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EFFECT ON THE TOTAL ANTIOXIDANT CAPACITY OF SUBSTITUTING WATER WITH ROOIBOS HERBAL TEAS IN POPULAR SOUP RECIPES

by

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DECLARATION

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SYNOPSIS

Oxidative stress had been linked to the development of certain chronic diseases, but can be delayed or prevented by the consumption of dietary antioxidants. Fruits, vegetables, wholegrains and beverages, such as, teas are the major dietary antioxidant contributors. The majority of South Africans do not consume adequate daily servings of fruits and vegetables, neither sufficient minimally processed grains nor wholegrains. One way to incorporate antioxidants in the South African diet is by adding antioxidant-rich foods or beverages to recipes as ingredients. The objective of this study was to determine the effect on the total antioxidant capacity (TAC) of substituting water with rooibos herbal tea in soup recipe formulations. Rooibos is a proudly South African beverage rich in antioxidants. Soup is a readily available and relatively inexpensive meal item regularly consumed during the winter months in South Africa. Three popularly consumed soups in the City of Cape Town Metropolitan Municipality namely chunky vegetable, butternut and chicken noodle were selected for the experimental study.

The water in each of the soup recipe formulations (control) was substituted with fermented and unfermented / "green" rooibos (experimental recipe formulations). The study was of comparative nature as the results (i.e. the TAC as the factor investigated) of three different soup recipe formulations on fluid manipulations of each (with fermented and unfermented rooibos) was compared to the control soup recipe formulations of each (no fluid manipulation). The results (i.e. the TAC) of the three prepared control and experimental soup recipe formulations were also compared to that of the raw soup mixtures of each of the soup recipe formulations to determine the effect of thermal processing on each.

The main variable identified in the preparation of the soup recipe formulations that may impact the TAC (the dependent variable) and needed to be controlled was the heat application. Other variables that may influence the results were the soup recipe formulation ingredients, the pre-preparation of the raw ingredients, the standing time of ingredients before use and the equipment used. Before determination of the heat applications and the fixed time allocations of the soup recipe formulations to ensure recipe standardisation, the pre-preparation procedures of the raw recipe ingredients were also standardised.

The hydrophilic (H) and lipophilic (L) oxygen radical absorbance capacity (ORAC) methods were chosen for the TAC determination as it has been used widely in the TAC determinations of foods and beverages. Firstly, samples of the raw ingredients were obtained and then analysed for their polyphenol content, H-ORAC, L-ORAC and carotenoid content.

Then the polyphenol content, H-ORAC, L-ORAC and carotenoid content of the combined raw ingredient samples obtained of each recipe formulation was analysed for both the control and experimental formulations. Lastly, the polyphenol content, H-ORAC, L-ORAC and carotenoid content of the samples obtained from the thermally processed recipe formulations was analysed.

The T-test was used for the statistical analysis of the data. The effect of the substitution of water with fermented and unfermented rooibos on the recipe formulation used the T-test for independent samples. The effect of thermal processing on the recipe formulations was compared using the T-test for paired samples. If the p value was greater than 0.05 ($p > 0.05$) then the result was considered not to be statistically significant different for both the paired and independent sample results.

The experimental study indicated the following:

(a) The substitution with fermented and unfermented rooibos significantly increased ($p < 0.05$) the polyphenol content and H-ORAC indicative of the increased TAC in all three raw soup recipe formulations. There was little effect on the carotenoid content and L-ORAC when water was substituted with fermented and unfermented rooibos in the raw soup recipe formulations.

(b) The polyphenol content on thermal processing was significantly ($p < 0.05$) higher for the cooked chunky vegetable and butternut control and both the cooked chunky vegetable and butternut experimental soup recipe formulations compared to the counterpart raw soup recipe formulations. The increase on thermal processing in the H-ORAC and TAC of the chunky vegetable soup recipe formulation was significant ($p < 0.05$ for each) for the experimental soup recipe formulation containing unfermented rooibos. Thermal processing also significantly ($p < 0.05$ for each) increased the H-ORAC and TAC of the cooked butternut experimental soup recipe formulations containing fermented and unfermented rooibos. In contrast thermal processing significantly ($p < 0.05$ for each) decreased the carotenoid content and L-ORAC of the cooked control and cooked experimental chunky vegetable and butternut soup recipe formulations. The longer thermal application significantly ($p < 0.05$ for each) decreased the polyphenol content, and H-ORAC, L-ORAC and thus TAC, of both the experimental chicken noodle soup recipe formulations.

(c) The substitution with unfermented rooibos generally caused a higher increase in the polyphenol content, H-ORAC and TAC compared to the substitution with fermented rooibos in the raw and thermally processed control and experimental soup recipe formulations.

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LIST OF OPERATIONAL TERMS AND CONCEPTS

Recipe formulation: An ingredient list and the procedure for preparing a food product (Bennion & Scheule, 2000:133).

Rooibos herbal tea / Rooibos: A beverage made from the leaves and fine stems of *Aspalathus linearis*, which is a leguminous shrub native to the Cederberg Mountains of the Western Cape in South Africa (Lee & Jang, 2004:285). For the purpose of this study the term rooibos will be used.

Soup: A liquid food made by boiling meat, fish or vegetables (Thompson, 1994:926).

Total antioxidant capacity (TAC): The measure of the moles of a given free radical scavenged by a test solution (Serafini & Del Rio, 2004:145).

Oxidative stress: The process in which the active redox balance between oxidants and antioxidants is shifted towards oxidative potentials (Serafini & Del Rio, 2004:145).

Free radical: Any molecule containing a single unpaired electron and has the ability of independent existence (McCord, 2000:652; Willcox, Ash & Catignani, 2004:276).

Antioxidant: Any substance that, when present at low concentrations in the presence of an oxidisable substrate, significantly delays or prevents oxidation of the substrate (Carr & Frei, 1999:1087).

LIST OF ABBREVIATIONS

A

AC	antioxidant capacity
AFR	ascorbyl free radical
AICR	American Institute of Cancer Research
AIDS	acquired immune deficiency syndrome
AUC	fluorescence decay curve
ApoB	apolipoprotein

C

˚C	degrees celsius
CAT	catalase
CHF	chronic heart failure
cm	centimeter
CPUT	Cape Peninsula University of Technology
CVD	cardiovascular disease

D

DNA	deoxyribonucleic acid
DHA	dehydroascorbic acid

E

EC	epicatechin
ECG	epicatechin gallate
EGC	epigallocatechin

F

FDA	Food and Drug Administration
FL	fluorescein
FW	fresh weight

G

g	gram
GAE	gallic acid equivalents
GSH	reduced glutathione

H

H	hydrophilic
HIV	human immunodeficiency virus
H-ORAC	hydrophilic oxygen radical absorbance capacity

I

ICAM	intra cellular adhesion molecule
IFN-y	interferon-y
IL	interleukins
IL-2	interleukin-2

K

Kg	kilogram

L

LDL	low density lipoprotein
L	lipophilic

L-ORAC	lipophilic oxygen radical absorbance capacity

M

mg	milligram
ml	milliliters
mRNA	messenger RNA
MRP	Maillard reaction products

N

nm	nanometer
N.D.	not detected
NF-KB	nuclear factor kappa-light-chain-enhancer of activated B cells
NO	nitric oxide
NOS	nitric oxide synthase

O

ORAC	oxygen radical absorbance capacity
OSRC	Oxidative Stress Research Centre

P

PUFA's	polyunsaturated fatty acids

R

RDA	recommended dietary allowance
REE	resting energy expenditure
ROS	reactive oxygen species
RONS	reactive oxygen and nitrogen species
RNA	ribonucleic acid

RNI	required nutrient intakes
RNS	reactive nitrogen species
S	
SMCS	smooth muscle cells
SOD	superoxide dismutase
T	
TAC	total antioxidant capacity
TE	trolox equivalents
TFs	theaflavins
TNF	tumour necrosis factor
TRs	thearubigens
U	
µg	microgram
µmole	micromole
USDA	United States Department of Agriculture
V	
VES	vitamin E succinate derivative

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CHAPTER 1

INTRODUCTION

Prevention of chronic diseases of lifestyle has initiated much scientific research. Population studies have shown that up to 80% of cardiovascular disease (CVD), 90% type II diabetes, and approximately 30% of cancers could be avoided by diet and lifestyle changes (Willcox *et al.,* 2004:275). Oxidative stress is considered a major cause of these chronic degenerative diseases (Tsukahara, 2007:340). Oxidative stress, the process in which the dynamic redox balance between oxidants and antioxidants is strongly shifted towards oxidative potentials (Serafini & Del Rio, 2004:145), can be prevented to some extent through a diet rich in antioxidants, as antioxidants delay the oxidation process (Gordon, 1996:265; Hercberg, Galan, Preziosi, Alfarez & Alfarex, 1998:513). The incidence of chronic diseases of lifestyle is on the increase in South Africa with poor dietary habits implicated in the development of these diseases (Steyn & Bradshaw, 2001:30).

Epidemiological studies have consistently shown that a high dietary intake of fruits, vegetables, herbs as well as wholegrains, is strongly associated with reduced risk of developing chronic diseases of lifestyle (Lui, 2004:3479S; Papas, 1999:1000). These foods provide not only essential nutrients needed for life, but also other bioactive compounds for health promotion and disease prevention (Liu, 2003:517S). Many of these bioactive compounds function as antioxidants (Tsao & Akhtar, 2005:10).

The majority of South Africans do not achieve the recommended daily intake of five servings of fruits and vegetables (Love & Sayed, 2001:S29). Available data also indicates that South Africans need to increase their intake of cereals and grains, especially cereals and grains in a whole or minimally processed form (Vorster & Nell, 2001:S19). This inadequate consumption of fruits, vegetables and wholegrains contribute to the low intake of antioxidant vitamins and probably other bioactive compounds by South Africans and their increased risk of chronic diseases of lifestyle.

Ways to incorporate antioxidants in the South African diet may have to be found other than increasing the intake of plant foods. One such way is the addition of antioxidant-rich food items or beverages as ingredients to recipe formulations to increase the overall antioxidant content. In

this study, the considered option was using rooibos to replace the water in soup recipe formulations. Soup is a commonly consumed, easily prepared and relatively inexpensive meal

item regularly consumed during the winter months in South Africa. A proudly South African beverage that is rich in antioxidants is rooibos. Rooibos contains the flavonol, quercetin, the flavone, luteolin and five additional glycosides (Jacobus, Steenkamp, Joubert, Burger & Ferreira, 1994:1559). Rooibos also contains aspalathin, a unique polyphenol, found only in rooibos. Rooibos is furthermore a herbal beverage containing volatile components, minerals, ascorbic acid, little caffeine and a low level of tannin (Lee & Jang, 2004:285). In soup recipe formulations water is generally the ingredient with the greatest percentage contribution to the total weight of the soup recipe formulation and is retained in the food portion to be consumed. Rooibos Ltd. (Clanwilliam, South Africa) has published recipe booklets that include soup recipe formulations with rooibos as the liquid ingredient but the effect on the antioxidant capacity of this liquid substitution had not been analytically investigated. This application was considered for this study with the soup recipe formulations the carriers of the incorporated antioxidant provision, in this case the rooibos inclusion as the recipe ingredient.

Using an experimental study design, three popular soup recipe formulations were chosen from South African recipe books and standardised. The water in the three control recipe formulations was substituted with fermented and unfermented rooibos respectively. The objective of this study was to determine the effect on the total antioxidant capacity (TAC) of substituting water with rooibos herbal teas in soup recipe formulations (to ultimately determine if substituting water with rooibos herbal teas in soup recipes will provide an antioxidant-richer meal for South African consumers to include in their household meal plan to aid in the prevention of the chronic diseases of lifestyle). The specific objectives were to determine the TAC of the three control and their experimental soup recipe formulations to form a comparison between the soup recipe formulations having water as an ingredient (control recipe formulations) and those with rooibos as an ingredient (experimental recipe formulations) and between the raw and cooked recipe formulations of these to determine the effect of thermal processing on the TAC of the soup recipe formulations. Processing and cooking conditions cause variable losses of antioxidants. Besides consumer preferences, the selected thermal processing method is an important factor affecting not only the food chemical composition, but also the intake of bioactive compounds under normal dietary conditions (Ruiz-Rodriguez & Marin, 2008:345).

Considering the objectives, the null hypothesis for the study was formulated as no significant difference between: (a) the TAC of the raw control soup recipe formulations containing water as the major ingredient (raw combined ingredient soup recipe formulation) and the correspondent

raw experimental soup recipe formulations containing fermented and unfermented rooibos as the major ingredient; and (b) the TAC of the raw control and experimental soup recipe formulations respectively containing water and fermented and unfermented rooibos as the major ingredients and the correspondent cooked control and experimental soup recipe formulations respectively containing water and fermented and unfermented rooibos as the major ingredients.

CHAPTER 2

LITERATURE STUDY

Generation of reactive oxygen species (ROS) and free radicals is a necessary and normal process that ideally is compensated for by a highly complicated endogenous antioxidant system. However, due to many environmental, lifestyle and pathological situations, excess radicals can accumulate that overwhelm the antioxidant system resulting in oxidative stress (Willcox *et al.*, 2004:275). Excessive oxidative stress over a prolonged period can impact cellular processes implicated in chronic disease development such as cancer, cardiovascular disease (CVD) and the pathogenesis of human immunodeficiency virus (HIV) (Hercberg *et al.*, 1998:513). Oxidative stress can be delayed or prevented by dietary antioxidants thereby assisting in the prevention of these chronic diseases (Willcox *et al.*, 2004:281). The process and link between oxidative stress and specific disease development will be discussed further, along with the major dietary antioxidants, how they can be consumed (i.e. natural sources, dietary supplements and / or other dietary means) and the effects of food processing on these major dietary antioxidants.

2.1 Oxidative stress

Oxidative stress is the process in which the active redox balance between oxidants and antioxidants is shifted towards oxidative potentials (Serafini & Del Rio, 2004:145). Oxidants can be produced within cells by many enzymes that use molecular oxygen as a substrate (Finkel, 2003:247). Oxidative stress involves any condition in which oxidant metabolites, such as free radicals, deploy their toxic effects because of increased production or changed cellular mechanisms of protection (Ceconi, Boraso, Cargnoni & Ferrari, 2003:217). This can be a major mediator of damage to important cell structures, such as proteins and deoxyribonucleic acid (DNA), and cell membranes that contain lipids (Valko, Leibfritz, Moncol, Cronin, Mazur & Telser, 2007:44). All cellular membranes are especially vulnerable to oxidation due to their high concentration of unsaturated fatty acids (Kohen & Nyska, 2002:626).

A free radical is any molecule containing a single unpaired electron and has the ability of independent existence (McCord, 2000:652; Willcox *et al.*, 2004:276). Examples of oxygen-

derived free radicals include super oxide and hydroxyl radicals and other common free radicals or ROS produced in the body, such as nitric oxide and the peroxynitrite anion (Kaur & Kapoor, 2001:704). The presence of unpaired electrons makes free radicals highly reactive because they need another electron to fill the orbital and become stable (Willcox *et al.*, 2004:276).

Both ROS and reactive nitrogen species (RNS) are well known for playing a dual role as both harmful and valuable species. ROS are small, highly reactive, oxygen containing molecules that are naturally generated in small amounts during the body's metabolic reactions and can react with and damage complex cellular molecules such as proteins, DNA and lipids (Wu & Cederbaum, 2003:277). The cell is exposed to a large variety of ROS and RNS from both exogenous sources, such as radiation, UV light, smog and tobacco smoke and endogenous sources (Kohen & Gati, 2000:150; Wu & Cederbaum, 2003:280; Willcox *et al.*, 2004:279). ROS and RNS are normally produced by tightly regulated enzymes, such as nitric oxide synthase (NOS) and NAD(P)H oxidase isoforms, respectively. An excess production of ROS (arising either from mitochondrial electron transport chain reactions or excessive stimulation of NAD(P)H) results in oxidative stress (Serafini & Del Rio, 2004:145; Valko *et al.*, 2007:44), which, as indicated above, is a harmful process. In contrast, beneficial effects of ROS / RNS occur at low / moderate concentrations and involve physiological roles in cellular responses to noxia, as for example in defense against infectious agents (Valko *et al.*, 2007:44).

Living organisms constantly form ROS (Ceconi *et al.*, 2003:217). ROS, namely hydrogen peroxide, superoxide anion and hydroxyl radical, may be formed in cells through several ways: (a) through the effect of radiation, both exciting (ultraviolet rays) and ionising (Xrays), in particular ionising radiation where electrons may be removed from water, (b) during xenobiotic or chemotherapeutic drug metabolism as the electron transport chain found in the smooth endoplasmic reticulum operates to hydroxylate different substrates such as steroids, drugs, carcinogens and other liposoluble substances to render them more hydrosoluble and more easily removable. Oxygen may be formed by the leakage of electrons from NADP(H) cytochrome P450 reductase and by the release from cytochrome P450 during the substrate hydroxylation and (c) during redox reactions, which characterise normal metabolic pathways (Poli, Leonarduzzi, Biasi & Chiarpotto, 2004:1163; Karihtala & Soini, 2007:82).

Under normal physiological conditions the balance between production and elimination of ROS is maintained by enzymes and antioxidants (Tsukahara, 2007:339). Cells can normally deal with mild oxidative stress by upregulating the synthesis of the antioxidant defense mechanism through changes in gene expression. But, at higher levels, cell injury may occur when adaptation is not adequate for the build up of oxidation products (Willcox *et al.*, 2004:281). This results in oxidative damage to important biomolecules including proteins,

DNA and lipids. The initial reaction generates a second radical, which reacts with a second macromolecule resulting in a continuing chain reaction (Kannan & Jain, 2000:155). The target of oxidative damage varies depending on the characteristic of the cell, the type and the amount of stress imposed (Willcox *et al.*, 2004:281). Excessive oxidative stress over a prolonged period can directly or indirectly impact cellular processes implicated in chronic disease development such as cancer, CVD and the pathogenesis of HIV (Hercberg *et al.*, 1998:513), as previously mentioned.

Oxidative stress may also result from the primary disease process. Tissue damage by infection, trauma, toxins, temperature extremes and other causes usually leads to the formation of increased amounts of free radicals that contribute to the disease pathology. Oxidative stress also plays an important role in furthering tissue damage in several diseases. The imbalance of reduction oxidation homeostasis is one of the processes that regulate gene expression in many pathological conditions to support the antioxidant defense mechanism but, as indicated above, this may not be adequate at high levels of oxidation product accumulation (Willcox *et al.*, 2004:281).

Antioxidants make up a diverse group of compounds with different properties. An antioxidant has been defined as any substance that, when present at low concentrations in the presence of an oxidisable substrate, significantly delays or prevents oxidation of the substrate (Carr & Frei, 1999:1087). Antioxidants operate by inhibiting oxidant formation, intercepting oxidants once they have formed and repairing oxidant-induced injury (Ergëuder, 2007:152). Antioxidants also neutralise free radicals by donating one of their own electrons (Kaur & Kapoor, 2001:704). There are two types of antioxidants namely endogenous and exogenous antioxidants. The endogenous type comprises of a large group of intracellular and extracellular antioxidants with different roles within each area of defense which includes the antioxidant enzymes, superoxide dismutase (SOD), glutathione (GSH) peroxidase and catalase (CAT) (Willcox *et al.*, 2004:275).

Diet plays a vital role in the production of the exogenous antioxidant defense system by providing essential nutrient and non-nutrient antioxidants such as vitamin E, vitamin C, β-carotene and other antioxidant plant phenols, including flavonoids, along with essential minerals that support the important endogenous antioxidant enzymes (Willcox *et al.*, 2004:281). Antioxidants act not in isolation but in combination and have synergistic and antagonistic effects (Collins, 2005:1924).

2.2 Oxidative stress in chronic disease development

Oxidative stress and ROS have been implicated in the development of various diseases as varied as the group of rheumatic diseases, respiratory infections, diabetes type 2, malignancies and CVD. A common occurrence in all is an imbalance between the rates of production and elimination of ROS (Ulrich-Merzenich, Zeitler, Vetter & Kraft, 2009:2). In South Africa the latest cause-of-death statistics for 1996 predominantly reflect the pre-acquired immune deficiency syndrome (AIDS) pattern. While death among men are dominated by injuries and tuberculosis and account for a large proportion of deaths in all ages, many chronic diseases such as stroke, ischaemic heart disease, diabetes and cancers play an important role, particularly in the 45 to 59 year age group (Steyn & Bradshaw, 2001:29). While the percentage of premature deaths due to chronic diseases can be expected to decrease as the AIDS epidemic proceeds, the risk profile suggests that there are already considerable numbers of people at risk for developing a range of chronic diseases (Steyn & Bradshaw, 2001:30). In the context of the South African disease mortality profile, oxidative stress and ROS will be discussed in the development of CVD, cancer and AIDS.

2.2.1 Cardiovascular disease

In Western societies, CVD is the main cause of death, causing 1.5 million deaths per year in the European Union. One in eight male deaths before the age of 65 are a result of CVD (Greaves & Channon, 2002:535). The magnitude of hyperlipidemia in South Africa has not been determined; however, rough estimates indicate that of South Africa’s 20 million adults, 4 million might have hyperlipidaemia (Steyn & Bradshaw, 2001:29). Atherosclerosis is the underlying pathological process of CVD that begins in the first decade of life (Greaves & Channon, 2002:535).

Atherosclerosis is a multifactorial, inflammatory degenerative, progressive disease of the arteries, characterised by the accumulation of lipids and fibrous elements in the large vessels (Cherubini, Vigna, Ruggiero, Senin & Fellin, 2005:2017). The level of low density lipoprotein (LDL), or specifically the level of apolipoprotein (ApoB) particles, are the primary determinants of the development of atherosclerosis (Kuller, 2006:S15). The fundamental processes underlying the development of vascular diseases, such as atherosclerosis, have their origins in an initial insult to the vessel wall (Cherubini *et al.*, 2005:2017). Many factors have been linked in causing this initial injury, including mechanical damage. An insult may

arise at regions of oscillatory shear stress by high blood pressure, viral infection and exposure to blood borne toxins, such as xenobiotics from cigarette smoke. Initial injury can also result from biological causes such as hypercholesterolemia, excess free radicals, diabetes, and increased concentrations of plasma homocysteiene or infectious agents (Papaharalambus & Gruendling, 2007:48).

The inflammatory response following an injury to the vessel wall includes a wide range of activities, including: an increase in oxidative stress; an increase in capillary permeability; build up of white blood cells; release of cytokines such as interleukins (IL) and tumour necrosis factor (TNF); induction of various enzyme activities (oxygenase, nitric oxide synthetase, peroxidases); induction of the arachidonic acid metabolism; and the release of cellular adhesion molecule (ICAM) and vascular cell adhesion molecule (Gross, 2004:22).

The fatty streak is the earliest and most common atherosclerotic lesion seen in CVD. Minor damage to the vascular endothelium attracts and provokes the adherence of white blood cells, known as monocytes (Willcox *et al.*, 2004:281). These processes lead to the tethering activation, and attachment of monocytes and T lymphocytes to the endothelial cells. Endothelial cells, leukocytes, and smooth muscle cells then secrete growth factors and chemoattractants, which effect the migration of monocytes and leukocytes into the subendothelial spaces. Blood leukocytes adhere poorly to the normal endothelium. When the endothelial monolayer becomes inflamed, it expresses adhesion molecules that bind cognate lignands on leukocytes. Once adherent to the endothelium, the leukocytes penetrate into the intima (Libby, Ridker & Miseri, 2002:1136). Monocytes subsequently develop into macrophages, taking up LDL particles that contain oxidised lipids. As a result, the macrophages are converted into lipid-laden foam cells. Fatty streaks are areas in the vessel wall that contain lipid deposits, but contain a lack of the cells and cellular debris that characterise more advanced lesions (Madamanchi, Hakim & Runge, 2004:255). The transport of oxidised LDL across the endothelium into the artery wall is necessary for the formation of the fatty streaks and this transport continues as more advanced lesions develop. These transformed macrophages are considered as precursors to the development of the occlusive plaque of atherosclerosis (Willcox *et al.*, 2004:281).

Oxidative stress has been recognised as a key mechanism in the development of vascular damage, particularly atherosclerosis (Minuz, Fava & Cominacini, 2006:774). This chronic condition develops through a series of stages that starts with fatty streak lesions, largely made up of lipid engorged macrophage foam cells (described earlier), and eventually progressing to complex plaques composed of a core of lipid and necrotic cell debris covered by a fibrous cap (Tribble, 1999:591). Fatty streaks are areas in the vessel wall that contain lipid deposits, but hardly contain any of the cells and cellular debris that identifies more

developed lesions (Madamanchi *et al*., 2004:255). The macrophages release factors (cytokines and prostanoids) that stimulate the proliferation of smooth muscle cells, and an atherosclerotic plaque develops, reducing the diameter of the blood vessel lumen and restricting blood flow. As the plaque develops over time, it can rupture and debris (a thrombus) can lodge in the restricted vessel causing either a heart attack or stroke depending on the vessel involved (Buttriss, Hughes, Kelly & Stanner, 2002:230).

Hypercholesterolemia, especially elevated levels of LDL cholesterol, is one major risk factor for coronary artery disease (Khovidhunkit & Memon, 2000:S463). LDL in its native state is not atherogenic (Singh & Jialal, 2006:130). However, after LDL is chemically modified, rapid uptake occurs, leading to cholesterol accumulation in the macrophage with subsequent foam cell formation (Khovidhunkit & Memon, 2000:S463).

Most lipoproteins, and in particular LDL, contain esterfied forms of polyunsaturated fatty acids (PUFA's). When these PUFA's undergo oxidation, they generate peroxidised fatty acids, commonly referred to as hydroperoxy fatty acids. These oxidised fatty acids may be present in LDL directly, as a result of oxidation of LDL lipids themselves, or they may be generated elsewhere in the circulation and then become acquired by LDL (Buttriss *et al*., 2002:230).

Blood contains antioxidants such as vitamin C, vitamin E, albumin, uric acid and GSH that provides protection against oxidative stress. Vascular cells also possess antioxidant systems, such as SOD, CAT and the GSH synthesising enzymes and GSH peroxidase (Kondo, Hirose & Kageyama*.,* 2009:532). GSH plays an important role in the antioxidant defence system by scavenging free radicals and regenerating other antioxidants (Campolo, De Maria, Caruso, Accinni, Turazza, Parolini, Roubina, De Chiara, Cighetti, Frigerio, Vitali & Parodi*,* 2007:49). GSH and GSH-related enzymes such as GSH peroxidase occur in extracellular spaces. The source of the enzymes is not entirely known; however, they are thought to play an important role in protecting the vascular system against oxidative stress. Oxidative stress modifies GSH to glutathione disulfide (GSSG) that modifies cell protein thiols (Kondo *et al.,* 2009:533). Such modifications of protein thiols by oxidative stress are speculated to occur in patients with arteriosclerosis obliterans (Kondo *et al.,* 2009:535). A study was done to assess the correlation between blood and plasma concentration of aminothiols and lipid peroxidation as a marker of oxidative stress in chronic heart failure (CHF) patients. The blood reduced GSH concentrations were significantly higher in CHF patients than in control patients and therefore abnormalities in intracellular GSH cycling are associated with increased lipid peroxidation in CHF (Campolo *et al.,* 2007:49). Oxidative stress and a weakened antioxidant enzyme defense system therefore contribute to vascular cell dysfunction that will not protect the atherosclerotic process (Kondo *et al.,* 2009:536).

2.2.2 Cancer

Cancer is a leading cause of death all over the world (Sugimura, 2002:17). According to the American Institute for Cancer Research there are more than 1.4 million new cancer diagnoses each year in the United States and approximately 600,000 deaths. Worldwide, about 10 million cancer diagnoses occur each year, and the number is increasing rapidly (Basu, Vecchio, Flider & Orthoefer, 2001:665). It is estimated that 88% to 90% of human cancers are environmentally induced and approximately 35% by diet (Willcox *et al.*, 2004:285). Dietary choices, together with exercise and a healthy body weight, could prevent three to four million cancer cases worldwide each year (Basu *et al.*, 2001:665). In South African males, the approximate lifetime risk of developing cancer, excluding skin cancer is one in seven persons for the black population, one in five for the coloured population and one in four for the white and Indian populations, while the approximate lifetime risk of developing cancer, excluding skin cancer in South African females, is one in eight persons for the black population, one in seven for the coloured population, one in five for the Indian population and one in four for the white population (Love & Sayed, 2001:S27).

Carcinogenesis is a complex multi-sequence process leading a cell from a healthy to a precancerous state and lastly to an early stage of cancer (Valko, Rhodes, Monocol, Izakovic & Mazur, 2006:19). Many common cancers develop as a consequence of years of chronic inflammation. Microbial presence in or around epithelial cells may lead to a chronic inflammatory state resulting in increased epithelial cell turnover. The joint effects of increased inflammation and epithelial cell turnover can promote the phenotypic and genotypic changes that may eventually progress to malignant transformation (Moss & Blaser, 2005:90). Cellular enzymes, structural proteins and membranes, simple and complex sugars, and DNA and ribonucleic acid (RNA) are all susceptible to oxidative damage that leads to tumour initiation (Lee & Lee, 2006:424). Cytokines, chemokines and free radicals initiate and spread inflammatory responses (Moss & Blaser, 2005:91).

