THE ADDITION OF ROOIBOS TEA EXTRACT (*ASPALATHUS LINEARIS*) AS A NATURAL ANTIOXIDANT TO SOUTH AFRICAN DROëWORS

by
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DECLARATION

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NOTES

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

Results from this study have been published in the following journals:

Hoffman, L.C., Jones, M., Muller, N., Joubert, E., & Sadie, A. (2013). Lipid and protein stability and sensory evaluation of ostrich (*Struthio camelus*) droëwors with the addition of rooibos tea extract (*Aspalathus linearis*) as a natural antioxidant. *Meat Science.* DOI: http://dx.doi.org/10.1016/j.bbr.2011.03.031

SUMMARY

The effect of rooibos tea (*Aspalathus linearis*) extract (RBTE) as a natural antioxidant on the lipid and protein stability and sensory profile of traditional South African droëwors (dried sausage) was investigated.

Ostrich meat (*Struthio camelus*) and pork back fat was used in the initial study as the meat and fat sources. Four treatments were prepared with each treatment increasing in concentration of RBTE: RBTE 0%, RBTE 0.25%, RBTE 0.50% and RBTE 1.0%. The lipid stability of the droëwors increased after drying with RBTE 0.25% having lower TBARS than the other treatments. The protein stability and heme-iron results of the droëwors did not differ ($P > 0.05$) between treatments.

The second study investigated the effect of added RBTE to droëwors of three different game species namely, blesbok (*Damaliscus pygargus phillipsi*), springbok (*Antidorcas marsupialis*) and fallow deer (*Dama dama*). No significant effects ($P > 0.05$) were seen between treatments in terms of the lipid and protein oxidation of the dried product within a species. Protein oxidation increased after drying but did not differ ($P > 0.05$) between the treatments within a stage (raw or dried) within species. Using different meat sources to the initial study and a shorter drying period did not result in any differences between treatments however, RBTE 0.25% did give the best results for lipid stability after drying. Heme-iron concentration differed ($P < 0.05$) between the RBTE treatments within the dried stage within a species with RBTE concentrations being inversely correlated with the levels of heme-iron.

The final study investigated the addition of RBTE to blesbok and springbok droëwors using an improved formulation, drying parameters and a different (beef) fat source. The results indicated that RBTE 1.0% significantly ($P < 0.05$) slowed down lipid oxidation after a two week storage. The added RBTE, however, did not result in any significant differences ($P > 0.05$) in protein oxidation and heme-iron concentration. A positive correlation between lipid oxidation and heme-iron concentration was noted.

Throughout the study the proximate composition analyses gave consistent results with the drying procedures. When the total moisture content decreased after drying, the fat and protein content became more concentrated. There were no differences ($P > 0.05$) between the moisture, protein and fat contents between treatments within a specific stage. High concentrations of oleic acid, stearic acid, linoleic acid and palmitic acid were detected. The fatty acid profile suggests that after drying there is a decrease in polyunsaturated fats which could explain the increase in lipid oxidation.

With the addition of RBTE, differences in sensory attributes between the different droëwors treatments were detected by a trained panel.

From these results it can be concluded that RBTE can be marketed as a natural antioxidant for use in droëwors. The composition of the RBTE particularly as pertaining to the levels of aspalathin and quercetin should however be considered when evaluating the level of RBTE to use.

OPSOMMING

Die studie het die invloed van rooibostee-ekstrak (*Aspalathus linearis*) (RBTE) as 'n natuurlike anti-oksidant op die oksidatiewe stabiliteit van tradisionele Suid-Afrikaanse droëwors ondersoek.

Aanvanklik is volstruis vleis (*Struthio camelus*) in kombinasie met varkrugvet gebruik. Daar is vier behandelinge voorberei met toenemende konsentrasies van RBTE (RBTE 0%, RBTE 0.25%, RBTE 0.50%, en RBTE 1.0%). Die lipiede het 'n hoër stabiliteit getoon na die drogingsproses, veral die 0.25% RBTE behandeling wat die beste resultate gelewer het. Die verskillende behandelings het geen uitwerking op die stabiliteit van die proteïene en die heem-yster konsentrasies gehad nie.

In die tweede studie is die invloed van RBTE in blesbok (*Damaliscus pygargus phillipsi*), springbok (*Antidorcas marsupialis*) en takbok (*Dama dama*) droëwors ondersoek. Die RBTE het geen effek getoon op die oksidatiewe stabiliteit van die verskillende gedroogde behandelings van elke spesie nie. Die proteïenoksidasie het wel toegeneem as gevolg van die vogverlies tydens die drogingsproses, maar het nie verskil ($P>0.05$) tussen die verskeie behandelings van die spesifieke spesies nie. Die 0.25% RBTE behandeling het weereens die beste resultate gelewer in terme van die lipiedstabiliteit na droging. Daar was verskille in die heem-yster konsentrasies tussen die verskeie gedroogde RBTE behandelings van elke spesie, wat weer goed gekorreleer het met die oksidasiestabiliteit.

Die finale studie het die insluiting van RBTE in blesbok en springbok droëwors ondersoek. Die formulasie en drogingsparameters is verbeter en 'n ander bron van vet (bees) is gebruik. Daar is gevind dat die insluiting van 1.0% RBTE die lipiedokisidasie betekenisvol verminder het na 'n stoortydperk van 2 weke. Verder het dit geen effek ($P>0.05$) op beide die proteïenoksidasie en heem-yster konsentrasie gehad nie. Die lipiedoksidasie en heem-yster het wel 'n positiewe korrelasie getoon.

In algeheel het die proksimale samestelling van die droëwors konsekwente resultate gelewer. Daar was 'n afname in die totale voginhoud as gevolg van die drogingsproses met 'n toename in vet- en proteïeninhoud. Geen verskille in terme van die vog-, proteïen- en vetinhoud is tussen die verskeie rou en gedroogde behandelinge gevind nie. Die vetsuurprofiel toon hoë konsentrasies oliensuur, steariensuur, linoliensuur en palmitiensuur. Daar was wel 'n afname in die poli-onversadigde vetsure na die drogings proses wat dien as 'n moontlike verklaring van die toename in lipiedoksidasie.

Die insluiting van RBTE in droëwors het gelei tot verskille in die sensoriese eienskappe van die verskeie droëwors behandelings.

In geheel het die studie bewys dat RBTE gebruik en bemark kan word as 'n natuurlike anti-oksidant in gedroogde vleis produkte. Dit is egter noodsaaklik dat die

samestelling van die RBTE in ag geneem moet word, veral met betrekking tot die vlakke van spesifieke anti-oksidante soos aspalatien en kwersetien.

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CHAPTER 1

Introduction

Consumers drive the food industry in that their preferences and needs are always considered when developing products. The average modern consumer wants a choice of convenient, healthy, value-added products (Resurreccion, 2003, Russell & Cox, 2004). Processed meat products are becoming more popular amongst these consumers, but these consumers are becoming more aware that synthetic additives, e.g. butylated hydroxyanisole (BHA), butylated hydroxyl toluene (BHT), tertiary butylhydroquinone (TBHQ) and sulphur dioxide (SO_2) are regularly added to such products (Karakaya *et al.,* 2011; Shahidi *et al.,* 1992; Tiwari *et al.,* 2009). These additives are added to prolong the shelf-life of the products (by acting as antioxidants), ensure colour stability and flavour improvement (Sánchez-Esalante *et al.,* 2003; Pokornỳ, 2001). The modern consumer, however, is wary of these synthetic additives and would prefer more natural additives to be used (Juntachote *et al.,* 2006; Markosyan *et al.,* 2009); this creates a need in the meat industry to shift towards the use of natural antioxidants. The reason for this study was to replace the synthetic additives used in commercial droëwors with a natural antioxidant to improve its oxidative stability. This would hopefully also result in an innovative product that would meet the modern consumer's requirements for the traditional South African droëwors.

Natural antioxidants are found in herbs, teas and spices (Vuorela *et al.,* 2005). Numerous studies have been conducted on the addition of natural antioxidants to meat products, in particular beef products. The sensory properties and oxidative stability of meat products were improved with the addition of natural antioxidants, especially when added in combination with a synthetic antioxidant (Crackel *et al.,* 1988; Bañón *et al.,* 2007; Liu *et al.,* 2010; Michalzyk *et al.,* 2012; Mathenjwa *et al.,* 2012). Green tea is commonly added to products to inhibit oxidation due to its favourable antioxidant profile. It has been successfully used in a variety of processed meat products (McCarthy *et al.,* 2001; Mitsumoto *et al.,* 2005; Nissen *et al.,* 2004; Tang *et al.,* 2001). Rooibos tea, a popular South African product, has a favourable antioxidant profile as it has a high level of polyphenolic compounds, which play an important role in the inhibition of oxidation (Joubert *et al.,* 2005). It is one of the most popular sources of antioxidants amongst South African consumers. Rooibos tea extract (RBTE) is a concentrated by-product when producing rooibos tea. RBTE has a high antioxidant profile and is commonly added as a food ingredient to various products (Joubert & De Beer, 2011). It was therefore used in this study as the added natural antioxidant whereby it could impart its antioxidant properties and distinctive flavour. Limited research has been conducted on using this product as an added antioxidant in meat products (Cullere *et al.* 2013), although it has

been demonstrated to inhibit lipid peroxidation in a number of assays (Joubert *et al.,* 2005; Snijman *et al.,* 2009).

South Africa is known for its dried meat products, droëwors and biltong. Both of which can be made from any meat source. Droëwors is a ready-to-eat dried meat sausage commonly made from beef and animal fat (Burnham *et al.,* 2008). Game meat however, is becoming a more popular option as a meat source for droëwors (Carr *et al.,* 1997). For this study, droëwors was used as it is a high fat product which is commonly stored for long periods by the consumer and therefore oxidation is likely to occur. Ostrich and game meat is gaining more attention amongst consumers in the marketplace due to its low fat and cholesterol content as well as its perceived minimal carbon footprint (Hoffman & Wiklund, 2006; Hoffman & Cawthorn, 2012; Sales & Horbanczuk, 1998). Game meat is harvested in South Africa for both local and international distribution. Research by Hoffman and Wiklund (2006) note that consumers are more likely to purchase game meat products as unprocessed (fresh) game meat is not accepted by all consumers.

Lipid and protein oxidation in meat results in undesirable aromas and flavours, especially in products with a high fat content such as droëwors. Oxidation occurs when the cellular structure is damaged which allows polyunsaturated fatty acids and natural pro-oxidants in the meat to interact (Vuorela et al., 2005). This study used meat sources that are known to have high polyunsaturated fatty acids and heme-iron (a pro-oxidant in meat) concentrations. Both lipid and protein oxidation were monitored as they are directly linked (Viljanen *et al.,* 2004). Sensory analyses by a trained panel was conducted to develop sensory attributes to compare the droëwors which is produced with and without RBTE.

This research project therefore investigates the development of droëwors from different meat sources (species) with the addition of rooibos tea extract (RBTE) to improve its oxidative stability and sensory attributes. Four meat sources were investigated namely ostrich (*Struthio camelus*), blesbok (*Damaliscus pygargus phillipsi*), springbok (*Antidorcas marsupialis*) and fallow deer (*Dama dama*) meat. Only the essential ingredients (such as meat, fat, salt and pepper) were used in the production as other additives and spices may mask any subtle differences that may occur due to the addition of the RBTE. It was envisaged that these experiments would result in the development of an improved, acceptable, uniquely South African alternative to the traditional South African droëwors. After the first two trials, a formulation trial was conducted using knowledge gathered from the previous trials so as to produce a droëwors with an optimised formulation and procedure.

The overall objective of this study was thus the development of a dried processed product (droëwors) with the addition of a natural antioxidant, rooibos tea extract (RBTE), to improve its lipid and protein stability.

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CHAPTER 2

Literature review

INTRODUCTION

South African game meat differs from venison from other areas of the world as it is defined as meat derived from wild animals found in their natural environment whilst venison is typically used to describe meat derived from cervids. Due to this it can be said that South African game meat is free from hormones, other growth promoters and human interaction (Radder & Le Roux, 2005). Game meat is becoming more popular among consumers due to its low fat content and favourable fatty acid profile (Schönfeldt, 1993; Viljoen, 1999; Hoffman & Wiklund, 2006; Hoffman & Cawthorn, 2012). The current needs of the average consumer are for convenient, healthy, value-added products (Resurreccion, 2003, Russell & Cox, 2004).

Antioxidants are becoming increasingly more popular in the food industry for addition into products for improvement of shelf-life, colour stability and quality of products. According to Halliwell and Gutteridge (1995), the definition of an antioxidant is "any substance that when present at low concentrations compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substrate." Antioxidants can be either of synthetic (chemical) or natural origin, both which have been successful in delaying/prolonging oxidation in meat and meat products (Attman *et al.,* 1986; Cross *et al.,* 1987; Powell *et al.,* 1986). Synthetic antioxidants currently used in the industry include butylated hydroxyanisole, butylated hydroxytoluene and propyl gallate. Sources of natural antioxidants include herbs, spices and teas (Karakaya *et al.,* 2011). Consumer demand for use of natural antioxidants on and/or in foods has increased over the years due to the perception and concern that synthetic antioxidants are toxic and have carcinogenic effects (Juntachote *et al.,* 2006). Green tea leaf extracts are commonly used as a natural antioxidant due to the antioxidative properties of the polyphenolic flavonoids found in green tea (Manzocco *et al.,* 1998). Studies have been conducted to evaluate the antioxidative properties of green tea leaf extract in beef, pork and poultry meats (McCarthy *et al.,* 2001; Mitsumoto *et al.,* 2005; Nissen *et al.,* 2004; Tang *et al.,* 2001). Limited research such as that of Cullere *et al.* (2013) has been conducted on the use of rooibos tea extract, which also contains polyphenolic compounds, in meat and meat products to inhibit oxidative processes.

Due to minimal research conducted, an opportunity has arisen to study the effects of adding a natural antioxidant to game meat after slaughtering to investigate whether this will improve the product quality and increase its shelf-life.

Red meat consumption in South Africa during 2010 – 2011 was estimated at 24.47 kg/person/year (DoA, 2012). This decreased from the previous year (2009 – 2010), which

was estimated at 24.96 kg/person/year (DoA, 2009). This decrease could be due to the increasing health concerns attached to the consumption of red meat and the increase in the consumers' intake of white meat, which during 2010 – 2011 was estimated at 34.91 kg/person/year (DoA, 2012) or it may be linked to the economic pressure that all consumers are presently experiencing.

Processed products such as sausages, minced meat, bacon, ham and other dried, salted and fermented products are becoming popular amongst South African consumers (Nel & Steyn, 2002).

ANTIOXIDANTS

It has been noted in research that antioxidants were first used as a form of food preservation in World War II (Pokorný, 2001). Over time, synthetic antioxidants replaced the natural antioxidants used as these were cheaper and had more consistent antioxidant properties (Pokorný, 2001). The trend has now returned to using natural antioxidants due to consumer demands. Consumers consider natural antioxidants as being more acceptable as dietary components (Tiwari *et al*., 2009). The trend is towards replacing synthetic antioxidants with more natural antioxidant substitutes. Natural antioxidants fall under two definite groups: a) natural oxidation inhibitors or b) ingredients with natural antioxidant activity (Pokorný, 2001). Rooibos tea extract would be considered an ingredient with natural antioxidant activity.

There are some factors in which antioxidant activity is dependent, for example, the antioxidant concentration, lipid composition, temperature, oxygen and the presence of other antioxidants (Pokorný, 1991).

Phenolic compounds play a role in the antioxidant activity in plant extracts (Kähkönen *et al.,* 1999; Vuorela *et al.,* 2005). There is an increasing interest in the addition of plant extracts to food products due to their ability to inhibit oxidative deterioration of lipids and proteins thus improving the product quality, nutritional value and sensory quality (Kähkönen *et al.,* 1999). The phenolic compounds have redox properties initiating them to act as reducing agents, singlet oxygen quenchers and hydrogen donators (Rice-Evans *et al.,* 1995).

Natural antioxidants have both advantages and disadvantages in comparison with synthetic antioxidants. Advantages include that natural antioxidants are more readily accepted by the consumer as they are regarded as safe and not chemically made, and they gain legislative approval more easily as no safety tests are required if the food component is already 'generally recognised as safe" (GRAS) (Pokorný, 1991). Disadvantages of using natural antioxidants are that they are usually more expensive than synthetic antioxidants, if they are not purified they could be less efficient, there is a large variety of antioxidant activity between different batches and they may add undesirable colour or flavour to the final product

(Pokornỳ, 1991). Similarly, the effectiveness of the natural antioxidants may be inhibited due to loss of synergistic effects after extraction.

Sources of natural oxidative inhibitors as shown in Table 2.1 are classified as either primary antioxidants or secondary antioxidants. Antioxidant activity depends on the mechanism with which it inhibits oxidation in foods. Primary antioxidants (donors) act by inactivating peroxides by reducing free radicals of fatty acids, where the antioxidant hydrogen interrupts the reaction sequence and loses its activity (phenolic compounds). Secondary antioxidants (acceptors) act by breaking the autoxidation chain reactions to delay lipid oxidation by means of: metal ions chelation, regeneration of primary antioxidants, oxygen scavengers, peroxides and non-radical products and quenching singlet oxygen (Gramza & Korczak, 2005).

Table 2.1 Sources of the main natural oxidation inhibitors (Pokornỳ, 1991).

Sources	Oxidation Inhibitors
Oils and oilseeds	Tocopherols and tocotrienols; sesamol and related substances; olive oil resins; phospholipids
Cereals	Various lignin-derived compounds
Fruits & vegetables	Ascorbic acid; hydroxycarboxylic acids, flavonoids, carotenoids
Spices, herbs, tea & cocoa	Phenolic compounds
Protein & protein hydrolysates	Amino acids; dihydropyridines; Maillard reaction products

Antioxidants are often added to processed meats in order to counteract the negative effects of processing aids such as drying, smoking and curing by delaying lipid oxidation and prolonging the shelf-life and quality of these products (Sánchez-Esalante *et al.,* 2003). Adding herbs, fruits, essential oils and other plant extracts in the processing of meat products will result in antioxidant and pro-oxidant actions to occur (Pokornỳ, 2001). Plant phenolics are commonly used as antioxidants. The compound's ability to act as an antioxidant depends on its own chemical structure, composition of the substrate and its characteristics (Pokornỳ, 2001).

Rooibos tea (*Aspalathus linearis)* extract as a natural antioxidant

Rooibos tea, an endemic South African fynbos plant, is a well-known herbal tea with high antioxidant activity (Joubert & De Beer, 2011). Rooibos tea is not only being utilised as a herbal beverage but also as an extract addition in value-added products ranging from food and beverages to the pharmaceutical and cosmetic markets (Joubert & De Beer, 2011).

Due to the variety of applications of rooibos tea extract, the raw material and type of extract used is dependent on what its final use will be. Extracts used in the production of

beverages and functional foods are produced mainly from fermented rooibos (Joubert & De beer, 2011).

Composition of Rooibos tea extract

Rooibos tea contains polyphenol antioxidants called flavonoids. Some of the flavonoids identified in rooibos tea include aspalathin, rutin, orientin, isoorientin, chrysoeriol, quercetin, vitexin and isovitexin, which can be classified as natural antioxidants (Joubert & De Beer, 2011; Pokorný, 2001). Natural antioxidants are usually low in active ingredients. The "active ingredient" of rooibos is assumed to be rooibos solids as there is no single compound in rooibos which is solely responsible for its antioxidant properties (Joubert & De Beer, 2011). Rooibos tea extract is prepared from the large quantities of 'dust' which is not suitable for drying and the production of tea (Pokorný, 1991).

Aspalathin is unique to rooibos and is classified as a dihydrochalcone glucoside (Joubert & De Beer, 2011). The level of aspalathin is dependent on the extraction and purification methods used (Joubert & De Beer, 2011). Fermentation of the rooibos plant material can also cause substantial quantitative changes in its phenolic composition such as the oxidation of aspalathin via its flavanone analogues to isoorientin and orientin (Joubert & De Beer, 2011).

The type of RBTE and the raw material from which it is produced depends on the final application (functional foods and beverages). A hot water extract of "fermented" (oxidised) rooibos is typically used as a food ingredient (Joubert & De Beer, 2011) which on average contains 0.58, 0.84 and 0.80% of its major flavonoids (aspalathin, orientin and isoorientin) (Joubert & De Beer, 2012). In Table 2.2 and 2.3, the flavonoids present in "fermented" (oxidised) rooibos extract can be seen showing the concentration in milligrams that can be found per gram.

Table 2.2 Flavonoids identified in fermented rooibos aqueous extract (Bramati *et al.,* 2002).

Flavonoid	Concentration (mg/g ± SD)
Aspalathin	1.234 ± 0.010
Isoorientin	0.833 ± 0.007
Orientin	1.003 ± 0.010
Quercetin-3-*O*-robinobioside	0.107 ± 0.002
Vitexin	0.330 ± 0.002
Isoquercitin & Hyperoside	0.429 ± 0.002
Rutin	1.269 ± 0.006
Isovitexin	0.265 ± 0.002
Luteolin - 7-O-glucoside	0.029 ± 0.001
Chrysoeriol	0.022 ± 0.001

Table 2.3 Contents of major phenolic compounds in fermented *Aspalathus linearis* plant material (Joubert & De Beer, 2011).

Compound	Concentration (mg/g ± SD)
Aspalathin	0.421±0.017
Nothofagin	0.040±0.022
Orientin	0.202±0.026
Iso-orientin	0.329±0.049
Vitexin	0.035±0.009
Isovitexin	0.035±0.012
Luteolin	0.010±0.005
Luteolin-7-O-β-D-glucoside	0.015±0.008
Chrysoeriol	0.007±0.002
Quercetin	0.010±0.001
Isoquercetin & Hyperoside	0.016±0.015
Rutin	0.173±0.016

The concentrations of flavonoids present in rooibos tea extract (Table 2.2) are approximates to illustrate the large variation in antioxidant activity between different batches of rooibos tea extract (Joubert & De Beer, 2011). Factors affecting the phenolic composition of rooibos tea extract include: variation in seedlings, harvest date and area, populations and plant types (wild/not wild) and natural/processed plant material (green and fermented) (Van Heerden *et al.,* 2003; Joubert *et al.,* 2008; Joubert & De Beer, 2011). Due to this variation, it is important to include the rooibos tea extract flavonoid concentrations in the research and analyses. It is also important to note that the antioxidant effect on both lipid and protein oxidation is dependent on the structure; composition and concentration of the phenolic compounds (Lund *et al*., 2011) in the rooibos tea extract. Research has shown that the concentrations of

aspalathin and quercetin present in a plant extract plays a role in whether the phenolic compounds will act as a pro-oxidant or antioxidant which will ultimately influence the results of studies in which it is being used (Joubert & De Beer, 2011).

Natural antioxidants are popular amongst consumers for their health-promoting properties in humans. Consumers are demanding for better quality food products and are placing increasing pressure on the food industry to re-evaluate the use of food additives (Finley & Given Jr., 1986). Flavonoids are under investigation as an alternative to synthetic antioxidants due to consumer resistance to the latter. The main reason for addition of antioxidants to food products is to improve product quality, such as decreasing oxidation and thereby increasing the shelf-life and flavour profile of these products, and in turn pleasing the consumers.

OXIDATION

Oxidation reactions are the main deterioration processes which result in decreased product quality, nutritional value and sensory quality (Pokorný, 2001). There are many methods to prevent oxidation such as inactivation of oxidative enzymes, reduction of oxygen and oxygen pressure, lowering of the temperature or to use oxidation inhibitors. Oxidation inhibitors are more commonly known as antioxidants (Pokorný, 2001).

Lipid peroxidation is the oxidative degradation of lipids present resulting in undesirable flavours and aromas (Ladikos & Lougovois, 1990). Protein oxidation is the covalent modification of a protein as a result of either reactive oxygen species or by reaction with secondary by-products of oxidative stress also resulting in the unwanted development of off-flavours and aromas (Shacter, 2000).

Lipid Oxidation

Lipid oxidation is a primary mechanism of quality deterioration in meat products. Quality deterioration includes adverse changes in flavour, colour, texture and nutritive value (Gray *et al.,* 1996). The presence or absence of antioxidants and prooxidants influences the stability of lipids in meat during processing and storage. A balance between antioxidants, unsaturated fatty acids and other fatty acids present results in the resistance of rancidity in meat (Ladikos & Lougovois, 1990).

Lipid oxidation is initiated by the disruption of the muscle membrane integrity by deboning, grinding, restructuring, processing and/or cooking. By altering the cellular compartmentalization, this assists the interaction of pro-oxidants with unsaturated fatty acids resulting in the production of free radicals and the propagation of oxidative reactions (Gray *et al.*, 1996). The unsaturated fatty acids in the meat react, via a free radical chain mechanism,

with molecular oxygen forming fatty acyl hydroperoxides (these are the primary reaction products of oxidation). Secondary reactions follow forming aldehydes and epoxides, leading to further lipid degradation and development of oxidative rancidity (Gray, 1978; Ladikos & Lougovois, 1990). Lipids of animal tissue comprise of both saturated and unsaturated fatty acids. The main unsaturated fatty acids of animal tissue are oleic acid, linoleic acid, linolenic acid and arachidonic acid (Mottram, 1987). There are a variety of factors which influence the rate of oxidation such as: the composition of the fat of the animal, processing and storage conditions, types of ingredients (added fat, preservatives, etc) and the concentration of pro- and antioxidants (Ladikos & Lougovois, 1990).

Pork back fat is known to have a favourable fatty acid profile for lipid oxidation as it has a higher unsaturated fatty acid content than beef fat (Wood & Enser, 1997). Pork back fat is commonly used in processed meat products which could explain high oxidation values in such products due to its composition (Fernández-Ginéz *et al.,* 2006). Therefore both pork back fat and beef fat need to be considered for the addition to meat products.

Other factors such as pH, temperature and water activity (a_w) also play an important role in lipid oxidation (Hall, 1987). Water activity influences oxidation of lipids and subsequent reactions. As seen in Fig. 2.1, lipid oxidation decreases between a_w 0 and 0.4, and then increases between a_w 0.5 and 0.8 (Finley & Given Jr., 1986).

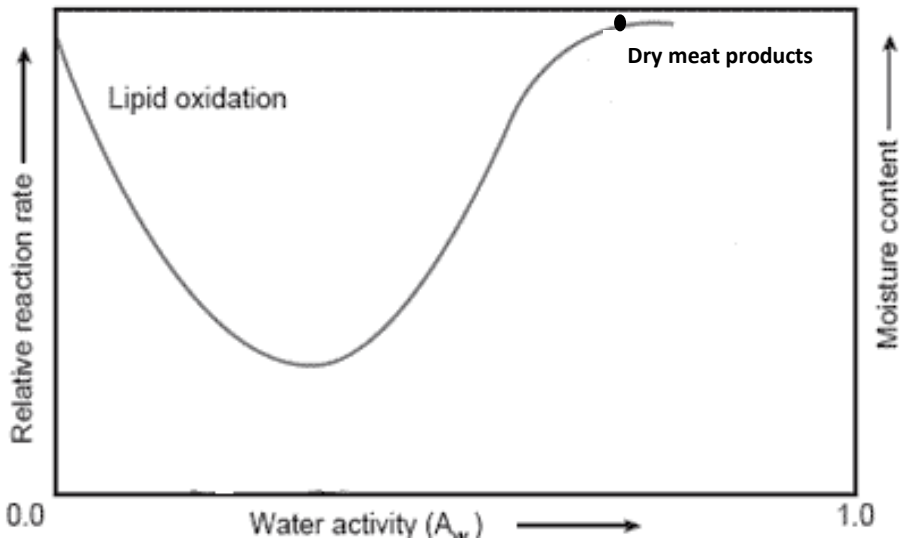

Figure 2.1 Reaction rates in foods as a function of water activity (Adapted from Finley & Given Jr., 1986).

Iron is a pro-oxidant. Pro-oxidants enhance lipid oxidation. Ferrous iron has greater pro-oxidant activity than ferric iron (Pearson *et al.,* 1977). Heme compounds also enhance lipid oxidation as they function as initiators of lipid oxidation and as pro-oxidants (catalysts) when in contact with lipids (Finley & Given Jr., 1986; Ladikos & Lougovois, 1990). Heme

pigments though, are found to be more active catalysts of lipid oxidation with iron in its ferric state (Greene & Price, 1975). These proteins are catalysts of the propagation step of lipid oxidation (Ladikos & Lougovois, 1990). On the other hand, research has shown that large concentrations of heme compounds will inhibit lipid oxidation (Hirano & Olcott, 1971). Therefore, it has been suggested that the level of lipid oxidation that occurs is dependent on the ratio of heme iron to unsaturated fatty acids (Lee *et al.,* 1975).

It has long been recognised that very dry fat-containing food products are vulnerable to lipid oxidation. During processing the cellular structure of the meat is disrupted which allows for greater exposure of the lipids in the product to the heme iron, which is a catalyst for lipid oxidation (Finley & Given Jr., 1986).

Health implications

Both the primary and secondary reaction products of lipid oxidation could have health implications. Lipid hydroperoxides and their decomposition products may affect vital cell functioning by damaging proteins, membranes and biological components of the cells (Frankel, 1984). Malonaldehydes are catalysts in the formation of *N*-nitrosamines which are known to cause mutagenesis (Jurdi-Haldeman, 1987; Pearson *et al.,* 1983; Sanders, 1987).

Methods for lipid oxidation determination

Chemical and physical methods for testing lipid oxidation include: Kreis test, peroxide value, conjugated diene method, ultraviolet spectrophotometry and thiobarbituric acid test.

Kreis test - The Kreis test was the first test used to evaluate fat oxidation. Using the principle that phloroglucinol reacts with oxidised fats in an acidic solution to produce a red colour, this test would be considered as outdated and even though it may be useful in indicating slight changes in the fat state, it does not provide a suitable index of rancidity in the samples (Gray, 1978).

Peroxide value determination - Peroxide value determination measures the primary products of lipid oxidation using an iodometric method or colourimetric method (Fernández *et al.,* 1997). This value is expressed as milliequivalents iodine per kilogram fat (Gray & Monohan, 1992). This method has been used to estimate lipid oxidation in various meat products (Gray & Monohan, 1992) but due to the decomposition of peroxides to secondary products, this could result in peroxide value determination underestimating the degree of lipid oxidation (Gray & Monohan, 1992).

Conjugated diene method - This test is not commonly used in the meat industry for measuring of lipid oxidation (Gray & Monohan, 1992). Oxidation of unsaturated fatty acids is accompanied by an increase in UV absorbance at 230-235 nm (Halliwell & Chirico, 1993). Fatty acids with conjugated unsaturation absorb strongly in the region 230-375 nm, diene unsaturation at 234 nm, and triene unsaturation at 268 nm. This method is not accurate to the degree of oxidation because of the various unsaturated fatty acids varying in quality and quantity. However, the changes in the ultraviolet spectrum of a given substance can be used as a relative measurement of oxidation (Gray, 1978). Greater sensitivity and specificity can be gained by use of different determination tests (Halliwell & Chirico, 1993).

Ultraviolet spectrophotometry - Ultraviolet spectrophotometry is used to determine malonaldehyde (MDA) values by using the absorbance difference between acidified and basified MDA solutions at 267 nm. This method is pH-dependent (3.0 and 7.0) and is sufficient to detect threshold levels of rancidity in meat (Kwon & Watts, 1963).

2-thiobarbituric acid reactive substances (TBARS) test - The 2-thiobarbituric acid reactive substances test (TBARS) test is the most widely used test to determine the level of oxidative deterioration. This method is based on spectrophotometric determination of extracted malonaldehydes (MDA). The suitability of this method is dependent on the type of product, manner of processing and its storage conditions. The test may be conducted either directly on the product, on an extract of the product or on a portion of a steam distillate of the product. TBARS values are said to increase with decreasing particle size as smaller particles are associated with greater cell membrane disruption. The extent of lipid oxidation is expressed as milligrams MDA per kilogram meat sample. This method measures secondary products, which is more appropriate for this study as after drying of the product the primary products would have decomposed and therefore the secondary products will give a better indication of the extent of lipid oxidation (Gray & Monohan, 1992).

