TOTAL ANTIOXIDANT CAPACITY OF STEWED TOMATO AND ONION FLAVOURED WITH PARSLEY: EFFECT OF THERMAL HOUSEHOLD PROCESSING

by

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ABSTRACT

Fruit and vegetables are the major antioxidant contributors to the diet. Antioxidants assist in the prevention of oxidative damage in the body and may as a result prevent the causation of degenerative diseases. Thermal household processing plays an integral part in South African consumers' lives, as most fruit and vegetables consumed are processed at home. Consumers' perceptions that food processing causes nutrient losses, especially of vitamin C, have been corroborated by studies that investigated thermal household processing of single foods or that of industrial processing. No studies have determined the effect of thermal household processing on mixed dishes. A popular consumed South African mixed dish, namely, stewed tomato and onion flavoured with parsley, was investigated by using three recipes, each using a different preparation method. The traditional recipe for the preparation of stewed tomato and onion was modified (control recipe) to contain parsley. Two other recipes (Recipe 1 and 2) were compiled based on the recipe formulation of the control recipe but differed in the preparation methods used. In Recipe 1, raw onion was added to cooked tomato and in Recipe 2, sautéed onions were added to cooked tomato. This study indicated the following:

i. Thermal household processing did not decrease the total antioxidant capacity (TAC) of the combined raw ingredients when it was compared to that of the end-products of the three respective recipes when either sunflower oil (SFO) or red palm oil (RPO) was used as ingredients in the recipe formulations. Thermal household processing, however, affected the recipes differently, as different preparation methods were used in each recipe. The TAC from the combined raw ingredients to the end-product in the control recipe significantly increased ($P < 0.05$) when both the oils were used. The TAC of the combined raw ingredients of the other two recipes (Recipes 1 and 2) remained similar after thermal household processing was applied.

ii. When the TAC of the end-products was compared it was found to be similar when using SFO. The TAC of the control recipe using RPO was significantly higher ($P < 0.05$) than when Recipe 1 was used, while the TAC of the control recipe was similar to that of Recipe 2 and the TAC of Recipe 1 similar to that of Recipe 2.

iii. The addition of parsley to the second-last preparation step of each recipe did not contribute to a higher TAC. This was found with both the oils used.

iv. When comparing the TAC of the three recipe end-products, using the two oils respectively in the recipe formulations, the TAC of the control recipe using RPO was

marginally higher than when using SFO in this recipe. Using RPO as ingredient in the formulation did not increase the TAC of the three recipes, but using the control recipe with RPO as ingredient provided the highest TAC.

DECLARATION

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

Signed MBraun

at Cape Town

on the 14 day of Sept 2006

DEDICATION

In memory of Jacques and Oupa Verus

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LIST OF OPERATIONAL TERMS AND CONCEPTS

Antioxidant activity (AOA): Corresponds to the rate constant of a single antioxidant against a given free radical (Ghiselli *et al.*, 2000: 1107).

Antioxidant capacity (AOC): Measure of the moles of a given free radical scavenged by a test solution, independently from the AOA of any one antioxidant present in the mixture (Ghiselli *et al.*, 2000: 1107).

Antioxidant status: Balance between antioxidants and pro-oxidants in living organisms (Papas, 1999: 93).

Combined raw ingredients: Homogenised sample consisting of raw tomato, onion, parsley and sunflower/red palm oil based on the recipe formulation's percentage weight contribution of each ingredient.

Dish: Foods in a combined form or a particular kind of food, prepared with multiple ingredients usually following a recipe.

Formulation: A particular list of ingredients to prepare a mixture of food.

Preparation method: Instructions in which edible ingredients are put together.

Percentage weight contribution: Individual ingredient weights represented in the actual step of a recipe in comparison to the final dish.

Recipe: List of ingredients and instructions for preparing food.

Sauté: A method of cooking and browning food in a small quantity of very hot fat in a frying pan. The food may be sautéed simply to brown it or to cook it through (Simon & Howe, 1970: 342). Freeland-Graves and Peckham (1996: 33) add that only thin pieces of food are sautéed and that these must be turned over to complete cooking.

Sensory evaluation: Scientific discipline used to evoke, measure, analyse and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch and hearing (Stone & Sidel, 2004: 13).

Synergism: Combined action of bioactive compounds, such as peptides and antioxidants, which results in an increased (e.g. antioxidant) potential more than that expected from a mere additive effect (Moure *et al.*, 2001: 158).

Total antioxidant capacity (TAC): Cumulative action of all the antioxidants present in plasma, body fluids or food, thus providing an integrated parameter of known and unknown antioxidants and their synergistic interaction (Ghisella *et al.*, 2000: 1105).

LIST OF ABBREVIATIONS

A	
AA	ascorbic acid
AAPH	2,2'-azobis(2-amidinopropane) dihydrochloride
AOA	antioxidant activity
AOC	antioxidant capacity
AUC	area under curve
AWA	acetone/water/acetic acid
C	
°C	degrees Celsius
CoQ	co-enzyme Q
cm	centimetre
CPUT	Cape Peninsula University of Technology
Cu	copper
D	
DCPIP	dichlorophenol-indophenol
DHA	dehydroascorbate
DPPH	1,1-diphenyl-2-picrylhydrazyl
E	
EtOH	ethanol
F	
Fe_2^+	iron
Fl	fluorescein
FRAP	ferric reducing antioxidant power
FW	fresh weight
G	
g	gram
GAE	gallic acid equivalents
H	
HCl	hydrochloric acid
HCA	hydroxycinnamic acid

H-ORAC$_{FL}$	hydrophilic oxygen radical absorbance capacity

L

l	litre
LDL	low-density lipoprotein
L-ORAC$_{FL}$	lipophilic oxygen radical absorbance capacity

M

µg	microgram
µl	microlitre
µmol	micromol
µM	micromilaar
mg	milligram
ml	millilitre
Mn	manganese
MP	metaphospharic acid
MPAA	metaphospharic acid/acetic acid
MPOPC	Malaysian Palm Oil Promotion Council

N

n	sample size
ND	not detected
nm	nanomilaar

O

OH	hydroxyl
ORAC$_{FL}$	oxygen radical absorbance capacity

P

PE	phycoerythrin
PUFA	polyunsaturated fatty acids

R

RDA	recommended dietary allowance
ROS	reactive oxygen species
rpm	rate per minute
RPO	red palm oil

S

SA	South Africa
SD	standard deviation
SFO	sunflower oil
SOD	superoxide dismutase

T

TAC	total antioxidant capacity
TE	Trolox equivalent
TEAC	Trolox equivalent antioxidant capacity
TOSC	total oxyradical scavenging capacity

U

UL	upper tolerable intake level
US	United States

Z

Zn	zinc

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CHAPTER 1

INTRODUCTION

An increased consumption of fruit and vegetables is associated with a lowered risk for the development of the degenerative diseases that accompany aging such as cancer, cardiovascular disease, macular eye disease, and brain and immune dysfunctions (Ames *et al.*, 1993: 7915; Eastwood, 1999: 527; Fang *et al.*, 2002: 872). The development of these diseases is linked to oxidative stress, which is inflicted by free radicals through damage on living systems (Willcox *et al.*, 2004: 291). The production of free radicals and reactive oxygen species (ROS) causes an increased utilisation of antioxidants, which in return affects the antioxidant status of the body (Papas, 1999: 23). Diet has a profound effect on the antioxidant status and ranks among the top factors under our control (Papas, 1999: 91). Antioxidants obtained through the diet or dietary supplements have the ability to significantly decrease the adverse effects of free radicals and other ROS on the body. Antioxidant dietary supplements, however, do not have the same health benefits as dietary antioxidants, because taken alone, the individual antioxidants studied in clinical trials do not appear to have consistent positive effects (Liu, 2003: 518S). Additional information is also required to ensure the efficacy and safety of antioxidant dietary supplements (Liu, 2003: 519S). In general, fewer than 3.0% of South African children and adults consume dietary supplements (Steyn *et al.*, 2003: 637).

Fruit and vegetables are the major source of antioxidants (Eastwood, 1999: 527). Only between 20.9 - 31.6% and 43.5 - 57.0% of the South African population respectively consume fruit and vegetables each day (Steyn *et al.*, 2003: 638-639). South Africans, however, also consume inadequate servings of fruit and vegetables (Bourne *et al.*, 1993: 238; Langenhoven *et al.*, 1995: 523), which contribute to their low intake of antioxidant vitamins (Langenhoven *et al.*, 1995: 523) and increased risk of disease. Inclusion of antioxidants through dietary intake therefore becomes extremely important. Most vegetables consumed are thermally processed, of which stewed tomato and onion is a popular dish consumed in South Africa (Steyn *et al.*, 2003: 642).

It is well known that many factors (i.e. antioxidant concentration, temperature, pH, and the occurrence of chemicals) can strongly influence the antioxidant capacity (AOC) of foods (Gazzani *et al.*, 1998: 4118). It is therefore expected that thermally processed vegetables should have a lower protecting capacity than fresh ones (Nicoli *et al.*, 1999: 95), owing to degradation and oxidation (Papas, 1999: 95). Several studies have investigated the effect of

thermal household processing on individual foods (i.e. sweet potato, tomato, asparagus) (Wu *et al.*, 2004a: 4030) and also on specific antioxidants (i.e. vitamin C, lycopene, flavonoids) (Crozier *et al.*, 1997: 594; Sahlin *et al.*, 2004: 635). However, experimental evidence is lacking on more complicated food systems such as ready-to-eat dishes (Pokorný & Schmidt, 2003: 310). Ninfali and co-workers (2005: 263) recently determined the total antioxidant capacity (TAC) of raw mixed salads and found that opportune combinations of vegetables increase the oxygen radical absorbance capacity ($ORAC_{FL}$) values. However, no research has determined the influence of thermal household processing on the TAC of a recipe containing more than one food item. Owing to the lack of scientific information of the effect of thermal household processing on the TAC of a mixed dish, it became the major objective of this study.

The ideal situation in the household is for foods to be served in a form that is preferred by the consumer, using preparation practices that minimise nutrient losses (Adams & Erdman, 1988: 582). It is further recommended to add natural antioxidants to food to compensate for processing losses and to increase the antioxidant content of foods (Nicoli *et al.*, 1999: 95). Large parts of the literature, however, stress the value and advantages of natural antioxidants as food preservatives (Pratt & Hudson, 1990: 171), and not how to increase the antioxidant content of mixed dishes during household food preparation. Two household customs that may increase the antioxidant content in foods are the addition of herbs, as most herbs are rich in antioxidants (i.e. rosemary, sage, thyme, parsley) (Craig, 1999: 492S), and also the use of antioxidant-rich cooking oils [i.e. red palm oil (RPO)] (Carotino®, 2006). The addition of herbs and the use of cooking oil are general practice in most recipes and consumers do not have to adjust their behaviour in terms of food preparation habits.

Using an experimental study design, three stewed tomato and onion recipes, using different preparation methods, were compiled and standardised to investigate the effect of thermal household processing on the TAC of a popular consumed South African dish. The traditional recipe (Addendum A) for the preparation of stewed tomato and onion was modified to contain parsley. The modified traditional recipe was used as control (control recipe). Two additional scientifically approached recipes (Recipes 1 and 2) were compiled by considering the literature on the effect of thermal household processing on antioxidants. Each of the recipes was flavoured with a standardised amount of parsley, and was prepared utilising sunflower oil (SFO) or RPO to increase the antioxidant content. Based on the above experimental design, the objectives of this study were to determine:

i. the effect that thermal household processing has on the TAC of stewed tomato and onion flavoured with parsley prepared using three different preparation methods in the respective recipes; and

ii. which of the three preparation methods in the respective recipes contributed to the highest TAC after thermal household processing was applied.

In addition to the above objectives, the following subsidiary objectives were also determined regarding the formulations of the three respective recipes:

i. whether the addition of parsley to the recipe formulations contributed to a higher TAC when added to the three recipes using different preparation methods; and
ii. which cooking oil (SFO or RPO) added to the formulation contributed to a higher TAC when using different preparation methods.

These objectives were investigated through the collection of additional samples for analysis during each step of the recipe preparations.

On meeting the stated objectives the dietary antioxidant content of stewed tomato and onion flavoured with parsley would also be provided. As a large proportion of the South African population consumes stewed tomato and onion, recommendations on the preparation of such a recipe to provide for a higher antioxidant content could aid in increasing their overall antioxidant intake.

Food processing has been found to influence the TAC of individual food items, moreover, it increases others. In this study it was hypothesised that:

i. thermal household processing would not negatively influence the TAC of the stewed tomato and onion flavoured with parsley when prepared with different preparation methods as the TAC of the end-products of the three respective recipes would be higher than that of the combined raw ingredients determined prior to the thermal processing;
ii. as three preparation methods were used in the respective recipes, the stewed tomato and onion flavoured with parsley prepared with one of the scientifically compiled recipes, namely Recipe 2, which contain sautéed onion and not raw onion as in Recipe 1, would have the highest TAC;
iii. the addition of parsley to the formulations would increase the TAC of stewed tomato and onion flavoured with parsley when the three recipes were prepared respectively; and
iv. stewed tomato and onion flavoured with parsley would have a higher TAC when RPO was used in the formulation as ingredient compared to using SFO.

CHAPTER 2

LITERATURE STUDY

2.1 Antioxidant defences and oxidative stress

In the body endogenous free radical production occurs accidentally or deliberately (Morissey & O'Brien, 1998: 464; Papas, 1999: 21; Evans & Halliwell, 2001: S67) to serve in important physiological functions. For example, regulation of vascular tone, sensing of oxygen tension and regulation of functions that are controlled by oxygen concentration, enhancement of signal transduction from various membrane receptors including the antigen receptor of lymphocytes, and oxidative stress responses that ensure the maintenance of redox homeostasis (Dröge, 2002: 49). Examples of free radicals include the ROS, such as hydroxyl ($OH^{\bullet}$), peroxyl ($RO^{\bullet}_2$), alkoxyl ($RO^{\bullet}$), superoxide ($O_2^{\bullet-}$) and hydroperoxyl ($HO_2^{\bullet}$) radicals, and reactive nitrogen species, such as nitric oxide ($NO^{\bullet}$) and nitrogen dioxide (NO_2) radicals (Halliwell & Gutteridge, 1999: 27, 38; Evans & Halliwell, 2001: S68). Other types of free radicals produced in the cells include carbon-centred radicals ($R^{\bullet}$) in membrane lipids and thiol radicals ($RS^{\bullet}$) in the oxidation of glutathione (Morissey & O'Brien, 1998: 463). Among these reactive species non-radicals also occur such as hydrogen peroxide (H_2O_2), ozone (O_3), singlet oxygen ($^1O_2$), nitroxyl anion (NO^-) and nitryl chloride (NO_2Cl) which are non-reactive when on their own. Two non-radicals can, however, react with one another to form a free radical (Evans & Halliwell, 2001: S68). Free radicals can damage surrounding cells as they are highly reactive but they can protect cells by either donating an electron to or extracting an electron from other molecules, therefore behaving as oxidants or reductants (Young & Woodside, 2001: 176).

Along with the endogenous production of free radicals, exogenous sources, such as cigarette smoke, environmental/air pollutants, radiation, chemicals also produce free radicals (Langseth, 1995: 5; Papas, 1999: 24; Doyle & Pariza, 2002: 2). A number of these exogenous sources may also increase the endogenous free radical load produced in the body (Morrissey & O'Brien, 1998: 464). Collectively, endogenous and exogenous free radical sources are referred to as pro-oxidant factors as they contribute to an increased formation of free radicals or other ROS (Biesalski *et al.*, 1997: 151).

Normally the production and inactivation of free radicals are in equilibrium (Dröge, 2002: 52). If free radicals are not inactivated, their chemical reactivity can damage all types of cellular macromolecules, including proteins, carbohydrates, lipids and nucleic acids. These

damaging effects have been implicated in the causation of degenerative diseases such as cancer, cardiovascular disease, and macular eye disease (Ames *et al.*, 1993: 7915; Fang *et al.*, 2002: 872). The free radicals that have been associated with many disease states are the oxygen derivatives, hydroxyl and superoxide radicals (Young & Woodside, 2001: 176).

For survival, cells have developed a complex antioxidant defence system for resistance against free radicals (Morrissey & O'Brien, 1998: 463; Doyle & Pariza, 2002: 2). This system can be divided into three main groups: i) endogenous antioxidant enzymes, ii) endogenous or exogenous chain-breaking antioxidants and iii) endogenous transition metal-binding proteins (Young & Woodside, 2001: 178). A summary of these antioxidant defences against free radical attack in the body is illustrated in Figure 2.1.

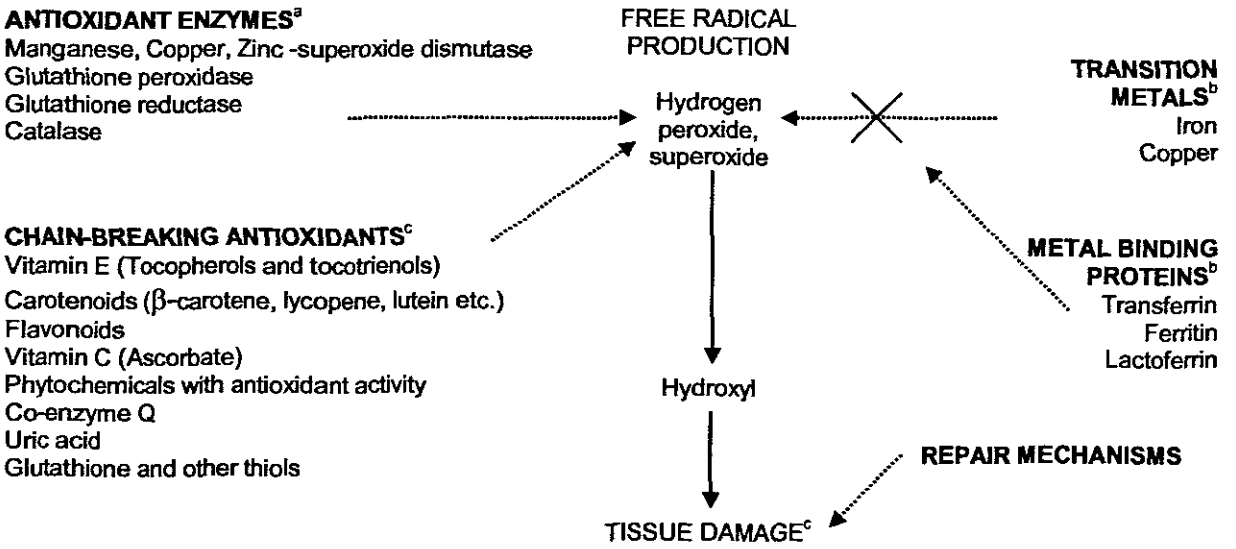


Figure 2.1: Summary of antioxidant defences against free radical attack

(Adapted from Papas, 1999: 24; Young & Woodside, 2001: 178)

[a]-Antioxidant enzymes catalyse the breakdown of free radical species.
[b]-Transition metal binding proteins prevent the interaction of transition metals with hydrogen peroxide and superoxide producing highly reactive hydroxyl radical.
[c]-Chain-breaking antioxidants are powerful electron donors and react preferentially with free radicals before important target molecules are damaged. In doing so, the antioxidant is oxidised and must be regenerated or replaced. By definition, the antioxidant radical is relatively unreactive and unable to attack further molecules.

These antioxidant defences are balanced, coordinated (Evans & Halliwell, 2001: S68) and complementary to one another because they act on different oxidants or in different cellular compartments (Langseth, 1995: 3). The activity of the exogenous antioxidant systems depends largely on dietary intake (Biesalski *et al.*, 1997: 151). Thus the diet plays an important role in the functioning of the antioxidant defences (Evans & Halliwell, 2001: S68). Several essential minerals including selenium, copper (Cu), manganese (Mn) and zinc (Zn) are involved in the structure or catalytic activity of the antioxidant enzymes. For example, CuZn and Mn are indispensable metals for the activities of CuZn-superoxide dismutase (SOD) and Mn-SOD, respectively. Inadequate dietary supply of these minerals may result in

peroxidative damage and mitochondrial disfunction (Fang *et al.*, 2002: 872), but only to a limited extent.

In health, the balance between the pro-oxidant factors and the antioxidant defences lies slightly in favour of the reactive species so that they can fulfil their biological functions (Evans & Halliwell, 2001: S68). When there is a disturbance or imbalance between the pro-oxidant and antioxidant defences, oxidative stress arises (Langseth, 1995: 4; Biesalski *et al.*, 1997: 151). Oxidative balance (Figure 2.2) can be achieved when pro-oxidant factors are reduced and the intake of dietary antioxidants is increased (Biesalski *et al.*, 1997: 151).

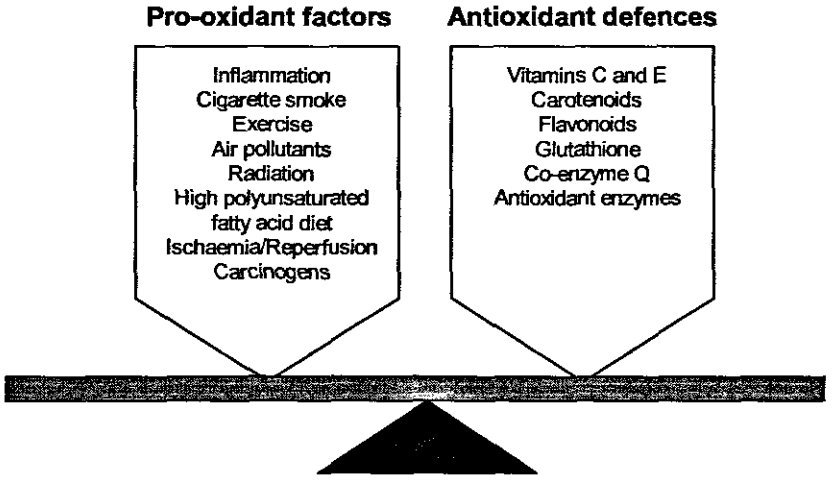


Figure 2.2: Balance between pro-oxidant factors and antioxidant defences
(Adapted from Langseth, 1995: 5; Papas, 1999: 24)

The diet has a profound effect on the antioxidant status and in addition it is one of the top factors under our control (Papas, 1999: 91). Therefore, an adequate intake of antioxidant rich foods (i.e. fruit, vegetables and grains) is important for diminishing the cumulative effects of oxidative damage over the long human lifespan (Halliwell, 1996: 43). These foods provide the body with an array of antioxidants that, as discussed, work complementary to one another against free radical attack. Antioxidants obtained through the diet are discussed below.

2.2 Dietary antioxidants

Natural and synthetic antioxidants are found in foods and beverages consumed by humans. In some cases, natural or synthetic antioxidants are added to foods for preservation purposes to slow down lipid peroxidation and retard the development of off-flavours (Ramis-Ramos, 2003: 266). This study will, however, only focus on dietary antioxidants present naturally in foods or beverages, as they are the major contributors of dietary antioxidants for the body's defences and not those extracted from natural sources.

Dietary antioxidants are multifunctional and can exert their antioxidant activity (AOA) by several different mechanisms. The chemical structure of the antioxidant and the number of physico-chemical factors of the food system determine which type of antioxidant mechanism will prevail. This in turn defines the AOC of foods (Shahidi & Naczk, 2004: 403). Furthermore, the exposure of foods to oxidation accelerators such as air, oxygen, heat, light and transition metals during processing and storage also affects the antioxidant mechanisms. As a result, oxidative damage may occur that impacts the antioxidant provision to the body (Papas, 1999: 95). This section will provide an overview of the structure and mechanism of the main dietary antioxidants present in the recipe ingredients of stewed tomato and onion flavoured with parsley.

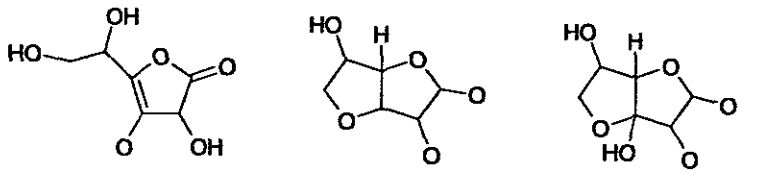
2.2.1 Water-soluble antioxidants

Dietary antioxidants are categorised into water-soluble (hydrophilic) and lipid-soluble (lipophilic) antioxidants. Water-soluble antioxidants include vitamin C and the polyphenols, which are present in the aqueous phase (Noguchi & Niki, 1999: 561).

2.2.1.1 Vitamin C

Vitamin C, also referred to as ascorbic acid, L-ascorbic acid, or ascorbate, is a water-soluble crystalline solid (Halliwell & Gutteridge, 1999: 200; Astley, 2003: 283; Kall, 2003: 316). Vitamin C plays a role in protecting cells and their components against free radical and oxidative damage owing to its electrochemical and physiological properties (Rumsey, *et al.*, 1999: 163). Its AOA is caused by the ease of its loss of electrons, making it very effective in biological systems (Rumsey *et al.*, 1999: 161; Kaur & Kapoor, 2001: 711).

During its antioxidant action, vitamin C undergoes a two-electron reduction, initially to the semidehydroascorbyl radical (also known as monodehydroascorbate or ascorbate free radical) and subsequently to dehydroascorbate (DHA) (Davey *et al.*, 2000: 827; Young & Woodside, 2001: 180; Kall, 2003: 317) (Figure 2.3).



a) Vitamin C b) Semidehydroascorbyl radical c)Dehydroascorbate

Figure 2.3: Structures of vitamin C in its different forms

(Obtained from Astley, 2003: 285)

The semidehydroascorbyl radical is relatively stable and can undergo disproportionation to reform vitamin C and DHA. Dehydroascorbate is relatively unstable at physiological pH and hydrolyses readily to diketogulonic acid, which is subsequently broken down via a series of complex reactions to oxalic and L-threonic acids (Rumsey *et al.*, 1999: 161; Davey *et al.*, 2000: 826-827; Young & Woodside, 2001: 180; Astley, 2003: 285).

Because vitamin C is an electron donor it serves as an outstanding antioxidant and a reducing agent to many ROS (Rumsey *et al.*, 1999: 161; Kaur & Kapoor, 2001: 711). Vitamin C provides electrons for enzymes, for chemical compounds that are oxidants, or for other electron acceptors. In most cases it provides electrons for enzymes that require prosthetic metal ions in a reduced form to achieve full enzymatic activity. In humans vitamin C acts as an essential cofactor for several enzymes catalysing hydroxylation reactions (Rumsey *et al.*, 1999: 161, 164; Young & Woodside, 2001: 180). Vitamin C donates electrons to both intercellular and extracellular reactions (Rumsey *et al.*, 1999: 163; Kall, 2003: 316) and is considered the most important antioxidant in the extracellular compartment (Kall, 2003: 316). This antioxidant has been shown to reduce hypochlorous acid, hydroxyl, superoxide (Rumsey *et al.*, 1999: 162; Young & Woodside, 2001: 180), aqueous peroxyl, hydrogen peroxide, and singlet oxygen (Young & Woodside, 2001: 180). Vitamin C has also been shown to inhibit oxidation synergistically with α-tocopherol. During this action vitamin C reduces the resulting α-tocopheroxyl radical efficiently to regenerate α-tocopherol and possibly to inhibit oxidation induced by α-tocopheroxyl radicals (Niki *et al.*, 1995: 1326S).

Humans are unable to synthesise vitamin C and are thus entirely dependent upon dietary sources (Davey *et al.*, 2000: 825). The recommended dietary allowance (RDA) for vitamin C is 75 mg/day for women and 90 mg/day for men. Smokers require an additional 35 mg/day owing to increased oxidative stress and other metabolic differences. The Upper Tolerable Intake Level (UL) is 2000 mg/day. Major food sources of vitamin C are citrus fruits and juices, tomatoes and tomato juice, potatoes, brussel sprouts, cauliflower, broccoli, strawberries, cabbage and spinach (National Academy of Sciences. Institute of Medicine of the National Academies, 2001).

Vitamin C is easily destroyed by oxygen, metal ions, increased pH, heat, and light. It is stable in a dry form, but in solution it is readily oxidised especially in the presence of trace amounts of iron, copper and alkali (Davey *et al.*, 2000: 827). Aqueous solutions of vitamin C are stable unless transition metal ions are present. Copper and iron both catalyse the oxidation of vitamin C, utilising molecular oxygen, to produce hydroxyl and hydrogen peroxide (Rumsey *et al.*, 1999: 161). It can, also, in the presence of free iron ions, generate

the dangerous ferrous ions, a crucial catalyst of oxidative damage (Kaur & Kapoor, 2001: 711).

2.2.1.2 Polyphenols

Polyphenols are a large group of secondary metabolites widespread in the plant kingdom. These compounds, also referred to as phenolic compounds or phenolics, are chemically defined as substances possessing an aromatic ring bearing one or more hydroxyl (OH) groups, including their functional derivatives ($R_{1\text{-}6}$) (Shahidi & Naczk, 2004: 1) (Figure 2.4). They are categorised into classes depending on their structure and subcategorised within each class according to the number and position of the OH group and the presence of other substituents. Examples of common phenolic antioxidant classes include the flavonoids and phenolic acids (Shahidi & Naczk, 2004: 421).

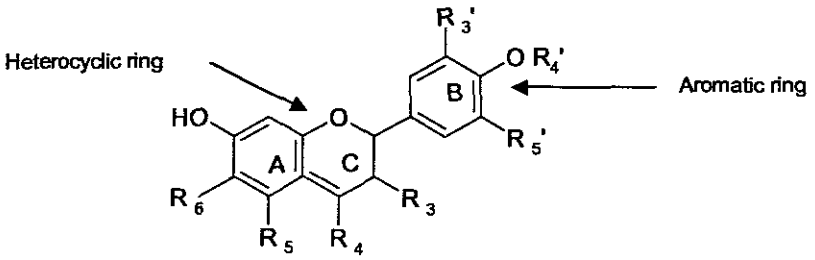


Figure 2.4: General structures and numbering pattern for common food flavonoids

(Obtained from Beecher, 2003: 3249S)

See Table 2.1 for unique linkages, unsaturation positions and functional groups of each of the flavonoid subclasses

Polyphenols are multifunctional and act as reducing agents, metal chelators and singlet oxygen quenchers (Shahidi & Naszk, 2004: 417). They possess an ideal structural chemistry for their antioxidant activities, namely the presence of i) the 3', 4' O-dihydroxy structure in the B ring, which confers higher stability to the radical form and participates in electron delocalisation, ii) the 2,3 double bond in conjunction with a 4-oxo function in the C ring which is responsible for electron delocalisation from the B ring (the antioxidant potency is related to the structure in terms of electron delocalisation of the aromatic nucleus). Where these compounds react with free radicals, the phenoxyl radicals produced are stabilised by the resonance effect of the aromatic nucleus, iii) the 3- and 5-OH groups with 4-oxo function in the A and C rings which is required for maximum radical scavenging potential (Rice-Evans *et al.*, 1996: 943). The availability of phenolic hydrogens as hydrogen donating radical scavengers or electron donating agents predicts the potential of polyphenols to act as antioxidants (Rice-Evans *et al.*, 1997: 153).

The most widespread and diverse group of polyphenols are the flavonoids, which generally consist of two aromatic rings, each containing as least one OH, which are connected through

a three-carbon "bridge" and become part of a six-member heterocyclic ring (Figure 2.4). The flavonoids are further divided into six subclasses based on the connection of an aromatic ring to the heterocyclic ring, as well as the oxidation state and functional groups (R_3, R_4) of the heterocyclic ring (C ring). Within each of the six subclasses, individual compounds are characterised by specific hydroxylation and conjugation patterns (Beecher, 2003: 3248S) (Table 2.1).

Table 2.1: Flavonoid subclasses, their chemical structures descriptions, names of prominent food flavonoids and typical food sources[a]

(Obtained from Beecher, 2003: 3249S)

Flavonoid subclass	B-ring connection to C-ring	C-ring unsaturation	C-ring functional groups	Prominent food flavonoids[b]	Typical rich food sources
Flavanol	2	None	3-hydroxy	(+)-Catechin (+)-Gallocatechin (-)-Epicatechin (-)-Epigallocatechin	Teas, red grapes and wines
			3-O-gallate	(-)-Epicatechin-3-gallate (-)-Epigallocatechin-gallate	
Flavanones	2	None	4-oxo	Eriodictyol Hesperetin Naringenin	Citrus foods
Flavones	2	2-3 double bond	4-oxo	Apigenin Luteolin	Parsley
Isoflavones	3	2-3 double bond	4-oxo	Daidzein Genistein Glycetein Biochanin A Formononentin	Soybeans, soy foods and legumes
Flavonols	2	2-3 double bond	3-hydroxy, 4-oxo	Isorhamnetin Kaempferol Myricetin Quercetin	Nearly ubiquitous in foods, e.g. quercetin
Anthocyanidins	2	1-2, 3-4 double bonds	3-hydroxy	Cyanidin Delphinidin Malvidin Pelargonidin Petunidin Peonidin	Red, purple and blue berries

[a]-see Figure 2.4 for general flavonoid structure and numbering pattern.
[b]-Flavonoids in highest concentration in foods and those reported in the USDA Database for the Flavonoids Content of Selected Foods and in the USDA-Iowa State University Database on the Isoflavone Content of Foods.
[c]-data in USDA flavonoid databases used as source of information.

The chemistry of flavonoids is predictive of their free radical scavenging activity because the reduction potential of flavonoid radicals is lower than those of alkyl peroxyl and superoxide (Rice-Evans *et al.*, 1996: 934). According to Firuzi and co-workers (2005: 181), quercetin and myricetin are the most active flavonoid antioxidants; rutin, catechin and kaempferol are active antioxidants and apigenin has no or a low activity in the ferric reducing antioxidant power (FRAP) assay. Quercetin is also the most effective 1,1-diphenyl-2-picrylhydrazyl

(DPPH) radical scavenger (Nuutila *et al.*, 2003: 489). Along with the radical scavenging potential of flavonoids, they (especially the flavonols) have also been shown to work synergistically with vitamin C. Flavonoids have a preserving effect on vitamin C mainly when copper is present (Kuhnau, 1976: 175-176).

Another class of polyphenols, the phenolic acids, are divided into two subclasses namely hydroxybenzoic and hydroxycinnamic acids (HCA). Of these two subclasses, HCA are the most effective (Rice-Evans *et al.*, 1996: 948). Hydroxycinnamic acids (i.e. caffeic acid, chlorogenic acid, *o*-, *m*-, *p*-coumaric acids, ferulic acid, gallic acid, synaptic acid and pyrogallol) are produced from either phenylalanine or L-tyrosine (Foley *et al.*, 1999: 1202). Dietary HCA consist predominantly of ferulic, caffeic and chlorogenic acids. These acids have become a subject of much interest since it was first recognised that they make up a significant proportion of the total polyphenols ingested in a normal diet and that they are readily absorbed in the digestive tract (Martinez-Valverde *et al.*, 2002: 326). Hydroxycinnamic acids (Figure 2.5) occur most commonly as simple esters with quinic acid or glucose and are present in a wide variety of fruit and vegetables such as tomatoes, spinach, broccoli, asparagus, white grapes, pears and peaches (Foley *et al.*, 1999: 1202).

The AOA of phenolic acids correlates positively with the number of OH groups bonded to the aromatic ring (Sroka & Cisowski, 2003: 753). The antioxidant properties of HCA have been demonstrated with regard to their ability to scavenge radicals generated in the aqueous phase (Rice-Evans *et al.*, 1996: 949) and to increase the resistance of low-density lipoprotein (LDL) to lipid peroxidation. The HCA, gallic acid, pyrogallol and caffeic acid have the strongest AOA on lipid peroxidation, hydrogen peroxide scavenging and DPPH scavenging tests (Sroka & Cisowski, 2003: 755-756).

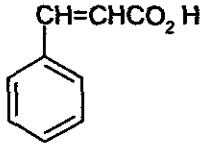


Figure 2.5: General structure of hydroxycinnamic acids
(Obtained from Sroka & Cisowski, 2003: 755-756)

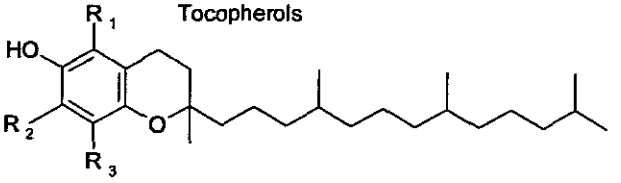
2.2.2 Lipid-soluble antioxidants

Vitamin E and the carotenoids are lipid-soluble antioxidants. These antioxidants are localised in lipophilic compartments of the body (i.e. membranes and lipoproteins) and are

also more effective antioxidants for scavenging radicals within the lipophilic domain (Noguchi & Niki, 1999: 561).

2.2.2.1 Vitamin E

Vitamin E in nature occurs in eight different forms, which differ greatly in their biological activity. Tocopherols and tocotrienols each exists in four isomers, namely α, β, γ, and δ (Halliwell & Gutteridge, 1999: 209; Papas, 1999: 190; Young & Woodside, 2001: 179; Astley, 2003: 286) (Figure 2.6). Tocopherols and tocotrienols both consist of a chroman ring but differ in their side chain structures. Tocopherols contain a long, saturated phytal side chain and tocotrienols an unsaturated chain with double bonds in the 3', 7', and 11' positions (Papas, 1999: 190; Young & Woodside, 2001: 179). The terms α-tocopherol and vitamin E are used interchangeably in the literature. This is, however, incorrect, as vitamin E is the nutritional term and α-tocopherol is only one of the forms of vitamin E, which is considered the most abundant and predominant form in human tissue (Halliwell & Gutteridge, 1999: 209; Doyle & Pariza, 2002: 4).



	R_1	R_2	R_3
α-Tocopherol and α-tocotrienol	CH_3	CH_3	CH_3
β-Tocopherol and β-tocotrienol	CH_3	H	CH_3
γ-Tocopherol and γ-tocotrienol	H	CH_3	CH_3
δ-Tocopherol and δ tocotrienol	H	H	CH_3

Figure 2.6: Structures of tocopherols and tocotrienols
(Obtained from Astley, 2003: 286)

The antioxidant and pro-oxidant properties differ between the various tocopherols and tocotrienols. It has been confirmed that the AOA of the tocopherols follows the same order as their biological potencies (α, β, γ, and δ) (Woolard & Indyk, 2003: 5789). In contrast to α-tocopherol, α-tocotrienol possesses 40 - 60 times higher AOA against iron (Fe_2^+) + ascorbate- and Fe_2^+ NADPH- induced lipid peroxidation in rat liver microsomal membranes

and 6,5 times better protection of cytochrome P-450 against oxidative damage (Serbinova *et al.*, 1991: 272).

Vitamin E is particularly important in tissues in direct contact with high concentrations of oxygen (i.e. lung) and cell organelles dealing with oxygen or oxygen-free-radical reactions (i.e. mitochondria). Most importantly, vitamin E is the major lipid-soluble chain-breaking antioxidant protecting tissues (i.e. brain and central nervous system) and foods (i.e. vegetable oils and margarine) containing relatively high levels of polyunsaturated fatty acids (PUFA) (Astley, 2003: 286-287). As a result vitamin E acts as the primary defence against lipid peroxidation (Dutta-Roy *et al.*, 1994: 562; Papas, 1999: 190; Astley, 2003: 287), which is considered to be the major antioxidant function of vitamin E (Papas, 1999: 199). Vitamin E reacts more rapidly with lipid peroxyl than PUFA and hence breaks the chain reaction of lipid peroxidation (Halliwell & Gutteridge, 1999: 209; Young & Woodside, 2001: 179). α-Tocopherol is most widely studied for the inhibition of lipid peroxidation (Papas, 1999: 190).

Tocopherols and tocotrienols also trap singlet oxygen (Kaiser *et al.*, 1990: 101) and other free radicals (Papas, 1999: 200). γ-Tocopherol has the ability to reduce NO_2 to NO or to react with NO_2 generating nitrosating species whereas α-tocopherol does the opposite and reacts with it (Cooney *et al.*, 1995: 259).

As discussed in 2.2.1.1, vitamin C acts synergistically with vitamin E. Vitamin E has furthermore been shown to exert an additive effect with β-carotene in oxidation. Their interaction is, however, not as significant as that between vitamins C and E. However, the combination of vitamins C and E and β-carotene may be effective in inhibiting oxidative damage, especially *in vivo* where oxygen concentrations are low (Niki *et al.*, 1995: 1326S).

The RDA for both men and women is 15 mg of α-tocopherol equivalents per day whereas the UL is 1000 mg of α-tocopherol equivalents per day. Sources of vitamin E include plant oils, unprocessed cereal grains, nuts, fruits, vegetables, and meats (National Academy of Sciences. Insitute of Medicine of the National Academies, 2001). Palm oil is the richest source of tocotrienols (Carotino®, 2006). Foods of animal origin are generally low sources of vitamin E. During ripening, processing and storage all foods containing vitamin E lose some of their activity (Abushita *et al.*, 1997: 211; Astley, 2003: 286). In foods, vitamin E is destroyed by oxygen while freezing causes degradation of the compound. In addition, oxidative processes of vitamin E are accelerated by heat and light (Astley, 2003: 286).

2.2.2.2 Carotenoids

Carotenoids are a group of over 600 naturally occurring plant pigments that provide the yellow, orange and red pigments seen in fruit and vegetables (Halliwell & Gutteridge, 1999: 220). Carotenoids are highly unsaturated tetraterpenes biosynthesised from eight isoprene units (Sambanthamurthi *et al.*, 2000: 525). These units are joined in such a manner that the arrangement is reversed at the centre of the molecule, placing the two central methyl groups in a 1,6 relationship (Thane & Reddy, 1997: 58) (Figure 2.7).