There are three distinct stages of the carcinogenesis process, namely initiation, promotion and progression. Initiation involves the formation of a mutated, preneoplastic cell from a genotoxic event. The formation of the preneoplastic, initiated cell is an irreversible, but dose dependent process. Promotion involves the selective clonal expansion of the initiated cell through an increase in cell growth through either an increase in cell proliferation and / or a decrease in apoptosis in the target cell population. The events of the promotion stage are dose dependent and reversible upon removal of the tumour promotion stimulus. Complete carcinogens are both initiators and promoters, whereas incomplete carcinogens require promoters to produce malignant transformations (Kovacic & Jacintho, 2001:774).

Progression involves cellular and molecular changes that occur from the preneoplastic to the neoplastic state. The progression stage is irreversible and involves genetic instability changes in nuclear structure, and disruption of chromosome integrity. Increased replicative DNA synthesis and subsequent cell division is important in each of the stages of carcinogenesis (Klaunig & Karmendulis, 2004:241).

Chemical carcinogens impact on various stages of the developmental process and function through modification of cellular and molecular events. To explain: Chemically induced tumour formation or neoplasia is a multistep process involving DNA damage (initiation process) and cell proliferation. Chemical carcinogens function in different ways in the carcinogenesis process and are either described as genotoxic or epigenetic (nongenotoxic) depending on how these chemicals function. Genotoxic agents usually refer to chemicals that directly damage genomic DNA, which in turn can result in mutation and / or clastogenic changes. Genotoxic chemicals are often activated in the target cell and produce a dose dependent increase in neoplasm formation. The nongenotoxic carcinogens often function during the promotion stage of the cancer process through either non-DNA reactive or indirect DNA reactive mechanisms. The nongenotoxic carcinogens modulate cell growth and cell death. Changes in gene expression and cell growth parameters are principal in the action of nongenotoxic carcinogens (Klaunig & Karmendulis, 2004:239).

Two key mechanisms have been proposed for the induction of cancer. One of the mechanisms involves an increased DNA synthesis and mitosis by nongenotoxic carcinogens that may induce mutations in dividing cells through misrepair. Mutations may then clonally expand from an initiated preneoplastic cell state to a neoplastic cell state. Another mechanism accounts for a balance between cell proliferation and cell death. If the damage to DNA is too vast, apoptosis occurs that eliminates altered cells selectively. In the process of apoptosis, which is a normal physiological process, cells initiate a programmed suicide mechanism to too many morphological changes (Valko, Rhodes, Monocol, Izakovic & Mazur, 2006:19).

An important step in the development of any tumour beyond a few millimetres is the generation of new blood supplies that feed the malignant cells. Angiogenesis is a multi-step process, involving degradation of the endothelial cell basement membrane, endothelial cell migration to the perivascular stroma and capillary sprouting (Valko *et al.*, 2006:21). Tumour cells grow by various mechanisms: (a) the host vascular network expands by budding of endothelial sprouts or formation of bridges; (b) tumour vessels remodel and expand by the insertion of interstitial tissue columns into the lumen of pre-existing vessels; (c) endothelial cell precursors come from the bone marrow or peripheral blood into tumours and contribute to the endothelial lining of the tumour vessels; and (d) lymphatic vessels around tumours

drain the interstitial fluid and provide a gateway for metastasising tumour cells (Carmeliet & Jain, 2000:250).

Experimental evidence supports an important role for ROS in the cancer process. ROS are now considered as a significant class of carcinogens involved in the start of cancer, promotion and progression (Klaunig & Karmendulis, 2004:241). The human cell is exposed to numerous oxidative hits a day from hydroxyl radicals and other such reactive species. The hydroxyl radical is known to react with all components of the DNA molecule damaging both the purine and pyrimidine bases and also the deoxyribose backbone. Permanent alteration of the genetic material resulting from these "oxidative damage" incidents represents the first step involved in mutagenesis, carcinogenesis and ageing. RNS such as peroxynitrites and nitrogen oxides, have also been implicated in DNA damage (Valko *et al.,* 2006:8). Increases in reactive oxygen in the cell, through either physiological modification or through chemical carcinogen exposure, greatly contribute to the carcinogenesis process. This may be via genotoxic effects resulting in oxidative DNA adducts or through modification of gene expression (Klaunig & Karmendulis, 2004:241). Evidence exists for the involvement of mitochondrial oxidative DNA damage in the carcinogenesis process. Hydrogen peroxide and other ROS have been implicated in the activation of nuclear genes that are involved in mitochondrial biogenesis, transcription and replication of the mitochondrial genome (Valko *et al.,* 2006:8).

A role for ROS production and oxidative stress has been proposed for both the stimulation of cell proliferation during the tumour promotion stage of carcinogenesis and for cell depletion by apoptosis (Klaunig & Karmendulis, 2004:249). The effects of ROS and oxidative stress within cells appear to be cell specific and dependent upon the form as well as the intercellular concentration of ROS. Therefore, the involvement of ROS in cell growth regulation is complex, and dependent on a number of cellular and biochemical parameters (Klaunig & Karmendulis, 2004: 250).

2.2.3 Human Immunodeficiency Virus

HIV infection is a worldwide problem and HIV / AIDS patients suffer from many opportunistic infections that occur because of their poor immune system functioning. The main characteristic of HIV infection is cellular CD4 T cell immunodeficiency (Gil, Martinez, Gonzalez, Tarinas, Alvarez, Giuliani, Molina, Tapanes, Perez & Leon, 2003:217). AIDS is the terminal phase of HIV infection when it runs its natural course (Stehbens, 2004:125).

The HIV virus is caused by two groups of cytopathic viruses, HIV – 1 and HIV – 2 (Stehbens, 2004:125). During the course of infection with HIV, three major phases may be distinguished within a few weeks post infection; firstly extensive viremia occurs, accompanied by large numbers of infected CD4 T cells with lastly serious clinical symptoms arising. During the first phase, an activation of the cellular immune system can be observed, for example by increased neopterin, which is secreted by interferon-y (IFN-y) activated monocytes or macrophages. When humoral and cellular immune responses to HIV become established, the amount of circulating virus declines, leading to the subclinical phase that is characterised by a lack of symptoms, low levels of virus and circulating infected cells, and moderately declining levels of CD4 T cells. The second phase is a latent phase of variable duration characterised by a constant amount of virus and infected CD4 T cells. This latent phase is not a period of inactivity, but a period of high turnover in CD4 T cells and, at the same time, a high mutation rate of the virus characterised by a marked immunodeficiency in which patients become highly at risk to opportunistic infections usually mild under normal circumstances. Constant virus production in this asymptomatic phase is still taking place and represents one of the most important forces causing AIDS pathogenesis (Baier-Bitterlich *et al.*, 1997:755). This late phase is characterised by a break down of the immune defences and a reduction in CD4 T cell numbers (Romero-Alvira & Roche, 1998:169). On average, ten years after infection AIDS develops, virus and infected cells significantly reappear in the periphery, associated with a sharp decrease in CD4 T cell numbers and eventual disruption of immunological functions (Baier-Bitterlich, Fuchs & Wachter, 1997:756).

Oxidative stress may lead to the progression of HIV infection due to impairment of the immune function, enhancement of HIV replication, or enhancement of apoptosis (Garland & Fawzi, 1999:1260). Oxidative stress occurs in HIV infection even at the early stages of disease. This pro-oxidant state is a result of an imbalance between the generation of reactive oxygen and nitrogen species (RONS) and the antioxidant system. Excessive production of RONS may occur following polymorphonuclear leukocyte activation in infectious conditions and by a pro-oxidant effect of TNF-a produced by activated macrophages. ROS's activate the nuclear transcription factor NF-KB, which is a needed for HIV replication (Treitinger, Spada, Verdi, Miranda, Oliveira, Silveira, Moreil & Abdalla, 2000:454).

The progressive loss of CD4 T cells in HIV infected patients may be related to an oxidative stress induced apoptosis (Treitinger *et al.*, 2000:455). Programmed cell death and latent virus activation may be linked closely to "oxidative stress" caused by excessive production of ROS and a resulting deficiency of antioxidant mechanisms in HIV infection. ROS are always produced during activation of phagocytes as a defense mechanism against environmental pathogens. ROS are equally important for the activation of T cells in the start of antigen specific immune responses (Baier-Bitterlich *et al.*, 1997:758). Chronic oxidative stress is

linked to abnormal immune function, particularly T-lymphocyte function, in HIV infected patients (Treitinger *et al*., 2000:455). A study was conducted to determine the significance of a novel early marker of oxidative stress which can reflect the TAC in HIV patients. Significantly increased levels of serum malondialdehyde were detected in the HIV-1 seropositive patients compared to the control group suggesting lipid peroxidation. Significantly decreased levels of serum vitamin E, vitamin C, SOD enzyme activity along with TAC were also detected in the HIV-1 seropositive patients compared to the control group, suggesting antioxidant depletion. The results clearly showed that severe oxidative stress occurs in HIV-1 seropositive patients in comparison with control subjects, and increased significantly with the progression of the disease (Suresh, Annam, Pratihba & Prasad, 2009:3).

Pace and Leaf (1995:524) provided a review of the possible roles of oxidative stress in the disease pathogenesis which are: (a) Viral replication – The redox imbalance to the oxidant state favours disease progression with increased viral replication, immune dysfunction and carcinogenesis (Stehbens, 2004:126). For example, HIV replication is enhanced under oxidative conditions *in vitro*. ROS activate a nuclear transcription factor NF-KB that is essential for HIV replication. NF-KB also acts as a transcription factor for many inflammatory cytokines of the immune system; (b) Immune response – The immune function is strongly influenced by redox potential (Pace & Leaf, 1995:524). Antioxidant depletion indicates a decrease in immune function. Cells of the immune system generally require a higher antioxidant concentration than other cells to retain redox balance, and preserve the condition and function of the cells (Stehbens, 2004:125). In lymphocytes *in vitro*, for example, oxidative stress and depressed glutathione levels lead to altered cell function, abnormal cytokine production, and an impaired proliferation response. Chronic oxidative stress is linked to abnormal immune function, particularly T-lymphocyte function, in HIV infected patients. Transport of cysteine via cystine into T cells is also inhibited. Further, the decreased intracellular cysteine may result in decreased intracellular glutathione levels, thereby fading resistance to intracellular oxidative stress. Oxidative conditions favour over expression of TNF-a, the IL-2 receptor, and certain cytokines, and decreased cell proliferation. TNF-a activates HIV replication in both T lymphocytes and mononuclear cells; (c) Oxidative stress may induce apoptosis (Pace & Leaf, 1995:524). Apoptosis of cells is fundamental to progression of the disease and correlates with a decrease in plasma zinc, selenium and vitamin E (Stehbens, 2004:125). Excess hydrogen peroxide combined with deficiencies in CAT and GSH peroxidise may lead to overproduction of hydroxyl radicals and lipid peroxides, and subsequently, induce apoptosis; (d) Disease progression – HIV and opportunistic infections directly or indirectly promote oxidative stress. The pro-oxidative conditions cause activation of free radical producing immune cells, enhancement of viral

replication, and weakening of the antioxidant defense system. This cycle becomes domineering and promotes disease progression; (e) Weight loss – Unintentional weight loss is one of the most common symptoms associated with HIV infection (lean body mass is consistently lost rather than fat). Both chronic and serious weight loss occurs during HIV infection. Many mechanisms contribute to the nature, rate and extent of the weight loss (Pace & Leaf, 1995:524). The gut is a major target for AIDS related diseases and conditions like diarrhoea, oral and oesophageal candidiasis, dysphagia and odynophagia further complicate the nutritional imbalance in the AIDS patient (Sepulveda & Watson, 2002:27). Culmative effects of oxidative stress and depletion increase anorexia that is accentuated by alcohol, other xenobiotics and periodic infections (Stehbens, 2004:125). The resting energy expenditure (REE) is elevated in infected patients and increases as the disease progresses. Elevated REE reflects a higher oxidant load and is observed in the absence of opportunistic infections and short term weight loss. The underlying cause of REE and hyper metabolism is possible due to the combination of effects of disturbed patterns in cytokine and hormone production, and oxidative stress (Pace & Leaf, 1995:524).

Patients with HIV infection develop a wide range of difficulties, some of which could be linked to the patient's chronic oxidative stress and / or GSH depletion. Oxidative stress also increases the probability of organ dysfunction. In HIV infected patients, renal and hepatic dysfunction may be exacerbated by GSH exhaustion pro-oxidant conditions (Pace & Leaf, 1995:524).

Low levels of antioxidants such as cysteine and GSH may aggravate this process. The high level of antigen and cytokine activity in HIV / AIDS results in an increased production of ROS, therefore superoxides, hydrogen peroxide and hydroxyl radicals. Antioxidant defences such as important reactive oxygen scavenger enzymes, manganese SOD and CAT, may thereby be overwhelmed. As a result, HIV infected patients often have decreased plasma cystine and cysteine concentrations, decreased intracellular GSH, which is an important radical scavenger, and elevated plasma concentrations of glutamate, which is a competitive inhibitor of the membrane transport of cystine. Elevated glutamate levels and decreased cystine and cysteine levels in HIV infected persons could even be responsible for the selective decrease of CD4 T cells (Baier-Bitterlich *et al.*, 1997:759).

2.3 Natural foods versus dietary supplements as dietary antioxidant sources

Food provides not only essential nutrients needed for life, but also other bioactive compounds for health promotion and disease prevention (Liu, 2003:517S). Epidemiological studies have consistently shown that a high dietary intake of fruits, vegetables, herbs as well as whole grains is strongly associated with reduced risk of developing chronic diseases, such as cancer and CVD (Papas, 1999:1000; Liu, 2004:3479S). Although plant-derived foods vary in their nutritional composition, they generally are good sources of important nutrients (i.e. fiber, vitamins and minerals) and of many less well-characterised bioactive compounds referred to as phytochemicals (Marchand, 2002:296). Phytochemicals are defined as bioactive nonnutrient compounds in fruits, vegetables, grains, and other plant foods that provide desirable health benefits beyond basic nutrition (Premier, 2002:S198; Liu, 2004:3479S). Phytochemicals could provide health benefits such as: (a) substrate for biochemical reactions; (b) cofactors for enzymatic reactions; (c) inhibitors of enzymatic reactions; (d) absorbents that bind to and reduce unwanted constituents in the intestine; (e) lignands that agonise or antagonise cell surface or intracellular receptors; (f) scavenging of reactive or toxic chemicals; (g) compounds that increase the absorption and / or stability of essential nutrients; (h) selective growth factors for useful gastrointestinal bacteria; (i) fermentation substrates for useful oral, gastric or intestinal bacteria; and (j) selective inhibitors of harmful intestinal bacteria (Rincon-Leon, 2003:2831). Plant-based foods, such as fruit, vegetables, whole grains and other plant foods, have been linked to reductions in the risk of major chronic diseases (Liu, 2003:517S).

Plant foods are valuable sources of antioxidant constituents like polyphenols and carotenoids, antioxidant vitamins and minerals (Marchand, 2002:296). These antioxidant nutrients and phytochemicals, when included in the diet, offer protection against a variety of oxidation related diseases (Kelawala & Ananthanarayan, 2004:511). A varied and balanced diet should provide sufficient amounts of all nutrients. A marginally deficient intake may happen when food choices results in an unbalanced diet, if the diet is naturally poor in specific nutrients, if a specific nutrient is lost during processing, handling or storage or because of a physiological condition, requirements of a specific nutrient are increased (Serra-Majem, 2001:101). This is also applicable to the provision and deficiency of phytochemicals.

The protective effects of fruits and vegetables against chronic diseases are attributed to a combination of several components in these foods (Williamson, 1996:6). The additive and

synergistic effects of phytochemicals in fruit and vegetables are generally responsible for their potent antioxidant and anticancer activities. This explains why no single antioxidant can replace the combination of natural phytochemicals in fruit and vegetables in achieving the health benefits (Liu, 2003:519S). The American Institute for Cancer Research (AICR) and the World Cancer Research Fund advises that five or more servings of fruits and vegetables must be consumed daily to reduce the risk of certain cancers (Norman, Butrum, Feldman, Heber, Nixon, Picciano, Rivlin, Simopoulos, Wargovich, Weisburger & Zeisel, 2003:3794S). A report by the World Cancer Research Fund and AICR in which extensive collection of worldwide research on this topic was reviewed, estimated that "diets high in vegetables and fruits (more than 400g per day) could prevent at least 20% of all cancer incidences" (Van Duyn & Pivonka, 2000:1512).

Many consumers are unaware of the health benefits of whole grains (Slavin, 2004:2). Whole grains are made up of the endosperm, the germ, and the bran of the grain. The endosperm makes up about 80% of the whole grain, while the germ and bran components vary among different grains (Slavin, Jacobs, Marquart & Wiemer, 2001:780). Whole grains are rich in phytonutrients, vitamins, minerals, unsaturated fatty acids, tocotrienols, tocopherols, insoluble fibre, phyosterols, stanols, sphingolipids, phytates, lignans and antioxidants like phenolic acids (Jones, Reicks, Adams, Fulcher, Weaver, Kanter & Marquart, 2002:294). Whole grains contain a unique nutrient content at a level similar to that of fruit and vegetables that may work synergistically together to reduce the risk of chronic diseases (Cleveland, Moshfegh, Albertson & Goldman, 2000:331S; Slavin, 2004:5). The protective effects of whole grains may depend on the presence or interaction of these numerous biologically active constituents (Slavin, Martini, Jacobs & Marquat, 1999:460S; Mc Keown, Meigs, Liu, Wilson & Jacques, 2002:390).

Whole grain products are relatively high in antioxidant activity (Cleveland *et al.*, 2000:331S; Slavin, 2004:5). Antioxidants found in whole grains are water soluble, fat soluble and approximately one half are insoluble. Antioxidant activity is also increased in grain based foods by browning reactions during the baking and toasting process (Slavin, 2004:5). In the refining process, the bran and germ are separated from the endosperm (lavin *et al.,* 2001:780). Refined grain foods contain lower amounts of vitamin E, fiber, magnesium and phytochemicals than do whole grain foods due to the removal of the bran (Slavin *et al.*, 1999:460S; Mc Keown *et al.,* 2002:390). Three of the recommended six servings of the food grouping bread, cereal, rice and pasta should be wholegrain (Jones *et al.*, 2002:294).

Drinking water is the preferred beverage to fulfil daily water needs. According to the proposed beverage guidance system the other beverages advised to be consumed on a daily basis is tea, coffee and fruit juice. Water consumption is necessary for metabolism and

for normal physiologic functions and may provide essential minerals such as calcium, magnesium and fluoride (Popkin, Armstrong, Bray, Caballero, Frei & Willet*,* 2006:530). Epidemiological studies show that a moderate consumption of coffee appears to benefit the cardiovascular system. Polyphenols are the components in coffee that have potential cardiovascular benefits via antioxidant mechanisms related to LDL oxidation as nitric oxide (NO) bioavailability and blood pressure lowering (Bonita, Mandarano, Shuta & Vinson*,* 2007:196). The recommended number of servings of coffee per day is one to two cups (Elhatton, 2002:21).

Dietary recommendations for healthy eating include the consumption of fruit juices with the beneficial health effects being due to the vitamin C content, which may inhibit the development of major clinical conditions including heart disease and certain cancers. Many fruit juices also contain phenolic compounds and carotenoids, some of which have antioxidant potential and whose intakes have also been inversely associated with heart disease and cancers. A study was conducted to determine the antioxidant potential of fruit juices. The carotenoid concentrations of the fruit juices were low, but all the fruit juices contained phenolics and the juices derived from citrus fruits were rich in vitamin C (Gardner, White, Mc Phail & Duthie*,* 2000:471). According to the 5-A-Day programme, one of the five or more daily servings of fruit and vegetables could be one serving of fruit juice (Popkin *et al.,* 2006:529).

Tea has been reported to posses various pharmalogical and anticancer effects (Hakim*,* Weisgerber, Harris, Balentine, van-Mierlo & Paetau-Robinson*,* 2000:1715). The anticancer properties of tea are well known, and the tumour inhibition potential of certain polyphenolic compounds from green and black tea has been well documented (Yao, Jiang, Shi, Tomas-Barberan, Datta, Singanusong & Chen, 2004:119). The flavonol compounds in tea epigallo- and epicatechins are the compounds responsible for the biological effects (Miller & Begona Ruiz-Larrea*,* 2002:43). The advised consumption for tea is four to six servings daily (Elhatton, 2002:21).

A dietary supplement is defined as a product (other than tobacco) intended to supplement the diet that bears or contains one or more of the following dietary ingredients: (a) a vitamin; (b) a mineral; (c) a herb or other botanical; (d) an amino acid; (e) a dietary substance for use by man to supplement the diet by increasing the total dietary intake; and (f) a concentrate, metabolite constituent, extract or combination of any ingredient described in clause (a), (b), (c), (d) or (e). There are three categories of dietary supplements: nutrient supplement ingredients, botanical supplement ingredients and other dietary substances (Marriott, 2000:1731S).

The use of dietary supplements in the United States has escalated in the past decade, motivated by the public's want to exert control over their health and by the mistaken belief that the safety of dietary supplements is assured by the Food and Drug Administration (FDA). The marketing of largely unregulated herbal dietary supplements presents a significant risk to public health. All persons taking non standardised, untested dietary supplements are thus at risk of encountering adverse effects, with certain populations being at significantly increased risk, such as those taking prescription drugs together with herbal remedies. A further concern is the abundance of misleading information on herbal supplements (Grollman, 2005:185).

It is now believed that dietary supplements do not have the same health benefits as a diet rich in fruits and vegetables because taken alone, the individual antioxidants, studied in clinical trials, do not appear to have consistent protective effects. Phytochemicals differ in molecular size, polarity, and solubility, and these differences may affect the bioavailability and distribution of each phytochemical in different macromolecules, subcellular organelles, cells, organs and tissues. The natural combination of phytochemicals in fruits and vegetables is responsible for its potent antioxidant activity (Liu, 2004:3483S). Pills or tablets simply cannot copy this balanced natural combination of phyochemicals present in fruit and vegetables. The isolated pure compound either loses its bioactivity or may not react the same way as the compound in whole foods (Liu, 2003:518S). Antioxidants act not in isolation but in combination, and synergistic and antagonistic effects are very hard to predict (Collins, 2005:1924). The health benefits of natural phytochemicals at the low levels in fruits and vegetables do not mean these compounds are more effective or safe when they are consumed at higher doses. Higher doses of these compounds increase the risk of toxicity (Liu, 2004:3484S). Optimal preventative effects may be expected with a combination of nutrients at levels similar to those found in a healthy diet (Hercberg *et al*., 1998:513).

2.4 Consumption of natural antioxidant sources by South Africans

The majority of South Africans do not achieve the recommended daily intake of five portions (400 g) of vegetables and fruits (Love & Sayed, 2001:S29). Regional and *ad hoc* food and nutrient studies of black rural and urban dwellers consulted by Love and Sayed (2001:S29) describe black rural dwellers as eating two main meals a day consisting of mealie meal with green leafy vegetables, wild spinach or pumpkin. When available, some fruits are eaten, and usually only by black rural women and children. Black urban dwellers also eat vegetables and fruits in small amounts, and also usually one small portion twice a day, with women

consuming notably more vegetables and fruits than men. Some studies consulted reported negligible vegetable and fruit intakes among black urban dwellers, such as in the Cape Peninsula where 29% of black adults (aged 15 to 64 years) reported eating no vegetables or fruits in the previous 24-hour period. Qualitative descriptions found by these authors also indicate that Indian and white urban dwellers eat vegetables in small amounts at the two main meals. A variety of fresh, frozen and canned vegetables as well as fresh fruit and fruit juices are eaten by the Indian and white urban dwellers, but as indicated in small amounts, and about two to three times a week (Love & Sayed, 2001:S29).

Available data indicate that especially white, coloured and Indian populations in South Africa could benefit by increasing their intakes of cereals and grains, and that all South Africans should eat more of their cereals and grains in an whole grain or minimally processed form (Vorster & Nell, 2001:S19). A 24-hour dietary recall study reported by Vorster and Nell (2001:S19) determined the average daily carbohydrate intake, among other nutrients, of adult South Africans aged 25 to 65 years. The black population still had the highest carbohydrate intake, followed by the coloured population. The black and coloured populations generally consume adequate amounts of carbohydrates. However, the white and Indian populations should be encouraged to consume more carbohydrates in relation to other macronutrients in their diet (Vorster & Nell, 2001:S19).

Food consumption results was summarised to determine the average intakes of foods and beverages commonly consumed by South Africans. The average g per person per day of those consuming the items were the following for the three beverage choices: coffee 436.5 g, tea 412.2 g and fruit juice 333.3 g (Nel & Steyn, 2002:92-95) which was above the daily suggested intakes of the proposed beverage guidance system for the age group ten and older for fruit juice as one serving (Popkin *et al.,* 2006:529) and coffee as one to two cups (Elhatton, 2002:21). The tea consumption of 2.29 servings (412.2 g) per day was less than the advised servings of four to six cups daily (720 g – 1080 g) (Elhatton, 2002:21) with one cup as a serving of 180 g.

2.5 Exogenous food antioxidants and disease prevention

Dietary antioxidants may be nutrients or non-nutrients (Lindley, 1998:336). The most abundant antioxidants contained in fruits and vegetables include vitamin C, carotenoids, and phenolics. Tocopherols and tocotrienols are also important phytochemical antioxidants, but they are present in relatively low levels in fruits and vegetables as compared to nuts and grains (Kalt, 2005:R11).

2.5.1 Non-nutrient antioxidants

Non-nutrient antioxidants include polyphenols, phenolic flavonoids and carotenoids (Lindley, 1998:336).

2.5.1.1 Flavonoids

(i) Description, types and food sources

Flavonoids belong to a vast group of polyphenolic compounds that are widely distributed in all foods of plant origin (Ross & Kasum, 2002:20). Polyphenols are secondary plant metabolites that give fruits and vegetables both desirable and undesirable food qualities (Kaur & Kapoor, 2001:706). Polyphenols arise biogenetically in plants from two main synthetic pathways: the shikimate pathway and the acetate pathway (Ross & Kasum, 2002:20). Flavonoids are a subclass of polyphenols, characterised as containing two or more aromatic rings, each bearing at least one aromatic hydroxyl ring and connected with a carbon bridge. For flavonoids, this bridge consists of three carbons that combines with an oxygen and two carbons of one of the aromatic rings (A ring) to form a third six-member ring (C ring). Flavonoids are further grouped into subclasses based on the connection of the B ring to the C ring, as well as the oxidation state and functional groups of the C ring. Within each subclass, individual flavonoids and isoflavones are identified and characterised by hydroxylation and conjugation patterns of the B ring, as well as the conjugation patterns of hydroxyls on the A and C rings (Beecher, 2003:3248S). Flavonoids are produced as the result of the secondary metabolism of plants and are frequently found attached to sugars (glycosides), rendering them water soluble (Ross & Kasum, 2002:20). It is known that the degree of glycosylation significantly affects the antioxidant properties of the compound, for example, aglycones of quercetin and myricetin are more active than their glycosides (Kaur & Kapoor, 2001:707). Occasionally, polyphenols also occur in plants as aglycones (Ross & Kasum, 2002:20).

Being plant phytochemicals, flavonoids cannot be synthesised by humans and animals. More than 5000 different plant derived flavonoids have been isolated from various plants. Flavonoids are classified into at least ten chemical groups (Yao *et al*., 2004:115). The six major subclasses of flavonoids include the flavones (apigenin, luteolin), flavonols (quercetin, myricetin), flavanones (naringenin, hesperidin), catechins or flavanols (epicatechin, gallocatechin), anthocyanidins (cyanidin, pelargonidin) and isoflavones (genistein, daidzein)

(Ross & Kasum, 2002:19). These six major subclasses are particularly common in the diet (Yao *et al*., 2004:115).

Flavonoid distribution in plants depends on a number of factors including variation according to plant family and population variations within species. The distribution pattern further depends on the degree of accessibility to light and previous illumination because formation of the higher oxidised flavonoids is accelerated by light. In leafy vegetables and fruits, flavonols are almost exclusively present as glycosides (Adherne & O'Brien 2002:76). Flavonols and flavones are located predominantly in the leaves and in the outer parts of the plants, while only trace amounts can be found below the soil surface. For example, approximately 90% of quercetin is localised in the first and second layers of the onion (Ewald, Fjelkner-Modig, Johansson, Sjoholm & Akesson, 1999:231).

Its been reported that tea, onions and apples make the greatest contribution of antioxidant flavonoids to the Western European diet by the asset of their content and their frequency of consumption (Kalt, 2005:R13). Other than water, tea (*Camellia Sinensis)* is consumed more than any other beverage worldwide (Kris-Etherton & Keen, 2002:42). Tea is made from the dried leaves of the plant *Camellia Sinensis*. There are three main types of tea: black tea, green tea and oolong tea. The first step in the production of black tea is allowing the tea leaves to dry. The black tea leaves are then rolled and crushed, which starts fermentation. Oolong tea is made in the same way as black tea, except that shortly after rolling the leaves, the leaves are fired to stop the oxidation process and dry the leaves. Green tea is made from freshly harvested leaves that are rapidly steamed or pan-fried. This inactivates the enzymes present in the leaves, which prevents fermentation (Brannon, 2006:2).