For this study, the 2-thiobarbituric acid (TBA) test was used as it is simple and most commonly used to determine the degree of lipid oxidation in general (Gray & Monohan, 1992). As sensory analysis will also be conducted and this method has been reported to have good correlations between TBARS results and sensory analysis to detect rancidity in meat products (Fernández *et al.,* 1997), it would benefit to use this test for this research.

Protein Oxidation

Protein oxidation is initiated by the naturally-occurring pro-oxidants present in the meat, namely heme proteins and metals and proceeds via a free radical chain reaction (Lund *et al.,* 2011). Oxidising lipids have also been reported to assist in protein oxidation (Estévez *et al.*, 2008a; Estévez *et al.*, 2008b). Myofibrillar proteins are targets for reactive oxygen species (ROS) which in the presence of oxygen alters the backbone and amino acid side chains of the proteins and peptides. Oxidative changes involved are cleavage of peptide bonds, modification of amino acid side chains (such as formation of protein carbonyl groups and protein hydroperoxides) and formation of covalent intermolecular cross-linked protein derivatives (explained as the formation of disulphide and dityrosine through the loss of cysteine and tyrosine residues) (Lund *et al.,* 2011). The products formed from protein oxidation are dependent on the amino acids present and the initiation of oxidation (Lund *et al.,* 2011).

The three main changes that occur by protein oxidation in meat are the following, a) formation of carbonyl derivatives impacting flavour, b) loss of sulphydryl groups and c) formation of protein cross-links (Lund *et al.,* 2011).

Protein oxidation is involved in quality deterioration in meat products. Quality deterioration includes reduced water-holding capacity and texture-forming ability (Xiong, 2000). Changes may also occur in protein hydrophobicity, protein conformation and solubility and modified susceptibility of protein substrates to proteolytic enzymes (Wolff & Dean, 1986; Davies *et al.,* 1987). The loss of essential amino acids and the decreased digestibility due to the protein susceptibility to enzymes results in a loss of nutritional value (Morzel *et al.,* 2006).

Research shows that processed meat is more susceptible to protein oxidation than raw meat due to its high concentrations of lipids liable to oxidation, heme-iron pigments and oxidative enzymes (Xiao *et al.,* 2011).

Protein oxidation determination

Protein carbonyls are commonly measured for the estimation of protein oxidation in meat samples (Shacter, 2000). The measurement of carbonyls involves the reaction of the carbonyl group with dinitrophenylhydrazine (DNPH) resulting in the formation of a stable dinitrophenylhydrozone product (Levine *et al.,* 1990). Various methods such as spectrophotometry (370nm), high performance liquid chromatography (HPLC), enzyme-linked immunosorbant assay (ELISA) and sodium dodecyl sufate (SDS) gel electrophoresis can be used to detect these products (Shacter, 2000). The spectrophotometry method will be used for the purpose of this research as it is a widely used method for a general overview of protein

oxidation in food systems, such as in meat and meat products (Estévez, 2011) and it's regarded as being accurate over time (Estévez *et al.* 2008).

CONSUMER PERCEPTION

Consumer needs are what drive the food industry. The current needs of the consumer are for more ready-to eat (RTE), quick, convenient food sources.

Red meat is associated with a high content of saturated fats (Nestle, 2007). Recently, red meat has been attached to increasing health concerns due to its relationship with high blood pressure, hypertension, obesity and cardiovascular diseases; this has led to a global decline of meat consumption over the last decade. These health concerns have resulted in consumers changing their diet from high-fat, high-protein diets to diets that include more fresh fruits and vegetables (Pollard *et al.,* 2002). Game meat, in comparison to beef, is lower in fat (with an average fat content ranging between two and three percent), lower in saturated fatty acids and higher in polyunsaturated fatty acids (USDA, 1986; Hoffman & Wiklund, 2006; Hoffman & Cawthorn, 2012). South African game meat is distinguished by its dark red colour, this could be result of myoglobin build-up as game animals are more active (Hoffman, 2001). Springbok (*Antidorcas marsupialis*) and blesbok (*Dameliscus dorcas phillipsi*), amongst others, were ranked by South African game farmers as the most favoured species to farm (Hoffman *et al.,* 2005). South African consumers consider game meat as an exotic seasonal product rather than a 'traditional' meat such as beef, lamb, chicken and pork. It was established that these consumers were not aware of the positive attributes of game meat but would not pay more for game meat regardless (Hoffman *et al.,* 2005). Tourists visiting South Africa (mainly German and Belgian tourists) stated that they enjoy game meat and are informed about the associated health benefits of game meat (Hoffman *et al.,* 2003).

Research shows that consumers readily judge meat quality by three sensory properties: appearance, flavour and texture (Gray *et al.*, 1996; Liu *et al.,* 1995; Meiselman & MacFie, 1996). Quality, according to Grunert (2004), is the multi-dimensional trend described by a set of characteristics that are subjectively perceived by the consumer. A consumer evaluates quality on the characteristics associated with a certain product. There are intrinsic and extrinsic indications of quality (Olsen & Jacoby cited in Bernués *et al.*, 2003). Intrinsic quality refers to the physical aspects of a product such as colour, shape, appearance, whereas extrinsic quality refers to the product information (brand, stamp, origin, packaging, etc.). Research by Hoffman *et al.* (2005) indicates that consumers consider the fat content, colour and freshness to be the most important qualities when purchasing any meat type.

Another approach to defining meat quality is by measuring product characteristics into four categories, as seen in Table 2.4.

Table 2.4 Categories of product characteristics measurements on meat quality (Becker, 2000).

Category	Product Characteristic
Nutritional value	Protein
	Fat
	Carbohydrate
Processing quality	Shear force
	pH-value
	Water-binding capacity
Hygienic-toxicological quality	Contaminants
	Microbacterial status
	Additives
Sensory quality	Texture (tenderness, juiciness)
	Flavour/Odour
	Colour appearance (marbling)

Nutritional value and sensory quality will be used as markers of meat quality in this study. The quality of meat and meat products is regulated by the National Department of Health (DoH) of South Africa (1990/2001). Temperature, water activity (a_w), pH, microbial composition and oxidation are all factors affecting the quality of meat and meat products (Eisel *et al.*, 1997; Garbutt, 1997; Morrissey *et al.,* 1998; Romans *et al.*, 2001).

Oxidation also affects the sensory attributes and shelf-life of a product. To improve the shelf-life of meat products preservatives are frequently added. However, consumers are becoming more aware of the negative health effects of chemical preservatives and are therefore looking for products which are preserved with natural preservatives or have no added preservatives (McDonald, 1992; Bañón *et al.,* 2007).

PROCESSED MEAT PRODUCTS

"Sausage" derives from the Latin word "*salsus"* which means salted, or meat preserved by salt (Rust, 1987). Since 900 B.C., sausage has been a known source of food preferred by the Romans (Steyn, 1989). During the Middle-Ages, countries around the world developed different types of sausages according to their national tastes, geographical location and climate (Steyn, 1989). Examples include the Italian Bologna sausage, the German Brutwurst, the French Lyons sausage and the South African boerewors and droëwors.

Boerewors is a South African sausage that contains a mixture of beef and pork, with added fat. A total meat content of 90% and a maximum of 30% fat is required. No other ingredients except: cereal products or starch; vinegar, spices, herbs, salt or other harmless flavourants; permitted food additives or water may be added. The meat is mixed with salt, pepper, coriander and various other spices (DoH, 1990/2001). Boerewors can be dried to

make what is known as droëwors (droëwors / dried sausage) (Steyn, 1989). Droëwors regulations, when produced using game species, follow those of raw species sausage/raw mixed-species sausage as set by the Department of Health (DoH) of South Africa (1990) containing minimum 75% total meat content and not exceeding more than 30% fat content. This corresponds with the SANS 885:2011 standard that droëwors, a processed meat product, should contain 80% total meat content, of which 55% is actual lean meat, with a maximum of 50% fat content. Formulations for game droëwors vary depending on the meat used. Typically it will consist of 60% game meat, 30% beef/sheep meat and 10% fat and spices (De Villiers, 1992). Commercially, a preservative is usually added so as to increase shelf-life and improve the colour stability of the product (Peña-Edgido *et al.*, 2005). Potassium and sodium nitrate (160 mg/kg max.) are the permitted preservatives used in processed meat products.

Droëwors

Droëwors is a ready-to-eat dried seasoned meat sausage commonly made from beef (Burnham *et al.,* 2008). However, droëwors can also be made from other meat sources such as game meat (springbok and kudu) (Carr *et al.,* 1997). Droëwors can be produced using any cuts of meat whether the premier cuts or less desirable cuts (Holm, 1969). This form of dried meat product was developed in South Africa (Burnham *et al.,* 2008) and is regularly consumed as a snack food.

The typical steps in the manufacturing process of droëwors are the following: cutting the meat and fat, grinding the meat and fat, seasoning, stuffing into natural casings and drying under ambient conditions (Burnham *et al.,* 2008). Droëwors is typically associated with acidic-vinegar flavours and coriander spices. Drying procedures influence the final product characteristics depending on the temperature, relative humidity and rate of air movement (Burnham *et al.,* 2008). Droëwors can be stored at low/medium temperatures for several months.

Over the years, consumers have increased their consumption of snack foods due to convenience. The food industry needs to take advantage of these consumption trends and develop and expand product lines to meet the current needs of the average consumer (Carr *et al.,* 1997; Miller *et al.,* 1988).

GAME MEAT SPECIES

Ostrich meat was used for the initial trial of the addition of rooibos tea extract to a processed meat product to make droëwors. Other trials to follow will utilize the meat of blesbok (*Damaliscus pygargus phillipsi*), springbok (*Antidorcas marsupialis*) and fallow deer (*Dama dama*). All these meats are known to contain high levels of iron (a pro-oxidant) in their

myoglobin and have favourable fatty acid profiles (low saturated fatty acids and high unsaturated fatty acids). Table 2.5 illustrates the averaged proximate composition of typical game species and domesticated species.

Table 2.5 Averaged proximate composition of typical game species meat in comparison with beef and chicken meat.

Component	Ostrich[1]	Blesbok[2]	Springbok[3]	Fallow Deer[4]	Beef[5]	Chicken[6]
Moisture (%)	76.27	75.09	73.14	76.02	71.6	75.46
Protein (%)	21.27	22.32	20.71	21.67	20.94	21.39
Intramuscular fat (%)	0.65	0.78	1.21	0.64	6.33	3.08
Ash (%)	1.07	1.29	1.28	1.13	1.03	0.96

[1] Sales & Hayes, 1996
[2] Hoffman *et al.*, 2008
[3] Hoffman *et al.*, 2007
[4] Volpelli *et al.*, 2003
[5] USDA, 1986
[6] USDA, 1979

As seen in Table 2.5, the game species (blesbok, springbok, fallow deer) all have a intramuscular fat content below 3%, with beef having a high intramuscular fat of more than 6%. Due to the low intramuscular fat content of game species in comparison with domesticated species, game species are expected to have higher moisture contents. This is based on the inverse correlation between intramuscular fat and moisture content of meats (Sale, 1995).

Ostrich meat (*Struthio camelus*)

General: marketing and utilization of ostrich

Ostrich (*Struthio camelus*) is marketed for sale as live-breeding birds, meat and by-products such as leather and feathers (Sheets, 1994).

Ostrich meat is marketed as a premium product as only the finest portion of the fresh cut meat is used in the food industry or establishment (McKenna *et al.,* 2003). A substantial portion of the meat and carcass is not used which, to increase profitability, could be sold as lean, raw material sources for processed meats (McKenna *et al.,* 2003).

Not many studies have been successful in using ostrich meat in processed meat products. In 1996, Bohme *et al.* successfully produced Italian-style salami using ostrich meat. Later, chopped ham-like products and Vienna sausages were produced using ostrich meat (Fisher *et al.,* 2000). Low fat ostrich patties have also been successfully produced as indicated by Hoffman and Mellet (2003). These studies concluded that processed meat

products using ostrich meat must be able to compete with other meat and meat products currently on the market both nutritionally and in sensory characteristics. Therefore utilization of ostrich meat as a raw material for the production of processed meat products will only be plausible if consumers find the finished products to be acceptable in comparison to traditional products (McKenna *et al.,* 2003).

Nutritional composition and health benefits

Ostrich (*Struthio camelus*) meat has become increasingly popular over the years due to its nutritional characteristics of being low in fat and cholesterol (McKenna *et al.,* 2003; Sales *et al.,* 1996; Sales & Hayes, 1996). It may be the most popular choice as an ideal alternative to beef (McKenna *et al.,* 2003). According to several reviews (Balog & Almeida Paz, 2007; Hoffman, 2005; Hoffman, 2008; Paleari *et al.,* 1995; Sales, 1999; Sales & Horbańczuk, 1998; Sales & Oliver-Lyons, 1996; Majewska *et al.,* 2009) it has also been concluded that ostrich meat relative to other meat species can be characterised as follows: high final pH (> 6.0), low intramuscular lipid content, low sodium content and high iron content. A high final pH can be both beneficial and undesirable for the meat as it improves colour and water-binding capacity but reduces its keeping quality and flavour (Majewska *et al.,* 2009). Ostrich meat is also known to have high levels of polyunsaturated fatty acids (Sales *et al.,* 1996) and higher heme-iron contents (Sales & Hayes, 1996) which make it more susceptible to oxidation than beef or chicken.

Table 2.5, illustrates the comparison of proximate composition between typical game species meat, beef and chicken meat. In terms of comparing the ostrich meat with beef and chicken meat, the results suggest that ash and protein contents are constant between the different meats with the difference between them being the intramuscular fat. The intramuscular fat of the ostrich meat is exceptionally lower than that of other domesticated meats. When compared with the other game meat species, ostrich meat has low intramuscular fat on par with fallow deer (Hoffman, 2005; Majewska *et al.,* 2009; Sales & Hayes, 1996).

Blesbok meat (*Damaliscus pygargus phillipsi*)

General: Local and commercial utilisation

Blesbok (*Damaliscus pygargus phillipsi*) is one of the top four of the most favoured game species to farm with in South Africa (Du Buisson, 2006). It is one of the most dominant game species exported from South Africa (Hoffman & Wiklund, 2006). Blesbok is most commonly used as fresh meat or in the production of biltong.

Blesbok is most commonly found in parts of Kwazulu-Natal, Eastern Cape as well as the Highveld of the Free State and Gauteng (Lloyd & David, 2008), mainly being present on privately owned farmlands (Watson *et al.,* 2011). Blesbok is used predominately for production of droëwors and biltong (a form of dried meat similar to jerky) in these farmland regions. Though well-known by South African consumers, blesbok is hunted for local utilisation each year and not for widespread use. There is still uncertainty for its use in commercially made processed meat products due to the consumers' perception of this meat having a "gamey taste" and lack of knowledge of the consumers regarding healthiness and benefits of consumption of game meat products (Hoffman *et al.,* 2005). In 2012, South African game meat exports was closed which could lead to an increase in commercial utilisation of game meat locally (Neethling, 2012) both as raw meat and processed meat products such as droëwors.

Nutritional composition

According to Hoffman *et al.* (2008), blesbok meat can be described as a red meat with a favourable fatty acid profile and fairly low lipid content. Studies show that blesbok meat contains 81.8% of the total essential amino acids needed for human development (Van Zyl & Ferreira, 2004). Table 2.5 shows the proximate composition of blesbok meat and other game meat species and popular domesticated meats. In terms of comparing the blesbok meat with beef and chicken meat, the results indicate that the intramuscular fat of the blesbok meat is much lower with a value under 1.0% whilst the other domesticated species have higher intramuscular fat content of greater than 3%. When compared with the other game meat species, blesbok meat has lower intramuscular fat than springbok but slightly higher content than ostrich meat and fallow deer.

Springbok meat (*Antidorcas marsupialis*)

General: Local and commercial utilisation

Research of Jansen van Rensburg (1992) showed that 39% of the South African population only eat game meat as biltong/droëwors (in a raw dried form). With springbok being the most favoured game species to be hunted in South Africa (Hoffman & Wiklund, 2006) it can be assumed that it is commonly consumed by South Africans as a processed meat product. Springbok has great potential for production due to its nutritional composition and well-known status among consumers (Hoffman *et al.,* 2007a). As with blesbok meat, it is used in biltong and droëwors production but is also commonly sold in commercial markets throughout South Africa. Springbok is also sold as raw meat and as processed meat products and is becoming increasingly popular as the meat of choice amongst consumers.

Nutritional composition

Springbok has a similar profile in terms of its fatty acids and lipid content as to blesbok and fallow deer meat (Table 2.5). Springbok meat when compared with the other game species has the highest intramuscular fat content but is still low being under 2%. Due to this low intramuscular fat content it is marketed as being a 'healthier' alternative to red meat (Hoffman *et al.,* 2007b).

Fallow Deer (*Dama Dama*)

Utilisation and nutritional composition

Fallow deer is not commonly eaten by South African consumers but the international farming of these animals has grown considerably over the past few decades (Hoffman & Cawthorn, 2012). Most fallow deer hunted and consumed in South Africa is derived from feral populations that escaped from introductions in the early 1900's. Studies have been conducted to determine the nutritional value of fallow deer meat (Table 2.5) although no studies have yet been conducted on deer found in South Africa. Protein is high with a percentage of greater than 20% with a fat content of less than 1% although observations have shown that local feral deer have substantially higher levels of fat with visible subcutaneous fat. Most African ungulates do not have a well established subcutaneous fat layer. None the less, the chemical composition proves that it could be a good replacement for red meat such as beef (Volpelli *et al.,* 2003). With the distribution of feral fallow deer becoming more extensive and its favourable nutritional content, it should be considered in the production of meat products such as droëwors. Limited research has been conducted on the sensory evaluation of fallow deer. It is not commonly found in the marketplace and the possibility exists that due to lack of knowledge of this species' meat by the local consumer, it would not sell well commercially. With increasing interest of this animal by hunters (due to its accessibility) the processing of fallow deer droëwors would be of interest.

CONCLUSION

With consumer preference leaning towards the consumption of snack and convenience foods the production of droëwors and improvement of its quality is of importance. Usually, in a commercial setting a synthetic preservative would be added to processed meat products in order to improve the shelf-life of a product as well as its colour stability and possibly flavour quality. With the increasing knowledge and awareness of consumers towards additives in foods, the food industry is starting to add natural antioxidants and preservatives to foods.

Rooibos tea is also gaining popularity with consumers and is known to originate from South Africa and to have high antioxidant potential. Therefore, it would be of interest to determine the effect of rooibos tea extract when added to droëwors (especially that made from game meat species) on its shelf-life, in terms of both lipid and protein oxidation, and its sensory attributes.

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CHAPTER 3*

Effect of rooibos tea extract (*Aspalathus linearis*) as a natural antioxidant on the lipid and protein stability and sensory profile of ostrich (*Struthio camelus*) droëwors

ABSTRACT

The effect of rooibos tea extract (RBTE 0%, 0.25%, 0.50%, 1.0%) as a natural antioxidant on the lipid and protein stability of ostrich droëwors after a 15 day drying period was investigated. The lipid stability of the droëwors increased with RBTE 0.25% having lower TBARS. The protein stability of the droëwors did not differ ($P \geq 0.05$) between treatments. The heme-iron results did not differ ($P \geq 0.05$) between the treatments and increased from day 0 to day 15. Drying resulted in a decrease in the total moisture content and a corresponding increase in all other components. There were no differences between the moisture, fat and ash contents between treatments within a specific day. The droëwors had high concentrations of oleic acid, palmitic acid and linoleic acid. The addition of RBTE also improved the sensory attributes and can thus be added and marketed as a natural antioxidant from 'out of Africa' for a traditional South African meat product.

Keywords: Aspalathus linearis; rooibos tea extract; natural antioxidant; lipid and protein stability; droëwors

*Hoffman, L.C., Jones, M., Muller, N., Joubert, E., & Sadie, A. (2013). Lipid and protein stability and sensory evaluation of ostrich (*Struthio camelus*) droëwors with the addition of rooibos tea extract (*Aspalathus linearis*) as a natural antioxidant. *Meat Science.* DOI: http://dx.doi.org/10.1016/j.bbr.2011.03.031

INTRODUCTION

Droëwors is a ready-to-eat dried seasoned meat sausage from Southern Africa commonly made from a mixture of ground beef and animal fat (Burnham *et al.,* 2008). However, droëwors can also be made from other exotic meat sources such as game meat (springbok and kudu) (Carr *et al.,* 1997) and ostrich meat. Ostrich meat, which is used in this study, is gaining more attention in the marketplace and is increasingly marketed as a healthy alternative to other red meats due to its leanness, low cholesterol content and favourable fatty acid profile (Sales & Horbanczuk, 1998).

Preservatives are frequently added to processed meat products so as to increase the shelf-life and improve the colour stability of the product (Pokorný, 2001). However, consumers are becoming more aware of the use of synthetic/chemical additives in food products (Tiwari *et al.,* 2009) and therefore the addition of natural antioxidants in products are becoming more popular as consumers consider natural antioxidants in products as being more acceptable as dietary components.

The antioxidant activity of plant extracts containing phenolic compounds against lipid oxidation has been investigated and plant extracts may provide an alternative for synthetic food additive use (Vuorela *et al.,* 2005). A plant extract that is increasingly used as a food ingredient is that of rooibos (*Aspalathus linearis*), an endemic South African fynbos plant traditionally used as a herbal tea. It is well-known for its antioxidant activity and the presence of aspalathin, a dihydrochalcone glucoside unique to rooibos (Joubert & De Beer, 2011). The antioxidant activity of rooibos flavonoids has been demonstrated in a number of assays, including their ability to inhibit lipid peroxidation (Von Gadow *et al.,* 1997; Joubert *et al.,* 2005; Snijman *et al.,* 2009). In natural products such as rooibos tea, the content of active antioxidants is usually low and therefore it is necessary to use a more concentrated form such as an extract of rooibos tea to obtain a concentrated amount of antioxidant to ensure significant improvement in oxidative stability (Pokorný, 2001). Hot water extract of "fermented" (oxidised) rooibos, typically used as a food ingredient (Joubert & De Beer, 2011), contains on average 0.58, 0.84 and 0.80% of its major flavonoids, aspalathin, isoorientin and orientin (Joubert & De Beer, 2012).

Lipid and protein oxidation in meat cause the damage of cellular structures which results in a decrease in meat quality and its shelf-life. The damage allows for the polyunsaturated fatty acids and pro-oxidants (iron in game and ostrich meat) to interact which causes oxidation to occur. Therefore, the higher the level of polyunsaturated fatty acids or pro-oxidants, the faster the oxidation process occurs (Vuorela *et al.,* 2005). There are two types of oxidation that occur in meat, lipid oxidation and protein oxidation. Lipid peroxidation is the oxidative degradation of lipids present resulting in undesirable flavours

and aromas (Ladikos & Lougovois, 1990). Protein oxidation is the covalent modification of a protein as a result of either reactive oxygen species or by reaction with secondary by-products of oxidative stress also resulting in the unwanted development of off-flavours and aromas (Shacter, 2000).

The aim of this study was to determine the effect of rooibos tea extract (RBTE) antioxidants on the quality and shelf-life stability of ostrich droëwors and it sensory attributes.

MATERIALS AND METHODS

Droëwors production

The experimental layout was a hierarchical (nested) design with three levels (Fig. 3.1). Ostrich meat was trimmed of all external fat and connective tissue and cut into approximately 10 cm x 10 cm cubes. Pork back fat was added to increase the amount of fat in the product. A total of 40 kg of raw product was used and divided into four batches of 10 kg each. Each 10 kg batch consisted of 75% ostrich meat and 25% pork back fat and combined with 2.4% (240 g) salt, 0.5% (50 g) pepper and varying amounts of hot water rooibos tea extract (Table 3.1) (RBTE: 0%, 0.25%, 0.50% and 1.0% concentrations). Each batch was minced through a 5 mm grinder after which the spices (salt and pepper) and varying concentrations of RBTE were added. The minced meat and spices were then mixed and minced through a 2 mm grinder. Natural sheep casings (22 mm diameter) were filled with the minced meat mixture. Half the sausage from each batch was dried in an environmentally controlled maturation chamber at 82% relative humidity (RH) for the first 4 days, dropping it to 75% RH thereafter. The temperature was kept at a constant 15°C. The drying process took 15 days in which the droëwors lost 45% of its mass. The rest of the sausage was left raw (first nested factor: 0 days or 15 days).

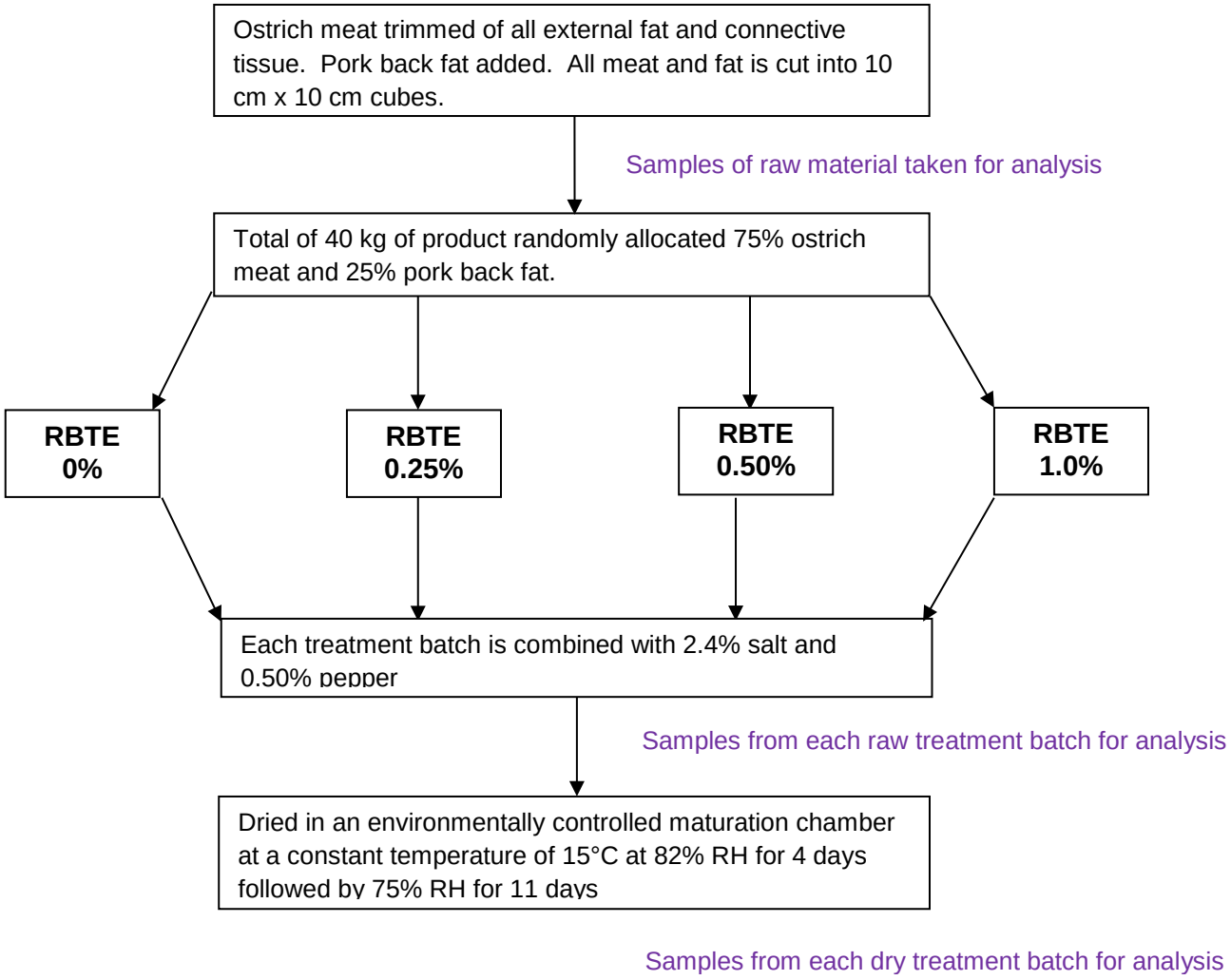

Fig. 3.1 Droëwors production procedure.

Table 3.1 Phenolic composition of fermented rooibos tea extract (RBTE) used in the samples analysed.

Flavonoid	Concentration (g compound/100 g extract)
Aspalathin	0.482
Nothofagin	0.056
Isoorientin	0.956
Orientin	0.823
Quercetin-3-*O*-robinobioside	0.637
Isoquercitrin	0.138
Vitexin	0.179
Hyperoside	0.167
Rutin	0.179
Isovitexin	0.144
Luteolin - 7-O-glucoside	0.196

Analysed according to the method of Beelders *et al.* (2012)

Sample preparation

Five replicate sub-samples (150-200 g) of both the raw and dried samples of each batch of droëwors were taken and individually homogenised in a blender for 3 min to ensure a representative sample was tested. The samples were stored in a -80°C freezer until analyzed. The homogenised samples were used for proximate analysis, and the analysis of fatty acid composition and heme-iron concentration, as well as for lipid and protein oxidation.

Proximate analysis

The samples were analysed to determine the moisture (Method 934.01) and ash (Method 942.05) content according to the AOAC (2002). The protein content used AOAC (1992) procedure 992.15, whereas the fat was determined using the chloroform/methanol (2:1) fat extraction method according to Lee *et al.* (1996). All analyses were performed in duplicate.

Fatty acid composition

The fatty acids were methylated and then analysed using gas chromatography to determine the fatty acid composition and percentages. Using a modified method of Folch *et al.* (1957), a 2 g sample was extracted with a chloroform/methanol (CM 2:1; v/v) solution containing a 0.01% butylated hydroxytoluene (BHT) antioxidant. An internal standard, heptadecanoic acid ($C_{17}H_{34}O_2$) (Sigma-Aldrich Inc., 3050 Spruce St., St. Louis, MO, 63103, USA; Cat. No. H3500) was added to quantify the individual fatty acids. The sample was homogenised within the extraction solvent using a polytron mixer (Kinematica, type PT 10-35, Switzerland).

A transmethylated reagent, methanol/sulphuric acid (19:1; v/v) was added to 250 µL of the isolated lipids and left for 2 h at 70°C. After cooling, the fatty acid methyl esters (FAME) were extracted with water and hexane and the supernatant transferred to a Kimax tube (125 x 16mm) and dried under nitrogen for ± 30 min at 45°C. Fifty µL hexane was added to the dried sample of which 1 µL was injected. Analysis was done on a Thermo Finnigan Focus GC (Thermo Electron S.p.A, Strada Rivoltana, Milan, Italy) equipped with a flame ionization detector using a BPX70 capillary column (60 m x 0.25 mm internal diameter, 0.25 µM film, SGE International Pty Ltd, 7 Argent Place, Ringwood, Victoria 3134, Australia). The carrier gas used was hydrogen (1 mL/min). The temperature settings were as follows: the initial temperature 60°C, the final temperature 160°C, the injector temperature 220°C and the detector temperature 260°C. The rate of temperature increase was 7°C per min. The FAME were identified by comparison of the retention times to those of a standard FAME mixture (Supelco 37 Component FAME mix, C4 – C24, Cat no. 47885-U , 595 North Harrison Rd, Bellefonte, PA, 16823-0048, USA) and the milligram fatty acid per gram of tissue sample was calculated.

Heme-iron

The heme-iron concentration was determined according to the method of Hornsey (1956). A 5 g homogenised sample was taken from each replicate and mixed with 1 mL distilled water, 20 mL acetone and 0.5 mL hydrochloric acid. Thereafter the flask was covered with parafilm and left in the dark overnight (±16 h). The sample was then filtered and the absorbance measured at 640 nm in a Cecil CE2021 2000 Series spectrophotometer (Lasec SA (Pty) Ltd). The heme-iron concentration values are expressed in mg heme-iron per g sample.

Lipid oxidation

A 1 g sub-sample was taken from each replicate and homogenised in a blender with 10 mL 0.15M potassium chloride buffer for 20 s. The lipid oxidation process was followed by measuring the thiobarbituric acid reactive substances (TBARS) using the spectrophotometric method described by Rosmini *et al.* (1996). The samples were tested in duplicate. The absorbance was measured at 532 nm in a Cecil CE2021 2000 Series spectrophotometer (Lasec SA (Pty) Ltd). The TBARS values are expressed as mg malonaldehydes (MDA) per kg product and mg MDA per gram fat.