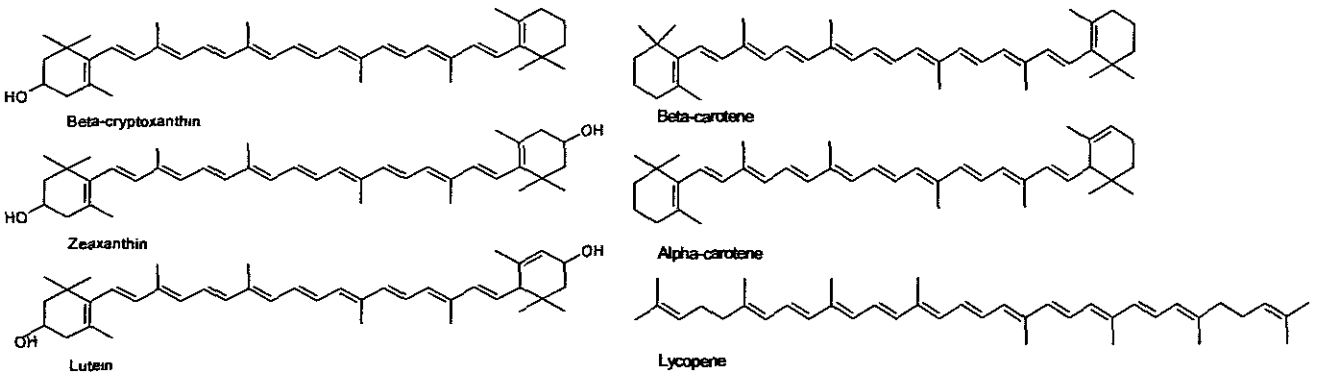


Figure 2.7: Structures of common carotenoids
(Obtained from Furr & Clark, 2003: 937)

Carotenoids can be divided into two main groups: i) carotenes or hydrocarotenoids - containing only carbon and hydrogen (i.e. lycopene, β-carotene and α-carotene) and ii) xanthophylls or oxycarotenoids - containing oxygen (i.e. β-cryptoxanthin, astaxanthin, canthaxanthin, lutein and zeaxanthin) (Thane & Reddy, 1997: 58-59; Sambanthamurthi *et al.*, 2000: 525) (Figure 2.7.) Depending on the number of double bonds present in a carotenoid, a number of *cis/trans* configurations are possible. In homogenous solutions carotenoids tend to isomerise and form a mixture of mono- and poly-*cis* isomers in addition to the all-*trans* form (Stahl & Sies, 2005: 101). Raw foods tend to have more of the *trans* form than the *cis* form. In heated foods more of the *cis* form is common, as they tend to isomerise on heating (Shi & Le Maguer, 2000: 307). Isomerisation is provoked by the release of constituent acids during slicing and pureeing of foods, heat treatment and exposure to light (Rodriguez-Amaya, 2003: 934).

The carotenes are the class of carotenoids most noted for their provitamin A activity, of which β-carotene (found in carrots, orange-fleshed sweet potato and pumpkin) has the highest pro-vitamin A value. Lycopene (found in tomato and processed tomato products) has no pro-vitamin A activity owing to the lack of a β-ionone ring structure (Shi & Le Maguar, 2000: 299; Böhm *et al.*, 2003: 385). Other common food carotenoids that do not exhibit pro-vitamin A

activity include astaxanthin (found in lobster, shrimp, and salmon), canthaxanthin (found in mushrooms) and lutein and zeaxanthin (found in egg yolks, potatoes, spinach, broccoli, and wheat) (Shi & Le Maguer, 2000: 299).

Carotenoids, in general, are able to function as chain-breaking antioxidants, much like vitamin E, and may protect cells and cellular components from oxidative damage. In the absence of vitamin E the carotenoids will protect lipids from oxidation. The quenching activity of individual carotenoids depends essentially on the number of conjugated double bonds in the molecule (Shi *et al.*, 2004: 206), of which carotenoids have been shown to scavenge singlet oxygen and peroxyl radicals (Stahl & Sies, 2005: 103). The presence of functional groups with increasing polarities in the thermal rings of carotenoids is also associated with more efficient radical scavenging activity (Shi *et al.*, 2004: 207). Combinations of carotenoids, which are more effective than the individual compounds, may be responsible for the observed biological activity (Khachik *et al.*, 2002: 846) in the prevention of oxidative damage (Shi *et al.*, 2004: 208).

Despite the similarities in the carotenoids structures, they have diverse biological functions and potentially different roles within the body. As stated above, carotenoids are effective quenchers of singlet oxygen, of which lycopene exhibits the highest singlet oxygen quenching activity (Di Mascio *et al.*, 1989: 532). Lycopene also has the highest Trolox-equivalent antioxidant capacity (TEAC) value of all carotenoids (Rice-Evans *et al.*, 1997: 152). Lycopene is also presumably the most effective in protecting against oxidative damage from free radicals (Ishida & Chapman, 2004: 8017), owing to it being a more efficient antioxidant than β-carotene, α-carotene, α-tocopherol or albumin-bound bilirubin (Di Mascio *et al.*, 1989: 534). At high oxygen tensions, such as those in the lungs, lycopene is more effective than β-carotene, which in turn is more efficient at low oxygen concentrations (Furr & Clark, 2003 937). Lycopene furthermore interacts synergistically with lutein, β-carotene and vitamin E and inhibits liposome oxidation (Shi *et al.*, 2004: 207).

The chemical structure of lycopene (Figure 2.7) is the key to its biological activities and ruby colour (Di Mascio *et al.*, 1989: 533; Shi & Le Maguer, 2000: 298; Shi *et al.*, 2004: 204). Lycopene is an unstructured open-chain carotenoid having 13 double bonds of which 11 are conjugated double bonds arranged in a linear configuration. It has an extremely hydrophobic structure as it only contains carbon and hydrogen. Seven of the conjugated double bonds can isomerise from the *trans*-form to the mono or poly-*cis* form under the influence of heat, light, oxygen and acids in degradation, and some metallic ions such as copper (Cu^{2+}) and iron (Fe^{3+}) catalyse its oxidation (Shi & Le Maguer, 2000: 299).

β-Carotene also acts as an antioxidant that scavenges free radicals (Rock, 1998: 1410). As illustrated above, lycopene is, however, a more efficient antioxidant than β-carotene. The additive effect of β-carotene along with vitamin E has been discussed previously (2.2.2.1).

No Dietary Reference Intakes have been assigned for the carotenoids, as it is believed that the current state of research on these compounds is not strong and consistent enough to support any recommendations (Rock, 1998: 1410). However, it has been recommended that 30 mg of tomato paste (± 4.5 tablespoons), a rich source of lycopene, should be consumed daily to lower the risk of prostate cancer (Ledesma, 2006: 8).

2.2.2.3 Co-enzyme Q

Co-enzyme Q (CoQ), also known as ubiquinone (Papas, 1999: 232, Sarubin-Fragakis, 2003: 94), is also a lipid-soluble antioxidant, which becomes amphiphillic in the process of translocating electrons and protons. Discrepancies in the literature occur as some authors refer to this compound as Co-enzyme Q_{10} (Sarubin-Fragakis, 2003: 94), while others only refer to it as CoQ. Human CoQ has ten isoprene units and is designated as CoQ_{10} (Papas, 1999: 232). In this study this compound will be referred to as CoQ.

The structure of CoQ (Figure 2.8) consists of a quinone ring attached to an isoprene side chain, which differs in animals and plants (Papas, 1999: 232). Co-enzyme Q functions independently as chain-breaking antioxidant and reduces peroxyl and alkoxyl radicals (Beyer, 1992: 390). Co-enzyme Q is also involved in a redox interaction with the vitamin E radical which regenerates vitamin E in the same manner as vitamin C. The major sources of CoQ are animal sources (i.e. beef, chicken), fish (Papas, 1999: 232, Sarubin-Fragakis, 2003: 94) and vegetables oils. With the exception of spinach and broccoli, vegetables are generally low sources (Papas, 1999: 232).

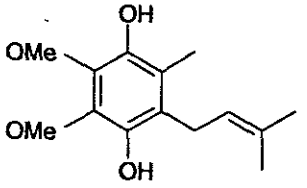


Figure 2.8: Structure of Co-enzyme Q
(Obtained from Papas, 1999: 232)

Most of the antioxidants and their classes discussed in 2.2 are mainly found in vegetable sources except for CoQ. Because of the importance of antioxidants in the diet, the intake of

fruit and vegetables among South Africans will be discussed below, as these foods are the major contributors to the dietary antioxidant intake.

2.3 Fruit and vegetables in the South African diet

Fruit and vegetables are the main sources of antioxidants, which make them essential to human health (Abushita *et al.*, 1997: 207). Vitamins C and E, carotenoids, and flavonoids are the most common antioxidants present in vegetables (Ou *et al.*, 2002: 3122). Not all South Africans, however, consume fruit and vegetables each day, while most of those who do, in general, consume more vegetables than fruit (Table 2.2). The majority of South Africans who consume fruit and vegetables, however, consume inadequate servings of fruit and vegetables (Bourne *et al.*, 1993: 238; Langenhoven *et al.*, 1995: 523). Five or more servings (400 g) of fruit and vegetables are recommended for consumption (Love & Sayed, 2001: S24); thus these consumers consume fewer than these recommendations. This contributes to their low micronutrient status, especially for the antioxidant vitamins, vitamin C and β-carotene (Langenhoven *et al.*, 1995: 523).

Table 2.2: Percentage of South Africans consuming fruit and vegetables each day
(Obtained from Steyn *et al.*, 2003: 638)

Fruit and vegetable consumption	Children aged 1-5 years[a]	Children aged 6-9 years[b]	Adults aged 10+ years[c]	Adults aged 10+ years[d]
Fruit	21.6	20.9	21.4	31.6
Vegetables	43.6	44.6	55.8	57.0

[a]-Estimated by taking the average values of the NFCS (South Africa, all), Lebowa study (Northern Province, rural blacks) and BRISK (Western Cape (urban blacks) data.
[b]-Estimated by taking the average values of the NFCS (South Africa, all), Lebowa study (Northern Province, rural blacks), BRISK (Western Cape (urban blacks) and THUSA Bana (North-West Province, all) data.
[c]-Estimated by taking the average values of the Dikgale study (Northern Province, rural blacks) and the Lebowa study (Northern Province, rural blacks) data.
[d]-Estimated by the CORIS data (Western Cape, urban & rural whites) and the BRISK (Western Cape, urban blacks) data.

The most common fruit and vegetables consumed as identified by food consumption studies are indicated in Table 2.3. Items consumed by at least 3.0% of the age groups are indicated. The majority of the vegetables commonly consumed are thermally processed, which indicates that thermal household processing plays an integral part of South African consumers' diets. Tomato and onion stew is one of the top ten commonly consumed fruit and vegetables by South Africans (Table 2.3). Its role as part of the South African diet is discussed below.

Table 2.3: Top ten commonly[a] consumed fruit and vegetables (grams) by South Africans
(Adapted from Steyn *et al.*, 2003: 641-642)

Fruit/Vegetables	Average portion (Per capita portion) 1 - 5 years[b]	Average portion (Per capita portion) 6 - 9 years[c]	Average portion (Per capita portion) Adults[d]	Average portion (Per capita portion) Adults[e]
Apple, fresh	132 (11)	160 (12)	209 (12)	227 (24)
Wild green leaves/spinach	151 (15)[f]	150 (14)[f]	185 (31)[g]	184 (18)[g]
Cooked potatoes	108 (24)	143 (29)	165 (28)	167 (52)
Boiled pumpkin	90 (7)	103 (7)	203 (8)	138 (9)
Banana, peeled	85 (7)	126 (6)	167 (11)	142 (10)
Tomato and onion stew	85 (5)	124 (7)	119 (20)	118 (15)
Boiled cabbage	79 (11)	91 (13)	114 (16)	105 (13)
Boiled carrots	76 (3)	77 (2)	56 (2)	59 (4)
Orange juice – Liquifruit / Ceres	254 (9)	287 (15)	[h]	[h]
Tomato, raw	[h]	[h]	103 (4)	102 (10)

[a]-Determined by taking the average consumed by the four indicated groups.
[b]-Estimated by taking the average values of the NFCS (South Africa, all), Lebowa study (Nothern Province, rural blacks) and BRISK (Western Cape (urban blacks) data.
[c]-Estimated by taking the average values of the NFCS (South Africa, all), Lebowa study (Nothern Province, rural blacks), BRISK (Western Cape (urban blacks) and THUSA Bana (North-West Province, all) data.
[d]-Estimated by taking the average values of the Dikgale study (Northern Province, rural blacks) and the Lebowa study (Northern Province, rural blacks) data.
[e]-Estimated by the CORIS data (Western Cape, urban & rural whites) and the BRISK (Western Cape, urban blacks) data.
[f]-Cooked.
[g]-Raw.
[h]-Item consumed by fewer than 3% of the group.

2.4 Stewed tomato and onion in the South African diet

Tomato and onion stew is a popular dish among all South Africans whether young or old or from a lower or higher socio-economic group (Steyn *et al.*, 2003: 642). This dish is traditionally served with maize (Langenhoven *et al.*, 1995: 527).

Several South African consumption studies (Langenhoven *et al.*, 1995: 527; Steyn *et al.*, 2003: 642) and food composition data (Kruger *et al.*, 1998: 38) refer to tomato and onion stew. However, a "stew" is a dish containing meat (Davidson, 1999: 754; Simon & Howe, 1970: 361-362) and the tomato and onion stew indicated in the published studies (Langenhoven *et al.*, 1995: 527; Steyn *et al.*, 2003: 642) and food composition data (Kruger *et al.*, 1998: 38) contains tomato and onion as the main ingredients. Furthermore, it is classified in the study data under vegetable consumption that further highlights that it does not contain meat. To clarify the ingredients it contains and the preparation technique it will henceforth be referred to as stewed tomato and onion.

The main ingredients of stewed tomato and onion are also commonly consumed vegetables worldwide (Hertog *et al.*, 1992: 2379; Vinson *et al.*, 1998: 3631; George *et al.*, 2004: 46). These vegetables are also the main ingredients of many other dishes such as pizza or pasta

(bolognaise) sauces, tomato-based meat sauces, and several stews of which the latter are considered as a potentially good carrier of micronutrients (Langenhoven *et al.*, 1995: 527).

At present there is an increasing interest in herbs, both in industry and in scientific research, because of their antioxidant properties (Calucci *et al.*, 2003: 927). Herbs, an excellent source of natural antioxidants (Justesen & Knuthsen, 2001: 248; Halvorsen *et al.*, 2002: 466), are added to tomato and onion-based recipes to add colour and flavour (Craig, 1999: 492S). Natural antioxidants in the form of herbs can also be added to food to compensate for processing losses (Nicoli *et al.*, 1999: 95). It has been recommended that the use of herbs should be increased, as herbs may contribute to the daily antioxidant intake (Justesen & Knuthsen, 2001: 245). A commonly consumed herb in the South African diet is parsley (Lizaar, 2005), which is added to soups, salads, sauces, stews, etc. (Paarman, 2001: 13, 33, 72; Human, 2002: 263). A Danish study found that the consumption of traditional food dishes containing a few grams of parsley could significantly contribute to the average daily antioxidant intake (Justesen & Knuthsen, 2001: 248). In addition, Ninfali and co-workers (2005: 264) found that the introduction of aromatic herbs such as marjoram and lemon balm into salads markedly increased the $ORAC_{FL}$ values of the whole salad.

In food preparation methods such as frying and sautéing the addition of cooking oils is general practice (Pokorný & Schmidt, 2003: 305). In the preparation of stewed tomato and onion, cooking oil is also required (Human, 2002: 263). The addition of cooking oils, such as SFO or RPO, to a green leafy vegetable relish could contribute to the recommended daily vitamin A intake as was found in an *in vitro* study simulating the digestion process. This was attributed to the provitamin A function of carotenoids which are also powerful antioxidants (Hedrén *et al.*, 2002: 450). Adding RPO instead of SFO resulted in twice as much accessible β-carotene, owing to the high accessibility of the RPO's β-carotene content. Furthermore, RPO contains high levels of β-carotene, while SFO does not. Lietz and co-workers (2001: 501) found that the consumption of RPO increased α and β-carotene significantly in both plasma and breast milk and maintained breast-milk retinol concentrations whereas SFO only conserved the former. The TAC of stewed tomato and onion flavoured with parsley may also be increased by using RPO and provide for antioxidant associated health benefits. In South Africa (SA), SFO is the most commonly used cooking oil because of its availability and low cost. South Africans consume an average of 8 g of SFO per day while a per capita portion of 0.5 g is consumed per day (Steyn *et al.*, 2003: 642). The Malaysian Carotino RPO, which is an excellent source of antioxidants (Carotino®, 2006), is also available to the South African consumer. Carotino Premium oil is considered to be a concentrated source of antioxidants as 20 g exceeds the United States (US) RDA for vitamins A and E (Carotino®, 2006).

Stewed tomato and onion is a popular dish in SA. Its consumption could contribute to the daily antioxidant intake of South African consumers. Furthermore, the addition of natural antioxidants such as herbs, parsley, and cooking oils, especially RPO, may increase the antioxidant content. These naturally added antioxidants may compensate for processing losses, but can also contribute to the TAC of stewed tomato and onion. This popular dish contains more than one recipe ingredient which may contribute to a higher antioxidant intake. Discussed below are the recipe ingredients and the antioxidants they will provide to the combined dish.

2.5 Antioxidant content and capacity of tomatoes, onion, parsley and cooking oils

The ingredients of stewed tomato and onion flavoured with parsley (tomato, onion, parsley, SFO or RPO) contain several antioxidants such as carotenoids, polyphenols, and vitamins C and E, which contribute to the TAC of the recipe ingredients (Halvorsen *et al.*, 2002: 466; Pelligrini *et al.*, 2003: 2815). Each of these food ingredients also contains one or more antioxidant components that make them unique as they are rich dietary sources thereof. These food ingredients also contain other antioxidants which contribute to the AOC and which may also result in possible synergistic interactions between the components. In order to evaluate the TAC of a given food system, it is necessary to know which antioxidants are present in the food.

2.5.1 Antioxidant content

Several factors influence the antioxidant content or the TAC of foods. They are the variety, maturity, growing conditions, part of the food (i.e. seeds, skin, leaves, pulp), agricultural practices and also the methods applied during the analyses of the antioxidant compounds (Leonardi *et al.*, 2000: 4727; Martinez-Valverde *et al.*, 2002: 323). The antioxidant content of the ingredients of stewed tomato and onion flavoured with parsley and the factors affecting the antioxidant content of the ingredients will be discussed below.

2.5.1.1 Tomatoes

Consumption of tomatoes (*Lycopersicon esculentum*), fresh or processed (tomato products), is considered a nutritional indicator of good dietary habits and healthy lifestyles (Martinez-Valverde *et al.*, 2002: 323; George *et al.*, 2004: 45). Tomatoes serve as a reservoir of diverse antioxidant molecules, such as carotenoids, polyphenols and vitamins C and E.

i Carotenoids

Over 20 carotenoids have been characterised in tomatoes, lycopene being the major carotenoid pigment along with lesser amounts of α, β, γ, and ε-carotene, phytoene, phytofluene, neurosporene and lutein (Shi & Le Maguer, 2000: 298; Khachic *et al.*, 2002: 845; Shi *et al.*, 2004: 203; Lewinsohn *et al.*, 2005: 3143). The second predominant carotenoid identified in tomatoes is lycopene epoxide, which is an oxidation product of lycopene (Abushita *et al.*, 2000: 2075). On average, lycopene constitutes about 79.0 - 85.0% of the total carotenoid content of tomatoes (Leonardi *et al.*, 2000: 4725).

A good estimate of lycopene content can be made by the dark red appearance of the tomato and/or tomato products (Ishida & Chapman, 2004: 8019). Since humans are unable to synthesise carotenoids *de novo*, they are exclusively obtained from the diet. At least 85.0% of dietary lycopene comes from fresh tomatoes and tomato-based products (Bramley, 2000: 234). More than 80.0% of tomatoes produced in America are consumed in the form of processed products such as tomato juice, paste, puree, catsup, sauce and salsa (Shi & Le Maguer, 2000: 314). Rao and co-workers (1998: 740) found that Canadians consume sufficient lycopene (25 mg/day), with 50.0% of the dietary lycopene being provided by fresh tomatoes.

The carotenoid content of tomato varieties, genotypes, typologies and cultivars differ as indicated in Table 2.4. The lycopene/carotenoid content also differs among the parts (i.e. skin/peel, pulp, seeds) of the tomato. George and co-workers (2004: 45) and Toor and Savage (2005: 490) studied the distribution of lycopene in tomatoes and found that the skin/peel contains the highest amount of lycopene and not the tomato pulp, which is the part mostly consumed. The concentration of lycopene in tomato skin is about 2 - 3 times higher than in the tomato pulp. This indicates that most of the lycopene is found attached to the insoluble fibre portion of tomato (Shi & Le Maguer, 2000: 296). The skin and seeds contribute 48.0% of the total lycopene content of the tomato (Toor & Savage, 2005: 493). Maturity and the environmental conditions under which the tomato fruit matures also play an important role in the carotenoid content (Shi & Le Maguer, 2000: 295).

Table 2.4: Summary of lipid-soluble antioxidant content (fresh weight) of tomatoes from different studies

Tomato description	Total carotenoid (mg/100 g)	Lycopene (mg/100 g)	β-carotene (mg/100 g)	Lutein (mg/100 g)	Lycopene epoxide (mg/100 g)	Reference
12 salad varieties	6.27 - 9.83	5.18 - 9.83	0.3 - 0.62	0.01 - 0.11	0.08 - 0.29	Abushita *et al.*, 2000: 2078 - 2079
15 industrial varieties	6.80 - 13.21	5.14 - 11.61	0.21 - 0.45	0.08 - 0.43	0.12 - 0.36	
5 tomato typologies	0.64 - 13.19	0.11 - 10.80	0.08 - 1.05	ND - 0.20	ND - 0.17	Leonardi *et al.*, 2000: 4725
9 varieties	-	1.76 - 7.01	-	-	-	Martinez-Valverde *et al.*, 2002: 323
25 lines and 5 cultivars	-	0.00 - 1.70	0.12 - 13.54	-	-	Minoggio *et al.*, 2003: 68
Skin of 12 genotypes	-	4.83 - 14.1	-	-	-	George *et al.*, 2004: 45
Pulp of 12 genotypes		2.04 - 6.94				
Skin of 3 cultivars	-	7.58 - 9.82	-	-	-	Toor & Savage, 2005: 490
Pulp of 3 cultivars		2.04 - 2.94				
Seeds of 3 cultivars		1.50 - 1.70				

Abbreviations: ND-Not detected, -Not determined.

Table 2.5: Summary of water-soluble antioxidant content (fresh weight) of tomatoes from different studies

Tomato description	Total polyphenols mg/100 g	Total flavonoid mg/100 g	Quercetin mg/100 g	Kaempferol mg/100 g	Chlorogenic acid mg/100 g	References
Peel of 12 genotypes	10.4 - 40.0[a]	-	-	-	-	George *et al.*, 2004: 48
Pulp of 12 genotypes	9.23[a]					
25 lines and 5 cultivars	4.04 - 28.69[b]	0.13 - 4.74	-	-	0.03 - 0.58	Minoggio *et al.*, 2003: 68
9 varieties	24.97 - 51.56[c]	-	0.68 - 43.99	ND - 2.42	1.39 - 36.55	Martinez-Valverde *et al.*, 2002: 325
Skin of 3 cultivars	33.37 - 36.03[b]	21.01 - 19.79[d]	-	-	-	Toor & Savage, 2005: 490
Pulp of 3 varieties	12.55 - 12.85[b]	7.83 - 8.57[d]				
Seeds of 3 varieties	21.41 - 29.59[b]	10.92 - 13.28[d]				

[a]-Expressed as catechin equivalents.
[b]-Expressed as gallic acid equivalents.
[c]-Expressed as ferulic acid equivalents.
[d]-Expressed as rutin equivalents
Abbreviations: ND-Not detected, -Not determined

ii Polyphenols

Tomatoes also contain polyphenols (Table 2.5), which are characterised as flavonols (quercetin, kaempferol) and HCA (caffeic, chlorogenic, ferulic and p-coumaric acids) (Martinez-Valverde, *et al.*, 2002: 323; Minoggio *et al.*, 2003: 68). The total polyphenol content of tomatoes is also dependent on the tomato variety and part of the tomato (Stewart *et al.*, 2000: 2667), as illustrated by the carotenoids.

Ninety-eight percent of the flavonols present in tomatoes are found in the skin where they primarily occur as the conjugates, quercetin and kaempferol (Stewart *et al.*, 2000: 2663). Quercetin is the most abundant flavonol in tomatoes (Martinez-Valverde *et al.*, 2002: 323) and contributes to 96.0% of the skin-derived flavonol with the remainder consisting of kaempferol. Furthermore, rutin was identified to be the main quercetin conjugate in tomatoes (Stewart *et al.*, 2000: 2663, 2665). According to Minoggio and co-workers (2003: 68), naringin and rutin were the main polyphenols found when different tomato lines and cultivars were analysed. In contrast, Martinez-Valverde and co-workers (2002: 323) found the naringin content to be smaller than 1.26 mg/100 g, and it was furthermore not detected in all tomato varieties.

Chlorogenic acid is the most encountered HCA in tomato fruits (Martinez-Valverde *et al.*, 2002: 326). Martinez-Valverde and co-workers (2002: 325) detected ferulic (0.16 - 0.54 mg/100 g) and caffeic acid (0.14 - 1.30 mg/100 g) levels in all the tomato varieties analysed while Minoggio and co-workers (2003: 69) detected much lower to no levels of these acids in 25 tomato lines and 5 tomato cultivars.

As evident from Table 2.5, the difference in the antioxidant content is primarily due to the use of different analytical methodologies. Different standards such as catechin, gallic acid and ferulic acid were used to determine the total polyphenol content in tomatoes. This makes it difficult to compare absolute values.

Studies have furthermore found that tomato lines characterised by low carotenoid content produce high levels of polyphenols, and consequently have the most powerful AOCs (Minoggio *et al.*, 2003: 69). As tomato fruit matures, the concentration of chlorogenic acid decreases and the content of ferulic acid, caffeic acid (Fleuriet & Macheix, 1981:669), lycopene (Leonardi *et al.*, 2000: 4725) and β-carotene (Abushita *et al.*, 1997: 210), which are the stronger antioxidants, increases.

iii Vitamins C and E

Tomatoes contain a considerable amount of vitamin C (20 mg/100 g) (FoodFinder™3, 2002). Abushita and co-workers (2000: 2077-2078) reported the vitamin C content of 27 tomato varieties to range between 15 and 22 mg/100 g fresh weight (FW).

The vitamin E content for raw tomatoes is 0.84 mg/100 g (FoodFinder™3, 2002). Several studies have reported the presence of tocopherols in Hungarian tomatoes (Abushita *et al.*, 1997: 210; Abushita *et al.*, 2000: 2075) (Table 2.6). Earlier work of Abushita and co-workers (1997: 211) indicated the α-tocopherol content of 15 tomato varieties to be lower (0.10 to 0.32 mg/100 g) than that indicated in Table 2.6.

Table 2.6: Tocopherol content (mg/100g) of salad and industrial tomato varieties on a fresh weight basis

(Obtained from Abushita *et al.*, 2000: 2077-2078)

Varieties	α-tocopherol	α-tocopherol quinone	β-tocopherol	γ-tocopherol
Salad[a]	0.12 - 0.61	0.09 - 0.39	Trace - 0.12	0.11 - 0.31
Industrial[b]	0.44 - 1.16	0.05 - 0.71	0.03 - 0.07	0.09 - 0.45

[a]-Range of 12 tomato varieties.
[b]-Range of 15 tomato varieties.

2.5.1.2 Onion

The characteristic flavour, aroma and associated health benefits of onion (*Allium cepa L.*) account for its popularity throughout the world (Patil & Pike, 1995: 643). Onion predominantly contains the flavonoid antioxidants and contains lesser amounts of organosulphur compounds (Shon *et al.*, 2004: 663), vitamin C (6mg/100g) (FoodFinder™3, 2002) and HCA (Herrmann, 1989: 334).

Onion is one of the major sources of flavonoids in the US, Finland, Greece and the former Yugoslavia (Hollman *et al.*, 1996: 45). A wide range of flavonols has been found to be present in onion (Rhodes & Price, 1996: 116), such as quercetin, kaempferol and myricetin (Yang *et al.*, 2004: 6788). Onions are known to contain a high flavonoid content (>0.5 mg/100 g), which is mainly represented by the sum of the quercetin, kaempferol, myricetin and apigenin content (Hertog *et al.*, 1995: 383).

Onions are unusual in their complexity of flavonol components. The major flavonols in onions are all derivatives of quercetin and monoglucoside, diglucoside, or free aglycones (Patil *et al.*, 1995: 912; Sellapan & Akoh, 2002: 5340). The majority are glucose derivatives of the aglycones, quercetin and kaempferol (Rhodes & Price, 1996: 116). Up to 20 minor

quercetin derivatives can be detected in onions of which the two major components are quercetin monoglucoside and quercetin diglucoside (Rhodes & Price, 1996: 115). Patil and co-workers (1995: 912) found spiraeoside to be the main quercetin-containing compound present in onions. Onions, like tomatoes, also contain conjugated quercetin (Crozier *et al.*, 1997: 592).

Great varietal diversity exists in both the onion colour and flavour, thereby influencing the composition and concentration of the polyphenols (Yang *et al.*, 2004: 6788) (Table 2.7). This is illustrated in the increased concentrations of total quercetin found in red, yellow and pink onions in contrast to white onions that contain only trace amounts (Patil *et al.*, 1995: 912). Furthermore, the physiology of flavonol accumulation is highly ring-specific and may be closely channelled and regulated within each ring. Nuutila and co-workers (2003: 488) found higher levels of polyphenols in the inedible outer skins than in the edible parts of the onion.

Table 2.7: Summary of flavonoid content (fresh weight) of onions from different studies

Onion description	Total quercetin mg/100 g	Total flavonoid mg/100 g	Total polyphenols GAE mg/100 g	References
Red skin	8059.8 - 8635.6	-	7952.7 - 8011.3	Nuutila *et al.*, 2003: 488
Red edible part	166.0 - 219.2		192.8 - 222.2	
Yellow skin	3221.9 - 3664.1		2520.7 - 2700.3	
Yellow edible part	93.9 - 122.1		1459.5 - 1640.5	
Red variety	0.9	177.8	-	Price & Rhodes, 1997: 335
Brown variety	3.9	151.6		
Pink variety	1.5	136.9		
White variety	0.3	8.9		
Red (6 entries)[a]	14.1 - 20.2	-	-	Patil *et al.*, 1995:
Pink (3 entries)[a]	11.8 - 15.8			
Yellow (55 entries)[a]	5.4 - 28.6			
White (11 entries)[a]	0.0 - 0.1			
5 Vidalia varieties	7.7 - 46.3	12.2 - 52.4.	73.3 - 180.8	Sellapan & Akoh, 2002: 5340

Abbreviations: GAE - gallic acid equivalents.
[a]-Different growing locations

Kaempferol is present in much smaller quantities than quercetin. Sellapan & Akoh (2002: 5340) found the kaempferol and myricetin content to be between 1.54 and 1.98 mg/100 g FW and 2.77 and 4.13 mg/100 g FW, respectively. Kaempferol is only detectable in certain onion varieties. Nuutila and co-workers (2003: 488) confirmed this by not being able to detect kaempferol in the red and yellow skin varieties analysed.

2.5.1.3 Parsley

Parsley (*Petroselinum crispum*) is a well-known herb and are often referred to as being a culinary (Zheng & Wang, 2001: 5166) and aromatic (Ninfali *et al.*, 2005: 258) herb. Parsley leaves are among the most valuable vegetables because of their biological properties (Lisiewska & Kmiecik, 1997: 633). It contains various antioxidants, namely flavonoids, tocopherols, β-carotene, vitamin C and antioxidant enzymes (Lisiewska & Kmiecik, 1997: 635; Fejes *et al.*, 1998: 150; Nielsen *et al.*, 1999: 454; Justesen & Knuthsen, 2001: 246; Calucci *et al.*, 2003: 929; Gomez-Coronado *et al.*, 2004: 231). It is therefore regarded as a good source of antioxidants (Halvorsen *et al.*, 2002: 465).

Parsley is a natural source of apigenin, which is the most abundant flavonoid in fresh parsley (510 - 630 mg/100 g) (Justesen & Knuthsen, 2001: 247). It is primarily present in parsley as apiin, the 7-O-apioside of apigenin (Nielsen *et al.*, 1999: 453). In addition, it is also a source of the flavonoids luteolin (0 - 4 mg/100 g) and quercetin (0 - 1 mg/100 g) (Justesen & Knuthsen, 2001: 247). Parsley is also a rich source of vitamin C as it contains 133 mg/100 g. The vitamin C content of parsley per weight is also much higher than the sum of the vitamin C content of raw tomatoes and onions (26 mg/100 g) (FoodFinder™3, 2002).

Calucci and co-workers (2003: 929) found the highest lutein and zeaxanthin content in parsley compared with that of basil, oregano, rosemary and sage. Parsley is also a source of β-carotene (2.83 mg/100 g) (FoodFinder™3, 2002). The presence of tocopherols was reported by Gomez-Coronado and co-workers (2004: 227) who indicated that parsley contains both α- (5.14 mg/100 g) and γ-tocopherols (3.27 mg/100 g). Parsley had the highest γ-tocopherol level when compared with other herbs (i.e. basil, oregano, thyme, rosemary, sage). South African data on the vitamin E content of raw parsley indicate a lower content (1.79 mg/100 g) (FoodFinder™3, 2002) than the tocopherol content found by Gomez-Coronado and co-workers (2004: 231). Fejes and co-workers (1998: 150) also found that the different parts (i.e. leaves, stems, seeds) of parsley contain different amounts of antioxidants.

Parsley has also been found to induce an unexpected increase in antioxidant enzymes (SOD and glutathione peroxidase), possibly reflecting an adaptive response by the endogenous antioxidant defence system. However, it cannot be excluded that other components in parsley, for example, lutein or furacoumarins, or a combination of parsley constituents, are responsible for the effects seen on the antioxidant enzymes (Nielsen *et al.*, 1999: 454).

2.5.1.4 Cooking oils

Refined SFO is widely used for cooking and frying, as salad oil or in margarine production (Silva *et al.*, 2001: 3936). Most studies investigating SFO were conducted on the stability of the oil (List *et al.*, 1972: 287; Huang *et al.*, 1981: 997; Silva *et al.*, 2001: 3936) and not as a source of antioxidants. Nevertheless, SFO contains several antioxidants such as CoQ and tocopherols (Cabrini *et al.*, 2001: 6026). This oil is, however, not regarded as a rich source of these antioxidants when it is compared with RPO (Carotino®, 2006).

Unrefined RPO is one of the most widely used cooking oils in West and Central Africa (Ebong *et al.*, 1999: 209). Palm oil is a lipid extracted from the fleshy orange-red mesocarp of the fruit of the oil palm tree (*Elaeis guineensis*), which contains 45.0 - 55.0% of oil (Edem, 2002: 319). Throughout the world, 90.0% of palm oil is used for edible purposes and included in products such as margarine, shortening, ice creams and cocoa butter substitutes in chocolate. Red palm oil-based cooking/salad oil is manufactured under the brand name Carotino and is available in more than ten countries worldwide. Carotino, a refined RPO, is utilised as a component or an ingredient in the food industry which can convert a normal food product into a functional food. There are two varieties of Carotino cooking/salad oil, namely i) Carotino Premium, which consists entirely of palm superolein with a premium level of natural carotenes and ii) Carotino Classic, a tailor-made blend of Carotino superolein and non-genetically modified canola oil endorsed by the Heart Foundations of Australia, Singapore and SA. Both Carotino Premium and Carotino Classic carry the Swiss Vitamin Institute endorsement for their antioxidant content of natural carotenes, vitamin E and CoQ (Carotino®, 2006). Of these two oils, only Carotino Classic is available for purchase by the South African consumer in health food stores.

Sunflower oil is an important source of linoleic acid, a PUFA (Huang *et al.*, 1981: 997; Silva *et al.*, 2001: 3936). In contrast, RPO contains 50.0% saturated fatty acids, 40.0% monounsaturated fatty acids and 10.0% PUFA (Rukmini, 1994: 126) having high oxidative stability due to the presence of natural antioxidants and the absence of linolenic acid (Edem, 2002: 322).

Both SFO and RPO are rich sources of vitamin E; however, RPO contains almost twice as much (Carotino®, 2006) (Table 2.8). The vitamin E content of RPO is unique, represented mainly as tocotrienols (70.0%) rather than tocopherols (30.0%) (Al-Saqer *et al.*, 2004: 579). Red palm oil in descending order contains 38.9% γ-tocotrienols, 28.6% α-tocotrienols and 23.5% α-tocopherol (Choo *et al.*, 1993: 79). Sunflower oil is a rich source of α-tocopherol (578±54 mg/l) when it is compared with other vegetable oils (i.e. corn, extra virgin olive,

peanut and soybean) but not when compared with RPO. Sunflower oil is also a source of γ-tocopherol (83 - 103 mg/l) (Cabrini *et al.*, 2001: 6028).

Table 2.8: Carotene and vitamin E content (mg per 100 ml) of Carotino and other plant oils (Obtained from Carotino®, 2006)

Oils	Carotene	Vitamin E
Carotino Premium	50	80
Carotino Classic	12.5	50
Sunflower	0	39
Safflower	0	27.4
Corn	0	20.7
Olive	0	7.6

The characteristic colour of crude palm oil is due to the abundance of carotenoids. Farombi and Britton (1999: 320) found that all carotenes in palm oil exhibited reactivity towards peroxyl radicals. The hydrocarbon carotenes (α-carotene, β-carotene) are more reactive than the xanthophylls (lutein and zeaxanthin). Sunflower oil contains insignificant amounts of carotenoids. When 12 g of SFO and RPO are consumed they contribute to 10.2 μg and 3496 μg total carotenoids respectively, per day (Lietz *et al.*, 2001: 502). This clearly indicates that RPO could be a major contributing source of carotenoids in the diet.

2.5.2 Antioxidant capacity

As indicated within the antioxidant content description of the ingredients, the variety, the part of the tomato, onion and parsley used, and the maturity will affect the antioxidant levels of these ingredients. Leonardi and co-workers (2000: 4727) and Martinez-Valverde and co-workers (2002: 323) confirmed the above and added that the individual antioxidants and the TAC of vegetables differ considerably as a result of the growing conditions and, in addition, the analytical antioxidant methodologies applied. Agricultural practices also seem to play a role, as organic compared to conventionally grown tomatoes have higher levels of several antioxidants (lycopene, total carotenoids) and also a higher TAC (Ishida & Chapman, 2004: 8020). Caris-Veyrat and co-workers (2004: 6503) also found higher levels of vitamin C, lycopene and total polyphenols for organic tomatoes than for conventional tomatoes. Although tomatoes, onions, parsley, SFO and RPO differ in their antioxidant content, these factors impact each of the antioxidants present in these food ingredients, which in return affect the TAC of the ingredients.

Comparisons between studies determining the TAC are difficult as the methodologies applied have different principles. Furthermore, researchers describe the AOC by using an array of terms. Terms used include total antioxidant "capacity" (or efficiency, power,

parameter, potential, potency, and activity). The "activity" of a chemical would be meaningless without the context of specific reaction conditions such as pressure, temperature, reaction media, co-reactants and reference points. Because the "AOA" measured by an individual assay reflects only the chemical reactivity under the specific conditions applied in that assay, it is inappropriate and misleading to generalise the data as indicators of "TAA" (Huang *et al.*, 2005: 1843). Many studies, however, do this. The other terms listed above are more independent of specific reactions and have similar chemical meanings (Huang *et al.*, 2005: 1843). To be consistent, the term "capacity" will be used to refer to the results obtained by the different assays.

Several studies have determined the TAC of tomato, onion, parsley and SFO (Cabrini *et al.*, 2001: 6029; Chu *et al.*, 2002: 6914; Exarchou *et al.*, 2002: 5298; Halvorsen *et al.*, 2002: 466; Ou *et al.*, 2002: 3125; Sellapan & Akoh, 2002: 5340; Campanella *et al.*, 2003: 1013; Dragland *et al.*, 2003: 1289; Ninfali & Bacchiocca, 2003: 2224; Pelligrini *et al.*, 2003: 2815; Wu *et al.*, 2004a: 4030; Yang *et al.*, 2004: 6790; Ninfali *et al.*, 2005: 260-261). These studies used different methods to determine the TAC, making it difficult to compare the absolute values.

Utilising the $ORAC_{FL}$ assay, the TAC of a typical onion serving (80 g or ½ cup) was found to contribute 823 μmol Trolox equivalents (TE), which is higher than that found for a serving tomatoes (415 μmol TE per 123 g) (Wu *et al.*, 2004a: 4030). Halvorsen and co-workers (2002: 466) determined the TAC using the FRAP assay for tomatoes (0.24 - 0.34 μmol/100 g), onions (0.67 - 0.70 μmol/100 g) and parsley (1.7 - 2.0 μmol/100 g). The TAC of parsley was higher than that of the tomato and onion. Dragland and co-workers (2003: 1289), however, found a higher TAC (3.00 μmol/100 g) for parsley when the same assay was used.

