 10ml skim, fresh milk and 5g honey[3]
Sample 74: Brand Cwith 10ml skim, fresh milk and 9g honey[3]
Sample 75: Brand Cwith 10ml skim, fresh milk and 15g honey[3]
Sample 76: Brand C with 20ml skim, fresh milk and 5g honey[3]
Sample 77: Brand Cwith 20ml skim, fresh milk and 9g honey[3]
Sample 78: Brand C with 20ml skim, fresh milk and 15g honey[3]
Sample 79: Brand Cwith 30ml skim, fresh milk and 5g honey[3]
Sample 80: Brand Cwith 30ml skim, fresh milk and 9g honey[3]
Sample 81: Brand C with 30ml skim, fresh milk and 15g honey[3]

Sample 82: Brand Fwith 10ml whole, fresh milk and 2g white sugar
Sample 83: Brand Fwith 10ml whole, fresh milk and 4g white sugar
Sample 84: Brand F with 10ml whole, fresh milk and 6g white sugar
Sample 85: Brand F with 20ml whole, fresh milk and 2g white sugar
Sample 86: Brand F with 20ml whole, fresh milk and 4g white sugar
Sample 87: Brand F with 20ml whole, fresh milk and 6g white sugar
Sample 88: Brand F with 30ml whole, fresh milk and 2g white sugar
Sample 89: Brand F with 30ml whole, fresh milk and 4g white sugar
Sample 90: Brand F with 30ml whole, fresh milk and 6g white sugar

Sample 91: Brand F with 10ml low fat/semi-skimmed/2% fat milk and 2g white sugar
Sample 92: Brand F with 10ml low fat/semi-skimmed/2% fat milk and 4g white sugar
Sample 93: Brand F with 10ml low fat/semi-skimmed/2% fatmilk and 6g white sugar
Sample 94: Brand F with 20ml low fat/semi-skimmed/2% fat milk and 2g white sugar
Sample 95: Brand F with 20ml low fat/semi-skimmed/2% fat milk and 4g white sugar
Sample 96: Brand F with 20ml low fat/semi-skimmed/2% fat milk and 6g white sugar
Sample 97: Brand Fwith 30ml low fat/semi-skimmed/2% fat milk and 2g white sugar
Sample 98: Brand F with 30ml low fat/semi-skimmed/2% fat milk and 4g white sugar
Sample 99: Brand F with 30ml low fat/semi-skimmed/2% fat milk and 6g white sugar
Sample 100: Brand F with 10ml skim, fresh milk and 2g white sugar
Sample 101: Brand F with 10ml skim, fresh milk and 4g white sugar
Sample 102: Brand F with 10ml skim, fresh milk and 6g white sugar
Sample 103: Brand F with 20ml skim, fresh milk and 2g white sugar
Sample 104: Brand F with 20ml skim, fresh milk and 4g white sugar
Sample 105: Brand F with 20ml skim, fresh milk and 6g white sugar
Sample 106: Brand F with 30ml skim, fresh milk and 2g white sugar
Sample 107: Brand F with 30ml skim, fresh milk and 4g white sugar
Sample 108: Brand F with 30ml skim, fresh milk and 6g white sugar
Sample 109: Brand F with 10ml whole, fresh milk and 2g brown sugar
Sample 110: Brand F with 10ml whole, fresh milk and 4g brown sugar
Sample 111: Brand F with 10ml whole, fresh milk and 6g brown sugar
Sample 112: Brand F with 20ml whole, fresh milk and 2g brown sugar
Sample 113: Brand F with 20ml whole, fresh milk and 4g brown sugar
Sample 114: Brand F with 20ml whole, fresh milk and 6g brown sugar
Sample 115: Brand F with 30ml whole, fresh milk and 2g brown sugar
Sample 116: Brand F with 30ml whole, fresh milk and 4g brown sugar
Sample 117: Brand F with 30ml whole, fresh milk and 6g brown sugar
Sample 118: Brand Fwith 10ml low fat/semi-skimmed/2% fat milk and 2g brown sugar
Sample 119: Brand F with 10ml low fat/semi-skimmed/2% fat milk and 4g brown sugar
Sample 120: Brand F with 10ml low fat/semi-skimmed/2% fatmilk and 6g brown sugar
Sample 121: Brand F with 20ml low fat/semi-skimmed/2% fat milk and 2g brown sugar
Sample 122: Brand F with 20ml low fat/semi-skimmed/2% fat milk and 4g brown sugar
Sample 123: Brand F with 20ml low fat/semi-skimmed/2% fat milk and 6g brown sugar
Sample 124: Brand F with 30ml low fat/semi-skimmed/2% fat milk and 2g brown sugar
Sample 125: Brand F with 30ml low fat/semi-skimmed/2% fat milk and 4g brown sugar
Sample 126: Brand F with 30ml low fat/semi-skimmed/2% fat milk and 6g brown sugar
Sample 127: Brand F with 10ml skim, fresh milk and 2g brown sugar
Sample 128: Brand F with 10ml skim, fresh milk and 4g brown sugar
Sample 129: Brand F with 10ml skim, fresh milk and 6g brown sugar
Sample 130: Brand F with 20ml skim, fresh milk and 2g brown sugar
Sample 131: Brand F with 20ml skim, fresh milk and 4g brown sugar
Sample 132: Brand F with 20ml skim, fresh milk and 6g brown sugar
Sample 133: Brand F with 30ml skim, fresh milk and 2g brown sugar
Sample 134: Brand F with 30ml skim, fresh milk and 4g brown sugar
Sample 135: Brand F with 30ml skim, fresh milk and 6g brown sugar
Sample 136: Brand F with 10ml whole, fresh milk and 5g honey[3]
Sample 137: Brand F with 10ml whole, fresh milk and 9g honey[3]
Sample 138: Brand F with 10ml whole, fresh milk and 15g honey[3]