Protein oxidation

A 1 g sub-sample was taken from each replicate and homogenised in a blender with 10 mL 0.15M potassium chloride buffer for 20 s. Carbonyls and proteins were measured. The

samples were tested in duplicate. The carbonyls were determined according to the method outlined by Oliver *et al.* (1987). The absorbance was measured at 562 nm in a Cecil CE2021 2000 Series spectrophotometer (Lasec SA (Pty) Ltd). The oxidised protein concentration values are expressed as µM carbonyl per mg protein.

Descriptive sensory analysis

A fourteen-member sensory panel was trained according to Lawless and Heymann (2010) using the generic descriptive analysis technique. The panel evaluated a 2 cm length 'stick' of each treatment for attributes pertaining to its aroma, flavor, appearance and texture. All attributes were scored on an unstructured 100-point scale (0 = no intensity, 100 = extreme intensity) (Table 3.2). The panel was trained over three training sessions of approximately 90 min each, mainly to generate sensory terminology and to develop the score sheet. After the training sessions, a blind test was conducted where the trained panelists would analyze each treatment. All samples were served in glass ramekins, labelled with 3-digit codes and presented to each panellist in a randomized order. The panelists were seated in booths equipped with *Compusense*® *five* software (Compusense, Guelph, Canada). The blind test was repeated three times per sample over a 10 day period. All analyses were conducted in an area with temperature and light control.

Table 3.2 Descriptions of the full range of sensory attributes as used in the descriptive sensory analyses.

Characteristic	Attribute	Description
Aroma*	Rooibos	Associated with sweet, woody aroma typical of rooibos
	Smoky	Associated with smoke essence
	Gamey, Ostrich	Associated with game meat, wild animal
	Fattiness	Associated with fresh non-oxidised fat
	Salami-like	Associated with salami attributes
	Fermented Meat	Associated with fermented smells
	Rancid	Associated with oxidised fat
Appearance	Colour intensity (meat)	Look at inside of the sample and outside surface *0=Red, 100=Brown*
	Fat colour	Look at fat on the inside of the sample *0=White, 100=Darker*
	Shiny casing	Associated with salami casing *0=Dull, 100=Shiny*
Flavour*	Rooibos	Associated with sweet, woody flavour typical of rooibos
	Smoky	Associated with smoke essence
	Acidity	Associated with acid, sour taste
	Saltiness	Associated with salty taste
	Fattiness	Associated with fresh, non-oxidised fat
	Peppery	Associated with pepper
	Rancidity	Associated with oxidised fat
	Gamey, Ostrich	Associated with game meat, wild animal
	Salami-like	Associated with salami attributes
	Fermented meat	Associated with fermented flavours

* Scale for descriptors: 0=No intensity, 100=Extreme intensity

Statistical analysis

The chemical composition data were analysed with SAS Enterprise Guide 4 (2006) using analysis of variance (ANOVA) for fitting a hierarchical model to the data (Quinn & Keough, 2002). The level of probability to determine a significant difference was 5%. Data were also subjected to tests for homoscedasticity and normality. In the case of treatment comparisons according to the ANOVA, treatment means were compared by means of multiply comparison testing using Bonferroni t-tests at $\alpha = 0.05$. The oxidative chemical and sensory analyses were analysed with Principal Component Analysis (PCA) using the correlation matrix (Lawless & Heymann, 2010) within the XLStat software package (Addinsoft, New York, USA).

RESULTS AND DISCUSSION

Proximate composition analysis

Results for per cent moisture, fat and protein values (on a wet weight basis) are presented in Table 3.3. Droëwors samples at day 15 were lower in moisture content than those at day 0. This is expected as the product was dried losing up to 45% of its original mass. Treatments did not influence the amount of mass lost which was expected as all the samples had been dried in the same chamber where there was sufficient airflow to ensure even drying. In terms of protein content, the samples at day 15 had a higher protein level than the samples at day 0. This was again expected due to the moisture loss during the drying process of the sausages. With lower levels of moisture, it would be expected that percent fat would be higher after drying (Nuñez de Gonzalez *et al.*, 2009), which can be seen in Table 3.3 as the fat content increases at day 15. The fat content ranged between 29.57 and 33.79% after drying. The ash content also increased after drying; the high levels of mineral (compared to raw meat) can be explained by the added spices (minerals). There were no major differences in the proximate analysis components between the treatments within the same day – a result consistent with the methodology followed.

Fatty acid profile

The fatty acid profiles (% of total fatty acids) of the ostrich droëwors with the various levels of RBTE added are presented in Table 3.4.

The ostrich meat in this investigation has high percentages of oleic acid, palmitic acid and linoleic acid (Table 3.4). In total, the ostrich meat had 39.21% saturated fatty acids (SFA) whilst the pork backfat added had 34.80% SFA. Ostrich meat has a low intramuscular fat content and favourable fatty acid profile, with intramuscular ostrich fat containing 16.5% polyunsaturated (PUFA) *n*-3 fatty acids (Fisher *et al.,* 2000; Sales, 1998; Sales *et al.,* 1996). Other studies have shown pork back fat with a fatty acid profile containing mainly oleic acid (39.6%), linoleic acid (24.7%) and palmitic acid (21.8%) (Koch *et al.,* 1968) but diet is known to influence the fatty acid profile of pork (Wood *et al.,* 2007). In this study, it was found that oleic acid, palmitic acid and linoleic acid were the most abundant fatty acids present in the pork back fat added (Table 3.4). The pork back fat had 214.08 mg/g meat SFA and 86.84 mg/g meat PUFA whilst the ostrich meat had very low values of 0.25 mg/g meat SFA and 0.18 mg/g meat PUFA. The dry droëwors samples ranged between 74.94 – 82.79 mg/g meat SFA and 29.91 – 46.55 mg/g meat PUFA which indicate that the samples had a more similar profile to the pork backfat than the ostrich meat.

The fatty acid profile of the droëwors, both raw and dried, is expected to change after drying. Oleic acid, palmitic acid and linoleic acid were found in the highest concentrations. Differences in the fatty acid profile within day 0 and within day 15 could be assumed to be due to the natural variation of fat content of a specific batch or random error in sampling.

When studying the total fatty acid profile, it can be seen that the saturated fatty acids (SFA) present are high for the ostrich meat and pork back fat (Table 3.4). The droëwors samples also had high saturated fat concentrations. This is because the added pork fat increased the SFA. The total polyunsaturated fatty acids are high in ostrich meat and therefore oxidation is expected to occur in the final product as in meat, the higher the level of polyunsaturated fatty acids (PUFA), the faster the oxidation process (Vuorela et al*.,* 2005).

According to Wood *et al.* (2004) and Simopoulos (2004), it has been recommended that to improve the health status of a population, the n-6:n-3 PUFA ratio should be less than 4% and the PUFA:SFA ratio should be more than 0.45. The polyunsaturated fatty acids and saturated fatty acid (PUFA:SFA) ratio, as well as the PUFA omega 6:omega 3 ratio (Table 3.4) were determined to assess the nutritional status of the droëwors. The PUFA:SFA ratio's across all treatments were all in accordance to the recommended 0.45 and the PUFA omega 6:omega 3 ratios were all less than 4% as recommended.

Heme-iron

Oxidation in meat and meat products is closely related to the breakdown of heme-iron and the release of iron from the porphyrin ring (Estévez *et al.,* 2005). The RBTE was added to protect the heme molecule from degradation and inhibit the increase of non-heme iron in the droëwors through primary and secondary antioxidant activity, i.e. scavenging of free radicals and chelation of non-heme iron by the flavonoids in RBTE (Snijman *et al.,* 2009). The amount of heme-iron, as with protein oxidation, remains fairly constant between treatments (Table 3.5) with low values of 0.59 mg heme-iron per 100 g meat and 2.51 mg heme-iron per 100 g meat at day 0 and day 15 respectively and high values of 0.67 mg heme-iron per 100 g meat and 2.67 mg heme-iron per 100 g meat at day 0 and day 15 respectively. The addition of RBTE should reduce the release of iron from the heme molecule which in turn will reduce protein oxidation.

According to the heme-iron results (Table 3.5), after drying, the addition of the RBTE did not differ significantly ($P < 0.05$) when comparing treatments within a day. Between concentrations of day 0 and day 15 when comparing the same treatments, it can be seen that there was not a reduction of the release of heme-iron molecule but a slight increase. Heme-iron levels increased from day 0 to day 15 which could be due to moisture losses that occur because of the drying period. Iron concentrations increase during cooking and drying

as the iron-containing proteins (myoglobin and haemoglobin) are denatured during the process (Campo *et al.,* 2003). The higher the heme-iron levels, the more susceptible the meat is to oxidation (Seydim *et al.,* 2006). RBTE 0% and RBTE 0.50% had a slightly higher heme-iron concentration at day 15 when compared with the other samples which is also depicted in Fig. 3.2.

Although no previous studies have been conducted on the addition of RBTE to ostrich meat, other red meats, with relatively high heme-iron have resulted in higher thiobarbituric acid reactive substances (TBARS) values than meats with lower heme-iron values (Fernandez-Espla & O'Neill, 1993). However, the results in Table 3.5 do not support this as in some cases, the lower TBARS values have higher heme-iron concentrations, and therefore this is an aspect that warrants further research.

Lipid oxidation

The results (Table 3.5) for lipid oxidation indicate that there were no significant differences ($P>0.05$) in oxidation values between the treatments but the last treatment (RBTE 1.0%) has a higher TBARS when compared with the other treatments at day 0. It was expected that with increasing concentrations of antioxidant (RBTE), there will be a decrease in oxidation. The TBARS values range between 1.43 and 2.84 mg MDA/kg meat and 0.007 and 0.013 mg MDA/g fat. At day 15 (dried final product), there was no significant difference ($P>0.05$) between the treatments. RBTE 1.0% had a high TBARS value of 0.036 mg MDA/g fat which differed from the other treatments on day 15. It is not clear whether this higher value is due to increased lipid oxidation or due to the fact that this treatment had an initial high TBARS value. Previous research has demonstrated pro-oxidant activity for RBTE when used in high concentration in a model system (Joubert *et al.,* 2005); this could explain the high association between RBTE 1.0% and lipid oxidation (Fig.3.2). The differences in TBARS of each treatment between day 0 and day 15 show that RBTE 0.25% has the lowest increase (0.0125 mg MDA/g fat) which could indicate that less oxidation occurred using this concentration of RBTE.

There is an association between lipid oxidation and the sensory profile of a product, the higher the TBARS value, the higher the rancidity of the product (Mathenjwa *et al.,* 2012). Research of Campo *et al.* (2006) and Suman *et al.* (2010) note that a TBARS value greater than or equal to 2 mg malonaldehydes (MDA) per kg beef meat can cause rancid off-flavours to be detectable to a sensory panel. The RBTE 0%, RBTE 0.25% and RBTE 0.50% (day 0) have values lower than 2 mg MDA/kg meat. However, the samples at day 15 are above the mentioned amount for rancidity flavor detection; this would indicate that rancidity should have been detected by the sensory panel. Even though the TBARS values indicated that

oxidation has progressed, it is clear from Fig. 3.2 and 3.3, that no rancidity aroma or flavor was detected by the trained sensory panel. A possible reason for this could be that the human palate does not perceive oxidation in droëwors at these TBARS levels, which could be attributed to the fact that South African consumers are so familiar with this rancid taste that it may go undetected.

As limited research such as that of Cullere *et al.* (2013) has been conducted on the effect of natural antioxidants in meat products; a comparison cannot be made in terms of the oxidation results. It must be noted that the results are relatively high in terms of TBARS values, which has been recorded to range between 0.1 and 6.0 MDA per kg meat in raw game meat (Okabe *et al.,* 2002). This could be due to the drying period as it took fifteen days to dry in ambient conditions or as previously mentioned the possible pro-oxidant activity of the RBTE at high concentrations. Also, it is known that natural antioxidants have a lesser effect on the inhibition of oxidative damage when added to a meat product after slaughtering of the animal. A reason for the decreased inhibition action of the natural antioxidant could be due to the antioxidants not being incorporated within the cell membranes as is when the antioxidants are fed to the animals (Descalzo & Sancho, 2007, Kerry *et al.,* 1999).

Protein oxidation

Results from the analysis of the oxidative deterioration of proteins in the ostrich droëwors are shown in Table 3.5. The amount of carbonyls from protein oxidation did not differ significantly ($P < 0.05$) between treatments on both day 0 (± 1.01 µM carbonyl/mg protein) and day 15 (± 0.92 µM carbonyl/mg protein). The antioxidant effect on protein oxidation was expected to decrease with higher levels of RBTE added, however, the level of µM carbonyl per mg protein remained fairly constant between treatments. However, as seen in Fig.3.2, protein oxidation can be better associated with RBTE 0% due to its high oxidation value (Table 3.5). The use of RBTE as a natural antioxidant in meat products has not been documented and therefore no comparisons or deductions can be made.

Lipid and protein oxidation are directly linked as the mechanisms and reaction pathways are affected by the same pro-oxidants and antioxidant factors (Estévez *et al.,* 2005). Phenolic compounds, such as those found in RBTE, can inhibit protein oxidation by binding to proteins and forming complexes, this in turn enhances lipid oxidation (Siebert *et al.,* 1996). Heme iron and oxidising lipids are known to play major roles in the initiation of protein oxidation in meat (Estévez *et al.,* 2008a; Estévez *et al.,* 2008b; Kroger-Ohlsen *et al.,* 2003; Salminen *et al.,* 2008). During droëwors production, the meat has to be minced (ground), which could enhance protein and lipid oxidation due to the increase in surface to volume ratio that the meat is exposed to (Ganhão *et al.,* 2010). The muscle tissue is also

disrupted resulting in the release of pro-oxidants, which are naturally present in the muscle, and the incorporation of oxygen during the whole procedure will enhance oxidation (Kristensen & Purslow, 2001; Schrickler & Miller, 1983). Protein oxidation products are also known to induce lipid oxidation (Viljanen *et al.,* 2004).

Sensory attributes

The sensory profiling results for aroma, flavour appearance and product characteristics are presented in Fig. 3.3. The droëwors was tested by the trained panel within 2 days after drying was completed. The trained panel could distinguish the aroma and flavour of the RBTE as the results indicate that RBTE 0% has a significantly lower ($P < 0.05$) rooibos aroma and flavour than the other three treatments. The mean scores increase as the concentration of RBTE increases in the product. During the sensory training sessions, the addition of RBTE to the ostrich droëwors was seen as positive and thus adding to the overall aroma and flavour of the product. A fresh, fatty aroma was detected in RBTE 0% (39.9%), this attribute was significantly lower ($P < 0.05$) in the samples with RBTE 1.0% (18.3%), therefore the mean scores showed a decrease in the fresh fatty aroma with an increase in RBTE concentration. A similar result is illustrated with the fresh fat flavour. Rancidity was also tested, however, as indicated in Fig. 3.3, no rancid aroma or flavour was detected in any of the treatments. As mentioned, whether this was an actual absence of any rancidity in the flavour is unclear.

The appearance results, which included scoring of colour intensity, fat colour and shiny casing, indicate that the addition of the RBTE does affect the overall look of the droëwors as there were significant ($P < 0.05$) differences among the results of RBTE 0% and the other three treatments with RBTE added. In terms of colour intensity, there was a significant ($P < 0.05$) difference between the RBTE 0% (42.0%) and RBTE 1.0% (71.3%) with the colour intensity becoming more intense. This is due to the intense red-brown colour of the RBTE that is added to the droëwors mixture.

Although the aroma and flavour intensity of the rooibos increases with increasing RBTE concentrations (Fig. 3.3), this was not seen as a negative attribute. There were minor differences in the product characteristics of the droëwors with the added RBTE which could indicate that each characteristic was seen as being the same or similar in these droëwors samples.

Table 3.3 Means (%) and standard deviations for proximate composition analyses of ostrich droëwors with added rooibos tea extract on a wet weight basis.

	Ostrich Meat	Pork back Fat	Day 0				Day 15			
			RBTE 0 %	RBTE 0.25 %	RBTE 0.50 %	RBTE 1.0%	RBTE 0 %	RBTE 0.25 %	RBTE 0.50 %	RBTE 1.0 %
Moisture	76.7	5.7	60.7 ± 1.17	60.5 ± 1.45	61.2 ± 1.055	59.6 ± 0.82	27.0 ± 2.00	26.7 ± 2.57	27.7 ± 1.19	27.7 ± 1.12
Protein	19.4	0.5	11.7 ± 0.21	12.3 ± 0.13	11.1 ± 0.067	12.6 ± 0.061	28.3 ± 0.61	28.9 ± 0.41	26.6 ± 0.40	31.8 ± 0.97
Fat	3.6	64.1	21.3 ± 2.13	21.2 ± 0.97	22.5 ± 0.72	22.4 ± 1.05	29.6 ± 1.32	33.0 ± 1.42	33.8 ± 1.99	31.8 ± 3.73
Ash	0.7	0.1	2.3 ± 0.042	2.8 ± 0.13	2.6 ± 0.087	3.3 ± 0.13	8.9 ± 0.47	8.5 ± 0.35	8.1 ± 0.33	9.1 ± 0.78

No significant differences ($P > 0.05$) were seen within a row per day (day 0/day 15)

Table 3.4 Fatty acid composition (%) of ostrich droëwors produced with increasing rooibos tea extract levels on a wet weight basis.

	Ostrich meat	Pork back fat	Day 0				Day 15			
			RBTE 0 %	RBTE 0.25 %	RBTE 0.50 %	RBTE 1.0 %	RBTE 0 %	RBTE 0.25 %	RBTE 0.50 %	RBTE 1.0 %
Saturated fatty acids										
C14:0	0.3	0.4	0.4^{a} ± 0.032	0.4^{a} ± 0.024	0.6^{c} ± 0.066	0.4^{b} ± 0.0076	0.3 ± 0.22	0.3 ± 0.75	0.3 ± 0.13	0.3 ± 0.17
C15:0	0.2	tr	tr	tr	tr	0.07 ± 0.016	tr	tr	tr	0.05 ± 0.17
C16:0	22.2	31.3	30.3^{a} ± 1.27	28.7^{a} ± 1.16	29.7^{a} ± 4.17	22.9^{b} ± 0.61	30.3 ± 2.61	29.2 ± 4.61	30.0 ± 0.18	30.2 ± 0.63
C18:0	15.3	3.04	2.9^{a} ± 0.069	2.9^{a} ± 0.024	4.0^{c} ± 0.49	3.3^{b} ± 0.090	2.6 ± 1.49	2.6 ± 1.51	2.5 ± 1.67	2.5 ± 1.49
C20:0	0.2	tr	tr	0.05 ± 0.092	0.07 ± 0.010	0.06 ± 0.013	tr	tr	tr	tr
C21:0	0.1	tr	tr	tr	tr	tr	tr	tr	tr	tr
C22:0	0.9	0.05	0.06^{a} ± 0.0065	0.07^{a} ± 0.0049	0.1^{b} ± 0.0096	0.09^{b} ± 0.0028	tr	tr	tr	tr
C24:0	0.09	tr	tr	tr	tr	tr	tr	tr	tr	tr
Monounsaturated fatty acids										
C16:1	4.0	0.6	0.7^{a} ± 0.042	0.8^{b} ± 0.036	1.2^{d} ± 0.039	1.0^{c} ± 0.017	0.6 ± 0.21	0.6 ± 0.26	0.7 ± 0.23	0.7 ± 0.23
C18:1n9t	0.3	0.06	0.09 ± 0.027	0.04 ± 0.033	0.07 ± 0.016	0.05 ± 0.0032	0.06 ± 0.25	0.07 ± 0.26	0.05 ± 0.069	0.06 ± 0.30
C18:1n9c	27.7	50.4	53.5^{a} ± 2.73	61.0^{b} ± 3.78	75.7^{c} ± 1.62	64.9^{b} ± 1.58	47.0 ± 9.52	51.1 ± 9.80	47.6 ± 9.80	48.1 ± 7.55
C20:1	0.08	tr	tr	tr	tr	tr	0.2 ± 0.13	0.3 ± 0.078	0.3 ± 0.0073	0.3 ± 0.0088
C22:1n9	0.1	tr	0.05 ± 0.0016	0.05 ± 0.0038	0.07 ± 0.0097	0.08 ± 0.0063	tr	tr	tr	tr
C24:1	0.2	tr	tr	tr	tr	tr	tr	tr	tr	tr

	Ostrich meat	Pork back fat	Day 0				Day 15			
			RBTE 0 %	RBTE 0.25 %	RBTE 0.50 %	RBTE 1.0 %	RBTE 0 %	RBTE 0.25 %	RBTE 0.50 %	RBTE 1.0 %
Polyunsaturated fatty acids										
C18:2n6t	0.05	tr	tr	tr	tr	tr	tr	tr	tr	tr
C18:2n6c	16.1	13.5	$11.^{d} \pm 0.60$	$4.8^{a} \pm 0.36$	$7.0^{b} \pm 0.32$	$5.7^{a} \pm 0.36$	18.0 ± 0.082	14.8 ± 0.10	17.7 ± 0.087	16.8 ± 0.025
C18:3n6	1.6	0.2	$0.3^{a} \pm 0.017$	$0.3^{a} \pm 0.017$	$0.4^{b} \pm 0.014$	$0.4^{b} \pm 0.068$	0.06 ± 0.077	tr	tr	0.06 ± 0.071
C18:3n3	0.4	0.2	$0.3^{a} \pm 0.0076$	$0.3^{b} \pm 0.015$	$0.4^{c} \pm 0.015$	$0.4^{c} \pm 0.0088$	0.2 ± 0.094	0.2 ± 0.096	0.2 ± 0.070	0.2 ± 0.89
C20:2	0.3	0.1	$0.2^{a} \pm 0.0036$	$0.2^{b} \pm 0.12$	$0.3^{b} \pm 0.0096$	$0.3^{b} \pm 0.0057$	tr	tr	tr	tr
C20:3n6	8.0	0.06	$0.1^{a} \pm 0.042$	$0.1^{a} \pm 0.030$	$0.2^{b} \pm 0.0026$	$0.2^{b} \pm 0.021$	0.2 ± 0.077	0.2 ± 0.045	0.2 ± 0.050	0.2 ± 0.038
C20:3n3	0.1	tr	tr	tr	tr	tr	0.06 ± 0.15	0.07 ± 0.0091	0.06± 0.0038	0.06 ± 0.058
C20:4n6	0.1	tr	tr	tr	tr	tr	0.1 ± 0.28	0.2 ± 0.11	0.2 ± 0.098	0.2 ± 0.056
C20:5n3	0.4	tr	tr	tr	tr	tr	0.05 ± 0.041	0.05 ± 0.021	0.04 ± 0.011	0.05 ± 0.011
C22:2	0.1	tr	tr	tr	tr	tr	tr	tr	tr	tr
Total	100	100	100	100	100	100	100	100	100	100
Total fatty acids profile										
SFA	39.2	34.8	$33.7^{a} \pm 1.32$	$32.2^{a} \pm 1.43$	$34.5^{a} \pm 4.73$	$26.9^{b} \pm 0.69$	33.3 ± 0.35	32.2 ± 0.45	32.9 ± 0.46	33.2 ± 0.35
MUFA	32.2	51.0	$54.4^{a} \pm 2.74$	$62.0^{b} \pm 3.80$	$77.0^{c} \pm 1.59$	$66.0^{b} \pm 1.59$	$47.7^{a} \pm 2.23$	$51.9^{b} \pm 2.17$	$48.3^{a} \pm 1.04$	$48.9^{a} \pm 1.82$
PUFA	28.3	14.1	$11.7^{a} \pm 2.37$	$5.5^{b} \pm 5.08$	$8.1^{b} \pm 3.37$	$6.7^{b} \pm 2.15$	18.7 ± 2.84	15.7 ± 5.11	18.5 ± 3.88	17.6 ± 5.09
PUFA:SFA	0.7	0.4	$0.4^{a} \pm 0.073$	$0.2^{b} \pm 0.16$	$0.6^{a} \pm 0.21$	$0.3^{a} \pm 0.032$	0.6 ± 0.042	0.5 ± 0.021	0.6 ± 0.041	0.5 ± 0.013
(n-6)/(n-3)	12.2	58.7	$1.3^{a} \pm 0.049$	$1.0^{b} \pm 0.049$	$1.2^{a} \pm 0.036$	$1.3^{a} \pm 0.042$	1.1 ± 0.025	1.3 ± 0.034	1.3 ± 0.040	1.4 ± 0.040

SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; (n-6)/(n-3) = (n-6)/(n-3) PUFA ratio; tr = trace (<0.05%)

^{a,b,c,d} Means within a row per day (day 0/day 15) with different superscripts are significantly different ($P < 0.05$)

Table 3.5 Means and standard deviations for chemical analyses of ostrich droëwors with added rooibos tea extract on a wet weight basis.

	Day 0				Day 15			
	RBTE 0 %	**RBTE 0.25 %**	**RBTE 0.50 %**	**RBTE 1.00%**	**RBTE 0 %**	**RBTE 0.25 %**	**RBTE 0.50 %**	**RBTE 1.00 %**
Lipid Oxidation (mg MDA/kg meat)	1.43 ± 0.194	1.59 ± 0.188	1.67 ± 0.245	2.84 ± 0.856	7.99 ± 1.266	6.70 ± 1.965	8.21 ± 1.423	11.28 ± 4.619
Lipid Oxidation (mg MDA/g fat)	0.0068 ± 0.000976	0.0075 ± 0.00111	0.0074 ± 0.000999	0.013 ± 0.00376	0.027 ± 0.00480	0.020 ± 0.00602	0.024 ± 0.00518	0.036 ± 0.0145
Protein Oxidation (µM carbonyl/mg protein)	1.05 ± 0.572	0.60 ± 0.180	1.03 ± 0.188	1.37 ± 0.924	1.19 ± 0.384	0.89 ± 0.268	0.98 ± 0.435	0.66 ± 0.403
Heme-Iron (mg heme-iron/100 g meat sample)	0.65 ± 0.137	0.67 ± 0.132	0.59 ± 0.146	0.67 ± 0.0996	2.67 ± 0.103	2.61 ± 0.281	2.67 ± 0.101	2.51 ± 0.152

No significant differences ($P > 0.05$) were seen within a row per day (day 0/day 15)

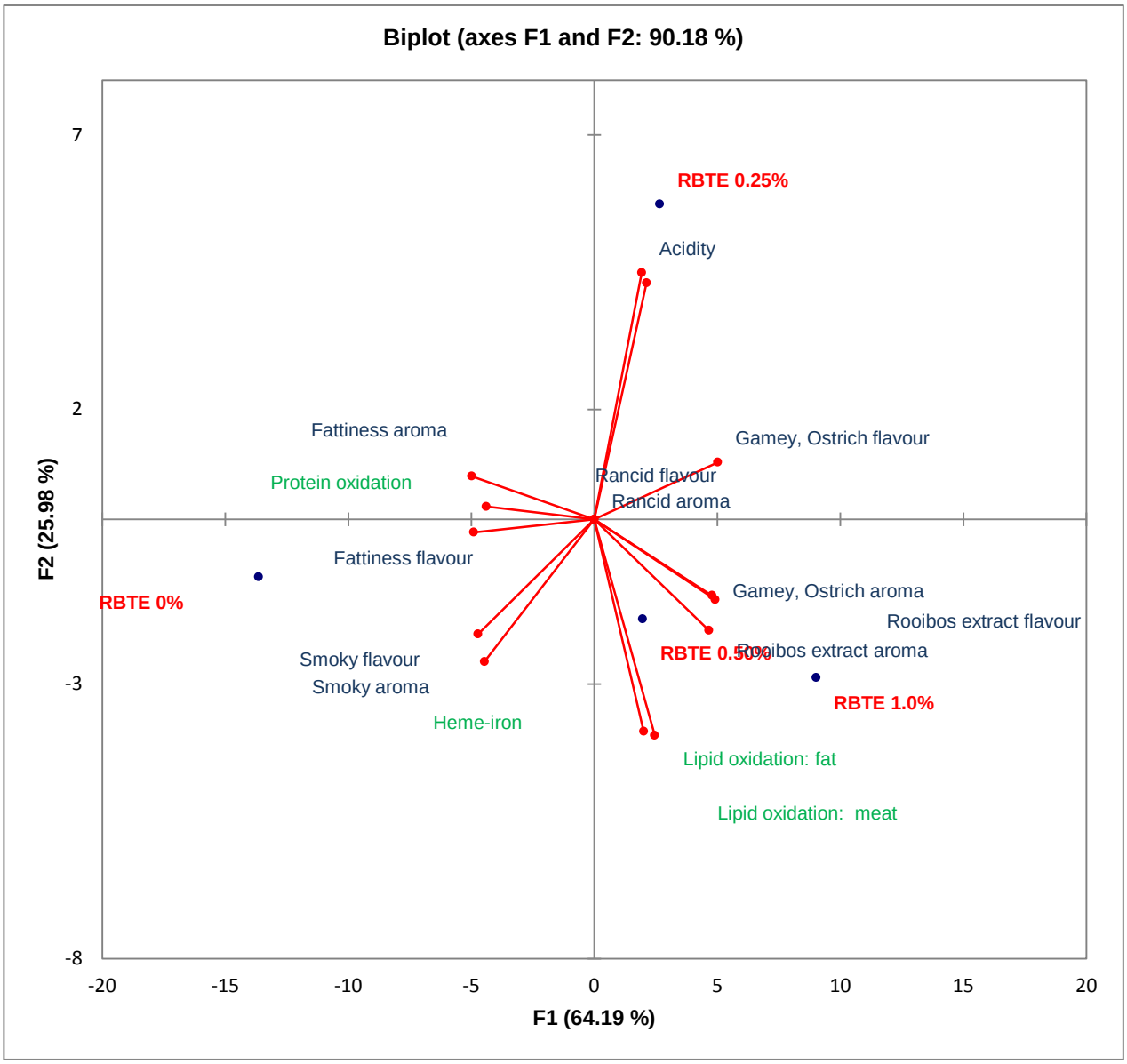

Fig. 3.2 PCA bi-plot illustrating the association between oxidative chemical and sensory attributes of dry droëwors.

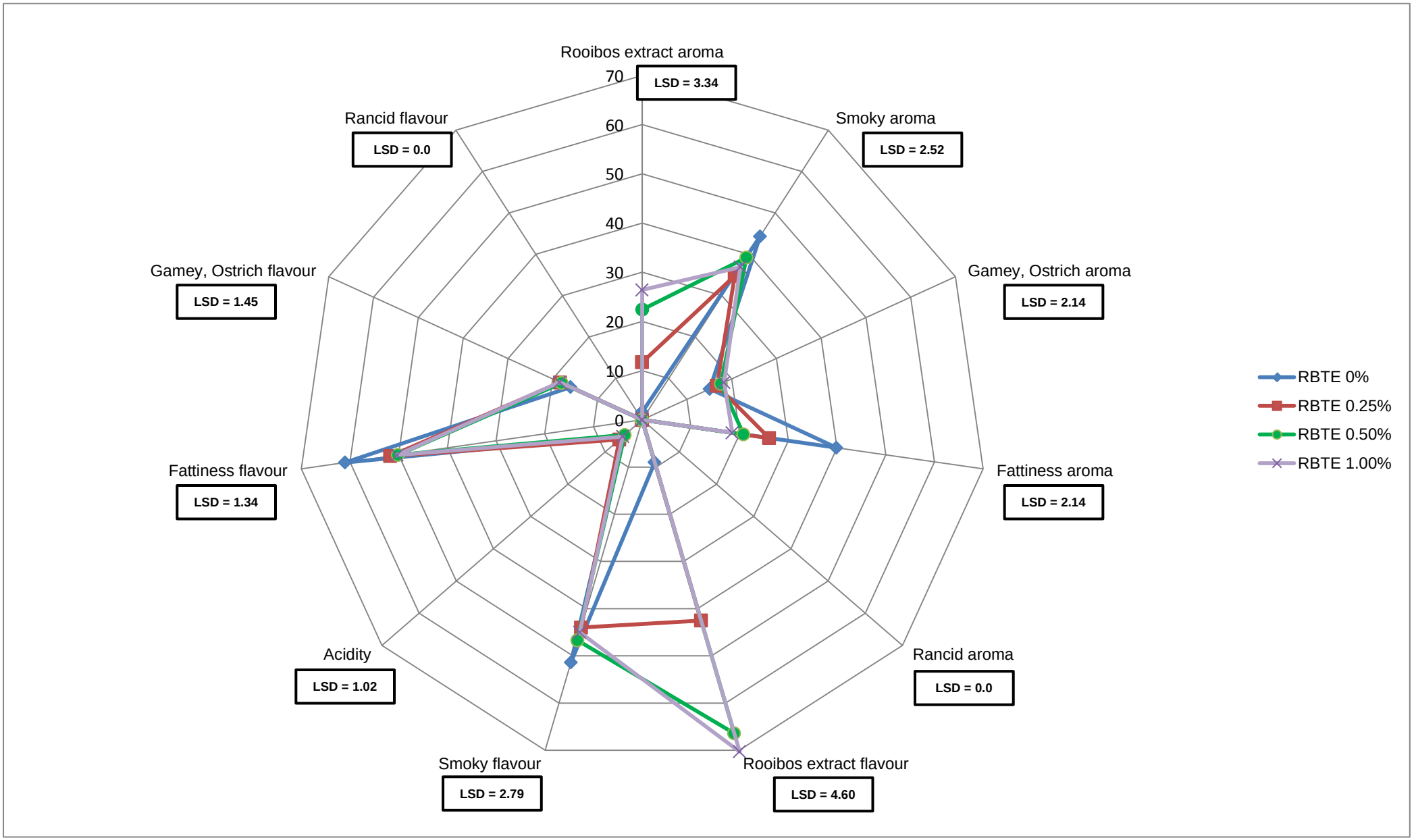

Fig. 3.3 Sensory results of the aroma and flavour attributes for the trained sensory panel (LSD = Least significant difference [P=0.05])

CONCLUSIONS

The results obtained from this study indicated that the addition of RBTE to ostrich droëwors did not result in the anticipated slowing down of lipid oxidation. This could be because of the long drying period or, seen with RBTE 1.0%, the RBTE acted as a pro-oxidant itself increasing the oxidation levels. There were no significant differences between the treatments within each day. RBTE 0.25% proved to give the best results in terms of lipids but with no effect on protein oxidation the RBTE cannot be said to slow down oxidation. Further research needs to be conducted to determine whether the drying method and duration plays a role in the oxidation rates and the possible effects of the RBTE on other game meat.