The main flavonoids in tea are the four catechins and gallocatechins namely, epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG). Together they constitute 30% to 50% of the solids in a green tea infusion, but decrease to less than or equal to ten percent after fermentation to black tea, with a simultaneous rise in the polymeric theaflavins (TFs) (three to six percent) and thearubigens (TRs) (ten percent to thirty percent). Flavonols and flavonol glycosides are also present at levels around five percent (Dreosti, 2000:692). The main flavonols in tea leaves are quercetin, kaempferol and myricetin (Wang, Provan & Helliwell, 2000:152) that are also the three most common flavonols (Yao *et al.,* 2004:115). These flavonols make up two to three percent of the water soluble extractive in tea (Wang *et al*., 2000:152).

Tea catechins are structurally, primarily flavanols, and these form 20% to 30% of the dry weight of green tea. Catechins are colourless, water soluble compounds which impart bitterness and astringency to green tea infusions. Almost all of the characteristics of manufactured tea, including its taste, colour, and aroma, are associated directly or indirectly

with modifications to the catechins. In the manufacture of black tea, catechins can be oxidised to form the typical colour and flavour of black tea. Characteristically the pigments of black tea have been divided into orange coloured TFs and brownish TRs. There are four main TFs, theaflavin, theaflavin 3-gallate, theaflavin 3-gallate, and theaflavin 3.3-digallate in black tea, formed through the reaction between quinines derived from a simple catechin and a gallocatechin. The content of TFs in black tea is minimal to two percent on a dry weight basis, while the TRs fraction comprises ten percent to twenty percent of the matter. TRs together with TFs contribute to the tea brew characteristics such as colour, strength and body (Wang *et al.*, 2000:153).

Rooibos (*Aspalathus Linearis)* leaves become reddish brown when the shredded plant material is “fermented” in heaps, before sun-dried. Shredding of the shoots starts enzymatic oxidation of polyphenols, leading to rapid browning. The wetting of the heap, followed by bruising is necessary to accelerate the “fermentation” process. In the manufacture of green / unfermented rooibos, oxidative changes are kept to a minimum (Joubert, Gelderblom, Louw & Beer, 2008:387). Unfermented rooibos is produced by spreading the shredded plant material in a thin layer in the sun for quick drying (Joubert *et al.,* 2008:388). In the manufacture of fermented (traditional) rooibos the dihydrochalcones, aspalathin and nothofagin, are oxidised rapidly as soon as the plant material is shredded. Aspalathin, a potent antioxidant, is oxidised to flavanones. Unfermented (green) rooibos herbal tea’s manufacturing process maximises the aspalathin content and the antioxidant potential of rooibos (Schulz, Joubert & Schutze, 2003:540). The monomeric flavonoid composition of rooibos is unique in that it contains aspalathin (Joubert *et al.,* 2008: 389). Rooibos is considered a low tannin beverage, especially when compared to *Camellia Sinensis* teas (Joubert *et al.,* 2008:389).

Onions have a high content of flavonoid antioxidants, in particular the flavonol quercetin and its glycosides. Among 12 onion cultivars, including yellow, red, and white types, the total flavonoid content ranged from about one to nine hundred and eighty milligram (mg) per kilogram (kg). Yellow onions had the highest flavonoid content, whereas white onions had the lowest levels (Kalt, 2005:R13). Flavanones are mainly found in citrus fruits and flavones in celery. Catechins are present in large amounts in red wine, whereas anthocyanins are found in strawberries and other berries. Isoflavones are almost exclusively found in soy foods (Yao *et al.*, 2004:115).

(ii) Absorption and storage uptake

Flavonoids present in foods were considered non-absorbable because flavonoids are bound to sugars as β-glycosides. Only free flavonoids without a sugar molecule were thought to pass through the gut wall. Hydrolysis, however, occurs in the colon by microorganisms, which at the same time degrade flavonoids (Hollman & Katan, 1997:305).

Flavonoids are absorbed by passive diffusion after glycosylated flavonoids are converted to their aglycones. The colon microflora play an important role in this conversion. The bioavailability of flavonoids is only partial with the proportion of the ingested amount that is absorbed varying from minimal to less than one percent for tea catechins to 20% of quercetin and isoflavones. A large amount of flavonoids remain unabsorbed and the gastrointestinal mucosa is exposed to particularly high concentrations of these compounds. After absorption, the flavonoids are conjugated in the liver by glucuronidation, sulfation or methylation or metabolised to smaller phenolic compounds (Marchand, 2002:297).

(iii) Beneficial health effects

Flavonoids have so far been found to demonstrate a wide variety of pharmacological properties, including antioxidative, antiallergic, anti-inflammatory, antidiabetic, hepato-and gastro-protective, antiviral and antineoplastic activities. Flavonoids have been given considerable attention because of the beneficial effect as antioxidants in the prevention of human diseases such as cancer and CVD, and some pathological disorders such as gastric and duodenal ulcers, allergies, vascular fragility, and viral and bacterial infections (Yao *et al.*, 2004:117). Many of the pharmacological effects of flavonoids are related to its interaction with several enzymes and to its antioxidant activity, which can be due to the ability to scavenge free radicals, to chelate metal ions and the synergistic effects with other antioxidants (Silva, Santos, Caroco, Rocha, Justino & Mira, 2002:1219).

In vitro and animal studies have demonstrated that flavonoids have antioxidant and antimutagenic activities and may respectively reduce the risk of CVD and stroke, as well as cancer. Flavonoids may therefore act as antioxidants to inhibit free radical-mediated cytotoxicity and lipid peroxidation, as antiproliferative agents to inhibit tumour growth or as weak estrogen agonists or antagonists to modulate endogenous hormone activity. Isoflavonoids, such as phytoestrogens, have a wide range of hormonal activities in animals or *in vitro*, suggesting potential human health benefits of diets rich in these compounds (Yao

et al., 2004:117). It has been demonstrated that flavonoid compounds in tea have very strong antioxidant and free radical scavenging activities, and are much more effective than vitamin C and vitamin E at protecting cells from free radical damage. Tea or tea catechins has reported effects on coronary heart disease and inhibition of carcinogenesis in experimental animals. These inhibitions were observed at all three levels of the cancer stages namely, initiation, promotion and transformation. This raises the possibility that tea drinking my reduce the incidence of both cancer and heart disease in humans. Residents of Shizuoka, Japan, where green tea is produced and consumed, were shown to have lower mortality rates from stomach, lung and liver cancer than a comparable population in non-green tea consuming areas (Wang *et al*., 2000:154).

2.5.1.2 Carotenoids

(i) Description, types and food sources

Carotenoids, the basic source of yellow, orange and red plant pigments, are widely distributed in nature (Basu, Vecchio, Flider & Orthoefer, 2001:665). Carotenoids are a group of more than 600 naturally occurring coloured pigments that are widespread in plants, but only 20 of which commonly occur in human food. Certain carotenoids are the precursors of vitamin A and those commonly occurring carotenoids in nature include α-, β- and y-carotene, lycopene and cryptoxanthin (Gayathri, Platel, Prakashi & Srinivasan, 2004:35). All carotenoids posses a polyisoprenoid structure, a long conjugated chain of double bonds and a near bilateral symmetry around the central double bond. Different carotenoids are derived essentially by modifications in the base structure by cyclisation of the end groups and by introduction of oxygen functions giving carotenoids their characteristic colours and antioxidant properties (Rao & Rao, 2007:208). These include β-carotene, lycopene and lutein (Hughes, 2001:823). β-carotene and lycopene are hydrocarbons and belong to a class of carotenoids called carotenes that are fat soluble. Lutein and zeaxanthin belong to a class of carotenoids known as xanthophylls. As xanthophylls contain at least one hydroxyl group, they are more polar than the carotenes (Krinsky & Johnson, 2005:461).

Similar to vitamin C, the content of various carotenoids varies considerably among fruits and vegetables (Kalt, 2005:R11). β-carotene accounts for more than 90% of total carotenoids in vegetables (Gayathri *et al*., 2004:35). Coloured antioxidants (for example, carotenoids and anthocyanins) often reach their highest level when the fruits are at their optimal ripeness (Kalt, 2005:R11). Typically 50 to 60 carotenoids are present in the human diet rich in green,

yellow/red, or yellow/orange vegetables (Dembinska-Kiec, 2005:93). In green vegetables β-carotene is incorporated in the carotenoid-protein-complexes in the chloroplasts. These carotenoproteins have an inhibitory effect upon carotenoid digestion and absorption. Whereas, in orange or red fruits β-carotene is dissolved in oil droplets in the chromoplast and can be easily removed during digestion (Bernhardt & Schlich, 2006:327).

The most abundant carotenoids in human plasma are β-carotene, lycopene, lutein, cryptoxanthin, α-carotene and zeaxanthin (Dembinska-Kiec, 2005:93). β-carotene is the most widely studied carotenoid and is one of the major carotenoids in the diet and in human blood and tissues (Krinsky & Johnson, 2005:461). Of the 50 different carotenoids that can be metabolised into vitamin A, β-carotene has the highest provitamin A activity. In carrots, β-carotene makes up about 45% to 80% of the total carotenoids present (Kalt, 2005:R13). Red fruits and vegetables, including tomatoes, watermelons, pink grapefruits, apricots and pink guavas contain lycopene (Rao & Agarwal, 2000:563). Dietary lycopene is derived predominantly from tomatoes and tomato products (Krinsky & Johnson, 2005:461). Processed tomato products, such as juice, tomato sauce, tomato paste and soup all are good dietary lycopene sources. Lycopene from processed tomato products appears to be more bioavailable than that of raw tomatoes. The release of lycopene from the food matrix due to processing, the presence of dietary lipids and heat induced isomerisation from an all trans to a cis conformation enhances lycopene availability. Lycopene, ingested in its natural trans form found in raw tomatoes is poorly absorbed (Rao & Agarwal, 2000:563). The bioavailibility of lycopene is also affected by the dosage and the presence of other carotenoids (Agarwal & Rao, 2000:742). The two foods found to have the highest amount of lutein and zeaxanthin are spinach and kale. Other major sources include broccoli, peas and Brussels sprouts (Krinsky & Johnson, 2005:461). Due to the unsaturated nature of the carotenoids they are subject to changes due mainly to oxidation (Rao & Rao, 2007:209).

(ii) Absorption and storage uptake

Other than β-carotene and lycopene the absorption of the other major carotenoids is not well known. Many factors influence the absorption of carotenoids. Food processing and cooking that causes mechanical breakdown of the tissue releasing the carotenoids improves their absorption. Carotenoids are absorbed into the gastrointestinal mucosal cells and appear unchanged in the circulation and tissues. In the intestine the carotenoids are absorbed by passive diffusion after being incorporated into the micelles that are formed by dietary fat and bile acids. The micellular carotenoids are then incorporated into the chylomicrons and

released into the lymphatic system (Rao & Rao, 2007:209). As lipid soluble compounds, carotenoids are absorbed with chylomicrons from the gut and incorporated into lipoproteins at the site of the liver and released into the blood stream. Different tissues absorb carotenoids differentially. The major site of tissue storage of carotenoids is the adipose tissue (Dembinska-Kiec, 2005:93; Rao & Rao, 2007:209).

(iii) Beneficial health effects

Carotenoids have been known to have beneficial properties for human health (Rao & Rao, 2007: 211). The only biological function of the carotenoids, which explains consideration of these compounds as micronutrients as well as phytochemicals, is their potential role as vitamin A precursors. Provitamin A carotenoids are the predominant dietary source of vitamin A, and deficiency of this vitamin remains a major nutrition problem in most economically disadvantaged areas of the world (Rock, Jacob & Bowen, 1996:699).

Results from numerous epidemiologic and laboratory studies suggest that carotenoids may be important in the prevention of several types of cancer and consumption of a carotenoid rich diet after a diagnosis of cancer has been associated with a more favourable prognosis (Rock *et al.,* 1996:699). The results of ten of seventeen case control studies show that a high intake of fruits and vegetables that are rich in carotenoids has been associated with decreased risk of cancer at a number of common sites. This association appears to be strongest for lung and stomach cancer. Although other types of cancers have been evaluated, for example breast cancer with a reduced risk with increased dietary intake, the relationship between β-carotene and lung cancer has been most studied and the data has been more consistent (Johnson, 2002:57).

In the past several years, evidence have supported a role of lycopene in the prevention of certain malignancies, especially prostate cancer (Giovannucci, 2005:2030S). Dietary lycopene may increase the lycopene status in the body and acting as an antioxidant may trap ROS, increasing the overall antioxidant potential thereby lowering oxidative stress and reducing the oxidative damage to lipid, proteins and DNA. This reduced oxidative stress may lead to reduced risk for cancer and CVD. Alternatively, the increased lycopene status in the body may regulate gene functions, improve intercell communication, modulate hormone and immune responses, and regulate the metabolism thus lowering the risk for chronic diseases (Agarwal & Rao, 2000:741; Giovannucci, 2005:2030S). One of the earliest case control studies examining the relationship between lycopene rich foods and cancer risk reported that weekly tomato consumption was associated with a 40% reduction in risk for oesophageal

cancer (Cook-Mozaffari, 1979:292). Several studies have also reported protective effects for diets rich in tomato products against gastric cancer (Tsugane, Tsuda, Gey & Watanabe, 1992:207; Franceschi, Bidoli, La Vecchia, Talamini, D' Avanzo & Negri, 1994:181). A study of prospectively collected serum samples from a cohort was used to compare serum micronutrients from those developing pancreatic cancer and matched controls. The greatest difference was observed for lycopene. However, because lycopene uptake and serum concentrations may be related to the digestibility of dietary lipids, diseases of the pancreas could significantly reduce absorption and may have confounded the results of such studies. In a single study to examine the role of tomatoes, lycopene and bladder cancer risk, serum micronutrient profiles were quantitated, and only lycopene and selenium were found to be inversely related to pancreatic cancer risk (Helzlsouer, Comstock & Morris, 1989:6144). In another study when tomato sauce was used as a source of lycopene, providing 30mg lycopene per day for three weeks preceding prostatectomy in men diagnosed with prostate cancer, serum and prostate lycopene levels were elevated significantly. Oxidative damage to DNA was reduced and serum prostate-specific antigen levels declined significantly by 20% with lycopene treatment. Other than prostate cancer there is now growing evidence in support of the protective role of lycopene in cancers of other sights including the breasts, lungs, gastrointestinal tract, cervix, ovaries and pancreas (Giovannucci, 1999:317).

There are many mechanisms by which carotenoids can function in cancer prevention. As provitamin A, carotenoids could have an effect on cellular differentiation and proliferation. Moreover, the antioxidant function could prevent free radical-induced damage to cellular DNA and other molecules (Krinsky & Johnson, 2005:489). Carotenoids are efficient quenchers of singlet oxygen and can directly scavenge free radicals (Rock *et al.,* 1996:699). The process of photoprotection, involves an electron exchange energy transfer between oxygen and a carotenoid to generate the triplet state of the carotenoid and ground state oxygen. The carotenoid formed can then return to its ground state by dissipating its energy through rotational and viberational interactions with the solvent system. Therefore, carotenoids can essentially act as catalysts to inactivate the very dangerous and reactive oxygen radical. However, carotenoids are not perfect catalysts for the above reactions. Chemical reactions between oxygen and carotenoids can also occur producing a variety of oxidised products. Its immunomodulatory effects could enhance immune surveillance in tumourigenesis and enhance cell-to-cell communication that would restrict clonal expansion of initiated cells (Krinsky & Johnson, 2005:489).

2.5.2 Nutrient antioxidants

The major nutrient antioxidants are vitamins C and E, with fruits and vegetables being the major sources of vitamin C and vitamin E compounds, found in wheat germ, nuts, green leafy vegetables and plant oils (Lindley, 1998:336).

2.5.2.1 Vitamin C

(i) Description, types and food sources

Plant foods vary greatly in their vitamin C content (Kalt, 2005:R13). Vitamin C is a water soluble vitamin and comprises of two major forms both present in biological tissue and food, namely L-ascorbic acid and L-dehydroascorbic acid (DHA). Of the two optical isomer forms, only the L form occurs naturally. The D form, isoascorbic or erythorbic acid, provides antioxidant, but little or no anti scorbutic activity (Jacob & Sotoudeh, 2002:67).

The richest sources of vitamin C are citrus and soft fruits, and the growing tips of plant shoots and buds. Animal tissues may be moderate sources (Bates, 1997:S28).

(ii) Absorption and storage uptake

Vitamin C is absorbed in the human intestine through an energy dependent active process that is saturable and dose dependent (Rock *et al.*, 1996:695). The dose dependent active transport process regulates the intestinal absorption of ascorbic acid. At nutritional intakes of 30 mg to 180 mg per day approximately 70% to 90% is absorbed, whereas absorption falls to less than 50% at intakes greater than one gram per day. Vitamin C is transported into cells primarily as DHA, and is reduced intracellularly to ascorbic acid. Vitamin C exists in the body primarily in the reduced form of ascorbic acid (Jacob & Soutoudeh, 2002:66).

(iii) Beneficial health effects

Vitamin C is a cofactor for several enzymes participating in the post translational hydroxylation of collagen, in the biosynthesis of carnitine, in the conversion of the neurotransmitter dopamine to norepinephrine, in peptide amidation and in the tyrosine metabolism. In addition, vitamin C is an important regulator of iron uptake. Vitamin C reduces ferric iron to ferrous iron, thus promoting dietary non-haem iron absorption from the gastrointestinal tract and stabilises iron binding proteins (Duarte & Lunec, 2005:672). Vitamin C is an essential micronutrient required for the normal metabolic functioning of the body. Vitamin C cannot be synthesised in the human body as a result of a mutation in the gene coding for L-gulonolactone oxidase, an enzyme required for the biosynthesis of vitamin C via the glucuronic acid pathway (Carr & Frei, 1999:1086). Thus the prolonged deprivation of vitamin C produces defects in the post translational modification of collagen that causes scurvy and ultimately death (Duarte & Lunec, 2005:672).

In addition to vitamin C's antiscorbutic action, vitamin C is a potent reducing agent and scavenger of free radicals in biological systems (Duarte & Lunec, 2005:672). Vitamin C readily scavenges RONS, such as superoxide and hydroperoxyl radicals thereby effectively protecting other substrates form oxidative damage (Carr & Frei, 1999:1087). The mono anion form (ascorbate) is the main chemical species at physiological pH. Ascorbate readily undergoes two consecutive, yet reversible, one electron oxidation to generate DHA and an intermediate, ascorbyl free radical (AFR). AFR is, however, a relatively unreactive free radical, with a reduction potential considerably low compared to the α-tocopherol radical, the GSH radical and almost all RONS that play a role in human disease (Duarte & Lunec, 2005:672). Vitamin C is an electron donor and therefore a reducing agent. All known physiological and biochemical actions of vitamin C are due to its action as an electron donor. Ascorbic acid donates two electrons from a double bond between the second and third carbons of the 6 – carbon molecule (Padayatty, Katz, Wang, Eck, Kwon, Lee, Chen, Corpe, Dutta, Dutta & Levine, 2003:19).

Vitamin C also has the ability to recycle other important antioxidant molecules such as α-tocopherol and GSH from antioxidant respective radical species. Ascorbate can also be recycled by chemical and enzymatic mechanisms. AFR can be converted back to vitamin C by an NADH-dependent reductase or by dismutation of two molecules of the radical into one molecule of vitamin C and one molecule of DHA. DHA, in turn, is unstable at physiological pH and unless DHA is reduced back to ascorbate, DHA may be irreversibly hydrolysed to 2, 3-diketogulonic acid. DHA can be reduced back to ascorbate either directly by GSH or

enzymatically by a GSH dependent DHA reductase, glutaredoxin, or the NADPH dependent thioredoxin reductase (Duarte & Lunec, 2005:672).

Low levels of plasma vitamin C are known to occur in several conditions of increased oxidative stress, such as cancer, diabetes mellitus, cataract and HIV infection. Vitamin C kills or inhibits growth of tumour cell lines and potentiates the cytotoxicity of radiosensitising drugs. According to Hercberg *et al.* (1998:514) there are several experimental studies showing that cancer cell lines are more sensitive to vitamin C than their non-malignant counterparts. Vitamin C may be useful in the prevention of cancer. The possible mechanisms by which vitamin C may affect carcinogenesis include antioxidant effects, blocking of formation of nitrosamines and fecal mutagens, enhancement of the immune response and acceleration of detoxification of the liver enzymes (Hercberg *et al.*, 1998:514).

Epidemiological evidence has also associated fruit and vegetable consumption with a lower risk of CVD. Notably, low levels of vitamin C were associated with death from CVD and it has been speculated that vitamin C may protect against CVD through several mechanisms. Vitamin C enhances endothelium dependent vasodilation, thereby preventing endothelial dysfunction associated with atherosclerosis, hypercholesterolemia, hypertension, diabetes and smoking. This process involves the ability of vitamin C to increase the atheroprotective NO. Thus vitamin C was shown to enhance the activity of endothelial NO synthase by keeping its cofactor, tetrahydrobiopterin, in a reduced state and thereby increasing its intracellular availability. In addition, vitamin C prevents oxidation of LDL, a critical process during atherosclerosis and CVD. Therefore it decreases damage caused by oxidised LDL to endothelial cells. The pre-treatment of cultured human arterial smooth muscle cells with vitamin C protected against apoptotic cell death induced by oxidised LDL. Vitamin C has also an anti inflammatory action in decreasing leukocyte adhesion to the endothelium. Therefore individuals with low plasma vitamin C levels have greater monocyte adhesion to endothelial cells and express higher levels of monocyte ICAM-1 mRNA (Duarte & Lunec, 2005:672).

2.5.2.2 Vitamin E

(i) Description, types and food sources

Vitamin E is the name given to a family of eight molecules, each consisting of a chromanol ring with an aliphatic side chain. Vitamin E consists of two groups, tocopherols and tocotrienols, based on the side chain being saturated or unsaturated. Within each group,

there are four isoforms α-, β-, y and δ named for specific methyl group substitutions at positions 5, 7 and 8 of the chromanol ring (Tucker & Townsend, 2005:381). Collectively vitamin E consists of a group of isoprenoid compounds of plant origin, which were first described as essential micronutrients for normal fertility in rats. Later studies established a wide range of functions for this vitamin family, especially the RRR-α-tocopherol stereoisomer, the most biologically active form (Mardones & Rigotti, 2004:252). The most important natural occurring compound with vitamin E activity is α-tocopherol (Bernhardt & Schlich, 2006:328). In contrast, y-tocopherol, the most abundant form of dietary vitamin E, has received much less attention, even though recent studies have indicated that it exhibits functional activities distinct from α-tocopherol. Besides vitamin E's role as a free radical chain-breaking antioxidant, α-tocopherol can also directly modulate cellular signalling pathways, mainly protein kinase C, leading to diverse biological responses in different cell types (Mardones & Rigotti, 2004:252).

Most dietary vitamin E is obtained mainly from plant sources including sunflower seeds, olive oil and almonds, which contain high amounts of α-tocopherol, while most other oils and seed oils are rich in y-tocopherol (Tucker & Townsend, 2005:380). In the leaves of the higher plants vitamin E occurs mainly as α-tocopherol, which is found in the chloroplasts. The concentration of α-tocopherol is higher in dark than in light green leaves (Bernhardt & Schlich, 2006:328). Although the majority of western dietary vitamin E consists of y-tocopherol, the α-isoform is predominant in vitamin E supplements in the form of RRR-α-tocopherol or molar equivalent tocopherol esters (Tucker & Townsend, 2005:380). Tocotrienols are usually found in the green parts of higher plants, although negligible amounts of α-tocotrienol have been found in leaves of some such species. Tocotrienols are rather found in high amounts in seeds, the pericarp and specialised cells, like the latex tuber (Munné–Bosch & Alegre, 2002:34).

(ii) Absorption and storage uptake

The absorption of tocopherols is rather inefficient. At physiologic doses, approximately 20% to 40% of α-tocopherol is absorbed, and the percentage of absorption decreases as the dose increases (Rock *et al.*, 1996:695). The biological activity of natural RRR-α-tocopherol is higher than that of the synthetic all *rac* α-tocopherol and other natural forms of vitamin E. Vitamin E is absorbed in the intestine and enters the circulation via the lymphatic system. Vitamin E is absorbed together with lipids, packed into chylomicrons, and transported to the liver and the remnants derived thereof. This process is similar for all forms of vitamin E. Only after passing through the liver does α-tocopherol appear in the plasma. Most of the ingested

β-, y and δ tocopherol is secreted into bile or not taken up and excreted in the faeces (Brigelius-Flohe & Traber, 1999:1149).

(iii) Beneficial health effects

In cell and molecular studies, it has been consistently reported that the oxidant-quenching action of vitamin E provides its beneficial effect by protecting cells from peroxyl radical induced oxidative damage (Dutta & Dutta, 2003:261). Vitamin E's function as an antioxidant is dependent upon its ability to break propagated chain reactions. As the main lipid soluble antioxidant in biological membranes, α-tocopherol reacts with many oxidant molecules. In turn, α-tocopherol helps protect cell membranes from lipid peroxidation by trapping peroxyl radicals. This protection involves the removal of a hydrogen atom from the OH group of tocopherol by a peroxyl (oxidant) molecule. Upon the formation of the tocopheroxyl radical, from a reaction between α-tocopherol and an oxidant molecule, it is now free to interact with another peroxyl radical. The reaction produces a stable tocopheroxyl radical, which does not spread radical chains and lipid hydroperoxides. As the rate constant for this reaction is several orders of magnitude greater then the reaction for peroxyl radical propagation, α-tocopherol is able to efficiently protect cellular membranes at levels as low as one α-tocopherol per 1000 phospholipids. The α-tocopherol can be regenerated from the tocopheroxyl radical by an electron donor, like ascorbic acid (vitamin C), and is thereby able to maintain cellular antioxidant protection over time (Dutta & Dutta, 2003:259).

Many lines of evidence support a role for oxidative stress in the pathogenesis of atherosclerosis (Jialal & Devaraj, 2005:348). Oxidative stress is known to affect cardiovascular function, increase sympathetic nervous activity and enhance atherosclerosis primarily via endothelial cell dysfunction (Dutta & Dutta, 2003:261). Furthermore, epidemiologic studies appear to suggest that low levels of α-tocopherol are associated with increased risk for CVD, and increased intakes appear to be protective (Jialal & Devaraj, 2005:348). Some epidemiological studies have shown an association between high dietary intake and high serum concentrations of vitamin E and lower rates of ischemic heart disease (Costa, Vianna, Aguila & Mandarim-de-Lacerda, 2005:251). Vitamin E is a powerful antioxidant able to prevent free radical - induced oxidations in biological membranes (Dutta & Dutta, 2003:261). While lipid peroxidation is important to the progression of the atherosclerotic process in blood vessels, vitamin E, as a strong antioxidant, has the ability to limit this process and slow down the unavoidable formation of arterial lesions in important blood vessels including the coronary arteries (Dutta & Dutta, 2003:261). A possible α-tocopherol action mechanism is to protect LDL from oxidation by peroxyl radicals (Jialal &

Devaraj, 2005:348). The administration of vitamin E seems to be linked with the inhibition of lipid peroxidation of LDL lipoprotein and apoprotein as well (Dutta & Dutta, 2003:261).

Studies *in vitro* showed that α-tocopherol, in addition to functioning as an antioxidant, inhibit smooth muscle cell proliferation, platelet adhesion and aggregation, and monocyte endothelial adhesion (Jialal & Devaraj, 2005:348). Tocopherol treated platelets presented a significant decrease of platelet aggregation induced by arachidonic acid, phorbol ester or adenosine 5-diphosphate, and as such would be independent of the antioxidant properties of vitamin E. Although there are these findings, vitamin E's effect on blood pressure is not clear yet (Costa *et al.*, 2005:251).

The vitamin E succinate derivative (VES) of α-tocopherol has received increasing attention for its potential use against cancer. *In vitro* studies of VES have shown inhibition of cell proliferation in a variety of cell lines, including A549 lung cancer cells and BEAS-2S bronchocarcinoma cells. Previous *in vivo* studies also suggest that vitamin E may promote dormancy and decrease metastatic spread in numerous cancers, including breast, melanoma and colon. Breast cancer is the principal site of new cancers in women and the second leading cause (after lung cancer) of cancer death in women (Quin *et al.*, 2005:1).

2.6 Other ways to incorporate antioxidants into the diet

As discussed earlier natural food sources such as fruits, vegetables, whole grains and certain beverage types are by far the ideal way to incorporate antioxidants into the diet. Nowadays antioxidants can also be incorporated into the diet through additional ways such as functional foods, nutraceuticals and fortified food products. The health benefits of functional foods, and nutraceuticals are generally focused on many areas, including prevention and treatment of CVD, various types of cancer, diabetes and inflammation, improvement of the immune system as well as retardation of the aging process and extension of a healthy lifetime (Shahidi, 2009:382).

Functional foods are similar in appearance to conventional foods, are consumed as part of a usual diet and are known to improve an individual's health status beyond the basic nutritional function expected of conventional foods. Nutraceuticals are products produced from foods but sold in the medicinal form of a capsule, tablet, powder, solution or potion which is not generally associated with food and have demonstrated physiological benefits and or provide protection against chronic diseases (Shahidi, 2004:R146).