Sample 139: Brand F with 20ml whole, fresh milk and 5g honey[3]
Sample 140: Brand F with 20ml whole, fresh milk and 9g honey[3]
Sample 141: Brand F with 20ml whole, fresh milk and 15g honey[3]
Sample 142: Brand F with 30ml whole, fresh milk and 5g honey[3]
Sample 143: Brand F with 30ml whole, fresh milk and 9g honey[3]
Sample 144: Brand Fwith 30ml whole, fresh milk and 15g honey[3]
Sample 145: Brand F with 10ml low fat/semi-skimmed/2% fat milk and 5g honey[3]
Sample 146: Brand F with 10ml low fat/semi-skimmed/2% fat milk and 9g honey[3]
Sample 147: Brand Fwith 10ml low fat/semi-skimmed/2% fat milk and 15g honey[3]
Sample 148: Brand Fwith 20ml low fat/semi-skimmed/2% fat milk and 5g honey[3]
Sample 149: Brand Fwith 20ml low fat/semi-skimmed/2% fat milk and 9g honey[3]
Sample 150: Brand Fwith 20ml low fat/semi-skimmed/2% fat milk and 15g honey[3]
Sample 151: Brand Fwith 30ml low fat/semi-skimmed/2% fat milk and 5g honey[3]
Sample 152: Brand Fwith 30ml low fat/semi-skimmed/2% fat milk and 9g honey[3]
Sample 153: Brand Fwith 30ml low fat/semi-skimmed/2% fat milk and 15g honey[3]
Sample 154: Brand Fwith 10ml skim, fresh milk and 5g honey[3]
Sample 155: Brand Fwith 10ml skim, fresh milk and 9g honey[3]
Sample 156: Brand F with 10ml skim, fresh milk and 18g honey[3]
Sample 157: Brand Fwith 20ml skim, fresh milk and 5g honey[3]
Sample 158: Brand Fwith 20ml skim, fresh milk and 9g honey[3]
Sample 159: Brand Fwith 20ml skim, fresh milk and 18g honey[3]
Sample 160: Brand Fwith 30ml skim, fresh milk and 5g honey[3]
Sample 161: Brand Fwith 30ml skim, fresh milk and 9g honey[3]
Sample 162: Brand F with 30ml skim, fresh milk and 18g honey[3]