The fatty acid profile had high concentrations of oleic acid, palmitic acid and linoleic acid in both the raw and dry droëwors samples. The high polyunsaturated fatty acids should result in a higher oxidative activity as noted.

Sensory attribute differences were observed among the different treatments when analysed by the trained sensory panel. The main attributes observed whilst tasting the ostrich droëwors were distinctive rooibos, smoky, fresh fatty and game aromas and flavours. The RBTE aroma and flavour increased with increasing concentrations of RBTE whilst the fresh fatty aroma and flavour decreased with increasing concentrations of RBTE. Rancidity did not appear to be detected during the sensory analyses. The addition of RBTE to the droëwors was seen as a positive attribute among the panel.

From these results, the subsequent study was formulated so to evaluate the effectiveness of RBTE as a natural antioxidant in different game species droëwors in terms of lipid and protein oxidation. The meat and fat sources, the meat:fat ratio as well as the drying parameters will differ from this study to determine if the RBTE will act in inhibiting lipid and protein oxidation.

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CHAPTER 4

Effect of rooibos tea extract (*Aspalathus linearis*) on the lipid and protein oxidation of blesbok (*Damaliscus pygargus phillipsi*), springbok (*Antidorcas marsupialis*) and fallow deer (*Dama dama*) droëwors

ABSTRACT

This study investigated the effect of added rooibos tea extract (RBTE) at 0%, 0.25%, 0.50% and 1.0% to improve the oxidative stability of blesbok, springbok and fallow deer droëwors (dried sausage). The RBTE treatments had no significant effects ($P > 0.05$) on the lipid oxidation and protein oxidation of the dried product. RBTE at 0.25% did however give the best result for lipid stability after drying as it decreased considerably. Heme-iron concentration differed ($P < 0.05$) between the RBTE treatments within the dried stage within species and increased after drying. A decrease in the total moisture content and increase in fat and protein content was observed after drying in all species. There were no differences ($P > 0.05$) between the moisture, protein and fat contents between treatments within a specific processing stage. With the high polyunsaturated fatty acid content of the sausages, a high level of oxidation occurred. Even though an addition of RBTE did not reduce oxidation significantly during the drying process, it could be a successful addition to the traditional South African meat product if it is shown to impart positive flavour attributes.

Keywords: *Aspalathus linearis,* natural antioxidant, lipid and protein stability, droëwors, venison

INTRODUCTION

Game that is hunted and harvested in South Africa is utilised both locally and internationally (Hoffman & Wiklund, 2006). South Africa is known for its dried meat products such as biltong and droëwors. Droëwors is traditionally made from ground beef and animal fat (5-30% on a wet mass basis) and is a ready-to-eat dried seasoned meat sausage (Burnham *et al.,* 2008). The droëwors market has expanded and is presently producing different game species droëwors (Carr *et al.,* 1997).

Game meat is ideal for the production of droëwors as it is a lean meat with a low intramuscular fat content and has a favourable fatty acid profile (low saturated fatty acids and high unsaturated fatty acids) (Hoffman & Wiklund, 2006; Hoffman & Cawthorn, 2012). Blesbok (*Damaliscus pygargus phillipsi*) and springbok (*Antidorcas marsupialis*) are harvested throughout Southern Africa. They are both used in the production of biltong and droëwors. It is becoming more popular to harvest feral fallow deer (*Dama dama*) and as this meat could also be used in the production of droëwors, it would be of interest to evaluate the suitability of this species for droëwors.

As droëwors is commonly stored at ambient temperatures for a long period and has added fat, oxidation is likely to occur. Lipid and protein oxidation in meat results in the development of off-flavours and aromas (Ladikos & Lougovois, 1990; Shacter, 2000). Natural antioxidants are commonly added to processed products to slow down oxidation and in turn, improve the shelf-life and flavour profile of the products (Pokorný, 2001; Sánchez-Esalante *et al.,* 2003). Rooibos tea extract (RBTE) is becoming more popular as a food ingredient being used in products such as yoghurt, ready-to-drink iced teas and jams (Joubert & De Beer, 2011). It is a high antioxidant powdered tea extract which is the waste product when producing commercial rooibos tea. The flavonoids found in rooibos have been shown to have the ability to inhibit lipid peroxidation in model systems (Joubert & De Beer, 2012).

In commercial markets, synthetic and natural antioxidants are added to meat products. Butylated hydroxyanisole (BHA), butylated hydroxyl toluene (BHT), tertiary butylhydroquinone (TBHQ), sulphur dioxide (SO_2) and tocopherol are commonly added as a blend to enhance shelf-life via inhibition of oxidation (Shahidi *et al.,* 1992). Consumers are more aware of the addition of synthetic antioxidants and therefore by replacing these with RBTE this should overcome the negative perception of some of the meat products currently sold in today's market. Numerous studies have been conducted on the addition of natural antioxidants (such as herbs, spices, teas and vitamin-rich plant extracts) to beef products. These results indicate that the addition most often has a positive effect on the sensory properties and when used in combination with a synthetic antioxidant at a reduced level give

the best results in terms of inhibiting lipid peroxidation (Crackel *et al.,* 1988; Bañón *et al.,* 2007; Mathenjwa *et al.,* 2012). Additional research indicated that a combination of natural antioxidants improves both flavour profile and oxidation stability of meat products (Bañón *et al.,* 2007; Liu *et al.,* 2010; Michalzyk *et al.,* 2012). According to Liu *et al.* (2010), teas have been used successful at low concentrations when in combination with tocopherol. This suggests that the use of RBTE at low concentrations should result in improved oxidative stability.

The aim for this study is therefore to investigate the effect of the addition of different levels of rooibos tea extract as a natural antioxidant to blesbok, springbok and fallow deer droëwors on its chemical properties and oxidation levels after drying.

MATERIALS AND METHODS

Droëwors production

The droëwors production procedure is given in Figure 4.1. Game meat of each species (blesbok, n=6; springbok, n=6 and fallow deer, n=3) was trimmed of all external fat and connective tissue and cut into approximately 10 cm x 10 cm cubes. No specific cuts were used. To increase the amount of fat in the product, sheep meat/fat (30 meat: 70 fat) was added. Three separate 36 kg replicates of droëwors from each species were made. Each 36 kg replicate was further divided into four treatment batches of 9 kg each. Each 9 kg batch consisted of 66.6% game meat and 33.3% sheep meat and fat. This was then combined with 1.5% (135 g) salt, 1% (90 g) pepper and varying amounts of powdered rooibos tea extract (RBTE: 0%, 0.25%, 0.50% and 1.0% w/w concentrations). The phenolic composition of the RBTE used is depicted in Chapter 3 (Table 3.1). Each treatment batch was minced through a 10 mm grinder after which the spices (salt and pepper) and varying concentrations of RBTE were added. The minced meat and spices were then mixed and minced through a 5 mm grinder. Natural sheep casings (22 mm diameter) were filled with the minced meat mixture. Half the sausage from each batch was dried in an environmentally controlled maturation chamber (Reich Unicontrol 2000S). Initially the sausage underwent a reddening stage at 30°C with a 60% relative humidity for 15 min followed by a drying period of 48 h at a constant temperature of 30°C and a relative humidity of 30%. The drying process took 2 days in which the droëwors lost 45-50% of its mass. The rest of the sausage was left raw for analyses.

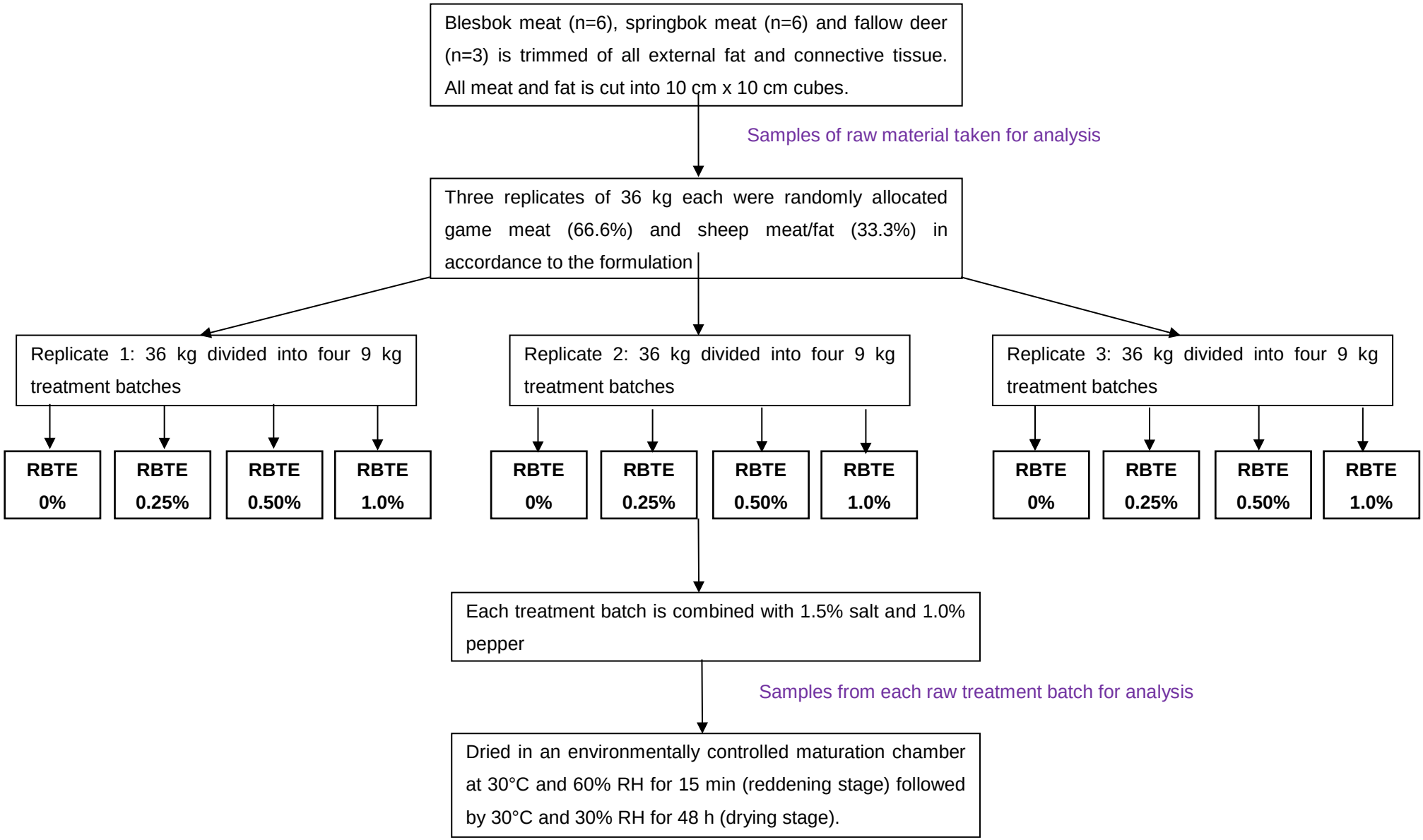

Figure 4.1 Droëwors production procedure.

Sample for analyses preparation

Five replicate sub-samples (150-200 g) of both the raw and dried droëwors of each batch of droëwors was taken and individually homogenised in a blender for 3 min to ensure a representative sample was tested. The dried droëwors samples were taken within 30 min after the drying process was completed. The samples were stored in a -80°C freezer until analyzed. The homogenised samples were used for proximate analysis, the analysis of fatty acid composition, heme-iron concentration, as well as for lipid and protein oxidation.

Proximate composition analysis

The samples were analysed to determine the moisture (Method 934.01) and ash (Method 942.05) content according to the AOAC (2002). The protein content was determined by multiplying the amount of N by a factor of 6.25, N levels were determined by the Dumas combustion method as described in procedure 992.15 of the AOAC (1992). Fat was determined using the chloroform/methanol (2:1) fat extraction method according to Lee *et al.* (1996). All analyses were performed in duplicate.

Fatty acid composition

The fatty acids were methylated and then analysed using gas chromatography to determine the fatty acid composition and percentages. A modified method of Folch *et al.* (1957) was used. An internal standard, heptadecanoic acid ($C_{17}H_{34}O_2$) (Sigma-Aldrich Inc., 3050 Spruce St., St. Louis, MO, 63103, USA; Cat. No. H3500) was added to quantify the individual fatty acids. The fatty acid composition was expressed as percentage. For the full chemical analytical method description, refer to Chapter 3.

Heme-iron

The heme-iron concentration was determined according to the method of Hornsey (1956). The heme-iron concentration values are expressed in mg heme-iron per g sample. For the full chemical analytical method description, refer to Chapter 3.

Lipid oxidation

The oxidation was followed by measuring the thiobarbituric acid reactive substances (TBARS) using the spectrophotometric method described by Rosmini *et al.* (1996). The samples were tested in duplicate. The TBARS values are expressed as mg malonaldehyde (MDA) per kg product and mg MDA per gram fat. For the full chemical analytical method description, refer to Chapter 3.

Protein oxidation

Carbonyls and proteins were measured on all samples. The samples were tested in duplicate. The carbonyls were determined according to the method outlined by Oliver *et al.* (1987). The protein concentration values are expressed as µM carbonyl per mg protein. For the full chemical analytical method description, refer to Chapter 3.

Statistical analysis

To test the effects of species, treatment and stage (raw or dried) on the various measurements, mixed model repeated measures ANOVA were used. The stage effect was the within subject repeated effect. For the mixed model, species, treatment and stage were treated as fixed effects and the batches to which the treatments were applied as a random effect. Fisher LSD post hoc test as used to further analyse significant differences when the main effects/interaction effects were significant. Statistical analyses were done using the VEPAC module of Statistica 11. A 5% significance level was used as guideline for determining significant differences.

RESULTS

Proximate composition analysis

Results of moisture, protein, fat and ash content for each species of droëwors (blesbok, springbok and fallow deer) are presented in Table 4.1. There were no differences ($P > 0.05$) amongst treatments between the raw and dried stages within species. Therefore the results indicate the proximate composition as averaged means of each species at a stage.

Fatty acid composition

The total fatty acid profile of each species droëwors with the various levels of RBTE added are presented in Table 4.2. The fatty acids composition should be the same for each treatment within a batch at the raw stage as no changes will have occurred; therefore the raw samples were tested using the control (RBTE 0%) sample for each batch for each species. After drying, the replicates of each treatment's batches were tested so that differences could be analysed.

For each species, the same fatty acids were found with the most prominent being oleic acid, stearic acid, linoleic acid and palmitic acid. As expected, the droëwors (irrespective of species) has a similar fatty acid profile to that of the sheep fat added. For the full fatty acid composition of each species, refer to Addendum A.

There were differences ($P < 0.05$) in the total polyunsaturated fatty acid (PUFA) profiles between the treatments of the springbok and fallow deer species droëwors after the drying process. When analysing the blesbok species droëwors (Table 4.2), the PUFA values increase with increasing concentration of RBTE. Springbok species droëwors (Table 4.2) does not follow a distinctive trend with the addition of the RBTE; RBTE 0.25% and RBTE 0.50% have increased PUFA values whilst the other treatments both have similar percentages which are also similar to that of the raw samples. With the fallow deer species droëwors (Table 4.2), the opposite trend to that of the blesbok species droëwors occurs; the PUFA values decrease with increasing RBTE concentration.

The (n-6)/(n-3) PUFA ratio of all treatments of each species fall under the recommended 4 % (Simopoulos, 2004; Wood *et al.,* 2004).

Heme-iron

The mean values for the heme-iron concentration in the droëwors samples are depicted in Table 4.3. The treatments of blesbok and springbok species droëwors have similar initial heme-iron concentrations that increase after drying. Furthermore, after drying all treatments have similar heme-iron concentrations over all species types. RBTE 0.25% have lower ($P < 0.05$) heme-iron concentrations after drying for all species as noted in Table 4.3 and Figure 4.2. There are no significant differences between the species ($P = 0.685$) therefore it would be acceptable to analyse the interaction ($P = 0.00926$) between stage and treatment regardless of species, as seen in Figure 4.2. RBTE 0.25% therefore had the lowest increase, which corresponds with the results of lipid oxidation given in Table 4.4. Although it was expected that the heme-iron would differ between raw and dry droëwors, when expressed on a dry mass basis, the droëwors still had higher heme-iron than the raw droëwors (Table 4.3).

Lipid oxidation

Lipid oxidation results for blesbok, springbok and fallow deer droëwors are depicted in Table 4.4. The results for each species differs significantly from each other ($P = 0.0231$). It is important to note that as droëwors is a dried meat product, it is expected to undergo a large level of oxidation and therefore the TBARS values are expected to be higher than those found in raw meat. Therefore, the results are compared across treatments rather than from raw to dried samples.

Blesbok species droëwors show a general trend that after drying each treatment has increased TBARS values, although the results also indicate that RBTE 0.25% resulted in the lowest lipid oxidation and heme-iron concentration (Table 4.3) in blesbok species droëwors.

The mean values for springbok species droëwors indicate that RBTE 0%, 0.25% and 0.50% all increase with drying. RBTE 1.0% decreases slightly (0.52 MDA per kg meat sample difference). As with the blesbok species droëwors, these results indicate that RBTE 0.25% gave the best results in terms of minimum lipid oxidation. Fallow deer species droëwors mean values differed slightly from blesbok and springbok species droëwors although the MDA levels fell within the same range of the blesbok and springbok droëwors of both the raw and dried stages except that the best result was shown to be at RBTE 1.0%.

The addition of RBTE to droëwors did not result in significant differences ($P > 0.05$) between treatments after drying within a species.

Protein oxidation

There were significant differences ($P = 0.0132$) in terms of the two stages, raw and dried, which is expected as during drying, protein oxidation will occur (Figure 4.3). However, each treatment did not always increase in carbonyls. When looking at the species individually within a stage, it can be seen that with the fallow deer species droëwors each treatment decreased in carbonyls after drying (Figure 4.3). Blesbok and springbok species droëwors follow the same trends for each treatment, staying at a constant for RBTE 0% and 0.25%, increasing for RBTE 0.50% and decreasing for RBTE 1.0%. As seen in Figure 4.3, there are visible differences between the species, mainly that of the fallow deer species droëwors in comparison with both blesbok and springbok species droëwors.

When evaluating within species, protein oxidation in blesbok species droëwors samples remained fairly constant with the addition of RBTE 0% and 0.25% with mean values (expressed as µM carbonyl per mg protein) of 0.00089 (raw); 0.00087 (dried) and 0.00070 (raw), 0.00069 (dried) respectively (Figure 4.3). The protein oxidation in samples with the addition of RBTE 0.50% increase over time from 0.00065 to 0.00085 µM carbonyl per mg protein. The samples with RBTE 1.0% decrease in carbonyls by 0.00026 which is a very small amount (not significant). Springbok species droëwors samples indicate similar results to blesbok species droëwors samples in that with the addition of RBTE 0.25% the protein oxidation products remain fairly equal over time with mean values (µM carbonyl per mg protein) of 0.00069 (raw) and 0.00069 (dried). Samples with RBTE 0.50% again increases in carbonyls after drying by 0.00018, whist RBTE 0% and RBTE 1.0% samples decrease by 0.000085 and 0.00021 µM carbonyl per mg protein. All treatments of the fallow deer species droëwors samples decrease after drying with an initial average mean value of 0.0035 and 0.00076 µM carbonyl per mg protein average mean values after drying. The differences between these values are not significant. There are no significant differences amongst the treatments at each stage (raw and dried) for the individual species.

Table 4.1 Average means* (%) ± SD for proximate composition analyses of raw and dried droëwors with added rooibos tea extract (RBTE) on a wet weight basis.

	Moisture		Protein		Fat		Ash	
	Raw	**Dried**	**Raw**	**Dried**	**Raw**	**Dried**	**Raw**	**Dried**
Blesbok	74.6 ± 0.82	38.7 ± 0.36	12.8 ± 0.24	34.6 ± 0.32	10.7 ± 1.68	20.3 ± 0.18	2.6 ± 0.11	5.3 ± 0.50
Springbok	68.7 ± 0.71	36.1 ± 1.73	13.2 ± 0.22	34.3 ± 0.35	12.4 ± 0.64	20.1 ± 0.33	2.7 ± 0.18	9.8 ± 1.35
Fallow Deer	72.1 ± 0.43	35.8 ± 1.70	14.5 ± 0.13	37.2 ± 0.22	10.7 ± 0.43	20.5 ± 0.47	2.2 ± 0.27	5.5 ± 0.62

*No differences ($P > 0.05$) amongst treatments between the raw and dried stages within species - results indicate the proximate composition as averaged means of each species at a stage.

Table 4.2 Total fatty acid profile (%) ± SD of fallow deer droëwors produced with increasing rooibos tea extract (RBTE) levels.

		SFA	MUFA	PUFA	PUFA:SFA	(n-6)/(n-3)
Blesbok						
Raw	RBTE 0%	57.7 ± 1.33	35.9 ± 2.08	6.5 ± 3.44	0.1 ± 0.061	0.7 ± 0.065
Dried	RBTE 0%	39.9 ± 13.80	56.7 ± 12.87	3.5^{b} ± 0.88	0.1 ± 0.060	0.3 ± 0.019
	RBTE 0.25%	44.9 ± 16.09	50.0 ± 17.83	5.2^{ab} ± 3.19	0.1 ± 0.060	0.3 ± 0.0094
	RBTE 0.50%	40.9 ± 15.46	53.1 ± 17.66	6.1^{a} ± 2.23	0.2 ± 0.0023	0.3 ± 0.0046
	RBTE 1.0%	38.4 ± 14.76	54.9 ± 17.52	6.9^{a} ± 2.80	0.2 ± 0.0066	0.3 ± 0.0061
Springbok						
Raw	RBTE 0%	48.8 ± 11.4	46.4 ± 11.48	4.9 ± 2.74	0.1 ± 0.056	0.6 ± 0.018
Dried	RBTE 0%	29.2^{c} ± 5.21	66.0^{a} ± 5.91	4.9^{b} ± 0.76	0.2 ± 0.010	0.6 ± 0.015
	RBTE 0.25%	57.2^{b} ± 1.36	32.6^{b} ± 1.27	10.4^{a} ± 0.17	0.2 ± 0.0065	0.6 ± 0.062
	RBTE 0.50%	60.4^{b} ± 0.64	29.5^{c} ± 0.51	10.3^{a} ± 0.16	0.2 ± 0.0042	0.7 ± 0.0085
	RBTE 1.0%	32.8^{c} ± 9.70	61.4^{a} ± 11.27	5.9^{b} ± 1.59	0.2 ± 0.0070	0.6 ± 0.0064
Fallow Deer						
Raw	RBTE 0%	54.7 ± 1.73	34.6 ± 2.88	10.8 ± 1.32	0.2 ± 0.020	0.6 ± 0.017
Dried	RBTE 0%	60.1 ± 7.68	29.7 ± 5.53	10.3^{a} ± 2.74	0.2 ± 0.064	0.2 ± 0.027
	RBTE 0.25%	53.2 ± 7.14	37.9 ± 10.11	9.1^{a} ± 2.96	0.2 ± 0.036	0.2 ± 0.010
	RBTE 0.50%	49.6 ± 15.77	46.6 ± 13.86	3.9^{b} ± 1.92	0.1 ± 0.084	0.2 ± 0.011
	RBTE 1.0%	62.5 ± 1.19	34.9 ± 1.25	2.7^{b} ± 0.14	0.1 ± 0.0019	0.2 ± 0.0099

SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; (n-6)/(n-3) = (n-6)/(n-3) PUFA ratio

^{a,b,c} Means within a column per species at the dried stage with different superscripts are significantly different ($P < 0.05$)

Table 4.3 Means ± SD for heme-iron (mg heme-iron/g meat sample) of droëwors with added rooibos tea extract (RBTE).

	Raw				Dried			
	RBTE 0 %	**RBTE 0.25 %**	**RBTE 0.50 %**	**RBTE 1.00%**	**RBTE 0 %**	**RBTE 0.25 %**	**RBTE 0.50 %**	**RBTE 1.00 %**
As is basis:								
Blesbok	0.30 ± 0.42	0.28 ± 0.75	0.24 ± 0.42	0.36 ± 0.55	0.95^{a} ± 0.21	0.73^{b} ± 0.091	0.99^{a} ± 0.19	0.97^{a} ± 0.036
Springbok	0.29 ± 0.44	0.28 ± 0.11	0.23 ± 1.27	0.39 ± 0.86	0.94^{a} ± 0.098	0.72^{b} ± 0.12	0.98^{a} ± 0.36	0.95^{a} ± 0.24
Fallow Deer	0.40 ± 0.51	0.36 ± 0.12	0.57 ± 0.33	0.31 ± 1.61	0.87^{a} ± 0.0026	0.76^{b} ± 0.081	0.93^{a} ± 1.15	0.90^{a} ± 0.20
Dry mass basis:								
Blesbok	0.96 ± 0.11	0.88 ± 0.085	0.77 ± 0.097	1.16 ± 0.19	1.45^{a} ± 0.30	1.14^{b} ± 0.15	1.62^{a} ± 0.28	1.51^{a} ± 0.18
Springbok	0.94 ± 0.12	0.87 ± 0.090	0.79 ± 0.10	1.14 ± 0.19	1.48^{a} ± 0.23	1.18^{b} ± 0.12	1.55^{a} ± 0.30	1.52^{a} ± 0.098
Fallow Deer	1.44 ± 0.22	1.28 ± 0.12	2.01 ± 0.33	1.10 ± 0.089	1.33^{a} ± 0.13	1.20^{b} ± 0.13	1.45^{a} ± 0.17	1.41^{a} ± 0.26

^{a,b,} Means within a row per species at the dried stage with different superscripts are significantly different ($P < 0.05$)

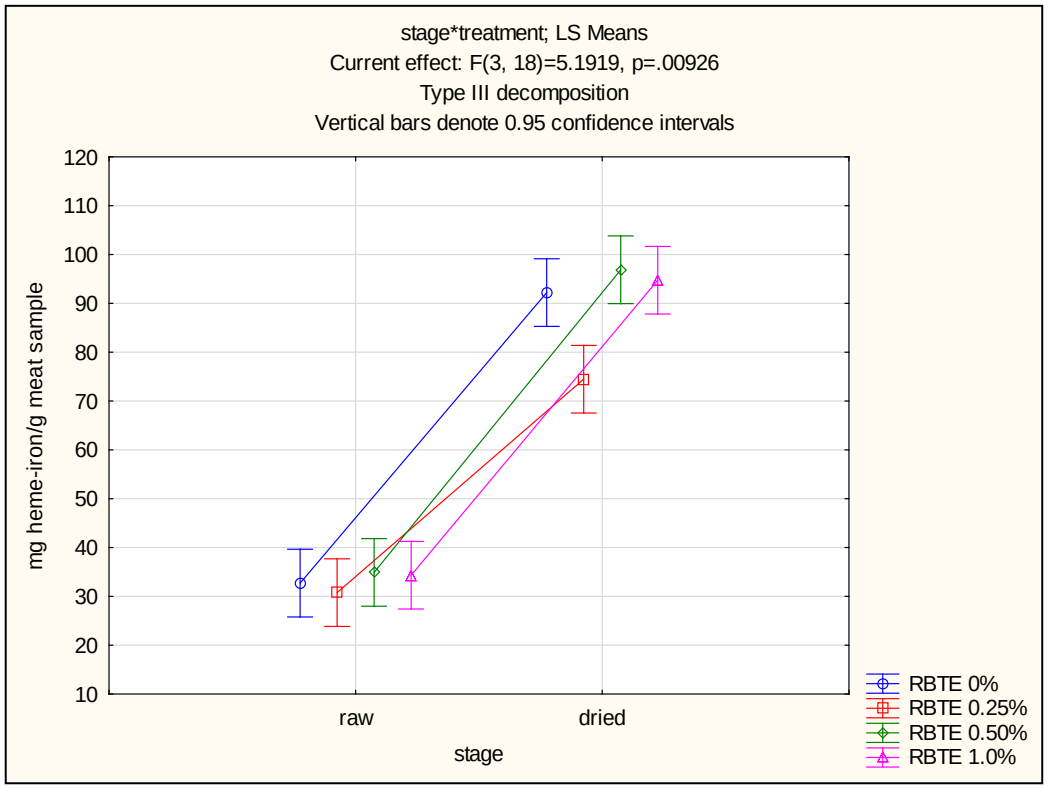

Figure 4.2 Heme-iron results indicating Stage*Treatment interaction apart from species ($P < 0.05$) for droëwors prepared with varying concentrations of rooibos tea extract (RBTE).

Table 4.4 Means ± SD for lipid oxidation (mg MDA/kg meat) of game droëwors with added rooibos tea extract (RBTE).