Antioxidant fortification is the addition of antioxidant vitamins and other antioxidants to foods (Papas, 1999:1004). Fortification is utilised for the following purposes: to help ensure that a population's daily intake of vitamins and minerals meet the required nutrient intakes (RNI's), when intake from unfortified foods do not reach the recommended levels and to provide levels of vitamins, minerals and trace elements greater than the current RNI's if there had been a demonstrated benefit against the manifestation of disease in healthy individuals or in the progression of a pre-existing disease in affected persons (Serra-Majem, 2001:104). Fortification of foods can help prevent nutritional inadequacies in populations where there is a risk of deficiencies and where intervention is needed to correct a proven deficiency in an identified segment of the population (Richardson, 1990:40).

The effectiveness of a programme of nutrient additions is influenced by whether the food that is to carry the nutrients is going to be acceptable, consumed by those who need or want it and be at a price people can afford. The nutrients must be bioavailable and sufficiently stable under normal condition of storage and use. The consumption of foods containing added nutrients should also not create a nutritional imbalance and ensure that an excessive intake of the nutrients will not occur, bearing in mind the collective amounts from other sources in the diet. Four categories of foodstuffs can be identified where the addition of vitamins and minerals is compulsory in some countries: (a) foods for special dietary uses; (b) foods having lost nutrients during manufacture; (c) food resembling a common food; and (d) staple foods representing ideal vehicles for nutrients (Richardson, 1990:40).

Another way to increase antioxidant intakes into the diet is through plant biotechnology. Plant biotechnology is a modern plant breeding technology used to produce higher levels of phytochemicals in fruits and vegetables (Lindsay, 2000:145; Grusak, 2002:508).

2.7 Effect of processing on antioxidants in food

Food quality often deals with the influences of primary agricultural production and industrial processing. Food preparation at home as the final step of the chain also has a large influence on quality determining parameters like sensory attributes and more importantly the content of vitamins, minerals and antioxidants (Ruiz-Rodriguez & Marin, 2008:345). Pre-preparation of fruits and vegetables such as peeling, cutting and slicing are expected to cause rapid enzymatic loss of several natural antioxidants (Nicoli, Anese & Parpinel, 1999:96). Cutting exposes the inner tissues to oxygen and light (Ruiz-Rodriguez & Marin, 2008:345). Besides consumer preferences, the selected thermal processing method is an important factor affecting not only the food chemical composition, but also the intake of

bioactive compounds under normal dietary conditions (Ruiz-Rodriguez & Marin, 2008:345). This final step of processing can change both the above in a positive or negative way (Bernhardt & Schlich, 2006:327); for example, with regard to antioxidants: (a) no effect on the antioxidant potential; (b) cause loss of naturally occurring antioxidants; (c) improvement of antioxidant properties of naturally occurring compounds; (d) formation of new compounds having antioxidant or pro-oxidant activity (i.e. Maillard reaction products); and (e) interaction among different compounds (Nicoli *et al.,* 1999:95). An increase in the radical scavenging activity of vegetables might be due to the thermal destruction of vegetable cell walls and subcellular components which releases more compounds and / or a thermal chemical reaction which produces more potent radical scavenging antioxidants (Yamaguchi, Katsuda, Oda, Terao, Kanazawan Oshima, Inakumam, Ishiguro, Takamura & Matoba, 2003:79). In addition to phenolic substances, there are other components present in foods that have no antioxidant activity of their own, but which increase that of phenolic antioxidants. These components in foods are known as synergists (Pokorny & Schmidt, 2003:38). During thermal treatments, beside the nutrient loss, foods can be subjected to other chemical changes such as those resulting from the Maillard reaction. This reaction, which occurs when sugars condense with free amino acids, peptides or proteins, leads to the formation of a large variety of brown melanoidins. Their antimutagenic activity has been attributed to the fact that certain Maillard reaction products (MRP) can act as antioxidants. Therefore the loss of natural antioxidants in heated foods could be minimised or compensated by the formation of non-nutrient antioxidants such as MRP's (Nicoli, Anese, Parpinel & Franceschi, 1997:72).

Most vegetables are for example cooked by boiling in water or microwaving before consumption. These cooking procedures would bring about a number of changes in the physical characteristics and chemical composition of vegetables (Turkmen, Sari & Velioglu, 2005:713). Processing and cooking conditions cause variable losses of antioxidants (Leskova *et al.*, 2006:258). Losses vary widely according to the cooking method used and the type of food. Degradation of antioxidants depends on specific conditions during the cooking process. Conditions such as temperature, presence of oxygen, light, moisture, pH and duration of heat treatment affect the degradation of antioxidants (Leskova, Kubikova, Kovacikova, Kosicka, Porubska & Holcokova, 2006:258). In some cases processing causes little or no change to the content and activity of naturally occurring antioxidants. Blanching represents a useful method in preventing enzymatic oxidation, which is the main cause of loss in naturally occurring antioxidants of plant origin. Fruit and vegetables subjected to blanching retain most of their original antioxidant properties (Nicoli *et al.*, 1999:95).

A study was conducted to determine the effects of boiling, steaming and microwaving on broccoli, cauliflower, cabbage and choy-sum. The type of cooking method used and the duration affected the antioxidant capacity. Steaming resulted in an increase in the antioxidant

capacity for all the above mentioned vegetables. The increase was minimal for cabbage (13%), but the antioxidant capacity of the cauliflower, broccoli and choy-sum doubled after five minutes of steaming compared to the raw uncooked control samples. When the vegetables were cooked for ten minutes there was a lower antioxidant capacity than those cooked for five minutes, but the effect of a longer cooking time was less in steamed vegetables. When comparing the effect of the three cooking methods, steaming resulted in the highest phenolic content, followed by boiling and microwaving resulting in the lowest phenolic content. Boiling and microwaving had major effects on cabbage, broccoli and choy-sum with a decrease of more than 60% in total phenolics whereas, for cauliflower, a loss of 39% was noticed after microwaving, four percent after boiling and an increase of 45% was noticed after streaming (Wachtel-Galor, Wing, Wang & Benzie, 2008:706).

2.7.1 Flavonoids

Although flavonoids are resistant to heat, oxygen and moderate degrees of acidity, household preparation will cause some flavonoid losses. The skin and leaves of most fruits and vegetables contain the greatest proportion of flavonols; therefore, preparation for consumption involving peeling, skinning, trimming and / or leaf selection may remove and reduce the total flavonoid content (Adherne & O'Brien, 2002:78). If bruising or cutting damages fruits and vegetables, polyphenols are involved in the process known as enzymatic browning. Polyphenols are oxidised to their corresponding phenol oxidase. The quinones are further polymerised with quinines or amines to form brown pigments, which causes a loss in nutritional value (Yamaguchi *et al.,* 2003:79).

The flavonol content in foods can also be modified by various cooking methods. Cooking tomatoes, broccoli and onions has been shown to reduce the level of quercetin found in these foods. However, the method of cooking has an impact on these levels (Adherne & O'Brien, 2002:78). Boiling, frying and microwave cooking can lower the quercetin concentrations in onions and tomatoes by 35% to 82%. An overall loss of 25% in the quercetin glucosides in onions following frying and boiling has also been reported. Blanching has been found to reduce quercetin and kaempferol levels in onions by 39% and 64%, respectively (Makris & Rossiter, 2001:3216). Boiling food results in the greatest reduction of the quercetin content. This decrease is probably due to the leaching action into the cooking water and / or chemical or thermal degradation. Microwave cooking also causes reduction in the flavonol content of these foods, whereas frying only causes a slight reduction in the flavonol levels. However, an increase in frying time significantly reduces the levels of quercetin glucosides (Adherne & O'Brien, 2002:78).

In a study, when different processing methods of onions were compared, the only significant losses of flavonoids took place during the peeling and trimming process (39%). As a result, the losses of flavonoids were very high (90%) in the onions where many layers were removed. Furthermore, the onion samples that had been held warm for one and two hours respectively, had the highest loss of flavonoids. The onion samples cooked in the microwave had lower losses of flavonoids than the samples cooked in water. The onion samples that were blanched had a higher loss of kaempferol than quercetin (64% versus 39%). The onions containing fat through the processing method had higher amounts of kaempferol than the reference sample. Kaempferol losses were again the greatest in the samples that had been held warm (Ewald *et al.*, 1999:234). In a further study the total phenolic content of squash, peas and leeks was significantly reduced with the reductions the same in all the cooking methods namely boiling, steaming and microwaving (Turkmen *et al.*, 2005:715). In another study comparing the effect of three cooking methods (microwaving, boiling and steaming) on broccoli, cauliflower, cabbage and choy sum, steaming showed the highest total phenolics retention value, followed by boiling and microwaving which showed the lowest retention value (Wachtel-Galor *et al.*, 2008:707).

2.7.2 Carotenoids

The bioavailability of β-carotene increases as a consequence of moderate heating and the enzymatic disruption of the vegetable's cell wall structure (Nicoli *et al.,* 1999:95). Carotenoids are extremely susceptible to degradation. Factors such as heat, light and oxygen exposure may have a detrimental effect on the carotenoid content due to the highly unsaturated carotenoid structure (Thane & Reddy, 1997:58; Leskova *et al.*, 2006:254). The influence of different methods of food preparation on the stability of carotenoids in carrots indicated lower retention levels of α-carotene (56% to 78%), whereas β-carotene is more stable (68.8% to 89.1%). β-carotene retention depends on the species of vegetable and the type of cooking method used (Leskova *et al.*, 2006:254). Compared with vitamin A, the provitamin carotenoids are more stable when exposed to light and oxidation. This may be due to the location of the carotenoids in the plant tissue (Gayathri *et al.*, 2004:35).

Food preparation, for example cutting and cooking, can increase the extractability and therewith the bioavailability of β-carotene from the food matrix by softening or disruption of plant cell walls and the destruction of carotenoid-protein-complexes (Bernhardt & Schlich, 2006:327). In a study the volume of water used when cooking the vegetables influenced the carotenoid retention. The highest retention of carotenoids was obtained when vegetables

were cooked without any addition of water and the lowest retention, with the use of a large amount of water during cooking. The moist/dry cooking method caused the greatest loss of carotenoids. Water cooking without pressure resulted in a higher retention of α- and ß-carotene (78% and 89% respectively). This effect can be explained by the lower temperature used in the cooking. However, the use of a higher temperature led to greater loss than the absence of water. Thus, the smallest losses of carotenoid content were observed in a case of water cooking without pressure (13.6%), followed by steam cooking (19.8%), water cooking with pressure (25%) and moist/dry cooking (34.3%) (Leskova *et al.*, 2006:254). But, heat, acid and light can also lead to isomerisation of the naturally occurring ß-carotene from the trans form to its cis-isomers. Cis-isomers are less bioavailable and the provitamin A activity is also lower (Gayathri *et al.*, 2004:36; Bernhardt & Schlich, 2006:328).

In a study the loss of ß-carotene from carrots was greater when the vegetables were pressure cooked for ten minutes (27%), than in boiling water for the same time (16%). Among two acidulants added in different samples, tamarind improved the retention of ß-carotene during pressure cooking, where the loss was only ten percent. The same occurred with the antioxidant spice turmeric where the retention was improved to 93% in pressure cooked carrots. Boiling onions with carrots had a similar beneficial influence (97.5% retention) (Gayathri *et al.*, 2004:37). In processing of tomatoes there is a positive effect on the lycopene activity. Lycopene occurs in an all-trans form in fresh tomatoes; however, processing may lead to the formation of a cis configured double bond. This cis configuration is more bioavailable and hence this example demonstrates a positive effect of processing on bioactivity in tomatoes (Shahidi, 2009:379).

2.7.3 Vitamin C

Ascorbic acid (vitamin C) is one of the most sensitive vitamins (Bernhardt & Schlich, 2006:328). Ascorbic acid, the reduced form of vitamin C, has strong radical scavenging activity. Ascorbic acid is oxidised to DHA by ascorbate oxidase, after a vegetable is cut and exposed to oxygen. DHA has no radical scavenging activity (Yamaguchi *et al.,* 2003:79). Cooking losses of L-ascorbic acid depend on the degree of heating, leaching into the cooking medium, food surface area exposed to water and oxygen, pH, presence of transition metals and any other factor that facilitates oxidation. Blanching as a pretreatment before freezing of vegetables ensures good preservation of vitamin C during storage of frozen products and also yields very good stability of the vitamin. Thawing vegetables before cooking causes a large vitamin C loss. The rate of ascorbic acid degradation is dependent on oxygen

availability, which in turn depend on the temperature and moisture content. A higher water activity results in a greater loss of vitamin C. Also, the type of vegetable contributes a significant source of variation with regard to retention value (Rumm-Kreuter & Demmel, 1990:7). Losses of ascorbic acid were minimal (eight to seventeen percent) when vegetables were cooked without any water (dry method), while maximum (20 to 40%) loss was associated with cooking in a large amount of water. Boiling caused the most extensive damage with losses of up to 75% in some cases, whereas the highest retention values of vitamin C were observed in vegetables prepared by steaming (Leskova *et al.*, 2006:259).

2.7.4 Vitamin E

Natural tocopherols are not very stable (Leskova *et al.*, 2006:257). Degradation of α-tocopherol in food is accelerated when exposed to heat, light and air (Bernhardt & Schlich, 2006:328). The vitamin E content of food also changes due to the processing and refining of food. The vitamin E content therefore depends on whether foods are consumed cooked or raw (Dutta & Dutta, 2003:259). Vitamin E in tomatoes is predominantly represented by α-tocopherol. Homogenisation and sterilisation of tomatoes during production of tomato juice results in a significant loss of α-tocopherol. In contrast, short term heating of tomato sauce, tomato soup and baked tomato slices led to a significant increase of α-tocopherol contents. In a model reaction, α-tocopherol was proven to be very stable against thermal treatment. Tomato juice, sunflower oil and a mixture of both were oven-heated to 180°C for 60 minutes. No significant changes in the α-tocopherol content were observed (Seybold, Frohlich, Bitsch, Otto & Bohm, 2004:7008).

2.3 Total antioxidant capacity

Antioxidants are a large diverse group of compounds with different chemical structures, distribution in nature, range of concentrations, possible sites of action, effectiveness against oxidative species and specificity (Porrini & Riso, 2008:647). Antioxidants can be physically classified by their solubility into two groups: (a) hydrophilic antioxidants, such as vitamin C and the majority of polyphenolic compounds; and (b) lipophilic antioxidants, mainly including vitamin E and the carotenoids (Huang, Ou, Hampsch-Woodill, Flanagan & Deemer, 2002:1815). A wide range of methods are currently used to assess the antioxidant capacity of foods, including that of fruits and vegetables (Kaur & Kapoor, 2001:715). The hydrophilic

antioxidant capacity of fruits and vegetables has been determined using the oxygen radical absorbance capacity (ORAC) analysis methodology. The hydrophilic antioxidants make up more than 85% of the total antioxidants in fruits and vegetables (Prior, 2003:3273).

The main factors affecting antioxidant bioavailability are the following: (a) specific species; (b) molecular linkage; (c) amount introduced; (d) interaction with other compounds; (e) nature of food matrix; (f) presence of effectors of absorption; (g) food processing; and (h) intestinal factors (Porrini & Riso, 2008:647). Antioxidants in foods can be essentially classified in the following categories: (a) low molecular weight compounds, which are free from chemical or physical interaction with other macromolecules; (b) compounds which are physically entrapped into different cellular structures; (c) low molecular weight compounds, which are chemically bound to other macromolecules; and (d) insoluble antioxidant material (Gokmen, Serpen & Fogliano, 2009:279). Food items have complex structures in which individual antioxidant compounds may be present in different forms that can be completely soluble in a solvent, but also insoluble. Most food items contain a mixture of hydrophilic and lipophilic compounds, which can be in free form or bound to other macromolecules. Antioxidants can also be divided according to the mechanism of action. The antioxidants having primary antioxidant groups, or single oxygen quenching groups, are present in low concentrations (Gokmen *et al.,* 2009:279).

Antioxidant compounds can also be formed during thermal processing as a result of chemical reactions during processing (Gokmen *et al.,* 2009:279). Browning reactions occur in food processing and storage. These browning reactions can involve different compounds and proceed through different chemical pathways. The major groups of reactions leading to browning are enzymatic phenol oxidation and non-enzymatic browning. Non-enzymatic browning is caused by heat treatments and includes a wide number of reactions such as the Maillard reaction, caramelisation, chemical oxidation of phenols and maderisation. In the Maillard reaction the resulting high antioxidant capacity is generally attributed to the formation of brown melanoidins (Manzocco, Calligaris, Mastrocola, Nicola & Lerici, 2001:340).

The TAC is a useful means to express the health potential of antioxidant rich products (Gokmen *et al.,* 2009:278). The antioxidant capacity is the measure of the moles of a given free radical scavenged by a test solution, independently from the antioxidant activity of any one antioxidant present in the mixture (Ghiselli, Serafini, Natella & Scaccini, 2000:1107). The antioxidant capacity gives the information about the duration of antioxidative action. The reactivity characterises the starting dynamics of antioxidation at a certain concentration of an antioxidant or antioxidant mixture (Roginsky & Lissi, 2005:236). The antioxidant activity corresponds to the rate constant of a single antioxidant against a given free radical (Ghiselli

et al., 2000:1107) and is the capability of a compound to inhibit oxidative degradation, such as lipid peroxidation (Roginsky & Lissi, 2005:236). The antioxidant capacity of plant foods is derived from the combined synergistic action of a wide variety of antioxidants such as vitamin C and vitamin E, polyphenols, carotenoids, terpenoids, Maillard compounds and trace minerals (Perez-Jimenez, Arranz, Tabernero, Diaz-Rubio, Serrano, Goni & Saura-Calixto, 2008:275).

Antioxidant capacity is usually measured in food samples obtained with chemical aqueous solvents (methanol, ethanol, acetone, etc.). The antioxidant content of plant foods, and hence their associated antioxidant capacity, depends on the variety and the degree of ripening. After harvesting some antioxidants in fruit and vegetables undergo certain reactions that may cause a decrease in the antioxidant capacity of the sample. The different post harvest treatments also affect the antioxidant capacity of fruit and vegetables. These treatments include storage (time and temperature), processing and the possible addition of synthetic antioxidants (Perez-Jimenez *et al.*, 2008:275).

A study was conducted determine the effects of different cooking methods (steaming, boiling and frying) on the nutritional and physiochemical characteristics of selected vegetables (carrots, broccoli and courgette). There was an overall increase of TAC observed which is in partial disagreement with the concept that processed vegetables have lower nutritional quality than raw vegetables (Miglio, Chiavaro, Visconti, Fogliano & Pellegrini, 2008:146).

2.9 Study approach

The incidence of chronic disease is a concern among the South African population. An underlying cause of many chronic diseases is oxidative stress. In this regard CVD, cancer and HIV/AIDS as such representative health concerns in South Africa, and their relationship with oxidative stress, was addressed in the preceding literature. The body's antioxidant defence system is the major means the body has to offset oxidative stress and the resulting physical damage caused by oxidants. The dietary antioxidant nutrients, vitamins C and E, and the non-nutrient polyphenols and carotenoids, is essential to the efficiency of this system. These dietary antioxidants, along with their food sources, were also addressed in the preceding literature. However, the preceding literature indicated that the major dietary sources of these antioxidants namely fruits, vegetables and whole grains, along with tea, are not consumed by South Africans in amounts that meet the guidelines for adequate intakes. A publication based on secondary dietary intake data obtained from summarised food consumption studies conducted across the provinces of South Africa provided an estimate of

the per capita South African dietary TAC which was found to fall far short of the estimated dietary TAC based on dietary recommendations (Louwrens, Rautenbach & Venter, 2009:195). In the preceding literature reference had also been made to the addition of antioxidant nutrients to foods through food fortification, the consumption of antioxidant dietary supplements and/or a change in dietary behaviour as possible methods to increase the South African dietary TAC to assist with the delay or prevention of oxidative stress. Another method to incorporate additional antioxidants in the diet of the South African population is recipe manipulation by including antioxidant-rich ingredients in recipe formulations to increase the TAC of the recipe and the resulting dietary antioxidant intake on such prepared food consumption. Recipe manipulation as a method to increase the TAC of the South African dietary intake formed the investigative approach of this study. The study considered the inclusion of rooibos as the antioxidant-rich source, due to its polyphenol contents, within soup recipe formulations as the food carrier. Recipe formulations most often require thermal processing as a preparation step. Both the dietary nutrient and non-nutrient antioxidants are susceptible to heat exposure. The effect of thermal processing on the nutrient and non-nutrient antioxidants was therefore also addressed in the preceding literature and also considered necessary to incorporate in the investigative study approach as the recipe formulation selected as the food vehicle for the study was soup, a food item requiring thermal processing. Thermal processing could have a major impact on the resulting TAC of the soup recipe manipulation that could cancel out the assumed probable increase in the TAC of the recipe formulation due to the antioxidant provision provided by the rooibos inclusion as a recipe ingredient.

CHAPTER 3

METHODOLOGY

3.1 Type of study and study design

An experimental study of comparative nature was conducted to determine the effect on the TAC of substituting water, as the major recipe ingredient, with unfermented (green) and fermented (traditional) rooibos herbal teas in popular soup recipe formulations. The study design, including its major methodological steps, are summarised in Figure 3.1. An experiment consists of an objective observation or measurement carried out under carefully controlled conditions. In any experiment the factor or phenomenon to be investigated must be clearly defined. Two groups must then be set up, namely an experimental group that receives the treatment and a control group that go untreated. The two groups must be similar in every respect, but for the factor or phenomenon investigated (Behr, 1983:108). In this study three soup recipe formulations with water were the control (or untreated) and the same soup recipe formulations where water was substituted with fermented and unfermented rooibos herbal teas the experimental (or treatment) groups. The study is of comparative nature as the results (i.e. the TAC as the factor investigated) of three different soup recipe formulations on fluid manipulations of each (with unfermented and fermented rooibos herbal teas) was compared to the control soup recipe formulations of each (no fluid manipulation). The results (i.e. the TAC) of the three prepared control and six experimental soup recipe formulations were also compared to that of six the raw soup mixtures to determine the effect of thermal processing on each (see data analysis in Figure 3.1) as heat was identified as the main variable that may affect the results (i.e. the TAC). Understanding unique cases can be deepened by comparative analysis. Comparisons can also be important in highlighting differences between evaluations (Patton, 2002:56).

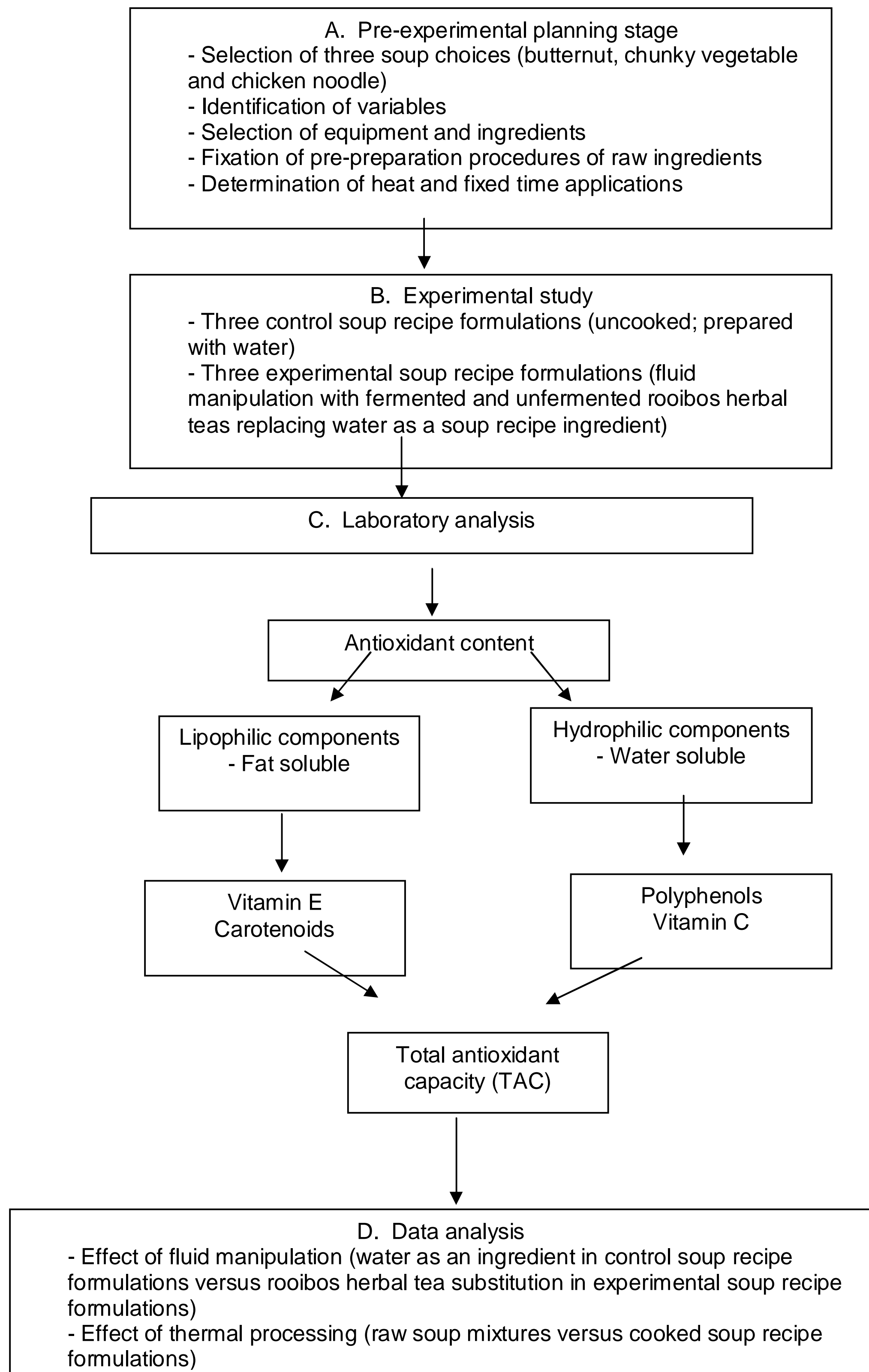

Figure 3.1: Flow diagram of study design depicting the major methodological steps

3.2 Selection of three soup choices and identification of variables

The three soup varieties that were selected are butternut, chunky vegetable and chicken noodle. In an unpublished study conducted by Fana (2005:42) the random sample of 64 customers in Cape Town who bought soup from a South African retail chain food store were asked to indicate the three soup flavours they liked most of all and soup flavours they were aware of or familiar with. A large variety of flavours were indicated with butternut (35.3%), chunky vegetable (35.3%) and chicken noodle (33.8%) being indicated as the top three flavours. These soup flavours represent a soup prepared from a single vegetable base, a large variety of vegetables as the base and a meat and starch base with minimal vegetables respectively.

The main variable identified in the preparation of the soup recipe formulations that may impact the TAC (the dependent variable) and needed to be controlled, is the heat application. A dependent variable is a variable that forms the focus of the research, and depends on another variable (the independent or explanatory variable) (Gray, 2009:576). The investigator has no control over the dependent variable and it occurs as the result of the influence of the independent variable (Leedy, 1989: 219). The two major variables linked to the application of heat (independent variable) that will influence the TAC are the degree of and the length of time heat is applied to the recipes. Heat application was therefore considered in the interpretation of the data as antioxidants are sensitive to heat. Other variables that may influence the results, are the soup recipe formulation ingredients, the pre-preparation of the raw ingredients, the standing time of ingredients before use and the equipment used (for example a large pot will cause greater evaporation than a smaller deeper pot). All these independent variables were as far as possible controlled for, in all the soup recipe formulations prepared, to minimise antioxidant oxidation. The control of these identified independent variables is described in the sub-sections of this chapter that follows below. If the investigator has control over the variable and is able to manipulate it or change it, then the variable is an independent variable (Leedy, 1989: 219).

3.3 Selection of the recipe formulation ingredients and equipment

All the ingredients were purchased at a specific retail chain food store in the City of Cape Town metropolitan area from the corresponding batch and on the same day and time. All the vegetables purchased were completely unprocessed. All the ingredients were accurately weighed off to the g on a calibrated Mettler Toled scale. The final raw, cooked and liquidised weights of all single and combined ingredients of each recipe formulation step were recorded in order to determine the TAC. The same two stainless steel pots with a base diameter of 20

centimeter (cm) were used for thermal processing of all the soup recipe formulations. Materials used in the construction of cooking utensils vary in their ability to conduct heat efficiently. Metals are good conductors including stainless steel, copper, aluminium and iron (Bennion & Scheule, 2000:142). The same tight fitting lid of each pot was applied throughout the thermal processing when stated in the recipe. A pot was the chosen vessel for the cooking of the soup due to the large volume of ingredients in a soup recipe formulation. The specific height of the pot (9.5 cm) was chosen to ensure all the ingredients of all the recipes could fit into the same pot and that the base diameter of the pot (20cm) would cover the diameter of the stove plate (18.5cm) for even cooking. The same pot was used throughout for each of the control and experimental recipe formulations to limit the variables affecting the results, for example the amount of evaporation. The same electric Defy stove was used and all recipes cooked on the two 18.5 cm stove plates. The electric stove was the chosen power source as data from the 1996 census indicated that 80% of the population in the Cape Metropolitan area use electricity for cooking purposes (Cape Metropolitan Council, 2001). The same hand peeler (Boardmans), chef's knives (Victorinox) and grater (Legend) were used for the ingredient preparation of all recipe formulations. A standard wooden spoon was used for the control and experimental recipe formulations, as a wooden spoon is a poor conductor of heat and therefore ideal to stir a pot of boiling soup. The different stages of the soup recipe formulations were timed to the second with a dual timer stopwatch (Merck) obtained from the Analytical Laboratory, Oxidative Stress Research Centre (OSRC), Cape Peninsula University of Technology (CPUT) and incorporated within each of the standardised soup recipe formulations. Before the food samples for the TAC analysis could be sampled the soups were liquidised until resembling a smooth puree in a Kenwood liquidiser, first speed, for the same time length per soup recipe formulation also incorporated within each of the standardised soup recipe formulations.