Sample 163: Brand G with 10ml whole, fresh milk and 2g white sugar
Sample 164: Brand Gwith 10ml whole, fresh milk and 4g white sugar
Sample 165: Brand G with 10ml whole, fresh milk and 6g white sugar
Sample 166: Brand G with 20ml whole, fresh milk and 2g white sugar
Sample 167: Brand G with 20ml whole, fresh milk and 4g white sugar
Sample 168: Brand Gwith 20ml whole, fresh milk and 6g white sugar
Sample 169: Brand G with 30ml whole, fresh milk and 2g white sugar
Sample 170: Brand G with 30ml whole, fresh milk and 4g white sugar
Sample 171: Brand G with 30ml whole, fresh milk and 6g white sugar
Sample 172: Brand G with 10ml low fat/semi-skimmed/2% fat milk and 2g white sugar
Sample 173: Brand G with 10ml low fat/semi-skimmed/2% fat milk and 4g white sugar
Sample 174: Brand Gwith 10ml low fat/semi-skimmed/2% fatmilk and 6g white sugar
Sample 175: Brand G with 20ml low fat/semi-skimmed/2% fat milk and 2g white sugar
Sample 176: Brand G with 20ml low fat/semi-skimmed/2% fat milk and 4g white sugar
Sample 177: Brand G with 20ml low fat/semi-skimmed/2% fat milk and 6g white sugar
Sample 178: Brand G with 30ml low fat/semi-skimmed/2% fat milk and 2g white sugar
Sample 179: Brand Gwith 30ml low fat/semi-skimmed/2% fat milk and 4g white sugar
Sample 180: Brand G with 30ml low fat/semi-skimmed/2% fat milk and 6g white sugar
Sample 181: Brand Gwith 10ml skim, fresh milk and 2g white sugar
Sample 182: Brand G with 10ml skim, fresh milk and 4g white sugar
Sample 183: Brand G with 10ml skim, fresh milk and 6g white sugar
Sample 184: Brand G with 20ml skim, fresh milk and 2g white sugar
Sample 185: Brand G with 20ml skim, fresh milk and 4g white sugar

Sample 186: Brand G with 20ml skim, fresh milk and 6g white sugar
Sample 187: Brand G with 30ml skim, fresh milk and 2g white sugar
Sample 188: Brand Gwith 30ml skim, fresh milk and 4g white sugar
Sample 189: Brand Gwith 30ml skim, fresh milk and 6g white sugar
Sample 190: Brand G with 10ml whole, fresh milk and 2g brown sugar
Sample 191: Brand G with 10ml whole, fresh milk and 4g brown sugar
Sample 192: Brand Gwith 10ml whole, fresh milk and 6g brown sugar
Sample 193: Brand G with 20ml whole, fresh milk and 2g brown sugar
Sample 194: Brand Gwith 20ml whole, fresh milk and 4g brown sugar
Sample 195: Brand Gwith 20ml whole, fresh milk and 6g brown sugar
Sample 196: Brand Gwith 30ml whole, fresh milk and 2g brown sugar
Sample 197: Brand G with 30ml whole, fresh milk and 4g brown sugar
Sample 198: Brand G with 30ml whole, fresh milk and 6g brown sugar
Sample 199: Brand G with 10ml low fat/semi-skimmed/2% fat milk and 2g brown sugar
Sample 200: Brand G with 10ml low fat/semi-skimmed/2% fat milk and 4g brown sugar
Sample 201: Brand G with 10ml low fat/semi-skimmed/2% fat milk and 6g brown sugar
Sample 202: Brand G with 20ml low fat/semi-skimmed/2% fat milk and 2g brown sugar
Sample 203: Brand Gwith 20ml low fat/semi-skimmed/2% fat milk and 4g brown sugar
Sample 204: Brand G with 20ml low fat/semi-skimmed/2% fat milk and 6g brown sugar
Sample 205: Brand G with 30ml low fat/semi-skimmed/2% fat milk and 2g brown sugar
Sample 206: Brand G with 30ml low fat/semi-skimmed/2% fat milk and 4g brown sugar
Sample 207: Brand G with 30ml low fat/semi-skimmed/2% fat milk and 6g brown sugar
Sample 208: Brand G with 10ml skim, fresh milk and 2g brown sugar
Sample 209: Brand G with 10ml skim, fresh milk and 4g brown sugar
Sample 210: Brand G with 10ml skim, fresh milk and 6g brown sugar
Sample 211: Brand G with 20ml skim, fresh milk and 2g brown sugar
Sample 212: Brand Gwith 20ml skim, fresh milk and 4g brown sugar
Sample 213: Brand G with 20ml skim, fresh milk and 6g brown sugar
Sample 214: Brand G with 30ml skim, fresh milk and 2g brown sugar
Sample 215: Brand G with 30ml skim, fresh milk and 4g brown sugar
Sample 216: Brand Gwith 30ml skim, fresh milk and 6g brown sugar
Sample 217: Brand G with 10ml whole, fresh milk and 5g honey[3]
Sample 218: Brand G with 10ml whole, fresh milk and 9g honey[3]
Sample 219: Brand Gwith 10ml whole, fresh milk and 15g honey[3]
Sample 220: Brand G with 20ml whole, fresh milk and 5g honey[3]
Sample 221: Brand G with 20ml whole, fresh milk and 9g honey[3]
Sample 222: Brand G with 20ml whole, fresh milk and 15g honey[3]
Sample 223: Brand G with 30ml whole, fresh milk and 5g honey[3]
Sample 224: Brand G with 30ml whole, fresh milk and 9g honey[3]
Sample 225: Brand Gwith 30ml whole, fresh milk and 15g honey[3]
Sample 226: Brand G with 10ml low fat/semi-skimmed/2% fat milk and 5g honey[3]
Sample 227: Brand G with 10ml low fat/semi-skimmed/2% fat milk and 9g honey[3]
Sample 228: Brand G with 10ml low fat/semi-skimmed/2% fat milk and 15g honey[3]
Sample 229: Brand G with 20ml low fat/semi-skimmed/2% fat milk and 5g honey[3]
Sample 230: Brand G with 20ml low fat/semi-skimmed/2% fat milk and 9g honey[3]
Sample 231: Brand G with 20ml low fat/semi-skimmed/2% fat milk and 15g honey[3]
Sample 232: Brand G with 30ml low fat/semi-skimmed/2% fat milk and 5g honey[3]
Sample 233: Brand G with 30ml low fat/semi-skimmed/2% fat milk and 9g honey[3]

