Moringa oleifera leaf powder as a functional antioxidant additive in pork droëwors

By

Felicitas Esnart MUKUMBO

Submitted in fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY IN ANIMAL SCIENCE

In the

Department of Livestock and Pasture Science

Faculty of Science and Agriculture

University of Fort Hare

Alice, South Africa

Main supervisor: Prof. V. Muchenje

Co supervisors: Dr E. Arnaud, Dr A. Descalzo, Prof. L. Hoffman, Prof. A. Collignan

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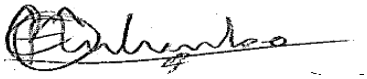
Declaration

I, Felicitas Esnart Mukumbo, vow that this dissertation has not been submitted to any University and that it is my original work conducted under the supervision of Prof. V. Muchenje. All assistance towards the production of this work and all references contained herein have been duly accredited.



13th April 2017

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13th April 2017

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Prof. V. Muchenje (Main-supervisor) Date

December 2016

Abstract

***Moringa oleifera* leaf powder as a functional antioxidant additive in pork droëwors**

By

Felicitas Esnart Mukumbo

The study investigated the effect of *Moringa oleifera* leaf powder (MLP) on physico-chemical characteristics, antioxidant activity, antioxidant compound content and lipid oxidation in pork droëwors. Firstly, the physico-chemical properties (proximate composition, salt content, water activity (a_w), pH) of commercially produced droëwors from different types of meat (beef, game, ostrich) were determined. In the second experiment beef and pork droëwors with similar fat content were produced. Physico-chemical properties and lipid oxidation (thiobarbituric reactive substances (TBARS)) during processing and 26 days of storage at 25 °C and 50% relative humidity (RH) were measured. In the third experiment, antioxidant compounds (Total Phenolic Compounds (TPC), *α*-tocopherol, *β*-carotene) in MLP were quantified. Thereafter, 4 treatments of pork droëwors were produced, with MLP included at 0, 0.5, 1 and 2 g/ 100 g. Physico-chemical properties and TBARS were measured at intervals during drying (0, 1.5, 5.75, 27.25, 72 h) and after 7 days of storage under ambient conditions. Antioxidant activity (ferric reducing antioxidant power (FRAP)), TBARS, *α*- and γ-tocopherol, and *β*-carotene contents were measured. In the fourth experiment, three batches of droëwors were produced (C: no antioxidant, M0.75: 0.75 g/ 100 g MLP, VE: 15 mg/ kg *α*-tocopherol oil) and stored at 25 °C and 50% relative humidity for 112 days. Drying kinetics and *α*-tocopherol contents of pork droëwors after drying were measured and the physico-chemical properties and TBARS were followed during storage. Results showed no differences ($P > 0.05$) in the physico-chemical characteristics of beef, game meat and ostrich droëwors; containing on average 25.8 ± 1.25 g/100 g moisture, 42.0 ± 0.10 g/100 g protein,

32.0 ± 1.68 g/100 g fat, 6.2 ± 0.13 g/100 g ash and 4.2 ± 0.10 g/100 g salt; with a_w and pH of 0.79 ± 0.015 and 5.3 ± 0.05, respectively. During processing and storage, TBARS were higher ($P < 0.05$) in pork droëwors (maximum 3.83 mg MDA/kg DM) than in beef (maximum 0.99 mg MDA/kg DM). *Moringa oleifera* leaf powder contained high levels of TPC (7.5 ± 0.2 mg gallic acid eq/g) and substantial levels of *α*-tocopherol (76.7 ± 1.9 mg/100 g) and *β*-carotene (23.2 ± 2.8 mg/100 g). The FRAP, *α*-tocopherol and *β*-carotene content of pork droëwors increased ($P < 0.05$) proportionally with increasing levels of MLP inclusion. Lipid oxidation occurred more rapidly ($P < 0.05$) when MLP was not added and was similar ($P > 0.05$) for all MLP treatments. There was no significant effect of the inclusion of 0.75 g/100 g MLP on the drying curves and physico-chemical characteristics of the droëwors. The *α*-tocopherol content was higher ($P < 0.05$) and TBARS during storage were lower ($P > 0.05$) with MLP addition. The results of the current study give an overview of the composition of commercial droëwors and showed higher susceptibility to lipid oxidation in pork droëwors. *Moringa oleifera* leaf powder exhibited antioxidant activity in pork droëwors, inhibited lipid oxidation and increased the content of *α*-tocopherol in the product. It can be concluded that MLP could be used as a functional antioxidant additive in pork droëwors.

Acknowledgments

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Abbreviations

AA	Antioxidant activity
a_w	Water activity
DM	Dry matter
FRAP	Ferric reducing antioxidant power
GC	Gas chromatography
HPLC	High performance liquid chromatography
LC-MS	Liquid chromatographic mass spectrophotometer
MDA	Malonaldehyde
MLP	*Moringa oleifera* leaf powder
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acids
PV	Peroxide value
RH	Relative humidity
ROS	Reactive oxygen species
SFA	Saturated fatty acid
TBA	Thiobarbuturic acid
TBARS	Thiobarbituric acid reacyive substances
TPC	Total Phenolic Content

VE	Vitamin E

Chapter 1. Introduction

1.1 Background

Feeding the world's rapidly growing population is a growing challenge, as exponential population growth puts an increasing strain on natural resources for food production and on food security (Godfray *et al.*, 2010). The challenge of food insecurity is widespread; with an estimated one in nine people worldwide lacking access sufficient food to maintain a healthy and active lifestyle (FAO, IFAD and WFP, 2014). In the developed and developing world alike, the challenge of maintaining a healthy and balanced diet has led to increased incidences of micronutrient deficiencies and chronic illnesses (Muchenje and Mukumbo, 2015). Meat production and processing has an important part to play in providing high-quality protein to consumers and regular income to producers. South Africa is known for being a nation of fervent meat eaters, ranking 11th in the world's top 15 meat eating countries (Muchenje and Njisane, 2015). The steady rise in the nation's meat consumption levels in the last 2 decades has been attributed to increments in the average income (Euromonitor, 2014). Generally, developing countries with emerging economies have in the last 20 years experienced an emphatic surge in the consumption of meat; especially poultry and pork meat (Sans and Combris, 2015).

Around 50% of pork produced in South Africa is sold fresh; while the other 50% goes for further processing (DAFF, 2012). A wide variety of processed meat products can be produced from pork. Meat processing is beneficial in that it improves product appearance, convenience, enhances food safety and improves product storage capability by inhibiting deterioration (Earle and Earle, 2008). Drying is amongst the earliest processing methods used for food preservation. Processing meat by drying prolongs its shelf life by reducing osmotic potential; hence preventing

microbial growth. Droëwors are shelf-stable ready-to eat dried sausages commonly made from beef, game or ostrich meat; developed in South Africa and fast becoming popular in many countries. Droëwors are produced from a mixture of ground meat and animal fat or trimmings, salted and spiced and stuffed into casings before being dried. The process of drying reduces the susceptibility of droëwors to microbial spoilage. However, the added animal fat results in susceptibility to spoilage from biochemical processes such as lipid oxidation.

One of the main causes of quality deterioration in muscle foods is oxidation, which is characterised by discolouration, the development of toxic compounds, off-flavours and nutrient losses (Descalzo *et al.*, 2005). Oxidative stability refers to the susceptibility of meat lipids to oxidation and is majorly influenced by the degree of saturation of meat lipids and is an important aspect that influences meat shelf life and consumer acceptability (Wood *et al*., 2003). In order to minimise the rate of lipid oxidation in processed meat products, antioxidants are incorporated during processing. Antioxidants are substances that are able to significantly prevent or delay oxidation in a substrate when added at low concentrations (Halliwell and Gutteridge, 1995). Currently, the majority of antioxidants used in meat processing are synthetic. However, there are natural sources of antioxidants; including micronutrients such as vitamin E, vitamin C and selenium; and plant phytochemicals such a polyphenols and flavonoids (Falowo *et al.*, 2014). *Moringa oleifera* Lam is a small, fast growing tree originally from Asia but now widely grown in tropic and sub-tropic areas worldwide. Studies have shown *Moringa oleifera* Lam to be of immense nutritional value, having high levels of crude protein, vitamins, amino acids, minerals and fatty acids, including notably high levels of *n*-3 (α-linolenic acid); as well as antioxidant properties (Verma *et al.*, 1999; Moyo *et al.*, 2011; Moyo *et al.*, 2012). Due to its nutritional and

functional properties, *Moringa oleifera* has the potential to be a valuable resource for meat processing.

1.2 Problem statement

In the last few decades, selection for leaner carcasses in pig breeding programmes has resulted in significant changes in the quality and composition of pork (Gous, 2000). A 2006 study by the USDA found that common cuts of fresh pork contain on average 16% less total fat and 27% less saturated fat than they did 15 years ago (USDA, 2009). The production of leaner pork carcasses has turned the fatty acid profile of pork towards being more unsaturated; which is as beneficial to consumers in terms of reduced levels of SFA but presents a challenge for pork processors (Wood *et al.*, 2003; Hugo and Roodt, 2015). Fatty acids with a higher degree of unsaturation are softer in consistency and have a lower melting point; producing products that are soft, inferior in quality, more susceptible to oxidation and less shelf stable (Warnants *et al.*, 1999). Increasing the PUFA profile of pork makes it more susceptible to oxidation which can lead to rancidity, off flavours, colour deterioration, reduced shelf life, inferior consistency, and economic losses during processing (Hugo and Roodt, 2007).