The antioxidants present in the ingredients (tomatoes, onions, parsley and oils) contribute differently to the TAC owing to their different chemical properties as was discussed in 2.2. Several studies investigated the contribution of antioxidant compounds to the AOC of these ingredients (Chu *et al.*, 2002: 6914; Martinez-Valverde *et al.*, 2002: 329; Sellapan & Akoh; 2002: 5340; Minoggio *et al.*, 2003: 68; Nuutila *et al.*, 2003: 491; Ishida & Chapman, 2004: 8019; Shi *et al.*, 2004: 208; Yang *et al.*, 2004: 6788; Toor & Savage, 2005: 492). Their results indicate that the contribution of an antioxidant to the TAC differs from fruit to fruit and from vegetable to vegetable as each of the foods differs in its antioxidant content. The interaction between carotenoids and the combined effects of each carotenoid with other antioxidants, such as vitamins C and E and flavonoids, may be more important in contributing to the overall capacity (Shi *et al.*, 2004: 204). Martinez-Valverde and co-workers (2002: 329) illustrated that no one compound or group of compounds sufficiently defines the

TAC of plant tissues in tomatoes and that a multifactorial approach is required, since other antioxidants and components could produce a synergistic effect on the AOC. This suggests that synergism plays an important role in the TAC of food.

Stewed tomato and onion flavoured with parsley contains a variety of antioxidants (i.e. vitamins C and E, carotenoids, flavonoids, HCA and CoQ), which would contribute to the TAC of the dish. As discussed in this section, many factors affect the antioxidant content and antioxidant capacity of the ingredients. Processing, furthermore, has a major influence on the TAC of foods (Papas, 1999: 95), as the composition and bioavailability of the antioxidants can be affected. It is therefore important to know what the effects of thermal processing are on the level and activity of antioxidants in foods (Dekker *et al.*, 1999: 797) especially those of household preparation methods.

2.6 Effect of thermal household processing on antioxidant components and capacity

Thermal household processing plays an important role in South African consumers' diets, as most of the vegetables they consume are processed (as discussed in 2.3). Some vegetables are commonly consumed in a cooked form such as potatoes, sweet potatoes and pumpkin, while others are consumed in raw or cooked forms such as tomatoes, onions and carrots (Wu *et al.*, 2004b: 419).

As with all forms of cooking, household processing (also known as domestic cooking) is intended to improve the palatability of the food to make it more appetising. Unlike commercial/industrial food preparation and mass catering or food service, household processing is carried out in the consumer household. Processing methods applied in the household can be with (i.e. cooking, baking) or without (i.e. peeling, chopping) the application of heat (Rosenthal, 2003: 1622). Thermal household processing methods include boiling, blanching and simmering; roasting, baking and drying; shallow frying, deep fat frying and sautéing; and microwave oven heating. Heat (energy) is transferred in these processing methods by water, air, oil or waves resulting in a rise in temperature (Pokorný & Schmidt, 2003: 300). The traditional route for energy transfer is by contact with a heated medium (i.e. water, oil), which causes heat to flow to the surface of the food and then on to the centre by conduction (Rosenthal, 2003: 1622). Figure 2.9 illustrates the thermal household processing techniques and their energy transfer.

Energy transferred by contact with heated:							Energy transferred by electromagnetic radiation Typical wavelength	
Solid	Liquid		Gas or vapour					
			Steam		Air convection		Short	Long
	Oil	Water	Atmospheric	Pressure	Natural	Forced	(0.03 mm)	(300 mm)
Griddling	Frying	Boiling Simmering	Steaming	Pressure cooking	Roasting/Baking		Grilling[a]	Microwave[b]
All these techniques achieve surface heating: heat flow to centre by conduction								Heat generated throughout

Figure 2.9: Thermal household processing techniques and the energy transfer source
(Obtained from Rosenthal, 2003: 1623)

[a]-Only able to penetrate a couple of millimetres below the surface of the food. The inner regions of the food are heated by conduction.
[b]-Microwaves have longer wavelengths, which are able to penetrate deep into foods, generating heat *in situ*. In many cases energy transfer during cooking is not by a single mechanism. For example, ovens absorb and emit infrared energy

Processing food with these various energy transfer media results in a number of simultaneous and interrelated processes which influence the flavour, texture, appearance, nutrient content, and safety of the food (Rosenthal, 2003: 1622). According to Adams and Erdman (1988: 557), the statement "it is impossible to separate most effects of commercial processing from home processing because the physical and chemical changes involved are identical", by Dr Agnes Fay Morgan (1960), is largely correct for the nutrient content of foods. This is partly true as structural integrity, the moisture content and some internal temperatures of foods would still be similarly affected. However, processing technologies applied commercially change regularly while those methods applied in the household do not change that often as traditional cooking methods are still mostly being applied. Most of the processing technologies applied commercially are also not reproducible in the household as the equipment is not available for household quantities. Furthermore the heat and pressure intensities applied commercially are much higher than those that will be achieved in the household. For example, during commercial steaming, steam is fed at a rate of approximately 5 kilometres per hour (Agüero *et al.*, 2005: 773). High temperatures and high-pressure intensities would have a greater impact on the antioxidants present in foods. In the light of these differences and considering that most South Africans prepare their own meals at home as they do not have the income to buy ready-prepared vegetable meals, the effect of thermal commercial processing will not be discussed and reference will only be made to the effect of thermal household processing.

Pokorný and Schmidt (2001 and 2003) reviewed the effect of several processing methods utilising water (boiling, blanching), oil (frying), air (baking, roasting) and microwaves as the heat transfer medium on antioxidants. The changes occurring in each of these processing

methods are based on three physical processes affecting the AOC of foods. They are i) structural integrity, ii) moisture content and iii) temperature (Diplock *et al.*, 1998: S98) (Figure 2.10). These three physical processes form a continuous and integrated system. The effect of thermal household processing will be discussed in the light of these three processes using temperature as the central point of discussion.

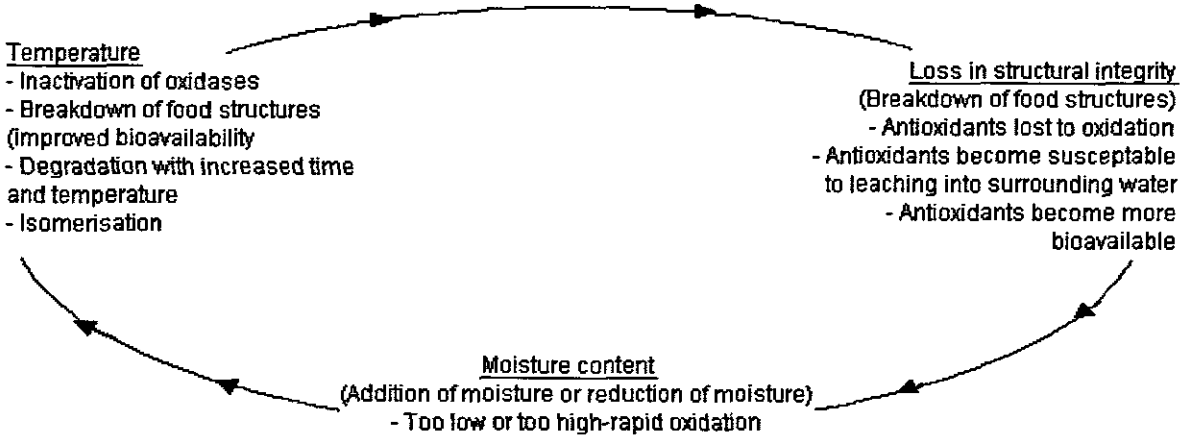


Figure 2.10: Physical processes affecting antioxidants during processing
(Adapted from Diplock *et al.*, 1998: S98)

With reference to the structural integrity, pre-preparation processes, such as peeling, cutting and grinding (without heat) and thermal household processing, such as boiling and frying break the structure of foods. Using pre-preparation processes without the application of heat, can lead to i) an increased oxidation of antioxidants, ii) an increased leaching of antioxidants into the surrounding water/liquid, and iii) it can make the antioxidants more bioavailable. After the application of these pre-preparation processes, thermal household processing is regularly applied in which further losses and/or gains in antioxidants occur (Diplock *et al.*, 1998: S98).

This can be explained by using a lipid-soluble antioxidant, such as lycopene as an example. Structural breakdown during pre-preparation of tomato cells gives rise to a greater bioavailability of lycopene owing to disruption or softening of the plant cell walls and lycopene-protein complexes (Hussein & El-Tohamy, 1990: 229). Applying thermal household processing in the form of boiling to the pre-prepared tomatoes enhances the nutritional value by increasing the bioaccessible lycopene content and the TAC (Dewanto *et al.*, 2002: 3010). This positive effect is against the notion that processed fruit and vegetables, have lower nutritional values than fresh produce. The effect of an increased nutritional content could be due to the isomerisation of lycopene caused by the temperature. Raw tomatoes contain *trans*-isomers while heated tomatoes contain *cis*-isomers. Although

some *trans*-isomers are present in raw tomatoes, thermal household processing increases the number of *cis*-isomers, which in return makes the lycopene from the tomato more bioavailable, as *cis*-isomers are more readily absorbed (Shi & Le Maguer, 2000: 307). Furthermore, degradation lowers the antioxidant content with increased time and temperature. Nevertheless, this effect is also different from antioxidant to antioxidant. The main cause of lycopene degradation in tomato processing is isomerisation and oxidation (Shi & Le Maguer, 2000: 314). Degradation of lycopene has been shown to be directly correlated with the intensity and duration of the thermal household processing (Shi *et al.*, 2004: 204). In general, losses of lipid-soluble antioxidants during processes where water acts as the heat transfer medium are relatively low, as there is limited oxygen access (Pokorný & Schmidt, 2003: 302).

Opposite effects are seen in water-soluble antioxidants. Pre-preparation processes assist in the structural breakdown which causes the antioxidants to leach into the surrounding medium (water or other liquid). This is aggravated with the application of heat. Dewanto and co-workers (2002: 3012) determined the TAC of tomatoes [determined by the total oxyradical scavenging capacity (TOSC) assay] and found lycopene and the TAC to increase with a longer cooking time. In contrast to the lycopene, the vitamin C content decreased with an increased cooking time from 2 minutes to 15 minutes and decreased even more when the cooking time was lengthened from 15 minutes to 30 minutes. Vitamin C also degrades as it is lost with an increased time and temperature. Quercetin conjugates are remarkably resistant to degradation during normal processing operations such as boiling and frying (Price *et al.*, 1997: 941). Up to 30.0% of the quercetin in processed tomato products occur in the free form which could be the result of enzymatic hydrolysis of rutin and other quercetin conjugates while fresh tomatoes contain almost exclusively conjugated quercetin (Stewart *et al.*, 2000: 2668). Discarding the surrounding medium also increases the loss in water-soluble antioxidants (Adams & Erdman, 1988: 382).

Furthermore, rapid heating of foods deactivates enzymes, such as lipoxygenases, which would otherwise catalyse lipid oxidation and would partially destroy natural antioxidants. The deactivation of polyphenoloxidases is also very useful for the protection of phenolics against enzyme-catalysed oxidation into respective quinines, the AOA of which is very low or non-existent (Pokorný & Schmidt, 2001: 335).

In the household many different processing methods are applied. Sahlin and co-workers (2004: 641) determined the effect of boiling, baking and frying on tomatoes. The TAC (determined by the TEAC assay) decreased by 10.0%, 7.0% and 37.1% for the respective processing methods as indicated above. Greater losses were also found for the individual

antioxidants (vitamin C, lycopene and total polyphenols) measured. Considering these thermal household processing methods (boiling, baking and frying), several of the physical processes as indicated in Figure 2.10 may occur in one method and not as much in the other. Food becomes susceptible to various chemical reactions during structural breakdown, as the most important loss of antioxidants present in foods is caused by oxidation (Pokorný & Schmidt, 2001: 333) (Table 2.9).

Table 2.9: Oxidative destruction of antioxidants in foods
(Obtained from Pokorný & Schmidt, 2001: 333)

Reaction type	Examples
Oxidation with lipid oxidation products	Oxidation with lipidic free radicals
	Oxidation with lipid hydroperoxides
	Oxidation with lipid dioxolanes
Oxidation with singlet oxygen	In presence of chlorophyll pigments
Oxidation with triplet oxygen	Oxidation of antioxidant free radicals
	Formation of quinines from phenolics
Oxidation with heavy metals	Metal ions in higher oxidation state

Oxidation depends largely on: i) availability of oxygen; ii) the antioxidants present and their physical state; iii) water activity; iv) exposure to light; v) presence of metals, enzymes and peroxides; and vi) severity of the processing treatment (Rodriguez-Amaya, 2003: 934). Those antioxidants most susceptible to oxidative destruction are the lipid-soluble antioxidants (Pokorný & Schmidt, 2003: 302). This is a contributing factor to why antioxidants are added to oils to retard their deterioration.

Processing of foods has been shown to increase the AOA as is illustrated in the Maillard reaction (Nicoli *et al.*, 1997: 74), partially due to its metal chelating capacity (Pokorný & Schmidt, 2003: 304). The Maillard reaction is a chemical process between amino acids and reducing sugars which is common during baking. Many chemical processes occur simultaneously and consecutively, and as a result sometimes very different and even opposite effects on the intrinsic antioxidant properties of foods are found (Nicoli *et al.*, 1999: 95). Other positive effects of food processing include transformation of antioxidants into more active compounds, such as the deglycosylation of onion quercetin, as well as an increase in the AOA owing to the inhibition of enzymes (Gazzani *et al.*, 1998: 4122). When the negative and positive effects counterbalance each other in the three processes mentioned above, no change in the AOC occurs (Pokorný & Schmidt, 2001: 332). These possible effects of food processing on the overall AOC of foods are indicated in Table 2.10.

Table 2.10: Possible effects of food processing on the overall antioxidant potential of foods
(Obtained from Nicoli *et al.*, 1999: 98)

No changes	• No changes in naturally occurring antioxidant concentration • Loss of naturally occurring antioxidants balanced by the simultaneous formation of compounds with novel or improved antioxidant properties
Increase	• Improvement of antioxidant properties of naturally occurring antioxidants • Formation of novel compounds having AOA (i.e. Maillard reaction products)
Decrease	• Loss of naturally occurring antioxidants • Formation of novel compounds having pro-oxidant activity (i.e. Maillard reaction products)

The AOA of polyphenols can also be increased by synergists (i.e. polyvalent organic amino acids, amino acids, phospholipids [lecithin] and various chelating agents) present in foods which do not exert AOA of their own. Proteins may modify the efficiency of antioxidants as they react with the reaction products of both polyphenols and synergists (Pokorný & Schmidt, 2003: 298). These effects have been seen as not all foods are negatively affected by processing as expected, and some foods' nutritional value actually increases when thermal household processing is applied, as is seen with lycopene in tomatoes. All foods are complex mixtures of components that have the potential to react and interact with one another (Davey *et al.*, 2000: 825).

Considering the above examples of lycopene and vitamin C, the effect of thermal household processing on individual antioxidants as seen by the work of Dewanto and co-workers (2002: 3012) and Sahlin and co-workers (2004: 641), and the use of different processing methods, it can be concluded that food undergoes several processes during thermal household processing which affect the TAC. Each antioxidant is affected differently and a major loss or gain may occur in one antioxidant but the rest may be affected differently because of their structure and the mechanism they implement. The number of physical processes a food undertakes will also determine the TAC. Fortunately during processing several other processes occur which have positive effects on the TAC such as the Maillard reaction and the effect of synergists. This is illustrated by Sahlin and co-workers (2004: 635), as they found that baking has a small effect on the TAC of tomatoes which could be due to new compounds formed during the Maillard reaction. It could be assumed that the greater loss in the TAC during frying could be due to oxidation. A large increase in total fat content from the cooking oil was also found (Sahlin *et al.*, 2004: 635). It was, however, not stipulated in the methodology whether the frying method used was deep fat frying or shallow frying, although the results indicate that it might be deep fat frying due to the short time and high temperatures applied. Although the work by Sahlin and co-workers (2004: 641) indicates that some thermal household processing methods are more harmful to antioxidants than others, factors during thermal household processing should also be considered.

Despite the great importance of food processing, relatively little has been published on the changes in antioxidants, their interactions with other food components, and the effect of these changes on food resistance against oxidation. Not all foods at home are consumed on their own. Many dishes are prepared where more than one ingredient is used. No studies have, however, investigated a recipe/dish to determine the effect of thermal household processing on its TAC. Table 2.11 lists several research areas identified during the literature search relating to food processing and antioxidant determinants confirming the absence of studies investigating a food recipe/dish.

Table 2.11: Food processing and antioxidant determination research studies analysis

Research areas	References
Retention of antioxidants after industrial processing methods.	Ishida & Chapman, 2004: 8017
Retention of single antioxidants such as lycopene, β-carotene, glucosinolates etc. during thermal household processing.	Verkerk *et al.*, 1997: 193
TAC determination of commercially processed dishes.	Arnao *et al.*, 2001: 240
TAC determination of single food items after thermal household processing.	Wu *et al.*, 2004a: 4030; Wu *et al.*, 2004b: 419
Retention of antioxidants after natural antioxidant (from herbs or spices) or synthetic antioxidant application for preservation purposes.	Huang *et al.*, 1981: 997; List *et al.*, 1972: 287

In many instances researchers would also use impractical applications when investigating the effect of thermal household processing. For example, in the boiling of 100 g of vegetables (i.e. savoy, green and black cabbage, cauliflower and broccoli), 500 ml of water would be used and the results would be that great losses occur during boiling (Ninfali *et al.*, 2005: 258). The principles of the processing methods are wrong, as great losses will occur with the use of such a large proportion of water to vegetables (5:1). The researchers, however, did not indicate whether it is common practice for Italian consumers to use such water quantities. In addition, the types of vegetables used are also those that do not require such large amounts of water. Studies like that of Ninfali and co-workers (2005: 258) furthermore make it difficult to determine the effect of thermal household processing on the TAC of foods.

Although some antioxidants are naturally lost during heating (Nicoli *et al.*, 1997: 74; Nicoli *et al.*, 1999: 98), suitable cooking procedures can be adopted, or specific ingredients can be included in food to minimise the loss (Gayathri *et al.*, 2004: 37) as new compounds with increased antioxidant properties are formed (Nicoli *et al.*, 1999: 98). Gayathri and co-workers (2004: 35) found that by adding antioxidant spices to food, it improves the β-carotene retention of the food during cooking. The findings can be attributed to the

synergistic effects between the antioxidant molecules. Dark soy sauce used in Asian cooking has also been recommended to be added to food as it can contribute to a higher TAC (Long *et al.*, 2000: 185). Furthermore, the use of oil has also been shown to increase the bioavailability of lycopene (Stahl & Sies, 1992: 2161). As a consequence, when tomatoes are processed, they should be co-ingested with an oil medium to extract lycopene into the lipidic phase.

It is theoretically possible to calculate the AOC of a food product by chemical analysis and the AOAs of individual components. However, this implies that no synergistic/antagonistic or other matrix effects play a role and that all components with AOA are known and detectible (Dekker *et al.*, 1999 cited in Dekker *et al.*, 1999: 798). Levels of single antioxidants in food do not necessarily reflect their TAC (La Vecchia *et al.*, 2001: 265), as synergism should be accounted for in any discussion of the AOC as the TAC depends on the synergistic and redox interactions among the different molecules present in the food (Pellegrini *et al.*, 2003: 2812).

In a recipe several ingredients are utilised providing a multi-component food. The effect of thermal household processing on the TAC of a dish can therefore not directly be related to these published studies as the activity of natural antioxidants is greatly affected by the complex interfacial occurrence in emulsions and multi-component foods (Pokorný & Schmidt, 2001: 331).

CHAPTER 3

METHODOLOGY

3.1 Type of study and study design

An experimental study of descriptive and comparative nature was conducted to determine the effect of thermal household processing on the TAC of stewed tomato and onion flavoured with parsley. In a descriptive experiment, a characteristic of a standard material is reported from the analysis of measurements on several test results (Greenfield, 1996: 97). The characteristic in this study was the effect of thermal household processing on the TAC. Three recipes, each using a different preparation method, were investigated following a descriptive experimental study design. The traditional household cooking procedures for the preparation of stewed tomato and onion flavoured with parsley (control recipe) were duplicated for analysis in the laboratory along with two other stewed tomato and onion flavoured with parsley recipes specifically designed for this study (Recipes 1 and 2). These experimental recipes differed in preparation method from and were compared with the control. In addition, a comparative experimental study design was applied to compare the TAC of the three recipes utilising SFO and RPO. The antioxidant content of the respective samples of the preparation steps was also determined to explain the AOA observed in the TAC of the end-product of the three respective recipes. Figure 3.1 illustrates a flow diagram of the design of the study.

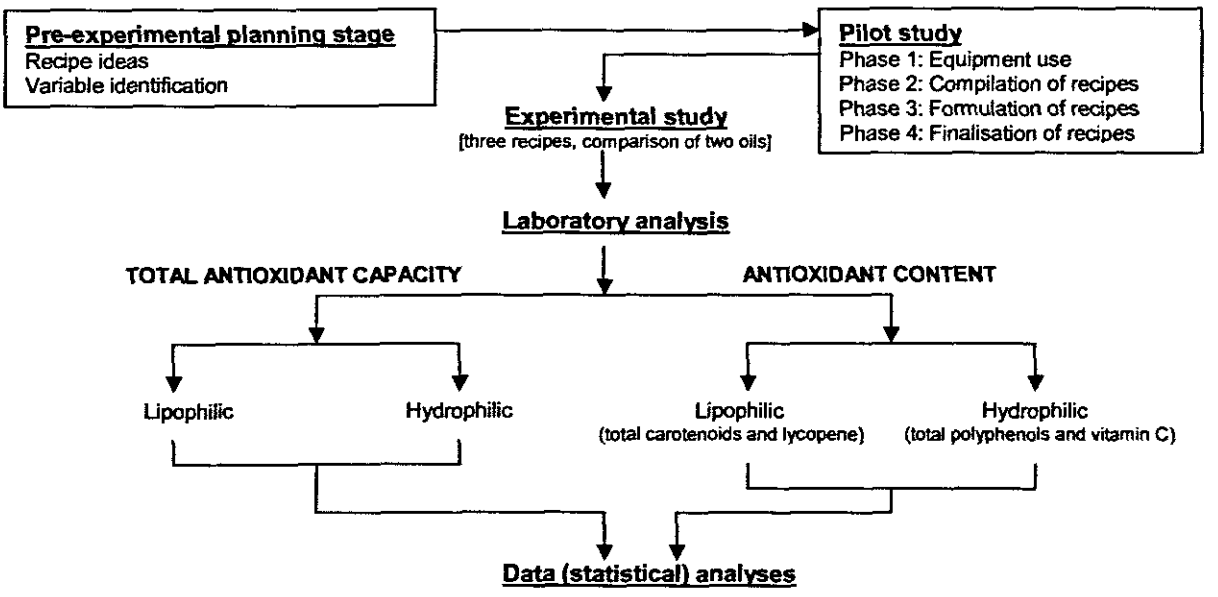


Figure 3.1: Flow diagram of study design

3.2 Pre-experimental planning stage

In the pre-experimental planning stage comprehensive planning was required. The recipe ideas were established and those variables identified that would surface during the study. The pilot study could only commence after this stage.

3.2.1 Recipe ideas

The nutritional value of foods and beverages has become an important consideration in food choice in the past few years due to the role of nutrition in disease prevention. Health consciousness has also been identified as a major trend driving the food industry in the development of functional foods (Sloan, 2005: 22). Consumers' diet choices are mostly guided by taste preferences and convenience (French, 2003: 841S). If foods are not prepared according to an individual's likes and dislikes, either small quantities of the product will be eaten, or the disliked food will not be eaten at all. Therefore, the ideal situation in the home is for foods to be served in a form that is preferred, using preparation practices that minimise nutrient losses (Adams & Erdman, 1988: 582).

In Chapter 2 it was revealed that stewed tomato and onion is a popular dish and food component utilised by South Africans (Steyn *et al.*, 2003: 642). According to Drewnowski (1997: 239), consumers first have to prefer a food before they will select to purchase and consequently consume it. Thus if the South African population is consuming stewed tomato and onion, it could be assumed that they enjoy it. Considering the latter, a traditional tomato and onion stew recipe (Addendum A) was used as a reference for the development of a control recipe for the preparation of stewed tomato and onion flavoured with parsley (Addendum B). Two scientifically approached recipes (Recipe 1: Addendum C and Recipe 2: Addendum D) were also developed, which formed the experiments compared with the control.

Curry powder, a spice and source of antioxidants, was used in the traditional tomato and onion stew recipe (Addendum A). Despite this, curry powder was not used in the recipes of this study as the addition of herbs to food is emphasised to increase the daily intake of antioxidants (Justesen & Knuthsen, 2001: 245). Furthermore, curry powder is also not indicated as an ingredient of stewed tomato and onion in the South African composition data (Kruger *et al.*, 1998: 35). Parsley, a popular herb in South Africa (Lizaar, 2005), was added to the recipes. The amount of fresh herbs added to recipes differs from recipe to recipe. For example, the amount of fresh herbs added to homemade sauces also differs depending on

the other ingredients present in the recipes (Van der Nest, 2000: 14, 58, 76; Paarman, 2001: 7, 8, 11, 45; Human, 2002: 138, 241). The amount of parsley added to each of the three recipe formulations for flavour and to determine whether it would contribute to a higher antioxidant content, was 10 ml. This amount was determined from the recipes published in Van der Nest (2000), Paarman (2001) and Human (2002).

In the development of the two scientifically approached recipes, with reference to the modified control recipe (Addendum B), the objectives were to (i) utilise the same ingredients, (ii) only change the recipe by incorporating research study findings, as discussed in 2.6, p.30, such as those factors enhancing the bioavailability of lycopene, which could result in a higher TAC and (iii) consider taste preferences of consumers, i.e., consumers enjoying the taste of the traditional preparation method of tomato and onion stew (Addendum A) in that sautéed onions are the first preparation step. Table 3.1 illustrates the three recipes, the changes made to the preparation methods and the supporting literature.

Table 3.1: Ideas and accompanying reasons for the experimental recipes

Change(s) made to recipe	Reason(s) for change	Reference
	Control recipe (Addendum B)[a]	
Addition of parsley	Increase antioxidant intake	Justesen & Knuthsen, 2001: 73
	Recipe 1 (Addendum C)[a]	
Cook tomato first with addition of oil	Heat treatment promotes isomerisation of lycopene	Stahl & Sies, 1992: 2161
	Processing breaks down structural integrity making lycopene more accessible	Stahl & Sies, 1992: 2161; Gartner *et al.*, 1997: 116; Nguyen & Schwartz, 1998: 101
	Addition of an oil medium increases lycopene bioavailability	Stahl & Sies, 1992: 2161
Addition of raw onion to cooked tomato	Raw onion has a higher antioxidant content	Crozier *et al.*, 1997: 594
Addition of parsley	Increase antioxidant intake	Justesen & Knuthsen, 2001: 73
	Recipe 2 (Addendum D)[b]	
Cook tomato first with addition of oil (pan 1)	Heat treatment promotes isomerisation of lycopene	Stahl & Sies, 1992: 2161
	Processing breaks down structural integrity making lycopene more accessible	Stahl & Sies, 1992: 2161; Gartner *et al.*, 1997: 116; Nguyen & Schwartz, 1998: 101
	Addition of a oil medium increases lycopene bioavailability	Stahl & Sies, 1992: 2161
Addition of sautéed onion to cooked tomato (pan 2)	Familiarity to consumers consuming traditional recipe (Addendum A)	Human, 2002: 263
Addition of parsley	Increase antioxidant intake	Justesen & Knuthsen, 2001: 73

[a]-Utilising one pan.
[b]-Utilising two pans.

In Recipe 2 (Addendum D), as indicated in Table 3.1, it was necessary to utilise two pans and not only one as in the other two recipes. The tomato and onion were cooked separately before assembling it in a later step. More oil was also used in this recipe, as the addition of oil was necessary for the cooking of the tomato and for the sautéing of the onion. The same amount was added to each pan as that used in the control recipe and Recipe 1.

3.2.2 Identification of variables

Published recipes, such as for tomato and onion stew (Addendum A), lack information on such aspects as the equipment to be used, the time to cook the food and the heat that should be applied. It was therefore not practicable to use such a tomato and onion stew recipe in its published format in an experimental study. This would have caused various aspects of the recipes to vary. Variables are any quantity or symbol that has no fixed value (McWilliams, 2001: 21). During this pre-experimental planning stage of the study it was essential to establish the necessary controls needed to eliminate errors resulting from variation in the sample preparation. Two types of variables important in the planning of an experiment are independent and dependent variables (Mitchell & Jolley, 2004: 554). An independent variable is presumed to cause or determine a dependent variable (Babbie, 2004: G5). The effect of thermal household processing was the variable to be manipulated (Mitchell & Jolley, 2004: 554) in this study and was called the independent variable. The TAC and antioxidant content were the dependent variables due to the effect and influence of the independent variable (thermal household processing).

Those variables that are not intended to be part of the experiment and which need to be eliminated from or controlled prior to the execution of the study are called the extraneous variables (McWilliams, 2001: 21). For example, the use of different tomato varieties, as this was not the focus of the experiment. According to Babbie (2004: G10) these variables are called the "test variables" as they are held constant in an attempt to further clarify the relationship of two other variables. The recognition of these undesirable variables, which will be referred to as extraneous variables, was important so that they could be eliminated and/or their influence prevented. Actual data collection could only begin after repeatable results were obtained and the problems caused by the extraneous variables solved. Figure 3.2 illustrates the extraneous variables identified that would affect the TAC and antioxidant content other than the thermal household processing. The elimination of these extraneous variables was done during the pilot study.

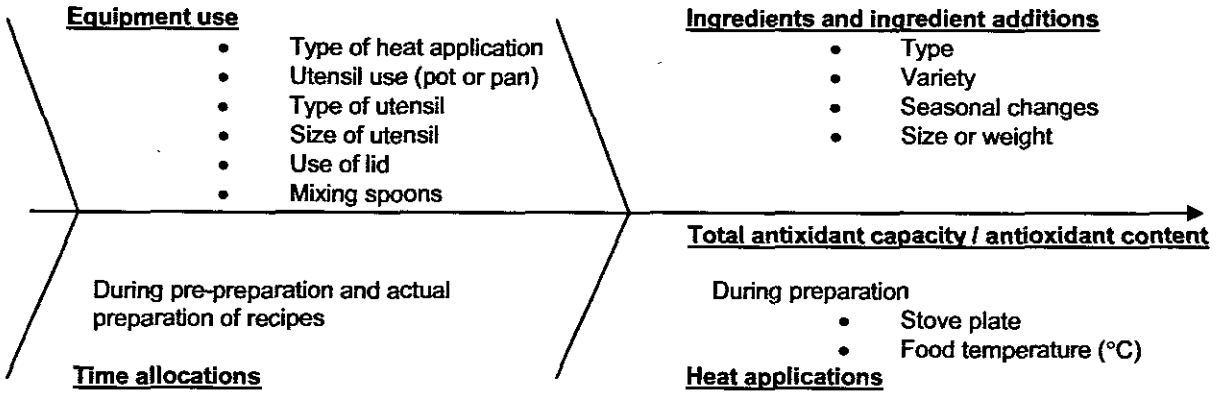


Figure 3.2: Extraneous variables affecting the total antioxidant capacity and antioxidant content in thermal household food processing

3.3 Pilot study

Careful consideration of the entire preparation process of the recipes and obtaining representative samples for analysis was necessary. During the pilot study the objective was to eliminate or fix all the extraneous variables as they could impact the above. This was done in four phases: (i) determination of equipment use; (ii) obtaining additional information for the recipe compilation; (iii) compilation of the recipes; and (iv) finalisation of the recipes. Prior to the compilation of the recipes it was necessary to determine which equipment consumers most often use for the preparation of stewed tomato and onion. This was determined first so that the information obtained could be applied and used for the recipe compilation.

3.3.1 Phase 1: Determination of equipment use

The equipment used in the pre-preparation of the recipes was a i) glass measuring jug (500 ml), ii) electric kettle (Pineware), iii) stainless steel chef's knife, iv) stainless steel peeling knife, v) glass bowls (1 x 20 ml, 1 x 500 ml, 1 x 1 l; 1 x 2 l), vi) plastic chopping board, and vii) calibrated scale (Mettler Toledo, Spider SW). The pre-preparation equipment was not determined prior to the study, as it would not influence the outcome of the recipes. The pre-preparation equipment used was, however, marked and used for the entire study. For example, the bowl used for the tomato was used consistently for the tomato. The type of utensil used for the preparation of the recipes was, on the contrary, determined as it is more frequently a factor of cost, availability, décor rather than nutrient retention (Adams & Erdman, 1988: 587). The particular utensil used may, however, affect nutrient value. The objective of

this phase was to determine the participant's behaviour during the preparation of stewed tomato and onion flavoured with parsley.

3.3.1.1 Type of study

A descriptive survey was utilised to determine the equipment use as it is appropriate for research questions about self-reported beliefs or behaviour. Surveys produce quantitative information about the social world and describe features of people or the social world (Babbie, 2004: 243; Neuman, 2006: 273). They are furthermore also used to explain or explore respondents' beliefs, opinions, characteristics, and post or present behaviour (Neuman, 2006: 273). An interview schedule was chosen as the instrument used in this survey being a set of questions read to the respondent by an interviewer, who also recorded the responses (Babbie, 2004: 263; Neuman, 2006: 305). A structured, face-to-face interview was used. Face-to-face interviews allow the interviewer to probe questions in response to unclear or incomplete answers to obtain the relevant response, and the use of visual illustrations/aids is possible. Telephone interviews and self-administered questionnaires do not include these advantages of the face-to-face interview (Neuman, 2006: 300, 306) and were, as a result, not chosen as the data collection method. Interview surveys also attain higher response rates, as participants are more inclined to throw away mailed questionnaires than turn down an interviewer standing in front of their doorstep (Babbie, 2004: 263; Neuman, 2006: 301).

3.3.1.2 Sample

The required criteria for the sample were i) females, ii) who consumed stewed tomato and onion and iii) prepared it from time to time. The sample furthermore had to include both white- and blue-collar workers to represent all working classes. Only females were included in the sample as it was assumed that most females prepare the food at home (Food Institute, 2003 as cited in Patterson & Cardona-Martinez, 2004: 8). A convenient cluster sampling method stratified to represent different professions of females was used to recruit the participants. Only volunteers in the respective buildings of the Cape Peninsula University of Technology (CPUT): Cape Town campus (n=6) who were available on the day of the survey were recruited. A total of 60 female employees participated in the survey representing a response rate of 96.8%. This is an extremely high response rate (Baruch, 1999: 429) as only two of the female participants approached were unwilling to participate. The professions of the females who participated were classified into three groups namely: i) professionals (lecturers and technicians) (33.3%), ii) administrative support (36.7%) and iii) security and cleaners (30.0%). According to Babbie (2004: 264), the interviewer can note the participants'

ethnicity (without asking), if it is considered a delicate question. The interviewer observed the ethnicity of the participating females because of its delicate nature in South Africa. Using observations has its limitations as the wrong observation can be made regarding the ethics of the participant. When the enthnicity of the participant was questioned by the researcher, it would have been asked in a polite and sensible manner by explaining the need of the question. This was, however, not necessary. Most of the participants were black (45.0%). Less than a third of the participants were coloured (21.7%), while the remaining participants were white and Indian (18.3% and 3.3%, respectively).

3.3.1.3 Interview questionnaire construction

A set of seven questions in the form of a questionnaire (Addendum E) was compiled to use in the interviews. The questionnaire included six questions (Addendum E, Questions 1-6) on the equipment use behaviour of the participants when preparing stewed tomato and onion and one threatening/sensitive demographic question (Addendum E, Question 7). The questions were compiled in a closed-ended format. By using the closed-ended format of questions, ambiguity, confusion and vagueness of the responses were avoided (Neuman, 2006: 279). Visual illustrations were used for Questions 3 and 4 (Addendum E) to eliminate guessing by the participants and possible errors. The researcher pre-recorded actual types and sizes (depth and diameter) of several pots and pans. The utensil sizes were based on the recipe ingredients, namely six tomatoes and two onions. Five actual types of utensils and several sizes (3 x depth and 6 x diameter) of pots and pans (sizes were cut out from paper into width and circular shapes) were shown to the participants to determine the type (Addendum E, Question 3) and the size of the utensil used (Addendum E, Question 4). These were all pre-coded and depending on the answer, the pre-coded number was recorded during the interview by the researcher.

Question 7 (Addendum E), in the form of an open-ended question, determined the residential area where the respondent lived. In an open-ended question the participant was asked to provide any answer (Babbie, 2004: 245; Neuman, 2006: 286). As the sample included both white- and blue-collar workers, this question was regarded as sensitive, as the participants could be ashamed, embarrassed, or afraid to give a truthful answer (Neuman, 2006: 283). This question was furthermore included to determine whether the participants represented most or all of the 20 sub-councils in the City of Cape Town. The City of Cape Town is a single municipal structure that has replaced the Cape Metropolitan Council and the six Metropolitan Local Councils (City of Cape Town, 2005). This question was also placed last in case any of the participants felt uneasy about it or had doubts about why it was important.

It could then be explained to the participants why this question was important to the study. The participant sample represented 18 of the 20 sub-councils (Table 3.2). No other demographic characteristic questions were asked in the interview.

Table 3.2: Pilot study participant sample residential area

Sub-council	Sub-council name	%	n
1	Blaauwberg	6.7	4
2	Name still to be allocated	1.7	1
3	Koeberg	5.0	3
4	Tygerberg	6.7	4
5	Central	1.7	1
6	Oostenberg	6.7	4
7	Name still to be allocated	3.3	2
8	Helderberg	3.3	2
9	Nxele Makana	0.0	0
10	Charlotte Maxeke	8.3	5
11	Looksmart Ngudle	10.0	6
12	Mitchell's Plain	6.7	4
13	David Mtheto Ntlanganiso	5.0	3
14	Miranda Ngculu	0.0	0
15	Name still to be allocated	13.3	8
16	Good Hope	6.7	4
17	Athlone & District	1.7	1
18	Rondevlei	5.0	3
19	South Peninsula	1.7	1
20	Protea	6.7	4

The training of interviewers was not necessary for this study as the researcher acted as the principal interviewer. Interview bias falls into six categories (Neuman, 2006: 309). Each of these errors during the interviews was avoided by the interviewer as she i) was not sloppy; ii) did not alter answers; iii) did not have preconceived ideas about the participants' answers; iv) probed questions properly; v) was neat and professional; and vi) interviewed one participant at a time.

3.3.1.4 Conducting the interview

On the day of the interview, the researcher introduced herself, stating briefly that she was conducting a survey to determine the equipment used by persons consuming and preparing stewed tomato and onion in their household. Participants were asked whether they would voluntarily participate in the survey if they had the requirements set by the criteria for the survey sample. The survey purpose (which equipment consumers used in their households for the preparation of stewed tomato and onion) and time to complete the interview (five minutes) were also explained to them. A letter was prepared which clearly explained the

purpose, reasons and background of the study (Addendum F), but none of the participants in the survey, however, requested such information. On completion of the five minute interview, each participant was rewarded with a gratituity consisting of wrapped candy.

3.3.1.5 Data analysis

The data was entered onto Microsoft® Excel 2000 (9.0.2720) spreadsheets and imported into SAS®STAT Software (9.1.2) for analysis. Basic descriptive statistics, such as frequencies, as well as the chi-square test to establish whether there was an association within variables or between data categories, were used. Data were considered significantly different if *p*-values were less than 0.05.

Electricity was the type of heat application used by the majority of the participants (91.7%). The other heat applications used (8.3%), were gas (5.0%) or paraffin (3.3%). Data from the 1996 census indicated that 80.0% of the population in the Cape Metropolitan area use electricity for cooking purposes (Cape Metropolitan Council, 2001). This corresponds with that found in this study. The participating sample's ethnicity ($P > 0.05$) or professions ($P > 0.05$) did not affect the type of heat application used.

The utensils and types used are indicated in Table 3.3. More participants (56.7%) used a pan rather than a pot (43.3%) during preparation. A significantly higher percentage of the participating sample (35.0%) used a stainless steel pan ($P < 0.05$). An equal number of participants used an aluminium (20.0%) or stainless steel (18.3%) pot.

Table 3.3: Type of utensils used to prepare stewed tomato and onion in the household

Utensils	Aluminium	Stainless steel	Other	Total
	% (n)	% (n)	% (n)	% (n)
Pot	20.0 (12)	18.3 (11)	5.0 (3)	43.3 (26)
Pan	8.3 (5)	35.0 (21)	13.3 (8)	56.7 (34)[a]
Total	28.3 (17)	53.3 (32)	18.3 (11)	100.0 (60)

[a]-Significant at $P < 0.05$

Significantly ($P < 0.05$) more participants (31.7%) used a pan with a 26 cm diameter. Those participants who used a pot, mostly (25.0%), used a 23 cm diameter pot. Most of the participant sample (35.3%) used a pan with a 12 cm depth, while those using a pot mostly (15.0%) used a 7 cm depth pot. The depth of the pan, as indicated by the participants, was much deeper and resembled the size of a pot. The reverse was encountered for the depth of the pots; here the depth resembled that of a pan. As significantly more participants used a pan with a 26 cm diameter, the depth of the pan was lowered to 7 cm to resemble an actual stainless steel pan.

An equal number of the participating sample (61.7%) respectively used a lid during preparation and stirred with a wooden spoon. A lid was used with a pot and pan by 31.7% and 30.0% of the participants respectively. Less than a third used a metal spoon (26.7%), while only 11.7% used a plastic spoon.

The equipment use identified in the pilot study and for use in the experimental study was i) electric stove (Defy FOUR TWENTY TWO); ii) stainless steel pan, with a depth of 7 cm and diameter of 26 cm; iii) use of a lid; and iv) use of a wooden spoon.