Sample 234: Brand G with 30ml low fat/semi-skimmed/2% fat milk and 15g honey[3]
Sample 235: Brand G with 10ml skim, fresh milk and 5g honey[3]
Sample 236: Brand Gwith 10ml skim, fresh milk and 9g honey[3]
Sample 237: Brand G with 10ml skim, fresh milk and 15g honey[3]
Sample 238: Brand G with 20ml skim, fresh milk and 5g honey[3]
Sample 239: Brand G with 20ml skim, fresh milk and 9g honey[3]
Sample 240: Brand G with 20ml skim, fresh milk and 15g honey[3]
Sample 241: Brand G with 30ml skim, fresh milk and 5g honey[3]
Sample 242: Brand G with 30ml skim, fresh milk and 9g honey[3]
Sample 243: Brand G with 30ml skim, fresh milk and 15g honey[3]
Control samples: Plain traditional/fermented rooibos herbal tea bags [from 1.1 Samples 1, 2 and 3 (Brands A, B and C)].

[1]Samples will be prepared with the specific brand rooibos herbal tea and 180ml boiling water, stirred for 30 seconds and steeped for three minutes the specific additions added after the three minute steeping period.
[2]Added milk and sugar amounts obtained from Langenhoven*et al*. (1991:9; 10; 140)
[3]Added average honey amounts obtained from Langenhoven *et al*. (1991:139) and the USDA Agricultural Research Service.

4.3 <u>Commercially flavoured</u> rooibos herbal tea samples[1]

4.3.1 Samples[1] with traditional / fermented **commercial flavoured rooibos herbal tea**

Sample 1: Brand B **camomile**
Sample 2: Brand B **lemon**
Sample 3: Brand C **lemon**
Sample 4: Brand C **camomile**
Sample 5: Brand C **honey**
Sample 6: Brand G **honey**
Sample 7: Brand G **camomile**
Control samples: Plain traditional / fermented rooibos herbal tea bags [from 1.1 Samples 1, 2 and 3 (Brands A, B and C)].

[1]Samples will be prepared with the specific brand rooibos herbal tea and 180ml boiling water, stirred for 30 seconds and steeped for three minutes the specific additions added after the three minute steeping period.