	Raw				Dried			
	RBTE 0 %	**RBTE 0.25 %**	**RBTE 0.50 %**	**RBTE 1.00%**	**RBTE 0%**	**RBTE 0.25 %**	**RBTE 0.50 %**	**RBTE 1.00 %**
As is basis:								
Blesbok	$0.57^{a} \pm 0.0025$	$0.45^{b} \pm 0.0036$	$0.37^{c} \pm 0.0064$	$0.58^{a} \pm 0.0015$	0.92 ± 0.44	0.75 ± 0.023	0.76 ± 0.31	0.97 ± 0.21
Springbok	$0.30^{c} \pm 0.021$	$0.40^{b} \pm 0.0056$	$0.33^{c} \pm 0.028$	$0.50^{a} \pm 0.023$	0.69 ± 0.29	0.42 ± 0.13	0.40 ± 0.15	0.45 ± 0.12
Fallow Deer	$0.37^{b} \pm 0.042$	$0.23^{c} \pm 0.0055$	$0.23^{c} \pm 0.0074$	$0.44^{a} \pm 0.0034$	0.73 ± 0.34	0.59 ± 0.24	0.70 ± 0.23	0.72 ± 0.19
Dry mass basis:								
Blesbok	$1.86^{a} \pm 0.31$	$1.40^{b} \pm 0.072$	$1.20^{c} \pm 0.22$	$1.83^{a} \pm 0.23$	1.42 ± 0.67	1.15 ± 0.18	1.26 ± 0.47	1.51 ± 0.36
Springbok	$0.99^{c} \pm 0.13$	$1.24^{b} \pm 0.13$	$1.06^{c} \pm 0.21$	$1.59^{a} \pm 0.32$	1.05 ± 0.39	0.65 ± 0.22	0.66 ± 0.25	0.70 ± 0.22
Fallow Deer	$1.36^{b} \pm 0.30$	$0.84^{c} \pm 0.068$	$0.84^{c} \pm 0.084$	$1.57^{a} \pm 0.078$	1.10 ± 0.41	0.94 ± 0.42	1.09 ± 0.43	1.12 ± 0.45

^{a,b,c} Means within a row per species at the raw stage with different superscripts are significantly different ($P < 0.05$)

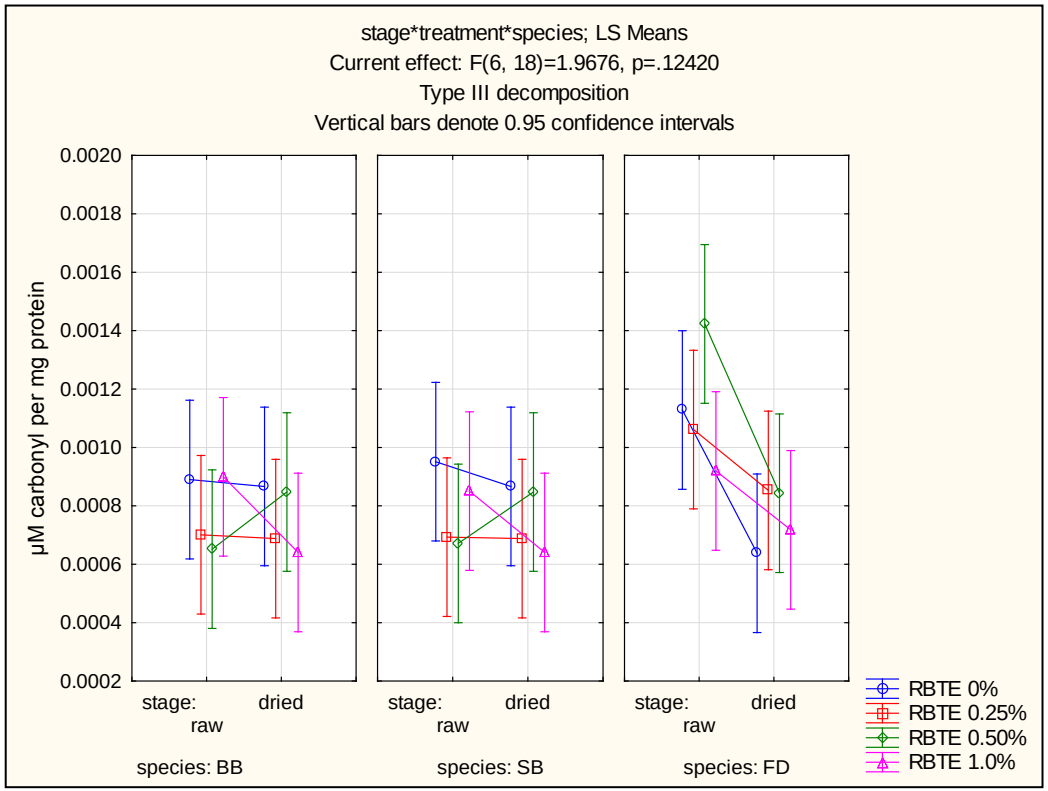

Figure 4.3 Protein oxidation results indicating Stage*Treatment*Species interaction ($P > 0.05$) for droëwors prepared with different concentration of rooibos tea extract (RBTE).

DISCUSSION

The proximate composition results indicate that the moisture lowers after drying by approximately 50% which is expected as the droëwors, as per the procedure used, was dried to lose 45 – 50% of its original mass. Due to the moisture loss after drying the protein and fat content is expected to become more concentrated (Nuñez de Gonzalez *et al.*, 2009). With the moisture loss experienced during the drying process, as expected the concentration of fat in the droëwors doubled (Table 4.2). The increase in concentration of the ash content with the drying can be explained by the drying process although it must be kept in mind that the higher than expected ash content would also be because of the added spices.

The fatty acid profile shows that the saturated fatty acids (SFA) initially make up approximately 50 – 60% of the sausages' total fatty acids. This is due to the addition of sheep meat/fat to the lean game meat species. Sheep fat is high in stearic acid which contributes to the high total saturated fatty acid of the droëwors. The total unsaturated fatty acids make up the remaining 40 – 50% of the fatty profile. After drying, the fatty acid profile of the droëwors is expected to change due to oxidation, however, if the RBTE functions as an antioxidant it is expected that the fatty acid profile will also differ in terms of percentage fatty acids between treatments within species. However, there were no significant differences between the PUFA values of the blesbok droëwors which would seem to indicate that the RBTE did not function as an antioxidant. The fatty acid profile, especially that of the low PUFA at treatments RBTE 0% and RBTE 1.0%, of the dried springbok species droëwors (Table 4.2) would seem to indicate that more oxidation may have occurred at these concentrations. Fallow deer droëwors' PUFA decreases with increasing concentrations of RBTE, which would indicate that there is increased oxidation with increasing RBTE levels.

The heme-iron levels, lipid oxidation and protein oxidation need to be discussed in conjunction with each other for an accurate conclusion to be made as heme-iron and oxidation are closely related to each other (Fernandez-Espla & O'Neill, 1993). Heme-iron is a catalyst for lipid oxidation therefore the higher the heme-iron content the more susceptible the product is to oxidation (Fernandez-Espla & O'Neill, 1993). Protein oxidation products are also known to induce lipid oxidation as the protein oxidation products react with free lipid radicals resulting in increased lipid oxidation (Viljanen *et al.,* 2004). Lipid oxidation however may also initiate protein oxidation (Lund *et al.,* 2011). A standard Pearson's correlation indicates that the heme-iron was positively linked to the TBARS of the dried droëwors in all the species (blesbok 0.52, springbok 0.24 and fallow deer 0.86).

During drying, protein oxidation occurs resulting in the formation of carbonyls (Lund *et al.,* 2011). Therefore it is expected that the oxidation products will increase after drying. Studies have shown that the DNPH method is used to obtain a general overall measurement

of protein oxidation in food systems (Estévez *et al.,* 2009; Estévez, 2011). This method is described as being accurate especially for quantification of overall protein oxidation levels over time (Estévez *et al*., 2008). Decreases which are visible in Figure 4.4 may be due to the protein carbonyls reacting with other cellular constituents. These cellular reactions result in the total protein carbonyls not being detected (Baron *et al.,* 2007). This corresponds with the fact that protein oxidation products are known to enhance lipid oxidation (Viljanen *et al.,* 2004) as the products will react with the free lipid radicals resulting in increased lipid peroxidation. Of importance are the changes in protein oxidation products over time per species. Due to the antioxidant effect of RBTE, protein oxidation was expected to decrease with higher levels of RBTE added, however, the level of µM carbonyl per mg protein, as seen with the blesbok and springbok species droëwors, remained fairly constant between treatments. Protein oxidation was thus not affected by the addition of RBTE at any of the concentrations although the results (Fig. 4.4) suggest that with the addition of RBTE higher than 1%, a large concentration of malonaldehydes will be detected (high oxidation level) with levels being similar to that of the control (RBTE 0%). Some studies have proved that conventional antioxidant strategies do not necessarily apply to muscle proteins, as compounds that are able to prevent lipid oxidation are not always able to prevent protein oxidation (Mercier *et al.,* 1998; Estévez & Cava, 2004; Viljanen *et al.,* 2004; Lund *et al.,* 2011).

Oxidation in meat and meat products is also closely related to the breakdown of heme-iron and the release of iron from the porphyrin ring (Estévez *et al.*, 2005). Heme-iron increases after drying which could be due to moisture loss of the product. However, the possibility exists that the heme-iron is not released from the porphyrin ring due to the drying process. The principle that is followed involves the interaction between lipid and protein oxidation which subsequently results in lowered heme-iron values. Lipid oxidation occurs though a free radical chain reaction whereby oxygen is the most important factor in the development of lipid oxidation in meat and meat products (Min & Ahn, 2005). Droëwors is a processed dried meat product which during its process includes a mincing step. The mincing of the meat disrupts the cell membranes releasing pro-oxidants (heme-iron) naturally found in meat which interact with the PUFA in the presence of oxygen and reactive oxygen species (ROS). This initiates lipid oxidation (Vuorela *et al.,* 2005). Lipid oxidation products include superoxide anions and hydrogen atoms which react to form hydrogen peroxide (H_2O_2). H_2O_2 acts as an oxidising agent by releasing the heme-iron molecule (via oxidative cleavage of the porphyrin ring) resulting in further lipid oxidation (Renerre and Labas, 1987; O'Grady *et al.,* 2001; Min & Ahn, 2005; Liu *et al.,* 2010). At this point however, if the antioxidants have bound to the binding sites of the myoglobin molecule in which the heme-iron is attached, this will be prevented.

At slaughter, reducing enzymes in meat are depleted, resulting in the accumulation of metmyoglobin. Metmyoglobin consists of heme-iron in its ferric state (Fe^{3+}), which has two binding sites (Min & Ahn, 2005). RBTE is made up of polyphenols (Joubert *et al.,* 2005), therefore it can be speculated that they will attach to these binding sites of metmyoglobin, which primarily bind oxygen. Therefore with the RBTE binding to these sites, a decrease in myoglobin oxidation occurs because oxygen cannot bind to the same sites. As the protein oxidation is a metal-ion catalysed reaction there is a decrease in protein oxidation thereby decreasing lipid oxidation via protein oxidation products.

Aspalathin is unique to rooibos tea (Joubert *et al.,* 2005; Joubert & De Beer, 2011). It acts as a potent antioxidant at low concentrations and a pro-oxidant at high concentrations. As an antioxidant it has a radical scavenging ability which protects against oxidative damage. Plant antioxidants added to meat and meat products have beneficial effects for the protection against excessive oxidative damage induced by ROS (Middleton *et al.,* 2000). High concentrations of total flavonoids, dihydrochalcones (aspalathin and nothofagin) and/or total polyphenols are a few reasons why RBTE could also act as a pro-oxidant (Joubert *et al.,* 2005). Assays have been demonstrated using RBTE to inhibit lipid peroxidation which concludes that its efficacy is dependent on the total polyphenol content rather than the individual flavonoids (Joubert *et al.,* 2005; Joubert & De Beer, 2012). However, the RBTE used in this study had high concentrations of aspalathin and quercetin (Table 3.1) which have been documented to have pro-oxidant properties when added in high concentrations. This could explain the increased lipid oxidation values with the addition of RBTE 0.50% and 1.0%.

The low results for heme-iron and lipid oxidation at RBTE 0.25% are due to the inhibition of heme-iron release via the binding of the antioxidant to its binding sites which inhibits protein oxidation and in turn minimises the level of lipid oxidation. Lipid oxidation does occur to some extent due to the initiation of lipid oxidation during processing. Only with the addition of RBTE 0.25% did this occur. The same trend was seen over all species. These results also relate to the protein oxidation results which show a minimal formation of carbonyls. With the addition of higher concentrations of RBTE, this resulted in higher oxidation levels. This could be due to the saturation of the binding sites of heme-iron. Once these sites have been saturated, the RBTE begins to act as a pro-oxidant as it has a high total polyphenol content and plays a role in the generation of hydroxide (OH^-), which is a reactive oxygen species. Therefore the more RBTE present, the higher the level of oxidation that will occur. Therefore it can be proposed that the addition of RBTE 0.25% would be the threshold for usage as an antioxidant in droëwors. The control (RBTE 0%) proved to have similar oxidation results as those of the RBTE at high concentrations.

Droëwors is considered a high in fat product and has a high level of PUFA (Table 3, Addendum A); the higher the PUFA, the faster the oxidation process (Vuorela *et al.,* 2005). It must be noted that the springbok species droëwors showed that RBTE 1.0% decreases slightly (0.52 MDA per kg meat sample difference). This could possibly be explained by experimental error; determination of MDA in a meat sample using the TBARS method is said to be inaccurate as it lacks specificity and sensitivity (Raharjo & Sofo, 1993; Shahidi & Zhong, 2005). Therefore it has been suggested that this method be used to assess the extent of lipid oxidation in general (Gray & Monahan, 1992). This method, which is specifically used for biological samples, muscle foods and fish oils (Dobarganes & Velasco 2002), should be regarded as a way to determine approximate lipid oxidation results. Variation in results has been seen in various studies which assess lipid oxidation in muscles food using the TBARS method (Rhee *et al.,* 1996; Soyer *et al.,* 2010; Sampaio *et al.,* 2012).

Both blesbok and springbok species droëwors show that the addition of RBTE 0.25% tended to slow down lipid oxidation. The addition of higher concentrations of RBTE (0.50% and 1.0%) did not give expected results as oxidation activity was higher than the control (RBTE 0%). Fallow deer species droëwors resulted in high oxidation activity with the addition of RBTE at 0.25% but this does not correspond with the other species droëwors as the addition of RBTE 1.0% gave the best results in terms of inhibition of oxidation activity after drying. This could be due to the proximate composition of the fallow deer; an aspect that warrants further research.

CONCLUSIONS

Although the results obtained from this study did not prove the inhibition of lipid oxidation to be significant, there were some interesting trends observed. Lipid and protein oxidation show that the addition of RBTE at 0.25% gave the best results. Heme-iron concentrations after drying showed significant differences with RBTE 0.25% resulting in a low heme-iron content in comparison with the higher RBTE concentrations. Though the differences for lipid oxidation weren't significant it is important to note that RBTE 0.50% and 1.0% gave higher oxidation results which may be due to the RBTE acting as a pro-oxidant when in high concentrations. As some results were inconsistent across species this indicates that species type (especially with the fallow deer droëwors) and formulation of the droëwors plays an important role in the antioxidant activity of the added RBTE. Therefore, further research needs to be conducted with an improved formulation and procedure for the production of droëwors, as well as conducting the chemical tests over a storage period. The fatty acid profile had high concentrations of oleic acid, stearic acid, linoleic acid and palmitic acid in both the raw and dry droëwors samples which is consistent with the sheep fat added. The

high polyunsaturated fatty acids resulted in a higher oxidative activity as noted. The formulation used contains a high fat content of 33.3%, by reducing the fat content and re-formulating the droëwors recipe, this should allow for an improved fatty acid profile (lower total saturated fatty acids and higher total unsaturated fatty acids), as the droëwors tends to be more similar to the added fat. Also, by improving the fatty acid profile, by increasing the polyunsaturated fatty acids, the antioxidant activity of the RBTE could be more clearly detected. A sensory panel analyses should also be conducted to determine whether the oxidation is detectable and to give an indication on whether RBTE will impart a specific flavour to the droëwors.

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ADDENDUM A

Percentage fatty acid composition (± SD) of blesbok droëwors produced with increasing rooibos tea extract (RBTE) levels on a wet weight basis.

	Blesbok meat	Sheep meat/fat	Raw	Dried			
			RBTE 0%	RBTE 0%	RBTE 0.25%	RBTE 0.50%	RBTE 1.0%
Saturated fatty acids							
C14:0	0.7	2.6	1.3 ± 0.72	0.9 ± 0.12	1.7 ± 1.39	2.0 ± 1.06	1.8 ± 0.81
C15:0	1.0	0.7	0.4 ± 0.24	0.3 ± 0.055	0.5 ± 0.32	0.5 ± 0.24	0.5 ± 0.21
C16:0	14.1	21.9	24.9 ± 0.65	24.5 ± 10.48	22.4 ± 8.32	19.0 ± 7.41	18.2 ± 7.12
C18:0	21.3	22.6	30.3 ± 1.98	13.9 ± 6.19	19.7 ± 6.78	18.7 ± 6.49	17.1 ± 6.39
C20:0	0.3	0.2	0.1 ± 0.041	0.04 ± 0.036	0.03 ± 0.012	0.04 ± 0.010	0.03 ± 0.016
C21:0	0.7	0.1	0.09 ± 0.041	0.05 ± 0.013	0.08 ± 0.031	0.07 ±0.030	0.09 ± 0.028
C22:0	0.1	0.2	0.2 ± 0.14	0.1 ± 0.041	0.3 ± 0.29	0.2 ± 0.10	0.2 ± 0.089
C24:0	0.5	0.2	0.3 ± 0.15	0.1 ± 0.053	0.2 ± 0.17	0.3 ± 0.12	0.3 ± 0.13
Monounsaturated fatty acids							
C14:1	0.06	0.2	0.1 ± 0.073	0.07 ± 0.011	0.1 ± 0.10	0.2 ± 0.080	0.1 ± 0.058
C15:1	0.1	0.2	0.2 ± 0.093	0.1 ± 0.025	0.2 ± 0.12	0.2 ± 0.079	0.2 ± 0.081
C16:1	0.7	1.9	0.8 ± 0.43	0.5 ± 0.13	0.8 ± 0.50	0.9 ± 0.34	0.9 ± 0.39
C18:1n9t	0.6	0.1	0.5 ± 0.30	0.2 ± 0.062	0.2 ± 0.14	0.3 ± 0.11	0.3 ± 0.20
C18:1n9c	21.8	36.1	34.1 ± 3.07	55.7 ± 12.73	48.5 ± 18.42	51.4 ± 18.33	53.1 ± 18.35
C20:1	0.1	0.1	0.09 ± 0.051	0.05 ± 0.023	0.1 ± 0.031	0.1 ± 0.038	0.1 ± 0.065
C22:1n9	0.1	1.0	0.04 ± 0.024	0.02 ± 0.0025	0.03 ± 0.026	0.03 ± 0.011	0.04 ± 0.014
C24:1	0.3	0.1	0.04 ± 0.025	0.09 ± 0.099	0.03 ± 0.017	0.04 ± 0.012	0.04 ± 0.020

	Blesbok meat	Sheep meat/fat	Raw	Dried			
			RBTE 0%	RBTE 0%	RBTE 0.25%	RBTE 0.50%	RBTE 1.0%
Polyunsaturated fatty acids							
C18:2n6t	1.6	1.2	0.5 ± 0.43	0.1 ± 0.051	0.2 ± 0.12	0.3 ± 0.085	0.3 ± 0.11
C18:2n6c	19.2	4.3	3.5 ± 1.78	1.9 ± 0.66	2.9 ± 1.81	3.4 ± 1.25	3.9 ± 1.59
C18:3n6	0.2	2.4	1.0 ± 0.52	0.5 ± 0.17	0.8 ± 0.49	0.9 ± 0.33	1.0 ± 0.42
C18:3n3	4.5	1.0	0.1 ± 0.051	0.05 ± 0.016	0.08 ± 0.037	0.1 ± 0.042	0.09 ± 0.038
C20:2	0.2	tr	0.05 ± 0.0037	0.03 ± 0.014	0.06 ± 0.032	0.07 ± 0.023	0.07 ± 0.031
C20:3n6	0.6	0.1	1.0 ± 0.52	0.04 ± 0.036	0.03 ± 0.012	0.04 ± 0.0098	0.03 ± 0.016
C20:3n3	5.2	1.0	0.04 ± 0.019	0.5 ± 0.20	0.8 ± 0.52	1.0 ± 0.36	1.2 ± 0.47
C20:4n6	2.4	1.2	0.07 ± 0.019	0.02 ± 0.0032	0.03 ± 0.024	0.04 ± 0.019	0.04 ± 0.016
C20:5n3	2.0	0.3	0.09 ± 0.041	0.06 ± 0.065	0.03 ± 0.026	0.04 ± 0.023	0.04 ± 0.018
C22:2	1.2	tr	0.07 ± 0.033	0.1 ± 0.15	0.07 ± 0.045	0.07 ± 0.023	0.08 ± 0.023
C22:6n3	0.5	0.2	0.09 ± 0.054	0.04 ± 0.018	0.08 ± 0.052	0.07 ± 0.039	0.08 ± 0.041
Total	100	100	100	100	100	100	100
Total fatty acids profile							
SFA	38.7	48.5	57.7 ± 1.33	39.9 ± 13.80	44.9 ± 16.09	40.9 ± 15.46	38.4 ± 14.76
MUFA	23.8	39.7	35.9 ± 2.08	56.7 ± 12.87	50.0 ± 17.83	53.1 ± 17.66	54.9 ± 17.52
PUFA	37.5	11.8	6.5 ± 3.44	3.5 ± 0.88	5.2 ±3.19	6.1 ± 2.23	6.9 ± 2.80
PUFA:SFA	1.0	0.2	0.1 ± 0.0.61	0.1 ± 0.060	0.1 ± 0.06	0.2 ± 0.0023	0.2 ± 0.0066
(n-6)/(n-3)	1.7	1.4	0.7 ± 0.065	0.3 ± 0.019	0.3 ± 0.0094	0.3 ± 0.0046	0.3 ± 0.0061

SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; (n-6)/(n-3) = (n-6)/(n-3) PUFA ratio ; tr = trace (<0.01%)

[a,b,c,d] Means within a row for the dry stage with different superscripts are significantly different ($P < 0.05$)

Percentage fatty acid composition (± SD) of springbok droëwors produced with increasing rooibos tea extract (RBTE) levels on a wet weight basis.

	Springbok meat	Sheep meat/fat	Raw	Dried			
			RBTE 0%	RBTE 0%	RBTE 0.25%	RBTE 0.50%	RBTE 1.0%
Saturated fatty acids							
C14:0	1.1	2.6	1.5 ± 0.87	1.5 ± 0.37	2.8 ± 0.27	2.5 ± 0.35	2.1 ± 0.80
C15:0	0.2	0.7	0.4 ± 0.22	0.4 ± 0.076	0.8 ± 0.055	0.7 ± 0.053	0.4 ± 0.15
C16:0	19.9	21.9	23.5 ± 6.28	14.1 ± 2.55	28.5 ± 1.01	29.7 ± 0.85	16.2 ± 4.85
C18:0	21.6	22.6	22.8 ± 5.24	12.7 ± 2.16	23.9 ± 0.47	26.4 ± 0.61	13.4 ± 3.67
C20:0	0.4	0.2	0.07 ± 0.044	0.07 ± 0.016	0.1 ± 0.033	0.1 ± 0.012	0.08 ± 0.39
C21:0	1.0	0.1	0.08 ± 0.045	0.06 ± 0.010	0.1 ± 0.0093	0.1 ± 0.013	0.06 ± 0.012
C22:0	0.2	0.2	0.1 ± 0.071	0.1 ± 0.034	0.4 ± 0.0062	0.4 ± 0.019	0.2 ± 0.062
C24:0	0.6	0.2	0.2 ± 0.11	0.2 ± 0.042	0.5 ± 0.045	0.4 ± 0.020	0.4 ± 0.14
Monounsaturated fatty acids							
C14:1	0.9	0.2	0.1 ± 0.058	0.1 ± 0.023	0.2 ± 0.022	0.2 ± 0.036	0.1 ± 0.045
C15:1	1.1	0.2	0.3 ± 0.20	0.2 ± 0.029	0.3 ± 0.022	0.4 ± 0.027	0.2 ± 0.072
C16:1	1.0	1.9	0.9 ± 0.55	0.8 ± 0.14	1.3 ± 0.050	1.0 ± 0.036	1.0 ± 0.31
C18:1n9t	0.5	0.1	0.3 ± 0.17	0.2 ± 0.032	0.4 ± 0.037	0.4 ± 0.0033	0.3 ± 0.15
C18:1n9c	17.8	36.1	44.7 ± 11.73	64.5 ± 6.16	30.2 ± 1.28	27.2 ± 0.61	59.6 ± 11.8
C20:1	0.2	0.1	0.07 ± 0.035	0.05 ± 0.0092	0.08 ± 0.0077	0.1 ± 0.0072	0.05 ± 0.013
C22:1n9	0.2	1.0	0.03 ± 0.016	0.03 ± 0.011	0.06 ± 0.0041	0.07 ± 0.013	0.04 ± 0.0095
C24:1	0.1	0.1	0.03 ± 0.021	0.05 ± 0.011	0.06 ± 0.0062	0.1 ± 0.012	0.04 ± 0.0071

	Springbok meat	Sheep meat/fat	Raw	Dried			
			RBTE 0%	RBTE 0%	RBTE 0.25%	RBTE 0.50%	RBTE 1.0%
Polyunsaturated fatty acids							
C18:2n6t	0.3	1.2	0.3 ± 0.16	0.2 ± 0.031	0.9 ± 0.41	1.1 ± 0.022	0.3 ± 0.064
C18:2n6c	20.2	4.3	2.7 ± 1.47	2.8 ± 0.39	5.7 ± 0.36	5.4 ± 0.15	3.4 ± 0.88
C18:3n6	0.2	2.4	0.9 ± 0.50	0.8 ± 0.14	1.4 ± 0.11	1.3 ± 0.042	1.0 ± 0.27
C18:3n3	3.4	1.0	0.1 ± 0.62	0.09 ± 0.012	0.2 ± 0.058	0.13 ± 0.010	0.09 ± 0.018
C20:2	0.4	tr	0.05 ± 0.030	0.03 ± 0.0040	0.06 ± 0.0083	0.07 ± 0.016	0.03 ± 0.0089
C20:3n6	1.1	0.1	0.6 ± 0.34	0.6 ± 0.11	1.5 ± 0.13	1.5 ± 0.041	0.8 ± 0.21
C20:3n3	4.9	1.0	0.03 ± 0.015	0.03 ± 0.0052	0.07 ± 0.010	0.1 ± 0.017	0.033 ± 0.011
C20:4n6	0.2	1.2	0.04 ± 0.021	0.04 ± 0.0086	0.08 ± 0.019	0.09 ± 0.0028	0.04 ± 0.012
C20:5n3	1.4	0.3	0.06 ± 0.038	0.06 ± 0.017	0.08 ± 0.015	0.2 ± 0.058	0.07 ± 0.034
C22:2	0.3	tr	0.05 ± 0.039	0.05 ± 0.011	0.2 ± 0.0091	0.3 ± 0.020	0.05 ± 0.020
C22:6n3	0.8	0.2	0.07 ± 0.048	0.07 ± 0.016	0.2 ± 0.016	0.1 ± 0.0090	0.1 ± 0.063
Total	100	100	100	100	100	100	100
Total fatty acids profile							
SFA	45.0	48.5	48.8 ± 11.4	29.2^{c} ± 5.21	57.2^{b} ± 1.36	60.4^{a} ± 0.64	32.8^{c} ± 9.70
MUFA	21.8	39.7	46.4 ± 11.48	66.0^{a} ± 5.91	32.6^{a} ± 1.27	29.5^{c} ± 0.51	61.4^{a} ± 11.27
PUFA	33.2	11.8	4.9 ± 2.74	4.9^{b} ± 0.76	10.4^{a} ± 0.17	10.3^{a} ± 0.16	5.9^{b} ± 1.59
PUFA:SFA	0.7	0.2	0.1 ± 0.056	0.2 ± 0.010	0.2 ± 0.0065	0.2 ± 0.0042	0.2 ± 0.0070
(n-6)/(n-3)	2.3	1.4	0.6 ± 0.018	0.6 ± 0.015	00.6 ± 0.062	0.7 ± 0.0085	0.6 ± 0.0064

SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; (n-6)/(n-3) = (n-6)/(n-3) PUFA ratio ; tr = trace (<0.01%)

^{a,b,c,d} Means within a row of a stage with different superscripts are significantly different ($P < 0.05$)

Percentage fatty acid composition (± SD) of fallow deer droëwors produced with increasing rooibos tea extract (RBTE) levels on a wet weight basis.

	Fallow deer meat	Sheep meat/fat	Raw	Dried			
			RBTE 0%	RBTE 0%	RBTE 0.25%	RBTE 0.50%	RBTE 1.0%
Saturated fatty acids							
C14:0	0.5	2.6	2.5 ± 0.30	3.7 ± 1.44	3.2 ± 1.47	1.1 ± 0.32	0.7 ± 0.057
C15:0	2.2	0.7	0.8 ± 0.048	1.1 ± 0.25	0.8 ± 0.26	0.3 ± 0.12	0.2 ± 0.0064
C16:0	11.5	21.9	25.1 ± 0.87	29.4 ± 3.94	28.0 ± 1.41	27.2 ± 11.01	31.9 ± 1.05
C18:0	19.1	22.6	25.3 ± 0.56	25.0 ± 2.86	20.3 ± 6.96	20.7 ± 6.74	29.4 ± 2.13
C20:0	0.5	0.2	0.1 ± 0.017	0.05 ± 0.020	0.06 ± 0.0066	0.04 ± 0.0056	0.02 ± 0.0068
C21:0	0.2	0.1	0.1 ± 0.054	0.2 ± 0.021	0.1 ± 0.039	0.06 ± 0.029	0.05 ± 0.019
C22:0	0.8	0.2	0.3 ± 0.072	0.3 ± 00.071	0.3 ± 0.027	0.08 ± 0.050	0.06 ± 0.011
C24:0	2.0	0.2	0.4 ± 0.050	0.5 ± 0.095	0.4 ± 0.13	0.1 ± 0.076	0.1 ± 0.020
Monounsaturated fatty acids							
C14:1	0.1	0.2	0.2 ± 0.017	0.2 ± 0.058	0.2 ± 0.062	0.06 ±0.036	0.03 ± 0.012
C15:1	1.3	0.2	2.8 ± 0.46	1.4 ± 1.81	0.7 ± 0.84	0.1 ± 0.076	0.07 ± 0.054
C16:1	0.6	1.9	1.3 ± 0.092	1.4 ± 0.35	1.3 ± 0.43	0.5 ± 0.22	0.4 ± 0.0027
C18:1n9t	2.1	0.1	1.0 ± 0.35	1.0 ± 0.85	0.6 ± 0.27	0.2 ± 0.10	0.2 ± 0.028
C18:1n9c	15.2	36.1	29.1 ± 3.27	25.5 ± 4.91	34.7 ± 9.88	45.5 ± 13.43	34.2 ± 1.20
C20:1	0.1	0.1	0.1 ± 0.0093	0.1 ± 0.042	0.2 ± 0.046	0.08 ± 0.022	0.04 ± 0.012
C22:1n9	0.2	1.0	0.05 ± 0.00046	0.06 ± 0.010	0.06 ± 0.0072	0.03 ± 0.0076	0.008 ± 0.0049
C24:1	0.2	0.1	0.08 ± 0.013	0.06 ± 0.018	0.06 ± 0.011	0.02 ± 0.012	0.02 ± 0.0091

	Fallow deer meat	Sheep meat/fat	Raw	Dried			
			RBTE 0%	RBTE 0%	RBTE 0.25%	RBTE 0.50%	RBTE 1.0%
Polyunsaturated fatty acids							
C18:2n6t	2.0	1.2	0.5 ± 0.021	0.5 ± 0.037	0.4 ± 0.14	0.2 ± 0.090	0.1 ± 0.0048
C18:2n6c	20.3	4.3	5.8 ± 0.72	5.7 ± 1.64	5.0 ± 1.70	2.1 ± 1.09	1.5 ± 0.057
C18:3n6	0.3	2.4	1.3 ± 0.12	1.3 ± 0.35	1.1 ± 0.39	0.5 ± 0.23	0.3 ± 0.0077
C18:3n3	5.6	1.0	0.2 ± 0.012	0.1 ± 0.031	0.1 ± 0.043	0.06 ± 0.031	0.04 ± 0.0014
C20:2	0.1	tr	0.07 ± 0.039	0.1 ± 0.017	0.1 ± 0.042	0.05 ± 0.026	0.03 ± 0.0013
C20:3n6	0.8	0.1	2.1 ± 0.38	0.05 ± 0.020	0.06 ± 0.0066	0.04 ± 0.0056	0.02 ± 0.0068
C20:3n3	5.1	1.0	0.08 ± 0.014	2.0 ± 0.67	1.8 ± 0.58	0.7 ± 0.39	0.5 ± 0.033
C20:4n6	2.9	1.2	0.09 ± 0.009	0.08 ± 0.0058	0.07 ± 0.029	0.03 ± 0.012	0.02 ± 0.0024
C20:5n3	2.2	0.3	0.2 ± 0.042	0.09 ± 0.0084	0.09 ± 0.040	0.04 ± 0.0056	0.02 ± 0.012
C22:2	1.2	tr	0.1 ± 0.028	0.1 ± 0.014	0.1 ± 0.0014	0.04 ± 0.021	0.03 ± 0.016
C22:6n3	2.9	0.2	0.2 ± 0.028	0.1 ± 0.039	0.1 ± 0.051	0.04 ± 0.021	0.04 ± 0.025
Total	100	100	100	100	100	100	100
Total fatty acids profile							
SFA	36.8	48.5	54.7 ± 1.73	60.1 ± 7.68	53.2 ± 7.14	49.6 ± 15.77	62.5 ± 1.19
MUFA	19.8	39.7	34.6 ± 2.88	29.7 ± 5.53	37.9 ± 10.11	46.6 ± 13.86	34.9 ± 1.25
PUFA	43.4	11.8	10.8 ± 1.32	10.3^{a} ± 2.74	9.1^{a} ± 2.96	3.9^{b} ± 1.92	2.7^{b} ± 0.14
PUFA:SFA	1.2	0.2	0.2 ± 0.020	0.2 ± 0.064	0.2 ± 0.036	0.1 ± 0.084	0.1 ± 0.0019
(n-6)/(n-3)	3.9	1.4	0.6 ± 0.017	0.2 ± 0.027	0.2 ± 0.010	0.2 ± 0.011	0.2 ± 0.0099

SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; (n-6)/(n-3) = (n-6)/(n-3) PUFA ratio ; tr = trace (<0.01%)

^{a,b,c,d} Means within a row of a stage with different superscripts are significantly different ($P < 0.05$)

CHAPTER 5

Refinement of recipe for game species droewors

INTRODUCTION

The results from the previous research chapters indicate the necessity for the formulation of a new recipe that will be used in the droëwors production. This formulation would take into account various factors such as the meat to fat ratio and the spice combinations, mainly pertaining to the salt level. The drying method also needs to be evaluated to ensure the most efficient method. Drying methods to be tested include air drying and drying in a maturation chamber. Using these factors, a formulation for droëwors could be decided on for the follow up trial (Chapter 6). This droëwors will be tested both by chemical analyses and a sensory panel. For this trial, no rooibos tea extract will be added as this is not necessary for the aim of the formulation trial. The ratios of game meat, beef meat, fat and spices ideal for droëwors would be tested. During this trial, samples of each droëwors formula will be evaluated by a focus group and their comments used as inputs in the determination of the final composition of the droëwors.