3.3.2 Phase 2: Obtaining additional information for the recipe compilation

Prior to the compilation of the recipes, it was necessary to obtain additional information regarding the recipe, such as the weight and type of recipe ingredients, other ingredient additions and the pre-preparation techniques of the ingredients used. This information had to be included in the recipes. No specific time allocations or heat applications for the preparation of the tomato and onion stew were indicated in the published recipe (Addendum A). It was assumed that consumers would prepare these ingredients until they seemed cooked. The time and temperature of samples, however, also had to be considered (McWilliams, 2001: 24). Several actions, as described below, were taken to obtain the necessary information to standardise the ingredient use and the preparation of the recipes.

3.3.2.1 Raw ingredient mass determinants

In the tomato and onion stew recipe published by Human (2002: 263) (Addendum A), no indications of the ingredient weights were supplied. Using a fixed number of six tomatoes in the recipes caused inconsistent weights from one recipe to another because of the size differences of tomatoes. Three batches of randomly selected tomatoes (six in each batch), and of onions (two in each batch) were taken to obtain an average weight. The average weight of the whole raw tomatoes and onions (both unpeeled) was 900 ± 1 g and 280 ± 1 g, respectively. The average weight of the peeled and chopped tomato and onion added to each recipe was 740 ± 1 g and 220 ± 1 g, respectively. These weights were then used in all the recipes as the standard. A standard amount of approximately 10 ml of fresh herbs is usually added to recipes (Van der Nest, 2000; Paarman, 2001; Human, 2002). In this study 3 g (± 10 ml) chopped parsley were added to the recipes as a standard. The oils were also weighed and 24 g of SFO and 27 g of RPO represented the 30 ml indicated in the traditional recipe (Addendum A).

3.3.2.2 Fixation of pre-preparation procedures of raw ingredients

The pre-preparation procedure of the raw recipe ingredients was also standardised. All the vegetables were pre-prepared according to the guidelines provided by Willan (1989: 13, 280, 291), an acknowledged chef and food writer whose food preparation techniques are those used and taught to students by many food lecturers. Parsley can be chopped by using a food processor, chef's knife or a two-handled mincing knife (mezzaluna) (Willan, 1989: 13). In this study a chef's knife was used as not all consumers would have food processors or a mezzaluna, but would have some sort of knife. Table 3.4 indicates the guidelines as described by Willan (1989: 13, 280, 291) in the peeling and preparation of the produce. These guidelines were changed slightly to save time during the pre-preparation stage.

Table 3.4: Pre-preparation of raw recipe ingredients based on the guidelines of Willan

Guidelines of Willan[a]	Pre-preparation applied in this study
Tomato	
Peeling: Bring a large pan of water to the boil. With a paring knife, cut the core out of the tomato. Turn the tomato over and lightly cross-hatch the bottom. Immerse the tomato in boiling water for 8-15 seconds, depending on ripeness, until the skin curls away from the cross-hatch. This shows that the skin will peel easily. Lift the tomato out, let it cool slightly, and peel it. Chopping: With a large knife, cut the tomato halves in slices, and then roughly chop them.	Peeling: One and a half litres of water was boiled in a kettle. The boiled water was poured over the cross-hatched tomato and left for one minute (timed). The tomato was removed from the water, cored and peeled. Chopping: With a chopping knife the tomato halves were sliced twice and then chopped three times providing a coarse texture.
Onion	
Peeling: Peel the onion, leaving the root on 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, but not through it. 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. Guide the blade of the knife with bent fingers. Continuous chopping will provide for finely chopped onion.	Peeling: Same as guidelines. Chopping: Same as guidelines; however, three horizontal cuts were made from the stalk towards the root (not through). Four lengthwise vertical cuts were made. Lastly, the onion was cut four times crosswise into a coarse dice (smaller than the tomato).
Parsley	
Chopping: Strip the leaves from the stalks and pile them on a cutting board. Cut the parsley into small pieces, holding the tip of the blade against the board and rocking the handle up and down. With continuous chopping the texture of the parsley will go from coarse to fine. Note: the knife should be sharp otherwise the parsley will be bruised rather than being cut.	Chopping: Same as guidelines into a coarse to fine texture.

[a]-Obtained from Willan, 1989: 13, 280, 291

Furthermore, it was necessary to prepare the ingredients in a specific order each time. The order used for this study is indicated in the flow diagram in Figure 3.3. The order of the pre-preparation was planned to perform two actions at once when possible, such as when boiling the water to also i) rinse the tomato and parsley, ii) cross the tomato and iii) remove the stalks of the parsley. This saved time and limited the exposure of the raw ingredients to air as exposure to oxygen decreases the antioxidant content of food (Papas, 1999: 96). In general, it is better to delay the preparation of foods to a few minutes before they are to be cooked or served (Adams & Erdman, 1988: 574) to limit loss of volatile components present in food. Therefore, it was planned to pre-prepare the ingredients, prepare the recipe and clean up before commencing the next recipe. This process was repeated for each recipe during the entire study. During the cleaning and pre-preparation of each step the stove plate was cooled. This was done by placing a pan on the stove plate filled with ice-cold water in order to remove the heat from the plate until it was cold before starting the next recipe.

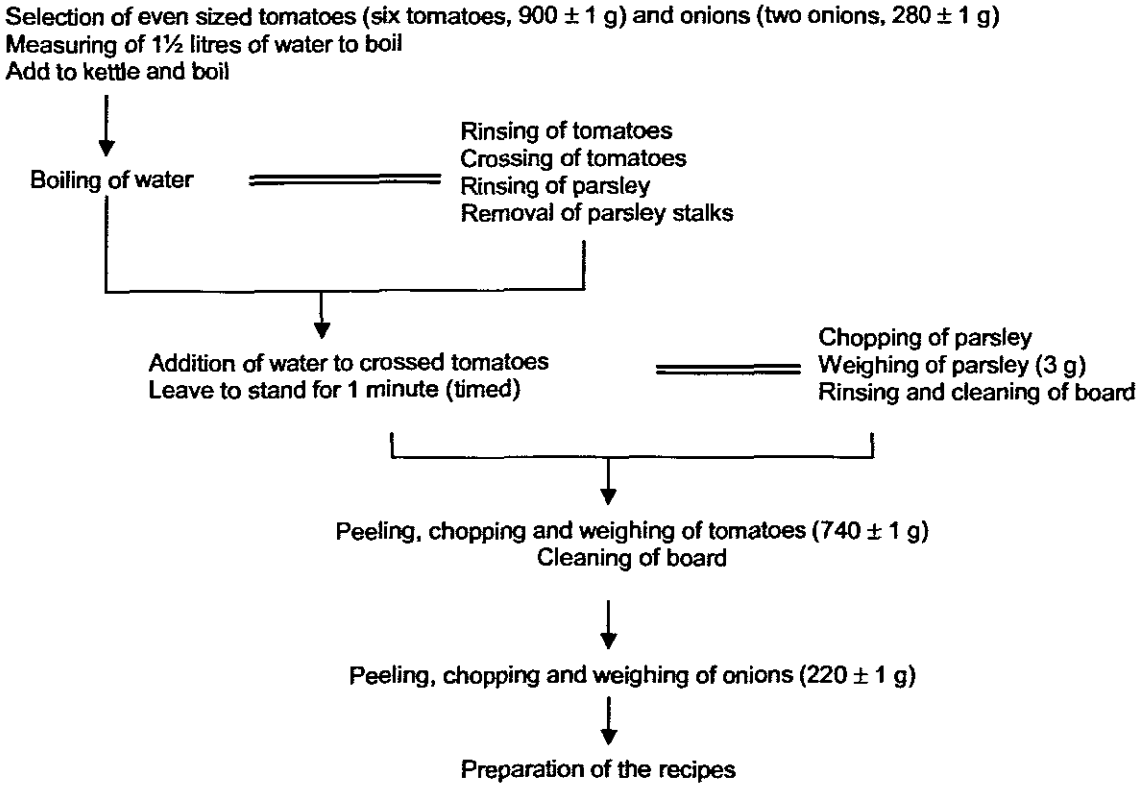


Figure 3.3: Flow diagram of ingredient pre-preparation procedure

The oils were obtained (in ml) directly from the oil container when a recipe step required its use. It was assumed that most consumers would measure it directly from the oil container to limit dirty dishes.

3.3.2.3 Recipe ingredients and ingredient additions

The potential variations resulting from the ingredients themselves had to be eliminated as much as possible (McWilliams, 2001; 20). The ingredients sought after were those that were the most used in South Africa. The raw ingredients, such as the tomato, onion and parsley, were obtained directly from farms supplying to leading supermarkets. Thus, the ingredients bought for this study were also those that were sold to consumers. Variables such as packaging, handling, storage and temperatures introduced from the supplier to the supermarket were excluded as the researcher collected the produce directly from the supplier/farmer. More important for the purpose of this study, the suppliers were able to supply the same variety of produce, harvest date and place of harvesting at each collection for the compilation of the recipes and the experimental study. A long shelf life tomato variety grown in tunnels, namely, Sevora, was used for this study and obtained from Rennie Farms (Paarl). A long shelf life tomato was used because most consumers would not buy jam/cooking tomatoes each month for the preparation of stewed tomato and onion. It would be more likely that they would buy produce according to availability and versatility. The onion variety used was Ergold, a brown onion and was obtained from Wildeklawer (Barkley West). The brown onion is the most popularly grown onion in South Africa (De Kock, 2005). Curly leaf parsley was used for this study as it is more popular than Italian parsley (Lizaar, 2005). Furthermore, the final experimental study was scheduled for execution on the same day to prepare all samples to eliminate other variables such as the daily decrease/increase in antioxidant values in produce due to ripening. BRANDNAME SFO, purchased from a leading supermarket, and Carotino Classic RPO, supplied by the Malaysian Palm Oil Promotion Council (MPOPC), was used. The bottle volumes were large enough to supply oil for the entire experiment.

The addition of salt, pepper and sugar to taste was not utilised in the three recipes. The exclusion of these ingredients can be supported by the fact that they were added in negligible amounts to the recipe (Addendum A) as described by Human (2002: 263). In the stewed tomato and onion recipes prepared in this study, parsley was added as a source of antioxidants, but may also have compensated for the flavour loss contributed by the traditional addition of salt, pepper and sugar.

Salt, pepper and sugar are mainly added to food to improve taste and for preservation purposes (Dodds, 1953: 1211). However, individual foods are chosen not because of a single sensory attribute, such as sweetness or saltiness, but for a number of attributes. Beliefs of nutritional benefits can also play a role in food choice. Many people may therefore

not add these ingredients to their food because of health reasons, while others may add one ingredient and not the other. Possible reasons for this could be:

i) High salt intake is the major dietary cause of hypertension and only a moderate salt intake through the diet is recommended. People suffering from high blood pressure are advised to limit the use of salt (Alderman, 2002: 315).

ii) Coarsely ground black pepper has the potential to act as an irritant in people with ulceration in the digestive tract. Pepper has also been found to take several years to digest as it is composed of a large proportion of unabsorbable fibre and other particles that are liable to be trapped in crevices of the digestive tract (Bryce, 1990: 279).

iii) Caramelisation occurs when onion is slowly sautéed over moderate heat in oil or fat, which gives the onion a characteristic sweet flavour. Natural sugars present in the onion undergo a series of chemical changes that begin with dehydration and end with polymerisation (Kroh, 1994: 374). Thus, the addition of sugar is not necessary when the correct cooking procedure for sautéing onion is used as it leads to caramelisation. Furthermore, sugar is a source of sucrose. The intake of sucrose and sucrose-containing foods by people with diabetes who do not adequately cover their intake with insulin or other glucose-lowering medication should be avoided. In such circumstances sugar elimination from the diet is better (American Diabetes Association, 2002: 110).

3.3.2.4 Determination of fixed time allocations and heat applications

Three higher education food and food science practical lecturers (Faculty of Applied Sciences, CPUT: Cape Town campus), with a food science background, and three higher education food practical lecturers (Hotel School, CPUT: Granger Bay campus), with a food service background, were approached to participate in the compilation of the recipes. These lecturers were invited to participate in this pilot study phase as it was assumed that they would have the necessary food knowledge and skills to prepare the recipes to the required stage of doneness so that the necessary information regarding the time allocations and heat applications could be obtained for the recipe formulations. Most importantly, the participants were asked to prepare the recipes as they would in the comfort of their own homes. Consumers from the public were not asked to participate in this phase as Recipes 1 and 2 would not be familiar to them and they would not be as comfortable preparing them as persons with training in practical food skills would be. All six lecturers participated voluntarily. Each participant was provided with the three recipes (Addenda B, C, and D) to familiarise themselves prior to the preparation thereof. An appointment was scheduled

individually with all six lecturers so that they all could use the same stove and utensils (as determined by the equipment use interview), which were also the stove and utensils that were used for the experimental study.

The lecturers were each supplied with the most often used utensils (as determined by the equipment use interview) and also the ingredients (as indicated in 3.3.2.3) necessary for the preparation of the stewed tomato and onion flavoured with parsley recipes. The ingredients were also pre-prepared in advance for each participant so that the weights of the ingredients and their size after being chopped were consistent throughout the pilot study.

During the preparation of the recipes by the participants, the researcher determined the fixed time allocations, by using a stopwatch and the heat applications. The researcher was pre-trained with the stopwatch to obtain accurate time allocations for each step. The participants were not asked to do these themselves in order to eliminate errors. The heat application was indicated as temperature mark 1 - 6 as on the temperature gauge of the stove. No comments were made to any of the participants in respect of whether they were correctly or incorrectly applying the methods during the preparation of the recipe to eliminate bias. Other observations made included the number of times the ingredient mixtures were stirred, whether a lid was used or not, along with the time allocations for the addition of the parsley (i.e. when the parsley was added).

Heat is conducted from the walls of a pan or from the cooking medium to the centre portions of the food (Freeland-Graves & Peckham, 1996: 27). It was thus important for the cooking utensil to be warmed sufficiently, as it would influence the time allocations of each step in the recipe (as was found in this study). Heating the pan and oil is an important step in the recipes. The procedure of how to heat the pan and oil (i.e. when to turn on the stove and when to place the pan on the stove) is not that important; however, the importance lies therein that it is well heated. These actions of the participants were observed for the three recipes as they differed.

All these observations (each participant prepared the methods once) were taken into account to formulate the final detailed recipes. Analysis of the results was done using basic descriptive statistics such as averages and/or median values in two steps. Firstly detailed descriptions of each participant's actions were determined and these are provided in Addenda G, H and I. Each participant's results were then summarised and used to compile the detailed recipes. These are discussed below.

It was assumed that the six participants would have made the stewed tomato and onion flavoured with parsley in similar fashion; however, only some similarities were found especially regarding the time allocations and the heat applications. Participant 3 prepared the stewed tomato and onion flavoured with parsley just until it was heated through but not until done for all three recipes. This furthermore indicates that not all persons apply the same time allocations and heat applications. The results of Participant 3 were excluded from the analysis as it was assumed that the heat application in the household would be applied until it provided a cooked product.

i Control recipe

Four of the participants turned the stove to temperature mark 6, placed the pan on the stove and added the oil (Addendum G). A high heat application such as temperature mark 6 is necessary for sautéing. Three of the participants lowered the temperature while sautéing the onion (Addendum G). A summary of the heat applications applied by the five participants is indicated in Table 3.5.

Table 3.5: Heat applications (mark) applied in the control recipe by the pilot study participants (n = 5)

	Start	Sauteing	Stewing	Total cooking time (minutes)
Participant 1	5 → 6	→ 5	→ 3 → 4 → 5 →	36.24
Participant 2	6	→	→	13.33
Participant 4	6	→ 3	6 →	25.33
Participant 5	6	→ 4	→	18.55
Participant 6	6	→	→	16.07

The time allocations were directly influenced by the heat applications. This is illustrated by comparing Participants 2 and 6 against Participants 1 and 4 (Table 3.5). Between Participants 2 and 6 there was a difference in the time they sautéed the onion (4.01 and 6.05 minutes, respectively) (Addendum G). This could be explained as follows: Participant 6 did not heat the pan long enough before adding the onion, while Participant 2 heated the pan and oil for longer but sautéed for a shorter time. This illustrates the importance of reaching the correct heat prior to starting the cooking process. Table 3.6 indicates a summary of the time allocations for the four major steps in the control recipe.

Table 3.6: Time allocations (minutes) of the steps in the control recipe by the pilot study participants (n = 5)

	Minimum	Maximum	Median
Heating (stove, pan, oil)	0.09	4.21	2.37
Sautéing the onion	2.41	11.30	4.43
Stewing the tomato and onion	6.42	24.45	11.01
Addition of parsley	0.08	0.24	0.18
Total cooking time	13.33	36.24	18.55

Four of the participants added the parsley before removing the stewed tomato and onion from the stove. The participants stirred the stewed tomato and onion flavoured with parsley on average only once for 18 seconds (Addendum G) in circular movements.

The use of a lid was not mentioned in the traditional published recipe instructions (Addendum A). From the equipment use interview, 52.9% of the participants (n = 19) using a pan reported that they also used a lid when preparing stewed tomato and onion. In the recipe preparation, three of the participants stewed the tomato and onion with the pan being covered with a lid, of whom one had the lid slightly open (Addendum G).

Stirring was necessary to prevent burning. Difficulty existed in measuring the times to stir and the average times owing to the diverse time allocations and heat applications. It was expected that the higher the heat application the more manipulation would be necessary. This was, however, only found for those participants who used a heat application of mark 6 and not with all the participants. The manipulation that was applied during the sautéing of the onion was in the range of six times (ten seconds at a time) to stirring continuously in circular movements. The manipulation that was applied during the stewing of the tomato and onion was in a range of eight to fifteen times (median = 10 times) for 16 to 23 seconds (median = 23 seconds) at a time (Addendum G).

ii Recipe 1

The heat applications of Recipe 1 (Addendum H) are summarised in Table 3.7. Four of the participants turned the stove to temperature mark 6, placed the pan on the stove and added the oil. Three of the participants adjusted the heat during the preparation of the recipe.

Table 3.7: Heat applications (mark) applied in Recipe 1 by the pilot study participants (n = 5)

	Start	Sauteing	Stewing	Total cooking time (minutes)
Participant 1	4			28.55
Participant 2	6		5	13.16
Participant 4	6		2 3 5	24.16
Participant 5	6	4	2 3	24.48
Participant 6	6			16.57

Table 3.8 indicates a summary of the time allocations for the four major steps in the recipe. During the preparation of Recipe 1, the participants realised that raw onion was added to the tomato. Owing to this, they applied a shorter cooking time for the tomato to allow for sufficient cooking of the raw onion.

Table 3.8: Time allocations (minutes) of the steps in Recipe 1 by the pilot study participants (n = 5)

	Minimum	Maximum	Median
Heating (stove, pan, oil)	0.38	2.29	1.09
Cooking of tomato	1.25	9.39	2.34
Stewing of tomato and onion	9.02	18.56	14.56
Addition of parsley	0.13	0.23	0.18
Total cooking time	13.16	28.55	21.38

Four of the participants added the parsley to the stewed tomato and onion before removing it from the stove. On average, the parsley was added and mixed once for 18 seconds in circular movements. Four of the participants cooked the tomato without the pan being covered with a lid while the remaining participant cooked the tomato with the pan being covered with a lid at a lower heat application (temperature mark 4). Four of the participants also stewed the tomato and onion with the lid (Addendum H).

The stirring applied by all the participants was similar for the cooking process of the tomato. The participants stirred the tomato between four and five times (median = 4) for 11 - 19 seconds at a time (median = 15 seconds) in circular movements. More diverse results were found when the stewed tomato and onion were stirred. The participants stirred the mixture five to thirteen times (median = 9 times) for 15 - 38 seconds (median = 19 seconds) at a time in circular movements.

iii Recipe 2

Recipe 2 requires two pans. In the first pan the tomato was cooked to which sautéed onion was added which was prepared in a second pan. During the preparation of Recipe 2 the

participants used both pans simultaneously, thus sautéing the onion while cooking the tomato.

Table 3.9 summarises the heat applications used in Recipe 2 for Pan 1 and 2 (Addendum I). Only one of the participants kept Pan 1 at a constant heat application (temperature mark 6). This participant also sautéed the onion in the control recipe at a constant temperature. The other four participants adjusted the heat applications.

Table 3.9: Heat applications (mark) applied in Recipe 2 by the pilot study participants (n = 5)

	Pan 1			Pan 2		
	Start	Cooking	Stewing	Start	Sauteing	Total cooking time[a] (minutes)
Participant 1	4		3	5	5	20.20
Participant 2	6	3	6	6		16.45
Participant 4	6	3		6	3	14.57
Participant 5	6	4		6	4	14.53
Participant 6	6			6		13.31

a-Time of both pans simultaneously cooked.

The time allocations applied by the participants for both the pans (Addendum I) are indicated in Table 3.10. The cooking time of the tomato was longer in Recipe 2 than that applied in Recipe 1 (Table 3.8). This is attributed to the fact that sautéed onion was added in Recipe 2 and not raw onion.

Table 3.10: Time allocations (minutes) of the steps in Recipe 2 by the pilot study participants (n = 5)

	Minimum	Maximum	Median
Pan 1			
Heating (stove, pan, oil)	0:00	4:05	0:51
Cooking of the tomato	6:17	10:23	7:44
Stewing of the tomato and onion	1:46	11:51	4:00
Addition of parsley	0.05	0.39	0.22
Pan 2			
Heating (stove, pan, oil)	0.09	4.21	2.37
Sautéing of the onion	2.41	11.30	4.43
Total cooking time	13.31	20.20	16.05

Three of the participants did not use a lid when the tomatoes were cooked. Four of the participants, however, used a lid when stewing the tomato and onion. The participants stirred the tomato in circular movements in a range of three to seven times (median = 4 times) for 9 - 19 seconds (median = 14 seconds) at a time. The stewed tomato and onion mixture was stirred in circular movements within a range of two to eight times (median = 5 times) for 14 - 25 seconds (median = 17 seconds) at a time (Addendum I).

Three of the participants added the parsley to the stewed tomato and onion before removing it from the stove. The parsley was added and stirred in circular movements within a range of 1 to 2 times (median = 1) and within a range of 5 to 39 seconds (median = 22 seconds) at a time (Addendum I).

During the sautéing of the onion in the control recipe and in Recipe 2, three of the participants commented that the amount of oil used in the recipes was too much. This was consequently reduced from 30 ml to 20 ml. Furthermore they also commented that the parsley could be increased from 10 ml to 15 ml, as it was too little compared with the other ingredients (tomato and onion).

iv Overall fixed time allocation and heat application considerations

The following conclusions were drawn which had to be considered for the formulation of the recipes:

a. Heat the stove properly and keep the heat as constant as possible to simplify the cooking process as household thermal processing recipes are being formulated.
b. Use a high temperature for the sautéing of the onion.
c. Alter heat application as most of the participants did, but keep to minimal adjustments, as household processing recipes are being investigated.
d. Cook the tomato for a shorter time in Recipe 1.
e. Cook the tomato for a longer time in Recipe 2.
f. Use the median time application for each step in each recipe.
g. Add the parsley just before removal from the stove/heat and stir once.
h. Use the lid when stewing the tomato and onion, but not when cooking the tomato in Recipes 1 and 2.
i. Reduce the oil content and increase the parsley content slightly.
j. Stir in circular movements.

3.3.3 Phase 3: Recipe compilation

The aim of the recipe compilation was to eliminate inconsistencies in the results of the experimental study. The information determined during phase 2 (Addenda G, H and I) was incorporated into the recipes and re-written into a recipe format. The recipes were then written for the experimental study into a format that stipulated each action for each step in the three recipes. A voice recorder was used to record the preparation steps and action for

each of the three methods to eliminate errors during the preparation of the recipes. The recipe formulations are provided in Addenda J, K, and L along with the percentage weight contributions of each ingredient and the recorded preparation steps for each of the recipe formulations as accompanying tables in Addenda J, K and L (Tables J-1, K-1 and L-1 and J-2, K-2 and L-2 respectively).

3.3.4 Phase 4: Recipe finalisation

After the recipes were compiled, the sensory evaluations followed. This was done to firstly determine whether the time allocations and heat applications applied were sufficient to provide for a cooked product and secondly to finalise the recipes. Each recipe was prepared strictly according to the formulated steps instructed by the voice recorder (Addenda J, K, and L).

3.3.4.1 Sensory evaluation method

Descriptive sensory analysis was used to determine whether the time allocations and heat applications were sufficient in the recipes of the stewed tomato and onion flavoured with parsley to provide for an acceptable cooked dish. There are several different methods of descriptive analysis, including the Flavour Profile Method, Texture Profile Method, Quantitative Descriptive Analysis (QDA®) Method, Spectrum™ Descriptive Method, and Free-choice Profiling (as discussed in Meilgaard *et al.*, 1999: 166-170). Another method, generic descriptive analysis, combines different approaches from the above-mentioned methods and is frequently employed during practical applications in order to meet specific project objectives (Murray *et al.*, 2001: 461-462).

Descriptive profiling, a term widely used to differentiate between the other types of descriptive methods, was used as the sensory method in this study. Descriptive profiling encompasses techniques based on quantitative descriptive analysis (QDA), which has been adapted and modified since 1974 to suit particular individual needs (Carpenter *et al.*, 2000: 47).

3.3.4.2 Sensory panel

Descriptive sensory analysis methods involve the detection and the description of all the sensory aspects of a product by trained panels of 5 to 100 panellists (Meilgaard *et al.*, 1999: 161). A trained panel is the most commonly used for conducting descriptive testing in sensory evaluation (Carpenter *et al.*, 2000: 49). In this study 10 trained panellists were used.

Smaller panels of 5 to 10 panellists are used for evaluation of the typical product on the grocery shelf, while larger panels are used for products of mass production, such as beers or soft drinks (Meilgaard *et al.*, 1999: 161). All the panellists had received training in sensory evaluation and had passed a panel-screening test. They were furthermore actively involved in the food industry in food product research and development, food product selection, buying and quality control.

3.3.4.3 Compilation of sensory evaluation sheets

All descriptive sensory analysis methods involve the detection (discrimination) and the description of both qualitative (phase 1) and quantitative (phase 2) sensory aspects (Meilgaard *et al.*, 1999: 161). The assessors develop descriptors for the sensory characteristics of a product (qualitative aspects) and then use these descriptors to quantify differences between products (quantitative aspects) (Carpenter *et al.*, 2000: 46). The qualitative aspects of a product (descriptors) include aroma, appearance, flavour, texture, after-taste and sound properties of a product, which distinguish it from others (Carpenter *et al.*, 2000: 47).

In descriptive profiling, assessors perform Phase 1 collectively but Phase 2 individually. The main distinctions between the descriptive method tests rest on whether assessors perform phase 1 and phase 2 collectively or individually. The collection of descriptors is the preliminary stage (phase 1) in descriptive profiling (Carpenter *et al.*, 2000: 47). The qualitative aspects of the stewed tomato and onion flavoured with parsley included appearance, texture, flavour and after-taste. Only aspects regarding the cooking of the recipe (namely texture) were of importance in this study. Flavour and after-taste were included as the preparation methods of the recipes differed and could influence the taste because of insufficient cooking.

A subgroup of the sensory panel contributed to the generation of the descriptive terms during a training session. The generated list of descriptive terms was then reviewed, refined and organised by the researcher and a draft list was drawn up. This list was presented to the panel for further discussion and refinement. Accurate definitions were prepared for each of the attributes, scale anchor points agreed upon and standards identified, discussed and rated. A standard set of descriptive terms was prepared, which all panellists understood and concurred with, and that could be used by them at each sensory evaluation session. The list was available to the panel to refer to as required (Addendum M). A standard evaluation sheet (Addendum N) was compiled for the three recipes. Only one standard sensory

evaluation sheet was compiled because the end product for all three recipes using the different recipes was stewed tomato and onion flavoured with parsley.

Selection of a scale for the sensory evaluation sheet is one of several tasks that needs to be completed before a sensory evaluation session can be organised (Stone & Sidel, 2004: 71). In this study, three-point and five-point scales were used depending on the attribute being measured. Scale length and number of scale categories are major variables that have an effect on scale sensitivity. Not all scales are equally sensitive for measuring differences. In effect, a three-point scale is less sensitive than a five-point scale (by about 30%), and both are less sensitive than a seven- or nine-point scale (Stone & Sidel, 2004: 72). Scale anchor points were provided for each of the scales. At each of the anchor points a descriptive term was assigned in logical progression to be evaluated by the panellist from left to right. These anchor points were provided with scale numbers (numerical scores to serve as the anchor points) to allow for statistical analysis. The scale numbers were not indicated on the panellists sensory evaluation form as this influences number biases (Stone & Sidel, 2004: 221). The scale numbers are, however, indicated in Addendum N to illustrate the scale numbers assigned for the analyses of the attributes. Appropriately constructed category scales enable the panellists to determine whether a product was more or less liked or the magnitude of differences for specific sensory attributes (e.g. colour, aroma) (Stone & Sidel, 2004: 70).

The order of the attributes (appearance, texture and flavour) was arranged in the same sequence in which they logically would be evaluated. This arrangement permits an orderly evaluation of the sample as the panellist simply works straight down the sensory evaluation sheet (McWilliams, 2001: 52). The evaluation sheet also evaluated only one item at a time.

3.3.4.4 Venue description

An air-conditioned sensory evaluation venue was used for the scoring of the recipe attributes. The venue is equipped with individual booths so that panellists do not have the opportunity to interact with one another. The temperature of the venue was comfortable (controlled at 22 - 24°C) with a relative humidity of 45 - 55%. No light (e.g. red light) other than the room light was used to reflect the colour of the samples, as the actual colour of the stewed tomato and onion flavoured with parsley was an attribute being scored.

3.3.4.5 Analytical sensory evaluation sessions

The final agreed upon sensory evaluation sheet (Addendum N) was provided to each of the ten panellists during the sensory evaluation sessions to rate the recipes according to the attributes. An evaluation sheet was provided for each of the recipes. Panellists signed a consent form (Addendum O) prior to the evaluation session indicating their willingness to participate. Only one of the panellists was a smoker and was asked not to smoke 30 minutes prior to the evaluation session. Furthermore, all the panellists were committed, motivated and present at each of the scheduled sessions. The samples were presented in a random, balanced order (one at a time) with random numbering so that the panellists could not identify which of the three recipes they tasted. The samples were also presented warm and a sufficient sample quantity was provided to each panellist to taste more than once if necessary. Water and crackers was provided to the participants to clean there palates after each sample evaluation.

3.3.4.6 Analysis of sensory evaluations

Basic descriptive statistics such as frequencies and means along with the mode and the median were calculated from the individual scores allocated for each sensory attribute of the recipes evaluated. An attribute was found acceptable when 70.0% or more of the panellists scored it as acceptable [three was the scale number considered as acceptable (Addendum N)] (Stone & Sidel, 2004: 258). When the recipes were found unacceptable (scored less than 70.0%) for any of the descriptive statistics, the recipe was reworked and re-evaluated by the panel until approved. The results of the recipe finalisation stage were applied to the methodology of the recipes prior to the preparation for the sampling and analysis of the TAC and antioxidant content.

i Control recipe

The overall acceptability of the control recipe was found not to be satisfactory in Trial 1 and it was re-formulated to produce an acceptable product in Trial 2 (Table 3.11). In Trial 1, five of the twelve attributes, namely onion texture, overall doneness, typical tomato flavour, typical onion flavour and after-taste were not scored as acceptable by 70.0% or more of the panellists. The onion texture was found to be too crunchy and affected the overall doneness and flavour of the stewed tomato and onion flavoured with parsley. The onion was sautéed for 1 minute longer during the recipe in Trial 2. All the sensory attributes were scored acceptable in Trial 2. The recipe of Trial 2 (Addendum P) was used for the experimental study.

Table 3.11: Sensory attributes of the control recipe as scored by the panellists (n = 10)

Attributes	Score							
	Mode		Median		Average± SD		Percentage of panellists who indicated an acceptable rating[a]	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Appearance								
Overall colour	3	3	3	3	2.7 ± 0.7	3.0 ± 0.0	70	100
Colour of onion	3	3	3	3	2.8 ± 0.4	3.2 ± 0.4	80	80
Oily surface	3	3	3	3	2.9 ± 0.3	3.1 ± 0.3	90	90
Texture								
Overall consistency	3	3	3	3	2.9 ± 0.3	2.9 ± 0.3	90	90
Overall coarseness	3	3	3	3	3.1 ± 0.7	3.1 ± 0.6	70	70
Oily mouthfeel	3	3	3	3	3.0 ± 0.0	2.8 ± 0.4	100	80
Onion texture	4	3	4	3	3.6 ± 0.5	2.9 ± 0.3	40	90
Overall doneness	3	3	3	3	2.6 ± 0.5	2.9 ± 0.3	60	90
Flavour								
Tartness	3	3	3	3	3.0 ± 0.0	2.7 ± 0.5	100	70
Typical tomato flavour	3	3	3	3	2.6 ± 0.5	3.2 ± 0.4	60	80
Typical onion flavour	3	3	3	3	3.4 ± 0.5	3.0 ± 0.5	60	80
After-taste	3	3	3	3	2.6 ± 0.5	2.8 ± 0.4	60	80

Abbreviations: SD-Standard deviation.
a-An acceptable rating was scored numerically as three.

ii Recipe 1

Four of the twelve attributes in Trial 1 were scored as unacceptable by 70.0% or more of the panellists. These included overall colour, onion texture, typical tomato flavour and typical onion flavour. The overall acceptability of Recipe 1 (Table 3.12) was as a result not satisfactory and the recipe was re-formulated. The onion texture was found to be too crunchy and affected the flavour of the stewed tomato and onion flavoured with parsley. In the re-formulation of Recipe 1 in Trial 2, the tomato was cooked less (30 seconds) and the onion was cooked longer (50 seconds) (Addendum Q). Results from the sensory evaluation session in Trial 2 indicated that the recipe attributes were acceptably scored (Table 3.12). The re-formulated recipe (Addendum Q) was used in the experimental study.

Table 3.12: Sensory attributes of Recipe 1 as scored by the panellists (n = 10)

Attributes	Score							
	Mode		Median		Average ± SD		Percentage of panellists who indicated on acceptable rating[a]	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Appearance								
Overall colour	3	3	3	3	2.7 ± 0.5	2.7 ± 0.5	60	70
Colour of onion	3	3	3	3	2.9 ± 0.3	2.8 ± 0.4	90	80
Oily surface	3	3	3	3	3.0 ± 0.0	2.9 ± 0.3	100	90
Texture								
Overall consistency	3	3	3	3	3.8 ± 0.4	2.6 ± 0.7	80	90
Overall coarseness	3	3	3	3	2.7 ± 0.7	2.9 ± 0.3	70	100
Oily mouthfeel	3	3	3	3	3.0 ± 0.6	3.0 ± 0.0	100	80
Onion texture	4	3	4	3	2.8 ± 0.5	2.7 ± 0.5	20	70
Overall doneness	3	3	3	3	3.0 ± 0.0	2.8 ± 0.4	70	70
Flavour								
Tartness	3	3	3	3	3.0 ± 0.0	2.7 ± 0.5	100	70
Typical tomato flavour	3	3	3	3	3.4 ± 0.5	2.8 ± 0.4	60	90
Typical onion flavour	3	3	3	3	2.7 ± 0.5	3.1 ± 0.3	50	80
After-taste	3	3	3	3	2.8 ± 0.5	2.9 ± 0.3	70	90

Abbreviations: SD-Standard deviation.
a-An acceptable rating was scored numerically as three.

iii Recipe 2

All twelve attributes evaluated were scored as acceptable by 70.0% or more of the panellists. The formulation of Recipe 2 was found satisfactory during the first trial (Table 3.13). No re-formulation of Recipe 2 was necessary and it was used as such for the experimental study (Addendum L).

Table 3.13: Sensory attributes of Recipe 2 as scored by the panellists (n = 10)

Attributes	Score			
	Mode	Median	Average ± SD	Percentage of panellists who indicated an acceptable rating[a]
Appearance				
Overall colour	3	3	3.1 ± 0.32	90
Colour of onion	3	3	3.0 ± 0.0	100
Oily surface	3	3	2.9 ± 0.32	90
Texture				
Overall consistency	3	3	3.0 ± 0.0	100
Overall coarseness	3	3	3.0 ± 0.0	100
Oily mouthfeel	3	3	2.9 ± 0.32	90
Onion texture	3	3	2.8 ± 0.42	80
Overall doneness	3	3	3.0 ± 0.47	80
Flavour				
Tartness	3	3	2.9 ± 0.32	90
Typical tomato flavour	3	3	2.9 ± 0.32	90
Typical onion flavour	3	3	3.1 ± 0.32	90
After-taste	3	3	2.9 ± 0.32	90

Abbreviations: SD-Standard deviation.
a-An acceptable rating was scored numerically as three.

3.4 Experimental study

The experimental study was conducted after completion of the recipe finalisation stage of the pilot study (Figure 3.1, p.38). Prior to the experimental study the final recipes were prepared once again to determine the temperature (°C) reached for each step. These temperatures were determined as another method to limit the heat variable. The temperatures are indicated in the final recipes in Addenda P, Q and L.

3.4.1 Food sampling methods

The ingredients were sampled from retail suppliers to obtain produce (tomato, onion and parsley) from the same variety and harvest date as discussed in 3.3.2.3. The SFO was purchased from a leading supermarket and the RPO was supplied by the MPOPC. Each recipe was cooked according to the recipe preparation steps [Addenda P (control recipe) and Q and L (Recipes 1 and 2, respectively)] as instructed by the pre-recorded tape. Raw samples of the tomato, onion, parsley and oils were collected from the recipe ingredients as well as combinations of the raw ingredients of each recipe according to their weight contribution [Addenda J, K and L (Tables J-1, K-1 and L-1)] of the recipe. Combined raw ingredients (represented by their weight contributions) and cooked samples were taken from each recipe as indicated in Table 3.14.

Table 3.14: Sampling procedure for stewed tomato and onion flavoured with parsley when using the three respective recipes

Utilising SFO	Utilising RPO	Time allocation (minutes per step)
	Control recipe	
Raw[a]	Raw[a]	0.00
SFO + onion	RPO + onion	5.53
SFO + onion + tomato	RPO + onion + tomato	11.43
SFO + onion + tomato + parsley	RPO + onion + tomato + parsley	0.22
		Total time: 17.58
	Recipe 1	
Raw[a]	Raw[a]	0.00
SFO + tomato	RPO + tomato	1.57
SFO + tomato + onion	RPO + tomato + onion	10.40
SFO + tomato + onion + parsley	RPO + tomato + onion + parsley	0.22
		Total time: 12.59
	Recipe 2	
Raw[a]	Raw[a]	0.00
SFO + tomato	RPO + tomato	6.54
SFO + onion	RPO + onion	5.54
SFO + tomato + onion	RPO + tomato + onion	2.54
SFO + tomato + onion + parsley	RPO + tomato + onion + parsley	0.22
		Total time: 19.56

Abbreviations: SFO-sunflower oil, RPO-red palm oil.
[a]-Raw samples consisted of a combination of oil, tomato, onion, and parsley based on the recipe formulation's percentage weight contributions as indicated in Addenda J (Table J-1), K (Table K-1) and L (Table L-1).

To obtain statistically significant data, it was necessary to include sufficient repetition to ensure that the results were due to the independent variable (thermal household processing) and not to chance (McWilliams, 2001: 20) Each recipe was therefore prepared three times to provide samples in triplicate. Other samples taken included those of cooked parsley (in oil), cooked tomato with parsley, sautéed onion with parsley and of the heated oils. These samples were prepared by the application of the time allocations and heat applications of the control recipe using both oils. Figure 3.4 indicates a schematic flow of the sampling procedure for the three recipes. Approximately 15 g of the food samples were collected in a 15 ml screw centrifuge cap tube and placed on ice. The food sample was then homogenised. Other studies investigating the TAC of food (Ninfali *et al.*, 2005: 258; Wu *et al.*, 2004a: 4027; Wu *et al.*, 2004b: 409) do not indicate how much of the initial food sample was taken. As samples were collected from each preparation step in this study, samples of approximately 15 g were collected to provide for the collection of all the required samples. After the preparation of each recipe, the end-product (stewed tomato and onion flavoured with parsley) was weighed to determine the water loss during thermal household processing.