4.3.2 Samples[1] with commercially flavoured **rooibos herbal iced teas**

Sample 1: Brand C **peach**
Sample 2: Brand C **pomegranate**
Sample 3: Brand C **apple and ginger**
Sample 4: Brand E **peach**
Sample 5: Brand E **lemon**
Control samples: Plain rooibos iced tea [from 2.1.3 Sample 2 (Brand E)]

[1]Samples will be prepared according to the instructions on the product packaging.

For the purpose of confidentiality, brandnames / suppliers are replaced with Brand A, Brand B, etc. or as Supplier A. The key for the brandname / supplier identification is indicated below for analytical purposes only.

Brand A –
Brand B –
Brand C –
Brand D –
Brand E –
Brand F –
Brand G –
Supplier A -

Addendum C

Ethical approval (Ref. 12 / 2013) from the Faculty of Applied Sciences Research Ethics Committee (CPUT)

Cape Peninsula University of Technology

Enquiries:
Dr M Opperman
Faculty of Applied Sciences
Chair: Ethics Committee
Tel: [redacted]
Email: oppermanm@cput.ac.za

5 February 2014

Mrs H Piek
Department of Agriculture and Food Science
Cape Peninsula University of Technology

Dear Mrs Piek

Effect of rooibos type, form, preparation method and added flavouring on the total polyphenol content and antioxidant capacity of the herbal tea (Ref. 12/2013)

The Ethics Committee has considered your application for Ethics approval for the above project and would like to advise that approval for the project is hereby granted.

We wish you every success with your research.

Kind regards

Dr MarethaOpperman (RD (SA))

Addendum D

Respondent consent form

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

Title of Research Study

Effect of rooibos type, form, preparation method and added flavouring on the total polyphenol content and antioxidant capacity of the herbal tea

Principal Investigator:

Hannelise Piek (Cell nr: [redacted])
Email: hannelisepiek@gmail.com
M Tech: Consumer Science: Food and Nutrition
Department: Agricultural and Food Sciences, Faculty of Applied Sciences,
Cape Peninsula University of Technology: Cape Town campus (Tennant Street, Cape Town)

Research study supervisors:

Dr I Venter and Prof JL Marnewick,
Cape Peninsula University of Technology

Introduction and Purpose of Study: Usually a sweetener such as sugar or honey and milk are added to rooibos herbal tea. Flavouring such as a slice of lemon, are also sometimes added. Various flavoured and green rooibos herbal teas are presently available on the market over and above the traditional rooibos herbal tea. As the leaves and stems of a shrub-like bush is used to manufacture rooibos and not the plant used to manufacture black and green teas, the prepared beverage should be referred to as 'rooibos' or 'rooibos herbal tea'.

The different forms (referring to tea bags, loose tea leaves, powdered tea etc.), types (referring to fermented or unfermented rooibos) and the ways in which rooibos herbal tea are prepared and flavoured for consumption will influence the total polyphenol content and the total antioxidant capacity (TAC) of this beverage. Polyphenols are substances found in plants which support human health in various ways. They are best known for their antioxidant function and therefore contribute to the TAC of the plant foods and beverages in which they occur. It seems that beverage consumption makes a large contribution to the daily polyphenolic intake and the dietary TAC. Consuming a cup of rooibos herbal tea brewed for optimal polyphenol extraction could make a notable contribution to the daily polyphenolic content and dietary TAC. An adequate dietary polyphenol and TAC provision have been proposed important dietary considerations as these dietary provisions support health through numerous antioxidant and other mechanisms reducing the risk of coronary heart disease, several common types of cancer and other chronic diseases of lifestyle.

Preparation methods and flavouring of rooibos herbal tea is done according to the specific taste of the consumer. The objective of this part of the research study is toassess the ways in which consumers usually drink rooibos herbal tea considering the different types, forms, preparation methods and flavouring additions and amounts added to prepare it as beverage.

Procedure: If you take part in this study, you will be asked to answer a detailed questionnaire on your beverage preferences, tea and in particular rooibos herbal tea consumption, as well as your demographic and lifestyle characteristics which will take about 5 to 10 minutes of your time to complete.

Benefits: There is no direct benefit to you. The information and results from this study may however benefit the consumer of rooibos herbal tea in that it could increase their daily dietary

TAC and total polyphenol intake by consuming a cup of rooibos herbal tea with a higher TAC and total polyphenol content.