Droëwors is known as a high fat dried meat product (Burnham *et al.,* 2008) and therefore the fat content should not be minimised too much as this will influence the sensory attributes. Droëwors is classified according to the SANS 885:2011 standard and as per definition should contain a minimum of 80% total meat equivalents (of which 55% is actual lean meat) with a maximum of 50% fat content. Lean game meat (typically <2% fat) is being used as the predominant meat source which therefore does not contribute considerably to the total fat content. The addition of less fat should enable better antioxidant activity of the RBTE and detect if there are any significant differences between the concentrations after drying of the droëwors.

In previous chapters, it was noted that the fatty acid profile of the droëwors was similar to that of the fat added. In Chapter 4, 33.3% sheep fat was used to produce the droëwors allowing oxidation to be pushed to the extreme. Minimising the amount of fat, assumes that this will be more pleasant for the consumer and possibly allow for the antioxidant activity of the RBTE to have a more noticeable effect. In the earlier trials, the unsaturated fats concentration was high (through use of pork back fat and sheep fat) and the results concluded that the RBTE at any concentration did not have a significant effect in inhibiting oxidation of droëwors. Therefore, by using beef fat, which is much lower in polyunsaturated fatty acids, it is envisaged that the antioxidant activity of the RBTE will function more efficiently.

Improvement of the formulation and procedures will allow for the most appropriate meat to fat ratio to be used and therefore show the potential of the added RBTE as a natural antioxidant in the chapter to follow.

MATERIALS AND METHODS

Droëwors production

The droëwors production procedure is given in Figure 5.1. Game meat, beef meat and fat were used in the formulation of the game species droëwors. A total of 22 kg of game meat, 13.5 kg of beef and 4.5 kg of beef fat were sourced for this trial. Prior to the start of the trial, each batch of meat/fat was cut into small blocks and thoroughly mixed. Two batches of 20 kg total product were produced. Batch 1 consisted of 55% game meat (no visible fat), 32.5% lean beef meat and 12.5% beef fat. Batch 2 consisted of 55% game meat, 35% lean beef meat and 10% beef fat. Each batch was further separated into two sub-batches of 10 kg each to which two different spice combinations were added. Spice A consisted of 90 g salt, 30 g pepper, 350 mL brown vinegar, 15 g brown sugar per 10 kg batch. Spice B consisted of 150 g salt, 30 g pepper, 350 mL brown vinegar, 15 g brown sugar per 10 kg batch. These sub-batches were further divided into two separate batches of 5 kg each, which would each undergo a different form of drying, either air drying or in the maturation chamber. Each separate batch was minced through a 10 mm grinder after which the spices were added. This was thoroughly mixed and re-minced through a 5 mm grinder. The minced meat mixture was filled into natural sheep casings (22 mm diameter). For air drying, the fresh sausage was hanged evenly on drying racks and left for 72 h in a closed space at an ambient temperature of 15°C with a constant airflow. The maturation chamber used was a Reich Unicontrol 2000S. Conditions were set at a temperature of 30°C and relative humidity of 60% for the first 15 min (reddening stage) followed by a 60 h period at 30°C and relative humidity of 30% (drying stage). The tables below show the different formulations that were produced for testing.

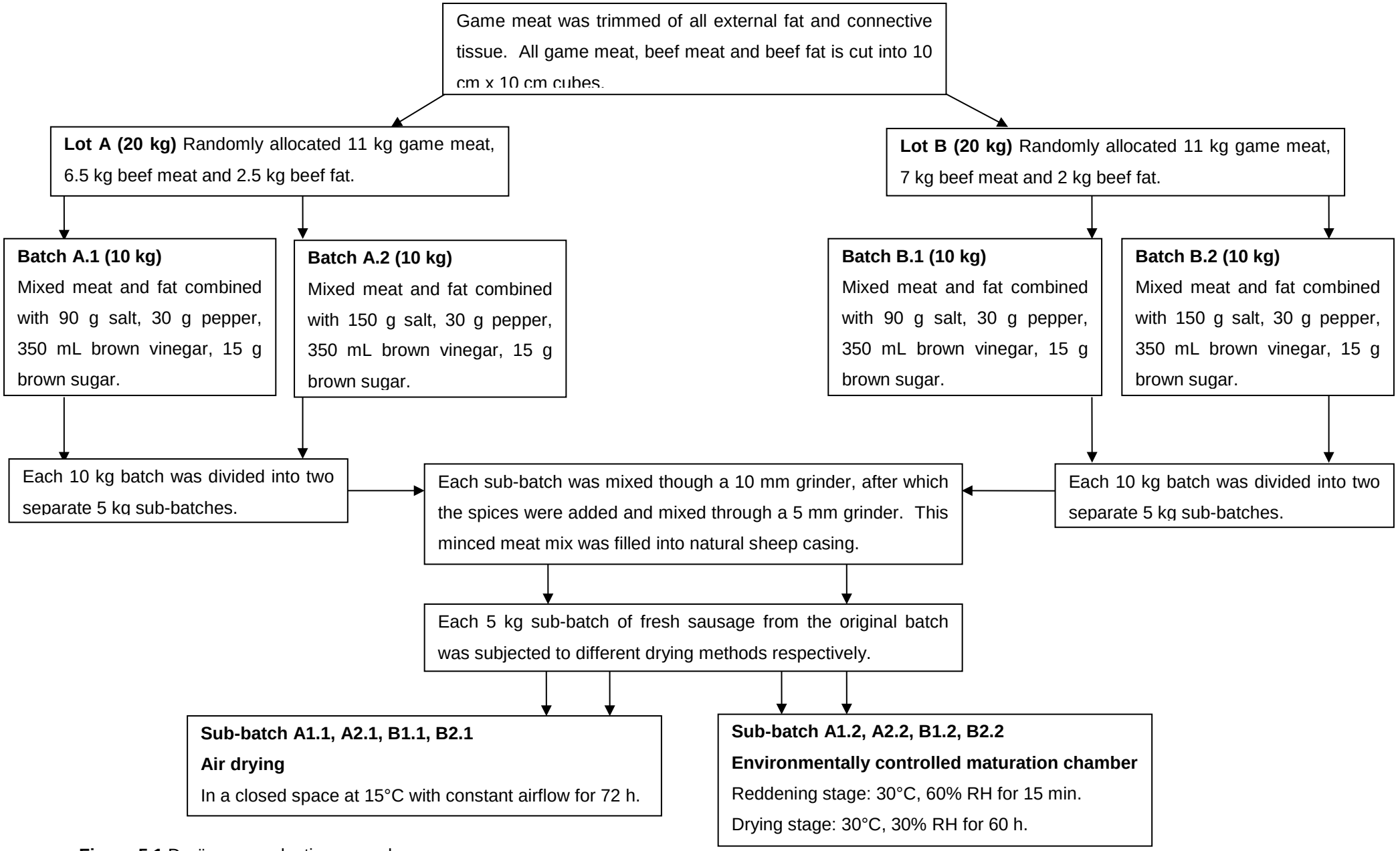

Figure 5.1 Droëwors production procedure.

The batches were weighed before and after drying to ensure that the product lost 45 – 50% of its initial wet mass. Table 5.1 indicates the masses before and after drying and the calculated mass loss.

Table 5.1 Mass before and after drying and the calculated mass loss.

	Drying method	Before drying (g)	After drying (g)	Mass loss (%)
Batch A.1	AD	5094.4	4204.3	17.5
Batch A.1	MC	3949.3	2073.3	47.5
Batch B.1	AD	5150.0	4214.0	18.2
Batch B.1	MC	5650.0	3023.9	46.5
Batch A.2	AD	4723.4	3903.0	17.4
Batch A.2	MC	5007.6	2781.9	44.4
Batch B.2	AD	4778.2	3981.4	16.7
Batch B.2	MC	5200.0	2870.9	44.8

AD: Air drying, MC: Maturation chamber

Informal preference test

The group of consumers consisted of 20 persons who regularly consume droëwors and are familiar with game meat products. Randomly numbered samples from each batch were given to each panel member from which each member indicated which sample was most preferred. This was followed by a discussion. No statistical data analysis was done as this was an informal test based on the opinions of the panel members.

DISCUSSION

Each batch was made separately on the same day and dried over the same period of time. This allowed for an accurate testing of both the best formulation to use and drying method as the conditions were the same throughout the process.

The differing fat content of 10% and 12.5% were chosen as the former option is what is commonly used in the production of commercial beef droëwors and the latter option was slightly increased because game meat, which is very lean (< 2% fat), would be used as the principle meat source and therefore the added fat would be the main fat source. The salt to pepper ratio's tested were a 3:1 ratio and 5:1 ratio with the pepper staying at a constant of 15 g for each spice combination. It was decided to increase the salt content for one of the spice combinations as this would decrease the pepper flavour which is picked up more easily by consumers. Droëwors is also known to have a high salt content (Burnham *et al.,* 2008).

Firstly, it is important to note that the air dried droëwors samples were eliminated from the trial after the 72 h drying period, as the product did not dry sufficiently (Table 5.1).

In the initial study (Chapter 3), it was concluded that the oxidation results increased considerably from the raw droëwors samples to the dried droëwors samples, which could be partially due to the long drying periods. It was therefore thought that by decreasing the drying period to 3 days this would eliminate the possibility of this factor interfering with the study further and 3 days is also sufficient time for the droëwors to lose up to 45 – 50% of its original mass. However, the air-dried droëwors samples had barely dried after the 72 h period (Table 5.1) which could be due to either the wrong conditions (too low temperatures or too high RH; the latter was not measured) or insufficient airflow between the fresh sausages. The maturation chamber batch samples dried sufficiently over the allotted period of time and lost up to 45 – 48% of its initial wet mass (Table 5.1). Therefore this drying method was the procedure of choice.

An informal discussion took place whereby the participant was given four samples of which they had to decide which they preferred. The results were then counted followed by a focused discussion.

This informal preference test was conducted amongst 20 persons (Table 5.2). The samples with the least salt were immediately eliminated as all participants described these samples as being too bland and needing more salt. There was a debate over the fat content as this could be more strongly linked to personal preference. The higher fat levels gave the droëwors samples less of a distinct gamey taste whilst the droëwors samples with less fat gave a more gamey flavour. The samples with higher fat content also left a fatty residue on the palate. As seen in Table 5.2, the majority of the persons preferred the samples with the higher salt and lower fat content.

Table 5.2 Preferences and comments of the group of regular droëwors consumers.

Sample	Vote	Comment	Verdict
Low salt, low fat	X	Too bland, needs more salt. No discussion needed.	Eliminated
Low salt, high fat	X	Too bland, needs more salt. No discussion needed.	Eliminated
High salt, low fat	75%	Gamey flavour-fat flavour balanced, good mouthfeel.	Accepted
High salt, high fat	25%	Less distinct gamey flavour, fatty residue on palate.	Eliminated

CONCLUSION

The formulation using 10% fat and the spice B combination with a higher salt content (Table 5.2) would be used for the next trial. This is mainly due to the results of the tasting of the persons that participated in the informal preference test. The meat to fat ratio will consist of 55% lean game meat, 35% beef meat and 10% beef fat, which follows guidelines, set out by SANS, as well as allows the research to show the potential of the addition of rooibos tea extract as an antioxidant by using beef fat at a lower percentage level. As the air drying was

unsuccessful, the maturation chamber drying method would be used for future droëwors production. The design of the chamber would also ensure an uniform environment whilst its size would allow for the experimental mixtures to be produced at the same time.

REFERENCES

Burnham, G.M., Hanson, D.J., Koshick, C.M., & Ingham, S.C. (2008). Death of *Salmonella serovars*, *Escherichia coli* H0157:H7, *Staphylococcus aureus* and *Listeria monocytogenes* during the drying of meat: A case study using biltong and droëwors. *Journal of Food Safety,* **28**, 198–209.

CHAPTER 6

Effect of rooibos tea extract (*Aspalathus linearis*) on lipid and protein oxidation over time and the sensory analysis of blesbok (*Damaliscus pygargus phillipsi*) and springbok (*Antidorcas marsupialis*) droëwors

ABSTRACT

The objective of this study was to investigate the addition of rooibos tea extract (RBTE) in blesbok and springbok droëwors to improve oxidative stability after storage. After the storage period of two weeks, inhibition of lipid oxidation proved to be successful with the addition of 1.0% of RBTE. The added RBTE, however, did not result in any significant differences ($P > 0.05$) in protein oxidation within the raw, dried or stored samples. The heme-iron results also show no significant differences within any stage (raw, dried or stored) however a positive correlation between lipid oxidation and heme-iron was noted. There are no differences ($P > 0.05$) between the moisture, protein and fat content between treatments within a stage. The moisture decreases after drying and storage whilst the other proximate components become more concentrated. The fatty acid profiles suggest that after drying the decrease in polyunsaturated fats could be linked to the increase in lipid oxidation. Flavour and aroma differences between the varying RBTE concentrations in the droëwors were detected by the sensory panel. A droëwors formulation using a combination of game meat and beef fat with the addition of RBTE at a 1.0% concentration could be a successful addition to the processed meat market in South Africa.

Keywords: *Aspalathus linearis,* rooibos tea extract, natural antioxidant, lipid and protein stability, game droëwors

INTRODUCTION

Blesbok meat (*Damaliscus pygargus phillipsi*) and springbok meat (*Antidorcas marsupialis*) have been indicated to be more acceptable by the South African consumer for consumption (Hoffman & Wiklund, 2006). Rooibos tea extract was added as a natural antioxidant to slow down oxidation over time.

When droëwors is purchased by a consumer it is usually stored in a brown paper bag over a period of time without refrigeration, primarily because the drying allows for preservation of the sausage. The brown paper bag also allows for air circulation which slows down fungal growth. Due to the long storage period that droëwors can undergo, oxidation will likely occur and therefore the addition of rooibos tea extract (RBTE) should slow down the oxidation of the added animal fat. Therefore it is necessary to evaluate the droëwors for oxidation stability over time. A two week storage period was chosen as this is common for commercial droëwors. Stability was evaluated by means of chemical analyses and descriptive sensory analyses. Chemical analyses included lipid and protein oxidation, heme-iron concentration, fatty acid composition and proximate analyses. The oxidation tests determined if the use of rooibos tea extract as a natural preservative is effective. The sensory analysis was conducted by means of a trained panel on the product after drying. It has been noted that consumers are prepared to purchase game meat products as unprocessed game meat is not readily accepted by all consumers (Crafford, 2002; Hoffman *et al.,* 2004; Hoffman & Wiklund, 2006). Over the years, dried game meat products have been commercialised and sold as an alternative to dried beef and other meat products in South Africa. The addition of rooibos tea extract to a comminuted and dried meat product will hopefully develop into an additional product for marketing to consumers as well as improve its chemical properties (inhibition of oxidation and improved fatty acid profile).

The aim for this study was therefore to investigate the effect of the addition of rooibos tea extract as a natural antioxidant to blesbok and springbok droëwors on its oxidative stability over time and to also evaluate the effect of this addition to its sensory attributes.

MATERIALS AND METHODS

Droëwors production

The droëwors production procedure is depicted in Figure 6.1. Game meat of each species, blesbok (n=10) and springbok (n=15) was trimmed of all external fat and connective tissue and cut into approximately 10 cm x 10 cm cubes. No specific cuts were used. Beef meat was added to increase the amount of fat in the product. Six separate 20 kg replicates of droëwors from each species were made. Each 20 kg replicate was further divided into four

treatment batches of approximately 5 kg each. Each 5 kg batch consisted of 55% game meat and 35% lean beef meat (90 meat: 10 fat) and 10% beef fat. Each batch had 45 g salt, 15 g pepper, 7.5 g brown sugar, 175 g brown vinegar and varying amounts of rooibos tea extract (RBTE: 0%, 0.25%, 0.50% and 1.0% concentrations) added. The phenolic composition of the RBTE used is depicted in Table 6.1. Each treatment batch was minced through a 10 mm grinder after which the spices and vinegar and varying concentrations of RBTE were added. The minced meat and spices were then mixed gently (so as to ensure that minimal salt soluble proteins are extracted as this would make the product's texture too "sticky") and minced through a 5 mm grinder. Natural sheep casings (22 mm diameter) were filled with the minced meat mixture. Samples (500 – 800 g) from each of the batches of raw droëwors were taken for analysis. The rest of the wet droëwors from each batch was dried in an environmentally controlled maturation chamber (Reich Unicontrol 2000S). Initially the droëwors underwent a reddening stage at 30°C with a 60% relative humidity (RH) for 15 min followed by a drying period of 60 h at a constant temperature of 25°C and a 30% RH. The droëwors lost 48-50 % of its initial mass (Table 6.2 and 6.3).

After drying, the droëwors samples from the control (RBTE 0%) and the RBTE 1.0% were placed in brown paper bags. Each bag was individually marked with RBTE treatment and batch number and stored at 23°C for two weeks. Sub-samples of each batch were taken after the two week storage period for chemical analyses.

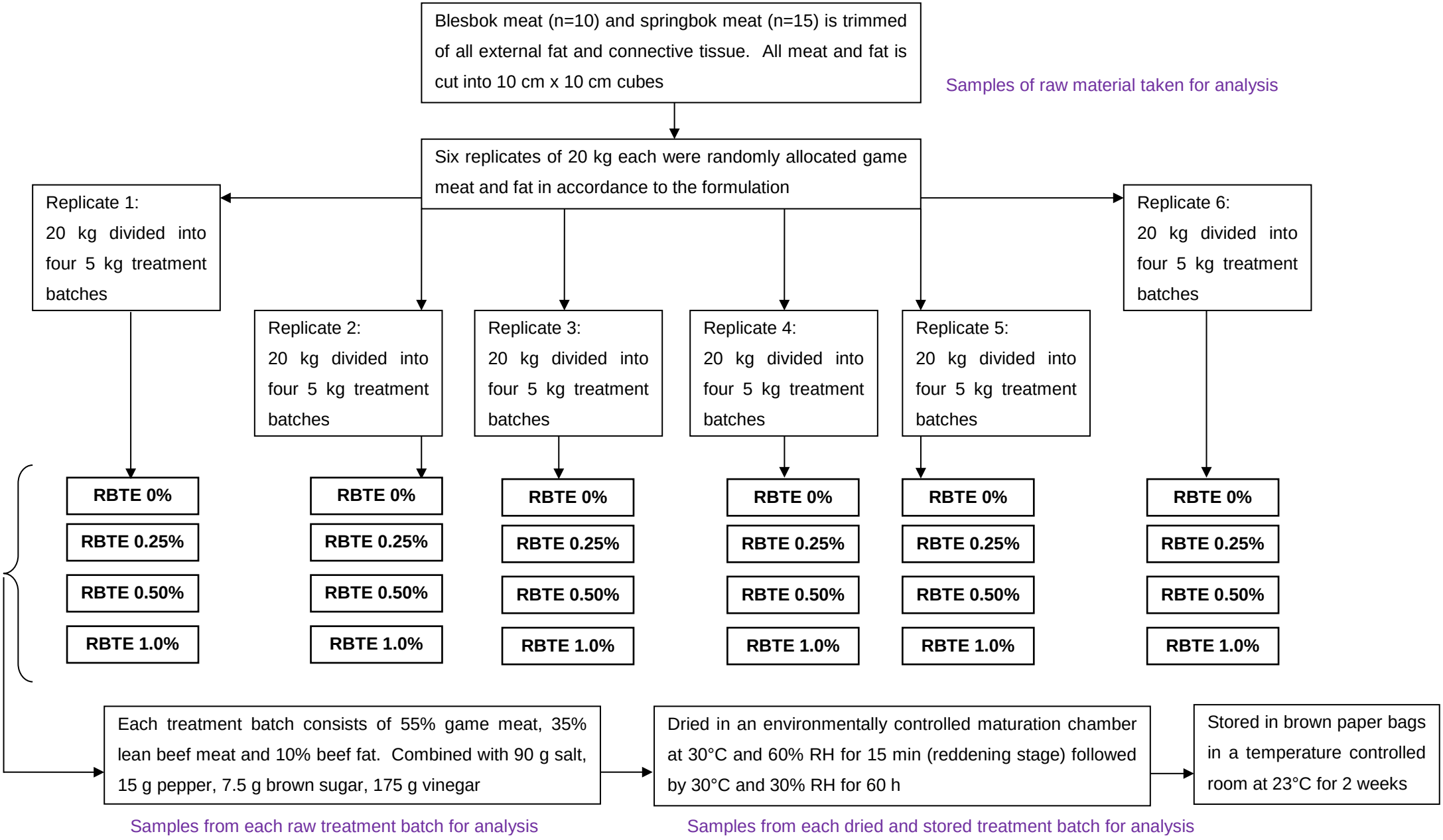

Figure 6.1 Droëwors production procedure.

Table 6.1 Phenolic composition of fermented rooibos tea extract (RBTE) used in the samples analysed.

Flavonoid	Concentration (g compound/100 g extract)*
Aspalathin	0.306
Nothofagin	0.038
Isoorientin	0.876
Orientin	0.576
Quercetin-3-*O*-robinobioside	0.442
Isoquercitrin	0.130
Vitexin	0.120
Hyperoside	0.161
Rutin	0.114
Isovitexin	0.076
Luteolin - 7-O-glucoside	0.196

*Analysed according to the method of Beelders *et al.* (2012)

Table 6.2 Averaged mass loss for each treatment batch of blesbok droëwors.

Treatment	Mass before drying (kg)*	Mass after drying (kg)	Moisture loss (%)
RBTE 0%	4.03	2.07	48.76
RBTE 0.25%	4.38	2.28	47.91
RBTE 0.50%	4.30	2.22	48.45
RBTE 1.0%	3.98	2.07	48.01

*These batches do not weigh 5 kg as per procedure as a sub-sample has been removed for chemical analyses

Table 6.3 Averaged mass loss for each treatment batch of springbok droëwors.

Treatment	Mass before drying (kg)*	Mass after drying (kg)	Moisture loss (%)
RBTE 0%	4.13	2.11	48.99
RBTE 0.25%	4.57	2.27	50.36
RBTE 0.50%	4.53	2.31	49.08
RBTE 1.0%	4.38	2.23	49.24

*These batches do not weigh 5 kg as per procedure as a sub-sample has been removed for chemical analyses

Sample for analyses preparation

Sub-samples of the raw materials and six sub-samples of both the raw and dried samples of each batch of droëwors were taken. The dried droëwors samples were taken within 30 min after the drying process was completed. Sub-samples were also taken after two weeks storage in a 23°C temperature controlled room. All samples were homogenised individually in a blender for 3 min to ensure a representative homogenous sample was analysed. The samples were stored in a -80°C freezer until analyzed. The homogenised samples were

used for proximate analysis, the analysis of fatty acid composition, heme-iron concentration, as well as for lipid and protein oxidation.

Water activity

Water activity was measured using the Novasina LabMASTER-aw. A sample from each batch was taken and measurements were taken every four h over the drying period. Figures 6.2 and 6.3 indicate that the product was sufficiently dried over the 60 h in terms of the water activity (a_w < 0.60) for blesbok and springbok droëwors, respectively.

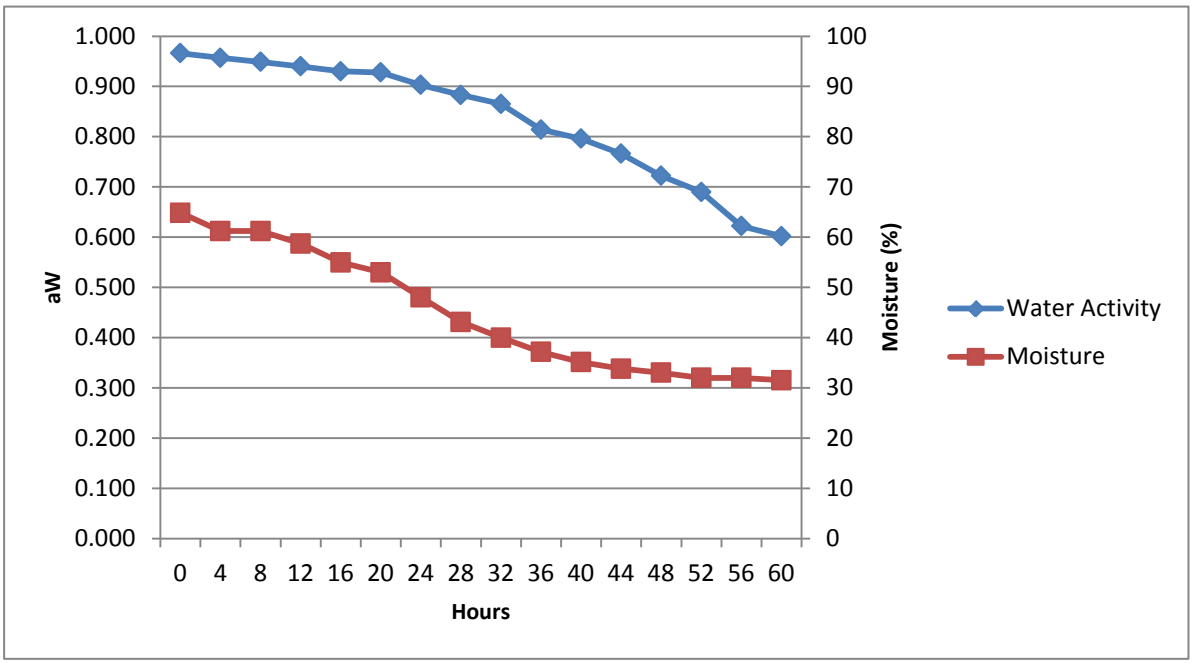

Figure 6.2 Water activity and moisture of blesbok droëwors over the drying period.

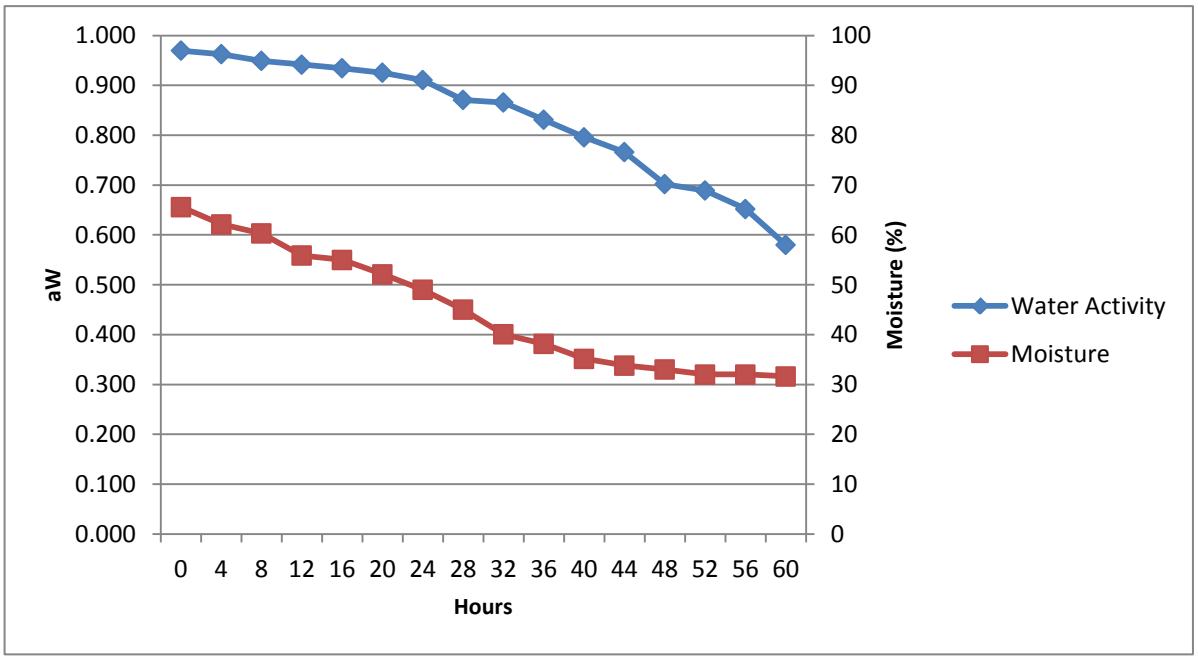

Figure 6.3 Water activity and moisture of springbok droëwors over the drying period.

Proximate composition analysis

The samples were analysed to determine the moisture (Method 934.01) and ash (Method 942.05) content according to the AOAC (2002). The protein content was determined according to AOAC (1992) procedure 992.15, whereas the total lipids was determined using the chloroform/methanol (2:1) fat extraction method of Lee *et al.* (1996). All analyses were performed in duplicate. For the full method descriptions, refer to Chapter 3.

Fatty acid composition

The fatty acids were methylated and then analysed using gas chromatography to determine the fatty acid composition and percentages. An internal standard, heptadecanoic acid ($C_{17}H_{34}O_2$) (Sigma-Aldrich Inc., 3050 Spruce St., St. Louis, MO, 63103, USA; Cat. No. H3500) was added to quantify the individual fatty acids. The fatty acid composition was expressed as percentage. For the full method description, refer to Chapter 3.

Heme-iron

The heme-iron concentration was determined according to the method of Hornsey (1956). The heme-iron concentration values are expressed in mg heme-iron per g sample. For the full method description, refer to Chapter 3.

Lipid oxidation

Lipid oxidation was followed by measuring the thiobarbituric acid reactive substances (TBARS) using the spectrophotometric method described by Rosmini *et al.* (1996). The samples were tested in duplicate. The TBARS values are expressed as mg malonaldehyde (MDA) per kg product. For the full method description, refer to Chapter 3.

Protein oxidation

Carbonyls and proteins were measured on all samples. The samples were tested in duplicate. The carbonyls were determined according to the method outlined by Oliver *et al.* (1987). The protein oxidation values are expressed as µM carbonyl per mg protein. For the full method description, refer to Chapter 3.

Descriptive sensory analysis

A ten-member sensory panel was trained according to Lawless and Heymann (2010) using the generic descriptive analysis technique. A 2 cm length ‘stick’ of each treatment of droëwors was tested by the panel for attributes pertaining to its aroma, flavour, appearance

and texture. All attributes were scored on an unstructured 100 mm line scale (0 = no intensity, 100 = extreme intensity) (Table 6.4). Three training sessions of approximately 90 min each allowed for the sensory terminology to be generated and the score sheet to be developed. Thereafter, a blind test was used for the trained panelists to analyze each droëwors treatment sample. The samples were served in glass ramekins labelled with 3-digit codes and presented in a randomized order. The panelists were placed in a booth equipped with *Compusense® five software* (Compusense, Guelph, Canada). The lighting and temperature of the room was controlled throughout the testing period. The blind test was repeated three times per sample over a 10 day period.