3.4 Fixation of pre-preparation procedures of raw ingredients

Before determination of the heat applications and the fixed time allocations of the soup recipe formulations to ensure recipe standardisation, the pre-preparation procedures of the raw recipe ingredients were also standardised. This step was necessary as the pre-preparation of the raw recipe ingredients were not described in the base recipe formulations selected (see Addenda A, B and C) and had been identified as independent variables that needed to be controlled. All the ingredients were pre-prepared according to the guidelines provided by Willan (1989: 13, 176, 177, 180, 259, 277, 278, 290, 291, 293, 294, 295, 287, 288, 460). Table 3.1 indicates the pre-preparation guidelines as described for all the raw ingredients of the chunky vegetable soup

recipe formulation, Table 3.2 for the butternut soup recipe formulation and Table 3.3 for the chicken noodle soup recipe formulation. Before the pre-preparation, all the vegetables were rinsed thoroughly for approximately 30 seconds each under cold running water to remove excess soil and debris and placed in colanders for the water to drip off.

Table 3.1: Pre-preparation of raw recipe ingredients for chunky vegetable soup based on the guidelines of Willan (adapted from Willan, 1989:278, 299, 295, 287, 460)

Pre-preparation guidelines of Willan	Pre-preparation applied in this study
Carrots	
Peeling: A vegetable peeler or small paring knife is the best utensil.	Peeling: Using a vegetable peeler peel 1 millimeter (mm) of peel off the carrot ensuring only outer peel is removed.
Dicing: Square off the sides. Slice the vegetables vertically, cutting thickly for large dice or thinly for small dice. Stack the slices and cut even strips of uniform thickness. Gather the strips together in a pile and slice them evenly crosswise to produce dice of the required size.	Dicing: Square off the sides by cutting 0.5 cm off the top and bottom of the carrots. Slice the vegetables vertically, cutting thickly for large dices. Gather the strips together in a pile and slice them evenly crosswise into dices of 0.5 cm x 0.5 cm.
Onions	
Peeling: Peel the onion, leaving the root on to hold the onion together.	Peeling: Peel the onion removing only the outer peel layer, removing 2 mm in thickness. Ensure the root remains intact to hold the onion together.
Chopping: Cut the onion in half and lay one half, cut side down, on a chopping board. With a chopping knife, make a series of horizontal cuts from the stalk towards the root. Cut just to the root, leaving the slices attached to the root end. Make a series of lengthwise vertical cuts, cutting almost but not quite through the root. Finally, cut the onion crosswise so that it falls into dice.	Chopping: Cut the onion in half and lay one half, cut side down, on a chopping board. With a chopping knife, make a series of horizontal cuts from the stalk towards the root. Cut just to the root, leaving the slices attached to the root end. Make a series of lengthwise vertical cuts, cutting almost but not quite through the root. Finally, cut the onion crosswise so that it falls into dices of 0.5 cm x 0.5 cm.
Cauliflower	
Preparing: Cut all the outer leaves from the cauliflower and trim the stalk near the base of the head. With a paring knife, cut around the core to remove it. Break the cauliflower into pieces, and then cut into florets.	Preparing: Cut all the outer leaves from the cauliflower and trim the stalk near the base of the head. With a paring knife, cut around the core to remove it. Break the cauliflower into pieces, and then break into small florets.
Celery	
Remove outer stalks. Break off any tough stalks or leaves and discard. Cut off and discard upper stalk and trim the root. Remove the leafy stalks and cut off green tops. Peel any strings from the outer sides of the stalks.	Remove outer stalks. Break off any tough stalks or leaves and discard. Cut off and discard upper stalk and trim the root. Remove the leafy stalks and cut off green tops. Peel any strings from the outer sides of the stalks. Chop each stalk into pieces 3 mm thick.

Table 3.1 continued

Pre-preparation guidelines of Willan	Pre-preparation applied in this study
Potatoes	
Preparation: Brush off dirt, wash and drain and peel thin skins with a vegetable peeler.	Preparation: Brush off dirt, wash and drain and peel thin skins with a vegetable peeler. Soak in cold water (15 minutes) and coarsely grate just before use.
Butternut	
Wash, trim stem and peel only if specifically required.	Wash, trim stem and peel with a vegetable peeler. Cut into rings 1cm thick and then dice into blocks 1.5 cm x 1.5 cm.
Parsley	
Chopping: Strip the leaves from the stalks and pile the leaves on a cutting board. Cut the herbs into small pieces, holding the tip of the blade against the board and rocking the handle up and down. Chop the herbs coarsely or finely.	Chopping: Strip the leaves from the stalks and pile the leaves on a cutting board. Cut the parsley and celery into small pieces, holding the tip of the blade against the board and rocking the handle up and down until a coarse texture is formed.
Lemon rind	
Using a zest tool scrape it against the surface of the fruit to remove the zest.	Using a zest tool scrape it against the surface of the fruit to remove the zest.
Fermented and unfermented rooibos herbal tea	
	Brewing: Place tea leaves and stems (19 g) in a measuring jug and pour required quantity of boiling water (1.345 kg) over. Allow to brew for 3 minutes. Pour the rooibos through a tea strainer to remove leaves and stems. Place in chiller to obtain same temperature (18ºC - 22ºC) as the control recipe formulations tap water.

Table 3.2: Pre-preparation of raw recipe ingredients for butternut soup based on the guidelines of Willan (adapted from Willan, 1989:295)

Pre-preparation guidelines of Willan	Pre-preparation applied in this study
Onions	
See Table 3.1	
Butternut	
See Table 3.1	
Potatoes	
Preparation: Brush off dirt, wash and drain and peel thin skins with a vegetable peeler.	Preparation: Brush off dirt, wash and drain and peel thin skins with a vegetable peeler. Soak in cold water (15 minutes) and dice into blocks, 2 cm x 2 cm, just before use.
Fermented and unfermented rooibos herbal tea	
	Brewing: Place tea leaves and stems (10 g) in a measuring jug and pour required quantity of boiling water (750 g) over. Allow to brew for 3 minutes. Pour the rooibos through a tea strainer to remove leaves and stems. Place in chiller to obtain same temperature (18ºC - 22ºC) as the control recipe formulation tap water.

Table 3.3: Pre-preparation of raw recipe ingredients for chicken noodle soup based on the guidelines of Willan (adapted from Willan, 1989:13, 176, 180, 258, 259, 290, 291, 294)

Pre-preparation guidelines of Willan	Pre-preparation applied in this study
Chicken pieces	
Skinning: Cut off the wing pinions and trim the drumstick. Slit the skin along the backbone from the head to the tail. Cut and peel the skin from one side of the bird, using short sharp strokes of the knife. Turn the leg and wing skin inside out as it is reached. Continue peeling away the skin, up to the breastbone. Repeat on the other side. Gently pull the skin from the carcass.	Skinning: Cut off the wing pinions and trim the drumstick. Slit the skin along the backbone from the head to the tail. Cut and peel the skin from one side of the bird, using short sharp strokes of the knife. Turn the leg and wing skin inside out as it is reached. Continue peeling away the skin, up to the breastbone. Repeat on the other side. Gently pull the skin from the carcass.
Cutting into eight pieces: To remove the legs use a knife or a pair of poultry shears to cut the skin between the leg and the breast. With the tip of the knife, locate the oyster meat lying against the backbone and cut round it so that it is attached to the thigh joint. Twist the leg sharply outwards to break the thigh joint, severing the leg with oyster meat attached. Turn the chicken round and remove the other leg, leaving the oyster meat attached to it. Cut off the wing pinions and discard them. Cut forward to remove the meat that lies along the backbone. Set the bird back down. Slit closely along the breastbone to loosen the meat, and then split the breastbone with a knife or shears. Turn the bird over, and with a knife or shears, cut the rib bones and backbone from the breast in one piece, leaving the wing joints attached to breast. Cut the breast in half with poultry shears or a knife. Divide each breast in half diagonally with poultry shears or a knife, cutting through the breast and rib bones so that a portion of breast meat is cut off with the wing.	Cutting into eight pieces: Remove the legs use a knife to cut the skin between the leg and the breast. With the tip of the knife, locate the oyster meat lying against the backbone and cut round it so that it is attached to the thigh joint. Twist the leg sharply outwards to break the thigh joint, severing the leg with oyster meat attached. Turn the chicken round and remove the other leg, leaving the oyster meat attached to it. Cut off the wing pinions and discard them. Cut forward to remove the meat that lies along the backbone. Set the bird back down. Slit closely along the breastbone to loosen the meat, and then split the breastbone with a knife. Turn the bird over, and with a knife, cut the rib bones and backbone from the breast in one piece, leaving the wing joints attached to breast. Cut the breast in half with a knife. Divide each breast in half diagonally with a knife, cutting through the breast and rib bones so that a portion of breast meat is cut off with the wing.
Shin	
Cutting: First pull apart muscles that are loosely connected with tissue and then cut the meat across the grain.	Cutting: First pull apart muscles that are loosely connected with tissue, and then cut meat, across the grain, into cubes 2 cm x 2 cm.

Table 3.3 continued

Pre-preparation guidelines of Willan	Pre-preparation applied in this study
Fermented and unfermented rooibos herbal tea	
	Brewing: Place tea leaves and stems (21 g) in a measuring jug and pour required quantity of boiling water (1.500 kg) over. Allow to brew for 3 minutes. Pour the rooibos through a tea strainer to remove leaves and stems. Place in chiller to obtain same temperature (18ºC - 22ºC) as the control recipe formulation tap water.
Carrots	
See Table 3.1	
Leeks	
Cleaning: Trim the top of the leek, leaving some green or removing it altogether depending on the recipe. Discard the outer leaves and trim the root. Split, the leek in quarters or halves almost through the root, depending on the size. Rinse the leek down to the root in cold water, shaking it to loosen any dirt. Reassemble the layers for cooking.	Cleaning: Trim the top of the leek, removing all green leaves. Discard the outer leaves and trim the root. Split, the leek in quarters almost through the root. Rinse the leek down to the root in cold water, shaking it to loosen any dirt. Reassemble the layers for cooking.
	Cutting: Cut into rings, 1 cm in thickness.
Onion	
See Table 3.1	
Soup celery and parsley	
See Table 3.1	
Turnip	
Preparation: Brush off dirt, wash and drain, trim tops and roots; peel the skin with a vegetable peeler.	Preparation: Brush off dirt, wash and drain, trim tops and roots; peel the skin with a vegetable peeler.
	Chopping: Dice into blocks approximately 1 cm x 1 cm.

3.5 Determination of soup recipe formulation heat applications and fixed time allocations for recipe formulation standardisation

Household recipe formulations routinely provide the amount and types of ingredients to be used, but do not fully describe the pre-preparation procedures of the raw ingredients, but the steps indicated in the method to prepare the food item or dish are also not specific as to the heat and time applications. Household recipe formulations for each of the selected soups were obtained from South African compiled recipe books and a base recipe formulation for each was selected (see Addenda A, B and C).

Before the soup samples were prepared for the TAC analysis, it was necessary to determine the fixed heat applications and time allocations for each step in the base recipe formulations (see Addenda A, B and C). The recipe formulations were also reduced to an average family household number of servings of four to six portions. Each base recipe formulation was tested and re-formulated to include fixed heat and time applications (see Addenda D, E and F). The recipe formulations were then finalised with slight amendments and adjustments on using the institutional laboratory electric Defy Stove (see Addenda G, H and I). Each step in the recipe formulations were controlled on a set heat application (number on the stove dial) fixed time allocation and this was maintained for each control and experimental recipe formulation. The soup temperatures were also taken using a Hanna HI 147-00 Merck thermometer obtained from the Analytical Laboratory (OSRC, CPUT) at the end of each step in the recipe formulation and noted.

The standardised base recipe formulations formed the control recipe formulations for each of the selected soup recipe formulations. The amount of water included in each of the control recipe formulations was replaced by (a) fermented rooibos and (b) unfermented rooibos to form the experimental recipe formulations for each of the selected soups. The heat and time applications in the soup recipe preparations had to be strictly adhered to as stated in the standardised base recipes as heat and time are the major independent variables that may influence the TAC. The rooibos used in the experimental recipe formulations were obtained from the same batch and had the same herbal tea to fluid ratio and fixed brewing times. The herbal tea to fluid ratio was 1.4 g per 100 ml. The rooibos herbal teas were brewed in all recipes for three minutes as that is the general household recommendation on the rooibos herbal tea packaging.

3.6 Food samples, sampling and extraction procedure for analysis

The effect on the TAC of substituting water with rooibos in popular soup recipe formulations was determined by using three popular soup recipe formulations prepared according to standardised recipe formulations (Addenda G, H, I). Firstly, samples of the individual raw ingredients were obtained and then analysed for their polyphenol content, hydrophilic (H)-ORAC, lipophilic (L)-ORAC and carotenoid content. Then the polyphenol content, H-ORAC, L-ORAC and carotenoid content of the combined raw ingredient samples for each recipe formulation (control and experimental formulations). Lastly, the polyphenol content, H-ORAC, L-ORAC and carotenoid content of the samples obtained from the thermally processed recipe formulations was also analysed. All samples for the analysis were taken in triplicate for each recipe formulation, (control and experimental) and for each of the selected soups that were prepared in triplicate (providing nine analysis samples). All food samples were stored at – 40°C until extraction and analysis. The hydrophilic antioxidants for H-ORAC and total polyphenol analysis were extracted using a solvent mixture consisting of water, acetone and acetic acid (70:29.9:0.1, v/v/v) (3 g / 30 ml). The lipophilic antioxidants for L-ORAC and carotenoids were extracted using hexane (3 g / 10 ml) (Prior, Hoang, Gu, Wu, Bacchiocca, Howard, Hampsch-Woodill, Huang, Ou & Jacob., 2003:3273).

3.7 Total antioxidant capacity and antioxidant content analysis

Antioxidants can be physically classified by their solubility into two groups (a) hydrophilic (water soluble) antioxidants, such as vitamin C and the majority of polyphenolic compounds, and (b) lipophilic antioxidants (fat soluble), mainly vitamin E and the carotenoids (Huang *et al.*, 2002:1815). The antioxidants present in the three soup recipes include both water and lipid solubles.

The ORAC method was chosen for the TAC determination as it has been used widely in TAC determinations of food (Wu, Gu, Holden, Haytowitz, Gebhardt, Beecher & Prior, 2004a:407; Wu, Beecher, Holden, Haytowitz, Gebhardt & Prior, 2004b:4026). The TAC is determined by analysing the hydrophilic and lipophilic antioxidant capacity separately, then adding the two values together to obtain a total value. The ORAC method measures the antioxidant scavenging activity against peroxyl radical induction by 2,2-azobis(2-amidinopropane) dihydrochloride at 37°C, pH of 7.4. In this assay, fluorescein (FL) is the fluorescent probe which is measured in a Fluoroskan Ascent plate reader (Thermo Electron Corporation, U.S.A). The loss of fluorescence

over time of FL is an indication of the extent of damage from its reaction with the peroxyl radicals. The protective effect of antioxidants present in the soup formulations are measured by assessing the area under the fluorescence decay curve (AUC) of the sample as compared to that of the blank in which no antioxidants are present (Ou, Hampsch-Woodill & Prior, 2001:4619). The unit of expression of the TAC is µmole (µ) Trolox equivalents (TE) per 100 g fresh weight (FW). All the samples were assayed in triplicate.

The total polyphenol content was analysed using the Folin Ciocalteu method. The amount of total phenolics was determined using Folin-Ciocalteu phenol reagent, as described by Singleton and Rossi (1965:144). Polyphenols in the food sample react with the Folin phenol reagent at alkaline pH and form a blue colour measured at a wavelength of 765 nanometer (nm). A Multiskan Spectrum plate reader (Thermo Electron Corporation, U.S.A) is used to determine the intensity of the blue colour formed during the reaction. Results were expressed as milligram (mg) gallic acid equivalents (GAE) per 100 g FW. All the samples were assayed in triplicate. Only the total polyphenol contents of the soup recipe formulations were determined, and not the vitamin C contents, to represent the hydrophilic component in the soup recipe formulations. The total polyphenol intake commonly reaches 1000 mg per day in people who eat several servings of fruits and vegetables per day (Manach, Scalbert, Morand, Remesy & Jimenez, 2004:732). In contrast, the current RDA for vitamin C is 75 milligram (mg) for women and 90 mg for men (Bsoul & Terezhalmy, 2004:1). The polyphenols therefore contribute much more to the dietary TAC than vitamin C. Vitamin C is furthermore a nutrient particularly sensitive to processing conditions. Temperature, as well as pH, water content, the presence of oxidising substances, oxygen, the presence and biological catalysts influence vitamin C destruction rate (Burg & Fraile, 1995:506). It was therefore assumed that the H-ORAC would largely be represented by the total polyphenol content of the soup recipe formulations.

Carotenoids are measured based on their ability to absorb visible light at wavelengths between 420 nm and 520 nm. A compromise wavelength of 450 nm was used to measure total carotenoids spectrophotometrically on a Nicolet Evolution spectrophotometer (Thermo Electron Corporation, U.S.A). An extinction coefficient of 2592 for β-carotene was used in the calculation and results were expressed as µg per 100 g FW. [Labelling of equation: A=: ϵl, A=absorbance (no unit), έ=extinction coefficient (M-1.cm-1), C=concentration (M of Molar), L=pathlength (cm)].

All the samples were assayed in triplicate (Rodriguez-Amaya & Kimura, 2004:13, 14, 36). The TAC, total polyphenol and carotenoid content analysis was performed by the Laboratory

Manager of the Analytical Laboratory (OSRC, CPUT). Only the carotenoid contents of the recipe formulations were determined as indicative of the lipophilic component in the soup recipe formulations. Vegetables are not commonly considered in the food source provision of dietary vitamin E, but food items such as sunflower seeds, olive oil and almonds, which contain high amounts of α-tocopherol, while most other oils and seed oils are rich in y-tocopherol (Tucker & Townsend, 2005:380). These food sources were not included among the soup recipe formulation ingredients. In addition, carotenoids are more sensitive to thermal processing than vitamin E. A study was conducted to determine the changes in the lycopene, ß-carotene and α-tocopherol during preparation of a tomato soup. The vitamin E was stable during the thermal processing. The ß-carotene and lycopene were more sensitive to thermal processing and decreased by about 50% during 50 minutes of cooking (Seybold *et al.,* 2004:7007). The ORAC assay furthermore does not measure the antioxidant activity of carotenoids, since chemically carotenoids are not chain breaking antioxidants. These antioxidants may act as the singlet oxygen scavengers and therefore follow a different reaction mechanism (Huang *et al.*, 2002:1820).

3.8 Statistical analysis

The MedCalc Software, Belgium (MedCalc Version 9.4.2.0) was used for the statistical analysis. The T-test was used for the statistical analysis of the data. The effect of the substitution of water with fermented and unfermented rooibos herbal tea on the recipe formulation used the T-test for independent samples. The effect of thermal processing on the recipe formulations was compared using the T-test for paired samples. If p was greater than 0.05 ($p > 0.05$) then the result was considered not to be statistically significant different for both the paired and independent sample results. The null hypothesis was therefore accepted that there was no effect on the TAC or antioxidant content of the soup recipe formulations on substituting the recipe ingredient water with unfermented (green) and fermented (traditional) rooibos herbal tea and on the TAC or antioxidant content of the raw soup recipe formulations after heat or thermal application to the soup recipe formulations. Pearson’s correlation coefficient was used to compare the TAC of the soup recipe formulations with the H-ORAC, L-ORAC, polyphenol and carotenoid content to determine the major antioxidant component contribution to the TAC of the soup recipe formulations on thermal processing.

CHAPTER 4

RESULTS

The total polyphenol content, H-ORAC, L-ORAC and carotenoid content were determined for each individual raw soup recipe formulation ingredient, the control and the experimental raw and thermally processed recipe formulations. The H-ORAC and L-ORAC were added together to obtain the TAC. The raw individual ingredients were analysed to determine the individual ingredient polyphenol and carotenoid contents and the TAC and how the different ingredients contributed to the total polyphenol and carotenoid contents and the TAC of the raw soup recipe formulations. The analysis of the raw recipe formulation controls was compared to the analysis of the raw recipe experimental formulations to determine the effect of the fermented and unfermented rooibos inclusions on the total polyphenol and carotenoid contents and the TAC. The analysis of the raw soup recipe formulations were also compared to the analysis of the thermally processed soup recipe formulations to determine the effect of thermal processing on the polyphenol and carotenoid contents and in particular the TAC of each recipe formulation.

4.1 Total polyphenol, carotenoid and TAC contributions of the ingredients to the raw soup recipe formulations

In all three raw soup recipe formulations, water or in the case of the experiments, fermented and unfermented rooibos, was the greatest single raw ingredient contributor in relation to the contribution of the other raw ingredients. In the chunky vegetable soup recipe formulation the liquid contribution was 52%, 39% in the butternut soup recipe formulation and 40% in the chicken noodle soup recipe formulation (see Table 4.1). The respective total polyphenol and carotenoid contents and the TAC of the raw recipe formulations varied greatly due to the varying phytochemical contents of the raw ingredients, as well as the quantity of each ingredient used the recipe formulations (see Table 4.2).

Table 4.1: Quantity and percentage contribution[a] of ingredients to the soup recipe formulations

Soup recipe formulation								
Chunky vegetable			Butternut			Chicken noodle		
Ingredient	Quantity (Kg)[b]	%	Ingredient	Quantity (Kg)	%	Ingredient	Quantity (Kg)	%
Carrots	0.130	4.99	Onions	0.225	11.73	Whole chicken	0.750	20.00
Onions	0.055	2.11	Sunflower oil	0.040	2.09	Chicken giblets	0.190	5.07
Cauliflower	0.225	8.63	Butternut	0.565	29.46	Chicken necks	0.190	5.07
Celery	0.060	2.30	Potatoes	0.225	11.73	Shin	0.190	5.07
Potatoes	0.225	8.63	Water	0.750	39.10	Chicken stock powder	0.030	0.80
Split peas	0.150	5.75	Vegetable stock powder	0.010	0.52	Water	1.500	40.00
Water (soaking of peas)	0.150	5.75	Lemon juice	0.005	0.26	Oil	0.040	1.07
Tomato paste	0.010	0.38	Cinnamon	0.001	0.05	Carrots	0.300	8.00
Butternut	0.225	8.63	Salt	0.001	0.05	Leeks	0.225	6.00
Vegetable stock powder	0.015	0.58	Coarse black pepper	0.001	0.05	Onions	0.115	3.07
Water	1.345	51.59	Full cream milk	0.095	4.95	Soup celery	0.040	1.07
Parsley	0.010	0.38				Parsley	0.040	1.07
Lemon rind	0.002	0.08				Coarse black pepper	0.005	0.13
Salt	0.002	0.08				Salt	0.005	0.13
Coarse black pepper	0.001	0.04				Vermicelli	0.040	1.07
						Turnip	0.090	2.40
Total	2.624	99.92 ~ 100.00	Total	1.918	99.99 ~ 100.00	Total	3.750	100.02 ~ 100.00

[a] The percentage contribution is the individual quantity of raw ingredient used in the recipe divided by the total mass of all the ingredients in the recipe formulation multiplied by 100 percent. The quantities of rooibos leaves and stems used (2.5 g per 180 ml water) were not included in the soup recipe formulations as the leaves were drained from the rooibos herbal teas before adding the liquid to the soup recipe formulations and therefore do not form part of the total weight of the soup recipe formulation.

[b] Kilogram

Table 4.2: Total polyphenol and carotenoid contents, H-ORAC, L-ORAC and TAC of the ingredients of the soup recipe formulations – chunky vegetable soup

Soup recipe formulation	Raw ingredient [a]	Total polyphenol contents (mg GAE [b] / 100g) mean +- SD	Calculated total polyphenol content (mg GAE) / actual weight of ingredient used	Carotenoid contents (ug [c] /100g) mean +-SD	Calculated carotenoid content (ug) / actual weight of ingredient used	H-ORAC [d] (µmole TE [e]/ 100g) mean +- SD	Calculated H-ORAC (µmole TE) / actual weight of ingredient used	L-ORAC [f] (µmole TE / 100g) mean +- SD	Calculated L-ORAC (µmole TE) / actual weight of ingredient used	TAC [g] (µmole TE / 100g) mean +-SD	Calculated TAC (µmole TE) / actual weight of ingredient used
Chunky vegetable	Carrots	9 ± 1	12	8 558 ± 718	11 125	976 ± 47	1 269	12 ± 1	16	988 ± 46	1 285
	Unfermented rooibos	61 ± 2	517	N.D. [h]	N.D.	1 637 ± 78	14 139	0 ± 0	0 ± 0	1 637 ± 78	13 835
	Fermented rooibos	44 ± 1	368	N.D.	N.D.	1 036 ± 36	8 757	0 ± 0	0 ± 0	1 036 ± 36	8 757
	Onions	78 ± 4	43	N.D.	N.D.	1 883 ± 104.	1 036	4 ± 0	2	1 887 ± 104	1 038
	Cauliflower	69 ± 2	156	N.D.	N.D.	1 440 ± 91	3 239	5 ± 0	10	1 444 ± 91	3 249
	Celery	13 ± 1	8	N.D.	N.D.	840 ± 54	504	7 ± 1	4	847 ± 54	508
	Potatoes	34 ± 2	77	N.D.	N.D.	914 ± 40	2 057	6 ± 0	13	920 ± 39	2 070
	Split peas	51 ± 2	76	N.D.	N.D.	1 522 ± 96	2 283	31 ± 3	46	1 553 ± 94	2 329
	Tomato paste	178 ± 7	18	N.D.	N.D.	1 5262 ± 1 121	1 526	394 ± 25	39	15 656 ± 1 094	1 566
	Butternut	46 ± 1	104	5 135 ± 148	11 554	1 283 ± 73	2 887	14 ± 1	32	1 298 ± 73	2 920
	Vegetable stock powder	57 ± 1	9	N.D.	N.D.	37 476 ± 2 010	5 621	196 ± 8	29	37 672 ± 2000	5 651
	Parsley	428 ± 20	43	5 976 ± 472	598	20 336 ± 1 086	2 034	59 ± 4	6	20 396 ± 1 083	2 040

Values are indicated as mean ± standard deviation for content analysis per 100g and as mean per calculated content
[a] Recipe ingredients not contributing to either the total polyphenol content and TAC not listed
[b] Milligram gallic acid equivalents (mg GAE)
[c] Microgram (µg)
[d] Hydrophilic oxygen radical absorbance capacity (H-ORAC)
[e] Micromole Trolox equivalents (µmole TE)
[f] Lipophilic oxygen radical absorbance capacity (L-ORAC)
[g] Total antioxidant capacity (TAC), calculated as L-ORAC + H-ORAC
[h] Not detected (N.D.)

Table 4.2 continued – Butternut soup

Soup recipe formulation	Raw ingredient [a]	Total polyphenol contents (mg GAE [b] / 100g) mean +- SD	Calculated total polyphenol content (mg GAE) / actual weight of ingredient used	Carotenoid contents (ug [c] /100g) mean +- SD	Calculated carotenoid content (ug) / actual weight of ingredient used	H-ORAC [d] (µmole TE [e]/ 100g) mean +- SD	Calculated H-ORAC (µmole TE) / actual weight of ingredient used	L-ORAC [f] (µmole TE / 100g) mean +- SD	Calculated L-ORAC (µmole TE) / actual weight of ingredient used	TAC [g] (µmole TE / 100g) mean +- SD	Calculated TAC (µmole TE) / actual weight of ingredient used
Butternut	Lemon rind	323 ± 10	7	N.D.	N.D.	30 179 ± 2 005	604	75 ± 4	2	30 254 ± 200	605
	Salt	0 ± 0	0 ± 0	N.D.	N.D.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Coarse black pepper	271 ± 10	3	N.D.	N.D.	55 793 ± 4 045	558	546 ± 30	6	56 340 ± 4 026	563
	Onions	90 ± 3	204	N.D.	N.D.	2 014 ± 134	4 532	11 ± 1	25	2 025 ± 133	4 557
	Unfermented rooibos	61 ± 2	459	N.D.	N.D.	1 637 ± 78	12 280	0 ± 0	0 ± 0	1 637 ± 78	12 2780
	Fermented rooibos	44 ± 1	327	N.D.	N.D.	1 036 ± 36.	7 773	0 ± 0	0 ± 0	1 036 ± 36	7 772
	Butternut	43 ± 2	244	5 135 ± 148	29 013	1 440 ± 113	8 135	20 ± 1	111	1 460 ± 111	8 247
	Potatoes	44 ± 1	99	N.D.	N.D.	1 276 ± 62	2 870	5 ± 0	11	1 280 ± 61	2 881
	Vegetable stock powder	57 ± 1	6	N.D.	N.D.	37 476 ± 2 010	3 748	196 ± 8	20	37 672 ± 2000	3 767
	Lemon juice	44 ± 1	2	N.D.	N.D.	644 ± 21	32	0 ± 0	0 ± 0	644 ± 21	32
	Cinnamon	4 5340 ± 211	45	N.D.	N.D.	120 674 ± 4 390	1 207	249 ± 19	3	120 923 ± 4 381	1 209
	Milk	10 ± 0	9	N.D.	N.D.	143 ± 3	136	0 ± 0	0 ± 0	143 ± 3	136

Values are indicated as mean ± standard deviation for content analysis per 100g and as mean per calculated content
[a] Recipe ingredients not contributing to either the total polyphenol content and TAC not listed
[b] Milligram gallic acid equivalents (mg GAE)
[c] Microgram (µg)
[d] Hydrophilic oxygen radical absorbance capacity (H-ORAC)
[e] Micromole Trolox equivalents (µmole TE)
[f] Lipophilic oxygen radical absorbance capacity (L-ORAC)
[g] Total antioxidant capacity (TAC), calculated as L-ORAC + H-ORAC
[h] Not detected (N.D.)