Risks: There are no risks involved in this study as it only entails the answering of the questionnaire.

Voluntary Participation / Withdrawal: Taking part in this study is voluntary.

Costs: There will be no costs to you during this study.

Compensation: You will not be paid to take part in this study.

Confidentiality: The results obtained from this study are for research purposes only. Your identity will be kept confidential in the research and any resulting publications. A participant number will be allocated to you in the research records.

Questions: If you have any questions, please contact Hannelise Piek at [redacted].

✂……

Consent to Participate in this Research Study:

I agree to take part in this research study and I retain the right to withdraw from the study at any time. My signature below indicates that I have read this consent form, including the details of the study, the risks and benefits, and have had all my questions answered. This consent form will be kept on file and be available on the day which the research project is conducted.

_____
Signature of Study Subject Date:

_____
Printed name of Study Subject Date:

_____
Signature of Investigator Date:

_____
Signature of Witness Date:

Addendum E

Consumer questionnaire

Cape Peninsula University of Technology

DEPARTMENT OF AGRICULTURAL AND FOOD SCIENCES
PROGRAMME: CONSUMER SCIENCE: FOOD AND NUTRITION
FACULTY OF APPLIED SCIENCES

ROOIBOS HERBAL TEA CONSUMPTIONQUESTIONNAIRE

Dear Participant,

This questionnaire assesses the ways in which consumers drink rooibos herbal tea. Consuming a cup of rooibos herbal tea brewed for optimal polyphenol extraction could make a notable contribution to the daily polyphenolic intake and dietary TAC. An adequate dietary polyphenol and TAC provision have been proposed important dietary considerations as these dietary provisions support health through numerous antioxidant and other mechanisms reducing the risk of coronary heart disease, several common types of cancer and other chronic diseases of lifestyle.

If you are willing to participate you will be asked a number of questions. The questions are related to your beverage preferences, tea and rooibos herbal tea consumption, as well as your demographic and lifestyle characteristics. Answers are provided for each of the questions for you to choose from. The completion of the questionnaire will take approximately 5 to 10 minutes of your time. Please answer each question as honestly as possible. There are no correct or incorrect answers. All information collected through the consumer questionnaires will be kept anonymous and confidential.

Participant code:

Date:

INSTRUCTIONS FOR COMPLETION

Please answer **ALL** the questions in both sections.
Only choose **ONE** of the answer choices provided by making a cross (x) in the block next to your answer, unless stated otherwise.

SECTION A: TEA AND HERBAL TEA CONSUMPTION

Office use

A1 Which one of the following non-alcoholic beverages is your preferred choice?

Coffee	1
Cold drinks / Squash (water added to cold drink mixture)	2
Fruit juice (pure, nectars)	3
Fruit juice concentrates (water added to concentrate)	4
Milk and milk beverages (milk, soured milk, flavoured milk etc)	5
Milk based hot beverages (e.g. hot chocolate; Milo etc)	6
Soda drinks (fizzy)	7
Tea / Herbal tea	8
Water (still, sparkling & flavoured)	9
Other non-alcoholic beverage (please indicate): ……..	10

A2 Which tea/herbal tea do you mostly drink?

Black tea, commercially flavoured (e.g. lemon flavoured black tea)	1
Black tea, plain (not commercially flavoured)	2
Green tea, commercially flavoured	3
Green tea, plain (not commercially flavoured)	4
Honeybush herbal tea, commercially flavoured	5
Honeybush herbal tea, plain (not commercially flavoured)	6
Iced tea, commercially flavoured	7
Iced tea, plain (not commercially flavoured)	8
Traditional rooibos herbal tea, commercially flavoured (e.g. honey flavoured rooibos herbal tea)	9
Traditional rooibos herbal tea, plain (not commercially flavoured)	10
Green rooibos herbal tea, plain (not commercially flavoured)	11
Other tea/herbal tea (please indicate): ……	12

A3 Why do you mostly drink this tea/herbal tea?