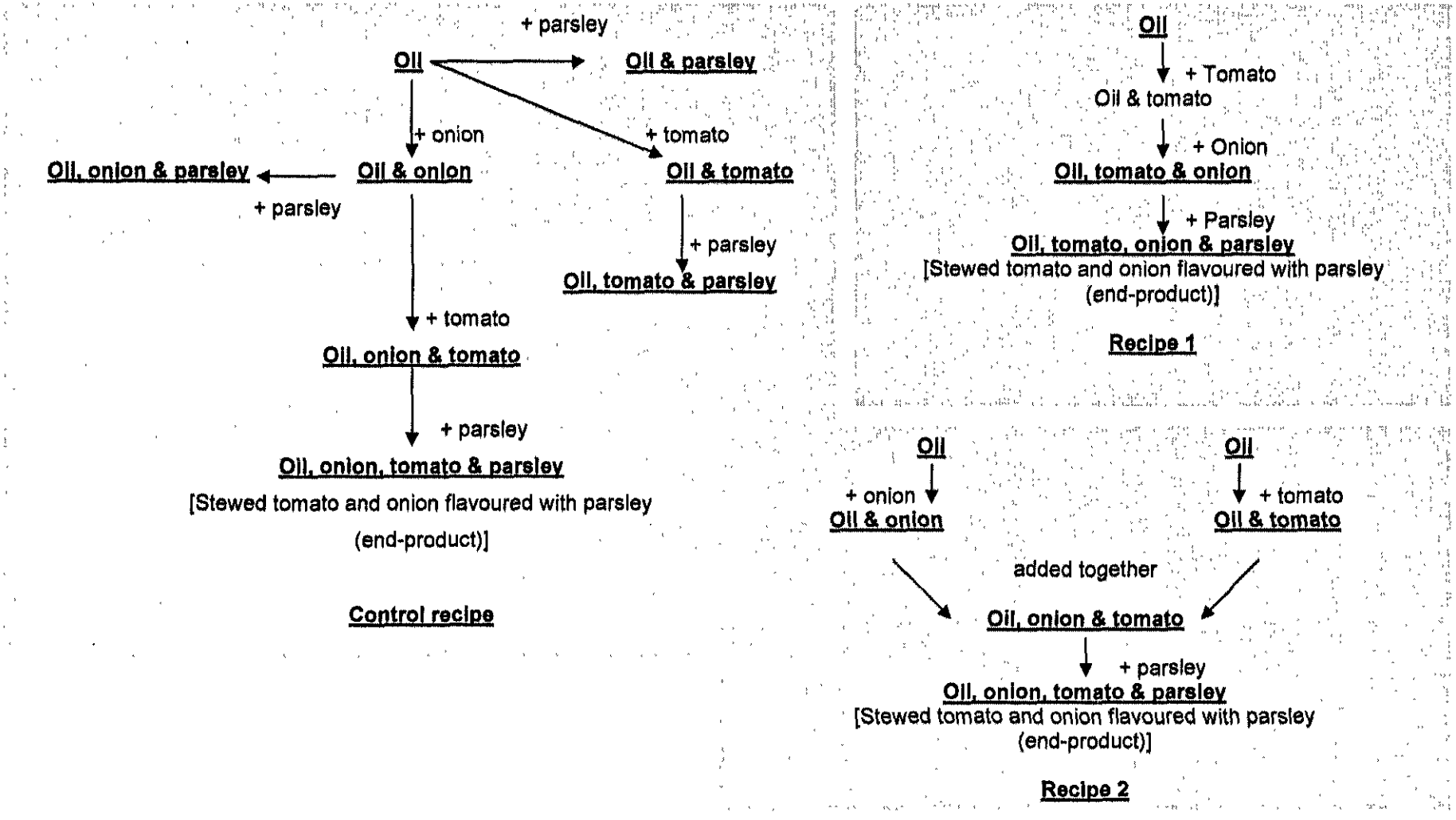


Figure 3.4: Schematic flow of the sampling procedures of the three recipes

3.4.2 Total antioxidant capacity analysis

The TAC for the samples was determined by using the $ORAC_{FL}$ assay as described by Prior and co-workers (2003: 3274). Some investigators have proposed that, in order to obtain a good measurement of the TAC for food, lipophilic components need to be separately analysed from those of the hydrophilic components using similar chemical principles (Cano *et al.*, 2000: 369; Arnao *et al.*, 2001: 244). In view of the above, the lipophilic (L-$ORAC_{FL}$) and hydrophilic (H-$ORAC_{FL}$) $ORAC_{FL}$ values were measured using the methodology applied by Wu *et al.*, (2004a: 4027) and Wu *et al.*, (2004b: 409), who determined the TAC of commonly consumed food in the US.

3.4.2.1 Principle of oxygen radical absorbance capacity assay

Originally developed by Cutler and Cao (1993), the first version of the $ORAC_{FL}$ assay employed B-phycoerythrin, a fuorescent protein (Cao *et al.*, 1993: 303). The fluorescence decay of B-phycoerythrin is an indication of damage from its reaction with the peroxyl radical. B-phycoerythrin was later replaced by fluorescein (Fl) after several disadvantages of B-phycoerythrin were found. The improved $ORAC_{FL}$ assay provides a direct measure of the lipophilic and hydrophilic chain-breaking AOC versus peroxyl radicals (Huang *et al.*, 2005: 1846). The $ORAC_{FL}$ assay has been broadly applied in academic institutions and the food and supplement industry as an assay of choice to quantify AOC. An antioxidant database in the US has been generated applying the $ORAC_{FL}$ assay (Wu *et al.*, 2004a: 4027; Wu *et al.*, 2004b: 409).

The $ORAC_{FL}$ assay is performed using a fluorescence spectrophotometer until zero fluorescence occurs. The results are reported as the $ORAC_{FL}$ value, which refers to the net protection area under the quenching curve of phycoerythrin (PE) (or Fl) in the presence of an antioxidant (Figure 3.5). Data reduction from the $ORAC_{FL}$ assay is achieved by i) calculating the area under the curve (AUC) and net AUC (AUCsample - AUCblank), ii) obtaining a standard curve by plotting the concentration of Trolox and the AUC (linear or quadratic fit between 5 and 25 µM Trolox), and iii) calculating the Trolox equivalents (TE) of a sample using the standard curve. These steps can be performed automatically on Microsoft Excel or a similar data processing program (Huang *et al.*, 2005: 1846). One ORAC unit is assigned as being the net protection area provided by 1 µM Trolox in the final concentration. When the AUC for the sample is compared with the AUC for Trolox, the result is given in TE.

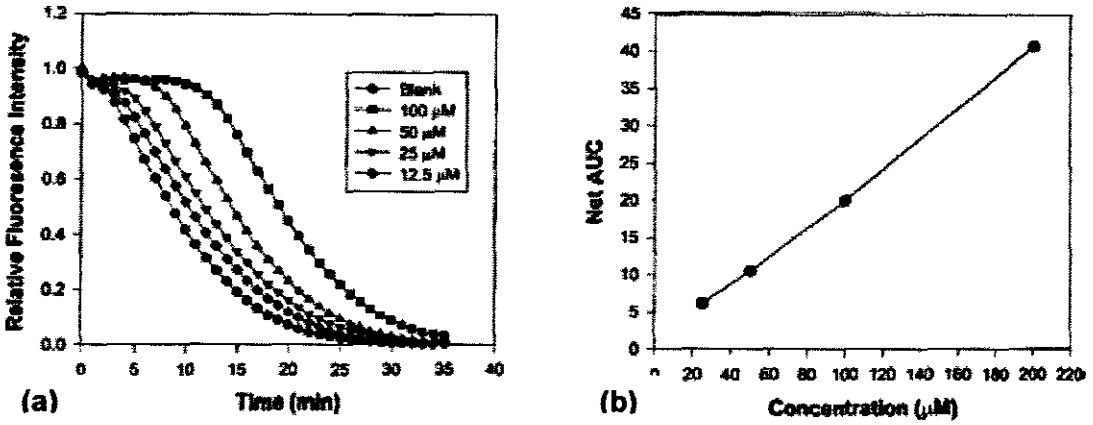


Figure 3.5 (a): Fluorescence decay curve of fluorescein in the presence of R-tocopherol and 2,2'-azobis(2-amidinopropane) dihydrochloride
(b): Linear plot of the net area under the curve versus R-tocopherol concentration
(Obtained from Huang *et al.*, 2005: 1847)

3.4.2.2 Chemicals and apparatus

2,2' Azobis(2-amidinopropane) dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and fluorescein (sodium salt) were purchased from Sigma-Aldrich (SA). Hexane, acetic acid and acetone were purchased from Merck (SA). ORAC analyses were carried out on a FLUOstar Galaxy plate reader (Bio-Tek SLx 800) (Bio-Tek Institute Inc. (Vermont, US). Fluorescence filters with an excitation wavelength of 485 nm and an emission wavelength of 520 nm were used. The 96 well black fluorescence microplates were purchased from Sigma-Aldrich (SA).

3.4.2.3 Sample preparation

Approximately 1 g of the homogenised food sample (discussed in 3.4.1) was taken and lyophillised using liquid nitrogen. The dried sample was then weighed and extracted in a 15 ml screw-cap centrifuge tube with 2 ml of hexane for 15 minutes. This was repeated three times. After each extraction the samples were centrifuged (3500 rpm for 1 minute) and the top hexane layer transferred to a new tube. All the hexane layers were combined in a glass tube and protected from light. The hexane was dried under nitrogen flow in a 30°C water bath, reconstituted with 1 ml acetone and transferred to a 1.5 ml eppendorf tube. This fraction was used to measure the L-$ORAC_{FL}$.

For the hydrophilic extract any residual hexane remaining in the original 15 ml tube was evaporated under nitrogen flow. The residue was then extracted with 5 ml of acetone/water/acetic acid (70:29.5:0.5, v/v/v) (AWA) for 15 minutes. This was repeated once

more. After each extraction the tubes were centrifuged (5500 rpm for 1 minute) and the supernatant transferred to a new tube. The two supernatant fractions were combined and protected from light. This fraction was used to measure the H-ORAC$_{FL}$.

3.4.2.4 Oxygen radical absorbance capacity assay on plate reader

Both H-ORAC$_{FL}$ and L-ORAC$_{FL}$ assays were carried out on a FLUOstar Galaxy plate reader, which was equipped with an incubator. The temperature of the incubator was set to 37°C. The procedures were based on the ORAC$_{FL}$ assay described by Prior and co-workers (2003: 3274). A Fl stock solution (stock #1) was prepared by dissolving 0.0225 g of Fl in 50 ml of 0.075 M phosphate buffer (pH 7.0). A second stock solution (stock #2) was prepared by diluting 50 µl of stock #1 in 10 ml of phosphate buffer. A 320 µl portion of stock #2 was added to 20 ml of phosphate buffer, of which 138 µl was added to each well. This provides 7.5 nmoles of Fl per well, or a final concentration of 14 µmol/l.

AAPH was used as peroxyl radical generator and Trolox (synthetic vitamin E, water-soluble) as a standard. Lipophilic and hydrophilic samples, blank (AWA for H-ORAC$_{FL}$ and acetone for L-ORAC$_{FL}$), and Trolox calibration solutions (12 µl each) were transferred to 96-well microplates in duplicate based upon a set layout. The plate reader was programmed to record the fluorescence of Fl on 5 minute cycles. Parameters of the assay for the plate reader were as follows: excitation 485 nm, emission 520 nm, reading every 5 minutes for 2 hours.

3.4.2.5 Preparation of calibration graphs

A stock standard of Trolox (500 µM) was dispensed into small vials for storage at –70°C until being used. In the standard assay, 12 µl Trolox calibration solutions (5, 10, 15, 20, 25 µM) in AWA (H-ORAC$_{FL}$) or acetone (L-ORAC$_{FL}$) (0.075 M) were pipetted into appropriate wells. A new stock of Trolox vials was removed from the freezer daily for use.

The final ORAC$_{FL}$ values were calculated using a quadratic regression equation ($y = ax^2 + bx + c$) between the Trolox standards or sample concentration and net area under the Fl decay curve. The ORAC$_{FL}$ value was expressed as micromoles of TE per gram (µmol TE/g), based on the wet weight of the sample. The AUC was calculated as

$$AUC + (0.5 + f_5/f_4 + f_6/f_4 + f_7/f_4 + \ldots + f_{24}/f_4 + f_{25}/f_4) \times CT \qquad (1)$$

where f_4 = initial fluorescence reading at cycle 4, f_1 = fluorescence reading at cycle 1, and CT = cycle time in minutes. The net AUC was obtained by subtracting the AUC of the blank from that of a sample. The data were analysed by Microsoft® Excel 2000 (9.0.2720) to apply Equation 1, to calculate the AUC.

3.4.2.6 Oxygen radical absorbance capacity assay of samples

For each specific sample, duplicate assays were performed and used for analyses. L-$ORAC_{FL}$ and H-$ORAC_{FL}$ values were measured on all samples taken during the preparation of the recipes. AAPH (50 µl) (17.2 mg/ml) was added to each well with a multichannel pipette for L-$ORAC_{FL}$ and H-$ORAC_{FL}$ to start the reaction.

3.4.3 Antioxidant content analyses

3.4.3.1 Total polyphenols

i Principle of total polyphenols assay

Polyphenols are a diverse class of chemicals produced by plants that are characterised by the presence of more than one aromatic bearing ring as described in Chapter 2 (2.2.1.2, p.9). This class of compounds can be subdivided into acetophenones, benzofurans, chromones, coumarins, flavonoids, phenolic acids, phenylacetic acids, phenylpropanoids, quinones, stilbenes and xanthones. Polyphenols have an absorption maximum at 280 nm and gallic acid is used as the standard (Sakakibara *et al.*, 2003: 575). The total polyphenols was determined by the method described by Prior and co-workers (2005: 495).

ii Chemicals and apparatus

Gallic acid was purchased from Sigma-Aldrich (SA) and ethanol (EtOH) and hydrochloric acid (HCl) from SAARCHEM (Merck, SA). Total polyphenol analyses were carried out on a Multiskan spectrum plate reader (Thermo Electron Corporation). Clear 96 well plates were purchased from Sigma-Aldrich (SA).

iii Sample preparation

Total polyphenols were determined using a plate reader at 280 nm with gallic acid as the standard. The hydrophilic supernatants obtained in 3.4.2.3 were used for the assay. These

extracted solutions were diluted 1:2 with 10.0% EtOH. Of this solution 12.5 µl were combined with 12.5 µl 0.1% HCl in 95% EtOH, 225 µl 2% HCl in a clear 96-well plate and incumbated at room temperature for 15 minutes before taking a reading.

iv Preparation of calibration graphs

A stock solution of 800 mg/l gallic acid (40 mg dissolved in 50 ml 10% EtOH) was freshly prepared and diluted to obtain standard solutions for the preparation of calibration graphs. A specified volume of the gallic acid stock solution was diluted to the required volume with 95% ethanol to obtain working solutions of 800, 400, 200, 100 and 50 mg/l. Of this solution, 12.5 µl were combined with 12.5 µl 0.1% HCl in 95% EtOH, 225 µl 2% HCl in a clear 96-well plate and incubated at room temperature for 15 minutes before taking a reading. The concentration of gallic acid in the sample solutions was deduced by means of regression equations of the related calibration graphs. The total polyphenols were expressed as mg gallic acid equivalents per gram (mg GAE/g).

3.4.3.2 Total carotenoids and lycopene

i Principle of carotenoid/lycopene assay

Carotenoids absorb strongly in the visible region between 400 and 500 nm. With a single wavelength detector total carotenoids can be measured at 450 nm, which is a compromise among the absorption maxima of various carotenoids (Feltl *et al.*, 2005: 98). In tomato, the major carotenoid present is lycopene, which has the longest wavelengths at 444, 470 and 520 nm (Rodriguez-Amaya, 2003: 930). The method used for the analysis of the total carotenoids and lycopene was obtained from Rodriguez-Amaya and Kimura (2004: 85).

ii Chemicals and apparatus

Total carotenoids and lycopene analyses were carried out on a Nicolet evolution 300 spectrophotometer (Thermo Electron Corporation).

iii Sample preparation

The lipophilic supernatants obtained in 3.4.2.3 were used for the assay and the absorbance read at 450 nm (total carotenoids) and 472 nm (lycopene) in a Nicolet evolution 300 double beam spectrophotometer. An extinction coefficient of 3450 for lycopene and 2460 for total

carotenoids was used in the calculations of the concentrations. Total carotenoids and lycopene concentrations of samples were expressed as micrograms per gram (µg/g).

3.4.3.3 Vitamin C

i Principle of vitamin C assay

Ascorbic acid (AA) is a reducing agent. The dye dichlorophenol-indophenol (DCPIP) is blue in base ($DCPIP^-$) and pink in acid (DCPIPH) and the pink form can be reduced by AA to a colourless form ($DCPIPH_2$). If a drop of blue DCPIP dye is added to a low pH solution (pH < 4.0), it will turn pink. If a suitable electron donor, such as AA, is present in that solution it will turn colourless. When all the AA in the solution has been oxidised to DHA (as discussed in 2.2.1.1, p. 7), no more electrons will be available to reduce the pink acid to the colourless form and the solution will remain pink. The amount of AA in the extract can be quantified by titrating a standard solution of dye (DCPIP) until the acid solution remains pink (Kall, 2003: 320). Horwitz (1980: 777) used the above principle to determine the vitamin C content of food. This method was used in this study.

ii Chemicals and apparatus

Ascorbic acid and metaphosphoric acid (MP) were supplied by Sigma (SA). All other chemicals and solvents were of analytical reagent grade and purchased from Merck (SA).

iii Sample preparation

A metaphosphoric acid (3%, w/v) and acetic acid (8%, v/v) (MPAA) solution was used as extraction solution. Approximately 1 g of the homogenised sample (from 3.4.2.3, p.68) was taken and lyophilised under liquid nitrogen. The dried sample was weighed and 1 ml of the MPAA solution was added immediately. The samples were stored at –80 °C until analysed. Samples were defrosted at room temperature for 30 minutes. Just before the assay, samples were centrifuged at 2500 rpm for 1 minute. The supernatant was analysed directly or after dilution (MPAA) to provide the necessary working concentration and titrated against DCPIP (0.8 mg/l) until the pink colour persisted for at least 10 - 15 seconds. The amount of dye added was recorded and this value used to calculate the AA concentration in the sample. Vitamin C concentrations of samples were expressed as µg/g.

iv Preparation of calibration graphs

A fresh stock solution of 50 µg/ml AA was prepared (5 mg of AA were dissolved in 100 ml of MPAA in a calibrated flask) and diluted to obtain standard solutions. A specified volume of the AA stock solution was diluted to the required volumes with MPAA in a 1 ml eppendorf tube to obtain working solutions (50, 40, 30, 20 and 10 mg/l). These solutions were titrated against DCPIP (0.8 mg/l) until the pink colour persisted for at least 10-15 seconds. The amount of dye added was recorded and the values used to prepare a calibration curve (Hernández *et al.*, 2006: 657).

3.4.4 Data analysis

The data was entered onto Microsoft® Excel 2000 (9.0.2720) spreadsheets and imported into SAS®STAT Software (9.1.2) for analysis. The data analysis was divided into two phases as adjustments were necessary to compare the data of the three recipes. Various samples were collected to represent the preparation steps of each recipe. Each sample collected, however, only contained the ingredients of that preparation step and did not on a percentage basis represent that contained in the stewed tomato and onion flavoured with parsley (end-product). The original data obtained for the combined raw ingredients (discussed in 3.4.1) and the end-product represented the recipe ingredients on a 100.0% basis, as all the ingredients were present according to the recipe percentage weight contribution [Addenda J, K and L (Tables J-1, K-1 and L-1)]. The data of the preparation steps between the combined raw ingredients and the end-products of the three recipes were as a result adjusted by multiplying the data with the ingredient percentage weight contribution [Addenda J, K and L (Tables J-1, K-1 and L-1)] of the end-product. For example, the sautéed onion in the control formulation only contained SFO (16 g) and onion (220 g), therefore it was multiplied with 24.06% [236 g (16 + 220 g) of 981 g] as this was the weight contribution of these two ingredients in the formulation. These percentage weight contribution adjustments were regarded as Phase 1. The data obtained after the adjustments in this data analysis phase (Phase 1) are indicated in Tables R-1 and S-1 (Addenda R and S).

Further analysis, however, indicated that significant differences existed in the data of the combined raw ingredient batches. For example the L-ORAC value of the control formulation with SFO was 0.13 µmol TE/g, while that of Recipe 1 was 0.21 µmol TE/g ($P < 0.05$). This was in spite of all the steps taken in order to control all the possible variables that could result in sample variations (discussed in 3.2.2.). These findings complicated comparisons

between the different recipes as the ingredient batches that were to be used to prepare the dishes contained different antioxidant contents.

In order to compare the data (after the Phase 1 adjustments) of the different recipes, the combined raw ingredient data of Recipes 1 and 2 were adjusted with a calculated factor (Table 3.15) to provide the same baseline data as the combined raw ingredient data of the control recipe. The calculated factor for Recipe 1 was then applied to all the data of steps 2, 3 and 4 in Recipe 1 while the calculated factor for Recipe 2 was applied to all the data of steps 2, 3, 4 and 5 in Recipe 2. Due to the use of these adjustment factors, the combined raw ingredient data of each recipe and assay provided the same standard deviation of 0.00. No standard deviations are therefore indicated in Tables 4.2, 4.5, 4.8, 4.9, 4.12 and 4. These adjustments to the data were regarded as Phase 2.

Table 3.15: Example of factor calculation adjustments for recipe comparisons of data[a]

Recipes	Original values of combined raw ingredients[b]	Calculation	Factor	Adjusted values of combined raw ingredients[c]	Calculation
Control recipe	**0.13**	(0.13 / **0.13**)	1	0.13	(0.13 / 1)
Recipe 1	0.21	(0.21 / **0.13**)	1.62	0.13	(0.21 / 1.62)
Recipe 2	0.19	(0.19 / **0.13**)	1.46	0.13	(0.19 / 1.46)

[a]-Extract of Table R-1 (Addendum R) for L-ORAC.
[b]-Adjusted values after Phase 1.
[c]-Adjusted values after Phase 2.

The above-mentioned phase could have been analysed differently by comparing the data differences between the combined raw ingredient and the end-product data of each recipe. In some cases, however, an unacceptably high standard deviation was calculated. For example, in Recipe 1 the average H-ORAC difference between the combined raw ingredients and the end-product was calculated to be 0.34 while the standard deviation was 1.62. This occurred because one of the triplicate samples had a negative difference while the other two samples had a positive difference. Owing to these large standard deviations this option of comparing the data was not pursued further.

The TAC and antioxidant content data obtained were expressed as the mean ± standard deviation (SD). Comparisons were made between the data of the preparation steps within the same recipe using a paired t-test to determine significant differences between the means. Comparisons were also made between the data of the preparation steps of the different recipes using a two-sample equal variance t-test to determine significant differences between these means. The Pearson correlation analysis was used to test for a significant relationship

between the data of the recipes for i) the effect of thermal household processing, and ii) the addition of parsley. A *p*-value of < 0.05 was considered significant.

The results in Chapter 4, sections 4.2.4, 4.3.4 and 4.4.2 exclude the effects of water loss during thermal household processing on the TAC and antioxidant content of the end-products. These results are therefore based on the wet weight of the combined raw ingredients. However, water loss will cause the antioxidants to become more concentrated (excluding the effect of thermal household processing). The weight of the end-products of each recipe was measured and the average weight calculated (samples collected during the preparation methods were included in the calculation). Therefore the antioxidant content and capacity of the end-products of the three recipes were adjusted to take into account the effect of the water loss during thermal household processing. This was also done in two phases. Table 3.16 indicates an example of the first phase in the water loss calculation. The second phase in the calculation was similar to that indicated in Table 3.15 and was done to make comparisons between the data of the recipes possible. These results are discussed in Chapter 4.

Table 3.16: Example of water loss calculation for comparisons of data

Recipes	Original value of stewed dish[a]	Weight percentage of stewed dish	Calculation	Adjusted value of stewed dish
Control recipe	0.19	62.5	(0.19 * 62.5%)	0.12
Recipe 1	0.22	71.9	(0.22 * 71.9%)	0.16
Recipe 2	0.32	60.3	(0.19 * 60.3%)	0.19

[a]-Adjusted values after Phase 2.

CHAPTER 4

RESULTS

During the experimental study the TAC (L-ORAC$_{FL}$, H-ORAC$_{FL}$ and TAC) and antioxidant content (total carotenoids, lycopene, total polyphenols and vitamin C) of the representative samples from the three recipes were determined. In this chapter these results (L-ORAC$_{FL}$, H-ORAC$_{FL}$, TAC, total carotenoid, lycopene, total polyphenol and vitamin C contents) will be discussed in four sections: i) the raw recipe ingredients, ii) the stewed tomato and onion flavoured with parsley using SFO as ingredient, iii) the stewed tomato and onion flavoured with parsley using RPO as ingredient, and iv) a comparison between the recipes when SFO and RPO was used as ingredients.

4.1 Raw recipe ingredients

The TAC and antioxidant content of the raw recipe ingredients are indicated in Table 4.1. No statistics are indicated in Table 4.1; they are, however, indicated in the text. The L-ORAC$_{FL}$ values as a percentage of the TAC was lower than 10% for all the ingredients used, except for the RPO (24.2%). As a result, the high H-ORAC$_{FL}$ values contributed most of the TAC of all the ingredients. Parsley had the highest TAC followed by onion, tomato, RPO and SFO (Table 4.1). The TAC of the raw ingredients differed significantly ($P < 0.05$). The TAC of the parsley was significantly ($P < 0.05$) higher than that of either the onion, the tomato, the SFO and the RPO; that of the onion significantly ($P < 0.05$) higher than either that of the tomato, the SFO and the RPO; that of the tomato significantly ($P < 0.05$) higher than that of the SFO and the RPO; and that of the RPO significantly ($P < 0.05$) higher than that of the SFO (see Table 4.1. for mean ± SD).

Lycopene was only detected in the tomato (Table 4.1) that contributed to 81.2% of the total carotenoids in the tomato. The total carotenoid content of the RPO was significantly ($P < 0.05$) higher (53.14 ± 4.24 µg/g) than that of the parsley (8.81 ± 0.61 µg/g) and the tomato (5.97 ± 6.36 µg/g). The carotenoids in SFO were not measured as it contains insignificant sources of carotenoids (Lietz *et al.*, 2001: 502).

Table 4.1: Total antioxidant capacity and antioxidant content of the raw recipe ingredients

	Tomato	Onion	Parsley	Sunflower oil	Red palm oil
L-ORAC$_{FL}$ µmol TE/g	0.24 ± 0.01	0.33 ± 0.06	**1.99 ± 0.17**	0.02±0.00	0.15 ± 0.01
L-ORAC$_{FL}$/TAC %	7. 7	3.6	3.8	7.7	**24.2**
H-ORAC$_{FL}$ µmol TE/g	2.89 ± 0.04	8.74 ± 2.51	**50.36 ± 9.63**	0.25±0.01	0.47 ± 0.04
H-ORAC$_{FL}$/TAC %	92.3	**96.4**	96.2	96.2	75.8
TAC µmol TE/g	3.13 ± 0.03	9.07 ± 2.55	**52.35 ± 9.70**	0.26±0.02	0.62 ± 0.04
Total carotenoids µg/g	8.81 ± 0.61	ND	5.97 ± 6.36	-	**53.17 ± 4.27**
Lycopene µg/g	**7.15 ± 0.50**	ND	ND	-	ND
Total polyphenols mg GAE/g	1.24 ± 0.04	1.27 ± 0.05	**5.31 ± 0.42**	-	ND
Vitamin C µg/g	**101.26 ± 4.96**	33.14 ± 16.04	51.11 ± 5.60	-	ND

Values in the columns represent mean ± standard deviation from samples in triplicate.
Bold values in the rows represent the ingredient with the highest value for the analysed parameter.
Statistics indicated in text.
Abbreviations: TE-Trolox equivalents, ND-Not detected, GAE-Gallic acid equivalents, -Not determined.

The total polyphenol content of the parsley was significantly ($P < 0.05$) higher (5.31 ± 0.42 mg GAE/g) than that of the tomato (1.24 ± 0.04 mg GAE/g) and the onion (1.27 ± 0.05 mg GAE/g). The total polyphenol content of the tomato and onion was similar ($P > 0.1$) (Table 4.1).

The vitamin C content of the tomato was significantly ($P < 0.05$) higher (101.26 ± 4.96 µg/g) than that of the parsley (51.11 ± 5.60 µg/g) and the onion (33.14 ± 16.04 µg/g). The vitamin C content of the onion and the parsley was similar ($P > 0.1$) (Table 4.1). No polyphenols or vitamin C was detected in the RPO, while the content of the SFO was not measured (Table 4.1).

4.2 Stewed tomato and onion flavoured with parsley using sunflower oil as ingredient

Table 4.2 indicates the TAC and antioxidant content of the three recipes and their preparation steps when SFO was used. The control recipe being the traditional recipe, Recipe 1 being the recipe in which raw onion were added to cooked tomato and Recipe 2 being the recipe in which sautéed onion were added to cooked tomato. The steps followed in each recipe are indicated from left to right with the control recipe and Recipe 1 each utilising three steps and Recipe 2 utilising four steps.

Table 4.2: Total antioxidant capacity and antioxidant content of the raw combined ingredients and the three recipes and their preparation steps using sunflower oil as ingredient

Recipe		Control recipe			Recipe 1			Recipe 2			
Step description	Combined raw ingredients[a]	Sautéed onion	Cooked onion and tomato	Stewed tomato and onion flavoured with parsley (end-product)	Cooked tomato	Cooked tomato and raw onion	Stewed tomato and onion flavoured with parsley (end-product)	Cooked tomato	Sautéed onion	Cooked tomato and onion	Stewed tomato and onion flavoured with parsley (end-product)
Steps		One	Two	Three	One	Two	Three	One	Two	Three	Four
L-ORAC$_{FL}$ µmol TE/g	0.13	0.02 ± 0.01	0.13 ± 0.05	0.13 ± 0.05	0.10 ± 0.03	0.11 ± 0.02	0.14 ± 0.00	0.10 ± 0.03	0 02 ± 0.00	0.11 ± 0.05	0.13 ± 0.08
L-ORAC$_{FL}$/TAC %	2.7	0.7	1.9	1.8	4.2	2.1	2.6	2.7	0.8	1.5	1.8
H-ORAC$_{FL}$ µmol TE/g	4.75	3.00 ± 0.30	6.85 ± 1.39	7.11 ± 0.21	2.26 ± 0.88	5.09 ± 1.06	5.20 ± 1.53	3.63 ± 0.89	2.56 ± 0.83	7.04 ± 2.84	7.12 ± 3.50
H-ORAC$_{FL}$/TAC %	97.3	99.0	98.1	98.3	95.8	97.9	97.4	97.3	99.2	98.6	98.2
TAC µmol TE/g	4.88	3 03 ± 0.31	6.98 ± 1.44	7.23 ± 0.16	2.36 ± 0.87	5.20 ± 1.08	5.34 ± 1.53	3.73 ± 0.88	2.58 ± 0.83	7.14 ± 2 82	7.25 ± 3.45
Total carotenoids µg/g	4.91	ND	7.42 ± 4.48	7.13 ± 0.93	6.18 ± 3.76	6.39 ± 1.25	6.62 ± 6.07	5.80 ± 2.39	ND	7.39 ± 2.80	7.16 ± 2.39
Lycopene µg/g	4.18	ND	6 01 ± 3.36	5.66 ± 1.03	4.90 ± 2.69	5.24 ± 0.80	7.14 ± 5.31	4.83 ± 2.06	ND	5.66 ± 2.34	5.76 ± 2.15
Lycopene/ Total carotenoids %	85.1	-	81.0	79.7	79.3	82.0	82.8	83.3	-	79.6	80.7
Total polyphenols mg GAE/g	1.58	0 41 ± 0.05	1.45 ± 0.40	1.63 ± 0.43	1.23 ± 0.31	1.33 ± 0.40	1.48 ± 0.10	1.33 ± 0.15	0.44 ± 0.01	1.67 ± 0.35	1.54 ± 0.30
Vitamin C µg/g	40.06	9 40 ± 0.64	76.01 ± 21.70	82.10 ± 15 91	57.35 ± 2.83	61.03 ± 3.22	72.98 ± 5.47	56.90 ± 14.45	9.59 ± 1 88	71.06 ± 17.40	73.86 ± 19.21

[a]-Values as obtained after factor adjustments as explained in 3.4.4.
Values in the columns represent mean ± standard deviation of the adjusted values from samples in triplicate.
Abbreviations: TE-Trolox equivalents, ND-Not detected, GAE-Gallic acid equivalents, -Not determined

Table 4.2 also indicates the results of the thermally processed stewed tomato and onion flavoured with parsley (end-product) (100.0% weight contribution) (the last step in each recipe) and the combined raw ingredients (100.0% weight contribution) in the left column of the table. No standard deviation is indicated for the combined raw ingredients, as it is 0.00 owing to the adjustments made as described in 3.4.4, p.73 to allow for comparisons between the recipes. No statistics are indicated in Table 4.2 either, as they are discussed and indicated later in this chapter.

The L-ORAC$_{FL}$ and H-ORAC$_{FL}$ values expressed as a percentage of the TAC are also indicated in this Table 4.2. The L-ORAC$_{FL}$ values as a percentage of the TAC was low for each step in all three the recipes (mostly less than 3%). The H-ORAC$_{FL}$ values contributed most (95.8 - 99.2%) to the TAC. The lycopene content as a percentage of the total carotenoid content is also indicated in Table 4.2. In general, the lycopene content contributed between 79.3 and 85.2% to the total carotenoid contents.

The comparison of the results indicated in Table 4.2 is described below, examining i) the effect of thermal household processing on the sautéed onion and cooked tomato, ii) the effect of thermal household processing on the stewed tomato and onion flavoured with parsley prepared using the three recipes, iii) the effect of the addition of parsley, and iv) the effect of water loss during the thermal household processing.

4.2.1 Effect of thermal household processing on the sautéed onion and cooked tomato

4.2.1.1 Sautéed onion

The control recipe and Recipe 2 both included sautéed onion in steps one and two. These recipes (control recipe and Recipe 2) differed in their time allocations (5.53 minutes and 6.09 minutes), heat applications (temperature gauge 6 and 5) and cooked content temperatures (100 °C and 86 °C) [Addenda P (Table P-1) and L (Table L-2)]. Furthermore, the control recipe used more oil (20 ml) than Recipe 2 (15 ml) owing to the use of two pans in Recipe 2.

No differences ($P > 0.1$) were found in the L-ORAC$_{FL}$ and H-ORAC$_{FL}$ values of the sautéed onion when the two recipes were used, respectively (Table 4.3). The TAC of the sautéed onion was also similar ($P > 0.1$) for the two recipes (Table 4.3).

The total carotenoid and lycopene contents were not detected while the total polyphenol content of the sautéed onion was similar ($P > 0.1$) when the control recipe and Recipe 2 was

used (Table 4.3). No differences (P > 0.1) were found in the vitamin C content for the sautéed onion when the two recipes respectively were used (Table 4.3).

Table 4.3: Effect of thermal household processing on the total antioxidant capacity and antioxidant content of the sautéed onion prepared by two different recipes using sunflower oil as ingredient

	Control recipe	Recipe 2
L-ORAC$_{FL}$ µmol TE/g	0.02 ± 0.01 a	0.02 ± 0.00 a
H-ORAC$_{FL}$ µmol TE/g	3.00 ± 0.03 a	2.56 ± 0.83 a
TAC µmol TE/g	3.03 ± 0.31 a	2.58 ± 0.83 a
Total carotenoids µg/g	ND	ND
Lycopene µg/g	ND	ND
Total polyphenols mg GAE/g	0.41 ± 0.05 a	0.44 ± 0.01 a
Vitamin C µg/g	9.40 ± 0.64 a	9.59 ± 1.88 a

Values in the columns represent mean ± standard deviation of the adjusted values from samples in triplicate.
Values in rows followed by the same letter do not differ significantly. If letters differ, then P < 0.05 while letters in parenthesis (), then P < 0.1.
Abbreviations: TE-Trolox equivalents, ND-Not detected, GAE-Gallic acid equivalents.

4.2.1.2 Cooked tomato

The preparation methods of Recipes 1 and 2 required the tomato to be cooked (step one of both recipes) before the addition of the onion. These two recipes differed in their time allocations (1.57 minutes and 7.02 minutes) and temperatures of the cooked contents (60°C and 98°C) [Addenda L (Table L-2) and Q (Table Q-1)]. Recipe 2 also used less oil (15 ml) than Recipe 1 (20 ml) as two pans were used in this preparation method.

The L-ORAC$_{FL}$ values of the cooked tomato for both these recipes were similar (P > 0.1) (Table 4.4), while the H-ORAC$_{FL}$ values differed. The H-ORAC$_{FL}$ value when Recipe 2 (3.63 ± 0.89 µmol TE/g) was used was marginally (P < 0.1) higher than that of Recipe 1 (2.26 ± 0.88 µmol TE/g). The TAC of the cooked tomato was also marginally (P < 0.1) higher for Recipe 2 (3.73 ± 0.88 µmol TE/g) than it was for Recipe 1 (2.36 ± 0.87 µmol TE/g). No differences (P > 0.1) were found in the antioxidant content, namely the total carotenoids, lycopene, total polyphenols and vitamin C content of the cooked tomato steps in Recipes 1 and 2 (Table 4.4).

Table 4.4: Effect of thermal household processing on the total antioxidant capacity and antioxidant content of the cooked tomato prepared by two different recipes using sunflower oil as ingredient

	Recipe 1	Recipe 2
L-ORAC$_{FL}$ µmol TE/g	0.10 ± 0.03 a	0.10 ± 0.03 a
H-ORAC$_{FL}$ µmol TE/g	2.26 ± 0.88a	3.63 ± 0.89 (b)
TAC µmol TE/g	2.36 ± 0.87 a	3.73 ± 0.88 (b)
Total carotenoids µg/g	6.18 ± 3.76 a	5.80 ± 2.39 a
Lycopene µg/g	4.90 ± 2.69 a	4.83 ± 2.08 a
Total polyphenols mg GAE/g	1.23 ± 0.31 a	1.33 ± 0.15 a
Vitamin C µg/g	57.35 ± 2.83 a	56.90 ± 14.45 a

Values in the columns represent mean ± standard deviation of the adjusted values from samples in triplicate.
Values in rows followed by the same letter do not differ significantly. If letters differ, then $P < 0.05$ while letters in parenthesis (), then $P < 0.1$.
Abbreviations: TE-Trolox equivalents, GAE-Gallic acid equivalents.

4.2.2 Effect of thermal household processing on the stewed tomato and onion flavoured with parsley

To determine the effect of thermal household processing, the combined raw ingredients (100.0% weight contribution) and the stewed tomato and onion flavoured with parsley (end-product) of each of the three recipes (100.0% weight contribution) were compared (Table 4.5). Henceforth, the term end-product will be used to refer to stewed tomato and onion flavoured with parsley. Each of the parameters investigated is discussed individually in this section.

4.2.2.1 Lipophilic oxygen radical absorbance capacity

When comparing the L-ORAC$_{FL}$ values of the combined raw ingredients (0.13 µmol TE/g) with that of the end-product (0.14 ± 0.00 µmol TE/g), thermal household processing resulted in a significant ($P < 0.05$) increase (4.9%) when Recipe 1 was used. Thermal household processing had no effect ($P > 0.1$) on the L-ORAC$_{FL}$ values when the control recipe or Recipe 2 was used (Table 4.5). When the end-products of the three recipes were compared, no differences ($P > 0.1$) were found in the L-ORAC$_{FL}$ values, despite the three different recipe preparation methods used.

Table 4.5: Effect of thermal household processing on the total antioxidant capacity and antioxidant content from the combined raw ingredients to the end-product using sunflower oil as ingredient

	Combined raw ingredients	Control recipe (end-product)	Recipe 1 (end-product)	Recipe 2 (end-product)
L-ORAC$_{FL}$ µmol TE/g	0.13 a	0.13 ±0.05 aA	0.14 ± 0.00 bA	0.13 ± 0.08 aA
H-ORAC$_{FL}$ µmol TE/g	4.75 a	7.11 ± 0.21 bA	5.20 ± 1.53 a(B)	7.12 ± 3.50 aA(B)
TAC µmol TE/g	4.88 a	7.23 ± 0.16 bA	5.34 ± 1.53 aA	7.25 ± 3.45 aA
Total carotenoids µg/g	4.91 a	7.13 ± 0.93 (b)A	8.63 ± 6.07 aA	7.16 ± 2.39 aA
Lycopene µg/g	4.18 a	5.68 ± 1.03 aA	7.14 ± 5.31 aA	5.78 ± 2.15 aA
Total polyphenols mg GAE/g	1.58 a	1.63 ± 0.43 aA	1.48 ± 0.10 aA	1.54 ± 0.30 aA
Vitamin C µg/g	40.06 a	82.10 ± 15.91 bA	72.98 ± 5.47 bA	73.86 ± 19.21 (b)A

Values in the columns represent mean ± standard deviation of the adjusted values from samples in triplicate.
Intra-recipe step comparisons are indicated by small letters in rows. The combined raw ingredients column is used as the reference to which the values in the end-product columns are compared.
Inter-recipe step comparisons are indicated by capital letters in rows. The end-products are compared with one another
Values followed by the same letter do not differ significantly. If letters differ, then $P < 0.05$ while letters in parenthesis (), then $P < 0.1$.
Abbreviations: TE-Trolox equivalents, GAE-Gallic acid equivalents.

4.2.2.2 Hydrophilic oxygen radical absorbance capacity

When comparing the H-ORAC$_{FL}$ values of the combined raw ingredients (4.75 ± 0.00 µmol TE/g) with that of the end-product (7.11 ± 0.21 µmol TE/g), thermal household processing resulted in a significant ($P < 0.05$) increase (49.8%) when the control recipe was used. Thermal household processing had no effect ($P > 0.1$) on the H-ORAC$_{FL}$ values from the combined raw ingredients to the end-product when either Recipe 1 or 2 was used (Table 4.5). The H-ORAC$_{FL}$ value of the end-product when the control recipe was used, was marginally ($P < 0.1$) higher than that for Recipe 1, that of the control recipe was similar ($P > 0.1$) to Recipe 2 and that of Recipe 1 similar ($P > 0.1$) to that of Recipe 2 (Table 4.5).

4.2.2.3 Total antioxidant capacity

When comparing the TAC of the combined raw ingredients (4.88±0.00 µmol TE/g) with that of the end-product (7.23 ± 0.16 µmol TE/g), thermal household processing resulted in a significant ($P < 0.05$) increase (48.2%) when the control recipe was used. Thermal household processing had no effect ($P > 0.1$) on the TAC from the combined raw ingredients to the end-product when Recipe 1 or 2 was used (Table 4.5). When the end-products of the three recipes were compared no differences ($P > 0.1$) were found in the TAC, despite the three different recipe preparation methods used (Figure 4.1).