Minced processed pork products are even more highly susceptible to oxidative deterioration. Meat processing techniques alter the physical properties and appearance of meat and disrupt the chemical integrity of the muscle cells (Warriss, 2010). The processing methods used in droëwors production include grinding or mincing the fresh meat to produce sausages and drying at relatively low temperatures (lower than 30°C) for 1 - 2 weeks under ambient conditions or for 2 - 3 days when dryers are used. Ground meat is especially susceptible to oxidation due to the incorporation of oxygen during grinding. This increases the rate and extent product deterioration

due to rancidity, the formation of off flavours and colour deterioration, resulting in a relatively short product shelf-life and limiting their potential value (Falowo *et al.*, 2016). Furthermore, droëwors is a fat enriched product, containing from 10 - 40 % fat in the finished product. The loss of moisture during drying concentrates the amount of fat in the finished product which can also increase its susceptibility to oxidative deterioration (Jones, 2013).

The use of synthetic antioxidants in pork products to delay lipid oxidation has been implicated with toxicity and carcinogenic effects on human health (Falowo *et al.*, 2014). Consequently there has been an increase in restrictions on what substances can be used as antioxidants because consumers are concerned about the nature of additives used, particularly those of synthetic origin (Troy and Kerry, 2010). Increasing consumer awareness and health-consciousness have resulted in pressure to avoid the use of synthetic additives in meat products. A distinct feature of today's modern consumers is that they are increasingly critical of the food they eat. As a result, there is a growing shift in the preference for natural food additives over synthetic. There is therefore an urgent need for natural alternatives to replace the synthetic antioxidants and additives. Research has shown that *Moringa oleifera* leaves possess strong antioxidant properties. However, no research has yet been done to determine the most effective way in which this plant may be used an antioxidant for meat products; hence its practical application by industry has been limited.

1.3 Justification

Pork is not typically used for droëwors production because of its susceptibility to oxidation. However, research on the use of a suitable natural antioxidant could minimise the extent of oxidative deterioration in dried pork products and open up a new and potentially viable market for pork droëwors. Consumer demand for convenient food products with new and exciting

flavours has been reported and innovation is critical for continual success in the meat industry (Barbut, 2015). Oyewumi and Jooste (2006) reported a preference for value added products in South African pork consuming-households and recommended that product research and innovation be a core imperative. This study will investigate a potential natural alternative to synthetic antioxidants, which could be used to enhance the oxidative stability of processed meat and the functional value. This will aid in responding to consumer demands for a shift towards natural antioxidant additives. Suitable natural alternatives to replace synthetic additives are currently sought after by many meat processors (Jayawardana *et al.*, 2015). Research has shown that *Moringa oleifera* leaves possess strong antioxidant properties (Verma *et al.*, 1999; Moyo *et al.*, 2012). Numerous studies have reported that supplementation of poultry and livestock feed with *Moringa oleifera* leaves resulted in improved oxidative stability and shelf life in the meat (Qwele *et al.*, 2013; Wapi *et al.*, 2013; Mukumbo *et al.*, 2014; Nkukwana *et al.*, 2014). However, limited research has been conducted on it's use as an antioxidant additive in meat processing (Jayawardana *et al.*, 2015), hence its practical application by the meat processing industry has been limited.

Additionally, droëwors is a product increasing in international popularity. However, there is limited information available on the reaction kinetics such as the rate of oxidative deterioration during processing and storage, its nutritional and physicochemical properties. Production and processing methods have a significant effect on the safety and nutritional value of meat products (De Smet and Vossen, 2016). Measuring physical, chemical, biological and nutritional changes during processing generates quantitative data that can be used to develop adequate models for practical processing purpose (Earle and Earle, 2008).

1.4 Aim and Objectives

The aim of this study is to evaluate the efficacy of *Moringa oleifera* leaf meal as a natural antioxidant in pork droëwors and the resultant implications on the product quality and functionality. The specific objectives of the study are to:

1. Determine the composition and physico-chemical characteristics of typical droëwors available in the South African market made from commonly used meat types.
2. Determine whether differences exist in the rate of lipid oxidation in pork droëwors and conventional beef droëwors.
3. Determine the antioxidant components (total phenolic compounds, carotenoids, tocopherols) of *Moringa oleifera* leaf powder (MLP) and the effect of varying levels on antioxidant activity, lipid oxidation, α-tocopherol and β-carotene content of pork droëwors.
4. Determine the effect of incorporating MLP in pork droëwors on drying kinetics, physico-chemical properties, α-tocopherol content and lipid oxidation of pork droëwors during processing and prolonged storage.

1.5 Hypotheses

The null hypotheses to be tested are:

$H_0$1 – There are no differences in physico-chemical characteristics of droëwors made from different commonly used meat types

$H_0$2 – There are no differences in lipid oxidation of beef and pork droëwors

$H_0$3 - *Moringa oleifera* leaf powder has no antioxidant properties and has no effect on the antioxidant activity, lipid oxidation, α-tocopherol and β-carotene content in pork droëwors

$H_0$4 – The inclusion of *Moringa oleifera* leaf powder in pork droëwors has no effect on physico-chemical properties, α-tocopherol content and lipid oxidation in pork droëwors during prolonged storage

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Chapter 2. Literature review

2.1 Introduction

Meat and meat products hold a prominent position in human diets as a rich source of high quality protein, essential amino acids, B vitamins and minerals (Zhang *et al.*, 2010; De Smet and Vossen, 2016). Meat supplies all the essential amino acids (histidine, threonine, valine, methionine, lysine, isoleucine, leucine and phenylalanine) which must be obtained by consumption because they cannot be synthesized in the human body (Pereira and Vicente, 2013; Domìnguez *et al.*, 2015). Regular meat consumption makes a valuable contribution towards meeting daily nutritional requirements for growth and maintenance of the human body (Hambidge *et al.*, 2011).

According to the FAO (2003), the world food economy has been characterised by a dietary shift towards the increased consumption of meat and livestock products. Exponential human population growth over the last few decades has caused a significant rise in the demand, production and consumption of meat (Rosegrant *et al.*, 2001); accompanied by rising expectations, concerns and demands from consumers concerning meat quality and safety. Although preferences tend to differ quite significantly amongst consumers from different socio-demographic backgrounds (Webb and O'Neill, 2008), meat quality aspects that are generally of importance include appearance, taste and nutritional value. In South Africa, product price is also a primary factor affecting the meat purchasing decisions of consumers (Rani *et al.*, 2013). Non-economic factors including meat quality and product consistency are also of importance to South African consumers; along with health related, nutritional and food safety concerns (Oyewumi and Jooste, 2006). In line with these demands, meat processing aims at enhancing product quality and safety.

2.2 Meat processing

Fresh meat is a rich nutrient and moisture matrix ideal for the growth and propagation of microorganisms, hence it is highly perishable and prone to spoilage (Zhou *et al.*, 2010). The primary aims of meat processing are preservation to extend product shelf life and the improvement of organoleptic properties (Boada *et al.*, 2016). Meat processing entails one of more of the following: removal of undesirable constituents and/or the addition of desirable constituents; the facilitation of flavour and texture development by enhancing enzyme action; inhibiting enzyme action to prevent undesirable changes; the controlled use of microorganisms for flavour and texture development; and the destruction of harmful microorganisms/inhibition of microbial growth to prevent spoilage, for consumer protection (Earle and Earle, 2008). A wide variety of processing techniques such as thermal processing, emulsification, marination, breading, drying, curing and smoking; have been extensively detailed in literature (Toldrá, 2010; Hui, 2012). For the purpose of this study, the focus will be placed on processing by drying.

2.2.1 Principles of drying

Drying is one of the oldest and simplest methods of food preservation (Mujumdar and Devahastin, 2004), dating back to the earliest records of human civilisation (Sabarez, 2016). The adaptability of drying to small, medium and large scale meat processing is one of the main advantages of this preservation method. In developing countries, dried meat is an important dietary protein source, as drying can be used when other preservation methods are too costly or unavailable due to lack of equipment/infrastructure (Santchurn *et al.*, 2012). Drying is typically used in combination with salting and for centuries, traditional salting and drying technologies have been used to produce dry cured meat products (Marino *et al.*, 2015).