Availability	1
Habit	2
Health reasons	3
Preferred taste	4
Other (please indicate): ……	5

A4 How often do you usually consume **tea/herbal tea** (traditional, green, plain and/or flavoured)? (1cup = 1 small mug = 180 mL)

Almost never / Seldom	1
1 - 3 cups per month	2

1 cup per week	3	
2 - 3 cups per week	4	
4 - 6 cups per week	5	
1 cup per day	6	
2 - 3 cups per day	7	
4 - 6 cups per day	8	
More than 6 cups per day	9	

A5 How often do you usually consume **rooibos herbal tea** (traditional, green, plain and/or flavoured)? (1cup = 1 small mug = 180 mL)

Almost never / Seldom	1	
1 - 3 cups per month	2	
1 cup per week	3	
2 - 3 cups per week	4	
4 - 6 cups per week	5	
1 cup per day	6	
2 - 3 cups per day	7	
4 - 6 cups per day	8	
More than 6 cups per day	9	

A6 What time of the day do you usually consume **rooibos herbal tea**?

Early morning (on waking up)	1	
During the morning	2	
Lunchtime	3	
Early afternoon	4	
Late afternoon (after work)	5	
In the evening	6	
Throughout the day	7	

A7 Which **commercially flavoured rooibos herbal tea/iced tea** do you usually drink?

Do not consume commercially flavoured rooibos herbal tea/iced tea	1	
Lemon flavoured tea bags	2	
Honey flavoured tea bags	3	
Camomile flavoured tea bags	4	
Ginger flavoured tea bags	5	
Lemon flavoured iced tea	6	
Peach flavoured iced tea	7	
Pomegranate flavoured iced tea	8	
Apple and ginger flavoured iced tea	9	
Berry flavoured iced tea	10	
Fruit punch flavoured iced tea	11	
Other (please indicate): ……..	12	

A8 What form of **rooibos herbal tea** do you usually consume?

Tea bags	1	
Loose tea leaves	2	
Iced tea - ready to drink (with rooibos extract)	3	
Iced tea - powder form (add water to powder)	4	

A9 How 'strong' do you usually consume your **rooibos herbal tea**?

Very weak (brewed for less than 1 minute)	1	
Weak (brewed for 1 minute)	2	
Weak to medium (brewed between 1 and 5 minutes)	3	
Medium (brewed for 5 minutes)	4	

Medium to strong (brewed between 5 and 10 minutes)	5	
Somewhat strong (brewed for 10 minutes)	6	
Strong (brewed for longer than 10 minutes)	7	

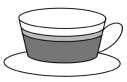
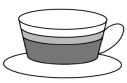
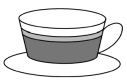
A10 How do you usually prepare your **rooibos herbal tea**?

Tea bag brewed in a cup or mug	1	
Tea bag (s) brewed in a teapot (teapot not kept warm)	2	
Tea bag (s) brewed in a teapot placed on the stove or warmer to keep warm	3	
Loose tea leaves brewed in a cup or mug	4	
Loose tea leaves brewed in a teapot (teapot not kept warm)	5	
Loose tea leaves brewed in a teapot placed on the stove or warmer to keep warm	6	

A11 How 'warm' do you usually consume your **rooibos herbal tea**?

Hot / Near boiling	1	
Warm / Still heated	2	
Cooled / Near cold	3	

A12 Follow **STEP 1 & STEP 2** to indicate the flavouring(s) and amounts in which you add these to your rooibos herbal tea.
Only choose **ONE type of milk and/or sweetening agent** (sweetener, sugar or honey) - the one you **usually** add.
If you **add no flavourings**, mark 'Do not add flavouring' and proceed to the next question.
If you add a **sweetener**, you need not indicate the amount added.