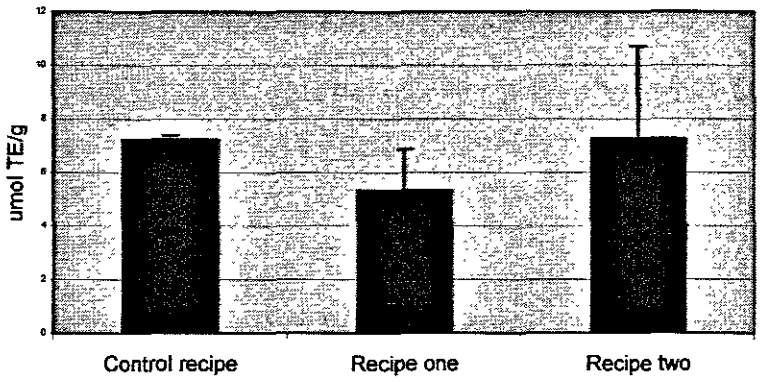


Figure 4.1: Total antioxidant capacity of the end-products using sunflower oil as ingredient

No significant difference, P > 0.05.
Abbreviation: TE-Trolox equivalents.

4.2.2.4 Total carotenoid content

When comparing the total carotenoid content of the combined raw ingredients (4.91 ± 0.00 µg/g) with that of the end-product (7.13 ± 0.93 µg/g), thermal household processing resulted in a marginally ($P < 0.1$) increase (45.2%) when the control recipe was used. Thermal household processing had no effect ($P > 0.1$) on the total carotenoid content when Recipes 1 and 2 were used (Table 4.5). The total carotenoid content of the end-product, despite using three respective recipes, was similar ($P > 0.1$) (Table 4.5).

4.2.2.5 Lycopene content

Thermal household processing had no effect ($P > 0.1$) on the lycopene content from the combined raw ingredients to the end-product on the three respective recipes (Table 4.5). No differences ($P > 0.1$) were found in the lycopene content for the end-products prepared by the three different recipes (Table 4.5).

4.2.2.6 Total polyphenol content

When the combined raw ingredients were compared to the end-products of the three respective recipes, thermal household processing had no effect ($P > 0.1$) on the total polyphenol content (Table 4.5). The total polyphenol content of the end-product when using the three recipes respectively were similar ($P > 0.1$) (Table 4.5).

4.2.2.7 Vitamin C content

When comparing the vitamin C content of the combined raw ingredients (40.06 ± 0.00 µg/g) with that of the end-product (82.10 ± 15.91 µg/g), thermal household processing resulted in a significant ($P < 0.05$) increase (104.9%) when the control recipe was used. When comparing the vitamin C content of the combined raw ingredients (40.06 ± 0.00 µg/g) with that of the end-product (72.98 ± 5.47 µg/g), thermal household processing also resulted in a significant ($P < 0.05$) increase (82.2%) when Recipe 1 was used. A marginal ($P < 0.1$) increase (84.4%) was found from the combined raw ingredients (40.06 ± 0.00 µg/g) to the end-product when Recipe 2 (73.86 ± 19.21 µg/g) was used. The vitamin C content of the end-products prepared by the three recipes was similar ($P > 0.1$) (Table 4.5). During thermal household processing when the control recipe was used, a positive correlation ($r = 0.94$) was found between the H-$ORAC_{FL}$ value and the vitamin C content.

4.2.3 Effect of the addition of parsley

At the end of step two in the control recipe and Recipe 1 and of step three in Recipe 2, parsley was added to determine if it contributed to a higher TAC and antioxidant content. Table 4.6 indicates the effect of the addition of parsley on the TAC and antioxidant content during the preparation of the three recipes. Only comparisons within a recipe are indicated in Tables 4.6 and 4.7.

The addition of parsley increased (12.7%) the total polyphenol content marginally ($P < 0.1$) when the control recipe was used and it increased (19.6%) the vitamin C content significantly ($P < 0.05$) when Recipe 1 was used. The remaining parameters (L-$ORAC_{FL}$, H-$ORAC_{FL}$, TAC, total carotenoid, lycopene, total polyphenol and vitamin C contents) for those two recipes remained similar ($P > 0.1$) (Table 4.6). The addition of parsley had no effect ($P > 0.1$) when Recipe 2 was used (Table 4.6).

Table 4.6: Effect of the addition of parsley on the total antioxidant capacity and antioxidant content during the preparation of the three recipes using sunflower oil as ingredient

	Control recipe		Recipe 1		Recipe 2	
	Cooked tomato and onion Step 3	End-product Step 4	Cooked tomato and onion Step 3	End-product Step 4	Cooked tomato and onion Step 4	End-product Step 5
L-ORAC$_{FL}$ µmol TE/g	0.13 ± 0.05 a	0.13 ± 0.05 a	0.11 ± 0.02 a	0.14 ± 0.00 a	0.11 ± 0.05 a	0.13 ± 0.08 a
H-ORAC$_{FL}$ µmol TE/g	6.85 ± 1.39 a	7.11 ± 0.21 a	5.09 ± 1.06 a	5.20 ± 1.53 a	7.04 ± 2.84 a	7.12 ± 3.50 a
TAC µmol TE/g	6.98 ± 1.44 a	7.23 ± 0.16 a	5.20 ± 1.08 a	5.34 ± 1.53 a	7.14 ± 2.82 a	7.25 ± 3.45 a
Total carotenoids µg/g	7.42 ± 4.48 a	7.13 ± 0.93 a	6.39 ± 1.25 a	8.63 ± 6.07 a	7.39 ± 2.80 a	7.16 ± 2.39 a
Lycopene µg/g	6.01 ± 3.36 a	5.68 ± 1.03 a	5.24 ± 0.08 a	7.14 ± 5.31 a	5.88 ± 2.34 a	5.78 ± 2.15 a
Total polyphenols mg GAE/g	1.45 ± 0.40 a	1.63 ± 0.43 (b)	1.33 ± 0.40 a	1.48 ± 0.10 a	1.67 ± 0.35 a	1.54 ± 0.30 a
Vitamin C µg/g	76.01 ± 21.70 a	82.10 ± 15.91 a	61.03 ± 3.22 a	72.98 ± 5.47 b	71.06 ± 17.40 a	73.86 ± 19.21 a

Values in the columns represent mean ± standard deviation of the adjusted values from samples in triplicate
Comparisons are indicated by small letters in rows. The first column for each investigated ingredient is used as the reference to which the values in the following column are compared.
Values in rows followed by the same letter do not differ significantly. If letters differ, then $P < 0.05$ while letters in parenthesis (), then $P < 0.1$
Abbreviations: TE-Trolox equivalents, GAE-Gallic acid equivalents, -Not determined.

With the addition of parsley a significant positive correlation was found in the control recipe between the L-ORAC$_{FL}$ value and the total carotenoid content ($r = 0.96$), and the L-ORAC$_{FL}$ value and the lycopene content ($r = 0.97$). In addition, with the addition of parsley when the control recipe was used, a significant positive correlation ($r = 0.84$) was found between the H-ORAC$_{FL}$ value and the vitamin C content.

The TAC and antioxidant content of the additional samples (sautéed onion and parsley, cooked tomato and parsley and heated parsley) (described in 3.4.1, p.64), using the time allocations and heat applications of the control recipe, are indicated in Table 4.7.

The addition of parsley to the sautéed onion resulted in a marginal ($P < 0.1$) increase in the L-ORAC$_{FL}$ value (28.6%), H-ORAC$_{FL}$ value (31.2%) and TAC (31.2%), while the vitamin C content increased (48.5%) significantly ($P < 0.05$). The total carotenoid and total polyphenol contents remained similar ($P > 0.1$) after the parsley was added (Table 4.7).

Table 4.7: Effect of the addition of parsley on the total antioxidant capacity and antioxidant content of the sautéed onion and the cooked tomato using sunflower oil as ingredient

	Sautéed onion		Cooked tomato		Parsley	
	Without parsley	Parsley added	Without parsley	Parsley added	Raw	Heated
L-ORAC$_{FL}$ µmol TE/g	0.05 ± 0.01 a	0.07 ± 0.01(b)	0.15 ± 0.02 a	0.17 ± 0.01 a	0.01 ± 0.00 a	0.04 ± 0.03 a
H-ORAC$_{FL}$ µmol TE/g	2.62 ± 0.25 a	3.83 ± 0.38 (b)	2.83 ± 0.22 a	4.23 ± 0.25 b	0.26 ± 0.05 a	0.93 ± 0.11 b
TAC µmol TE/g	2.67 ± 0.25 a	3.88 ± 0.39 (b)	2.98 ± 0.23 a	4.40 ± 0.26 b	0.27 ± 0.05 a	0.97 ± 0.14 b
Total carotenoids µg/g	1.44 ± 1.53 a	1.05 ± 0.86 a	10.35 ± 1.94 a	10.01 ± 1.80 a	0.03 ± 0.03 a	0.70 ± 0.20 b
Lycopene µg/g	-	-	7.77 ± 1.48 a	7.51 ± 1.26 a	-	-
Total polyphenols mg GAE/g	0.35 ± 0.06 a	0.33 ± 0.01 a	1.07 ± 0.11 a	1.08 ± 0.03 a	0.03 ± 0.00 a	0.11 ± 0.01 b
Vitamin C µg/g	8.63 ± 0.52 a	16.76 ± 1.44 b	88.19 ± 10.65 a	95.45 ± 7.03 a	0.26 ± 0.03 a	8.76 ± 0.43 b

Values in the columns represent mean ± standard deviation of the adjusted values from samples in triplicate.
Comparisons are indicated by small letters in rows. The first column for each investigated ingredient is used as the reference to which the values in the following column are compared.
Values in rows followed by the same letter do not differ significantly. If letters differ, then $P < 0.05$ while letters in parenthesis (), then $P < 0.1$.
Abbreviations: TE-Trolox equivalents, GAE-Gallic acid equivalents, -Not determined

When adding the parsley to the cooked tomato the H-ORAC$_{FL}$ value (33.1%) and the TAC (32.3%) increased significantly ($P < 0.05$), while the L-ORAC$_{FL}$ value, total carotenoid, lycopene, total polyphenol and vitamin C contents remained similar ($P > 0.1$) (Table 4.7).

The thermal household processing of the parsley with sunflower oil significantly ($P < 0.05$) increased the H-ORAC$_{FL}$ value (72.0%), TAC (72.2%), total carotenoid (95.7%), total polyphenol (72.7%) and vitamin C (97.0%) contents. Only the L-ORAC$_{FL}$ value remained similar ($P < 0.1$) (Table 4.7) while the lycopene was not determined.

4.2.4 Effect of water loss during thermal household processing

During thermal household processing water was lost owing to evaporation and as a result the concentration of the other matter in the end-product increased. The results indicated so far did not take into consideration this water loss. When considering the water loss during the preparation of the recipes, decreases were found for some of the parameters investigated. The calculation of the water loss was discussed in 3.4.4, p.73. When the control recipe was used, the H-ORAC$_{FL}$ value decreased (-6.3%) marginally ($P < 0.1$) while the TAC decreased (-7.8%) significantly ($P < 0.05$). When Recipe 1 was used, the L-ORAC$_{FL}$ value and total polyphenol content significantly ($P < 0.05$) decreased (-24.6% and –32.5%, respectively), while the vitamin C content increased significantly ($P < 0.05$) with 31.0%. Using Recipe 2

marginally ($P < 0.1$) decreased the total polyphenol content (41.2%). For the remaining parameters the content remained the same ($P > 0.1$) for all three the recipes (Table 4.8).

In summary, only two of the parameters decreased when the control recipe (H-ORAC$_{FL}$, TAC) and Recipe 1 (L-ORAC$_{FL}$, total polyphenols) were used, while one decreased (total polyphenols) when Recipe 2 was used. In contrast, vitamin C increased when Recipe 1 was used.

Table 4.8: Effect of water loss on the total antioxidant capacity and antioxidant content when the three recipes were prepared using sunflower oil as ingredient

	Combined raw ingredients	Control recipe (end-product)	Recipe 1 (end-product)	Recipe 2 (end-product)
L-ORAC$_{FL}$ µmol TE/g	0.13a	0.08 ± 0.03 a	0.10 ± 0.00 b	0.08 ± 0.05 a
H-ORAC$_{FL}$ µmol TE/g	4.75 a	4.45 ± 0.13 (b)	3.74 ± 1.10 a	4.29 ± 2.11 a
TAC µmol TE/g	4.88 a	4.50 ± 0.08 b	3.84 ± 1.09 a	4.23 ± 1.95 a
Total carotenoids µg/g	4.91 a	4.46 ± 0.58 a	6.20 ± 4.36 a	4.31 ± 1.44 a
Lycopene µg/g	4.18 a	3.55 ± 0.65 a	5.13 ± 3.82 a	3.48 ± 1.30 a
Total polyphenols mg GAE/g	1.58 a	1.02 ± 0.27 a	1.07 ± 0.07 b	0.93 ± 0.18 (b)
Vitamin C µg/g	40.06 a	51.39 ± 9.96 a	52.50 ± 3.94 b	44.51 ± 11.58 a

Values in the columns represent mean ± standard deviation of the adjusted values from samples in triplicate.
Comparisons are indicated by small letters in rows. The combined raw ingredients column is used as the reference to which the values in the end-product column are compared.
Values followed by the same letter do not differ significantly. If letters differ, then $P < 0.05$ while letters in parenthesis () then $P < 0.1$.
Abbreviations: TE-Trolox equivalents, GAE-Gallic acid equivalents

4.3 Stewed tomato and onion flavoured with parsley using red palm oil as ingredient

Table 4.9 indicates the TAC and antioxidant content of the three recipes and their preparation steps when RPO was used as ingredient. This table is similar to Table 4.2, on p.78. The layout of the results discussed below is based on section 4.2 when SFO was used as an ingredient.

The L-ORAC$_{FL}$ values expressed as a percentage of the TAC was, in general, low in all three the recipes, being less than 3%. The H-ORAC$_{FL}$ values contributed most to the TAC of the three recipes (Table 4.9). The lycopene content contributed between 73.9 and 95.1% to the total carotenoid content of the three recipes steps (Table 4.9).

Table 4.9: Total antioxidant capacity and antioxidant content of the raw combined ingredients and the three recipes and their preparation steps using red palm oil as ingredient

Recipe		Control recipe			Recipe 1			Recipe 2			
Step description	Combined raw ingredients[a]	Sautéed onion	Cooked onion and tomato	Stewed tomato and onion flavoured with parsley (end-product)	Cooked tomato	Cooked tomato and raw onion	Stewed tomato and onion flavoured with parsley (end-product)	Cooked tomato	Sautéed onion	Cooked tomato and onion	Stewed tomato and onion flavoured with parsley (end-product)
Steps		One	Two	Three	One	Two	Three	One	Two	Three	Four
L-ORAC$_{FL}$ (µmol of TE/g)	0.13	0.02 ± 0.00	0.10 ± 0.02	0.10 ± 0.01	0.07 ± 0.02	0.09 ± 0.01	0.12 ± 0.01	0.09 ± 0.05	0.04 ± 0.01	0.14 ± 0.02	0.14 ± 0.03
L-ORAC$_{FL}$/TAC (%)	2.7	0.7	1.3	1.2	2.9	1.9	2.2	2.9	1.8	2.2	2.0
H-ORAC$_{FL}$ (µmol of TE/g)	4.75	2.80 ± 0.32	7.44 ± 1.28	8.06 ± 0.67	2.29 ± 0.54	4.56 ± 0.33	5.39 ± 0.32	3.02 ± 0.03	2.22 ± 0.40	6.14 ± 0.89	7.04 ± 2.51
H-ORAC$_{FL}$/TAC (%)	97.3	99.9	98.7	98.8	97.0	98.1	97.8	97.1	98.7	97.8	98.1
TAC (µmol of TE/g)	4.88	2.82 ± 0.32	7.54 ± 1.28	8.16 ± 0.66	2.36 ± 0.53	4.65 ± 0.32	5.51 ± 0.32	3.11 ± 0.08	2.25 ± 0.40	6.28 ± 0.91	7.18 ± 2.54
Total carotenoids (µg/g)	4.91	ND	9.63 ± 3.20	8.61 ± 2.55	4.70 ± 2.70	6.29 ± 2.31	7.58 ± 2.65	4.79 ± 1.53	ND	6.49 ± 1.41	8.04 ± 0.56
Lycopene (µg/g)	4.18	ND	7.12 ± 2.22	6.92 ± 2.01	4.40 ± 1.95	5.74 ± 1.05	7.21 ± 2.51	4.04 ± 1.27	ND	5.49 ± 1.21	6.91 ± 0.68
Lycopene/Total carotenoids (%)	85.1	-	74.0	80.4	93.6	91.3	95.1	84.3	-	84.6	85.9
Total polyphenols (mg GAE/g)	1.58	0.37 ± 0.06	1.43 ± 0.34	1.57 ± 0.26	1.19 ± 0.09	1.59 ± 0.03	1.55 ± 0.13	1.20 ± 0.15	0.40 ± 0.04	1.51 ± 0.05	1.62 ± 0.03
Vitamin C (µg/g)	40.06	9.19 ± 0.99	65.70 ± 6.81	65.89 ± 5.76	35.30 ± 5.33	39.30 ± 6.30	46.81 ± 3.02	51.74 ± 12.86	7.97 ± 1.67	66.43 ± 24.79	70 61 ± 25.98

[a]-Values as obtained after factor adjustments as explained in 3.4.4.
Values in the columns represent mean ± standard deviation of the adjusted values from samples in triplicate.
Abbreviations: TE-Trolox equivalents, ND-Not detected, GAE-Gallic acid equivalents, -Not determined.

4.3.1 Effect of thermal household processing on the sautéed onion and cooked tomato

4.3.1.1 Sautéed onion

The control recipe and Recipe 2 both included sautéed onion in step one and step two respectively. The differences in these two recipes were previously discussed in 4.2.1.1.

The L-ORAC$_{FL}$ value of the sautéed onion was significantly ($P < 0.05$) higher when Recipe 2 (0.04 ± 0.01 µmol TE/g) was used in comparison to that when the control recipe (0.02 ± 0.00 µmol TE/g) was used. The H-ORAC$_{FL}$ value of the control recipe (2.80 ± 0.32 µmol TE/g) was marginally ($P < 0.1$) higher than that of Recipe 2 (2.22 ± 0.40 µmol TE/g). The TAC was also marginally ($P < 0.1$) higher for the control recipe (2.82 ± 0.32 µmol TE/g) than for Recipe 2 (2.25 ± 0.40 µmol TE/g).

Table 4.10: Effect of thermal household processing on the total antioxidant capacity and antioxidant content of the sautéed onion prepared by two different recipes using red palm oil as ingredient

	Control recipe	Recipe 2
L-ORAC$_{FL}$ µmol TE/g	0.02 ± 0.00 a	0.04 ± 0.01 b
H-ORAC$_{FL}$ µmol TE/g	2.80 ± 0.32 a	2.22 ± 0.40 (b)
TAC µmol TE/g	2.82 ± 0.32 a	2.25 ± 0.40 (b)
Total carotenoids µg/g	ND	ND
Lycopene µg/g	ND	ND
Total polyphenols mg GAE/g	0.37 ± 0.06 a	0.40 ± 0.04 a
Vitamin C µg/g	9.19 ± 0.99 a	7.97 ± 1.67 a

Values in the columns represent mean ± standard deviation of the adjusted values from samples in triplicate.
Values in rows followed by the same letter do not differ significantly. If letters differ then $P < 0.05$ while letters in parenthesis (), then $P < 0.1$.
Abbreviations: TE-Trolox equivalents, ND-Not detected GAE-Gallic acid equivalents

The total polyphenol content of the sautéed onion was similar ($P > 0.1$) for the two recipes (Table 4.10). No difference ($P > 0.1$) was found in the vitamin C content of the sautéed onion for the two recipes (Table 4.10).

4.3.1.2 Cooked tomato

The L-ORAC$_{FL}$ value of the cooked tomato was similar ($P > 0.1$) for Recipes 1 and 2 (Table 4.11). The H-ORAC$_{FL}$ value was marginally ($P < 0.1$) higher for Recipe 2 (3.02 ± 0.03 µmol TE/g) than for Recipe 1 (2.29 ± 0.54 µmol TE/g). The TAC of the cooked tomato was also marginally ($P < 0.1$) higher for Recipe 2 (3.11 ± 0.08 µmol TE/g) than for Recipe 1 (2.36 ± 0.53 µmol TE/g).

Table 4.11: Effect of thermal household processing on the total antioxidant capacity and antioxidant content of the cooked tomato prepared by two different recipes using red palm oil as ingredient

	Recipe 1	Recipe 2
L-ORAC$_{FL}$ µmol TE/g	0.07 ± 0.02 a	0.09 ± 0.05 a
H-ORAC$_{FL}$ µmol TE/g	2.29 ± 0.54 a	3.02 ± 0.03 (b)
TAC µmol TE/g	2.36 ± 0.53 a	3.11 ± 0.08 (b)
Total carotenoids µg/g	4.70 ± 2.70 a	4.79 ± 1.53 a
Lycopene µg/g	4.40 ± 1.95 a	4.04 ± 1.27 a
Total polyphenols mg GAE/g	1.19 ± 0.09 a	1.20 ± 0.15 a
Vitamin C µg/g	35.30 ± 5.33 a	51.74 ± 12.86 (b)

Values in the columns represent mean ± standard deviation of the adjusted values from samples in triplicate.
Values in rows followed by the same letter do not differ significantly If letters differ, then $P < 0.05$ while letters in parenthesis (), then $P < 0.1$.
Abbreviations: TE-Trolox equivalents, GAE-Gallic acid equivalents.

The total carotenoid content of the cooked tomato was similar ($P > 0.1$) in the two recipes (Table 4.11). Similar ($P > 0.1$) contents for lycopene were also found after using the two recipes (Table 4.11).

The total polyphenol content of the cooked tomato was similar ($P > 0.1$) for Recipes 1 and 2 (Table 4.11). The vitamin C content of the cooked tomato in Recipe 2 (51.74 ± 12.86 µg/g) was marginally ($P < 0.1$) higher than that of Recipe 1 (35.30 ± 5.33 µg/g).

4.3.2 Effect of thermal household processing on the stewed tomato and onion flavoured with parsley

Table 4.12 indicates the effect of thermal household processing on the TAC and antioxidant content from the combined raw ingredients to the end-product for the three recipes.

Table 4.12: Effect of thermal household processing on the total antioxidant capacity and antioxidant content from the combined raw ingredients to the end-product using red palm oil as ingredient

	Combined raw ingredients	**Control recipe** (end-product)	**Recipe 1** (end-product)	**Recipe 2** (end-product)
L-ORAC$_{FL}$ µmol TE/g	0.13 a	0.10 ± 0.01 bA	0.12 ± 0.01 aA	0.14 ± 0.03 aA
H-ORAC$_{FL}$ µmol TE/g	4.75 a	8.06 ± 0.67 bA	5.39 ± 0.32 (b)B	7.04 ± 2.51 aAB
TAC µmol TE/g	4.88 a	8.16 ± 0.66 bA	5.51 ± 0.32 aB	7.18 ± 2.54 aAB
Total carotenoids µg/g	4.91 a	8.61 ± 2.55 (b)A	7.58 ± 2.65 aA	8.04 ± 0.56 bA
Lycopene µg/g	4.18 a	6.92 ± 2.01 aA	7.21 ± 2.51 aA	6.91 ± 0.68 bA
Total polyphenols mg GAE/g	1.58 a	1.57 ± 0.26 aA	1.55 ± 0.13 aA	1.62 ± 0.03 aA
Vitamin C µg/g	40.06 a	65.89 ± 5.76 bA	46.81 ± 3.02 (b)B	70.61 ± 25.98 (b)AB

Values in the columns represent mean ± standard deviation of the adjusted values from samples in triplicate
Intra-recipe step comparisons are indicated by small letters in rows. The combined raw ingredients column is used as the reference to which the values in the end-product columns are compared.
Inter-recipe step comparisons are indicated by capital letters in rows. The end-products are compared with one another
Values followed by the same letter do not differ significantly. If letters differ, then $P < 0.05$ while letters in parenthesis (), then $P < 0.1$.
Abbreviations: TE-Trolox equivalents, GAE-Gallic acid equivalents.

4.3.2.1 Lipophilic oxygen radical absorbance capacity

When comparing the L-ORAC$_{FL}$ values of the combined raw ingredients (0.13 ± 0.00 µmol TE/g) with that of the end-product (0.10 ± 0.01 µmol TE/g), thermal household processing resulted in a significant ($P < 0.05$) decrease (-24.5%) when the control recipe was used. Thermal household processing had no effect ($P > 0.1$) on the L-ORAC$_{FL}$ values from the combined raw ingredients to the end-product when Recipes 1 and 2 were used (Tables 4.12). The L-ORAC$_{FL}$ values of the end-products was similar ($P > 0.1$) for the three recipes used (Table 4.12). During thermal household processing of the control recipe a negative correlation was found between the L-ORAC$_{FL}$ value and the total carotenoid content ($r = -0.81$), and between the L-ORAC$_{FL}$ value and the lycopene content ($r = -0.76$).

4.3.2.2 Hydrophilic oxygen radical absorbance capacity

When comparing the H-ORAC$_{FL}$ values of the combined raw ingredients (4.75 ± 0.00 µmol TE/g) with that of the end-product (8.06 ± 0.67 µmol TE/g), thermal household processing resulted in a significant ($P < 0.05$) increase (69.7%) when the control recipe was used. A marginal ($P < 0.1$) increase (13.6%) in the H-ORAC$_{FL}$ value was also found from the combined raw ingredients (4.75 ± 0.00 µmol TE/g) to the end-product when Recipe 1 (5.39 ± 0.32 µmol TE/g) was used. Thermal household processing had no effect ($P > 0.1$) on the H-ORAC$_{FL}$ value when Recipe 2 was used (Tables 4.12). The H-ORAC$_{FL}$ value of the end-product when the control recipe was used was significantly ($P < 0.05$) higher than that of Recipe 1, that of the control recipe was similar ($P > 0.1$) to that of Recipe 2, and that of Recipe 1 was similar ($P > 0.1$) to that of Recipe 2 (Table 4.12).

4.3.2.3 Total antioxidant capacity

When comparing the TAC of the combined raw ingredients (4.88 ± 0.00 µmol TE/g) with that of the end-product (8.16 ± 0.66 µmol TE/g), thermal household processing resulted in a significant ($P < 0.05$) increase (67.1%) when the control recipe was used. Thermal household processing had no effect ($P > 0.1$) on the TAC from the combined raw ingredients to the end-product when Recipes 1 and 2 were used (Tables 4.12). The TAC of the end-product when using the control recipe (8.16 ± 0.66 µmol TE/g) was significantly ($P < 0.05$) higher than that of Recipe 1 (5.51 ± 0.32 µmol TE/g). The TAC of the end-product when the control recipe was used was similar ($P > 0.1$) to that of Recipe 2 (7.18 ± 2.54 µmol TE/g), and that of Recipe 1 was similar ($P > 0.1$) to that of Recipe 2 (Figure 4.2).

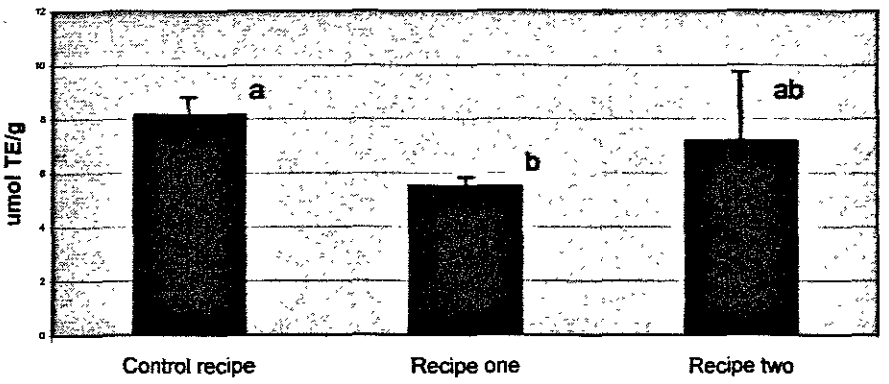


Figure 4.2: Total antioxidant capacity of the end-products using red palm oil as ingredient

Letters followed by the same letter do not differ significantly. If letters differ, then $P < 0.05$.
Abbreviation: TE-Trolox equivalents.

4.3.2.4 Total carotenoid content

When comparing the total carotenoid content of the combined raw ingredients (4.91 ± 0.00 µg/g) with that of the end-product (8.61 ± 2.55 µg/g), thermal household processing resulted in a marginal ($P < 0.1$) increase (75.5%) when the control recipe was used. Thermal household processing had no effect ($P > 0.1$) when Recipe 1 was used (Tables 4.12). When comparing the total carotenoid content of the combined raw ingredients (4.91 ± 0.00 µg/g) with that of the end-product (8.04 ± 0.56 µg/g), thermal household processing resulted in a significant ($P < 0.05$) increase (63.8%) when the Recipe 2 was used. The total carotenoid content of the end-product was similar ($P > 0.1$) when the three recipes respectively were used (Table 4.12).

4.3.2.5 Lycopene content

Thermal household processing had no effect ($P > 0.1$) on the lycopene content from the combined raw ingredients to the end-product when the control recipe or Recipe 1 was used (Table 4.12). When comparing the lycopene content of the combined raw ingredients (4.18 ± 0.00 µg/g) with that of the end-product (6.91 ± 0.68 µg/g), thermal household processing resulted in a significant ($P < 0.05$) increase (65.6%) when Recipe 2 was used. The lycopene content was similar ($P > 0.1$) for the end-product when the three recipes respectively were used (Table 4.12).

4.3.2.6 Total polyphenol content

Thermal household processing had no effect ($P > 0.1$) on the total polyphenol content from the combined raw ingredients to the end-product when the three recipes respectively were used (Table 4.12). No significant differences ($P > 0.1$) were found for the total polyphenol content of the end-product when the three recipes respectively were used (Table 4.12). During the thermal household processing a significant positive correlation was found between the H-$ORAC_{FL}$ value and the total polyphenol content for the three recipes respectively (control recipe: $r = 0.75$, Recipe 1: $r = 0.86$, and Recipe 2: $r = 0.75$).

4.3.2.7 Vitamin C content

When comparing the vitamin C content of the combined raw ingredients (40.06 ± 0.00 µg/g) with that of the end-product (65.89 ± 5.76 µg/g), thermal household processing resulted in a significant ($P < 0.05$) increase (64.5%) when the control recipe was used. Thermal household processing marginally ($P < 0.1$) increased the vitamin C content when Recipes 1

(46.81 ± 3.02 µg/g) and 2 (70.61 ± 25.98 µg/g) were used (Table 4.12). The vitamin C content of the end-product when the control recipe was used, was significantly ($P < 0.05$) higher than that of Recipe 1 but similar ($P > 0.1$) to that of Recipe 2 (Table 4.12). The vitamin C content of the end-products when Recipes 1 and 2 were used was also similar ($P > 0.1$) (Table 4.12). During the thermal household processing a significant positive correlation was found between the H-ORAC$_{FL}$ value and the vitamin C content when the control recipe ($r = 0.96$) and Recipe 2 ($r = 0.77$) were used.

4.3.3 Effect of the addition of parsley

Table 4.13 indicates the effect of the addition of parsley on the TAC and antioxidant content when RPO was used as ingredient. Comparisons indicated in this table are only those within a recipe. None of the parameters investigated changed ($P > 0.1$) with the addition of parsley when the control recipe was used (Table 4.13). The addition of parsley contributed to a significant ($P < 0.05$) increase in the L-ORAC$_{FL}$ value (29.6%) when Recipe 1 was used. The addition of parsley also contributed to a marginal ($P < 0.1$) increase in the H-ORAC$_{FL}$ value (18.3%), TAC (18.5%) and vitamin C content (19.1%) when Recipe 1 was used. When using Recipe 2 the addition of parsley significantly ($P < 0.05$) increased the total polyphenol content (7.1 %). With the addition of the parsley a significant positive correlation was found between the L-ORAC$_{FL}$ value and the total carotenoid ($r = 0.77$), and between the L-ORAC$_{FL}$ value and the lycopene content ($r = 0.92$) when the control recipe was used.

Table 4.13: Effect of the addition of parsley on the total antioxidant capacity and antioxidant content during the preparation of the three recipes using red palm oil as ingredient

	Control recipe		Recipe 1		Recipe 2	
	Cooked onion and tomato Step 3	End-product Step 4	Cooked tomato and raw onion Step 3	End-product Step 4	Cooked tomato and onion Step 4	End-product Step 5
L-ORAC$_{FL}$ µmol TE/g	0.10 ± 0.02 a	0.10 ± 0.01 a	0.09 ± 0.01 a	0.12 ± 0.01 b	0.14 ± 0.02 a	0.14 ± 0.03 a
H-ORAC$_{FL}$ µmol TE/g	7.44 ± 1.28 a	8.06 ± 0.67 a	4.56 ± 0.33 a	5.39 ± 0.32 (b)	6.14 ± 0.89 a	7.04 ± 2.51 a
TAC µmol TE/g	7.54 ± 1.28 a	8.16 ± 0.66 a	4.65 ± 0.32 a	5.51 ± 0.32 (b)	6.28 ± 0.91 a	7.18 ± 2.54 a
Total carotenoids µg/g	9.63 ± 3.20 a	8.61 ± 2.55 a	6.92 ± 2.31 a	7.58 ± 2.65 a	6.49 ± 1.41 a	8.04 ± 0.56 a
Lycopene µg/g	7.12 ± 2.22 a	6.92 ± 2.01 a	5.74 ± 1.05 a	7.21 ± 2.51 a	5.49 ± 1.21 a	6.91 ± 0.68 a
Total polyphenols mg GAE/g	1.43 ± 0.34 a	1.57 ± 0.26 a	1.59 ± 0.03 a	1.55 ± 0.13 a	1.51 ± 0.05 a	1.62 ± 0.03 b
Vitamin C µg/g	65.70 ± 6.81 a	65.89 ± 5.76 a	39.30 ± 6.30 a	46.81 ± 3.02 (b)	66.43 ± 24.79 a	70.61 ± 25.98 a

Values in the columns represent mean ± standard deviation of the adjusted values from samples in triplicate.
Comparisons are indicated by small letters in rows. The first column for each investigated ingredient is used as the reference to which the values in the following column are compared.
Values in rows followed by the same letter do not differ significantly. If letters differ, then $P < 0.05$ while letters in parenthesis (), then $P < 0.1$.
Abbreviations: TE-Trolox equivalents, GAE-Gallic acid equivalents.

The TAC and antioxidant content of the additional samples (sautéed onion and parsley, cooked tomato and parsley and heated parsley), using the time allocations and heat applications of the control recipe, are indicated in Table 4.14. Adding parsley to the sautéed onion contributed to a significant ($P < 0.05$) decrease in the total carotenoid content (-10.5%). The addition of parsley, however, marginally ($P < 0.1$) increased the H-ORAC$_{FL}$ value (32.8%) and TAC (32.5%) of the sautéed onion.

The addition of parsley contributed to a significant ($P < 0.05$) increase in the L-ORAC$_{FL}$ value (33.3%), H-ORAC$_{FL}$ value (36.2%), TAC (35.9%) and vitamin C content (19.3%) of the cooked tomato. The total carotenoid (23.0%) and lycopene (21.8%) contents increased marginally ($P < 0.1$) while the total polyphenol content remained similar ($P > 0.1$) (Table 4.14).

Thermal household processing significantly ($P < 0.05$) increased all the parameters (L-ORAC$_{FL}$ value: 65.8%, H-ORAC$_{FL}$ value: 20.1%, TAC: 21.4%, total carotenoid content: 92.5%, total polyphenol content: 1.1%, and vitamin C content: 87.8%) of the heated parsley. The lycopene content was not determined.

Table 4.14: Effect of the addition of parsley on the total antioxidant capacity and antioxidant content of the sautéed onion and the cooked tomato using red palm oil as ingredient

	Sautéed onion		Cooked tomato		Parsley	
	Without parsley	Parsley added	Without parsley	Parsley added	Raw	Heated
L-ORAC$_{FL}$ µmol TE/g	0.05 ± 0.01 a	0.06 ± 0.00 a	0.14 ± 0.01 a	0.21 ± 0.01 b	0.01 ± 0.00 a	1.20 ± 0.04 b
H-ORAC$_{FL}$ µmol TE/g	2.21 ± 0.20 a	3.29 ± 0.35 (b)	3.03 ± 0.15 a	4.75 ± 0.50 b	0.26 ± 0.05 a	41.94 ± 4.73 b
TAC µmol TE/g	2.26 ± 0.20 a	3.35 ± 0.35 (b)	3.18 ± 0.14 a	4.96 ± 0.51 b	0.27 ± 0.05 a	43.14 ± 4.96 b
Total carotenoids µg/g	1.69 ± 0.14 b	1.53 ± 0.17 b	12.11 ± 2.36 a	15.73 ± 4.16 (b)	0.03 ± 0.03 a	79.58 ± 7.34 b
Lycopene µg/g	-	-	9.21 ± 2.14 a	11.78 ± 3.41 (b)	-	-
Total polyphenols mg GAE/g	0.36 ± 0.04 a	0.33 ± 0.02 a	1.00 ± 0.06 a	1.17 ± 0.05 a	0.03 ± 0.00 a	0.13 ± 0.03 b
Vitamin C µg/g	18.17 ± 1.21 a	21.84 ± 2.60 a	87.89 ± 1.62 a	108.85 ± 6.63 b	0.26 ± 0.03 a	419.48 ± 14.11 b

Values in the columns represent mean ± standard deviation of the adjusted values from samples in triplicate.
Comparisons are indicated by small letters in rows. The first column for each investigated ingredient is used as the reference to which the values in the following column are compared.
Values in rows followed by the same letter do not differ significantly. If letters differ, then $P < 0.05$ while letters in parenthesis (), then $P < 0.1$.
Abbreviations: TE-Trolox equivalents, GAE-Gallic acid equivalents, -Not determined

4.3.4 Effect of water loss during thermal household processing

Table 4.15 indicates the TAC and antioxidant content of the end-products when water loss was considered. When the control recipe was used the H-ORAC$_{FL}$ value (-53.6%) and total polyphenol content (-38.9%) decreased significantly ($P < 0.05$). The remaining parameters (L-ORAC$_{FL}$, TAC, total carotenoids, lycopene and vitamin C) remained the same when the control recipe was used ($P > 0.1$) (Table 4.15). When Recipe 1 was used, the L-ORAC$_{FL}$ value (-36.9%), H-ORAC$_{FL}$ value (-19.5%), TAC (-20.7%), total polyphenol (-30.5%) and the vitamin C (-17.4%) contents significantly ($P < 0.05$) decreased. The total carotenoid and lycopene contents remained similar ($P > 0.1$) when Recipe 1 was used. Using Recipe 2 significantly ($P < 0.05$) decreased the total polyphenol content (-38.6%) while a marginal decrease in the L-ORAC$_{FL}$ value (-37.1%) was found. For the remaining parameters the content remained the same ($P > 0.1$) (Table 4.15).

Table 4.15: Effect of water loss on the total antioxidant capacity and antioxidant content when the three recipes were prepared using red palm oil as ingredient

	Combined raw ingredients	Control recipe (end-product)	Recipe 1 (end-product)	Recipe 2 (end-product)
L-ORAC$_{FL}$ µmol TE/g	0.13 a	0.06 ± 0.01 a	0.08 ± 0.01 b	0.08 ± 0.02 (b)
H-ORAC$_{FL}$ µmol TE/g	4.75 a	4.95 ± 0.41 b	3.82 ± 0.23 b	4.22 ± 1.50 a
TAC µmol TE/g	4.88 a	4.96 ± 0.38 a	3.87 ± 0.21 b	4.28 ± 1.51 a
Total carotenoids µg/g	4.91 a	5.30 ± 1.57 a	5.37 ± 1.88 a	4.82 ± 0.33 a
Lycopene µg/g	4.18 a	4.26 ± 1.24 a	5.11 ± 1.78 a	4.15 ± 0.41 a
Total polyphenols mg GAE/g	1.58 a	0.97 ± 0.16 b	1.10 ± 0.09 b	0.97 ± 0.02 b
Vitamin C µg/g	40.06 a	40.52 ± 3.54 a	33.18 ± 2.14 b	42.35 ± 15.58 a

Values in the columns represent mean ± standard deviation of the adjusted values from samples in triplicate.
Comparisons are indicated by small letters in rows. The combined raw ingredients column is used as the reference to which the values in the end-product columns are compared.
Values followed by the same letter do not differ significantly. If letters differ, then $P < 0.05$ while letters in parenthesis (), then $P < 0.1$.
Abbreviations: TE-Trolox equivalents, GAE-Gallic acid equivalents

In summary, when the control recipe and Recipe 2 were used, both found decreases in the L-ORAC$_{FL}$ value and total polyphenol content. When Recipe 1 was used, five of the parameters (L-ORAC$_{FL}$, H-ORAC$_{FL}$, TAC, total polyphenols and vitamin C) decreased.

4.4 Comparison between the recipes when sunflower and red palm oil were used as ingredients

4.4.1 Effect of thermal household processing on the stewed tomato and onion flavoured with parsley

Only the values of the end-product in each recipe will be referred to when the results of the oils used are compared. Table 4.16 indicates the TAC and antioxidant content of the end-products after thermal household processing. Only comparisons within a recipe are indicated in Table 4.16 and text while those comparisons between the recipes are only indicated in the text.