Microbial growth and biochemical reactions are the main causative agents of spoilage, resulting in deterioration of meat quality (Mills, 2004). Microbes require water in order to

survive, grow and reproduce (Hui, 2012). Drying works on the principal that lowering the moisture content and water activity (a_w) of food creates an unconducive environment for microbial growth. Water activity is equal to the relative humidity of the air in equilibrium with the product, and measures the availability of water in a product (Young *et al.*, 2010). When water in food is not bound to food molecules, it supports the growth of spoilage microorganisms. Water activity is lowered by the removal of water (drying) and/or increasing solute concentration, e.g. through the addition of salt (Hui, 2012). In solution, sodium chloride dissociates into ions (Na^{2+} and Cl^-) which surround polar water molecules (H_2O) exerting a partially negative charge around the hydrogen atoms and a positive charge around the oxygen atom; thereby immobilising them and preventing their ability to act in chemical and enzymatic reactions (Honikel, 2010). At high salt concentration (6% and above), products become shelf stable due to reduced a_w (Mills, 2004). Increasing osmotic potential draws water out of the cells of spoilage organisms, causing them to shrink (Hui, 2012). As a result, the shelf life of the meat is extended. For storage stability at ambient temperatures, the a_w of meat must be reduced below the level at which microorganisms can survive; which is organism specific (Zukál and Incze, 2010). The minimum a_w for the growth of bacteria, yeasts and molds and fungi are presented in Table 2.1. The majority of microbial spoilage organisms are inhibited when the a_w is reduced to 0.7 and below (Mujumdar and Devahastin, 2004). Most bacteria and pathogens do not grow at $a_w < 0.9$ which is the limit for a meat product to be stored without chilling. The a_w can be increased to 0.95 if pH < 5.2, but that some patghogens like *Staphylococcus aureaus* grow at lower a_w and yeasts and moulds grow until a_w is decreased to 0.6.

Speaking of dry meat products, Burnham *et al*. (2008) explained that drying procedures for different processed meats vary greatly due to differences in the temperature and relative humidity, rate of air movement and the desired characteristics of the final products. This will

affect the efficacy of drying. In fresh meat, the moisture content ranges from 70-80 %, and the targeted moisture content is product dependant; and further on whether other processing methods will be applied. Drying is often used in combination with other preservation methods such as salting, as previously mentioned, but also fermentation or smoking, in the processing of speciality meat products. In addition to preservation, the combination of these treatments is done with the aim of improving palatability and flavour development to suit consumer requirements (Santchurn *et al.*, 2012). Under controlled dying conditions, it is possible to monitor the rate of water evaporation over time. Drying curves are used to illustrate the transfer phenomena that occur during meat drying, hence drying kinetics are illustrations of the decrease in moisture content plotted as a function of drying time (Santchurn *et al.*, 2012). Drying kinetics data is obtained by either weighing the product periodically at points during drying, continuously throughout drying, intermittently at points when airflow is cut off to obtain more accurate weights, or indirectly by calculating the rate of evaporation from the humidity of the air emerging from the drying chamber (Kemp *et al.*, 2001). Choice of method depends on a number of factors including the level of accuracy required, the availability of equipment, the particle size of the product, the initial and final moisture content, the air velocity and the drying regime (Kemp *et al.*, 2001).

Table 2.1 Minimum water activity for development of microorganisms

Bacteria	Yeasts	Molds	a_w
E. coli			0.99
Str. fecalis			0.98
Vib. metschnikovii			0.97
Pse. fluorescens			0.97
Clo. botulinum			0.97
Campylobacter ssp.			0.97
Shighella			0.97
Yersinia enterocolitica			0.97
Clo. perfringens			0.96
Bac. cereus			0.96
Bac. subtilis			0.95
Sal. newport			0.95
Ent. aerogenes			0.94
Microbacterium			0.94
Vib. parahaemolyticus			0.94
Lac. viridescens	*Schizosaccharomyces*	*Rhisopus*	0.93
		Mucor	0.93
	Rodotorula		0.92
Mic. roseus	*Pichia*		0.91
Anaer. Staphylococcus			0.91
Lactobacillus	*Saccharomyces*		0.90
Pediococcus	*Hansenula*		0.90
	Candida	*Asp. niger*	0.88
		Debaryomyces	0.88
	Torulopsis	*Cladosporium*	0.87
Staphylococcus aureus	*Torulaspora*	*Paecilomyces*	0.86
Listeria monocyt.			0.83
		Penicillium	0.80
		Asp. ochraceus	0.80
Halophilic bacteria			0.75
		Asp. glaucus	0.72
		Chrysosporium fastidum	0.70
Zygosaccharomyces rouxii		*Monascus bisporus*	0.60

a_w Water activity
Source: (Zukál and Incze, 2010)

2.3 Droëwors

Droëwors (Figure 2.1) are traditional South African dried, seasoned, ready-to-eat sausages (Burnham *et al.*, 2008). The droëwors preparation process is schematically represented in Figure 2.2. Trimmings or meat and animal fat mixture are ground, salted and spiced, stuffed into casings and dried (Burnham *et al.*, 2008, Hoffman *et al.*, 2014). When lean meat is used, animal fat such as pork backfat (Hoffman *et al.*, 2014), beef fat (Jones *et al.*, 2015a) or sheep fat (Jones *et al.*, 2015b) is added to increase the amount of fat. Beef and game meat are most commonly used in droëwors production; however the use of exotic meats such as ostrich has become more common (Hoffman *et al.*, 2014). Traditionally, droëwors are made from pieces of meat that were not suitable for biltong production (CSIR, 2001). Typical drying conditions are 1-2 weeks in a well ventilated area or a few days in a controlled drying chamber (Santchurn *et al.*, 2012). Scientific studies in which droëwors were dried in drying chanbers mention temperature and relative humidity (RH) of 15°C and 75-82% respectively for 15 days (Hoffman *et al.*, 2014) but also higher temperature and lower RH for a shorter time (30 °C, RH 30% for 2 days, Jones *et al.*, 2015a). Drying times are driven by the targeted moisture loss of 45-50% which seems to be also commonly used by droëwors producers. Processing methods have a significant effect on the safety and nutritional value of meat (De Smet and Vossen, 2016). Under the temperature conditions described for droëwors production, there is minimal protein denaturation and minimal degradation of vitamins and functional compounds (Guerrero-Legarreta and García-Barrientos, 2012). Limited research has been conducted on droëwors, hence reports on the composition and physico-chemical properties of the product are limited; and are summarised in Table 2.2. The average moisture content of droëwors ranges from 27.2 – 38.7 g/100 g. When drying is the only preservation method used, it is recommended that moisture content should be reduced to 12-15% (Santchurm *et al.*, 2012). However, if drying is combined with salting (or other processing methods); moisture content

in the range of 28-50% is sufficient for product stability at ambient temperature. Sodium chloride is the main curing agent used in droëwors production, although the salt content has not been not reported in literature. Petit *et al.* (2014) reported that the use of nitrites in traditional biltong production is uncommon. This is also the case in droëwors. South African legislation regulating the use of preservatives and antioxidants in Section 15.1 of the foodstuffs, cosmetics and disinfectants act (Act 54 of 1972) permits the use of 160-200 mg total nitrate expressed as sodium nitrate. In addition to lowering a_w and inhibiting microbial growth, nitrites are involved in the generation of nitrosylmyoglobin, which gives the characteristic pink colour to cured products (Deda *et al.*, 2007; Toldrá and Reig, 2011); and droëwors are brown in colour (Figure 2.1). The average a_w (0.6 - 0.74) supports reports that droëwors can be stored at ambient temperatures for long periods (Jones *et al.*, 2015a). Average fat content ranges from 20.1 – 35.6 g/ 100 g, which is consistent with the addition of 10 - 20% of animal fat added before drying. The fat content increases after drying because the concentration of fat increases, as the product loses 45-50% of its initial weight during drying (Hoffman *et al.*, 2014). The protein content ranges on average from 28.3-37.2 g/100 g.

The droëwors preparation process is schematically represented in Figure 2.1 Physical and biochemical changes occur during processing at as a result of the preparation that the meat is subjected to before drying; and the chemical nature of the meat itself (Santchurn *et al.*, 2012). The processing methods entail reducing meat particle size by cutting and mincing, adding ingredients (salt and spices), mixing, mincing again and stuffing the minced meat into casings and hanging to dry (Burnham *et al.*, 2008). During drying, weight loss due to the evaporation of moisture results in changes in the physical appearance; causing shrinkage and hardening of the product. Water loss automatically results in a concentration of dry solids, causing fat, protein, and carbohydrate contents to increase. Hoffman *et al.* (2014) and Jones *et al.* (2015 a; b) have reported concentration of dry solids in droëwors production. Water loss leads to a

decrease in a_w which in turn affects biochemical and enzymatic reactions occurring in the meat. The colour of the meat generally changes from red to brown (Santchurn *et al.*, 2012).