Flavouring added	STEP 1: Indicate with a cross (X) all flavouring(s) usually added	STEP 2: Amount added of flavouring(s) usually used (i.e. those crossed)					Office use
			Small amount	Medium amount	Large amount		
Do not add flavouring(s)	1	NOT APPLICABLE					
SWEETENER, (tablet or powder form)	2	NOT APPLICABLE					
SUGAR, brown	3	Add less than small amount 1	 Level teaspoon 2	 Heaped teaspoon 3	 Level + heaped teaspoon 4	Add more than large amount 5	12.1
SUGAR, white	4	Add less than small amount 1	 Level teaspoon 2	 Heaped teaspoon 3	 Level + heaped teaspoon 4	Add more than large amount 5	12.2
HONEY	5	Add less than small amount 1	 1/2 teaspoon 2	 Level teaspoon 3	 Heaped teaspoon 4	Add more than large amount 5	12.3
MILK, whole, fresh (full cream)	6	Add less than small amount 1	10ml Milk  180ml Tea 2	20ml Milk  180ml Tea 3	30ml Milk  180ml Tea 4	Add more than large amount 5	12.4
MILK, fat/semi-skimmed, 2% fat	7	Add less than small amount 1	10ml Milk  180ml Tea 2	20ml Milk  180ml Tea 3	30ml Milk  180ml Tea 4	Add more than large amount 5	12.5
MILK, skim, fresh	8	Add less than small amount 1	10ml Milk  180ml Tea 2	20ml Milk  180ml Tea 3	30ml Milk  180ml Tea 4	Add more than large amount 5	12.6
LEMON JUICE	9	Add less than small amount 1	 Level teaspoon 2	 2x level teaspoons 3	 Level tablespoon 4	Add more than large amount 5	12.7

A13
1,2

SECTION B: DEMOGRAPHIC AND LIFESTYLE INFORMATION

B1	What is your gender?	Male	1
		Female	2
B2	Indicate your age category.	25-29 years	1
		30-34 years	2
		35-39 years	3
		40-44 years	4
		45-49 years	5
		50-54 years	6
		55-59 years	7
		60-64 years	8
		65-69 years	9
		70-74 years	10
		75 years and older	11
B3	Under which racial group do you fall?	Black	1
		Coloured	2
		Indian	3
		White	4
B4	What is your highest level of education?	Standard 9/Grade 11 and lower	1
		Standard 10/Grade 12	2
		Grade 12 + Certificate	3
		Grade 12 + Diploma	4
		Grade 12 + Degree	5
		Postgraduate (Masters/Doctorate)	6
B5	What is your marital status?	Married / living together with children	1
		Married / living together without children	2
		Single and living with children	3
		Single and living without children	4
B6	Which one of the following options best describes your own food and beverage intake?	Consume foods/beverages popular with and consumed by most adults of your age (similar food and beverage intakes as most of your friends, family and/or colleagues)	1
		Consume foods/beverages considered healthier choices than those consumed by most adults of your age (or most of your friends, family and/or colleagues)	2
B7	Are you currently physically active? (being active means regular moderate exercise [e.g. walking or cycling] or strenuous exercise [jogging, football and vigorous swimming] for 4 hours or more per week)	Yes	1
		No	2

B8	How often do you take dietary supplements (a vitamin, mineral, herbal, plant extract, amino acid, metabolite, constitute, or extract, or a combination of any of these substances)?	Never	1	
		Seldom	2	
		When I remember	3	
		Fairly regularly	4	
		Regularly	5	
B9	What is your smoking status?	Non-smoker (have never smoked)	1	
		Current smoker (smoked in the last 12 months or quit in the past year)	2	
		Former smoker (quit smoking more than a year ago)	3	
B10	How would you describe your body weight status?	Underweight	1	
		Optimal/Normal body weight	2	
		Slightly overweight/Overweight	3	
		Obese	4	
B11	Do you have any of the following chronic diseases of lifestyle?			
B11.1	Cardiovascular disease	Yes	1	
		No	2	
		Don't know/Unsure	3	
B11.2	Diabetes mellitus type 2	Yes	1	
		No	2	
		Don't know/Unsure	3	
B11.3	Inflammatory conditions (e.g. arthritis)	Yes	1	
		No	2	
		Don't know/Unsure	3	
B11.4	Cancer (skin, lung, breast, liver, prostate)	Yes	1	
		No	2	
		Don't know/Unsure	3	
B12	Do you have a family history of any of the following chronic diseases of lifestyle?			
B12.1	Cardiovascular disease	Yes	1	
		No	2	
		Don't know/Unsure	3	
B12.2	Diabetes mellitus type 2	Yes	1	
		No	2	
		Don't know/Unsure	3	
B12.3	Inflammatory conditions (e.g. arthritis)	Yes	1	
		No	2	
		Don't know/Unsure	3	
B12.4	Cancer (skin, lung, breast, liver, prostate)	Yes	1	

No	2	
Don't know/Unsure	3	

Thank you for your participation in this study.