Table 4.2 continued – Chicken noodle soup

Soup recipe formulation	Raw ingredient [a]	Total polyphenol contents (mg GAE [b] / 100g) mean +- SD	Calculated total polyphenol content (mg GAE) / actual weight of ingredient used	Carotenoid contents (ug [c] /100g) mean +- SD	Calculated carotenoid content (ug) / actual weight of ingredient used	H-ORAC [d] (µmole TE [e]/ 100g) mean +- SD	Calculated H-ORAC (µmole TE) / actual weight of ingredient used	L-ORAC [f] (µmole TE / 100g) mean +- SD	Calculated L-ORAC (µmole TE) / actual weight of ingredient used	TAC [g] (µmole TE / 100g) mean +- SD	Calculated TAC (µmole TE) / actual weight of ingredient used
Chicken noodle	Chicken stock powder	276 ± 15	83	N.D.	N.D.	21 844 ± 938	6 553	84 ± 7	25	21 928 ± 934	6 578
	Unfermented rooibos	61 ± 2	918	N.D.	N.D.	1 637 ± 78	24 560	0 ± 0	0 ± 0	1 637 ± 78	24 560
	Fermented rooibos	44 ± 1	654	N.D.	N.D.	1 036 ± 36	15 545	0 ± 0	0 ± 0	1 036 ± 36	15 545
	Carrots	6 ± 0	18	8 558 ± 718	25 674	1 189 ± 103	3 568	16 ± 1	47	1 205 ± 101	3 616
	Leeks	16 ± 0	36	N.D.	N.D.	1 942 ± 103	4 368	25 ± 2	55	1 966 ± 102	4 424
	Turnip	26 ± 1	23	N.D.	N.D.	1 281 ± 108	1 153	7 ± 0	6.0	1 287 ± 107	1 159
	Onions	109 ± 4	126	N.D.	N.D.	2 271 ± 167	2 611	6 ± 0	6.8	2 277 ± 166	2 619
	Soup celery	18 ± 1	7	N.D.	N.D.	1 072 ± 68	429	11 ± 1	4.5	1 083 ± 68	433
	Parsley	351 ± 14	140	5 976 ± 472	2 390	19 799 ± 921	7 919	74 ± 4	30	1 9873 ± 917	7 949
	Coarse black pepper	271 ± 10	14	N.D.	N.D.	114 051 ± 4 045	5 703	547 ± 30	27	114 597 ± 4 026	5 730
	Salt	0 ± 0	0 ± 0	N.D.	N.D.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Vermicelli	72 ± 2	29	N.D.	N.D.	15 682 ± 832	6 273	54 ± 4	22	15 736 ± 829	6 295

Values are indicated as mean ± standard deviation for content analysis per 100g and as mean per calculated content
[a] Recipe ingredients not contributing to either the total polyphenol content and TAC not listed
[b] Milligram gallic acid equivalents (mg GAE)
[c] Microgram (µg)
[d] Hydrophilic oxygen radical absorbance capacity (H-ORAC)
[e] Micromole Trolox equivalents (µmole TE)
[f] Lipophilic oxygen radical absorbance capacity (L-ORAC)
[g] Total antioxidant capacity (TAC), calculated as L-ORAC + H-ORAC
[h] Not detected (N.D.)

The fermented and unfermented rooibos as ingredients did not have the highest polyphenol content of the soup recipe formulation ingredients when compared on the same weight basis. The fermented rooibos total polyphenol content was 44 ± 1 mg GAE/100 g and that of the unfermented rooibos 61 ± 2 mg GAE/100 g (see Table 4.2). However, although the fermented and unfermented rooibos did not have the highest total polyphenol content, it was the greatest polyphenol contributor to the raw recipe formulations due to the highest ingredient quantity used in all three recipe formulations (see Table 4.1). When comparing the ingredients on the same weight basis, the herbs, spices and flavourings showed the highest polyphenol contents. Cinnamon contained 4 540 ± 211 mg GAE/100 g, parsley a mean of 389 mg GAE/100 g across the two ingredient use determinations, lemon rind 323 ± 10 mg GAE/100 g, chicken stock powder 276 ± 15 mg GAE/100 g and coarse black pepper 271 ± 10 mg GAE/100 g (see Table 4.2). However, these herbs, spices and flavourings made a very small percentage contribution to the recipe formulations (all less than 2%) (see Table 4.1). Each ingredient's contribution to the total polyphenol content of the various recipe formulations are shown in Table 4.2 expressed as mg GAE/relevant weight used in the formulations.

A carotenoid content was only detected in three of the raw ingredients. Carrots had the highest carotenoid contents (8 558 ± 718 µg /100 g) followed by parsley (5 976 ± 472 µg /100 g) and butternut (5 135 ± 148 µg /100 g) (see Table 4.2).

The herbs, spices and flavourings also had the highest H-ORAC of the raw ingredients. Cinnamon had the highest H-ORAC followed by coarse black pepper, parsley and vegetable stock powder. The H-ORAC of cinnamon (120 674 ± 4 390 µmole TE/100 g) is nearly double the H-ORAC of coarse black pepper (55 793 ± 4 045 µmole TE/100 g) (see Table 4.2). The H-ORAC of the raw recipe ingredients varied among the different vegetables. Onions had the highest H-ORAC (a mean of 2 056 µmole TE/100 g across the three soup recipe formulation use determinations) followed by leeks (1 942 ± 103 µmole TE/100 g), cauliflower (1 440 ± 91 µmole TE/100 g) and butternut (a mean of 1 362 µmole TE/100 g across the two ingredient use determinations). Celery in the chunky vegetable soup recipe formulation was the raw vegetable that had the lowest H-ORAC (840 ± 54 µmole TE/100 g) (see Table 4.2). The fermented and unfermented rooibos H-ORAC was higher than that of some of the vegetable types, but not as high as that of the herbs, spices and flavourings. The fermented rooibos H-ORAC was lower (1 036 ± 36 µmole TE/100 g) than the unfermented rooibos H-ORAC (1 637 ± 78 µmole TE/100 g) (see Table 4.2).

The fermented and unfermented rooibos made no contribution to the L-ORAC of the raw recipe formulations. The raw ingredients with the highest L-ORAC were coarse black pepper, tomato paste, cinnamon and the vegetable stock powder with values ranging from just below 200 µmole TE/100 g (196 ± 8 µmole TE/100 g for the tomato paste) up to nearly 550 µmole TE/100 g (547 ± 30 µmole TE/100 g) for the coarse black pepper (see Table 4.2). All the raw vegetable ingredients, although greatly varied, showed a L-ORAC capacity but some vegetables a very minimal L-ORAC. Leeks, butternut and carrots had a far higher L-ORAC than the other vegetables (25 µmole ± 2 TE/100 g and a mean of 17 µmole TE/100 g and 14 µmole TE/100 g, respectively, across the two ingredient use determinations) (see Table 4.2). The vegetables with the next highest L-ORAC was onions (a mean of 7 µmole TE/100 g across the three soup recipe formulation use determinations) and celery (7 ± 1 µmole TE/100 g). The H-ORAC of all the raw ingredients was much higher than the L-ORAC (see Table 4.2). The TAC of the raw ingredients comprised mainly of the H-ORAC of the raw ingredients.

4.2 Effect of rooibos inclusion on the total polyphenol and carotenoid contents and the TAC of the raw and cooked soup recipe formulations

The effect of the fermented and unfermented rooibos inclusions on the total polyphenol and the carotenoid contents and the TAC of the raw recipe formulation controls compared to the raw recipe experimental formulations are firstly discussed in this section. These results are followed by the results on the total polyphenol and the carotenoid contents and the TAC of the effect of the rooibos inclusion on the cooked experimental soup recipe formulations versus the cooked control soup recipe formulations. The TAC and antioxidant content results of the raw and cooked experimental, fermented versus unfermented, soup recipe formulations are also compared. These results were only compared for the total polyphenol content, H-ORAC and TAC as there was no carotenoid content or L-ORAC detected in the fermented and unfermented rooibos and therefore any difference between the two experimental formulations in the carotenoid content or L-ORAC would not be caused by the herbal teas. However, these results must be interpreted with caution as these are vertical comparisons and variances could have occurred with the distribution of the raw vegetables as ingredients for the preparation of the different soup recipe formulations. These sets of results for each of the soup recipe formulations are indicated in Table 4.3.

4.2.1 Chunky vegetable soup

The substitution of water with either fermented or unfermented rooibos in the raw recipe formulation, significantly ($p < 0.05$ for each) increased the total poyphenol content, with the control having a content of 20 ± 0 mg GAE/100 g, the fermented rooibos 37 ± 1 mg GAE/100 g and the unfermented rooibos 41 ± 1 mg GAE/100 g. There was furthermore a significant difference ($p < 0.05$) in the polyphenol content between the raw fermented and unfermented rooibos raw soup recipe formulations with, as indicated above, the raw unfermented rooibos experimental soup recipe formulation having the higher content (Table 4.3).

Table 4.3: Effect of rooibos inclusion and thermal processing on the total polyphenol and carotenoid contents and the TAC of the soup recipe formulations

Chunky vegetable soup						
Biochemical analysis	**Raw control mean +-SD**	**Raw fermented rooibos mean +-SD**	**Raw unfermented rooibos mean +-SD**	**Cooked Control mean +-SD**	**Cooked fermented rooibos mean +-SD**	**Cooked unfermented rooibos mean +-SD**
Total polyphenols (mg GAE^{i}/100g)	20 ± 0 $^{a c}$	37 ± 1 $^{b c e}$	41 ± 1 $^{b c f}$	22 ± 0 $^{a d}$	40 ± 1 $^{b d g}$	49 ± 2 $^{b d h}$
Carotenoid (ug^{j}/100g)	1 091 ± 49 $^{a c}$	1 032 ± 31 $^{a c}$	1 159 ± 59 $^{a c}$	528 ± 25$^{a d}$	568 ± 18 $^{b d}$	650 ± 83 $^{b d}$
H-ORAC^{k} (µmole TE^{l}/100g)	988 ± 113 $^{a c}$	1 538 ± 103 $^{b c e}$	1 680 ± 106 $^{b c f}$	1 017 ± 89 $^{a c}$	1 660 ± 134 $^{b c g}$	1 963 ± 25 $^{b d h}$
L-ORAC^{m} (µmole TE/100g)	10 ± 1 $^{a c}$	12 ± 4 $^{a c}$	12 ± 1 $^{a c}$	6 ± 1 $^{a d}$	7 ± 0$^{a d}$	7 ± 1 $^{a d}$
TAC^{n} (µmole TE/100g)	997 ± 114 $^{a c}$	1550 ± 107 $^{b c e}$	1 692 ± 106 $^{b c f}$	1 023 ± 88 $^{a c}$	1 666 ± 134 $^{b c g}$	1 970 ± 24 $^{b d h}$
Butternut soup						
Total polyphenols (mg GAE/100g)	26 ± 1 $^{a c}$	42 ± 2 $^{b c e}$	49 ± 0 $^{b c e}$	30 ± 0 $^{a d}$	48 ± 1 $^{b d g}$	51 ± 95 $^{b d h}$
Carotenoid (ug/100g)	900 ± 48 $^{a c}$	847 ± 22 $^{a c}$	872 ± 84 $^{a c}$	467 ± 33 $^{a d}$	596 ± 13 $^{b d}$	650 ± 52 $^{b d}$
H-ORAC (µmole TE/100g)	1 045 ± 134 $^{a c}$	1 473 ± 133 $^{b c e}$	1 684 ± 66 $^{b c e}$	1 080 ± 68 $^{a c}$	2 224 ± 125 $^{b d g}$	2 244 ± 154 $^{b d g}$
L-ORAC (µmole TE/100g)	8 ± 3 $^{a c}$	6 ± 1$^{a c}$	6 ± 1 $^{a c}$	2 ± 0 $^{a d}$	2 ± 2 $^{a d}$	1 ± 1 $^{a d}$
TAC (µmole TE/100g)	1 053 ± 137 $^{a c}$	1 479 ± 133 $^{b c e}$	1 690 ± 67 $^{b c e}$	1 083 ± 68 $^{a c}$	2 227 ± 123 $^{b d g}$	2 245 ± 152 $^{b d g}$

Table 4.3 continued

Biochemical analysis	**Raw control mean +-SD**	**Raw fermented rooibos mean +-SD**	**Raw unfermented rooibos mean +-SD**	**Cooked control mean +-SD**	**Cooked fermented rooibos mean +-SD**	**Cooked unfermented rooibos mean +-SD**
Chicken noodle soup						
Total polyphenols (mg GAE/100g)	27 ± 2 [a c]	48 ± 2 [b c e]	57 ± 1 [b c f]	33 ± 1 [a d]	43 ± 0 [b d g]	47 ± 1 [b d g]
Carotenoid (ug/100g)	1 600 ± 118 [a]	1 642 ± 149 [a]	1 525 ± 142 [a]	0 ± 0	0 ± 0	0 ± 0
H-ORAC (µmole TE/100g)	1 305 ± 187 [a c]	2 453 ± 328 [b c e]	2 555 ± 261 [b c e]	1 692 ± 93 [a d]	1 738 ± 132 [a d g]	1 889 ± 158 [a d g]
L-ORAC (µmole TE/100g)	22 ± 3 [a c]	22 ± 2 [a c]	20 ± 2 [a c]	0 ± 0 [a d]	1 ± 0 [a d]	1 ± 1 [a d]
TAC (µmole TE/100g)	1 326 ± 186 [a c]	2 475 ± 329 [b c e]	2 575 ± 262 [b c e]	1 692 ± 93 [a d]	1 739 ± 132 [a d g]	1 891 ± 159 [a d g]

Values in columns are indicated as mean ± standard deviation

[a, b] Results for rooibos inclusion: Control and experimental contents per raw or cooked soup recipe formulation indicated by the same symbol ([a]) are not significantly different ($p > 0.05$). Raw or cooked control and experimental contents per soup recipe formulation indicated with different symbols ([a] and [b]) are significantly different ($p < 0.05$).

[c, d] Results for thermal processing: Raw and cooked control and experimental contents per soup recipe formulation indicated by the same symbol (c) are not significantly different ($p > 0.05$). Raw and cooked control and experimental contents per soup recipe formulation indicated with different symbols ([c] and [d]) are significantly different ($p < 0.05$).

[e, f] Results for raw experimental soup recipe formulations (fermented versus unfermented rooibos): Raw experimental soup recipe formulation (fermented versus unfermented rooibos) contents per soup recipe formulation indicated by the same symbol ([e]) are not significantly different ($p > 0.05$). Raw experimental soup recipe formulation (fermented versus unfermented rooibos) contents per soup recipe formulation indicated with different symbols ([e] and [f]) are significantly different ($p < 0.05$).

[g h] Results for cooked experimental soup recipe formulations (fermented versus unfermented rooibos): Cooked experimental soup recipe formulation (fermented versus unfermented rooibos contents per soup recipe formulation indicated by the same symbol ([g]) are not significantly different ($p > 0.05$). Cooked experimental soup recipe formulation (fermented versus unfermented rooibos) contents per soup recipe formulation indicated with different symbols ([g] and [h]) are significantly different ($p < 0.05$).

[i] Milligram gallic acid equivalents (mg GAE)

[j] Microgram (µg)

[k] Hydrophilic oxygen radical absorbance capacity (H-ORAC)

[l] Micromole Trolox equivalents (µmole TE)

[m] Lipophilic oxygen radical absorbance capacity (L-ORAC)

[n] Total antioxidant capacity (TAC), calculated as L-ORAC + H-ORAC

The inclusion of fermented or unfermented rooibos in place of water in the raw soup recipe formulation had little effect on the carotenoid content of the raw control soup recipe formulation ($p > 0.05$ for each). An increase in the carotenoid content (+ 68 ug/100 g) occurred in the raw soup recipe formulation when water was replaced with unfermented rooibos and a near equivalent decrease in the carotenoid content (- 58 ug/100 g) in the raw soup recipe formulation when water was replaced with fermented rooibos.

The H-ORAC of the raw chunky vegetable soup recipe formulation (988 ± 113 µmole TE/100 g) also increased significantly ($p < 0.05$ for each) when water was substituted with fermented (1 538 ± 103 µmole TE/100 g) and unfermented (1 680 ± 106 µmole TE/100 g) rooibos, with a significant difference ($p < 0.05$) also found between the fermented and unfermented rooibos raw soup recipe formulations. The unfermented rooibos raw soup recipe formulation also had the higher H-ORAC, as it had the higher polyphenol content.

The inclusion of the fermented and unfermented rooibos in the place of water in the raw soup recipe formulation had little effect on the L-ORAC of the raw control soup recipe formulation ($p > 0.05$ for each). The increase in the L-ORAC was only 2 µmole TE/100g and 3 µmole TE/100 g in the soup recipe formulation when water was replaced with unfermented and fermented rooibos, respectively.

The TAC of the raw recipe formulations was substantially increased with the substitution of water with the fermented and unfermented rooibos as soup ingredients. The increase in the TAC of the raw control soup recipe formulation was higher (+ 695 µmole TE/100 g) with the substitution of unfermented rooibos compared to the increase (+ 553 µmole TE/100 g) with the substitution of fermented rooibos. The TAC, with the substitution of the unfermented and fermented rooibos in the raw recipe formulations compared to that of the raw control recipe formulation, was significantly higher ($p < 0.05$) for both the experimental recipe formulations. There was furthermore a significant difference ($p < 0.05$) between the fermented and unfermented rooibos raw soup recipe formulations with the unfermented rooibos experimental recipe formulation having the higher increase in the TAC and the higher TAC.

The polyphenol contents was also significantly ($p < 0.05$ for each) higher for the cooked fermented and unfermented rooibos soup recipe formulations (40 ± 1 mg and 49 ± 2 mg GAE/100 g, respectively) when compared to the cooked control recipe formulation (22 ± 0 mg GAE/100 g). The substitution with fermented rooibos in the cooked recipe formulation,

increased the polyphenol content by nearly double (81%), while substitution with unfermented rooibos doubled (119%) the polyphenol content. This resulted in a significant difference ($p < 0.05$) between the cooked fermented and unfermented rooibos raw soup recipe formulations (Table 4.3).

There was a significant difference ($p < 0.05$ for each) in the carotenoid content of both the cooked experimental soup recipe formulations versus that of the cooked control soup recipe formulation. The increase when the unfermented rooibos was included as an ingredient (+ 122 ug/100 g) was higher compared to the fermented rooibos herbal tea inclusion as the ingredient (+ 39 ug/100 g) replacing water in the cooked experimental soup recipe formulations.

The cooked chunky vegetable experimental soup recipe formulations resulted in a significantly ($p < 0.05$ for each) higher H-ORAC for the fermented (1659 ± 134 µmole TE/100 g) and unfermented (1963 ± 25 µmole TE/100 g) rooibos soup recipe formulations when compared to the cooked control (1 017± 89 µmole TE/100 g) chunky vegetable soup recipe formulation. There was also a significant ($p < 0.05$) increase in the H-ORAC on the substitution of water with unfermented rooibos (+ 946 µmole TE/100 g) compared to the substitution of fermented rooibos (+ 642 µmole TE/100 g) in the cooked chunky vegetable soup recipe formulation. The H-ORAC of the thermally processed unfermented rooibos formulation (1 963 ± 25 µmole TE/100 g) was almost double the H-ORAC of the thermally processed control formulation (1 017 ± 89 µmole TE/100 g).

As with the raw recipe formulations, the inclusion of the rooibos had little effect on the cooked recipe's L-ORAC in the chunky vegetable soup recipe formulations, with no significant differences ($p > 0.05$ for each) in the L-ORAC of the cooked control versus both that of the cooked experimental soup recipe formulations.

There was a significant difference ($p < 0.05$ for each) in the TAC of the cooked control soup recipe formulation versus both the cooked rooibos herbal tea experimental soup recipe formulations. The TAC of the cooked unfermented rooibos formulation (1 970 ± 24 µmole TE/100 g) was almost double that of the cooked control soup recipe formulation (1 023 ± 88 µmole TE/100 g), a result also found for the H-ORAC of these cooked soup recipe formulations. The TAC of the cooked fermented rooibos soup recipe formulation was 63% higher than the TAC of the cooked control soup recipe formulations. This resulted in a significant difference ($p < 0.05$) in the TAC of the

cooked unfermented rooibos formulation versus the cooked fermented rooibos soup recipe formulation.

4.2.2 Butternut soup

The inclusion of fermented or unfermented rooibos in the place of water as an ingredient in the raw butternut recipe formulation, increased the total polyphenol content in both. There was a significant increase ($p < 0.05$ each) in the total polyphenol content of the raw control soup recipe formulation (26 ± 1 mg GAE/100 g) when fermented (42 ± 2 mg GAE/100 g) and unfermented (49 ± 0 mg GAE/100 g) rooibos were added to the soup recipe formulations. The increase when unfermented rooibos (106 %) was included in the place of water as an ingredient was higher compared to the fermented rooibos (88 %) inclusion, but this did not result in the total polyphenol content of the two raw experimental soup recipe formulations being significantly ($p > 0.05$) different (Table 4.3).

The substitution of water with either fermented or unfermented rooibos in the butternut soup raw recipe formulation had no significant ($p > 0.05$ for each) effect on the carotenoid content when compared to the raw control soup recipe formulation. Substitution with fermented rooibos resulted in a non-significant decrease of 53 µg/100 g in the carotenoid content, while unfermented rooibos substitution resulted in a non significant decrease of 29 µg/100 g when compared with the raw control soup recipe formulation.

The inclusion of the fermented or unfermented rooibos in the place of water as an ingredient in the raw butternut recipe formulation significantly ($p < 0.05$) increased the H-ORAC in both. The raw recipe formulation with the unfermented rooibos inclusion only had a slightly higher H-ORAC (1 684 ± 66 µmole TE/100 g) compared to the raw recipe formulation with the fermented rooibos inclusion (1 473 ± 133 µmole TE/100 g) ($p > 0.05$). The inclusion of the fermented and unfermented rooibos in the butternut soup raw recipe formulation had little effect on the L-ORAC of the raw control soup recipe formulation ($p > 0.05$ for each).

The fermented or unfermented rooibos substitution of water as an ingredient, significantly ($p < 0.05$ for each) increased the TAC of both the raw experimental soup recipe formulations compared to the control soup recipe formulation containing water. Although there was a greater increase in the TAC with the substitution of the unfermented rooibos (+ 637 µmole TE/100 g) compared to the

substitution with the fermented rooibos (+ 426 µmole TE/100 g) to the raw control soup recipe formulation there was no significant ($p > 0.05$) difference in the TAC of the two raw experimental soup recipe formulations.

The cooked experimental soup recipe formulations also had a significantly ($p < 0.05$ for each) higher total polyphenol content for the fermented and unfermented rooibos soup recipe formulations (48 ± 1 and 51 ± 1 mg GAE/100 g, respectively) compared to the cooked control soup recipe formulation (30 ± 0 mg GAE/100 g). The slightly greater increase in the polyphenol content on the substitution of water with unfermented rooibos (+ 21 mg GAE/100 g) compared to the substitution with fermented rooibos (+ 19 mg GAE/100 g) in the cooked butternut soup recipe formulation did not result in the polyphenol content of the two cooked experimental soup recipe formulations being significantly ($p > 0.05$) different (Table 4.3).

In the cooked soup recipe formulations the inclusion of the fermented or unfermented rooibos also significantly ($p < 0.05$ for each) increased the carotenoid content when compared to the cooked control. The increase was higher with the substitution of unfermented rooibos (+ 183 ug/100 g) compared to the substitution with fermented rooibos (+ 129 ug/100 g) in the cooked soup recipe formulations.

The cooked butternut soup recipe formulation had a significantly ($p < 0.05$ for each) higher H-ORAC for the fermented and unfermented rooibos soup recipe formulations (2 224 ± 125 and 2 244 ± 154 µmole TE/100 g, respectively) when compared to the cooked control (1 080 ± 68 µmole TE/100 g) soup recipe formulation. The H-ORAC of the cooked unfermented rooibos formulation (2 244 ± 154 µmole TE/100 g) and fermented rooibos formulations (2 224 ± 125 µmole TE/100 g) was double the H-ORAC of the cooked control soup recipe formulation (1 080 ± 68 µmole TE/100 g). There was a slightly greater increase in the H-ORAC on the substitution of water with unfermented rooibos (+ 1 164 µmole TE/100 g) compared to the substitution of fermented rooibos (+ 1 144 µmole TE/100 g) in the cooked butternut soup recipe formulation but this did not result in a significant ($p > 0.05$) difference in the H-ORAC of the two cooked experimental soup recipe formulations. As with the raw recipe formulations, the inclusion of the rooibos had a non significant ($p > 0.05$ for each) effect on the cooked butternut soup recipe formulation's L-ORAC.

In the cooked soup recipe formulation the inclusion of the fermented or unfermented rooibos also increased the TAC significantly ($p < 0.05$ for each). The increase in the TAC was more than 100%

when water was substituted with fermented and unfermented rooibos in the cooked soup recipe formulation. The cooked control soup recipe formulation had a TAC of 1 083 ± 68 µmole TE/100 g, while the TAC of the cooked unfermented rooibos soup recipe formulation was 2 245 ± 152 µmole TE/100 g and the TAC of the cooked fermented rooibos soup recipe formulation 2 227 ± 123 µmole TE/100 g. There was however, no significant ($p > 0.05$) difference in the TAC of the two cooked experimental soup recipe formulations.

4.2.3 Chicken noodle soup

The inclusion of fermented or unfermented rooibos in the place of water as an ingredient in the raw chicken noodle soup recipe formulation, significantly ($p < 0.05$) increased the total polyphenol content in both experiments. There was a significant difference ($p < 0.05$) in the polyphenol content of the raw control (27 ± 2 mg GAE/100 g) versus both the raw fermented (48 ± 2 mg GAE/100 g) and unfermented (57 ± 1 mg GAE/100 g) rooibos experiments. There was a significant increase ($p < 0.05$) when the unfermented rooibos substituted water (+ 30 mg GAE/100 g) compared to the substitution with fermented rooibos (+ 21 mg GAE/100 g) in the raw soup recipe formulations (Table 4.3).

There was no significant differences ($p > 0.05$ for each) in the carotenoid content and L-ORAC of the raw control versus both the raw fermented and unfermented rooibos soup recipe formulations. The inclusion of fermented and unfermented rooibos in place of water as the fluid ingredient in the raw chicken noodle soup recipe formulation, also significantly ($p < 0.05$ for each) increased the H-ORAC (by 1 148 µmole TE/100 g and 1 250 µmole TE/100 g, respectively) when compared to the control. In the raw recipe formulation, the substitution of water as an ingredient with fermented and unfermented rooibos, also significantly ($p < 0.05$) increased the TAC (+ 1149 µmole TE/100 g for the fermented and + 1249 µmole TE/100 g for the unfermented) when compared to the control soup recipe formulation. There was, however, no significant ($p > 0.05$ for each) difference in the H-ORAC and the TAC of the two raw experimental soup recipe formulations.

The cooked experimental soup recipe formulations also resulted in a significantly ($p < 0.05$ for each) higher total polyphenol content for the cooked fermented and unfermented rooibos soup formulations (43 ± 0 and 47 ± 1 mg GAE/100 g, respectively) when compared to the cooked control soup recipe formulation (33 ± 1 mg GAE/100 g). The total polyphenol content of the two cooked experimental soup recipe formulations was not significantly ($p > 0.05$) different (Table 4.3).

The cooked experimental soup recipe formulations had a slightly higher H-ORAC compared to the cooked control formulation. The increase was higher with the substitution of unfermented rooibos (+ 197 µmole TE/100 g) compared to the substitution with fermented rooibos (+ 46 µmole TE/100 g) in the cooked soup recipe formulation, but there was no significant ($p > 0.05$ for each) difference in the H-ORAC of the soup recipe formulations. Neither was there a significant difference ($p > 0.05$) in the L-ORAC of the cooked control versus both the thermally processed fermented and unfermented rooibos soup recipe formulations. No carotenoids could be detected in the cooked control and experimental soup recipe formulations, due to the removal of all the vegetables from the cooked control and experimental soup recipe formulations (see Addendum I for the soup recipe formulation).