Table 4.16: Comparison of the total antioxidant capacity and antioxidant content of the end-products prepared by the different recipes using sunflower and red palm oil as ingredients

	Control recipe		Recipe 1		Recipe 2	
	SFO	RPO	SFO	RPO	SFO	RPO
L-ORAC$_{FL}$ µmol TE/g	0.13 ± 0.05 a	0.10 ± 0.01 a	0.14 ± 0.00 a	0.12 ± 0.01 b	0.13 ± 0.08 a	0.14 ± 0.03 a
H-ORAC$_{FL}$ µmol TE/g	7.11 ± 0.21 a	8.06 ± 0.67 b	5.20 ± 1.53 a	5.39 ± 0.32 a	7.12 ± 3.50 a	7.04 ± 2.51 a
TAC µmol TE/g	7.23 ± 0.16 a	8.16 ± 0.66 b	5.34 ± 1.53 a	5.51 ± 0.32 a	7.25 ± 3.45 a	7.18 ± 2.54 a
Total carotenoids µg/g	7.13 ± 0.93 a	8.61 ± 2.55 a	8.62 ± 6.07 a	7.58 ± 2.65 a	7.16 ± 2.39 a	8.04 ± 0.56 a
Lycopene µg/g	5.68 ± 1.03 a	6.92 ± 2.01 a	7.14 ± 5.31 a	7.21 ± 2.51 a	5.78 ± 2.15 a	6.91 ± 0.68 a
Total polyphenols mg GAE/g	1.63 ± 0.43 a	1.57 ± 0.26 a	1.48 ± 0.10 a	1.55 ± 0.13 a	1.54 ± 0.30 a	1.62 ± 0.03 a
Vitamin C µg/g	82.10 ± 15.91a	65.89 ± 5.76 a	72.98 ± 5.47 a	46.81 ± 3.02 b	73.86 ± 19.21 a	70.61 ± 25.98 a

Values in the columns represent mean ± standard deviation of the adjusted values from samples in triplicate.
Comparisons are indicated by small letters in rows. The first column for each investigated recipe using SFO is used as the reference to which the values in the following column using RPO are compared.
Values in rows followed by the same letter do not differ significantly. If letters differ, then $P < 0.05$ while letters in parenthesis (), then $P < 0.1$.
Abbreviations: SFO-sunflower oil, RPO-red palm oil, TE-Trolox equivalents, GAE-Gallic acid equivalents

In general, no differences ($P > 0.1$) were found in the parameters investigated when SFO or RPO was used in a recipe. Using RPO in the control recipe provided for a marginally ($P < 0.1$) higher H-ORAC$_{FL}$ value and TAC (8.06 ± 0.67 µmol TE/g and 8.16 ± 0.66 µmol TE/g) than when SFO (7.11±0.21µmol TE/g and 7.23 ± 0.16 µmol TE/g) was used. Using SFO in Recipe 1 provided for a marginally ($P < 0.1$) higher L-ORAC$_{FL}$ value (0.14 ± 0.00 µmol TE/g) and a significantly ($P < 0.05$) higher (72.98 ± 5.47 µg/g) vitamin C content than when RPO (0.12 ± 0.01 µmol TE/g and 46.81 ± 3.02 µg/g, respectively) was used. No differences ($P > 0.1$) were found for Recipe 2.

Comparing the three recipes using SFO and RPO with one another indicated more differences. Using SFO in Recipe 1 provided a significantly ($P < 0.05$) higher L-ORAC$_{FL}$ value (0.14 ± 0.00 µmol TE/g) than using RPO in the control recipe (0.10 ± 0.01 µmol TE/g). The L-ORAC$_{FL}$ values were similar ($P > 0.1$) for the other recipes (Table 4.16).

The H-ORAC$_{FL}$ value when SFO was used in the control recipe (7.11 ± 0.21 µmol TE/g) was significantly ($P < 0.05$) higher than when RPO was used in Recipe 1 (5.39 ± 0.32 µmol TE/g). The H-ORAC$_{FL}$ value was significantly ($P < 0.05$) higher when the control recipe (8.16 ± 0.66 µmol TE/g) was prepared with RPO than it was using Recipe 1 with SFO (5.34 ± 1.53 µmol TE/g). No other differences ($P > 0.1$) were found between H-ORAC$_{FL}$ values for the different recipes when the two oils were used (Table 4.16).

The TAC in the control recipe when SFO (7.23 ± 0.16 µmol TE/g) was used was significantly ($P < 0.05$) higher than in Recipe 1 when RPO (5.51 ± 0.32 µmol TE/g) was used. The TAC was significantly ($P < 0.05$) higher when the control recipe was prepared with RPO (8.06 ± 0.67 µmol TE/g) than it was using Recipe 1 with SFO (5.20 ± 1.53 µmol TE/g). No other differences ($P > 0.1$) were found in the TAC between the recipes (Table 4.16).

No differences ($P > 0.1$) were found in the total carotenoid content when the oils were used in the three recipes (Table 4.16). Using RPO in Recipe 2 (6.91 ± 0.68 µg/g) provided for a marginally ($P < 0.1$) higher lycopene content than when SFO was used in the control recipe (5.68 ± 1.03 µg/g). For the other recipes, when the two oils were used, the lycopene content was similar ($P > 0.1$) (Table 4.16).

The total polyphenol content of Recipe 2 using RPO (1.62 ± 0.03 mg GAE/g) was marginally ($P < 0.1$) higher than that of Recipe 1 when SFO (1.48 ± 0.10 mg GAE/g) was used. No other differences ($P > 0.1$) were found in these antioxidants between the oils and the recipes used (Table 4.16). The vitamin C content of the control recipe using SFO (82.10 ± 15.91 µg/g) was significantly ($P < 0.05$) higher than Recipe 1 using RPO (46.81 ± 3.02 µg/g). No other differences ($P > 0.1$) were found between the oil uses in the recipes for the vitamin C content (Table 4.16).

4.4.2 Effect of water loss during thermal household processing

The comparisons between the recipes and the use of the two oils when water loss is considered are based on those when the effect of thermal household processing (4.4.1) was considered. Only comparisons within a recipe are indicated in the table while those comparisons between the recipes are indicated in the text. The TAC and antioxidant content of the end-products after water loss was considered are indicated in Table 4.17.

Table 4.17: Comparison of the total antioxidant capacity and antioxidant content of the recipes using sunflower and red palm oil when water loss during thermal household processing is considered

	Control recipe		Recipe 1		Recipe 2	
	SFO	RPO	SFO	RPO	SFO	RPO
L-ORAC$_{FL}$ µmol TE/g	0.08 ± 0.03 a	0.06 ± 0.01 a	0.10 ± 0.00 a	0.08 ± 0.01 b	0.08 ± 0.05 a	0.08 ± 0.02 a
H-ORAC$_{FL}$ µmol TE/g	4.45 ± 0.13 a	4.95 ± 0.41 a	3.74 ± 1.10 a	3.82 ± 0.23 a	4.29 ± 2.11 a	4.22 ± 1.50 a
TAC µmol TE/g	4.50 ± 0.08, a	4.96 ± 0.38 a	3.84 ± 1.09 a	3.87 ± 0.21 a	4.23 ± 1.95 a	4.28 ± 1.51 a
Total carotenoids µg/g	4.46 ± 0.58 a	5.30 ± 1.57 a	6.20 ± 4.36 a	5.37 ± 1.88 a	4.31 ± 1.44 a	4.82 ± 0.33 a
Lycopene µg/g	3.55 ± 0.65 a	4.26 ± 1.24 a	5.13 ± 3.82 a	5.11 ± 1.78 a	3.48 ± 1.30 a	4.15 ± 0.41 a
Total polyphenols mg GAE/g	1.02 ± 0.27 a	0.97 ± 0.16 a	1.07 ± 0.07 a	1.10 ± 0.09 a	0.93 ± 0.18 a	0.97 ± 0.02 a
Vitamin C µg/g	51.39 ± 9.96 a	40.52 ± 3.54 a	52.50 ± 3.94 a	33.18 ± 2.14 b	44.51 ± 11.58 a	42.35 ± 15.58 a

Values in the columns represent mean ± standard deviation of the adjusted values from samples in triplicate.
Comparisons are indicated by small letters in rows. The first column for each investigated recipe using SFO is used as the reference to which the values in the following columns using RPO are compared
Values in rows followed by the same letter do not differ significantly. If letters differ then $P < 0.05$ while letters in parenthesis (), then $P < 0.1$.
Abbreviations: SFO-sunflower oil, RPO-red palm oil, TE-Trolox equivalents, GAE-Gallic acid equivalents

No differences ($P > 0.1$) were found in the control recipe and in Recipe 2 when the two oils were used, respectively. Using SFO provided for a marginally ($P < 0.1$) higher L-ORAC$_{FL}$ value (0.10 µmol TE/g) and a significantly ($P < 0.05$) higher vitamin C content (52.50 µg/g) in Recipe 1 than when RPO (0.08 µmol TE/g and 33.18 µg/g, respectively) was used (Table 4.17).

Comparing the three recipes and the oil use with one another provided no differences ($P > 0.1$) in the L-ORAC$_{FL}$ value, and the total carotenoid and lycopene contents (Table 4.17). The H-ORAC$_{FL}$ value and TAC when SFO was used in the control recipe (4.45 µmol TE/g and 4.50 µmol TE/g, respectively) were significantly ($P < 0.05$) higher than when RPO was used in Recipe 1 (3.82 µmol TE/g and 3.87 µmol TE/g, respectively). No other differences ($P > 0.1$) were found between the different recipes when the two oils were used in the H-ORAC$_{FL}$ value and TAC (Table 4.17).

The total polyphenol content of Recipe 1 using SFO (1.07 mg GAE/g) was marginally ($P < 0.1$) higher than that of Recipe 2 when RPO (0.97 mg GAE/g) was used. No other differences ($P > 0.1$) were found in the total polyphenol content between the oils and the recipes used (Table 4.17). The vitamin C content of the control recipe using SFO (51.39 µg/g) was significantly ($P < 0.05$) higher than that of Recipe 1 with RPO (33.18 µg/g). No

other differences ($P > 0.1$) were found between the oil uses in the recipes for the vitamin C content (Table 4.17).

CHAPTER 5

DISCUSSION

The contents [TAC (with reference to the L-ORAC$_{FL}$ and H-ORAC$_{FL}$ values) and the antioxidant content (total carotenoids, lycopene, total polyphenols and vitamin C)] of the three recipes were presented in four sections in Chapter 4: i) raw recipe ingredients, ii) stewed tomato and onion flavoured with parsley using SFO as ingredient, iii) stewed tomato and onion flavoured with parsley using RPO as ingredient, and iv) a comparison between the recipes when SFO and RPO were used as ingredients. The discussion of these results will be done according to the primary and subsidiary objectives investigated in this study. In this field of antioxidants, no similar studies have been published that could be compared to those results found in this study.

5.1 Raw recipe ingredients

Five raw recipe ingredients were used for the preparation of the recipes. In general, the H-ORAC$_{FL}$ values of the raw recipe ingredients contributed most to the TAC. This finding is consistent with previous studies on raw vegetables (Halvorsen *et al.*, 2002: 466; Wu *et al.*, 2004a: 4030; Ninfali *et al.*, 2005: 260) as the water-soluble polyphenols contribute most to the TAC of raw vegetables (Wu *et al.*, 2004a: 4030).

Parsley was the raw recipe ingredient with the highest TAC, followed by onion and tomato. Halvorsen and co-workers (2002: 466) determined the TAC for tomatoes, onion and parsley and also found the TAC of parsley to be the highest, followed by the onion and then the tomato. It is, however, not possible to compare the absolute values of this study with that of Halvorsen and co-workers (2002: 466) as different analytical methods were applied. Parsley's high TAC could be attributed to the fact that it had the highest L-ORAC$_{FL}$, H-ORAC$_{FL}$ and total polyphenol content. Although, tomato had the highest vitamin C and lycopene content, the onion had a higher TAC which could be ascribed to the higher total polyphenol content of the onion. This confirms that the total polyphenols are the major contributor to the H-ORAC$_{FL}$ values and as a result to the TAC.

Wu and co-workers (2004a: 4030) found similar L-ORAC$_{FL}$ (0.24 ± 0.07 µmol TE/g), H-ORAC$_{FL}$ (3.13 ± 0.69 µmol TE/g) and TAC (3.37 µmol TE/g) values for US tomatoes as those found in this study (the same analytical methods were used). However, the total polyphenol content found in the US tomatoes (0.80 ± 0.12 mg GAE/g) was slightly lower than that found in this study. The lycopene content accounted for most of the total carotenoid content of the

tomatoes, which is consistent with previous findings that indicate that lycopene on average constitutes about 79 - 85% of the total carotenoid content of tomatoes (Leonardi *et al.*, 2000: 4725).

The TAC of the yellow and sweet onion varieties grown in the US is 10.29 µmol TE/g (H-$ORAC_{FL}$: 0.12 ± 0.03 µmol TE/g and L-$ORAC_{FL}$: 10.17 ± 1.89 µmol TE/g) and 6.15 µmol TE/g (H-$ORAC_{FL}$: 0.21 ± 0.09 µmol TE/g and L-$ORAC_{FL}$: 5.94 ± 0.74 µmol TE/g), respectively (Wu *et al.*, 2004a: 4030). The TAC and H-$ORAC_{FL}$ value of the brown South African onion were within the range of that of the yellow and sweet US onions, but the L-$ORAC_{FL}$ value was higher than that of the US onions. The total polyphenol content found in the US yellow (0.91 ± 0.09 mg GAE/g) and sweet (0.74 ± 0.20 mg GAE/g) onion varieties (Wu *et al.*, 2004a: 4030) was slightly lower than that found in this study. This was also the tendency with the total polyphenol content of the tomatoes. The L-$ORAC_{FL}$ value and total polyphenol content of raw US onions (sweet and yellow), however, differed from the brown onion used in this study. These differences could be ascribed to varietal diversity found in both the onion colour and flavour (Yang *et al.*, 2004: 6788) that would influence the composition and concentration of the total polyphenol content and the TAC of the three onion varieties involved. Other factors influencing the antioxidant content of vegetables are seasonal changes and agricultural practices (Leonardi *et al.*, 2000: 4727; Martinez-Valverde *et al.*, 2002: 323), which may be responsible for the lower total polyphenol content found in the tomatoes. The research results of Wu and co-workers (2004a: 4030) confirm those found in this study for the TAC except for the total polyphenol content.

The antioxidant content (total carotenoid, lycopene, total polyphenol and vitamin C contents) of the SFO was not determined as it is not regarded a source of these antioxidants (Lietz *et al.*, 2001: 502; Carotino®, 2006). This was confirmed by SFO being the raw recipe ingredient with the lowest TAC. In contrast to SFO not being a good source of antioxidants, RPO had the highest total carotenoid content of all the raw recipe ingredients. Red palm oil was also superior to SFO for its TAC and antioxidant content. One of the aspects emphasised in the marketing of RPO by Carotino® is its natural carotene, tocopherol, tocotrienol and CoQ content (Carotino®, 2006), which are all fat-soluble antioxidants. These antioxidants present in RPO may be responsible for its higher TAC.

The above-mentioned raw recipe ingredients were used in the preparation of the three stewed tomato and onion flavoured with parsley recipes. The results of the three recipes using SFO or RPO as ingredient are discussed below according to the primary and subsidary objectives of this study as indicated in Chapter 1.

5.2 Effect of thermal household processing on the total antioxidant capacity of stewed tomato and onion flavoured with parsley

Thermal household processing was applied to the above-mentioned raw recipe ingredients. These ingredients were assembled during the three respective methods for the preparation of stewed tomato and onion flavoured with parsley. With the addition of each ingredient to the dish, the TAC increased owing to the antioxidant contribution of each recipe ingredient. This suggests that using more than one antioxidant rich ingredient in a dish would contribute to a higher TAC. To determine the effect of thermal household processing on the TAC and antioxidant content, the end-product of each recipe was compared with the combined raw ingredients representing the end-product (without thermal household processing).

During the preparation of the control recipe with SFO as ingredient, the TAC significantly increased on thermal household processing from the combined raw ingredients to the end-product. This could be ascribed to the increase in the vitamin C content, which positively correlated with the H-$ORAC_{FL}$ value. As found with the raw recipe ingredients, the H-$ORAC_{FL}$ contributed most to the TAC and could be the contributing factor responsible for the increase in the TAC when this recipe was used. This effect of thermal household processing on the vitamin C content was also found when RPO was used as ingredient (except for Recipe 1). In addition to this effect, it was also found that as the L-$ORAC_{FL}$ value decreased from the combined raw ingredients to the end-product when RPO was used, the total carotenoid content and the lycopene content increased on thermal household processing in the control recipe. This effect of thermal household processing could be ascribed to the RPO as it is a lipid-soluble antioxidant rich oil. It could be speculated that the total carotenoid and lycopene contents were not the major contributors to the L-$ORAC_{FL}$ value of this recipe and that other lipid-soluble antioxidants such as vitamin E and CoQ were more responsible for the decrease in the L-$ORAC_{FL}$ value. These antioxidants could be more susceptible for loss during thermal household processing and could as a result decrease the L-$ORAC_{FL}$ value. No information is, however, available to confirm the above.

Thermal household processing increased fewer of the parameters in Recipe 1 than the control recipe when SFO was used as ingredient. The TAC remained the same after thermal household processing was applied. This could be due to that no positive correlation was found between the vitamin C content and the H-$ORAC_{FL}$ value. The L-$ORAC_{FL}$ value increased significantly during thermal household processing, but did not contribute to an increase in the TAC as it is not the major contributor to the TAC. Using RPO as ingredient in Recipe 1 marginally increased the vitamin C content and the H-$ORAC_{FL}$ value, but not the

TAC which could also possibly be due to the finding that no correlation was found between the vitamin C content and the H-$ORAC_{FL}$ value.

During the preparation of Recipe 2 when SFO was used as ingredient, the TAC did not increase. This could be due to the marginal increase in the vitamin C content with no increase found in the H-$ORAC_{FL}$ value. When RPO was used, a marginal increase was also found for the vitamin C content. The total carotenoid content and lycopene content increased significantly which could be due to the use of the RPO.

During the thermal household processing of the three respective recipes when RPO was used as ingredient, the total polyphenol content remained the same for all the recipes, although a positive correlation was found between the total polyphenol content and the H-$ORAC_{FL}$ value in the three recipes. No effect was seen when SFO was used as ingredient. This suggests that RPO provide antioxidants to the recipe which protect the polyphenols during thermal household processing. No information is, however, available on the synergistic effects of polyphenols to confirm these results. The similar total polyphenol content after thermal household processing could be due to the small amount of RPO used. More (>3.0%) RPO would possibly be necessary to cause an increase in the total polyphenol content.

The vitamin C content seems to play an important role in the outcome of the TAC. The increase in the vitamin C content could be ascribed to the ingredients used and the antioxidants they contain. The effect of thermal household processing, however, affected the vitamin C content among the recipes differently which could be due to the difference in the recipe preparation methods. This was not only seen with the variations in the vitamin C content between the recipes, but also in the total carotenoid, lycopene, and total polyphenol content, and as a result the TAC. Each step of the recipes was compiled using different preparation method principles. The specific actions applied in the recipe steps during thermal household processing could have contributed to the different effects seen. No liquid was also discarded during the preparation of the recipes. This could be another possible explanation for the increase in the vitamin C content in the recipes. Discarding the cooking liquid is a factor that decreases the vitamin C content during thermal processing (Adams & Erdman, 1988: 585). Even when the water loss was considered, the vitamin C content increased which indicates that the recipe contents concentrated during the thermal household processing of the recipe. These results are in contrast with that of previous studies indicating that the vitamin C content of food significantly decreases during thermal processing (Adams & Erdman, 1988: 584).

In general, the increase or no effect of thermal household processing on the TAC and antioxidant content indicates that thermal household processing does not negatively affect the dish, stewed tomato and onion flavoured with parsley. This is against the notion that thermal household processing negatively effect antioxidants in food (tested only on single food items). This finding could be ascribed to the improvement of antioxidant properties of the antioxidants present in the food system and the formation of novel compounds exerting AOA (i.e. Maillard reaction products) (Nicoli *et al.*, 1999: 95). Combining different food ingredients in recipes could thus be an important factor in preserving or increasing the TAC of thermal processed foods.

The raw recipe ingredients (tomato, onion, parsley) contain a large amount of water (FoodFinder™3, 2002) which on heating is liable to be lost. The water loss when accounted for does suggest that some of the parameters decrease, especially in the case of water-soluble polyphenols. Although water loss contributed to decreases in some of the parameters such as the total polyphenol content and H-ORAC$_{FL}$ value, thermal household processing is a form of food preservation that is required to drive off free water to limit microbial growth thereby increasing food product shelf life and decreasing the risk of microbial food poisoning. It can furthermore improve the flavour, texture and appearance of food (Rosenthal, 2003: 1622) and should as a result not be regarded as a negative food preparation technique.

5.3 Effect of different preparation methods in the recipes on the total antioxidant capacity of the end-products

The three recipes were specifically compiled to determine which recipe contributes to the highest TAC. These results will be discussed by referring only to the end-products of the recipes when SFO and RPO were respectively used.

As indicated in 5.2, several of the parameters increased during thermal household processing from the combined raw ingredients to the end-product when the three recipes were used. This suggests that when different preparation methods are used in a recipe, with the same recipe ingredients, it may contribute differently to the TAC and antioxidant content on thermal household processing. These different effects found, especially for the antioxidant content, makes it difficult to discuss the results, as limited information are available on the antioxidant actions in food systems. As a result when comparing the end-products with one-another, reference will only be made to the TAC, as this includes all the combined actions of the antioxidants present.

When the TAC of the respective end-products are compared with one-another the differences in the recipes does not seem to influence the outcome, as there was no difference in the TAC when SFO was used as ingredient. However, when RPO was used as ingredient the TAC of the control recipe was significantly higher than in Recipe 1. Using the control recipe therefore provided for a higher TAC than Recipe 1, while the TAC in the control recipe was similar to that of Recipe 2 and that of Recipe 1 was similar to that of Recipe 2. It was hypothesised that using Recipe 2 would provide the highest TAC of the recipes. This was, however, not found in this study. The time allocations of the recipes and the different preparation methods in the recipes could play an important role in the above-mentioned results. However, owing to the limited amount of research in this field of antioxidants, it could not be confirmed. The different results found between the two oils used, again suggests that RPO provide antioxidants to the recipe which protect more of the antioxidants present in the food system during thermal household processing.

5.4 Effect of the addition of parsley

In general, the addition of parsley did not increase the TAC and antioxidant content of the stewed tomato and onion recipes when the respective oils (SFO and RPO) were used. In the control recipe an increase in the L-ORAC$_{FL}$ value positively correlated with the increase in the total carotenoid and the lycopene contents. Furthermore, an increase in the vitamin C content in the same recipe positively correlated with the increase in the H-ORAC$_{FL}$ value with the addition of parsley. Although these correlations were found when the control recipe was used, none of these individual parameters increased, suggesting that a higher proportion of parsley should be used to increase these parameters. In contrast to when SFO was used as ingredient, the use of RPO contributed to a decrease in the L-ORAC$_{FL}$ value which positively correlated with the decrease in the total carotenoid content and the lycopene content. This suggests that when an antioxidant rich oil, such as RPO, is used other synergistic interactions between the antioxidants or other components takes place. Limited information is, however, available to suggest which of the antioxidants would be responsible for the decreased effect.

When Recipe 1 was used with RPO, the L-ORAC$_{FL}$ value significantly increased but this increase did not correlate with the total carotenoid content and the lycopene content. This result found with the addition of parsley could be due to the differences in the recipe preparation methods. In Recipe 1 raw onion was added to the cooked tomato and not raw tomato to the sautéed onion as was done in the control recipe. Wide speculations could be made for the antioxidant reactions within the recipe but, as was discussed in 5.3, at the end the various reactions within and between the antioxidants does not affect the TAC as they

are generally the same. This should, however, be confirmed. When Recipe 2 was used, only the total polyphenol content significantly increased, which again suggests that the difference in the recipe preparation methods could affect the antioxidants differently.

However, adding parsley to smaller amounts of food (sautéed onion on its own and cooked tomato on its own) increased more parameters when both oils were used respectively. In these preparations parsley constituted more of the total weight contribution in comparison with the three recipes. It could as a result be assumed that by increasing the parsley content of the recipes, the TAC and the antioxidant content of the recipes could increase. Heating the fresh parsley with SFO and also RPO did not negatively impact the antioxidants present in the herb as most of the measured parameters increased significantly. It could thus be assumed that several of the antioxidants present in the parsley become more accessible on heating and by adding parsley to a recipe could, because of its antioxidant contribution to the recipe, compensate for possible thermal household processing antioxidant losses.

It was hypothesised that the addition of parsley would contribute to a higher TAC, but this was however not found. Increasing the parsley in a recipe to a higher weight proportion could, however, increase the TAC. This weight proportion on the other hand was not a component of this study.

5.5 Contribution of sunflower and red palm oil on the stewed tomato and onion flavoured with parsley

A comparison between the recipes using SFO and RPO are discussed below. Reference will only be made to the end-product of each recipe for the effect of thermal household processing. The comparison will furthermore only compare the TAC of the recipes, as the TAC represents all the actions of the individual antioxidants present in the recipes.

The TAC of the recipe end-products using SFO as ingredient, was similar (5.3), while the TAC of the control recipe was significantly higher than that of Recipe 1 when RPO was used as ingredient (5.3). Using the control recipe with RPO resulted in the highest TAC of all the recipes investigated and compared. This suggests that RPO can be used to increase the TAC. Using RPO in Recipes 1 and 2, however, did not provide for a higher TAC than when SFO was used. A recommendation on the use of RPO as ingredient can as a result not commonly be made. In this study Carotino Classic was used. It could be assumed that when Carotino Premium, a more concentrated source of antioxidants, is used significant increases in the TAC could be provided. Nevertheless, this oil is not available to the South African consumer. Consuming stewed tomato and onion flavoured with parsley prepared by

using the control recipe with RPO as ingredient, however, could contribute to between 750 - 822 µmol TE per 100 g. A TAC of between 3 000 and 5 000 µmol TE is recommended daily for humans (McBride, 1999: 17). Consuming a 100 g of stewed tomato and onion flavoured with parsley could contribute to approximately 25.0% of this recommended daily antioxidant intake.

CHAPTER 6

CONCLUSIONS

Three recipes were developed to determine the effect of thermal household processing on the TAC and antioxidant content. During the experimental study the antioxidant content were determined along with the TAC for all the applied preparation steps to obtain a better understanding of the antioxidant changes in the recipes caused by thermal household processing and the addition of parsley. However, no pattern emerged when comparing the antioxidant content of the three recipes. This emphasises the fact that during food preparation, various reactions and/or influctuations occur resulting in positive and/or negative effects on the bioactive compounds (i.e. peptides, antioxidants). Such interactions between the antioxidants may also have no effect on the TAC of the combined ingredients. This happens when the loss of naturally occurring antioxidants is balanced by the simultaneous formation of compounds with novel and improved antioxidant properties. Owing to the inconsistent results in the antioxidant content, the TAC is the best measure to investigate the antioxidant contribution to the food. The H-ORAC$_{FL}$ values as a percentage of the TAC contributes most to the TAC, which is in agreement with previous research findings (Halvorsen *et al.*, 2002: 466; Wu *et al.*, 2004a: 4030; Ninfali *et al.*, 2005: 260) investigating food. Thus for this study water-soluble antioxidants (polyphenols and vitamin C) are more important when considering the TAC of the various recipes.

In general, thermal household processing did not decrease the TAC from the combined raw ingredients to end-product when SFO was used as ingredient, but it affected the TAC of the three recipes differently. Thermal household processing significantly increased the TAC in the control recipe, while the TAC of the other two recipes remained unchanged. Similar results were found when RPO was used as ingredient. This implicates the control recipe as the recipe to be used when preparing stewed tomato and onion flavoured with parsley in the household.

During the thermal household processing of the sautéed onion using SFO as ingredient the TAC remained the same irrespective of the differences between the preparation methods applied. When the tomatoes were cooked, the TAC increased with a longer cooking time in Recipe 2. The TAC was affected differently when RPO was used as ingredient in the sautéing of the onion and cooking of the tomato. The TAC of the sautéed onion was higher when the control recipe was used. More oil was used in this recipe. Using RPO, which is a rich source of antioxidants, could have contributed to the increase. The thermal household processing of the tomato for a longer time allocation also increased the TAC as was seen in

the cooked tomato when SFO was used. Previous studies also found the TAC of tomato to increase with cooking (Dewanto *et al.*, 2002: 3012; Sahlin *et al.*, 2004: 641). It could be concluded that the antioxidants present in tomato become more available during longer thermal household processing which increases the TAC. Unfortunately no combined raw ingredient samples were taken for the sautéed onion and the cooked tomato. As a result no comparisons were made to compare the combined raw ingredients with the thermally processed samples to determine the effect of thermal household processing from the combined raw ingredients to the thermally processed samples. Previous studies that investigated the effect of thermal household processing, for example, compared fried onion, which includes the use of oil, with raw onion (Sahlin *et al.*, 2004: 641). This, however, is not a correct comparative procedure as both the oil and onion will be consumed after frying. Therefore it is imperative to use the appropriate sampling methods for the above-mentioned comparisons that were a major limitation of this study and those previously conducted.

The TAC of the end-products of the three recipes when SFO was used as ingredient was similar. It could therefore be concluded that when using SFO it does not matter which recipe is used. When RPO was used as ingredient the TAC of the control recipe was higher than that of Recipe 1. The possible reasons for this result could be the longer time allocation applied in the control recipe, and the use of sautéed onion in its preparation method and not raw onion as was used in the preparation method of Recipe 1. Between the recipes investigated and the oils used, the TAC was the highest for the control recipe using RPO as ingredient. To obtain the highest TAC from this dish again implies that it should be prepared by using the control recipe which is based on the traditional preparation method used by South Africans. Despite that RPO may have positively contributed to the TAC in this recipe, it did not increase the TAC of all the recipes (and it is more expensive oil for South African consumers to purchase than SFO). Therefore it cannot be concluded that RPO should be the oil of choice in preparing stewed tomato and onion flavoured with parsley. If this dish is consumed with RPO it could, however, provide consumers with 750 - 822 µmol TE per 100 g than when prepared with SFO (707 - 739 µmol TE per 100 g) which in fact would contribute more to the daily antioxidant intake.

Water loss is common during thermal household processing. Although water is lost, thermal household processing actually preserves the dish and should not be regarded as a negative food preparation technique. The water loss was inconsistent as it was not found in all the recipes and the most significant loss was found when RPO was used in Recipe 1. However, it could be concluded that thermal household processing does not negatively affect antioxidants present in these recipes during preparation as the TAC in general was higher for the stewed dish than it was for the combined raw ingredients. Several factors that could be

responsible for the increases are: i) the concentration of the dish due to water loss, ii) the use of more than one ingredient in the recipes that caused each ingredient to contribute its own "unique" antioxidants to the recipe, and iii) several physical processes (temperature, structural integrity and moisture content) which make antioxidants more accessible for possible interactions such as synergism.

The addition of parsley to the cooked tomato and onion did not contribute to a higher TAC. With the addition of parsley to the sautéed onion and cooked tomato, the TAC, however increased, which could be attributed to its higher weight contribution. When RPO was used as ingredient the TAC increased with the addition of the parsley to the cooked tomato and onion and to the cooked tomato on its own. The TAC of the sautéed onion, however, did not increase and remained similar. This possibly illustrates that not all vegetables are opportune combinations of herbs as was illustrated with the onion and parsley as opposed to the tomato and parsley. Adding herbs, such as parsley, to recipes could increase the TAC of a recipe dish and alternatively contribute to the consumers' overall antioxidant intake. Heating parsley on its own with oil did not lower the TAC but significantly increased the TAC as many antioxidants become more accessible on heating. A sufficient amount of herbs should, however, be added to a recipe to increase the TAC. This study, however, was not designed to determine how much parsley would be sufficient to increase the TAC significantly.

The following limitations were identified from this study:

i. The published stewed tomato and onion recipe could be different from that used in most South African households. As a supporting measure for the recipe used, the 60 participants who were interviewed regarding the equipment used, could have been asked to comment on the recipe selected with reference to their own household practice; and

ii. The number of representative samples collected at each step should have been more than one. However, this would have influenced the reliability and validity of the TAC measured of the end-products as the number of samples collected in-between would have decreased the weight of the end-products significantly. Heat would therefore have affected the end-products more than it would have with a larger quantity of end-product.

CHAPTER 7

RECOMMENDATIONS

Limited research on the effect of food processing on antioxidants naturally present in food (not natural food additives) has been completed, yet various inconsistencies occur in these reports of the studies conducted. Besides supporting further research in this field, the following recommendations are made for such research:

i. Determine how recipes are prepared (includes amount of ingredients used) by the consumer prior to the investigation of thermal household processing or other household processing methods involving recipes (use of more than one ingredient). The results obtained from the analysis of such recipes would represent the antioxidant intake more accurately.

ii. Use preparation methods, food quantities and the heat transfer media as the consumer would use them. Many studies use large quantities of water for boiling food (Ninfali *et al.*, 2005: 258). Smaller water quantities should be used or the quantity of the food used in the boiling process should be adjusted appropriately for the amount of water used.

iii. Include salt, pepper and sugar (flavourants) in the amounts consumers would add on a daily basis in their food preparation as this would reflect consumer household practices more accurately.

iv. Accurately duplicate the household processing methods applied by consumers in the laboratory (or a nearby kitchen) using household equipment to represent the environment of the consumer.

v. Provide detailed information on the preparation methods used in method descriptions. Most studies do not provide sufficient information of how the food was prepared (Wu *et al.*, 2004: 409). This makes comparisons difficult.

vi. Compare the end product of the prepared food with a representative sample of the combined raw ingredients of the food to be prepared. Studies investigating the effect of thermal household processing compare the thermally processed food with a raw sample of the main ingredient which does not represent the food prepared (Sahlin *et al.*, 2004: 641).

vii. Determine the TAC of foods. As indicated in this study the individual antioxidants (lycopene, vitamin C) or classes of antioxidants (total carotenoids, total polyphenols) gave inconclusive results. This is due to the possible interactions/synergism between the antioxidants. Determining the TAC includes all the known and unknown antioxidants and their synergistic interactions.

viii. Analyse duplicate samples of each prepared dish (triplicate) to obtain reliable measurements.

ix. Only analyse representative samples of the combined raw ingredients of the dish and the end-product of the prepared dish as the data of the sample analysis of the preparation steps inbetween is difficult to interpret. There is currently only limited information available on how food processing and preparation influences the release and uptake of dietary antioxidant compounds from the food matrix (Astley & Lindsay, 2002: 290).

x. Lastly, research should aim to determine the effect of household processing methods on the release of the dietary antioxidant components from the food matrix as this is a research area not thoroughly investigated. The results of this study could not be compared with other studies owing to the lack of similar published studies.

Further research may contribute to providing more significant recommendations to the consumer. Research on recipes could provide a better understanding of food antioxidants and may result in sufficient information to construct recipes with opportune vegetable combinations in which antioxidants work synergistically to obtain the highest TAC. The most important recommendation for the consumer from this study is that thermal household processing did not decrease the TAC of stewed tomato and onion flavoured with parsley, which may also be applicable to other cooked vegetable dishes, and that consuming plant foods raw does not necessarily provide for a higher antioxidant intake.

Household food preparation procedures currently applied seem justifiable which may mean that adjusted preparation methods to retain the antioxidants present in food may not be necessary. However, to increase the daily antioxidant intake to assist the body's antioxidant defences, herbs can be added. Using parsley in a recipe can increase the TAC of a recipe, but more than 5 g should be added to a recipe weighing less than 1 kg. It has already been recommended that herbs should be added to food to increase the antioxidant intake (Craig, 1999: 492S; Justesen & Knuthsen, 2001: 248). Consuming stewed tomato and onion flavoured with a larger amount of parsley could increase the antioxidant intake of South Africans and provide a larger variety of antioxidants, which may be more beneficial. It is therefore recommended that future studies also investigate the amount of herbs that should be added to a dish to increase the antioxidant intake.

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ADDENDA

Addendum A:

Traditional recipe of tomato and onion stew

6 – 8 medium ripe tomatoes, peeled and chopped

2 medium onions, peeled and chopped

30 ml sunflower oil

1 ml curry powder

Pinch of white sugar

Salt and pepper

1. Sauté the onion in the oil until soft.
2. Add the curry to the onion and sauté further.
3. Add the chopped tomato and simmer over a low heat until thickened. Mix often.
4. Sweeten the stew with sugar and add salt and pepper to taste.

(Obtained from Human, 2002: 263)

Addendum B:

Control recipe of stewed tomato and onion flavoured with parsley

2 medium onions, peeled and chopped

6 medium ripe tomatoes, peeled and chopped

30 ml sunflower oil

10 ml fresh parsley, chopped

1. Sauté the onion in the oil until soft.
2. Add the chopped tomato and simmer over a low heat until thickened. Mix often.
3. Add the parsley.

Addendum C:

Recipe 1 of stewed tomato and onion flavoured with parsley

6 medium ripe tomatoes, peeled and chopped

2 medium onions, peeled and chopped

30 ml sunflower oil

10 ml fresh parsley, chopped

1. Add the chopped tomato and the oil to the pan and cook.
2. Add the chopped onion and cook until soft, stirring often.
3. Add the parsley.

Addendum D:

Recipe 2 of stewed tomato and onion flavoured with parsley

6 medium ripe tomatoes, peeled and chopped

2 medium onions, peeled and chopped

60 ml sunflower oil

10 ml fresh parsley, chopped

1. Add the chopped tomato and 30 ml of oil to the pan and cook. Stir often.
2. In a separate pan sauté the onion in the remaining 30 ml of oil until soft, while stirring often.
3. Add the cooked onion to the tomato and cook further until thickened.
4. Add the parsley.

Addendum E:

Equipment use interview outline

Respondent code		

Instructions:

Please answer the following questions with your household in mind in the preparation of stewed tomato and onion (tomato and onion stew).

1. Which type of heat application do you use?

Gas	1	
Electricity	2	
Paraffin	3	1

2. Which cooking utensil do you use?

Pot	1	
Pan	2	
Other	3	2

3. Which type of utensil do you use?

Aluminium	1	
Stainless steel	2	
Cast iron	3	
Pointerware	4	
Le Creuset	5	
Other	6	3

4. If you are using 6 tomatoes and 2 onions in your recipe, what size of utensil do you use? (Illustrate by indicating the approximate diameter and depth with use of visuals.)

Diameter		4
Depth		5

5. Do you use a lid?

Yes	1	
No	2	6

6. What type of spoon do you use for mixing?

Wooden spoon	1	
Plastic	2	
Metal/stainless steel	3	
Other	4	7

7. In which residential area do you stay?

_____ 8

Addendum F:

Letter accompanying researcher for equipment use interview

Cape Peninsula
University of Technology

To: Whom It May Concern

From: Ms I Venter

Date: 7 June 2005

Subject: Determination of household utensils used to prepare stewed tomato and onion

Ms M Braun (student number: 201003155) is an MTech Consumer Science: Food and Nutrition student at the Faculty of Applied Sciences. She is determining the effect of thermal household processing on the total antioxidant capacity of stewed tomato and onion flavoured with parsley for her Master's degree. As thermal household processing is investigated, she needs to determine which household utensils are most often used by South Africans in the preparation of this recipe.

Your participation in answering the short questionnaire (7 questions) in this regard will be highly appreciated. Your participation is voluntary and anonymous.

Yours sincerely

Ms I Venter
Supervisor
Programme: Consumer Science: Food and Nutrition
Department Food and Agricultural Sciences
Faculty of Applied Sciences: Cape Town Campus

Addendum G:

Heat applications and time allocations of the control recipe as indicated by the pilot study participants (n = 5)

Participant 1

1. The pan was placed on the stove and the stove temperature turned to mark 5. The pan was heated for 1.38 minutes.
2. The oil was added to the pan and the pan with the oil was heated for a total of 2.41 minutes. After 2.14 minutes into the heating of the oil the heat was raised to temperature mark 6.
3. The onion was added and sautéed for a total of 7.16 minutes while stirring continuously in circular movements. The heat was lowered after 5.54 minutes to mark 5.
4. The tomato was added and stewed for a total of 24.45 minutes with the pan being covered with a lid. During the cooking process the tomato and onion mixture was stirred 15 times (an average of 24 seconds at a time) in circular movements. The heat was lowered to temperature mark 3, raised to temperature mark 4 and 5 after 54 seconds, 9.46 and 11.37 minutes respectively into the cooking of the tomato and onion.
5. The pan was removed from the heat.
6. The parsley was added and was stirred once for 24 seconds in circular movements.

(Total cooking time: 36.24 minutes; total duration: 37.19 minutes)

Participant 2

1. The stove temperature was turned to mark 6 and the pan placed on the stove. The oil was added to the pan, swirled and heated for 2.37 minutes (actions were consecutive).
2. The onion was added and sautéed for a total of 4.01 minutes while stirring continuously in circular movements (onion was added in small amounts at a time).
3. The tomato was added in small amounts at a time and stewed for a total of 6.42 minutes with the pan not being covered with a lid. During the cooking process the tomato and onion mixture was stirred ten times (an average of 23 seconds at a time) in circular movements.
4. The parsley was added and stirred once for 23 seconds in circular movements.
5. The pan was removed from the heat.

(Total cooking time: 13.33 minutes; total duration: 14.45 minutes)

Participant 4

1. The stove temperature was turned to mark 6 and the pan placed on the stove. The oil was added to the pan and was heated for 9 seconds (actions were consecutive).
2. The onion was added and sautéed for a total of 11.30 minutes while it was stirred 11 times (an average of ten seconds at a time) in circular movements. The heat was lowered to temperature mark 3 and raised to temperature mark 6 after 4.44 minutes and 6.07 minutes, respectively.
3. The tomato was added and stewed for a total of 13.46 minutes with the pan being covered with the lid opened slightly. During the cooking process the tomato and onion mixture was stirred ten times (an average of 16 seconds at a time) in circular movements.
4. The parsley was added and stirred once for eight seconds in circular movements.
5. The pan was removed from the heat.