Figure 2.1 Droëwors

Table 2.2 Physico-chemical properties of droëwors

Meat type	Moisture	Protein	Fat	Ash	aW	pH	Source
Beef	-	-	35.6	-	0.6 – 0.74	5.4 – 5.5	Burnham *et al.*, 2007
Ostrich[$]	27.2 ± 0.51	28.3 ± 2.16	29.3 ± 1.83	8.7 ± 0.44	-	-	Hoffman *et al.*, 2014
Blesbok	38.7 ± 0.36	34.6 ± 0.32	20.3 ± 0.18	5.3 ± 0.50	-	-	Jones *et al.*, 2015a
Springbok	36.1 ± 1.73	34.3 ± 0.35	20.1 ± 0.33	9.8 ± 1.35	-	-	
Fallow deer	35.8 ± 1.70	37.2 ± 0.22	20.5 ± 0.47	5.5 ± 0.62	-	-	

Moisture, protein, fat, ash and salt contents are expressed in g/100 g; a_w: Water activity; - Not measured;
[$] Values were calculated as the mean ± standard deviation of all droëwors in the study

2.4 Lipid oxidation

Meat products, especially those with a high fat content, are prone to deterioration by lipid oxidation (Descalzo *et al.*, 2005). It involves the loss of at least one electron when unsaturated lipids in meat are exposed to oxygen in the air (Falowo *et al.*, 2014). Reactive oxygen species (ROS) degrade polyunsaturated fatty acids (PUFAs) by disrupting the double bond between carbon atoms (Young *et al.*, 2010). Fatty acids consist of a hydrocarbon chain with a single carboxyl group at one end, and can either be saturated, (i.e. containing no double bonds), monounsaturated, (i.e. having one double bond) or polyunsaturated (i.e. having more than one double bond (Willian, 2013); as illustrated in Figure 2.3. Free radicals are frequently produced by normal biological redox-reactions in the body which involve the transfer of one electron and the can also be induced as a result of exposure to external factors such as drugs, pollutants, heavy metals, heat, ultraviolet and visible light and other forms of ionising radiation (Wang and Quinn, 1999). The process of lipid oxidation has been detailed extensively (Kanner, 1994; Mills, 2004; Jacobsen, 2010; Young *et al.*, 2010), and is depicted in Figure 2.4. Oxidation of unsaturated fatty acids is characterised by an initiation stage, a propagation stage and a termination stage (Kanner, 1994). The process is initiated by oxygen and a metal catalyst such as iron, producing a free radical. These free radicals react with oxygen forming lipid peroxyl radicals and continue to react with other lipid molecules forming peroxides, the primary products of oxidation. Peroxides decompose into secondary volatile compounds, including aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids, and epoxy compounds (Shahidi and Zhong, 2005). These compounds are responsible for the formation of off-flavours in the product and, in excess, can contribute to aging, cancers and cardiovascular diseases (Toldrá and Reig, 2011). The reactions produce new free radicals that go on to repeat the process on other PUFAs.