Table 6.4 Descriptions of the full range of sensory attributes as used in the descriptive sensory analyses.

Characteristic	Attribute	Description
Aroma*	Rooibos	Associated with sweet, woody aroma typical of rooibos
	Gamey	Associated with game, wild animal
	Fatty	Associated with fresh non-oxidised fat
	Rancid	Associated with oxidised fat
Appearance	Colour intensity (meat)	The inside of the sample and outside surface
		0=Red, 100=Brown
	Fat colour	The fat on the inside of the sample
		0=White, 100=Darker
Flavour*	Rooibos	Associated with sweet, woody flavour typical of rooibos
	Sweet-associated	Associated with sugar/honey
	Gamey	Associated with game, wild animal
	Saltiness	Associated with salty taste
	Acidity	Associated with acid, sour taste
	Peppery	Associated with pepper
	Rancid	Associated with oxidised fat
Texture	Toughness – casing	The ease to bite through the casing
		0=Not tough, 100=Very tough
	Chewiness – meat	The ease which the wors falls apart when biting
		0=Soft, 100=Chewy
	Dryness	*0=Wet, 100=Dry*
	Fattiness	Leaves a fatty mouthfeel on the palate
		0=None, 100=Extreme
	Residue	Pieces that stay in your mouth after chewng
		0=None, 100=Extreme

***Scale for descriptors: 0=No intensity, 100=Extreme intensity

Statistical analysis

To test the effects of species, treatment and stage (raw, dried and stored) on the various measurements, mixed model repeated measures ANOVA were used. The stage effect was the within subject repeated effect. For the mixed model, species, treatment and stage were treated as fixed effects and the batches to which the treatments were applied as a random effect. For the sensory analyses, mixed model repeated measures ANOVA was again used with judge and treatment entered into the model as fixed main effects, and the batches as random effects. Fisher LSD post hoc test were used to further analyse significant differences when the main effects/interaction effects were significant. Statistical analyses were done using the VEPAC module of *Statistica 11*. A 5% significance level was used as guideline for determining significant differences.

RESULTS

As it was not the intention of the experiment to compare the droëwors between species, the results are given per species, but where applicable general trends across species will be discussed.

Proximate composition

The drying of the product was efficient (Figures 6.1-2). The results of the proximate composition (Table 6.5 and 6.6) were uniform across the samples which did not differ significantly ($P > 0.05$) between treatments. Therefore, the results will be discussed as averaged means over each time period for each species. Results for percent moisture, protein and fat values of each species of droëwors (blesbok and springbok) are presented in Table 6.5 and 6.6 respectively. The blesbok droëwors had a moisture content which lowered from an averaged 64.7% to 31.7% after drying as the product lost approximately 48% of its moisture content (Table 6.2 and 6.5) during the drying process. The water activity also decreased over time (Figure 6.2). The protein and fat is expected to increase in concentration after drying. During the two week storage period, the droëwors dried further losing more moisture, decreasing from 31.7% to 24.5%, causing it to be slightly more concentrated in the protein and fat content. A difference is seen between the protein content of the raw samples and that of the dried samples at week 0, with an increase in concentration from an averaged 11.5 to 30.8%. No difference in protein content is observed between the dried samples and those after the two week storage period. The fat in the raw samples was fairly high at 18.5% becoming more concentrated ($P < 0.05$) - 34.8% after drying. A slight increase in concentarion, less than 1%, is seen after the two week storage period. The ash content becomes more concentrated with the drying and storage period,

which could be explained by the added spices (mineral content) and lowered moisture content. As with the blesbok droëwors results, the moisture content of the springbok droëwors decreased over time from 64.8% (raw samples) to 31.5% (after drying) and finally 25.7% (after storage). Protein concentration increased by more than 50% after drying, increasing from 11.6% to 30.9%, with a further increase to 32.8% after storage. The fat concentration increased from 18.6% which was detected in the raw samples to 31.7% after drying. A slight increase in concentration was also seen after the storage period.

Fatty acid composition

The major fatty acids and total fatty acid profile of each species droëwors with the various levels of RBTE added are presented in Table 6.7 for blesbok species droëwors and Table 6.8 for springbok species droëwors. The full fatty acid composition of the droëwors for each species is given in Addendum A.

Blesbok meat in this investigation has 17.0% oleic acid, followed by 19.6% palmitic acid, 20.3% linoleic acid and 23.8% stearic acid with a saturated fatty acid (SFA) percentage of 46.3% and a polyunsaturated fatty acid (PUFA) percentage of 39.4% (Table 6.7). Springbok meat in this investigation has 15.1% palmitic acid, 18.7% oleic acid, 19.2% linoleic acid and 27.0% stearic acid with a saturated fatty acid (SFA) percentage of 41.7% and a polyunsaturated fatty acid (PUFA) percentage of 37.9% (Table 6.8). The beef fat added to the mixture had a high level of SFA (59.1%) and a very low PUFA (1.2%); it is thus expected that the fatty acid profile of the wet droëwors will closely resemble that of the beef fat as 10% beef fat was added to the initial mixture (Figure 6.1).

Results differ between the species when studying the total fatty acid profile of the raw droëwors although these differences are small due to the predominate effect of the added beef fat, as mentioned. Generally, the saturated fatty acids (SFA) present are high with an initial concentration of 57.1% and 48.7% for the blesbok droëwors and springbok droëwors, respectively. Differences in the fatty acid profile of the droëwors after drying were slight (not significant), the PUFA decreased from an initial 8.5% to ± 1.8% for blesbok droëwors and 4.9% to ±1.8% for springbok droëwors. The differences between the RBTE 0% and RBTE 1.0% at the end of the drying period and the end of the two week storage period showed no fixed trend ($P > 0.05$).

Heme-iron

The heme iron concentrations detected in the respective raw samples are similar in both species. The results after drying and storage are slightly lower for the springbok droëwors samples than those of the blesbok droëwors samples. There are no differences ($P > 0.05$)

between the heme-iron concentrations of all the dried samples for both species but a clear change between the raw and dried samples of both species. The results for the heme-iron concentration for both the blesbok and springbok droëwors samples are given in Table 6.9.

For blesbok droëwors, the heme-iron values range between 0.39 mg per 100 g meat and 0.51 mg per 100 g meat for the raw blesbok samples and 2.14 mg per 100 g meat and 2.38 mg per 100 g meat for the dry samples. Samples after the two week storage period were not significantly ($P > 0.05$) different with only a slight increase in heme iron concentration. No significant differences ($P > 0.05$) are seen between treatments of each stage. According to Table 6.9, heme iron concentrations for springbok droëwors increase across the drying period from an averaged 0.47 mg per 100 g meat to 1.67 mg per 100 g meat. As with the blesbok droëwors, heme iron concentration increased slightly over the storage period increasing to an averaged 2.07 mg per 100 g meat. No significant differences ($P > 0.05$) are seen between treatments of each stage.

Lipid oxidation

The results for lipid oxidation in blesbok and springbok species droëwors can be seen in Table 6.10. As the results differ significantly from each other ($P < 0.05$), each species will be discussed separately.

Blesbok droëwors shows differences within the raw samples, which was not expected, as these samples were taken before drying at the same time. It is important to note that the blesbok RBTE 0% and RBTE 1.0% had higher ($P < 0.05$) values than the other two concentrations of RBTE. After drying, although the mg MDA/kg meat increased, the RBTE 0.25% had the lowest ($P < 0.05$) values. This trial included a storage period of two weeks whereby lipid oxidation was again measured; the RBTE 1.0% samples showed lower ($P < 0.05$) oxidation than the control (RBTE 0%).

Significant differences ($P < 0.05$) are also seen in the springbok droëwors between the treatments on the raw samples; again the control (RBTE 0%) and RBTE 1.0% have the highest oxidation levels. However, after drying, the RBTE 1.0% had the lowest levels of MDA – why this result is lower than that measured in the raw sample is unknown but could be due to experimental error and/or sampling error. After the two week storage period, the lipid oxidation results showed that similar to the blesbok droëwors, the springbok droëwors with the addition of RBTE 1.0% had oxidised the least ($P = 0.000577$) in terms of mg MDA/kg meat (2.84 mg MDA/kg meat for RBTE 0% and 1.68 mg MDA/kg meat for RBTE 1.0%).

It was expected that the lipid oxidation would differ between raw, dried and stored droëwors. When expressed on a dry mass basis, the droëwors still had significant differences between treatments within a stage (Table 6.10).

Protein oxidation

Results from the analysis of the oxidative deterioration of proteins in the blesbok and springbok droëwors are shown in Table 6.11. The trends of the addition of RBTE 0% and 1% over each stage (raw, dried and storage) can be seen.

According to Table 6.11, carbonyl concentration in the blesbok droëwors did not differ significantly ($P > 0.05$) over time or between treatments. The carbonyls detected from protein oxidation were very low with values ranging over a small interval of ± 0.0003 to ± 0.0009 mg carbonyls per mg protein over both time and treatments.

Sensory attributes

The sensory profiling results for the blesbok and springbok droëwors are depicted in Table 6.12 and 6.13 and displayed in Figures 6.4 and 6.5, respectively.

The blesbok droëwors shows a significant increase ($P < 0.05$) in rooibos aroma and flavour with increasing concentrations of RBTE. These results correspond with the game aroma and flavour. As the RBTE increases in concentration, the game aroma and flavour decreases ($P < 0.05$). A slight fatty aroma was detected, which also tended ($P > 0.05$) to decrease with increasing RBTE. No rancidity was detected throughout the trained panel trial. There were no significant differences ($P > 0.05$) in the appearance (colour intensity and fat colour) and texture attributes of the droëwors. The colour intensity of the droëwors was on the upper end of the intensity scale being perceived as being a more brownish colour than a red colour. In terms of the texture, the fattier the droëwors the more residue was left in the mouth after chewing. A standard Pearson's correlation indicates that the fatty texture was positively-linked to the droëwors residue left in the mouth after chewing for species, with a correlation (r=value) for blesbok 0.98 and springbok 0.56. A fattier mouthfeel was detected in the control samples. The springbok droëwors show the same trends as the blesbok droëwors with that the rooibos aroma and flavour increase significantly ($P < 0.05$) with increasing RBTE addition, and the game aroma and flavour decreasing significantly ($P < 0.05$). The colour intensity increased ($P < 0.05$) with added RBTE. The texture remained the same over the four treatments and the residue after swallowing decreased slightly with added RBTE (Figure 6.6).

Table 6.5 Means (%) ± SD for proximate analyses of blesbok droëwors with added rooibos tea extract.

	Week 0 (Raw)				Week 0 (Dried)				Week 2 (Dried)	
	RBTE 0%	**RBTE 0.25%**	**RBTE 0.50%**	**RBTE 1.0%**	**RBTE 0%**	**RBTE 0.25%**	**RBTE 0.50%**	**RBTE 1.0%**	**RBTE 0%**	**RBTE 1.0%**
Moisture	64.7 ± 1.08	65.1 ± 0.77	65.0 ± 0.79	64.1 ± 0.36	32.0 ± 1.27	31.7 ± 0.55	31.5 ± 0.49	31.7 ± 0.68	24.5 ± 0.99	24.4 ± 0.56
Protein	11.6 ± 0.13	11.4 ± 0.11	11.4 ± 0.093	11.6 ± 0.20	30.8 ± 0.34	30.8 ± 0.20	30.9 ± 0.35	30.9 ± 0.21	32.9 ± 0.088	33.0 ± 0.032
Fat	18.5 ± 0.21	18.5 ± 0.15	18.5 ± 0.12	18.6 ± 0.14	34.9 ± 0.67	34.9 ± 0.78	34.7 ± 0.52	34.8 ± 0.56	36.0 ± 0.78	35.6 ± 0.59
Ash	2.1^{a} ± 0.028	2.2^{a} ± 0.089	2.2^{a} ± 0.052	2.6^{b} ± 0.54	4.3^{a} ± 0.17	4.3^{a} ± 0.19	4.5^{b} ± 0.20	4.5^{b} ± 0.20	6.2 ± 0.041	6.2 ± 0.079

^{a,b,c} Means within a row per stage (raw, week 0, week 2) with different superscripts are significantly different ($P < 0.05$)

Table 6.6 Means (%) ± SD for proximate analyses of springbok droëwors with added rooibos tea extract.

	Week 0 (Raw)				Week 0 (Dried)				Week 2 (Dried)	
	RBTE 0%	**RBTE 0.25%**	**RBTE 0.50%**	**RBTE 1.0%**	**RBTE 0%**	**RBTE 0.25%**	**RBTE 0.50%**	**RBTE 1.0%**	**RBTE 0%**	**RBTE 1.0%**
Moisture	64.5 ± 1.08	65.2 ± 1.05	65.7 ± 1.18	63.6 ± 1.22	31.6 ± 0.49	31.1 ± 1.47	31.8 ± 0.52	31.7 ± 0.56	25.7 ± 0.58	25.6 ± 0.58
Protein	11.5 ± 0.23	11.6 ± 0.20	11.6 ± 0.17	11.6 ± 0.22	30.8 ± 0.34	30.9 ± 0.23	30.9 ± 0.33	30.9 ± 0.26	32.9 ± 0.34	32.7 ± 0.22
Fat	19.1 ± 1.13	18.3 ± 0.32	18.2 ± 0.37	18.6 ± 1.00	31.5 ± 1.12	31.7 ± 0.61	31.5 ± 0.61	32.0 ± 0.81	36.1 ± 0.82	35.6 ± 0.52
Ash	2.4 ± 0.097	2.4 ± 0.17	2.5 ± 0.10	2.5 ± 0.26	4.3 ± 0.16	4.4 ± 0.14	4.4 ± 0.092	4.4 ± 0.093	6.3 ± 0.11	6.3 ± 0.14

^{a,b,c} Means within a row per stage (raw, week 0, week 2) with different superscripts are significantly different ($P < 0.05$)

Table 6.7 The major fatty acids present and the total fatty acid profile (%) ± SD of blesbok droëwors produced with increasing rooibos tea extract levels.

Fatty acids	Blesbok meat	Beef fat	Week 0 (Raw)	Week 0 (Dried)				Week 2 (Dried)	
			RBTE 0%	RBTE 0%	RBTE 0.25%	RBTE 0.50%	RBTE 1.0%	RBTE 0%	RBTE 1.0%
C16:0	19.6	29.5	29.3 ± 7.52	30.0 ± 1.19	30.7 ± 5.58	31.4 ± 2.65	30.3 ± 0.34	30.2 ± 2.26	30.6 ± 0.59
C18:0	23.8	15.4	25.0 ± 6.37	26.2 ± 1.53	30.9 ± 3.25	24.1 ± 1.27	26.9 ± 2.66	26.9 ± 1.82	26.0 ± 1.40
C18:1n9c	17.0	39.7	32.2 ± 0.54	40.5 ± 0.92	34.0 ± 6.81	40.0 ± 2.14	38.6 ± 2.49	39.1 ± 1.69	39.7 ± 0.98
C18:2n6c	20.3	1.4	4.4 ± 0.36	0.7 ± 0.052	1.2 ± 0.13	1.1 ± 0.047	1.1 ± 0.74	1.1 ± 0.069	0.9 ± 0.021
C18:3n6	0.06	0.4	1.2 ± 0.16	0.1 ± 0.013	0.3 ± 0.036	0.2 ± 0.019	0.3 ± 0.021	0.3 ± 0.019	0.2 ± 0.014
SFA	46.3	59.1	57.1 ± 1.24	57.1 ± 0.97	62.7 ± 6.97	56.8 ± 2.19	58.45 ± 2.57	58.1 ± 1.85	57.7 ± 1.07
MUFA	17.5	37.0	34.4 ± 0.58	41.7 ± 0.91	35.1 ± 6.85	41.2 ± 2.15	39.7 ± 2.46	40.1 ± 1.73	40.7 ± 1.02
PUFA	39.4	1.7	8.5 ± 0.93	1.2 ± 0.096	2.2 ± 0.25	2.0 ± 0.10	1.9 ± 0.15	1.9 ± 0.14	1.5 ± 0.095
PUFA:SFA	0.9	0.03	0.2 ± 0.019	0.02 ± 0.0010	0.03 ± 0.0052	0.04 ± 0.0055	0.03 ± 0.0041	0.03 ± 0.0052	0.03 ± 0.0052
(n-6)/(n-3)	3.8	2.5	9.2 ± 0.14	7.3 ± 1.25	8.5 ± 0.57	6.9 ± 1.38	6.7 ± 0.57	6.7 ± 0.59	5.9 ± 1.00

SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; (n-6)/(n-3) = (n-6)/(n-3) PUFA ratio

Table 6.8 The major fatty acids present and the total fatty acid profile (%) ± SD of springbok droëwors produced with increasing rooibos tea extract levels.

Fatty acids	Springbok meat	Beef fat	Week 0 (Raw)	Week 0 (Dried)				Week 2 (Dried)	
			RBTE 0%	RBTE 0%	RBTE 0.25%	RBTE 0.50%	RBTE 1.0%	RBTE 0%	RBTE 1.0%
C16:0	15.1	29.5	23.3 ± 5.61	34.0 ± 2.81	34.3 ± 1.71	37.2 ± 2.97	36.2 ± 5.90	33.4 ± 2.13	31.6 ± 1.15
C18:0	27.0	15.4	23.0 ± 5.45	20.3 ± 3.74	17.5 ± 2.88	17.3 ± 5.07	18.3 ± 6.73	17.6 ± 1.26	22.8 ± 0.98
C18:1n9c	18.7	39.7	44.7 ± 11.75	41.3 ± 2.31	44.6 ± 2.26	47.6 ± 3.87	29.5 ± 12.3	43.8 ± 2.41	42.0 ± 0.68
C18:2n6c	19.2	1.4	2.73 ± 1.45	1.1 ± 0.12	0.7 ± 0.026	0.8 ± 0.046	1.6 ± 0.51	0.7 ± 0.029	0.8 ± 0.032
C18:3n6	0.11	0.4	0.6 ± 0.50	0.2 ± 0.018	0.1 ± 0.0075	0.2 ± 0.0084	0.3 ± 0.11	0.3 ± 0.40	0.2 ± 0.011
SFA	41.7	59.1	48.7 ± 10.81	55.7 ± 2.55	52.9 ± 2.31	49.7 ± 4.01	66.2 ± 11.29	52.7 ± 0.79	55.5 ± 0.64
MUFA	19.2	37.0	46.4 ± 11.48	42.4 ± 2.37	45.9 ± 2.33	49.0 ± 3.96	31.0 ± 12.01	45.2 ± 2.25	43.1 ± 0.65
PUFA	37.9	1.7	4.9 ± 2.75	1.9 ± 0.22	1.2 ± 0.039	1.4 ± 0.069	2.8 ± 0.88	2.1 ± 2.20	1.4 ± 0.040
PUFA:SFA	0.9	0.03	0.1 ± 0.05	0.04 ± 0.0055	0.02 ± 0.0020	0.03 ± 0.0041	0.04 ± 0.0082	0.04 ± 0.045	0.03 ± 0.052
(n-6)/(n-3)	2.4	2.5	7.7 ± 0.18	7.2 ± 0.67	5.6 ± 0.49	5.7 ± 0.24	7.0 ± 0.59	4.6 ± 2.13	5.57 ± 0.31

SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; (n-6)/(n-3) = (n-6)/(n-3) PUFA ratio

Table 6.9 Means ± SD for heme-iron (mg heme-iron/100 g meat sample) of game droëwors with added rooibos tea extract.

	Week 0 (Raw)				Week 0 (Dried)				Week 2 (Dried)	
	RBTE 0%	**RBTE 0.25%**	**RBTE 0.50%**	**RBTE 1.0%**	**RBTE 0%**	**RBTE 0.25%**	**RBTE 0.50%**	**RBTE 1.0%**	**RBTE 0%**	**RBTE 1.0%**
As is basis:										
Blesbok	0.39 ± 0.047	0.45 ± 0.067	0.51 ± 0.022	0.45 ± 0.049	2.14 ± 0.33	2.38 ± 0.11	2.37 ± 0.13	2.21 ± 0.18	2.24 ± 0.094	2.33 ± 0.16
Springbok	0.42 ± 0.12	0.50 ± 0.096	0.54 ± 0.11	0.42 ± 0.11	1.77 ± 0.33	1.53 ± 0.66	1.55 ± 0.56	1.81 ± 0.22	2.06 ± 0.15	2.08 ± 0.24
Dry mass basis:										
Blesbok	1.11 ± 0.35	1.28 ± 0.39	1.45 ± 0.28	1.26 ± 0.31	3.16 ± 0.54	3.49 ± 0.27	3.45 ± 0.27	3.23 ± 0.40	2.97 ± 0.22	3.08 ± 0.30
Springbok	1.19 ± 0.42	1.45 ± 0.42	1.58 ± 0.46	1.56 ± 0.39	2.59 ± 0.55	2.21 ± 0.89	2.27 ± 0.79	2.65 ± 0.51	2.78 ± 0.28	2.80 ± 0.39

Table 6.10 Means ± SD for lipid oxidation (mg MDA/kg meat) of game droëwors with added rooibos tea extract.

	Week 0 (Raw)				Week 0 (Dried)				Week 2 (Dried)	
	RBTE 0%	**RBTE 0.25%**	**RBTE 0.50%**	**RBTE 1.0%**	**RBTE 0%**	**RBTE 0.25%**	**RBTE 0.50%**	**RBTE 1.0%**	**RBTE 0%**	**RBTE 1.0%**
As is basis:										
Blesbok	0.87^{a} ± 0.066	0.60^{b} ± 0.12	0.65^{b} ± 0.11	0.85^{a} ± 0.10	1.04^{a} ± 0.29	0.69^{b} ± 0.14	0.82^{ab} ± 0.066	0.87^{a} ± 0.21	1.23^{a} ± 0.041	1.15^{b} ± 0.066
Springbok	1.48^{a} ± 0.44	0.76^{b} ± 0.072	0.64^{b} ± 0.049	1.55^{a} ± 0.43	1.51^{a} ± 0.31	1.43^{a} ± 0.11	1.13^{b} ± 0.11	0.95^{c} ± 0.14	2.84^{a} ± 0.67	1.68^{b} ± 0.099
Dry mass basis:										
Blesbok	2.47^{a} ± 0.51	1.71^{b} ± 0.56	1.86^{b} ± 0.70	2.44^{a} ± 0.65	1.53^{a} ± 0.48	1.01^{b} ± 0.44	1.20^{ab} ± 0.21	1.25^{a} ± 0.40	1.62^{a} ± 0.53	0.67^{b} ± 0.21
Springbok	4.21^{a} ± 1.58	2.19^{b} ± 0.47	1.87^{b} ± 0.55	4.29^{a} ± 1.41	2.21^{a} ± 0.50	2.08^{a} ± 0.32	1.66^{b} ± 0.28	1.39^{c} ± 0.31	3.82^{a} ± 0.85	0.92^{b} ± 0.19

^{a,b,c} Means within a row within a stage (raw, week 0, week 2) with different superscripts are significantly different ($P < 0.05$)

Table 6.11 Means ± SD for protein oxidation (µM carbonyl/mg protein) of game droëwors with added rooibos tea extract.

	Week 0 (Raw)				Week 0 (Dried)				Week 2 (Dried)	
	RBTE 0%	**RBTE 0.25%**	**RBTE 0.50%**	**RBTE 1.0%**	**RBTE 0%**	**RBTE 0.25%**	**RBTE 0.50%**	**RBTE 1.0%**	**RBTE 0%**	**RBTE 1.0%**
Blesbok	0.0009 ± 0.0004	0.0009 ± 0.0004	0.0007 ± 0.0004	0.0007 ± 0.0003	0.0004 ± 0.0001	0.0006 ± 0.0002	0.0006 ± 0.0004	0.0005 ± 0.0002	0.0005 ± 0.0003	0.0007 ± 0.0005
Springbok	0.0005 ± 0.0002	0.0005 ± 0.0002	0.0004 ± 0.0002	0.0004 ± 0.0002	0.0003 ± 0.0002	0.0005 ± 0.0005	0.0005 ± 0.0003	0.0005 ± 0.0004	0.0007 ± 0.0002	0.0005 ± 0.0002

No significant differences ($P > 0.05$) were seen within a row per stage (raw, week 0, week 2)

Table 6.12 Mean values ± SD for the sensory attributes evaluated from blesbok droëwors (week 0) with increasing concentrations of RBTE.

	RBTE 0%	RBTE 0.25%	RBTE 0.50%	RBTE 1.0%
Rooibos aroma	1.4^{d} ± 4.32	17.3^{c} ± 9.60	38.1^{b} ± 13.70	62.3^{a} ± 13.02
Gamey aroma	53.7^{a} ± 8.18	37.5^{b} ± 11.42	24.6^{c} ± 10.60	9.0^{d} ± 10.73
Fatty aroma	19.2 ± 4.67	13.4 ± 5.14	11.0 ± 5.52	6.5 ± 6.09
Rancid aroma	0	0	0	0
Rooibos flavour	1.6^{d} ± 4.42	16.7^{c} ± 8.56	34.4^{b} ± 8.75	50.0^{a} ± 7.22
Sweet-associated flavour	1.9^{d} ± 3.72	12.6^{c} ± 6.89	21.3^{b} ± 5.52	31.8^{a} ± 5.40
Gamey flavour	53.1^{a} ± 10.21	34.2^{b} ± 12.55	22.8^{c} ± 8.70	12.4^{d} ± 5.61
Rancid flavour	0	0	0	0
Colour intensity	69.6 ± 2.98	71.5 ± 3.29	72.7 ± 2.97	74.3 ± 1.90
Fat colour	21.5 ± 3.27	22.7 ± 3.43	23.6 ± 3.97	26.2 ± 4.20
Toughness – casing	50.3 ± 10.00	42.8 ± 8.90	37.0 ± 9.08	35.4 ± 8.25
Chewiness – meat	28.0 ± 3.60	24.9 ± 3.91	25.4 ± 3.91	23.1 ± 3.90
Dryness	31.9 ± 19.90	35.5 ± 19.91	37.5 ± 18.73	39.5 ± 19.21
Fattiness	19.0 ± 3.84	15.9 ± 4.88	15.5 ± 5.51	14.1 ± 5.82
Residue	7.7 ± 3.60	6.1 ± 3.74	5.2 ± 3.84	4.3 ± 4.24

^{a,b,c,d} Values in the same row with different superscripts differ significantly ($P < 0.05$)

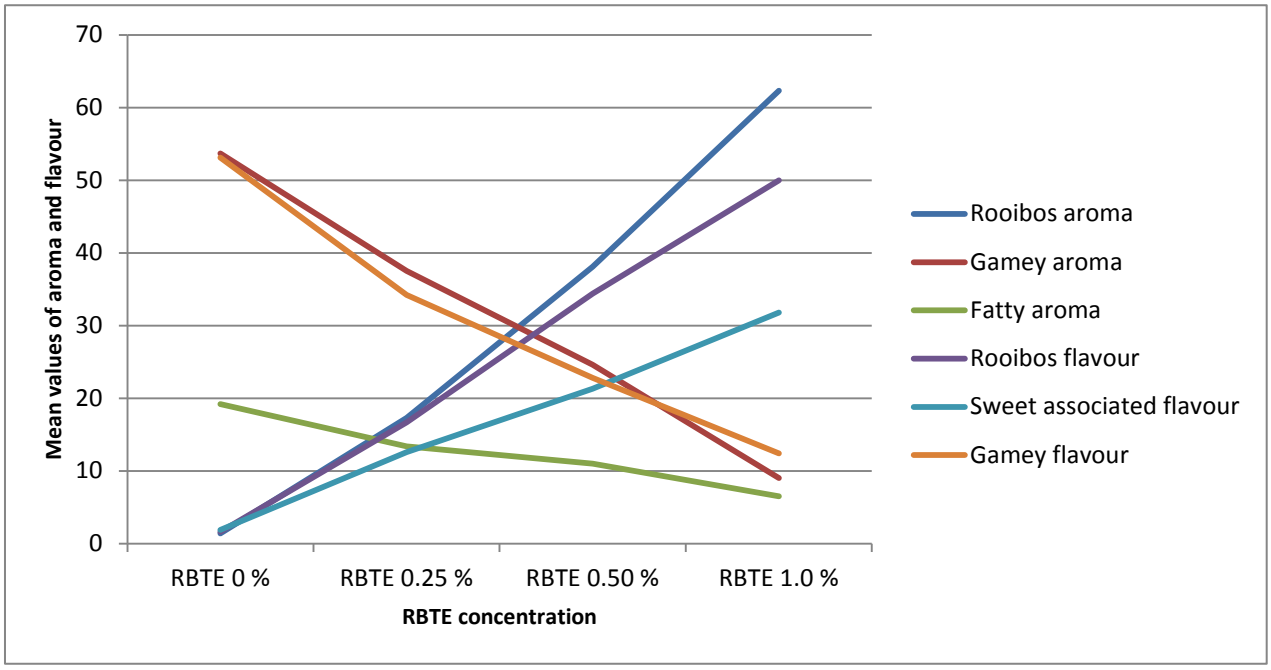

Figure 6.4 Sensory attributes for blesbok droëwors with increasing concentrations of RBTE.

Table 6.13 Means ± SD for the sensory attributes evaluated from springbok droëwors (week 0) with increasing concentrations of RBTE.

	RBTE 0%	RBTE 0.25%	RBTE 0.50%	RBTE 1.0%
Rooibos aroma	4.8^{c} ± 10.92	19.3^{b} ± 12.27	25.9^{b} ± 14.23	47.3^{a} ± 8.36
Gamey aroma	19.0 ± 5.43	14.5 ± 3.82	14.0 ± 3.93	10.4 ± 2.22
Fatty aroma	13.5 ± 4.29	9.1 ± 4.91	7.4 ± 4.78	3.3 ± 4.11
Rancid aroma	0	0	0	0
Rooibos flavour	4.7^{c} ± 12.61	30.9^{b} ± 19.94	40.1^{b} ± 22.00	74.6^{a} ± 13.49
Sweet-associated flavour	1.8^{c} ± 4.96	12.6^{b} ± 7.46	16.1^{b} ± 77.78	28.7^{a} ± 5.43
Gamey flavour	46.2^{a} ± 6.38	33.4^{b} ± 8.65	29.2^{b} ± 8.48	19.9^{c} ± 6.09
Saltiness	8.9 ± 2.31	9.5 ± 2.25	9.3 ± 1.95	9.4 ± 2.25
Acidity	6.3 ± 2.59	6.6 ± 2.61	7.0 ± 2.50	6.4 ± 2.25
Peppery	6.5 ± 2.62	7.0 ± 2.57	6.73 ± 2.73	7.0 ± 2.47
Rancid flavour	0	0	0	0
Colour intensity	61.6^{c} ± 5.06	69.4^{b} ± 5.90	70.1^{b} ± 5.81	77.6^{a} ± 4.42
Fat colour	21.4^{b} ± 3.53	23.8^{b} ± 3.51	24.3^{b} ± 3.77	28.3^{a} ± 3.12
Toughness – casing	20.9 ± 9.93	26.1 ± 7.78	26.5 ± 8.75	33.1 ± 7.88
Chewiness – meat	22.3 ± 10.58	26.9 ± 7.57	29.3 ± 7.34	33.8 ± 7.78
Dryness	37.1 ± 7.66	38.9 ± 5.70	39.24 ± 5.86	44.3 ± 7.86
Fattiness	31.0 ± 11.84	29.1 ± 8.97	30.7 ± 9.91	29.5 ± 9.75
Residue	9.1 ± 2.63	8.8 ± 2.63	8.7 ± 3.18	8.5 ± 2.60

^{a,b,c,d} Values in the same row with different superscripts differ significantly ($P < 0.05$)

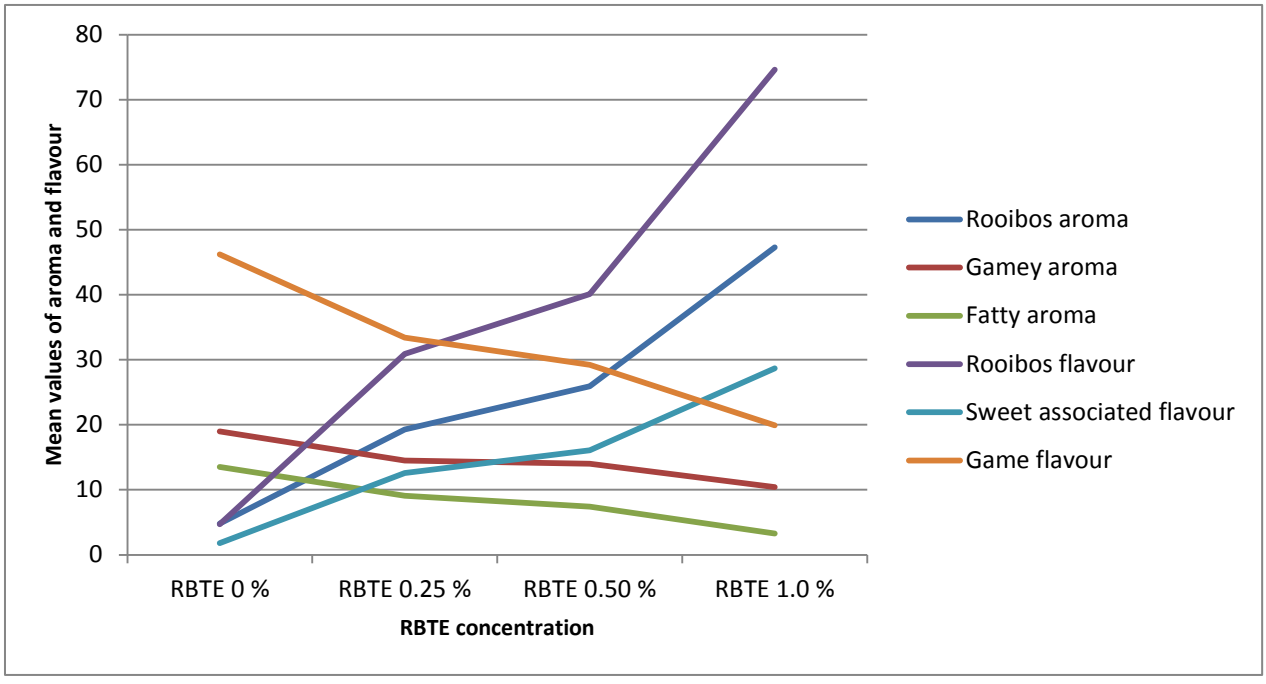

Figure 6.5 Sensory attributes for springbok droëwors with increasing concentrations of RBTE.