The inclusion of fermented and unfermented rooibos in the cooked recipe formulations increased the TAC of both although not significantly ($p > 0.05$). Although the increase in the TAC was higher with the inclusion of unfermented rooibos (+ 199 µmole TE/100 g) compared to the recipe formulation with the fermented rooibos (+ 47 µmole TE/100 g) inclusion, there was no significant ($p > 0.05$) difference in the TAC of the two cooked experimental soup recipe formulations.

4.3 Effect of thermal processing on the total polyphenol and carotenoid contents and the TAC of the soup recipe formulations

Thermal processing did not greatly reduce the moisture content of the soup recipe formulations (Table 4.4). The moisture reduction percentage ranged from approximately 1% (between the raw and cooked control and unfermented rooibos experimental butternut soup recipe formulations) and 1% (between the raw control and both the experimental chunky vegetable soup recipe formulations versus these cooked recipe formulations) up to between 2.3% and 4.5% (between the raw control and both the experimental chicken noodle soup recipe formulations versus these cooked recipe formulations). The higher percentage moisture content loss that occurred in the thermal processing of the chicken noodle soup recipe formulation could be due to the longer thermal processing time involved (see Addendum I). No corrections were made to the small deviations in the moisture content and the total polyphenol, carotenoid contents and the H-ORAC, L-ORAC and TAC results reported, as the results are reported as the soup recipe formulations would be consumed (FW basis). Other studies have also reported on the "as eaten" basis with no corrections made for the moisture content deviations (Volden *et al.*, 2008:603). Normalisation for the moisture content by reporting on the basis of the dry matter may be important for comparison purposes when the thermal processing of single vegetables is reported due to the varying water content of vegetables and the amount of water used and leaching needs to be intensively considered (Turkmen *et al.*, 2005:714). In the thermal processing of soup recipe formulations the water used is retained and not discarded as with the wet thermal processing of single vegetables.

Table 4.4: Effect of thermal processing on the moisture contents of the soup recipe formulations

Soup recipe formulation	Moisture content of soup recipe formulations					
	Chunky vegetable		Butternut		Chicken noodle	
	%	SD[a]	%	SD[a]	%	SD[a]
Raw control	90	1	90	0	93	0
Raw fermented rooibos herbal tea	89	1	90	0	92	0
Raw unfermented rooibos herbal tea	89	1	89	0	91	0
Cooked control	89	0	90	0	90	0
Cooked fermented rooibos herbal tea	88	0	87	0	88	0
Cooked unfermented rooibos herbal tea	88	0	88	0	87	0

[a] Standard deviation (SD)

4.3.1 Chunky vegetable soup

As shown in Table 4.3 thermal processing resulted in a significant ($p < 0.05$) increase in the total polyphenol content of the thermally processed control chunky vegetable soup recipe formulation when compared to the raw control soup recipe formulation. The experimental soup recipe formulations also both increased in total polyphenol contents after thermal processing with the total polyphenol contents in the control and both the experimental recipe formulations significantly higher ($p < 0.05$ for each) when compared to the raw soup recipe formulations containing either water, fermented or unfermented rooibos. The increase in polyphenol content after thermal processing in the experimental soup recipe formulations was the highest with the use of unfermented rooibos as the liquid ingredient. On thermal processing the polyphenol content increased with 3 mg GAE/100 g in the fermented rooibos soup recipe formulation and with 8 mg GAE/100 g in the unfermented rooibos soup recipe formulation.

Thermal processing had a detrimental effect on the carotenoid content of the control and both the experimental chunky vegetable soup recipe formulations (Table 4.3). The carotenoid content with thermal processing significantly decreased ($p < 0.05$ for each) in the raw and cooked control and experimental soup recipe formulations. The decrease in the carotenoid content was the greatest with thermal processing of the raw control soup recipe formulation (- 563 µg/100 g), followed by the unfermented rooibos experimental soup recipe formulation (- 509 µg/100 g) and with the lowest decline occurring in the raw fermented rooibos experimental soup recipe formulation (- 465 µg/100 g).

There was a slight although not significant ($p > 0.05$) increase in the H-ORAC after thermal processing of the cooked control chunky vegetable soup recipe formulation compared to the raw control soup recipe formulation. Thermal processing of the soup recipe formulation when water was substituted with fermented rooibos did not result in a significant ($p > 0.05$) increase in H-ORAC when compared to the raw recipe formulation, while thermal processing of the experimental recipe formulation substituted with unfermented rooibos, resulted in a significantly ($p < 0.05$) higher H-ORAC, when compared to the raw experimental soup recipe formulation. Thermal processing significantly ($p < 0.05$ for each) reduced the L-ORAC of the cooked control and experimental chunky vegetable soup recipe formulations when compared to the raw control and the experimental recipe formulations. Thermal processing increased the TAC of the control and experimental chunky vegetable soup recipe formulations; however, the increase was only significant ($p < 0.05$) for the thermally processed soup recipe

formulation containing unfermented rooibos versus the raw recipe formulation containing unfermented rooibos as was found for the increase in the H-ORAC (Table 4.3).

A strong correlation was found between the TAC and the total polyphenol content ($r = 0.999$) and the H-ORAC ($r = 1.000$) of the chunky vegetable soup recipe formulation, whereas the correlation between the TAC and the L-ORAC ($r = 0.088$) and the carotenoid content ($r = 0.073$) was low.

4.3.2 Butternut soup

As shown in Table 4.3 thermal processing also resulted in a significant ($p < 0.05$) increase in the total polyphenol content of the cooked control butternut soup recipe formulation when compared to the raw control recipe formulation. The experimental soup recipe formulations also both increased in total polyphenol contents after thermal processing with the total polyphenol contents in the control and both the experimental recipe formulations significantly higher ($p < 0.05$ for each) when compared to the raw soup recipe formulations containing either water, fermented or unfermented rooibos. The increase in the total polyphenol content on thermal processing was the highest (+ 6 mg GAE/100 g) for the experimental recipe formulation containing fermented rooibos.

Thermal processing significantly ($p < 0.05$ for each) reduced the carotenoid content of the cooked control and both the cooked experimental butternut soup recipe formulations compared to the raw control and the raw experimental soup recipe formulations (Table 4.3). This detrimental effect on the carotenoid content of the control and both the experimental butternut soup recipe formulations was also found with the chunky vegetable soup control and experimental recipe formulations (see 4.3.1).

There was a slight although not significant ($p > 0.05$) increase in the H-ORAC after thermal processing of the cooked control butternut soup recipe formulation compared to the raw control butternut soup recipe formulation. The experimental soup recipe formulations also both increased in H-ORAC after thermal processing with the H-ORAC of both experimental recipe formulations significantly higher ($p < 0.05$ for each) when compared to the raw soup recipe formulations containing either fermented or unfermented rooibos. Thermal processing significantly ($p < 0.05$ for each) decreased the L-ORAC of the cooked control and both experimental butternut soup recipe formulations when compared to the raw control and the experimental recipe formulations as was found for the carotenoid contents (Table 4.3) and the L-ORAC of the cooked control and both the experimental chunky vegetable soup recipe formulations (see 4.3.1).

Thermal processing increased the TAC of the control butternut soup recipe formulation; however, this increase was not significant ($p > 0.05$). The experimental soup recipe formulations' TAC also both increased after thermal processing, with the TAC of both experimental recipe formulations significantly higher ($p < 0.05$ for each) when compared to the raw soup recipe formulations containing either fermented or unfermented rooibos (Table 4.3).

As for the chunky vegetable soup recipe formulation a strong correlation existed between the TAC and the total polyphenol content ($r = 0.909$) and the H-ORAC ($r = 1.000$) for the butternut soup recipe formulation with the correlation between the TAC and the L-ORAC ($r = -0.577$) and the carotenoid content ($r = -0.199$) again low.

4.3.3 Chicken noodle soup

Thermal processing significantly ($p < 0.05$) increased the total polyphenol content of the cooked control chicken noodle soup recipe formulation compared to the raw soup mixture. Thermal processing of the chicken noodle soup experimental recipe formulations when water was substituted with fermented or unfermented rooibos resulted in significantly decreased ($p < 0.05$ for each) total polyphenol contents of these soup recipe formulations. The loss in the polyphenol content after thermal processing in the unfermented rooibos experimental soup recipe formulation was double (- 10 mg GAE/100 g) compared to the loss in the fermented rooibos experimental soup recipe formulation (- 5 mg GAE/100 g) (Table 4.3).

No carotenoids could be detected in either the thermally processed control or experimental chicken noodle soup recipe formulations (Table 4.3). Thermal processing therefore extensively reduced the carotenoid content of the cooked control and both the experimental chicken noodle soup recipe formulations compared to the raw control and the raw experimental soup recipe formulations carotenoid content.

There was a significant ($p < 0.05$) increase in the H-ORAC after thermal processing of the cooked control chicken noodle soup recipe formulation compared to the raw control soup recipe formulation. The opposite results occurred for the experimental recipe formulations. The H-ORAC of the chicken noodle experimental soup recipe formulations on thermal processing was significantly lower ($p < 0.05$ respectively) than that of the raw soup mixtures. The decrease in the H-ORAC on thermal processing was somewhat higher in the experimental soup recipe formulation with fermented rooibos with it decreasing 715 µmole

TE/100 g compared to the experimental soup recipe formulation with unfermented rooibos that decreased by 666 µmole TE/100 g with thermal processing (Table 4.3).

Thermal processing significantly ($p < 0.05$ for each) decreased the L-ORAC of the cooked control and both experimental chicken noodle soup recipe formulations when compared to the raw control and the experimental recipe formulations. Thermal processing resulted in a decrease of the L-ORAC to between 0 and 1 µmole TE/100 g measured in the three cooked soup mixtures. The highest decrease on thermal processing occurred in the fermented rooibos (- 22 µmole TE/100 g) followed by the control (- 22 µmole TE/100 g) soup recipe formulation and the lowest decrease in the unfermented rooibos (-19 µmole TE/100 g) soup recipe formulation (Table 4.3).

The TAC of the raw control soup recipe formulation significantly ($p < 0.05$) increased on thermal processing by 366 µmole TE/100 g. There was a significant ($p < 0.05$ for each) decrease in the TAC of the experimental soup recipe formulations on thermal processing compared to the TAC of the raw experimental recipe formulations. The TAC of the experimental soup recipe formulation with the fermented rooibos herbal tea decreased by 736 µmole TE/100 g and the TAC of the experimental soup recipe formulation with the unfermented rooibos herbal tea by 684 µmole TE/100 g after thermal processing (Table 4.3). These changes in the TAC of the control and experimental soup recipe formulations correspond to the changes in the H-ORAC of the soup recipe formulations on thermal processing.

A strong correlation also existed between the TAC ($r= 0.900$) and the H-ORAC ($r = 1.000$) for the chicken noodle soup recipe formulation with the correlation between the TAC and the L-ORAC ($r = 0.38$) and the carotenoid content ($r = 0.39$) again low.

CHAPTER 5

DISCUSSION

The results of the experimental study are discussed in conjunction with other similar study findings. The polyphenol content and TAC of the raw recipe ingredients are firstly compared to the published values in the United States Department of Agriculture (USDA) database. The effect of the rooibos inclusion in the soup recipe formulations is hereafter discussed and compared to other studies where antioxidant rich food items had also been added to recipe formulations. The effect of thermal processing on the soup recipe formulations is lastly compared to the effect of thermal processing on individual vegetables.

5.1 Raw ingredient total polyphenol content and TAC

Obvious differences in the phytochemical contents of food occur due to the different cultivars and growing conditions, making interpretations and comparisons between studies difficult (Volden *et al.,* 2008:596). The total polyphenol content and the TAC determined for the raw recipe ingredients in this study was therefore only compared to that published in the ORAC database of selected foods as published by the USDA (USDA, 2007).

In this study the spice, herb and flavouring ingredients had the highest polyphenol contents when compared on the same weight basis to the other soup recipe ingredients. It has already been recommended that herbs should be added to recipes to increase individuals' antioxidant intake (Craig, 1999:492S; Justesen & Knuthsen, 2001:245). It has also been proposed that specific reference should be made to the inclusion of herbs and spices in dietary guidelines. This proposal is based on evidence of mechanisms through which herbs and spices may provide protection against oxidative mechanisms and the very high phenolic content of herbs and spices that may increase the antioxidant capacity of meals with the addition of just a few grams to food (Tapsell, 2008:132). The total polyphenol content determined for cinnamon was 4 540 ± 211 mg GAE/100 g, while the USDA database reported a polyphenol content of 15 718 mg GAE/100 g. Su, Yin, Charles, Zhou, Moore & Yu (2007:996) obtained extractions from black peppercorn, nutmeg, cinnamon and oregano leaf with 50% acetone and 80% methanol and

evaluated their radical scavenging activities against cation, peroxyl and hydroxyl radicals. For each spice extraction, the total phenolic content and chelating activity was also determined. The extracts of all the samples showed significant radial scavenging capacities and chelating abilities. Cinnamon had the highest natural phenolic content and the strongest antioxidant properties (Su *et al.,* 2007:996). In contrast, the polyphenol content determined for parsley was 389 mg GAE/100 g in comparison to the USDA database reported polyphenol content of only 77 mg GAE/100 g for parsley. The polyphenol content of 178 mg GAE/100 g determined for tomato paste was the same as that reported for tomato paste in the USDA database. The USDA database reported a much higher polyphenol content (175 mg GAE/100 g) for lemon juice compared to the content determined in this study (44 mg GAE/100 g).

Previous studies (Velioglu, Mazza, Gao & Oomah, 1998:4113; Sevick, Kondrashov, Kvasnicka, Vacek, Hamouz, Jiruskova, Voldrich & Cizkova, 2009:171) as well as this study have shown a strong correlation between the total polyphenol content and the TAC. Cinnamon had a TAC of 120 923 µmole TE/100 g while in the USDA database the TAC for cinnamon was much higher at 267 536 µmole TE/100 g. Coarse black pepper had a TAC of 114 597 µmole TE/100 g while in the USDA database the TAC was far lower (27 618 µmole TE/100 g). Parsley had a TAC of 20 134 µmole TE/100 g while in the USDA database a TAC of only 1 301 µmole TE/100 g was reported. The TAC of the tomato paste was 15 656 µmole TE/100 g while in the USDA database the TAC was only 694 µmole TE/100 g. The analysed total polyphenol content and TAC of the herbs, spices and flavourings were either far lower or higher compared to the USDA's total polyphenol content and TAC of the herbs, spices and flavourings. Although herbs and spices are used in small quantities in recipe formulation their contribution to the total polyphenol content and the TAC can be substantial.

The total polyphenol content of the four vegetables used across the soup recipe formulations is published in the USDA database. The different vegetable inclusions had varying polyphenol contents. Raw carrots had a polyphenol content of 8 mg GAE/100 g while in the USDA database the polyphenol content is recorded as 35 mg GAE/100 g. Raw potatoes had a polyphenol content of 39 mg GAE/100 g while in the USDA database the polyphenol content was higher (163 mg GAE/100 g). The USDA database also had a higher polyphenol content (111 mg GAE/100 g) for raw cauliflower compared to this study (69 mg GAE/100 g). However, for the raw onion the polyphenol content determined of 93 mg GAE/100 g was somewhat higher than that reported for raw onion in the USDA database (23 mg GAE/100 g). No polyphenol contents were reported in the USDA database for the other vegetable ingredients included in

the soup recipe formulations. Three of the four vegetable ingredients analysed had a lower total polyphenol content compared to the total polyphenol content published in the USDA database. The lower total polyphenol contents in the analysed vegetables compared to the USDA database values is possibly due to different cultivars used for analysis and different environmental growing conditions (Volden *et al.,* 2008:596). Different pre-preparation techniques could also possibly cause variances between the analysed values in this study compared to the USDA database values. Pre-preparation of fruits and vegetables such as peeling, cutting and slicing are expected to cause rapid enzymatic loss of several natural antioxidants (Nicoli *et al.,* 1999:96). Cutting exposes the inner tissues to oxygen and light (Ruiz-Rodriguez & Marin, 2008:345).

A previous study reported on the phenolic content of five vegetables, raw and thermally processed (Ismail, 2004:581). Among all the fresh vegetables, spinach had the highest phenolic content, followed by swamp cabbage, kale, shallots and cabbage (Ismail, 2004:581). Spinach was not a vegetable ingredient used in the soup recipe formulations of the current study. Raw onion was the vegetable ingredient with the highest polyphenol content (93 mg GAE/100 g) in this study. The USDA database vegetable with the highest polyphenol content across the four vegetables used in the soup recipe formulations for which a polyphenol content was published, was raw cauliflower (111 mg GAE/100 g). Raw carrot was the vegetable ingredient with the lowest polyphenol content (8 mg GAE/100 g) in this current study. The USDA database vegetable with the lowest polyphenol content across the four vegetables used in the soup recipe formulations for which a polyphenol content was published, was raw onion (23 mg GAE/100 g).

The TAC of the six vegetables used in the current study across the soup recipe formulations is also published in the USDA database. Raw carrots had a TAC of 1 097 µmole TE/100 g while in the USDA database the TAC was 666 µmole TE/100 g. Raw cauliflower had a TAC of 1 444 µmole TE/100 g while in the USDA database the TAC for raw cauliflower was much lower (829 µmole TE/100 g). Raw celery had a TAC of 1 083 µmole TE/100g while in the USDA database the TAC was 497 µmole TE/100 g. Raw leeks had a TAC of 1 966 µmole TE / 100 g which was more than 200% higher than the USDA database TAC for raw leeks (490 µmole TE/100 g). The TAC for raw onions was 2 063 µmole TE100 g while in the USDA database the TAC was 1 034 µmole TE/100 g. Raw potatoes had a TAC of 1 100 µmole TE/100 g while in the USDA database the TAC was 1 058 µmole TE/100 g. Raw onion was the vegetable ingredient with the highest TAC (2 063 µmole TE/100 g) in this study. Onions have relatively high scavenging properties among the different vegetables (Pinilla *et al.,* 2005:S60). The USDA database

vegetable with the highest TAC across the six vegetables used in the soup recipe formulations, for which a TAC was published, was raw potatoes (1058 µmole TE/100 g). Raw celery was the vegetable ingredient with the lowest TAC (847 µmole TE/100g) in this study. The USDA database vegetable with the lowest TAC across the six vegetables used in the soup recipe formulations for which a TAC was published, was raw leeks (490 µmole TE/100 g). The TAC of the six vegetables used in the current study across the soup recipe formulations were all higher compared to the TAC values published in the USDA database. The South African vegetable cultivation environment may be partly be responsible for this finding. A previous study (Stewart, Bozonnett, Mullen, Jenkins, Lean & Crozier, 2000:2668) found that tomatoes originating from warm, sunny climates such as Spain, Israel and South Africa have a far higher phenolic flavonol concentration than tomatoes originating from growing conditions with lower light and sunlight exposure, such as from greenhouses (Stewart *et al.*, 2000:2668).

Split peas had an analysed total polyphenol content of 51 mg GAE/100 g, while in the USDA database the polyphenol content reported was somewhat higher (74 mg GAE/100 g). Split peas had a TAC of 1 553 µmole TE/100 g while in the USDA database split peas had a TAC of about a third of this capacity (524 µmole TE/100 g).

5.2 Effect of rooibos inclusion on the TAC of the soup recipe formulations

Rooibos herbal teas contain bioactive ingredients, such as flavonoids (Schulz *et al.*, 2003:540), that will add to the antioxidant properties of the soup recipe formulations. In all three raw soup recipe formulations the inclusion of the fermented or unfermented rooibos as the major ingredient significantly increased ($p < 0.05$ for each) the TAC of the raw control soup recipe formulations containing water as the major ingredient. The unfermented rooibos herbal tea inclusion in all three raw soup recipe formulations resulted in a greater increase in the TAC compared to the inclusion of the fermented rooibos herbal tea in the raw soup recipe formulation.

In all three the cooked soup recipe formulations the inclusion of the fermented or unfermented rooibos as the major ingredient also increased the TAC of the cooked control soup recipe formulations containing water as the major ingredient. This increase was significant ($p < 0.05$) for the cooked chunky vegetable and butternut soup recipe formulations. The unfermented rooibos in all three cooked soup recipe formulations also resulted in a greater increase in the TAC compared to the inclusion of the fermented rooibos in the cooked soup recipe formulations.

The TAC of the raw and the cooked unfermented rooibos chunky vegetable soup recipe formulations was significantly ($p < 0.05$ for each) higher than the TAC of the raw and the cooked fermented rooibos chunky vegetable soup recipe formulations.

The total polyphenol content and composition of the unfermented (green) rooibos differs from that of the fermented (traditional) rooibos. A substantial loss of flavonoids occurs as a result of oxidation taking place during the fermentation process of the fermented (traditional) rooibos (Schulz *et al.*, 2003:540). The processing steps entail heap fermentation of the comminuted leaves and fine stems for several hours during which the desired oxidative changes take place, followed by sun-drying and sieving to remove coarse material consisting mainly of woody stems. The dry product is sterilised by steam pasteurization before bulk packaging (Standley, Winterton, Marnewick, Gelderblom, Joubert & Britz, 2001:114).

Using recipe manipulations to possibly add to or improve the antioxidant properties of food has already been tried in bread baking where a green tea extract was added as an ingredient (Wang, Zhou & Isabelle, 2007:471) and barley replaced 40% of the wheat flour used as an ingredient (Holtekjølen, Baevre, Rødbotten, Berg & Knutsen, 2008:414). The green tea extract study focused on the quality of the bread and not as such on the antioxidant properties (Wang *et al.*, 2007:471) whereas the study where barley was incorporated in the bread, focused on the antioxidant properties (Holtekjølen *et al.*, 2008:414). In this study the incorporation of barley increased the antioxidant properties of the breads compared to the control bread as barley contains bioactive components (Wang *et al.*, 2007:471). The study where green tea extract was added to bread found that the tea catechins were relatively stable in breadmaking, having 84% of the total tea catechins remaining after baking as well as during the shelf life (Wang *et al.*, 2007:471). Mango peel, which is a by-product obtained during processing of mango products, have also been incorporated into macaroni. Macaroni products with incorporated dried and powdered mango peel also exhibited significantly ($p < 0.05$) increased polyphenol contents and improved antioxidant activity (Ajila, Aalami, Leelavathi & Rao, 2010:219).

5.3 Effect of thermal processing on the total polyphenol content, H-ORAC and the TAC of the soup recipe formulations

Vegetables are frequently subjected to various forms of processing to make them more suitable for consumption (Volden *et al.,* 2008:596). When vegetables are submitted to cooking processes variations appear in their antioxidant activity or scavenger capacity. These variations depend on the vegetables themselves, the cooking method, the bioavailability of the phenolics, the temperature, localisation of the structures in the vegetables, cutting, stability of the structure to heat, the synergic activity of the structures and on the reaction systems assayed (Jimenez-Monreal*,* Garcia-Diz, Martinez-Tome, Mariscal & Murcia*,* 2009:H102).

Wet thermal processing can affect the phytochemical contents by thermal breakdown, as the integrity of the cell structure is lost by migration of components, leading to losses by leakage or breakdown by enzymatic action and non enzymatic factors, such as light and oxygen (Volden *et al.,* 2008:596). After various cooking procedures, the total phenolic content of squash, peas and leeks was significantly ($p < 0.05$) reduced in a study by Turkmen *et al.* (2005:715) and the reductions were the same in all the cooking methods applied. A study conducted to determine the phenolic content of five vegetables, raw and thermally processed, also found reduced levels. Swamp cabbage lost the highest amount of phenolic content (26%), followed by cabbage (20%), spinach (14%), shallots (13%) and kale (12%) after one minute blanching in water (Ismail, Marjan & Foong*,* 2004:584).

However, when optimised, thermal treatment will rapidly inactivate enzymes, prevent oxygen access and minimise leakage, thus increasing the retention of plant constituents (Volden *et al.,* 2008:596). The total phenolic content of pepper, broccoli and green beans was significantly ($p < 0.05$) increased to various extents in a study by Turkmen *et al.* (2005:715), depending on the type of cooking method used. A minimal increase in the total phenolics of spinach resulted when cooked by all methods (Turkmen *et al.,* 2005:715). A study was also conducted to investigate how heat treatments affect the polyphenols as well as the hydrophilic antioxidant capacity of tomato products. The results showed an increase in the total phenolics with thermal processing of tomatoes, possibly due to the liberation of the phenolics from the food matrix. The hydrophilic antioxidant activity increased during the preparation of baked tomatoes, tomato sauce and tomato soup despite that a continuous loss of the water-soluble vitamin C was observed with increased heating time and processing steps (Gahler, Otto & Bohm*,* 2003:7966). A study was

also conducted to determine the effect of thermal processing on sweet corn. The vitamin C content declined with increased heating time at 115˚C. There were, however, significant increases in the content of total phenolics in sweet corn following thermal treatment with both increased heating times and temperatures (Dewanto, Wu & Liu*,* 2002:4959).

The concentration of phenolic acids is highest in the outer layers of some vegetables and these outer layers are extremely exposed to the cooking water, reducing antioxidant power of some vegetables such as peas, spinach, cauliflower and cabbage. However, although total phenolics are usually stored in vegetables in pectin or cellulose networks and can be released during thermal processing, individual phenolics may sometimes increase because heat can break supramolecular structures, releasing the phenolic sugar glycosidic bonds (Jimenez-Monreal *et al.,* 2009:H102). Blanching and boiling red cabbage significantly ($p < 0.05$) reduced the polyphenol contents by 43% and 16% respectively, however, when the cumulative levels of total polyphenols in both the water and solids were assessed no overall degradation occurred. The cumulative values for boiling and steaming for 10 minutes were significantly higher than the fresh samples, indicating an overall increase in the content of polyphenols (Volden *et al.,* 2008:601).

In this study, thermal processing had a significant ($p < 0.05$ for each) impact on the total polyphenol content of all three control soup recipe formulations versus the raw control soup recipe formulations. Thermal processing increased the total polyphenol contents in the chunky vegetable and butternut experimental soup recipe formulations versus the raw butternut and chunky vegetable experimental soup recipe formulations. The polyphenol contents in the control and both the experimental recipe formulations were significantly ($p < 0.05$ for each) different to the polyphenol content of the raw soup recipe formulations containing either water, fermented or unfermented rooibos herbal tea versus the cooked soup recipe formulations containing either water, fermented or unfermented rooibos. The increase in polyphenolic content (and antioxidant capacity) after cooking are probably the result of a balance between enhanced release of polyphenols from the plant matrix and degradation of the released components. As most polyphenols will at least initially degrade to other polyphenol components, the antioxidant capacity of cooked samples can remain higher than the raw samples (McDougall, Dobson & Jordan-Mahy*,* 2010:764). Thermal processing, however, decreased the polyphenol content in the chicken noodle experimental soup recipe formulations versus the raw chicken noodle experimental soup recipe formulations.

Most studies on antioxidants in food have concentrated on the measurement of specific antioxidants (Hunter & Fletcher, 2002:399). In contrast, the total antioxidant activity of vegetables has been discussed only in a few publications (Hunter & Fletcher, 2002:399). The antioxidant activity of vegetables is increased by boiling. This suggests that the pro-oxidant activity was due to peroxidases which were inactivated at high temperatures. Cooking had no deleterious effect on the total antioxidant activity and total phenolic contents of vegetables with the exception of some losses of phenolics in only squash, peas and leeks. The total antioxidant activity of pepper, green beans, broccoli and spinach significantly increased after cooking, while the antioxidant activity of squash, peas and leeks remained the same (as for the fresh samples) (Turkmen *et al.,* 2005:716).

In this study, thermal processing had an impact on the H-ORAC of all three control soup recipe formulations versus the raw control recipe formulations. Thermal processing increased the H-ORAC in the control chunky vegetable, butternut and chicken noodle soup recipe formulations versus the raw control chunky vegetable, butternut and chicken noodle soup recipe formulations. Thermal processing of the chunky vegetable experimental recipe formulation on substitution of water with unfermented rooibos resulted in a significantly different ($p < 0.05$) H-ORAC. Thermal processing also resulted in a significant difference ($p < 0.05$ for each) in the H-ORAC of the cooked butternut experimental soup recipe formulations containing fermented and unfermented rooibos respectively compared to the raw experimental recipe formulations containing these rooibos herbal teas with the cooked butternut soup having the higher capacity. The H-ORAC of the control chicken noodle soup recipe formulation on thermal processing was significantly different ($p < 0.05$) to that of the raw chicken noodle soup control recipe formulation. As was found for the polyphenol content, thermal processing also decreased the H-ORAC of both the chicken noodle experimental soup recipe formulations compared to the raw chicken noodle experimental soup recipe formulations. The H-ORAC of the chicken noodle experimental soup recipe formulations on thermal processing was significantly lower ($p < 0.05$ for each) than that of the raw soup mixtures.