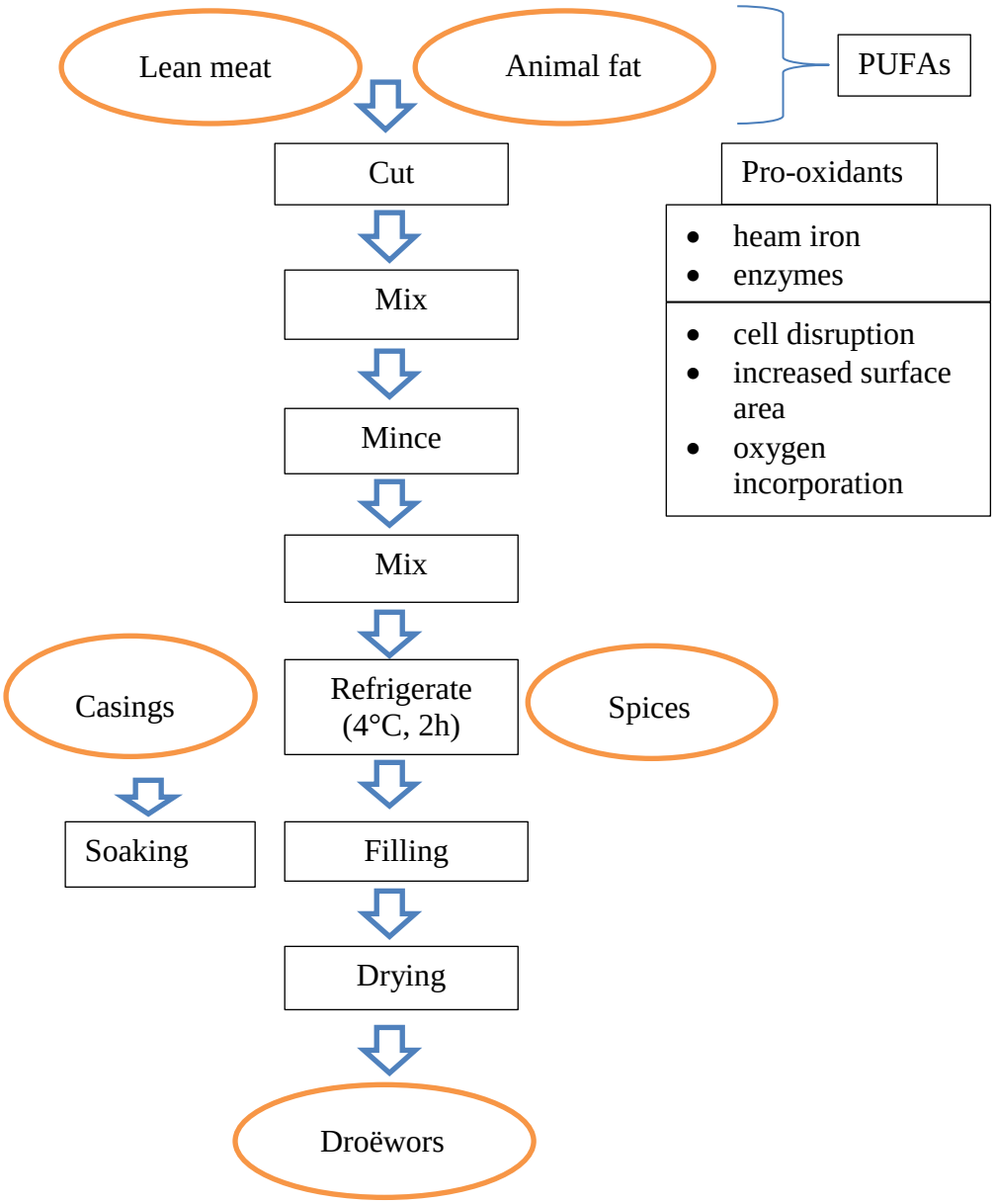


Figure 2.2 Pro-oxidants in the droëwors processing procedure

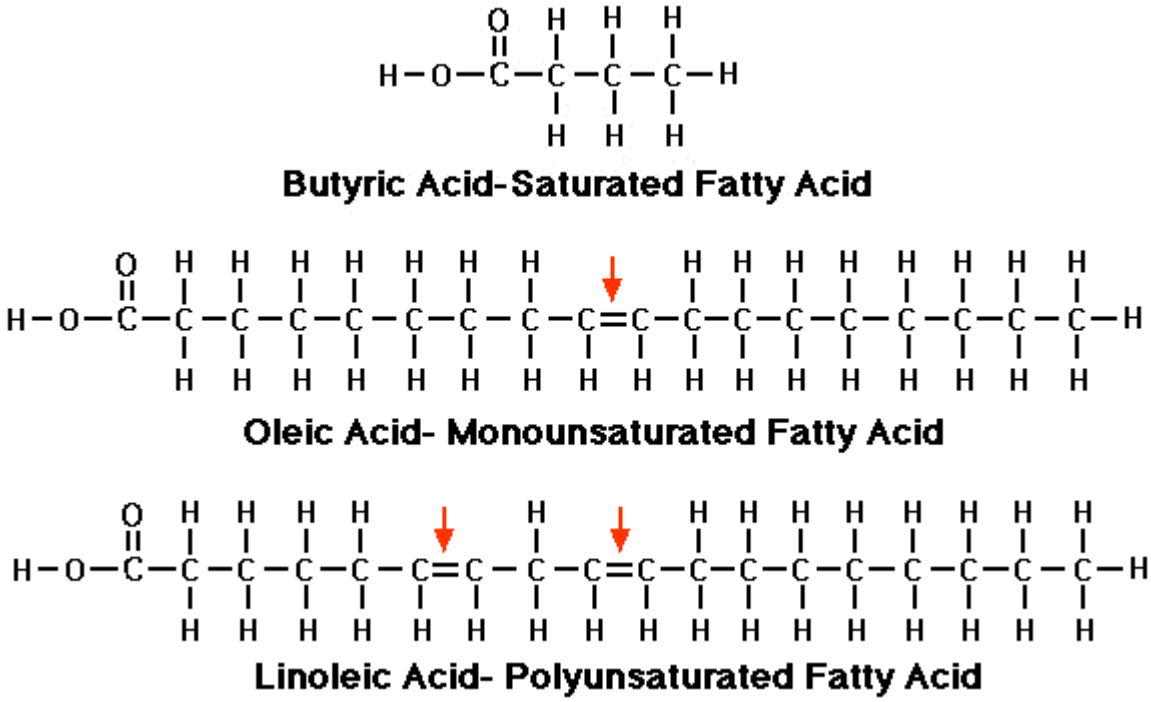
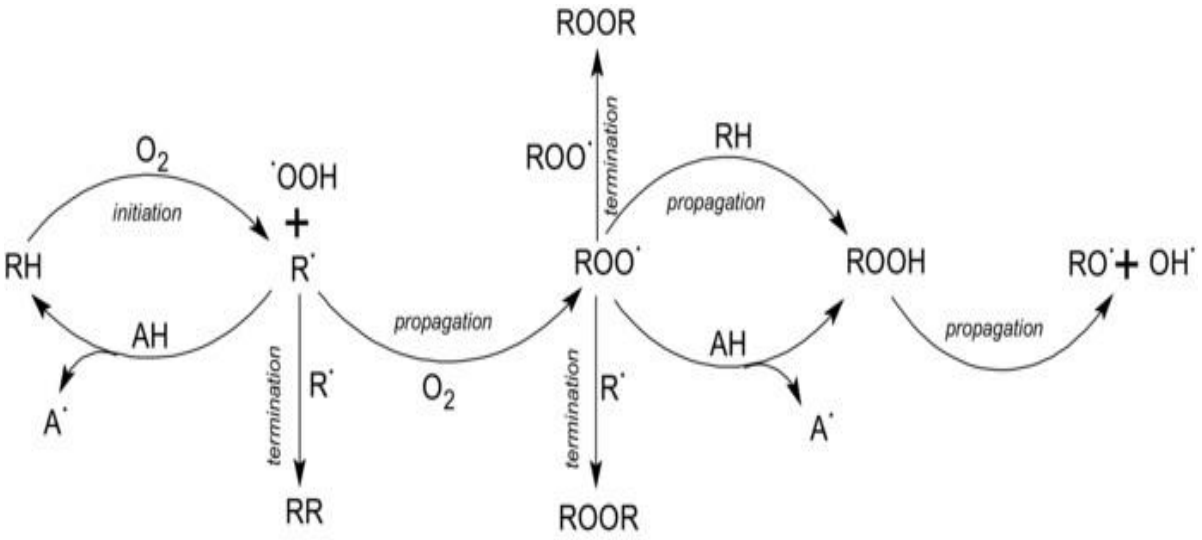
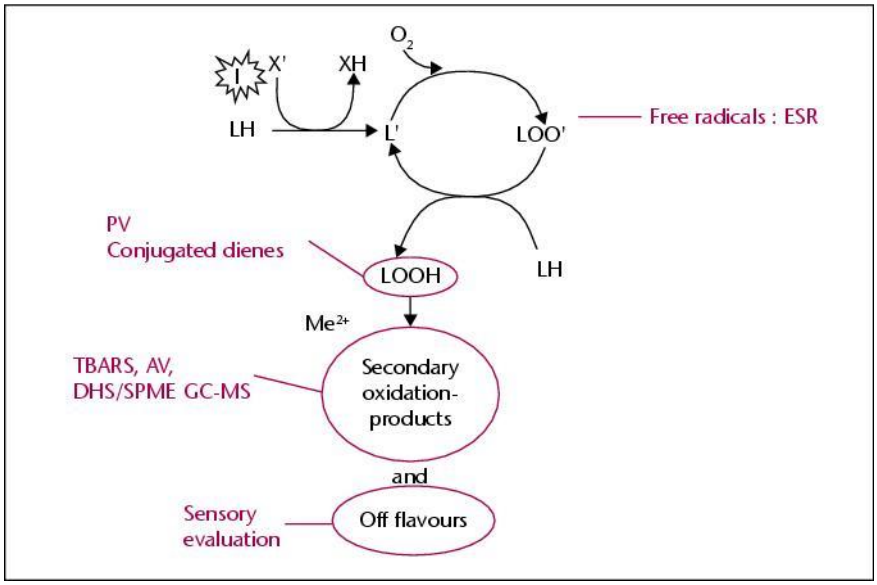


Figure 2.3 Chemical structure of saturated, monounsaturated and poly unsaturated fatty acids

Source: (http://antranik.org/organic-compound-2-lipids/)



a



b

Figure 2.4 Reactions (a) and products (b) involved in lipid oxidation

Source: (Jacobsen, 2010)

Lipid oxidation is the main non-microbial cause of quality deterioration in muscle foods (Gray *et al.*, 1996). It results in colour deterioration, of off odour and flavours, and nutrient losses (Kanner, 1994), poor shelf life, and the development of toxic compounds (Contini *et al.*, 2014). Consequences of lipid oxidation include rancidity, which lowers the functional, sensory and nutritive values of meat products; and therefore, consumer acceptability (Bou *et al.*, 2004). Wood et al. (2003) reported that pork back fat is known to have a more unsaturated fat profile than beef and is therefore it is more susceptible to lipid oxidation. Being highly oxidisable substrates, PUFAs may act as pro-oxidants in meat and meats products (Morrissey *et al.*, 1998). The main factors that define the susceptibility of lipids to peroxidation in tissue are the proportion of PUFA in lipid bilayers, the amount of reactive oxygen species produced and the level of endogenous or nutritional antioxidants (Brenes *et al.*, 2008).

Lipid oxidation in muscle foods is initiated by stressors arising from both internal and external sources (Descalzo *et al.*, 2005). Both intrinsic meat factors and the processes involved in droëwors preparation are sources of pro-oxidants; as has been illustrated the schematic representation of droëwors preparation (Figure 2.4). When meat is minced, the disruption of lipid membranes promotes contact between lipids and pro-oxidant agents; casing the generation of free radicals and the propagation of oxidative reactions (Guyan *et al.*, 2016; Kiliç *et al.*, 2016). The resultant disruption damages natural antioxidant systems and the development of oxygenated free radicals which initiate the oxidation of polyunsaturated fatty acids (Hygreeva *et al.*, 2014).

Shahidi and Zhong (2005) classify the methods available to monitor lipid oxidation in foods into five groups on the basis of what they measure: the absorption of oxygen, the loss of initial substrates, the formation of free radicals, and the formation of primary and secondary oxidation products. The most commonly used techniques include measuring the values of

peroxide (PV), thiobarbituric acid-reactive substances (TBARS), sulphydryl and carbonyl groups generated during the process (Falowo *et al.*, 2014). These analyses are carried out using spectrophotometric or chromatographic (head space gas chromatographic (GC), high-performance liquid chromatography (HPLC), liquid chromatographic mass spectrophotometer [(LC–MS) and 2,4 dinitrophenylhy-drazine (DNPH)] methods. Since peroxide is a primary product of oxidation, peroxide values can sometimes underestimate the extent of lipid oxidation due to the decomposition of peroxides into secondary products (Gray and Monohan, 1992). Thiobarbituric acid-reactive substances the most commonly used methods for measuring lipid oxidation (Toldrá and Reig, 2011). Degradation of PUFAs produces malonaldehyde (MDA), which is used as an indicator of lipid oxidation because it is produced at an early stage in the oxidation reaction. Malonaldehyde reacts with thiobarbituric acid (TBA) to form a pink complex that can be measured spectrophotometrically at 530-535 nm (Shahidi and Zhong, 2005).

2.5 Antioxidants

The balance between pro-oxidants and antioxidants determines the oxidative stability of meat lipids (Pouza *et al.*, 2016). Antioxidants are used extensively in the food industry to improve the shelf life, colour and flavour stability of processed meat products (Falowo *et al.*, 2014). They aid in food preservation by scavenging the active forms of oxygen involved in the initiation or progression of oxidation; thereby inhibiting the process and protecting cells from damage caused by free radicals (Shahidi and Zhong, 2014). In practise the addition of antioxidants, which are classified as being either synthetic or natural in origin, is capable of is the major preventive measure against lipid oxidation in meat and meat products (Nkukwana *et al.*, 2014). Synthetic antioxidants, such as butylated hydroxyanisole, butylated hydroxyl toluene, tertiary butlylhydroquinone are commonly used in meat processing to reduce the detrimental effects of lipid oxidation on product quality (Shahidi and Wanasundara, 1992).

However, it has been reported that synthetic antioxidants may increase the risk of cardiovascular diseases, obesity and cancer (Hygreeva *et al.*, 2014).

2.5.1 Antioxidants and human health

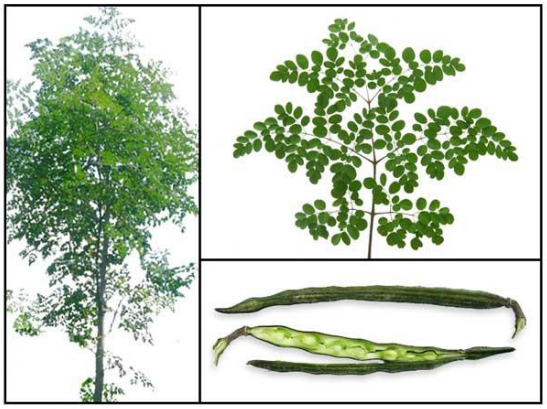
In addition to food preservation, the health promoting properties of antioxidants are another significant benefit (Shahidi and Zhong, 2014). The uncontrolled generation of reactive species from oxidation can cause significant reversible or irreversible damage to a wide range of biological molecules including DNA, proteins, carbohydrates, and lipids (Wang and Quin, 1999). Consumption of foods rich in antioxidants provides the physiological functionality of protecting living organisms from oxidative damage (Sánchez-Machado *et al.*, 2006). This contributes towards the prevention of diseases such as cancer, cardiovascular diseases, and diabetes. Consumption of plant derivatives containing antioxidant components such as vitamins A, C, E, minerals, polyphenols, flavonoids and terpenoids may decrease the risk of degenerative diseases (Hygreeva *et al.*, 2014). There is an increasing demand for effective antioxidants from natural sources because they are increasingly viewed as being safer for human consumption (Sánchez-Machado *et al.*, 2006).