DISCUSSION

As the droëwors production and formulation was the same, no differences in the proximate analyses were expected nor analysed (Table 6.5). Similarly, the drying processes and storage of the droëwors was done under the same controlled conditions and should thus not have had any influence on, the proximate composition within a stage (raw, dried or storage).

Blesbok meat and springbok meat have high percentages of oleic acid, palmitic acid, linoleic acid and stearic acid (Table 6.7 and 6.8). The total saturated fatty acid (SFA) percentages are similar to the total polyunsaturated fatty acid (PUFA) percentages in the game meat. The game meat used had similar fatty acid profiles to each other with the beef fat being higher in total SFA's (59.1%) than the game meat. Beef fat added to the product had oleic acid (39.7%), palmitic acid (29.5%) and stearic acid (15.4%) as the most abundant fatty acids.

As expected, the fatty acid composition of the droëwors of both species was closely related to that of the beef fat, especially in terms of the PUFA content, and as the blesbok and springbok meat used had low fat levels and therefore did not make a large contribution to the total fat content of the final product. The fatty acid profile of the droëwors is expected to change after drying due to the moisture loss (as they will also become more concentrated) and potentially used for lipid oxidation. The total SFA of both species droëwors remained constant after drying, the total MUFA were inconclusive whilst the PUFA showed substantial decreases after drying. As oxidation occurs, the polyunsaturated fatty acids begin to deteriorate thereby decreasing after drying (Vuorela *et al.,* 2005).

The definition of protein oxidation in biological samples is the covalent modification of a protein induced either directly by reactive oxygen species or indirectly by reaction with secondary by-products of oxidative stress such as pro-oxidants and processing (Shacter, 2000). Over time, carbonyl levels in protein-rich foods will increase as the proteins begin to oxidise (Lund *et al.,* 2011). This occurs regularly in processed meat and meat products. The addition of RBTE was expected to result in the inhibition of protein oxidation (Cullere *et al.,* 2013). In principle, with higher concentrations of RBTE added to the droëwors, lower carbonyl concentrations should be detected. Baron *et al.* (2007) suggest that by using the dinitrophenylhydrazine (DNPH) method for carbonyl concentration determination, the total protein carbonyl content is not detected as some protein carbonyls react with other cellular constituents; this influences the results because only the protein carbonyls still present after these reactions are measured.

The results (Table 6.11) indicate that the RBTE antioxidant action had no effect on protein oxidation, as the carbonyl level (µM per mg protein) remained fairly constant over time and between treatments ($P > 0.05$). Prevention of protein oxidation is expected to some

extent when adding antioxidants that will inhibit lipid oxidation. Minimising the formation of secondary lipid oxidation products will prevent their interaction with proteins (Lund *et al.,* 2011). However, this is sometimes not the case. Some antioxidants, dependant on their chemical structure, concentration and composition of the substrate, may prevent lipid oxidation but are ineffective at preventing protein oxidation (Mercier *et al.,* 1998; Frankel & Meyer, 2000; Estévez & Cava, 2004; Baron *et al.,* 2005).

Results from the previous studies (Chapter 3 and 4) also resulted in protein oxidation with no significant differences between treatments within a stage. It is known that oxidising lipids assist with the initiation of protein oxidation in meat (Estévez *et al.*, 2008a; Estévez *et al.*, 2008b; Kroger-Ohlsen *et al.,* 2003; Salminen *et al.,* 2008), and protein oxidation products are known to induce lipid oxidation (Viljanen *et al.,* 2004), therefore it can be said that lipid and protein oxidation are directly linked.

The lipid oxidation results show differences within the raw samples, which was not expected as these samples were taken before drying at the same time. Over time, oxidation will occur and therefore the amount of MDA per kg meat is expected to increase. It has been proposed that with the addition of RBTE, there will be a decrease in oxidation with an increase in antioxidant concentration. Due to the changes in the droëwors formulation (Chapter 5) such as the use of beef fat at a lower ratio of 10%, this could possibly enable the antioxidant action of the added RBTE to act above the threshold of RBTE 0.25% as concluded in Chapter 4. Lipid oxidation will still occur, initially from the exposure of the minced meat to oxygen during processing and drying but also due to the interaction with protein oxidation products. After drying, the results indicate that the oxidation levels do decrease with increasing RBTE concentrations.

Previous results from this study show that RBTE 1.0% acted as a pro-oxidant but in this case this concentration gives the best results. There is a significant difference between the droëwors samples with no added RBTE and the addition of RBTE 1.0% after storage whereby the added RBTE results in slowing down lipid oxidation (Table 6.10). After storage refers to the 2 week storage period in which the droëwors was kept in brown paper bags at 23°C. The RBTE used was analysed for its composition (concentration of polyphenols present, Table 6.1). This shows that aspalathin and quercetin are present in high concentrations. These concentrations, however, are lower than the RBTE used in the previous chapters. It has been reported that these polyphenols at high concentrations can act as pro-oxidants (Joubert *et al.,* 2005) but this did not occur in this investigation as they are lower than the earlier batch of RBTE used. Therefore, the combination of lower fat content and RBTE composition are both main factors in the threshold of RBTE and its use in droëwors. It has been previously noted that RBTE at 0.25% has acted as a pro-oxidant but due to the differing composition may act differently at increased concentrations. Previous

research has shown that a longer time period may be required for lipid oxidation to be inhibited effectively by flavonoids, (Deng *et al.,* 1997; Cullere *et al.,* 2013) which could also explain the low oxidation levels after storage.

Processed meat products are susceptible to oxidation as the high bio-available heme-iron degrades into less available non-heme iron which is a catalyst for oxidation (Clark *et al.,* 1997; Schricker & Miller, 1983). Therefore the higher the heme-iron levels, the more susceptible the meat is to oxidation (Seydim *et al.,* 2006). The increased concentration in heme-iron after drying and storage is due to the moisture loss and not the release of the heme-iron from the porphyrin ring. Metmyoglobin, which is formed in meat by the depletion of reducing enzymes at slaughter, has two binding sites for oxygen which results in the release of the heme-iron from the porphyrin ring of myoglobin (Min & Ahn, 2005). It has been proposed that RBTE interferes with the oxygen-myoglobin binding, by binding to these sites and thereby inhibiting the release of heme-iron. Heme-iron is a catalyst for protein oxidation and without its release, protein oxidation is minimal. Any protein oxidation that does occur is via the presence of reactive oxygen species and these products are typically used to induce lipid oxidation.

Although there were no significant differences amongst the treatments within a stage for heme-iron concentration, a positive Pearson's correlation of heme-iron with lipid oxidation (blesbok 0.99 and springbok 0.94 after storage) was noted.

Descriptive sensory analysis was used to analyse the droëwors samples for aroma, flavour, appearance and texture attributes after drying. Many consumers do not like game meat due to its very distinct aroma and flavour (Hoffman *et al.,* 2005) and with the addition of increasing RBTE, the game flavour and aroma was detected at decreasing levels. Game meat flavours and aromas scores were higher for the blesbok droëwors than the springbok droëwors. Differences in the blesbok and springbok meat in terms of gender, vegetation differences and even time of death can influence its flavour profile (Hoffman *et al.,* 2004). The fatty aroma was also influenced by the added RBTE in the same manner, such that with increasing RBTE, there was a decrease in this attribute. The appearance of the droëwors, as expected, showed no difference amongst the droëwors samples with/without added RBTE.

It would seem as if the addition of RBTE could be acceptable to consumers as the trained panel received the droëwors with added RBTE positively and that the rooibos extract could be a positive attribute as it minimises the gamey aroma and flavour as well as the fatty mouth feel of the droëwors.

CONCLUSION

The results obtained from this study indicated that the addition of RBTE to blesbok and springbok resulted in the slowing down of lipid oxidation when added at a concentration of RBTE 1.0%. The results in this study could be said to be dependent on the droëwors formulation used and drying parameters and due to addition of beef fat which has a more favourable fatty acid profile as opposed to pork back fat or sheep fat. Significant differences between the treatments within a stage are clearly seen. Heme-iron results showed an increase after drying over time which corresponds with the increasing lipid oxidation results after drying over time. The level of lipid oxidation significantly decreases with increasing concentration of RBTE. No effect on protein oxidation at any RBTE concentration was detected. Research has indicated that the addition of antioxidants to inhibit lipid oxidation will not necessarily inhibit protein oxidation. From these results it can be postulated that RBTE 1.0% would be effective in slowing down oxidation when using this together with beef meat and fat in the formulation of a game species droëwors. The fatty acid profile had high concentrations of oleic acid, palmitic acid, linoleic acid and stearic acid in both the raw and dry droëwors samples. The high polyunsaturated fatty acids should result in a higher oxidative activity as noted. Further research could be conducted on the addition of RBTE in combination with other natural antioxidants on meat products as this might prove more effective with improved results.

Sensory attribute differences were observed among the different treatments. The main attributes observed whilst tasting both species droëwors were distinctive rooibos and game aromas and flavours. The rooibos aroma and flavour increased with increasing concentrations of RBTE whilst the game and fatty aroma and flavour decreased. Rancidity did not appear to be detected during the sensory analyses. The addition of RBTE to the droëwors was noted to be a positive attribute among the panel in terms of the addition of RBTE due to no rancidity being detected, but also the fact that the RBTE lowered the game meat flavour.

No rancidity was detected by the sensory panel but high TBARS were measured when analysing the occurance of lipid oxidation. When using natural antioxidants it is important to evaluate both the sensory profile and oxidation products being formed. As there is no research on the correlation between sensory profiling and oxidation analyses in game meat products, this therefore warrants further research.

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ADDENDUM A

Fatty acid composition (%) ± SD of blesbok droëwors produced with increasing rooibos tea extract levels on a wet weight basis.

	Blesbok meat	Beef fat	Week 0 (Raw)	Week 0 (Dried)				Week 2 (Dried)	
			RBTE 0%	RBTE 0%	RBTE 0.25%	RBTE 0.50%	RBTE 1.0%	RBTE 0%	RBTE 1.0%
Saturated fatty acids									
C14:0	0.8	2.3	1.6 ± 0.22	0.7 ± 0.033	0.9 ± 0.063	0.9 ± 0.10	0.9 ± 0.068	0.8 ± 0090	0.7 ± 0.070
C15:0	1.0	0.8	0.6 ± 0.074	0.1 ± 0.0072	0.2 ± 0.011	0.2 ± 0.014	0.1 ± 0.0078	0.1 ± 0.0084	0.1 ± 0.0048
C16:0	19.6	29.5	29.3 ± 7.52	30.0 ± 1.19	30.7 ± 5.58	31.4 ± 2.65	30.3 ± 0.34	30.2 ± 2.26	30.6 ± 0.59
C18:0	23.8	15.4	25.0 ± 6.37	26.2 ± 1.53	30.9 ± 3.25	24.1 ± 1.27	26.9 ± 2.66	26.9 ± 1.82	26.0 ± 1.40
C20:0	0.4	0.2	0.2 ± 0.074	0.03 ± 0.013	0.04 ± 0.025	0.04 ± 0.012	0.05 ± 0.021	0.04 ± 0.0094	0.04 ± 0.012
C21:0	0.6	0.1	0.1 ± 0.0053	0.03 ± 0.0045	0.03 ± 0.021	0.03 ± 0.0086	0.03 ± 0.0073	0.02 ± 0.0070	0.05 ± 0.026
C22:0	0.1	0.3	0.3 ± 0.053	0.06 ± 0.024	0.1 ± 0.016	0.1 ± 0.0058	0.1 ± 0.011	0.09 ± 0.0085	0.1 ± 0.12
Monounsaturated fatty acids									
C14:1	0.1	0.2	0.2 ± 0.024	0.1 ± 0.0056	0.1 ± 0.0094	0.1 ± 0.016	0.1 ± 0.0087	0.1. ± 0.015	0.1 ± 0.013
C15:1	0.1	0.2	0.2 ± 0.014	0.06 ± 0.0048	0.08 ± 0.0052	0.08 ± 0.0065	0.07 ± 0.0041	0.06 ± 0.0034	0.06 ± 0.0045
C16:1	0.7	2.1	1.0 ± 0.13	0.8 ± 0.019	0.6 ± 0.039	0.7 ± 0.081	0.6 ± 0.032	0.5 ± 0.026	0.6 ± 0.017
C18:1n9t	0.9	0.4	0.6 ± 0.17	0.1 ± 0.019	0.2 ± 0.019	0.2 ± 0.038	0.2 ± 0.016	0.2 ± 0.023	0.2 ± 0.029
C18:1n9c	17.0	39.7	32.2 ± 0.54	40.5 ± 0.92	34.0 ± 6.81	40.0 ± 2.14	38.6 ± 2.49	39.1 ± 1.69	39.7 ± 0.98
C20:1	0.1	0.1	0.1 ± 0.016	0.02 ± 0.0012	0.04 ± 0.0037	0.03 ± 0.0019	0.03 ± 0.0017	0.03 ± 0.0015	0.03 ± 0.0041
C22:1n9	0.1	1.0	0.05 ± 0.012	0.02 ± 0.0096	0.01 ± 0.0025	0.01 ± 0.0040	0.02 ± 0.0057	0.01 ± 0.0076	0.01 ± 0.0014
C24:1	0.2	0.1	0.04 ± 0.021	0.08 ± 0.0014	0.08 ± 0.0027	0.05 ± 0.0023	0.07 ± 0.091	0.03 ± 0.0036	0.03 ± 0.0047

	Blesbok meat	Beef fat	Week 0 (Raw)	Week 0 (Dried)				Week 2 (Dried)	
			RBTE 0%	RBTE 0%	RBTE 0.25%	RBTE 0.50%	RBTE 1.0%	RBTE 0%	RBTE 1.0%
Polyunsaturated fatty acids									
C18:2n6t	1.6	1.2	0.6 ± 0.35	0.06 ± 0.0060	0.08 ± 0.0067	0.08 ± 0.0076	0.06 ± 0.0045	0.06 ± 0.0025	0.06 ± 0.00071
C18:2n6c	20.3	1.4	4.4 ± 0.36	0.7 ± 0.052	1.2 ± 0.13	1.1 ± 0.047	1.1 ± 0.74	1.1 ± 0.069	0.9 ± 0.021
C18:3n6	0.06	0.4	1.2 ± 0.16	0.1 ± 0.013	0.3 ± 0.036	0.2 ± 0.019	0.3 ± 0.021	0.3 ± 0.019	0.2 ± 0.014
C18:3n3	4.8	0.8	0.1 ± 0.010	0.04 ± 0.0034	0.05 ± 0.0040	0.05 ± 0.0011	0.04 ± 0.0017	0.05 ± 0.0039	0.05 ± 0.016
C20:4n6	2.0	1.0	0.1 ± 0.035	0.01 ± 0.0035	0.03 ± 0.012	0.04 ± 0.0065	0.03 ± 0.0098	0.03 ± 0.010	0.02 ± 0.012
C20:5n3	2.3	0.3	0.1 ± 0.0051	0.03 ± 0.0083	0.04 ± 0.012	0.07 ± 0.048	0.05 ± 0.013	0.07 ± 0.0099	0.06 ± 0.030
C22:2	1.2	0.05	0.09 ± 0.0029	0.02 ± 0.0040	0.03 ± 0.0082	0.04 ± 0.011	0.03 ± 0.011	0.02 ± 0.0084	0.02 ± 0.00080
C22:5n3	1.0	0.6	0.4 ± 0.037	0.04 ± 0.0090	0.09 ± 0.014	0.08 ± 0.0048	0.08 ± 0.0084	0.08 ± 0.0062	0.05 ± 0.0030
C22:6n3	0.5	0.2	0.1 ± 0.031	0.02 ± 0.0058	0.02 ± 0.0081	0.03 ± 0.011	0.03 ± 0.010	0.02 ± 0.0080	0.02 ± 0.0094
Total	100	100	100	100	100	100	100	100	100
Total fatty acids profile									
SFA	46.3	59.1	57.1 ± 1.24	57.1 ± 0.97	62.7 ± 6.97	56.8 ± 2.19	58.45 ± 2.57	58.1 ± 1.85	57.7 ± 1.07
MUFA	17.5	37.0	34.4 ± 0.58	41.7 ± 0.91	35.1 ± 6.85	41.2 ± 2.15	39.7 ± 2.46	40.1 ± 1.73	40.7 ± 1.02
PUFA	39.4	1.7	8.5 ± 0.93	1.2 ± 0.096	2.2 ± 0.25	2.0 ± 0.10	1.9 ± 0.15	1.9 ± 0.14	1.5 ± 0.095
PUFA:SFA	0.9	0.03	0.2 ± 0.019	0.02 ± 0.0010	0.03 ± 0.0052	0.04 ± 0.0055	0.03 ± 0.0041	0.03 ± 0.0052	0.03 ± 0.0052
(n-6)/(n-3)	3.8	2.5	9.2 ± 0.14	7.3 ± 1.25	8.5 ± 0.57	6.9 ± 1.38	6.7 ± 0.57	6.7 ± 0.59	5.9 ± 1.00

SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; (n-6)/(n-3) = (n-6)/(n-3) PUFA ratio

Fatty acid composition (%) ± SD of springbok droëwors produced with increasing rooibos tea extract levels on a wet weight basis.

	Springbok meat	Beef fat	Week 0 (Raw)	Week 0 (Dried)				Week 2 (Dried)	
			RBTE 0%	RBTE 0%	RBTE 0.25%	RBTE 0.50%	RBTE 1.0%	RBTE 0%	RBTE 1.0%
Saturated fatty acids									
C14:0	0.8	2.3	1.5 ± 0.87	1.0 ± 0.10	0.8 ± 0.061	0.9 ± 0.13	1.3 ± 0.29	0.8 ± 0.074	0.8 ± 0.068
C15:0	1.0	0.8	0.4 ± 0.22	0.2 ± 0.013	0.1 ± 0.0051	0.1 ± 0.016	0.2 ± 0.048	0.1 ± 0.0081	0.1 ± 0.0069
C16:0	15.1	29.5	23.3 ± 5.61	34.0 ± 2.81	34.3 ± 1.71	37.2 ± 2.97	36.2 ± 5.90	33.4 ± 2.13	31.6 ± 1.15
C18:0	27.0	15.4	23.0 ± 5.45	20.3 ± 3.74	17.5 ± 2.88	17.3 ± 5.07	18.3 ± 6.73	17.6 ± 1.26	22.8 ± 0.98
C20:0	0.5	0.2	0.1 ± 0.044	0.03 ± 0.022	0.04 ± 0.023	0.04 ± 0.017	0.05 ± 0.020	0.1 ± 0.18	0.04 ± 0.011
C21:0	0.6	0.1	0.1 ± 0.045	0.04 ± 0.013	0.04 ± 0.0030	0.04 ± 0.013	0.04 ± 0.0070	0.2 ± 0.29	0.03 ± 0.0072
C22:0	0.08	0.3	0.1 ± 0.071	0.1 ± 0.015	0.07 ± 0.0029	0.08 ± 0.0067	0.1 ± 0.041	0.5 ± 0.84	0.08 ± 0.0055
Monounsaturated fatty acids									
C14:1	0.1	0.2	0.1 ± 0.058	0.08 ± 0.0073	0.2 ± 0.010	0.2 ± 0.031	0.1 ± 0.033	0.2 ± 0.016	0.1 ± 0.013
C15:1	0.1	0.2	0.3 ± 0.20	0.09 ± 0.0073	0.07 ± 0.0042	0.07 ± 0.0059	0.1 ± 0.028	0.08 ± 0.010	0.06 ± 0.0040
C16:1	0.9	2.1	0.9 ± 0.55	0.7 ± 0.043	0.9 ± 0.10	1.0 ± 0.076	0.8 ± 0.21	0.8 ± 0.037	0.7 ± 0.030
C18:1n9t	0.5	0.4	0.3 ± 0.17	0.2 ± 0.015	0.09 ± 0.014	0.1 ± 0.011	0.3 ± 0.11	0.2 ± 0.056	0.1 ± 0.014
C18:1n9c	18.7	39.7	44.7 ± 11.73	41.3 ± 2.31	44.6 ± 2.26	47.6 ± 3.87	29.5 ± 12.3	43.8 ± 2.41	42.0 ± 0.68
C20:1	0.1	0.1	0.1 ± 0.035	0.04 ± 0.0037	0.02 ± 0.00049	0.02 ± 0.0018	0.05 ± 0.013	0.07 ± 0.11	0.02 ± 0.0093
C22:1n9	0.1	1.0	0.03 ± 0.0016	0.02 ± 0.0097	0.01 ± 0.0017	0.01 ± 0.0034	0.02 ± 0.0088	0.02 ± 0.024	0.01 ± 0.0037
C24:1	0.2	0.1	0.03 ± 0.0021	0.01 ± 0.0069	0.02 ± 0.0058	0.03 ± 0.013	0.02 ± 0.0047	0.03 ± 0.0065	0.04 ± 0.0022

	Springbok meat	Beef fat	Week 0 (Raw)	Week 0 (Dried)				Week 2 (Dried)	
			RBTE 0%	RBTE 0%	RBTE 0.25%	RBTE 0.50%	RBTE 1.0%	RBTE 0%	RBTE 1.0%
Polyunsaturated fatty acids									
C18:2n6t	1.4	1.2	0.3 ± 0.16	0.07 ± 0.0071	0.06 ± 0.00085	0.06 ± 0.0025	0.1 ± 0.029	0.08 ± 0.050	0.06 ± 0.0031
C18:2n6c	19.2	1.4	2.7 ± 1.47	1.1 ± 0.12	0.7 ± 0.026	0.8 ± 0.046	1.6 ± 0.51	0.7 ± 0.029	0.8 ± 0.032
C18:3n6	0.11	0.4	0.9 ± 0.50	0.2 ± 0.018	0.1 ± 0.0075	0.2 ± 0.0084	0.3 ± 0.11	0.3 ± 0.40	0.2 ± 0.011
C18:3n3	3.5	0.8	0.1 ± 0.062	0.05 ± 0.0043	0.05 ± 0.0017	0.05 ± 0.0037	0.07 ± 0.037	0.1 ± 0.13	0.05 ± 0.0025
C20:4n6	2.6	1.0	0.04 ± 0.021	0.02 ± 0.010	0.02 ± 0.0043	0.02 ± 0.0057	0.03 ± 0.010	0.03 ± 0.013	0.02 ± 0.0036
C20:5n3	2.3	0.3	0.06 ± 0.038	0.05 ± 0.015	0.04 ± 0.0052	0.04 ± 0.0040	0.06 ± 0.021	0.08 ± 0.10	0.05 ± 0.0064
C22:2	1.2	0.05	0.05 ± 0.039	0.02 ± 0.0073	0.02 ± 0.0091	0.02 ± 0.0063	0.03 ± 0.011	0.01 ± 0.0047	0.03 ± 0.0080
C22:5n3	1.0	0.6	0.06 ± 0.038	0.08 ± 0.015	0.05 ± 0.0054	0.06 ± 0.0039	0.1 ± 0.052	0.2 ± 0.38	0.06 ± 0.0024
C22:6n3	0.5	0.2	0.07 ± 0.048	0.03 ± 0.018	0.02 ± 0.011	0.02 ± 0.0084	0.04 ± 0.016	0.4 ± 0.94	0.02 ± 0.0084
Total	100	100	100	100	100	100	100	100	100
Total fatty acids profile									
SFA	41.7	59.1	48.7 ± 10.81	55.7 ± 2.55	52.9 ± 2.31	49.7 ± 4.01	66.2 ± 11.29	52.7 ± 0.79	55.5 ± 0.64
MUFA	19.2	39.0	46.4 ± 11.48	42.4 ± 2.37	45.9 ± 2.33	49.0 ± 3.96	31.0 ± 12.01	45.2 ± 2.25	43.1 ± 0.65
PUFA	37.9	1.7	4.9 ± 2.74	1.9 ± 0.22	1.2 ± 0.039	1.4 ± 0.069	2.8 ± 0.88	2.1 ± 2.20	1.4 ± 0.040
PUFA:SFA	0.9	0.03	0.1 ± 0.055	0.04 ± 0.0055	0.02 ± 0.0020	0.03 ± 0.0041	0.04 ± 0.0082	0.04 ± 0.045	0.03 ± 0.052
(n-6)/(n-3)	2.4	2.5	7.7 ± 0.18	7.2 ± 0.67	5.6 ± 0.49	5.7 ± 0.24	7.0 ± 0.59	4.6 ± 2.13	5.57 ± 0.31

SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; (n-6)/(n-3) = (n-6)/(n-3) PUFA ratio

CHAPTER 7

General conclusions and recommendations

The full study was conducted on a variety of meat sources, namely ostrich (*Struthio camelus*), blesbok (*Damaliscus pygargus phillipsi*), springbok (*Antidorcas marsupialis*) and fallow deer (*Dama dama*) meat. Droëwors was produced with added rooibos tea extract (RBTE) to analyse its effect to inhibit lipid and protein oxidation.

The initial trial was conducted on ostrich meat. These results showed that the RBTE did not make a significant impact to the inhibition of oxidation of both lipid and protein. This was concluded to be due to the long drying period of 15 days and the use of pork back fat which is very high in polyunsaturated fatty acids and becomes rancid more quickly than other animal fats.

The following chapter focused on three different game species, namely blesbok, springbok and fallow deer. As the ostrich meat trial showed no significant differences amongst the treatments after drying, other meat sources that are high in iron and polyunsaturated fatty acids were sourced. The formulation of the droëwors was changed slightly, using sheep fat instead of pork fat and altering the meat to fat ratios. These results proved that the added RBTE still did not give any significant differences across treatments after drying for the lipid oxidation results but a significant difference in the heme-iron results indicate that RBTE 0.25% had the lowest concentration of heme-iron after drying. Heme-iron is known to be a pro-oxdidant. A RBTE concentration of 0.25% can therefore be said to give the best results as the correlations between lipid oxidation and heme-iron were high. The addition of RBTE 1.0% resulted in an increase in oxidation which could be due to its pro-oxidant effect. The results suggested that with improving and optimising the droëwors formulation, the addition of RBTE could possibly have a significant effect. It was also decided that it was important to test oxidation not only after drying but after a short storage period of two weeks. However, before the final trial were to ensue, the formulation needed to be optimised.

Pork back fat and sheep fat were added to the formulation of ostrich droëwors and game droëwors, respectively. The amount of fat used in the formulation of the droëwors changed as the research progressed. Pork back fat and sheep fat both proved to be unsuitable for the droëwors. The pork back fat is more unsaturated than other animal fats and is known to become rancid more rapidly than other fats. Therefore in the initial study this was used to see if RBTE would help to extend the shelf-life of droëwors and to see whether the pork back fat stayed “fresh” for longer. Sheep fat was used for the next trial as its fatty acid profile has a more favourable (slightly lower) unsaturated fatty acid profile. However,

sheep fat left an unpleasant smell after drying which would not be appealing to consumers. After a formulation trial utilising different meat:fat ratios was conducted, it was decided that beef fat would be used in the formulation and this resulted in the RBTE having a significant effect on the oxidation of the droëwors. This could be due to the smaller percentage of total polyunsaturated fatty acids found in the beef fat. It also proved to be successful in the sensory study that was conducted. The results of this research study show that by using an optimised formulation (with lower fat levels) and drying parameters, that the addition of RBTE had a significant effect on the droëwors oxidative stability both after drying and storage. This was seen with the addition of RBTE 1.0% in terms of lipid oxidation. Previous results in this study had actually shown that RBTE 1.0% resulted in the highest lipid oxidation results, which were concluded to be due to the pro-oxidant effect of RBTE when added at too high concentrations within a specific droëwors formulation.

Consumers tend to store droëwors before consumption and therefore a two week storage period was included in this study. The added RBTE was effective in inhibiting lipid oxidation after the 2-week storage period. It could, however, be speculated that by testing oxidation after a longer storage period, the RBTE could prove to be even more effective in further slowing down lipid oxidation. Therefore it would be recommended that a longer shelf-life study be conducted.

Sensory attribute differences were observed among the different treatments when analysed by a trained descriptive sensory panel for both the ostrich droëwors trial and the game (springbok and blesbok) droëwors trials. The main attributes observed were distinctive rooibos, game and fatty aromas and flavours. With increasing concentrations of added RBTE, the panel noted increasing rooibos aromas and flavours and decreasing game and fatty aromas and flavours. The distinctive rooibos flavour and aroma in the droëwors were regarded as positive attributes by the trained panel. An informal conclusion can be made that a consumer would respond positively to the added RBTE to game droëwors due to the sensory profile that was developed though this aspect warrants further research.

When using natural antioxidants it is important to evaluate both the sensory profile and oxidation products present, however, the human palate will not always pick up rancidity, especially at the onset of rancidity when rancid aroma and flavour notes are present at extremely low intensities. This was seen in the results as no rancidity was detected by the sensory panel but high TBARS were measured when analysing lipid oxidation. As mentioned, there is no research on the correlation between sensory profiling and oxidation analyses in game meat products and therefore this warrants further research. This research would be beneficial to the food industry as by using natural antioxidants in meat products could prolong a rancid flavour by inhibition of lipid oxidation. Research is also needed to

determine the level of TBARS in game meat products that would be detected by a trained descriptive sensory panel.

It can be concluded that the analysis of the composition of the RBTE used is important, as when using different batches of RBTE, there were varying results in terms of which concentration was most effective. Therefore it would be suggested that the concentrations of the flavonoids in RBTE, especially aspalathin and quercetin, be studied further, primarily to determine an effective RBTE dosage level.

A formulation with the optimised meat:fat ratio and the use of beef fat in the droëwors production should be used as this influences the antioxidant action of the added RBTE. In this study it was seen that the batch of RBTE with lower concentrations of aspalathin and quercetin resulted in inhibition of lipid oxidative. The addition of RBTE could therefore be perceived as a positive concept by consumers and could be a valuable addition to the South African meat industry. Based on this research, it would also be beneficial to investigate the combination of RBTE with other natural antioxidants in dried processed meat products.