(Total cooking time: 25.33 minutes; total duration: 26.07 minutes)

Participant 5

1. The stove temperature was turned to mark 6 and the pan placed on the stove. The oil was added to the pan and was heated for 3.03 minutes (actions were consecutive).
2. The onion was added and sautéed for a total of 4.43 minutes while it was stirred six times (an average of 13 seconds at a time) in circular movements. The heat was lowered to temperature mark 4 after nine seconds after the onion was added to the pan.
3. The tomato was added and was stewed for a total of 11.01 minutes with the pan being covered with a lid. During the cooking process the tomato and onion mixture was stirred eight times (an average of 16 seconds at a time) in circular movements.
4. The parsley was added and stirred once for eight seconds in circular movements.
5. The pan was removed from the heat.

(Total cooking time: 18.55 minutes; total duration: 19.05 minutes)

Participant 6

1. The stove temperature was turned to mark 6 and the pan was placed on the stove and heated for 31 seconds.
2. The oil was added and heated for 26 seconds.
3. The onion was added and sautéed for a total of 6.05 minutes while it was stirred six times (an average of 23 seconds at a time) in circular movements.

4. The tomato was added and stewed for a total of 7.27 minutes with the pan not being covered with a lid. During the cooking process the tomato and onion mixture was stirred nine times (an average of 16 seconds at a time) in circular movements.
5. The parsley was added and stirred twice for an average of 18 seconds at a time in circular movements. The total cooking process of the parsley took 1.38 minutes.
6. The pan was removed from the heat.

(Total cooking time: 16.07 minutes; total duration: 16.39 minutes)

Addendum H:

Heat applications and time allocations of Recipe 1 as indicated by the pilot study participants (n = 5)

Participant 1

1. The pan was placed on the stove and temperature turned to mark 4. The oil was added to the pan and heated for 1.09 minutes (actions were consecutive).
2. The tomato was added to the pan and cooked with the pan being covered with a lid for 9.39 minutes. During the cooking process it was stirred four times (an average of 16 seconds at a time) in circular movements.
3. The raw onion was added to the cooked tomato and stewed for a total of 17.54 minutes also with the pan being covered with a lid. During the stewing process the tomato and onion mix was stirred ten times (an average of 38 seconds at a time) in circular movements.
4. The pan was removed from the heat.
5. The parsley was added and stirred once for 13 seconds in circular movements.

(Total cooking time: 28.55 minutes; total duration: 29.07 minutes)

Participant 2

1. The stove temperature was turned to mark 6 and the pan placed on the stove. The oil was added to the pan, swirled and heated for 1.04 minutes (actions were consecutive).
2. The tomato was added to the pan and cooked with the pan not being covered with a lid for 1.25 minutes. During the cooking process it was stirred four times (an average of 19 seconds at a time) in circular movements.
3. The raw onion was added to the cooked tomato and stewed for a total of 10.34 minutes with the pan being covered with a lid. During the stewing process the tomato and onion mix was stirred 13 times (an average of 19 seconds at a time) in circular movements. The heat was lowered to temperature mark 5 after 1.58 minutes.
4. The parsley was added and stirred once for 13 seconds in circular movements.
5. The pan was removed from the heat.

(Total cooking time: 13.16 minutes; total duration: 13.30 minutes)

Participant 4

1. The stove temperature was turned to mark 6 and the pan placed on the stove. The oil was added and heated for 2.23 minutes (actions were consecutive).
2. The tomato was added and cooked for a total of 2.34 minutes with the pan not being covered with a lid. During cooking it was stirred four times (an average of 15 seconds at a time) in circular movements.

3. The raw onion was added to the cooked tomato and stewed for 18.56 minutes with the pan being covered with the lid opened slightly. During the stewing process the tomato and raw onion were stirred nine times (an average of 18 seconds at a time) in circular movements. The heat was lowered to temperature mark 2 and was raised to temperature mark 3 and 5 after 3.25 minutes, 1.13 minutes and 10.51 minutes, respectively.
4. The parsley was added and stirred once for 23 seconds in circular movements.
5. The pan was removed from the heat.

(Total cooking time: 24.16 minutes; total duration: 24.48 minutes)

Participant 5

1. The stove temperature was turned to mark 6 and the pan placed on the stove. The oil was added and heated for 2.29 minutes (actions were consecutive).
2. The tomato was added and cooked for a total of 2.27 minutes with the pan not being covered with a lid. During the cooking process it was stirred four times (an average of 11 seconds at a time) in circular movements. The heat was lowered to temperature mark 4 after 59 seconds.
3. The raw onion was added to the cooked tomato and stewed for 19.35 minutes with the pan being covered with a lid. During the stewing process the tomato and raw onion were stirred five times (an average of 15 seconds at a time) in circular movements. The heat was lowered to temperature mark 2 and raised to temperature mark 3 after 16 seconds and 4.47 minutes, respectively.
4. The parsley was added and stirred once for 17 seconds in circular movements.
5. The pan was removed from the heat.

(Total cooking time: 24.48 minutes; total duration: 27.15 minutes)

Participant 6

1. The stove temperature was turned to mark 6, the pan placed on the stove and heated for 17 seconds.
2. The oil was added and heated for 21 seconds.
3. The tomato was added to the pan and cooked for a total of 4.39 minutes with the pan not being covered with a lid. During the cooking process it was stirred five times (an average of 12 seconds at a time) in circular movements.
4. The raw onion was added to the cooked tomato and stewed for 9.02 minutes with the pan not being covered with a lid. During the stewing process the tomato and raw

onion were stirred eight times (an average of 19 seconds at a time) in circular movements.

5. The parsley was added and stirred three times for an average of 18 seconds at a time in circular movements. The total cooking process of the parsley took 2.38 minutes.
6. The pan was removed from the heat.

(Total cooking time 16.57 minutes; total duration: 17.20 minutes)

Addendum I:

Heat applications and time allocations of Recipe 2 as indicated by the pilot study participants (n = 5)

Participant 1

1. The pan was placed on the stove and temperature turned to mark 4. The oil was added and heated for 27 seconds (actions were consecutive).
2. The tomato was added and cooked for 7.57 minutes with the pan being covered with a lid. During the cooking process it was stirred three times (an average of 14 seconds at a time) in circular movements.
3. While the tomato was cooking, the onions were sautéed.
4. The pan was placed on the stove and turned to temperature mark 5. The pan and plate were heated for 1.38 minutes.
5. The oil was added and heated for a total of 2.41 minutes. After 2.14 minutes the heat was raised to temperature mark 6.
6. The onion was sautéed for a total of 7.16 minutes while stirring continuously in circular movements. After 5.54 minutes the heat was lowered to mark 5.
7. The sautéed onion was added to the cooked tomato and stewed for 11.51 minutes with the pan being covered with a lid. During the stewing process of the tomato and onion it was stirred eight times (an average of 25 seconds at a time) in circular movements. The heat was lowered to temperature mark 3 after 6.12 minutes during the cooking.
8. The pan was removed from the heat.
9. The parsley was added and stirred once for five seconds in circular movements.

(Total cooking time: 20.20 minutes; total duration: 20.55 minutes)

Participant 2

1. The stove was turned to temperature mark 6; the pan was placed on the stove and heated for 2 minutes (actions were consecutive).
2. The oil and tomato were added in the pan consecutively. The tomato was cooked with the pan not being covered with a lid for 6.17 minutes. It was stirred four times (an average of nine seconds at a time) in circular movements. After 3.43 minutes the heat was lowered to temperature mark 3.
3. While the tomato was cooking the onions were sautéed.
4. The stove temperature was turned to mark 6 and the pan placed on the stove. The oil was added to the pan, swirled and heated for 2.37 minutes (actions were consecutive).
5. The onion was added and sautéed for a total of 4.01 minutes while stirring continuously (onion was added in small amounts at a time) in circular movements.

6. The sautéed onion was added to the cooked tomato and stewed for another 7.02 minutes with the pan being covered with the lid. The heat application was also raised to mark 6 when the onion was added to the tomato. During the stewing process the tomato and onion mixture was stirred eight times (an average of 17 seconds at a time) in circular movements.
7. The pan was removed from the heat.
8. The parsley was added and was stirred once for 26 seconds in circular movements.

(Total cooking time: 16.45 minutes; total duration: 16.50 minutes)

Participant 4

1. The pan was placed on the stove, the stove temperature turned to mark 6 and heated for 43 seconds.
2. The oil was added and heated for 1.26 minutes.
3. The tomato was added to the pan and cooked for 10.23 minutes with the pan being covered with the lid. It was stirred seven times (an average of 19 seconds at a time) in circular movements. After 3.18 minutes the heat was lowered to mark 3.
4. While the tomato was cooking the onions were sautéed.
5. The stove temperature was turned to mark 6 and the pan placed on the stove. The oil was added to the pan and heated for 9 seconds (actions were consecutive) in circular movements.
6. The onion was added and sautéed for a total of 11.30 minutes while it was stirred 11 times (an average of ten seconds at a time). The heat was lowered to temperature mark 3 and raised to temperature mark 6 after 4.44 minutes and 6.07 minutes, respectively.
7. The sautéed onion was added to the cooked tomato and stewed for 1.46 minutes with the pan being covered with the lid. During the stewing process the tomato and onion were stirred two times (an average of 21 seconds at a time) in circular movements.
8. The parsley was added and stirred once for 39 seconds in circular movements.
9. The pan was removed from the heat.

(Total cooking time: 14.57 minutes; total duration: 15.03 minutes)

Participant 5

1. The pan was placed on the stove and the stove temperature turned to mark 6. The oil was added and heated for 4.05 minutes (actions were consecutive).
2. The heat was lowered to temperature mark 4 and the tomato added and cooked for 6.30 minutes with the pan not being covered with a lid. It was stirred four times (an average of 18 seconds at a time) in circular movements.
3. While the tomato was cooking, the onions were sautéed.
4. The stove temperature was turned to mark 6 and the pan placed on the stove. The oil was added to the pan and heated for 3.03 minutes (actions were consecutive).
5. The onion was added and sautéed for a total of 4.43 minutes while it was stirred six times (an average of 13 seconds at a time) in circular movements. After nine seconds the heat was lowered to temperature mark 4.
6. The sautéed onion was added to the cooked tomato and stewed for four minutes with the pan being covered with a lid. During the stewing process the tomato and onion were stirred three times (an average of 16 seconds at a time) in circular movements.
7. The parsley was added and stirred once for 18 seconds in circular movements.
8. The pan was removed from the heat.

(Total cooking time: 14.53 minutes; total duration: 15.01 minutes)

Participant 6

1. The pan was placed on the stove and the stove temperature turned to mark 6. The oil was added and heated for 51 seconds (actions were consecutive).
2. The tomato was added to the pan and cooked for 7.44 minutes with the pan not being covered with a lid. It was stirred four times (an average of 11 seconds at a time) in circular movements.
3. While the tomato was cooking, the onions were sautéed.
4. The stove temperature was turned to mark 6 and the pan placed on the stove and heated for 31 seconds.
5. The oil was added and heated for 26 seconds.
6. The onion was added and sautéed for a total of 6.05 minutes while it was stirred six times (an average of 23 seconds at a time) in circular movements.
7. The sautéed onion was added to the cooked tomato and stewed for another 3.23 minutes with the pan not being covered with a lid. During the stewing process the tomato and onion were stirred five times (an average of 14 seconds at a time) in circular movements.

8. The parsley was added and stirred twice for 21 seconds at a time in circular movements. The total cooking process of the parsley took 1.33 minutes.
9. The pan was removed from the heat.

(Total cooking time: 13.31 minutes; total duration: 13.43 minutes)

Addendum J:

Control recipe compilation

6 medium ripe (740 g ± 1 g) tomatoes, peeled and chopped

2 medium (220 g ± 1 g) onions, peeled and chopped

20 ml sunflower oil (16 g)/red palm oil (18 g)

5 g fresh parsley, chopped

1. Pre-prepare tomato, onion and parsley as described in 3.3.2.2.
2. Turn stove to temperature mark 6, place pan on stove and add oil. Heat for 2.30 minutes.
3. Add onion to sauté and stir continuously [approximately 33 times in circular movements forming an 8 (3 seconds) with a resting period of 5 seconds in between] for 4.30 minutes.
4. Turn stove to temperature mark 5.
5. Add raw tomato to sautéed onion.
6. Stir approximately 6 times in circular movements forming an 8 (20 seconds). Simmer tomato and onion, with the lid opened slightly, for 50 seconds at a time. In between, stir approximately 6 times in circular movements forming an 8 (20 seconds). Repeat the simmering and stirring nine times.
7. Add parsley to stewed tomato and onion and stir for approximately 6 times in circular movements forming an 8 (20 seconds).
8. Remove from heat.

Table J-1: Ingredient percentage weight contribution of the control recipe for both oils

		Recipe utilising sunflower oil			
Ingredients	Tomato	Onion	Parsley	Sunflower oil	Total
Weight (g)	740	220	5	16	981
% weight contribution	75.43	22.43	0.51	1.63	100
		Recipe utilising red palm oil			
Ingredients	Tomato	Onion	Parsley	Red palm oil	Total
Weight (g)	740	220	5	18	983
% weight contribution	75.28	22.18	0.51	1.83	100

Table J-2: Recorded preparation steps of the control recipe preparation method

	Instructions		Time (minutes)
Step 1	Turn stove temperature to mark 6, place pan on stove and add oil		0.12
	Heat		2.30
Step 2	Add onion to pan		0.05
	Sauté onion, stirring continuously [approximately 33 times in circular movements forming an 8 (3 seconds) with a resting period of 5 seconds in between]		4.30
	Turn stove temperature to mark 5		0.02
Step 3	Add tomato to sautéed onion		0.05
	Stir approximately 6 times in circular movements forming an 8		0.20
	Replace lid	Repeat nine times	0.02
	Simmer		0.50
	Remove lid		0.02
	Stir approximately 6 times in circular movements forming an 8		0.20
Step 4	Add parsley to stewed tomato and onion		0.02
	Stir approximately 6 times in circular movements forming an 8		0.20
Step 5	Remove from heat		0.02

Addendum K:

Recipe 1 compilation

6 medium ripe (740 g ± 1 g) tomatoes, peeled and chopped

2 medium (220 g ± 1 g) onions, peeled and chopped

20 ml sunflower oil (16 g)/red palm oil (18 g)

5 g fresh parsley, chopped

1. Pre-prepare tomato, onion and parsley as described in 3.3.2.2.
2. Turn stove temperature to mark 6 and place pan on stove.
3. Heat pan and stove for 2.00 minutes.
4. Add oil and heat for 30 seconds.
5. Add tomato. Stir five times in circular movements forming an 8 (15 seconds). Cook the tomato for 50 seconds at a time without the lid. After simmering stir five times in circular movements forming an 8 (15 seconds). Repeat the cooking and stirring twice.
6. Turn stove temperature to mark 5.
7. Add raw onion.
8. Stir approximately 6 times in circular movements forming an 8 (20 seconds). Simmer tomato and raw onion, with the lid opened slightly for 1.30 minutes at a time. In between stir approximately 6 times in circular movements forming an 8 (20 seconds). Repeat simmering and stirring five times.
9. Add parsley to stewed tomato and onion and stir approximately 6 times in circular movements forming an 8 (20 seconds).
10. Remove from heat.

Table K-1: Ingredient percentage weight contribution of Recipe 1 for both oils

		Recipe utilising sunflower oil			
Ingredients	Tomato	Onion	Parsley	Sunflower oil	Total
Weight (g)	740	220	5	16	981
% weight contribution	75.43	22.43	0.51	1.63	100
		Recipe utilising red palm oil			
Ingredients	Tomato	Onion	Parsley	Red palm oil	Total
Weight (g)	740	220	5	18	983
% weight contribution	75.28	22.18	0.51	1.83	100

Table K-2: Recorded preparation steps for the preparation method of Recipe 1

	Instructions		Time (minutes)
Step 1	Turn stove temperature to mark 6 and place pan on stove		0.05
	Heat		2.00
Step 2	Add oil to pan		0.08
	Heat		0.30
Step 3	Add tomato to pan		0.05
	Stir 5 times in circular movements forming an 8		0.15
	Cook		0.50
	Stir 5 times in circular movements forming an 8		0.15
	Turn stove temperature to mark 5		0.02
Step 4	Add raw onion to cooked tomato		0.05
	Stir approximately 6 times in circular movements forming an 8		0.20
	Replace lid	Repeat five times	0.02
	Simmer		1.30
	Remove lid		0.02
	Stir approximately 6 times in circular movements forming an 8		0.20
Step 5	Add parsley to stewed tomato and onion		0.02
	Stir approximately 6 times in circular movements forming an 8		0.20
Step 6	Remove from heat		0.02

Addendum L:

Recipe 2 compilation

6 medium ripe (740 g ± 1 g) tomatoes, peeled and chopped

2 medium (220 g ± 1 g) onions, peeled and chopped

30 ml sunflower oil (24 g)/red palm oil (27 g)

5 g fresh parsley, chopped

1. Pre-prepare tomato, onion and parsley as described in 3.3.2.2.
2. Turn stove temperature to mark 6 and place pan on stove.
3. Heat pan and stove for 2.00 minutes.
4. While tomato is being cooked, start heating another pan for the sautéing of the onion (after one minute into the heating of Pan 1). Turn stove temperature to mark 6, place pan on stove and add 10ml oil. Heat for 2.30 minutes.
5. Add 10ml oil to Pan 1 and heat for 30 seconds.
6. Add tomato to Pan 1. Stir five times in circular movements forming an 8 (15 seconds). Cook for 2.00 minutes without the lid at a time.
7. Add onion and sauté, stirring continuously for 8 times in circular movements forming an 8 (3 seconds) with a resting period of 5 seconds in between for 1.00 minute while cooking the tomato.
8. Stir Pan 1 five times in circular movements forming an 8 (15 seconds).
9. Stir Pan 2 15 times in circular movements forming an 8 (3 seconds) with a resting period of 5 seconds in between for 2.00 minutes while cooking the tomato.
10. Stir Pan 1 five times in circular movements forming an 8 (15 seconds).
11. Stir Pan 2 15 times in circular movements forming an 8 (3 seconds) with a resting period of 5 seconds in between for 2.00 minutes while cooking the tomato.
12. Stir Pan 1 five times in circular movements forming an 8 (15 seconds).
13. Turn stove temperature of Pan 1 to mark 5.
14. Add sautéed onion to cooked tomato.
15. Stir approximately 6 times in circular movements forming an 8 (20 seconds).
16. Simmer tomato and sautéed onion, with the lid opened slightly for 35 seconds at a time. In between stir approximately 6 times in circular movements forming an 8 (20 seconds). Repeat simmering and stirring.
17. Add parsley to stewed tomato and onion and stir approximately 6 times in circular movements forming an 8 (20 seconds).
18. Remove from heat.

Table L-1: Ingredient percentage weight contribution of Recipe 2 for both oils

		Recipe utilising sunflower oil			
Ingredients	Tomato	Onion	Parsley	Sunflower oil	Total
Weight (g)	740	220	5	24	989
% weight contribution	74.82	22.24	0.51	2.43	100
		Recipe utilising red palm oil			
Ingredients	Tomato	Onion	Parsley	Red palm oil	Total
Weight (g)	740	220	5	27	992
% weight contribution	74.60	22.18	0.50	2.72	100

Table L-2: Recorded preparation steps for the preparation method of Recipe 2

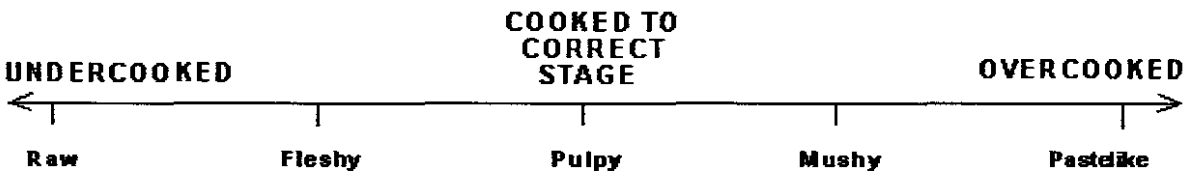
	Instructions: Pan 1	Time (minutes)		Instructions: Pan 2	Time (minutes)
Step 1	Turn stove temperature to mark 6 and place pan on stove.	0.05			
	Heat	2.30	**Start Pan 2's actions after 1 minute into Pan 1's cooking**		
Step 2	Add oil to pan	0.08	Step 1	Turn stove temperature to mark 5 and place pan on stove	0.05
	Heat	0.30		Heat	2.30
Step 3	Add tomato to pan	0.05	Step 2	Add oil to pan	0.08
	Stir 5 in circular movements forming an 8	0.15		Heat	0.30
	Cook	2.00	Step 3	Add onion to pan	0.05
				Stir approximately 8 times in circular movements forming an 8 (3 seconds) with a resting period of 5 seconds in between]	1.00
				Remove spoon	0.03
				Leave	0.20
	Stir	0.15			
	Remove spoon	0.03			
	Cook	2.00		Stir 15 times in circular movements forming an 8 (3 seconds) with a resting period of 5 seconds in between]	2.00
				Remove spoon	0.03
				Leave	0.20
	Stir	0.15			
	Remove spoon	0.03			
	Cook	2.00		Stir 15 times in circular movements forming an 8 (3 seconds) with a resting period of 5 seconds in between]	2.00
				Remove spoon 86°C	0.03
	Stir	0.15		Leave	
	Remove spoon 96°C	0.03			
	Turn stove temperature to mark 5	0.02			
Step 4	Add sautéed onion to cooked tomato	0.16			
	Stir	0.20			
	Replace lid	0.02			
	Simmer (Repeat twice)	0.35			
	Remove lid	0.02			
	Stir 92°C	0.20			
Step 5	Add parsley to stewed tomato and onion	0.02			
	Stir	0.20			
Step 6	Remove from heat 90°C	0.02			

Addendum M:

Sensory descriptors for the texture of stewed tomato and onion flavoured with parsley

Use the following descriptors to guide you in the evaluation of the tomato and onion texture.

Tomato texture



Clarification of above terms:

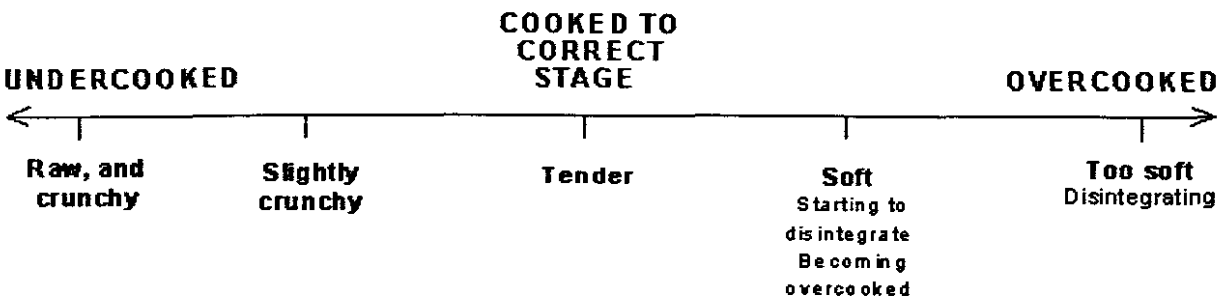
Fleshy: refers to the edible pulpy part of the raw tomato's flesh (thick and soft). If chopped and uncooked, fruit pieces and some juice are visible.

Pulpy: refers to a soft, wet, shapeless mass of material.
Furthermore it for example resembles the soft fleshy part of a fruit such as a tomato that has been squashed. Soft fruit pieces visible, but very little or no liquid.

Mushy: refers to a soft, wet, pulpy mass with no visible fruit pieces.

Pastelike: refers to a thick, soft, moist substance, typically produced by overcooking soft fruit pieces, with evaporation of any surrounding liquid and becoming concentrated.

Onion texture



Addendum N:

Analytical sensory evaluation form

ANALYTICAL SENSORY EVALUATION	STEWED TOMATO AND ONION FLAVOURED WITH PARSLEY

Name: _____ Date: _____

Panellist number: _____ Sample number: _____

EVALUATE THE DIFFERENT ATTRIBUTES OF THE SAMPLE BY PLACING A CIRCLE AROUND THE BLOCK WHICH BEST DESCRIBES YOUR PERCEPTION OF THE ATTRIBUTE (Indicate only one attribute per item). USE THE ATTACHED PAGE WHEN EVALUATING THE TEXTURE OF THE TOMATO AND ONION.

1. APPEARANCE

1.1 COLOUR OF TOMATO

2	3	4
Pale yellow-red	Typical of stewed tomatoes red-yellow to red ACCEPTABLE	Synthetic red (artificial)

1.2 COLOUR OF ONION

1	2	3	4	5
Typical of raw onion	Opaque – white	Slightly opaque to translucent ACCEPTABLE	Slightly dark – caramelised	Too dark - caramelised may even be burnt

1.3 OILY SURFACE

1	2	3
Oily surface	Some oily droplets on the surface	No oily droplets on the surface ACCEPTABLE

2. TEXTURE

2.1 ONION TEXTURE

1	2	3	4	5
Too crunchy and undercooked	Slightly crunchy	Tender ACCEPTABLE	Soft becoming overcooked	Too soft and overcooked

2.2 OVERALL CONSISTENCY

1	2	3	4	5
Watery and chunky – runs from the spoon UNDERCOOKED	Slightly watery and chunky – immediately falls from the spoon	Acceptable consistency – slightly thick falls from the spoon in phases consecutively ACCEPTABLE	Pastelike and thick	Pastelike and very thick – clings to the spoon OVERCOOKED

2.3 OVERALL COARSENESS

1	2	3	4	5
Large and chunky pieces of tomato (>2cm) and onion (>1,5cm) UNDERCOOKED	Slightly large	Medium pieces of tomato (1 - 1.5cm) and onion (1/2 - 1cm) ACCEPTABLE	Slightly small	Too small and very finely chopped tomato (<1cm) and onion (<1/2cm) pieces OVERCOOKED

2.4 OVERALL DONENESS

1	2	3	4	5
Raw tomato and too crunchy onion pieces UNDERCOOKED	Fleshy tomato and slightly crunchy onion pieces	Typical of stewed tomato and onion flavoured with parsley - some visible tomato pieces, mostly pulpy with tender onion pieces ACCEPTABLE	Tomatoes too pulpy and onions soft becoming overcooked	Tomatoes mushy with no visible tomato pieces and too soft onions OVERCOOKED

2.5 OILY MOUTHFEEL

1	2	3
Unacceptable oil residue left on the palate	Slight oil residue left on the palate	No oil residue left on palate ACCEPTABLE

3. FLAVOUR

3.1 TARTNESS

1	2	3
Very sour	Slightly sour	Moderate tartness ACCEPTABLE

3.2 TYPICAL TOMATO FLAVOUR

1	2	3	4	5
Typical flavour is absent – very weak and not identifiable	Slight typical flavour – too weak	Well-balanced tomato flavour ACCEPTABLE	Slightly prominent	Too prominent - overpowering

3.3 TYPICAL ONION FLAVOUR

1	2	3	4	5
Typical flavour is absent – very weak and not identifiable	Slight typical flavour – too weak	Well-balanced onion flavour ACCEPTABLE	Slightly prominent	Too prominent - overpowering

3.4 AFTER-TASTE

1	2	3
** Strong after-taste – overpowering	** Slight after-taste – too prominent	No definite after-taste - well-balanced ACCEPTABLE

** Please identify the prominent aftertaste (refer to comment section)

COMMENTS: _____

_____

_____

_____

_____

_____

Please activate the green indicator light when you have completed your evaluation of the sample.

Addendum O:

Analytical sensory evaluation informed consent

Informed Consent

Title of Research Study:

Analytical descriptive sensory evaluation of stewed tomato and onion flavoured with parsley.

Principal Investigator:
Marlè Braun
Cape Peninsula University of Technology: Cape Town campus

Introduction and Purpose:

With the increased emphasis on antioxidants in disease prevention the aims of this study are to determine the total antioxidant capacity (TAC) of a recipe such as stewed tomato and onion flavoured with parsley and the effect thermal household processing has on the TAC of the ingredient use in the recipe formulations.

The aim of this analytical descriptive sensory evaluation is to determine whether the time allocations and heat applications allocated to each recipe step in the three recipe formulations for the experimental study allow for sensorically acceptable dishes. The recipes provided to you for evaluation do not contain any flavouring such as salt, pepper and/or sugar. These flavourings are variables that need to be limited in the experimental study.

You are invited to participate in this research study because your acceptance or not of these recipes is very important for the purpose of this research project.

Procedure:

If you participate in this study, you will be asked to evaluate and taste three recipes prepared with different processing methods in order to indicate if these recipes are acceptable in terms of appearance, taste and texture. Analytical descriptive sensory evaluation forms will be provided for each of the developed recipes.

Benefits:

There may be no direct benefit to you.

Risks:

There are no known risks at this time to participation in this study.

Voluntary Participation / Withdrawal:

Taking part in this study is voluntary. You may choose not to take part in this study, or if you decide to take part, you can later change your mind and withdraw from the study.

Costs:

There will be no costs to you for participation in this research study.

Compensation:

All the necessary utensils and evaluation forms will be supplied during the sensory evaluation sessions. You will not be paid to participate in this study.

Confidentiality:

All information collected during the course of this study will be kept confidential to the extent permitted by law. A panellist number will identify you in the research records.

Questions:

If you have any questions in the future, you may contact Marlè Braun: Cell: .....

✂.....

Consent to Participate in this Research Project:

To voluntarily agree to take part in this study, you must sign on the line below. If you choose to take part in this study, you may withdraw at any time. You are not giving up any of your legal rights by signing this form. Your signature below indicates that you have read this entire consent form, including the risks and benefits, and have had all your questions answered. You will be given a copy of this consent form.

_____ _____

Signature of Study Subject Date

_____

Printed name of Study Subject

_____ _____

Signature of Witness (if applicable) Date

_____ _____

Signature of Investigator / Designee obtaining consent Date

Addendum P:

Control recipe re- compilation

6 medium ripe (740 g ± 1 g) tomatoes, peeled and chopped

2 medium (220 g ± 1 g) onions, peeled and chopped

20 ml sunflower oil (16 g)/red palm oil (18 g)

5 g fresh parsley, and chopped

1. Pre-prepare the tomato, onion and parsley as described in 3.3.2.2.
2. Turn stove temperature to mark 6, place pan on stove and add oil. Heat for 2.30 minutes.
3. Add onion and sauté and stir continuously [approximately 41 times in circular movements forming an 8 (3 seconds) with a resting period of 5 seconds in between] for 5.30 minutes.
4. Turn stove temperature to mark 5.
5. Add raw tomato to sautéed onion.
6. Stir approximately 6 times in circular movements forming an 8 (20 seconds). Simmer tomato and onion, with the lid opened slightly, for 50 seconds at a time. In between, stir approximately 6 times in circular movements forming an 8 (20 seconds). Repeat the simmering and stirring nine times.
7. Add parsley to stewed tomato and onion and stir for approximately 6 times in circular movements forming an 8 (20 seconds).
8. Remove from heat.

Table P-1: Recorded preparation steps of the control recipe re- compilation

	Instructions		Time (minutes)
Step 1	Turn stove temperature to mark 6, place pan on stove and add oil to pan		0.12
	Heat		2.30
Step 2	Add onion to pan		0.05
	(Sauté onion, stirring continuously [approximately 33 times in circular movements forming an 8 (3 seconds) with a resting period of 5 seconds in between] (100°C)		5.30
	Turn stove temperature to mark 5		0.02
Step 3	Add tomato to sautéed onion		0.05
	Stir approximately 6 times in circular movements forming an 8		0.20
	Replace lid	Repeat nine times	0.02
	Simmer		0.50
	Remove lid		0.02
	Stir approximately 6 times in circular movements forming an 8 (90°C)		0.20
Step 4	Add parsley to stewed tomato and onion		0.02
	Stir approximately 6 times in circular movements forming an 8 (88°C)		0.20
Step 5	Remove from heat		0.02

Addendum Q:

Recipe 1 re- compilation

6 medium ripe (740 g ± 1 g) tomatoes, peeled and chopped

2 medium (220 g ± 1 g) onions, peeled and chopped

20 ml sunflower oil (16 g)/red palm oil (18 g)

5 g fresh parsley, chopped

1. Pre-prepare the tomato, onion and parsley as described in 3.3.2.2.
2. Turn stove temperature to mark 6 and place pan on stove.
3. Heat pan and stove for 2.00.
4. Add oil and heat for 30 seconds.
5. Add tomato. Stir five times in circular movements forming an 8 (15 seconds). Cook the tomato for 35 seconds at a time without the lid. After simmering stir five times in circular movements forming an 8 (15 seconds). Repeat the cooking and stirring twice.
6. Turn stove temperature to mark 5.
7. Add raw onion.
8. Stir approximately 6 times in circular movements forming an 8 (20 seconds). Simmer tomato and raw onion, with the lid opened slightly for 1.40 minutes at a time. In between stir approximately 6 times in circular movements forming an 8 (20 seconds). Repeat simmering and stirring five times.
9. Add parsley to stewed tomato and onion and stir approximately 6 times in circular movements forming an 8 (20 seconds).
10. Remove from heat.

Table Q-1: Recorded preparation steps of the re-compilation of Recipe 1

	Instructions		Time (minutes)
Step 1	Turn stove temperature to mark 6 and place pan on stove.		0.05
	Heat		2.00
Step 2	Add oil to pan		0.08
	Heat.		0.30
Step 3	Add tomato to pan		0.05
	Stir 5 times in circular movements forming an 8		0.15
	Cook	Repeat twice	0.35
	Stir 5 times in circular movements forming an 8 (60°C)	Repeat twice	0.15
	Turn stove temperature to mark 5		0.02
Step 4	Add raw onion to cooked tomato		0.05
	Stir approximately 6 times in circular movements forming an 8		0.20
	Replace lid	Repeat five times	0.02
	Simmer	Repeat five times	1.40
	Remove lid	Repeat five times	0.02
	Stir approximately 6 times in circular movements forming an 8 (92°C)	Repeat five times	0.20
Step 5	Add parsley to stewed tomato and onion		0.02
	Stir approximately 6 times in circular movements forming an 8 (92°C)		0.20
Step 6	Remove from heat		0.02

Addendum R:

Original values of the representative samples of stewed tomato and onion flavoured with parsley utilising sunflower oil as ingredient before the factor calculation adjustments

Table R-1: Original values of the representative samples of stewed tomato and onion flavoured with parsley utilising sunflower oil as ingredient before the factor calculation adjustments

Recipe	Control recipe				Recipe 1				Recipe 2				
Step description	Combined raw ingredients	Sautéed onion	Cooked onion and tomato	Stewed tomato and onion flavoured with parsley (end-product)	Combined raw ingredients	Cooked tomato	Cooked tomato and raw onion	Stewed tomato and onion flavoured with parsley (end-product)	Combined raw ingredients	Cooked tomato	Sautéed onion	Cooked tomato and onion	Stewed tomato and onion flavoured with parsley (end-product)
Steps		One	Two	Three		One	Two	Three		One	Two	Three	Four
L-ORAC$_{FL}$ µmol TE/g	0.18 ± 0.05	0.03 ± 0.00	0.17 ± 0.03	0.16 ± 0.02	0.18 ± 0.04	0.13 ± 0 01	0.15 ± 0.02	0.19 ± 0 04	0 27 ± 0.08	0.19 ± 0.03	0.04 ± 0.01	0.19 ± 0.05	0.23 ± 0.08
H-ORAC$_{FL}$ µmol TE/g	4.53 ± 0.36	2.88 ± 0.49	6.54 ± 1.53	6.78 ± 0.54	5.17 ± 0 72	2.39 ± 0.65	5.46 ± 0 86	5.51 ± 0 91	3 58 ± 1.08	2.60 ± 0.20	1.82 ± 0.31	4.90 ± 0.80	4.91 ± 1.29
TAC µmol TE/g	4 71 ± 0 35	2.91 ± 0.50	6 71 ± 1.54	6.94 ± 0.56	5.35 ± 0.74	2.52 ± 0 64	5.61 ± 0.85	5.70 ± 0.89	3.84 ± 1.00	2.79 ± 0.18	1.86 ± 0.32	5.09 ± 0.81	5.14 ± 1.25
Total carotenoids µg/g	6.17 ± 1.10	-	9 22 ± 6.16	0.03 ± 2.45	7.10 ± 2.58	7.73 ± 3.87	8.82 ± 1.47	10.69 ± 3.85	10.41 ± 3 46	11.31 ± 1.92	-	14.46 ± 2.63	14.23 ± 1.92
Lycopene µg/g	5.19 ± 0 88	-	7.48 ± 4 80	7.11 ± 2.06	5 87 ± 1.84	6.22 ± 2.88	7.13 ± 1.26	8.60 ± 3.46	8.42 ± 3.14	8.79 ± 1.37	-	10.79 ± 2.11	10 77 ± 1.76
Total polyphenols mg GAE/g	1631 44 ± 407.62	411.77 ± 50.09	1423.53 ± 49 56	1612.70 ± 115.14	1426.71 ± 208.54	1089.19 ± 190.41	1179 21 ± 293.77	1330 68 ± 113.84	1385.19 ± 185.90	1154.39 ± 80.25	387.68 ± 63 35	1439.36 ± 124.25	1328 26 ± 115.09
Vitamin C µg/g	47.95 ± 10 79	11.14 ± 1.69	87.38 ± 9.36	95 41 ± 1.17	57.34 ± 2.60	81 97 ± 1.76	87.36 ± 6.70	104.56 ± 11.11	68.14 ± 16.40	92.84 ± 1.62	15.82 ± 1.55	116.13 ± 6.53	120.73 ± 15.95

Values in the columns represent mean ± standard deviation of the adjusted values from samples in triplicate
Abbreviations: TE-Trolox equivalents, ND-Not detected, GAE-Gallic acid equivalents, -Not determined

Addendum S:

Original values of the representative samples of stewed tomato and onion flavoured with parsley utilising red palm oil as ingredient before the factor calculation adjustments

Table S-1: Original values of the representative samples of stewed tomato and onion flavoured with parsley utilising red palm oil as ingredient before the factor calculation adjustments

Recipe	Control recipe				Recipe 1				Recipe 2				
Step description	Combined raw ingredients	Sautéed onion	Cooked onion and tomato	Stewed tomato and onion flavoured with parsley (end-product)	Combined raw ingredients	Cooked tomato	Cooked tomato and raw onion	Stewed tomato and onion flavoured with parsley (end-product)	Combined raw ingredients	Cooked tomato	Sautéed onion	Cooked tomato and onion	Stewed tomato and onion flavoured with parsley (end-product)
Steps		One	Two	Three		One	Two	Three		One	Two	Three	Four
L-ORAC$_{FL}$ µmol TE/g	0 23 ± 0.05	0 04 ± 0 07	0.17 ± 0.07	0.18 ± 0 05	0.31 ± 0.05	0.16 ± 0 07	0.21 ± 0.07	0.27 ± 0.05	0.23 ± 0.05	0.15 ± 0.07	0.06 ± 0.07	0.25 ± 0.05	0.24 ± 0.01
H-ORAC$_{FL}$ µmol TE/g	4 76 ± 0.47	2.81 ± 0.49	7.43 ± 2 46	8.04 ± 1.55	4.20 ± 0.36	1.99 ± 0.43	4.02 ± 2.27	4.79 ± 1.45	4.30 ± 0.39	2.73 ± 0.42	2.02 ± 2.03	5.53 ± 1.47	6.28 ± 1.76
TAC µmol TE/g	5.00 ± 0.47	2.85 ± 0.45	7.60 ± 2.50	8.21 ± 1.52	4.51 ± 0.37	2.15 ± 0 40	4.24 ± 2.31	5.06 ± 1.42	4.54 ± 0.40	2 89 ± 0 39	2 08 ± 2.08	5.78 ± 1.44	6 52 ± 1.77
Total carotenoids µg/g	4.90 ± 3 96	-	8.93 ± 2.74	8.01 ± 5.14	11.62 ± 3.73	9 65 ± 3.19	13.55 ± 2.52	17 27 ± 5.12	10.56 ± 4.00	10 47 ± 3.19	-	13.69 ± 5.12	17.41 ± 3.49
Lycopene µg/g	4.28 ± 2.03	-	6.80 ± 1.96	6.61 ± 3 53	7.48 ± 1.78	7.56 ± 2.42	10.13 ± 1.83	12.75 ± 3.54	7.91 ± 2.00	7.76 ± 2.42	-	10.19 ± 3.54	13.19 ± 2 88
Total polyphenols mg GAE/g	1773.42 ± 230.69	408.64 ± 322.18	1577.77 ± 540.72	1750.24 ± 266.79	1309.01 ± 245.50	983.60 ± 330.50	1316.57 ± 521.79	1283.78 ± 218.27	1485.13 ± 174 53	1130.09 ± 260 67	373.31 ± 524.59	1419.26 ± 184 47	1519.50 ± 117.18
Vitamin C µg/g	59.34 ± 18.12	13.64 ± 38.41	96.96 ± 38.86	97.42 ± 13 18	88.34 ± 18.45	77.35 ± 35.86	85 99 ± 39 41	102.99 ± 12.29	79 28 ± 17.85	97.20 ± 35.55	15.10 ± 38.85	121.79 ± 12 19	129.28 ± 1.84

Values in the columns represent mean ± standard deviation of the adjusted values from samples in triplicate
Abbreviations: TE-Trolox equivalents, ND-Not detected, GAE-Gallic acid equivalents, -Not determined