The importance of consumer health and well-being has fuelled research efforts towards improving the nutritional value of meat with the aim of minimising unhealthy substances and promoting the presence of substances with health benefits (Toldrá and Reig, 2011). In response to consumer concerns, the food processing industry is exploring the use of phytochemicals from plants with antioxidant and antimicrobial properties to be used in place of synthetic chemical additives (Singh *et al.*, 2015). The demand for food containing bioactive or functional components with additional health benefits is on the increase (Cofrades *et al.*, 2008); creating a viable market for the development of healthier, functional food products (Hygreeva *et al.*, 2014). Researchers, food technologists and nutritionists have been working on the development of novel meat products with decreased levels of

undesirable components and increased levels of healthier components (Decker and Park, 2010; Hygreeva *et al.*, 2014). In recent years, special attention has been paid to a number of medicinal plants that could be used as potential sources of antioxidants for muscle food preservation and nutritional quality improvement (Faseas *et al.*, 2007; Georgantelis *et al.*, 2007; Nkukwana *et al.*, 2014; Falowo *et al.*, 2016). Antioxidant vitamins (A, C, E) and phenolic compounds (poly phenols, flavonoids, carotenoids) are the major constituents of plant materials that contribute to their antioxidant capacity (Falowo *et al.*, 2014).

2.6 *Moringa oleifera*: antioxidant potential and functional value

Moringa oleifera (Figure 2.5) is a fast growing, drought resistant, multi-functional plant; originating from sub-tropical regions of Asia including India, Pakistan and Nepal (Sidduhraju and Becker, 2003; Iqbal and Banger, 2006). *Moringa oleifera* has now become naturalised to many other tropical and sub-tropical regions of Asia, Africa, the Mediterranean and the Arabian Peninsula, South and Central America and the Caribbean Islands. Furthermore, it is now being propagated and cultivated in many countries across the globe (HDRA, 2002; ECHO, 2007; Roloff *et al.*, 2009), including South Africa. *Moringa oleifera* is considered to be one of the most valuable and useful tropical trees on earth (NRC, 2006; Ashfaq *et al.*, 2012). It is commonly referred to as "the miracle tree" (Ashraf and Gilani, 2007), "the tree of life" (Djakalia *et al*., 2011), and as "nature's medicine cabinet" (Paliwal *et al.*, 2011). Research on *Moringa oleifera*, a previously an unknown plant species, has increased in the last three decades because of its nutritional value and its medicinal use for the preparation of natural remedies in regions where it natively grows (Sauveur and Broin, 2010). Studies have shown *Moringa oleifera* Lam to be of immense nutritional value, having high levels of crude



a

b

c

Figure 2.5 *Moringa oleifera* leaves (a), leaf powder (b) and nutritional value (c)

protein, vitamins, amino acids, minerals and fatty acids (Moyo *et al.*, 2011). The antioxidant and biological activities of *Moringa oleifera* plants have been attributed to the presence of phytochemicals including flavonoids, phenolics and vitamin E in their leaves (Falowo *et al.*, 2014). The leaves, seeds, pods and roots of this plant have all been shown to be of nutritional and medicinal value, suitable for both human and animal consumption. Qwele *et al*. (2013) reported higher antioxidant activity in meat from goats supplemented with *Moringa oleifera* leaves. Nkukwana *et al*. (2014) reported that supplementing broiler chickens with *Moringa oleifera* leaf meal reduced lipid oxidation in chicken. *Moringa oleifera* is also a valuable source of nutraceuticals with the potential for wide application in the food processing industry (Singh *et al.*, 2015). In terms of anti-nutritional factors, Makkar and Becker (1997) reported that the leaves had negligible amounts (12 gkg^{-1}or 1.2%) of tannins, a phytate content of 21gkg^{-1} (2.1%) and a saponin content of 80 gkg^{-1} (8%) as diosgenin equivalent that did not show any haemolytic activity. Tannins are natural water-soluble phenolic compounds that bind with proteins forming stable complexes that are not easily degraded (Waterman, 2000), thus reducing the amount of protein available for utilisation by the ingesting animal. Makkar and Becker (1997) reported that they were unable to detect any trypsin and amylase inhibitors, lectins, cyanogenic glucosides and glucosinolates.

In some regions of India and the Phillipines, fresh *Moringa oleifera* leaves are traditionally used in the preparation of fatty foods and has been reported to significantly increase the shelf life of these foods because it is a rich source of natural antioxidant (Siddhuraju and Becker, 2003). *Moringa oleifera* is a rich source of vitamin E, the leaves reportedly containing 77 mg/ 100g (Moyo *et al*., 2011). The major role of vitamin E in cells is to act as an antioxidant to protect tissue cells and unsaturated fatty acids from free radical damage (Traber and Packer, 1995; Wang and Quinn, 1999; Traber and Atkinson, 2007) and that it also performs other roles besides its antioxidant activity such cellular growth, gene transcription and protein

kinase C inhibition (Azzi and Stocker, 2000). Vitamin E encompasses a group of eight isomeric fat-soluble molecules: α-, β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol (Wang and Quin, 1999; Higdon, 2000). The homologues of tocopherol are α-, β-, γ- and δ-tocopherol all possess antioxidant properties, with α- tocopherol being the most biologically active form of vitamin E; as γ- tocopherol and δ- tocopherol have only 10% and 1% of the activity of α- tocopherol respectively (Sánchez-Machado *et al.,* 2006). Both *α*-tocopherol and γ-tocopherol are essential components of cellular defence mechanisms against endogenous and exogenous oxidants, as their action is non-enzymatic and rapid (Wang and Quinn, 1999). High-performance liquid chromatography (HPLC) methods are widely used for the determination of α- and γ- tocopherol (Sánchez-Machado *et al.,* 2006).

2.7 Summary of review

Processed meat products are an important component of modern human diets. Processing methods such as drying, have been developed for meat preservation, to extend its shelf life; enable its storage at ambient conditions and to create convenient products suited to consumers' needs. The biochemical changes involved in meat processing, such as lipid oxidation, can have detrimental effects on product quality in terms of off flavour development generation of toxic compounds that pose human health risks. Synthetic antioxidants that have been used to minimise the effects of lipid oxidation in processing have also been implicated with negative impacts on human health. Increasing research has consequently been carried out on suitable natural alternatives to synthetic antioxidants. *Moringa oleifera* leaves, by virtue of having high nutritional value and bioactive components, show promise as a natural alternative to synthetic antioxidants and has the additional potential benefit of imparting nutritional value to the product.

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Chapter 3: Physico-chemical characteristics of South African droëwors

Abstract

This investigation determined the physico-chemical characteristics (proximate composition, salt content, water activity (a_w), pH) of South African droëwors produced from commonly used meat types (beef, game meat, ostrich). Commercially produced beef, game meat and ostrich droëwors (n=20) were purchased and the product packaging conditions recorded. Physico-chemical properties measured were proximate composition (moisture, protein, fat, ash), salt content, a_w and pH. The results showed that the physico-chemical characteristics of commercially available droëwors on the South African market are similar across different meat types; as no differences ($P > 0.05$) in the physico-chemical composition of beef, game meat and ostrich droëwors were recorded. Similarly, the packaging conditions had no effect on the physico-chemical characteristics of the product. A strong correlation (0.936, $P < 0.001$) was found between moisture content and a_w. There was, however, a wide variation in the moisture (17.6 – 35.3 g/100 g), protein (32.4 – 51.4 g/100 g) and fat contents (16.8 – 47.0 g/100 g) and a_w (0.67 – 0.86); and some variation in pH (4.9 – 5.7) across and within droëwors of different meat types. Droëwors were composed of on average 25.8 ± 1.25 g/100 g moisture, 42.0 ± 0.10 g/100 g protein, 32.0 ± 1.68 g/100 g fat, 6.2 ± 0.13 g/100 g ash and 4.2 ± 0.10 g/100 g salt; with a a_w and pH on average of 0.79 ± 0.015 and 5.3 ± 0.05, respectively. Water activity showed that droëwors can be stored at ambient temperatures.

3.1 Introduction

Drying preserves meat by reducing its moisture content and its a_w to create a non-conducive environment for microbial growth (Earle and Earle, 2008). Apart from preservation, the drying process is also a valuable means of transformation to produce speciality processed meat products (Burnham *et al.*, 2008). The chemical reactions that occur during drying result in the development of new flavours and can be used to create economically viable meat products which are lighter, easier to transport and are shelf stable. Since drying is one of the oldest methods of food preservation (Santchurn *et al.*, 2012), many traditional delicacies from different regions of the world are produced using this time tested technique. South African dried meat products such as droëwors and biltong, traditionally dried under ambient conditions (Burnham *et al.*, 2008), are now produced industrially, frequently under controlled environmental conditions and are marketed widely internationally and locally.