Four possibilities are suggested for the increase in antioxidant activity of some vegetables after cooking: (a) the liberation of high amounts of antioxidant components due to the thermal destruction of the cell walls and sub cellular compartments, (b) the production of stronger radical-scavenging antioxidants by thermal chemical reaction, (c) suppression of the oxidation

capacity of antioxidants by thermal inactivation of oxidative enzymes and (d) production of new nonnutrient antioxidants or the formation of novel compounds such as Maillard reaction products with antioxidant activity (Jimenez-Monreal *et al.,* 2009:H102). In this study the TAC of the raw chicken noodle control soup recipe formulation increased on thermal processing that caused a significant difference ($p < 0.05$) in the capacity of the raw control compared to the cooked control soup recipe formulation. Thermal processing also increased the TAC of the chunky vegetable and butternut experimental soup recipe formulations versus the raw chunky vegetable and butternut experimental soup recipe formulations. The TAC of the raw chunky vegetable soup recipe formulation containing unfermented rooibos herbal tea versus the thermally processed chunky vegetable soup recipe formulation containing unfermented rooibos herbal tea was significantly different ($p < 0.05$). The thermally processed butternut experimental soup recipe formulation containing fermented rooibos herbal tea, also had a significantly different ($p < 0.05$) TAC compared to the raw butternut soup recipe formulation containing fermented rooibos herbal tea. Thermal processing caused a decrease in the TAC of the experimental chicken noodle soup recipe formulations compared to the raw chicken noodle soup recipe experimental formulations. This decrease in the TAC after thermal processing resulted in a significant difference ($p < 0.05$ for each) in the TAC of the raw experimental soup recipe formulations compared to the TAC of both the cooked experimental soup recipe formulations. The TAC increases observed in this study considering the above mentioned suggestions could be due to: the release of antioxidant compounds from the vegetable matrix, the formation of new antioxidant compounds and the conversion of polyphenols into very active chemical species (Pellegrini, Miglio, Del Rio, Salvatore, Serafini & Brighenti*.,* 2009:20).

In this study thermal processing in summary caused an increase of the polyphenol content of the control and experimental chunky vegetable and butternut soup recipe formulations compared to the raw control and experimental soup recipe formulations. Thermal processing also caused an increase in the chicken noodle control polyphenol content compared to the raw control soup recipe formulation. The increase in the polyphenol content is possibly due to the liberation of the phenolics from the food matrix (Gahler, Otto & Bohm 2003:7966) and the resultant accumulation in the soup mixture. Thermal processing furthermore caused an increase of the H-ORAC of the control and experimental chunky vegetable and butternut soup recipe formulations compared to raw control and experimental soup recipe formulations. The H-ORAC of the chicken noodle control soup recipe formulation also increased on thermal processing compared to the raw control soup recipe formulation. The TAC of the control and experimental

chunky vegetable and butternut soup recipe formulations also increased after thermal processing compared to the raw control and experimental soup recipe formulations. With optimal thermal treatment, enzymes will be rapidly inactivated thereby preventing oxygen access and minimizing leakage, thus increasing the retention of plant constituents (Volden *et al.,* 2008:596). The peroxidases are inactivated at high temperatures (Turkmen *et al.,* 2005:716). Antioxidant compounds can also be formed during thermal processing as a result of a chemical reaction during processing (Gokmen *et al.,* 2009:279). Browning reactions occur in food processing and storage. These browning reactions can involve different compounds and proceed through different chemical pathways. The major groups of reactions leading to browning are enzymatic phenol oxidation and non-enzymatic browning. Non-enzymatic browning is caused by heat treatments and includes a wide number of reactions such as the Maillard reaction, caramelisation, chemical oxidation of phenols and maderisation. In the Maillard reaction the resulting high antioxidant capacity is generally attributed to the formation of brown melanoidins (Manzocco *et al.,* 2001:340). In this study on thermal processing of the soup recipe formulations, the retention of antioxidant compounds most possibly also occurred due to enzyme inactivation along with possible antioxidant compound formation as a result of some chemical reactions that may have occurred. These actions along with the polyphenol accumulation may contribute to the increased H-ORAC and TAC found on thermal processing of most of the soup recipe formulations.

Thermal processing of the experimental chicken noodle soup recipe formulations caused a decrease in the polyphenol content, H-ORAC and TAC compared to the raw experimental soup recipe formulations. The chicken noodle soup recipe formulation was subjected to thermal processing far longer than the other two soup recipe formulations. One step in the chicken noodle soup recipe formulation also involved removal of the vegetables (and the bones), a step that removed the phytochemical source of this recipe formulation that may possibly also contribute, with the longer heat exposure, to the decrease found.

Thermal processing significantly ($p < 0.05$ for each) reduced the L-ORAC and carotenoid content of all three thermally processed control and experimental soup recipe formulations compared to the three raw control and experimental soup recipe formulations. Carotenoids are extremely susceptible to degradation. Factors such as heat, light and oxygen exposure may have a detrimental effect on the carotenoid content due to the highly unsaturated carotenoid structure (Leskova *et al.*, 2006:254; Thane & Reddy, 1997:58). However, boiling of fresh broccoli has been reported to promote the release of β-carotene from the matrix due to

denaturation of carotenoproteins, leading to better extractability and higher concentrations in cooked samples (Pellegrini *et al.,* 2009:20). The TAC of the soup recipe formulations comprised mainly of the H-ORAC. Due to the above the L-ORAC and carotenoid content has not been extensively addressed in the discussion.

5.4 Limitations and strengths of the study

The limitations of the study were the following. The effect of thermal processing on the TAC of the soup recipe formulations was not fully addressed (the vitamin E and vitamin C contents and the bound versus the free phenolics were for example not determined). The release of the dietary antioxidant components from the food matrix is a research area not intensively investigated and an intensive field to research due to (a) the synergistic/antagonistic or other food matrix effects (Gahler, 2003:7966) and (b) all the possible components with antioxidant activity may not be known that may play a role (Dewanto *et al.,* 2002:4964). Even data on the total phenolic content in cooked green vegetables itself is very limited (Turkmen *et al*., 2005:715) with even less information available on the thermal processing of composite dishes. This also impacted the discussion of the results found in this study as it could not be compared to a wide range of findings and in particular that of similar studies utilising composite dishes.

However, the study provided evidence that (a) the addition of an antioxidant rich food item or beverage can increase the TAC of a recipe formulation, in particular recipe formulations where the whole formulation, including the cooking liquid, is consumed, and (b) that thermal processing can maintain or even increase food nutritive value in the form of its TAC. These findings can be considered strengths of the study as (a) it adds to the limited information available on the effect of household food processing on dietary antioxidants in a food matrix and (b) confirms that the antioxidant nutritive value of vegetables is not necessarily destroyed or even reduced on wet thermal processing. Knowledge of the effects of wet thermal processing on vegetables is important from a dietary perspective because cooked vegetables make up a large portion of the dietary vegetable intake (Volden *et al*., 2008:596). The common belief from a consumer perspective that thermal processing destroys the antioxidant content of vegetables (Dewanto *et al.,* 2002:4959) can, as a result, also be questioned as it was not confirmed in this study in two of the soup recipe formulations.

CHAPTER 6

CONCLUSIONS

Three soup recipe formulations were used to determine the effect of the substitution of water with rooibos. The total polyphenol content and the carotenoid content, along with the H-ORAC, L-ORAC and TAC were determined for the raw recipe ingredients, the raw control and experimental soup recipe formulations and the correspondent thermally processed control and experimental soup recipe formulations.

There was no similarity or trend in the USDA published polyphenol content and the TAC for the raw soup recipe formulation ingredients utilised in this study and their analysed values. In some cases the published values were far lower or higher compared to the analysis in this study. The only identical value found was for the polyphenol content of the tomato paste. Differences in the phytochemical contents and the TAC of foods occur due to the diverse factors such as the conditions of growth, harvesting, handling, storage, preparation and processing which influence the food value (Kalt, 2005:209).

In all three the raw soup recipe formulations, water or in the case of the experiments, fermented and unfermented rooibos, was the greatest raw single ingredient contributor in relation to the contribution of the other raw ingredients. The fermented and unfermented rooibos did not have the highest polyphenol content, H-ORAC and TAC, but it was the greatest contributor to the raw recipe formulations due to the highest ingredient quantity used in all three soup recipe formulations. The ingredients with the highest polyphenol content and H-ORAC were the herbs, spices and flavourings. The vegetables had a varied polyphenol contents and H-ORAC. Onions had the highest polyphenol content and H-ORAC of all the raw recipe vegetable ingredients. The fermented and unfermented rooibos made no contribution to the L-ORAC of the raw recipe formulations. The H-ORAC of all the raw ingredients was much higher than the L-ORAC. The TAC of the raw ingredients comprised mainly of the H-ORAC of the raw ingredients.

The substitution of water with fermented and unfermented rooibos in all three raw soup recipe formulations resulted in a significant increase in the polyphenol content and H-ORAC. The inclusion of the fermented and unfermented rooibos herbal teas in place of water in the raw soup recipe formulations had little effect on the L-ORAC and carotenoid content of all three raw experimental soup recipe formulations. The TAC of all three raw recipe formulations was also

significantly increased with the substitution of water with fermented and unfermented rooibos as soup ingredients. The null hypothesis stated for the study of no significant difference between the TAC of the raw control soup recipe formulations containing water as the major ingredient (raw combined ingredient soup recipe formulation) and the correspondent raw experimental soup recipe formulations containing fermented and unfermented rooibos herbal teas as the major ingredient, was thus rejected. The liquid (water) substitution with fermented and unfermented rooibos is therefore a worthwhile recipe manipulation to increase the overall TAC of the recipe formulation in particular when the liquid forms part of the whole food to be consumed as with soup. By adding rooibos the nutraceutical properties of the recipes was increased as well, as shown by the increased antioxidant activity. Development and utilisation of foods with functional food characteristics can be used to improve the nutritional status of the population (Ajila *et al.,* 2010:223).

The long-held belief that thermal processing of food decreased and even destroyed the food nutritive value (Dewanto *et al.,* 2002:4959) was not confirmed in this study when using the TAC as index. Thermal processing resulted in a significant increase in the total polyphenol content of all the cooked control versus the raw control soup recipe formulations. The chunky vegetable and butternut experimental soup recipe formulations both increased in total polyphenol content after thermal processing providing for the polyphenol content on thermal processing to be significantly different for the cooked controls and the cooked chunky vegetable and butternut experimental soup recipe formulations compared to the raw soup recipe formulations.

The H-ORAC of all the control soup recipe formulations also increased on thermal processing. Both the experimental chunky vegetable and butternut soup recipe formulations containing unfermented rooibos also had a significantly increased H-ORAC after being thermally processed compared to the raw experimental soup recipe formulation of each. The H-ORAC of the experimental butternut soup recipe formulation containing fermented rooibos also increased significantly on thermal processing.

Thermal processing caused a significant decrease in the L-ORAC of all the control and experimental soup recipe formulations. Thermal processing had a similar effect on the carotenoid content. The carotenoid content of all the control and experimental soup recipe formulations significantly decreased after thermal processing.

Thermal processing resulted in an increase in the TAC of all three cooked control soup recipe formulations compared to the raw soup recipe formulations. Thermal processing caused a significant increase in the TAC of the chunky vegetable experimental soup recipe formulation

containing unfermented rooibos compared to the raw experimental soup recipe formulation containing unfermented rooibos. The TAC of both the raw experimental butternut soup recipe formulations increased significant on thermal processing. The null hypothesis stated for the study (of no significant difference between the TAC of the raw control and experimental soup recipe formulations respectively containing water and fermented and unfermented rooibos herbal teas as the major ingredients and the correspondent cooked control and experimental soup recipe formulations respectively containing water and fermented and unfermented rooibos herbal teas as the major ingredients) was also rejected for the chunky vegetable and the butternut soup recipe formulations, in particular for the experimental soup recipe formulations, but not for the chicken noodle experimental soup recipe formulations. The H-ORAC of the chicken noodle experimental soup recipe formulations on thermal processing was significantly lower than that of the raw soup mixtures. There was also a significant decrease in the TAC of the chicken noodle experimental soup recipe formulations on thermal processing compared to the TAC of the raw chicken noodle experimental soup recipe formulations. The prolonged heat exposure in the experimental chicken noodle soup recipe formulation possibly caused the decrease in the H-ORAC and TAC. Processing and cooking conditions cause variable losses of antioxidants. Losses vary widely according to cooking method used and the type of food (Leskova *et al.*, 2006:258)

CHAPTER 7

RECOMMENDATIONS

A number of recommendations can be made based on this study. The first being the development of a non nutrient antioxidant database for South African grown foods, reporting at least the polyphenol content and the TAC. This is necessary due to the varying antioxidant content of fruits and vegetables as a result of climate, growing condition and post harvest storage conditions (Stewart *et al*., 2000: 2666; Volden *et al*., 2008:596). There was no similarity or trend in the USDA published polyphenol content and the TAC for the raw soup recipe formulation ingredients utilised in this study and their analysed values. In some cases the published values were far lower or higher compared to the analysis in this study.

Other ways must furthermore be found to support and increase the South African dietary TAC. An exploratory study was conducted by Louwrens *et al.* (2009:195) on the TAC of the South African dietary intake. The TAC for the South African dietary intake was calculated and compared to that which would be provided, when consuming a diet based on the dietary guidelines and recommendations. The TAC of the South African dietary intake in comparison was found to be relatively low (Louwrens *et al.,* 2009:195). It has been suggested that the dietary TAC could be a useful research tool in assessing antioxidant intake (Puchau, Zulet, Gonzalez de Echavarri, Hermsdorff & Martinez, 2010:534).

This study furthermore supports the argument that food rather than supplements should be the first consideration in addressing nutritional requirements of the population (Jacobs, Gross & Tapsell, 2009:1546S). One way of increasing the dietary TAC is incorporation of antioxidant rich food sources or beverages into recipe formulations (as done in this study). Recipe development incorporating rooibos (proudly South African product) in household recipes must therefore be furthered. This will be valuable for consumers due to the bioactive ingredients rooibos contains. The addition of rooibos will therefore incorporate more antioxidants in the dietary intake. In recent years, tea has attracted more and more attention because of reported health benefits, in particular as an antioxidant, but also as an anticarcinogenic and antiarteriosclerotic agent (Wang *et al*., 2000:152). The rooibos manufacturers should also distribute recipe booklets more readily. Rooibos Ltd (Clanwilliam, RSA) has published a set of recipe booklets with the inclusion of

rooibos in recipes such as soups and cooldrinks. The use of rooibos in cooking will increase consumers overall TAC in the diet with beneficial health effects.

Consumers also need to be educated that soups, although mostly thermally processed for longer than single cooked vegetable ingredients, can make a valuable contribution to their dietary TAC. Processed fruits and vegetables have long been perceived to have lower nutritional value than fresh fruits and vegetables due to the loss of vitamin C content after processing (Dewanto *et al.,* 2002:4959). The current consumer's belief that thermal processing generally decreases nutrients in foods must as a result be changed as thermal processing resulted in an increase in the TAC of all three cooked control soup recipe formulations compared to the raw soup recipe formulations in this study. Thermal processing also caused an increase in the TAC of the chunky vegetable and butternut experimental soup recipe formulations compared to the raw soup recipe chunky vegetable and butternut experimental soup recipe formulations.

Further studies are required to determine the effect of thermal processing on the TAC of other soups and commonly consumed South African composite dishes. Currently most literature available focuses on thermal processing of individual vegetable items. These further studies undertaken to determine the effect of thermal processing should focus on the TAC of composite dishes, instead of determination of an individual antioxidant in an individual food item. This should in time also be followed by research about cooking's impact on the food chemical efficacy *in vivo* (Jimenez-Monreal *et al.,* 2009:H103).

In addition to the above, further studies on the effect of rooibos inclusion on the TAC where rooibos does not replace a major ingredient and the cooking liquid is not largely maintained, should also be embarked upon. Consumer acceptability should also be determined of recipe formulations with the inclusion of rooibos as an ingredient. Below is a list of commonly consumed composite dishes consumed in South Africa where rooibos can be added or substituted for the water in the recipe formulation:

- Meat products and dishes for example bobotie, cottage pie, pies and meatballs
- Samoosa, vetkoek, chili bites and savoury tarts
- Sweet potato cooked without skin
- Tomato and onion stew
- Samp and rice dishes
- Chicken stews and dishes and pies (Nel & Steyn, 2002:92-95).

One of the major nutritional health problems in South Africa is iron deficiency, especially among women and children (Breet, Kruger, Jerling & Oosthuizen, 2005:984). Fortunately rooibos does not seem to significantly affect dietary iron absorption (Breet *et al.,* 2005:984) which provides support for embarking on the incorporation of tea, but in the form of rooibos herbal tea, as an ingredient in recipe formulations such as that of the above proposed commonly consumed South African foods.

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Addendum A

Base recipe formulation:
Chunky vegetable soup
(obtained from Niehaus, 2001:72)

Chunky vegetable soup

Serves 16

6 medium carrots, scraped and diced
1 medium onion, finely chopped
1 small cauliflower, broken into florets
4 stalks celery, finely chopped
4 medium potatoes, grated
500ml dried peas, soaked in water for 30 minutes
2 tomato paste cubes
1 medium butternut, peeled and diced
45ml vegetable stock powder
250ml pasta shapes – optional
2 handfuls chopped fresh parsley
grated rind of 1 lemon
salt and freshly ground pepper to taste

Place all the ingredients, except the parsley and seasonings, in a large saucepan. Add about 7x250ml water or enough to cover the vegetables and bring to the boil. Reduce the temperature and simmer slowly until the peas are soft and done. Add more water if preferred. Add the parsley and lemon rind, season generously with salt and black pepper and serve hot.

Addendum B

Base recipe formulation:
Butternut soup
(obtained from Shippel, 2005:33)

Butternut soup

Serves 6 – 8

2 large onions
Or 1 bunch leeks
50g butter
750g butternut
1 – 2 potatoes
750ml chicken or vegetable stock
squeeze of fresh lemon juice
or other desired flavourings such as ground nutmeg, cinnamon or cumin to taste
125ml cream or milk

1. Sauté sliced onions or leeks in butter.
2. Add vegetables, potatoes and stock and simmer until vegetables are soft.
3. Puree the mixture in a liquidiser or food processor, or press through a sieve.
4. Add the cream or milk, heat through, check seasoning and serve.

Addendum C

Base recipe formulation:
Chicken noodle soup
(obtained from Shippel, 2005:35)

Chicken and noodle soup

Serves 6 – 8

1 chicken, cut into portions
250g chicken giblets
250g chicken necks
1 large piece of beef shin, cut into pieces
2 – 3 Telma chicken cubes, crumbled or stock powder of your choice
2 litres water
45ml oil
4 – 5 carrots, chopped
1 bunch leeks, chopped
1 turnip, chopped
2 large onions, chopped
½ bunch soup celery with leaves, chopped
½ bunch parsley, with stalks, chopped
3ml coarse black pepper
5ml salt
4 extra carrots for serving, thinly sliced
50g vermicelli – optional

1. In a large pot sauté onions and leeks in oil. Add remaining vegetables and sauté until glossy.
2. Remove from heat. Place remaining ingredients in the pot and allow to soak for a few hours before cooking.
3. Bring to the boil and simmer for 2 hours.
4. Remove chicken pieces from soup, take all the meat off the bones, break up the bones and return them to the soup.
5. Continue simmering the soup, covered, for a further 2 to 3 hours.
6. Place pot in cold water until cool. When cool enough, place in fridge overnight.
7. Remove from fridge and remove all the fat from the top of the soup.
8. Warm the soup and strain through a colander or strainer to remove all the solids. Discard solids.
9. Return the soup to the pot and add sliced carrot. Bring to the boil and break in vermicelli.
10. Check seasoning and simmer until carrots and noodles are soft.

Addendum D

Standardised recipe formulation: Chunky vegetable soup

Chunky vegetable soup

Serves 4 – 6

130 g carrots*
55 g onion*
225 g cauliflower*
60 g celery*
225 g potatoes*
150 g dried peas
150 g water
10 g tomato paste
225 g butternut*
15 g vegetable stock powder
1.345 kg water
20 g fresh parsley*
10 g lemon rind*
1 g salt
1 g ground black pepper

* All fresh ingredients prepared as per indicated pre-preparation procedure.

Step Number	Instructions	Time (minutes)
Step 1	Soak dried peas in cold water.	30.00
Step 2	Turn stove temperature to mark 6, place pot on stove and place all ingredients in the pot, except the parsley, water and seasonings.	0.15
Step 3	Add water and bring to the boil on mark 6 on stove.	4.00
Step 4	Turn stove temperature to mark 2 and simmer until peas are soft and done.	20.00
Step 5	Add the parsley, lemon rind, salt and pepper.	0.15

Addendum E

Standardised recipe formulation: Butternut soup

Butternut soup

Serves 4 – 6

225 g onions*
40 g sunflower oil
565 g butternut*
225 g potatoes*
750 g water
10 g vegetable stock powder
5 g lemon juice
5 g cinnamon
5 g salt
5 g ground black pepper
95 g full cream milk

* All fresh ingredients prepared as per indicated pre-preparation procedure.

Step Number	Instructions	Time (minutes)
Step 1	Turn stove temperature to mark 6 and place pot on stove.	0.05
Step 2	Heat	2.00
Step 3	Add oil to pan.	0.08
Step 4	Saute´ onions in oil with no lid on pot.	6.00
Step 6	Add butternut, potatoes, water and simmer until vegetables are soft.	60.00
Step 7	Puree the mixture in a liquidiser, food processor or push through a sieve.	05.00
Step 8	Add milk, heat through and add seasoning.	05.00

Addendum F

Standardised recipe formulation: Chicken noodle soup

Chicken noodle soup

Serves 4 – 6

750 g whole chicken*
190 g chicken giblets*
190 g chicken necks*
190 g shin*
30 g chicken stock powder
1.500 kg water
40 g oil
300 g carrots*
225 g leeks*
115 g onions*
40 g soup celery*
40 g parsley*
5 g coarse black pepper
5 g salt
40 g vermicelli
90 g turnip*

* All fresh ingredients prepared as per indicated pre-preparation procedure.

Step Number	Instructions	Time (minutes)
Step 1	Turn stove temperature to mark 8 and place large pot on stove.	0.05
Step 2	Heat	2.00
Step 3	Add oil to pan.	0.08
Step 4	Saute´ onion and leeks in oil. Stir 5 times in circular movements forming an 8. No lid on pot.	5.00
Step 5	Place the all the remaining ingredients in the pot. Stir 4 times in circular movements forming an 8.	1.00
Step 6	Bring to the boil while stove temperature on mark 8	10.00
Step 7	Simmer on temperature mark 1 on the stove.	150.00
Step 8	Remove all chicken pieces from soup, take all meat off the bones and put aside. Break up bones and return all the bones to the soup.	10.00
Step 9	Continue simmering soup on temperature mark 1 on the stove.	150.00
Step 10	Place pot (with lid off) in cold water until cool. When cold enough place in fridge overnight.	25.00
Step 11	Remove from fridge and remove the entire fat layer from top of the soup.	3.00
Step 12	Warm the soup and strain through a colander to remove all the solids. Discard solids.	06.00
Step 13	Bring to the boil and break in vermicelli.	05.00
Step 14	Simmer until vermicelli are soft.	4.00

Addendum G

Adjusted (actual) standardised recipe formulation: Chunky vegetable soup

Chunky vegetable soup
Serves 4 – 6

130 g carrots*
55 g onion*
225 g cauliflower*
60 g celery*
225 g potatoes*
150 g dried peas
150 g water
10 g tomato paste
225 g butternut*
15 g vegetable stock powder
1.345 kg water / 1.345 kg fermented rooibos / 1.345 kg unfermented rooibos
20 g fresh parsley*
10 g lemon rind*
1 g salt
1 g ground black pepper

* All fresh ingredients prepared as per indicated pre-preparation procedure.

Step Number	Instructions	Time (minutes)
Step 1	Soak dried peas in cold water.	30.00
Step 2	Turn stove temperature to mark 6, place pot on stove and place all ingredients in the pot, except the parsley, water, lemon rind and seasonings.	00.45
Step 3	Add water and bring to the boil on mark 6 on stove with lid on. Stir after 5 minutes in circular movements forming an 8, three times.	07.46
Step 4	Turn stove temperature to mark 2 and simmer until peas are soft and done. Stir after every 15 minutes in circular movements forming an 8 three times.	80.00
Step 5	Add the parsley, lemon rind, salt and pepper.	0.15
		Total: 118.46

Temperatures at various steps and weights after cooking and liquidising

	Control	Fermented rooibos	Unfermented rooibos
Temperature of water or tea before adding	18.5°C	21.1°C	19.8°C
Temperature at end of step 3	89.0°C	98.6°C	91.0°C
Temperature at end of step 5	99.6°C	97.5°C	96.0°C
Final mass	2328 g	2068 g	2184 g
Mass after liquidising	2272 g	1970 g	2098 g

Addendum H

Adjusted (actual) standardised recipe formulation: Butternut soup

Butternut soup

Serves 4 – 6

225 g onions*
40 g sunflower oil
565 g butternut*
225 g potatoes*
750 g water / 750 g fermented rooibos / 750 g unfermented rooibos
10 g vegetable stock powder
5 g lemon juice
5 g cinnamon
5 g salt
5 g ground black pepper
95 g full cream milk
* All fresh ingredients prepared as per indicated pre-preparation procedure.

Step Number	Instructions	Time (minutes)
Step 1	Turn stove temperature to mark 4, place pot on stove, add oil and heat.	2.00
Step 2	Add onions and sauté in oil leaving the stove temperature on mark 4 with no lid. Stir every second minute in circular movements forming an 8 three times.	6.00
Step 3	Adjust the temperature mark to 4. Add butternut, potatoes, water and vegetable stock powder and simmer with lid on pot until vegetables are soft. Stir every ten minutes in circular movements forming an 8 three times.	45.00
Step 4	Remove pot and pour contents into liquidiser (Speed 1) and puree.	2.00
Step 5	Place pot back on stove on temperature mark 4 and add the pureed soup.	01.00
Step 6	Add the milk, lemon juice and seasoning. Heat through with lid on and stir every minute in circular movements forming an 8 three times.	02.00
	Total:	58:00

Temperatures at various steps and weights after cooking and liquidising

	Control	Fermented rooibos	Unfermented rooibos
Temperature of water or tea before adding	19.4°C	17.8°C	20.4°C
Temperature at end of step 2	97.4°C	100.1°C	98.2°C
Temperature at end of step 3	99.8°C	99.7°C	100.0°C
Temperature at end of step 6	98.4°C	100.1°C	100.0°C
Final mass	1658 g	1300 g	1518 g
Mass after liquidising	1601 g	1182 g	1467 g

Addendum I

Adjusted (actual) standardised recipe formulation: Chicken noodle soup

Chicken and noodle soup

Serves 4 – 6

750 g whole chicken*
190 g chicken giblets*
190 g chicken necks*
190 g beef shin*
30 g chicken stock powder
1.500 kg water / 1.500 kg fermented rooibos / 1.500 kg unfermented rooibos
40 g oil
300 g carrots*
225 g leeks*
115 g onions*
40 g soup celery*
40 g parsley*
5 g coarse black pepper
5 g salt
40 g vermicelli
90 g turnip*

* All fresh ingredients prepared as per indicated pre-preparation procedure.

Step Number	Instructions	Time (minutes)
Step 1	Turn stove temperature to mark 6, place large pot on stove, and add oil and heat.	02.00
Step 2	Sauté onion and leeks in oil with no lid on the pot. Stir once in three circular movements forming an 8.	05.00
Step 3	Place all the remaining ingredients in the pot.	01.00
Step 4	Simmer on temperature mark 3 on the stove with the lid on. Stir nine times every ten minutes in three circular movements forming an 8.	90.00
Step 5	Remove all chicken pieces from soup, take all meat off the bones and put aside. Break up bones and return all the bones to the soup.	10.00
Step 6	Continue simmering soup on temperature mark 1 on the stove with lid on. Stir three times every thirty minutes in three circular movements forming an 8 each time.	90.00
Step 7	Place pot in fridge overnight.	
Step 8	Remove from fridge and remove the entire fat layer from top of the soup.	02.10
Step 9	Warm the soup on temperature mark 6 with the lid on. Stir twice after every two minutes in circular movements forming an 8 three times.	05.18
	Strain through a colander to remove all the solids. Discard solids.	02.29
Step 10	Place back into the pot and bring to the boil on temperature mark 4 and break in vermicelli and place lid on.	03.00
Step 11	Simmer until vermicelli is soft. Stirring consistently for four minutes.	04.00
	Total:	217:57

Temperatures at various steps and weights after cooking and liquidising

	Control	Fermented rooibos	Unfermented rooibos
Temperature of water or tea before adding	20.0°C	17.0°C	22.5°C
Temperature at end of step 2	95.0°C	95.0°C	95.0°C
Temperature at end of step 4	94.0°C	99.3°C	93.0°C
Temperature at end of step 6	98.0°C	93.6°C	98.0°C
Temperature after removal from fridge	3.6°C	4.2°C	2.6°C
Temperature at end of step 9	32.1°C	32.1°C	32.1°C
Final temperature	86.0°C	96.3°C	98.7°C
Mass of bones and other solids removed	1310 g	1389 g	1109 g
Mass after bones removed	1307 g	1026 g	984 g
Mass after liquidising	1317 g	1036 g	990 g