Droëwors are shelf-stable, ready to eat salted and dried sausages; produced and consumed widely in South Africa and growing in popularity on the international market (Jones *et al.*, 2015b). To produce droëwors, sausages are prepared using thin natural casing to ensure even drying of the product; and are dried in a way similar to biltong (CSIR, 2001). They are commonly made from offcuts and trimmings of beef and animal fat, although they may also be made from game meat and ostrich meat (Hoffman *et al.*, 2014; Jones *et al.*, 2015a). There are many variations in the recipes and formulations used to process droëwors. Some differences in the stability of biltong, another traditional South African dried-meat product, have been attributed to differences in the type of meat used, different spice combinations, the extent of salting and drying and the use of preservatives (Petit *et al.*, 2014).

Scientific literature detailing the physico-chemical properties of commercial droëwors is lacking as is information describing the different processing procedures. Data on the moisture

content, salt content and a_w is required as these parameters are used to determine whether the product is shelf-stable and will give indications on the amount of salt to be added in the formulation and the extent of drying to be realized. Other information needed is the typical fat content of the final product; which will allow processors, after determining the fat content of the trimmings to be used, to calculate the amount of fat to be added in the formulation. This investigation aimed at providing information on the physico-chemical composition of commercially available South African droëwors made from the most commonly used meat types.

3.2 Materials and methods

3.2.1 Sampling

Droëwors processed from three different types of meat were analysed in this study, with at least 3 replicates for each meat type. Packets of beef (n=9), game meat (n=8) and ostrich (n=3) droëwors were randomly purchased from supermarkets, butcheries and retail outlets in Stellenbosch, South Africa. The packaging conditions of the purchased samples were recorded (Appendix 2). A 100 g sub sample was taken from each packet, ground using a blender (Braun PowerMax MX2050) for 1 min and vacuum sealed. Samples were stored at -20 °C until analysed.

3.2.2 Proximate analysis

The proximate composition of the droëwors was determined in duplicate according to the procedures outlined in AOAC (2002). Crucibles were pre-weighed and approximately 5 g of each sample was weighed out into crucibles, dried in an oven at 103 °C ± 1 °C for 24 h, allowed to cool in a desiccator for 30 min and re-weighed to calculate the moisture content and dry matter content. Thereafter, the water-free sample was placed in a furnace at 500 °C for 6 h, allowed to cool down for 2 h and then re-weighed to determine the ash content.

Total fat was extracted according to the method of Lee *et al.* (1996). Samples (5 g) were weighed into a beaker with 50 mL of chloroform/methanol (2:1), homogenised for 1 min and filtered through a separation funnel (the residue on the filter paper was kept and dried to be used later for protein analysis). To the filtrate, 20 mL of 0.5% sodium chloride (NaCl) was added, the mixture was shaken gently 4 times and allowed to stand for 30-60 min to allow for separation. Thereafter, 5 mL of the bottom layer of liquid was pipetted into a pre-weighed glass fat beaker, heated on a sand bath for 45 min, allowed to cool in a desiccator for 30 min and re-weighed to calculate the fat content.

For protein analysis, the fat-free residue collected from the filter paper was placed in a container and dried overnight in an oven at 60 °C. Thereafter, 0.15 g of residue was weighed out onto a tin foil cup and nitrogen content was measured (Leco FP528) and used to calculate the protein content using a factor of 6.25.

3.2.3 Salt content

For salt determination, 0.3 g of sample was weighed out in duplicate, 50 mL of 0.3M nitric acid was added and the solution was stirred with a magnetic stirrer for at least 2 hrs. Thereafter, the chloride content (mg/L) was measured using a chloride analyser (Model 962, Sherwood, Cambridge, UK). Salt content was calculated as:

$$Salt\ content = \frac{1.648\text{x}10^{-4}\ \text{x}\ [Cl^-]\text{x}\ HNO3\ volume}{M_{sample}} \qquad \text{(g/100 g)}$$

Where [CL] = chloride content (mg/L)

HNO_3 = nitric acid volume in mL

M_{sample} = sample mass in g

3.2.4 Water activity

The a_w was measured using an a_w meter (AquaLab 4TE, accuracy 0.003) at 18 °C ± 0.5 °C. Readings were done in duplicate.

3.2.5 pH

Three grams of samples were weighed in duplicate into 50 mL beakers, thereafter filled with distilled water until the weight of 30 g was reached. The beakers were then placed on magnetic stirrers for 30 min. The pH was measured during continuous agitation on the magnetic stirrers with a pH meter (Crison 25 Glass Probe).

3.2.6 Statistical analysis

Data on physico-chemical properties of beef, game meat and ostrich droëwors were analysed using PROC GLM procedures of SAS (2003) with the main effect of meat type and pair wise comparisons of least square means were done using t-tests (PDIFF option). Differences were significant at $P < 0.05$. The statistical model used was:

$$Y_{ij} = \mu + \alpha_i + e_{ij}$$

Where Y_{ij} = dependant variable (Moisture, protein, fat, ash, salt content, a_w, pH,)

μ = overall mean

α_i = i^{th} effect of meat type (beef, game meat, ostrich)

e_{ij} = random error.

Pearson's correlation coefficients (PROC CORR) were analysed between the physico-chemical traits of beef, game meat and ostrich droëwors.

3.3 Results and discussion

The physico-chemical characteristics of beef, game meat and ostrich droëwors are showed in Table 3.1. No significant differences were found in the moisture, protein, fat, ash and salt contents, a_w and pH of all three droëwors types. There were, however, wide variations in the physico-chemical characteristics of the commercial droëwors as shown by the minimum and maximum values. This variation is also an indication that the industry needs to develop standard operating procedures (SOP's) to ensure a more consistent end product. The mean values of moisture, protein, fat and ash in droëwors made from game meat (Blesbok, Springbok and Fallow deer) reported by Jones *et al*. (2015a) are 36.9, 35.4, 20.3 and 6.9 g/100 g respectively; and fall within the ranges recorded on game meat in this study (Table 3.1). Hoffman *et al.* (2014) reported similar moisture content but less protein and more fat and ash (on average 27.3, 28.9, 32.1 and 8.65 respectively) compared to the composition reported in this study (on average 29.2, 44.0, 26.2 and 5.9 g/100 g respectively for moisture, protein, fat and ash). On beef droëwors, Burnham *et al.* (2008) reported lower a_w ranging from 0.60 to 0.74 and similar fat content (35.5 g/100 g) and pH (on average 5.5).

The physico-chemical properties of the analysed samples were consistent with the requirements for shelf stability at ambient temperatures and inhibition of microbial growth. In non-fermented meat products, the a_w, moisture content and salt content are key determinants to prolong shelf life without the use of refrigerated storage (Earle and Earle, 2008). The a_w of the analysed samples ranged, on average, from 0.67 – 0.86 which complies with the requirements (a_w of < 0.91) for meat to be storable at ambient temperature (Rodel, 1975 in Girard, 1988). The minimum a_w required for the growth of most bacteria and yeast is 0.91 and 0.88 respectively (Mujumdar and Devahastin,

Table 3.1 Physico-chemical characteristics of beef, game meat and ostrich droëwors (ls means ± standard error; min-max range)

Physico-chemical characteristics	Beef (n=9)			Game meat (n=8)			Ostrich (n=3)			P value
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	
Moisture	26.3 ± 1.82	19.9	31.7	23.7 ± 1.93	17.6	35.3	29.2 ± 3.15	27.3	31.5	0.25
Protein	41.3 ± 1.82	33.1	48.2	42.0 ± 1.93	32.4	51.4	44.0 ± 3.15	41.7	45.9	0.77
Fat	32.9 ± 2.57	25.0	40.3	33.1 ± 2.72	16.8	47.0	26.2 ± 4.45	23.5	28.8	0.38
Ash	6.1 ± 0.20	5.5	7.5	6.5 ± 0.21	6.0	7.4	5.9 ± 0.34	5.4	6.9	0.34
Salt	4.0 ± 0.13	3.5	4.9	4.4 ± 0.14	4.0	4.9	4.0 ± 0.23	3.5	4.7	0.09
a_w	0.81± 0.021	0.71	0.86	0.76 ± 0.022	0.67	0.86	0.82 ± 0.036	0.81	0.86	0.10
pH	5.3 ± 0.07	5.1	5.7	5.4 ± 0.08	4.9	5.6	5.0 ± 0.13	4.9	5.5	0.22

Moisture, protein, fat, ash and salt contents are expressed in g/100 g; a_w: Water activity

2014). Specific microorganisms of concern in droëwors production and storage are *Salmonella serovars, Escherichia coli, Listeria monocytogenes and Staphylococcus aureus* reportedly known to be able to withstand high salt concentrations and reduced a_w (Burnham *et al.*, 2008). Under aerobic conditions, *S. aureus* and *L. monocytogenes* growth is inhibited when the a_w is $\leq$ 0.85 and 0.92, respectively (Burnham *et al.*, 2008). Based on dietary guidelines recommending a daily allowance of 4-6 g salt/day (Bertram *et al.*, 2012), droëwors has a high salt content (3.5 - 4.9 g/100 g). At these levels, consumption of 100 g of droëwors/ day will constitute almost the entire recommended daily consumption limit. Droëwors is known to have a high salt content (Burnham *et al.*, 2008). However, to the author's knowledge, no scientific literature detailing the salt content of droëwors is available. In comparison with biltong, a similarly dried meat product, there was less variation in the salt content of the three types of droëwors than in the salt content of biltong, reported by Petit *et al.* (2014) to be between 1.9 and 7.9 g/100 g. Salami is another shelf-stable sausage product with low moisture content and a_w. According to Pretorius and Schönfeldt (2016), average sodium content of South African salami (1.7 g/100g) converted to sodium chloride content (by a multiplication factor of 2.5) is 4.2 g/100g; which is comparable to that of droëwors. Excessive sodium consumption has been linked as a risk factor for hypertension, cardiovascular diseases and osteoporosis (Damez and Clerjon, 2003).

The Pearson's correlation coefficients are presented in Table 3.2. Positive correlations were found between a_w and moisture ($P < 0.001$); salt and ash ($P < 0.001$) and pH and ash ($P < 0.05$). Negative correlations were found between a_w and ash ($P < 0.01$); a_w and salt ($P < 0.01$); pH and moisture ($P < 0.05$); ash and moisture ($P < 0.05$); pH and a_w ($P < 0.05$); moisture and fat ($P < 0.05$) and protein and fat ($P < 0.05$). A strong positive correlation between moisture content and a_w as well as a negative one between moisture content and salt content was expected, as a_w measures the amount of free water in the product (Earle and Earle, 2008).

The packaging materials and conditions of the purchased droëwors samples are presented in Table 3.3. The results show that a variety of packaging materials are used for commercially produced droëwors. According to Nortjé *et al.* (2005), biltong is usually sold unpackaged. The same is true of droëwors. Typically, droëwors are displayed unpackaged in butcheries and shops and are packaged into open paper bags once sold. The packaging conditions were grouped and classified as open (brown paper bag; n=10) and sealed (n=10). The sealed packages were sub-classified according to difference in the interior atmosphere conditions, as detailed on the packaging material (styrofoam tray with a modified atmosphere overwrapped with plastic (n=6), sealed plastic bags (n=1) with a modified atmosphere and an oxygen absorbent pack (n=2) or vacuum sealed (n=1). There were no significant differences between the physico-chemical characteristics of open and sealed droëwors although it could have been hypothesised that the unpacked one could have been drier as when stored uncovered for a prolonged period of time, the product continue to lose moisture.

Table 3.2 Correlation between physico-chemical characteristics of beef, game meat and ostrich droëwors

	Fat	Moisture	Ash	Salt	aW	pH
Protein	-0.490*	-0.126^{NS}	0.030^{NS}	-0.109^{NS}	-0.285^{NS}	0.152^{NS}
Fat		-0.448*	-0.283^{NS}	-0.072^{NS}	-0.165^{NS}	-0.083^{NS}
Moisture			-0.450*	-0.392^{NS}	0.932***	-0.509*
Ash				0.870***	-0.647**	0.459*
Salt					-0.563**	0.364^{NS}
a_w						-0.575*

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ^{NS} Not significant ($P > 0.05$); a_w: Water activity

Table 3.3 Packaging conditions of of beef, game meat and ostrich droëwors

Packaging condition	Beef	Game meat	Ostrich
Open (n=10)			
Brown paper bag	4	5	1
Sealed (n=10)			
Styrofoam tray, plastic over wrap and modified atmosphere	3	1	2
Plastic bag with modified atmosphere and oxygen absorbent	0	2	0
Plastic bag	1	0	0
Plastic bag with vacuum	1	0	0

3. 4 Conclusion

The study provided data on the physico-chemical properties of commercially available South African droëwors. In terms of mean moisture, protein, fat, salt, a_w and pH; commercially produced droëwors from beef, game meat and ostrich did not differ significantly. However, there was a wide range of values for individual droëwors indicating that the industry needs to develop industrial processing standards to ensure consistent products. On average and irrespective of packaging conditions, the moisture content and a_w of all analysed samples were found to be in the recommended range for storage at ambient temperatures. The results of the study provides information on the physico-chemical characteristics of droëwors, which is lacking in published literature; and outlines the target composition parameters to be used in droëwors preparation.

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Chapter 4. Lipid oxidation in beef and pork droëwors

Abstract

The aim of the study was to compare the levels of lipid oxidation in similarly produced beef and pork droëwors during processing and storage. Raw materials (lean beef, beef fat, lean pork, pork fat) were purchased and analysed for moisture and fat content. Twelve 2 kg batches of beef (n=6) and pork (n=6) droëwors were prepared with similar fat content and dried at 30 °C and 40% relative humidity for 48 h and stored in a controlled environment (25 °C, 50% relative humidity) for a 26 day period. Physico-chemical properties (proximate, salt content, a_w and pH) were measured at day 0 (before drying) and day 5 (after drying and 3 days of storage); with some of them (moisture content, a_w, pH) being followed during storage (day 12, 21 and 26). During processing and storage weight loss was recorded and lipid oxidation (TBARS) was followed. The moisture, fat, protein and ash content of beef and pork droëwors was similar ($P > 0.05$) at day 0 and day 5. Significant differences were found in the TBARS values of beef and pork droëwors from day 5 and throughout storage; with higher ($P < 0.05$) TBARS in pork droëwors. The results revealed that lipid oxidation was significantly higher in pork droëwors than beef droëwors.

4.1 Introduction

Droëwors is a traditional dried meat sausage from South Africa. It is commonly made from a mixture of meat and fat (usually beef but also ostrich and game meat), salted and dried in a few days. Spices (usually pepper and coriander) and vinegar but no nitrates/nitrites or any other preservatives are added to the formulation. It is a high fat product with fat content ranging from 16.8 to 40.3 g/100g as shown in chapter 3. It is often sold unpackaged in ambient conditions and commonly stored by consumers for several days to a few weeks, during which it is susceptible to oxidative deterioration (Hoffman *et al.*, 2014). Deteriorative changes that manifest as a consequence of lipid oxidation include adverse changes in colour, flavour, texture, nutritive value and possible production of toxic compounds (Gray *et al.*, 1996; Dai *et al.*, 2014). The use of pork in traditional droëwors recipes is not common. One reason for this is that most droëwors recipes date back to the Great Trek, the historic migration in 1836-1838 of semi-nomadic pastoralists and farmers away from the "Cape Colony" (modern day Eastern Cape, Western Cape and Northern Cape provinces) into the interior of South Africa (SAHO, 2011). During this time, hunted game meat and beef were the most commonly used meat sources; as pigs were not suited to the nomadic lifestyle of the trekkers (http://www.biltongmakers.com/biltong20_drywors.html). Currently, pork is not commonly used for droëwors production because it is considered to be more prone to rancidity when dried (http://www.biltongmakers.com/biltong20_drywors.html).

Research has indeed reported that the ratio of polyunsaturated fatty acids has increased in modern pork as a consequence of the efforts to obtain lean carcasses, these 2 parameters being inversely correlated (Wood *et al.*, 2003; USDA, 2009). This makes it more susceptible to oxidation (Rosenvold & Andersen, 2003). Furthermore, pigs are highly susceptible to pre-slaughter stress (Gajana *et al.*, 2013); which can cause the overproduction of reactive oxygen species in muscle tissues and accelerate the rate of oxidation (Falowo *et al.*, 2014). However,

limited research has been conducted on lipid oxidation in droëwors made from different meat types, resulting in no substantive data validating that pork droëwors are indeed more susceptible to oxidation than the commonly used meat sources (beef and game meat). Pork droëwors could be a lucrative and value-added product and add to the variety of droëwors available in local and international markets. The price/kg of pork in South Africa is generally lower than that of beef and game meat; and the shelf-stability of the product would make pork droëwors a dietary protein source that would meet consumer demands for convenience and affordability. The aim of this study was to compare the level of lipid oxidation in similarly produced beef and pork droëwors to validate whether droëwors made from pork is more prone to rancidity; and to serve as a point of reference for pork droëwors preparation.

4.2 Materials and methods

4.2.1 Raw materials

Lean beef (10 kg), beef fat (2 kg), lean pork (10 kg) and pork fat (2 kg), dehydrated natural sheep casings (stored in salt and rehydrated in water prior to use) (22 mm diameter, Freddy Hirsch), salt and ground black pepper were purchased for droëwors preparation. The lean meat and fat were cut into cubes (2 x 2 cm) and a 100 g representative sample of each (lean beef, lean pork, beef fat, pork fat) was taken, blended (Ampa Cutter CT 35 N, Golasecca, Italy) and analysed in duplicate for moisture and fat content.

4.2.2 Droëwors preparation

Thereafter, 12 separate 2 kg batches of beef (n=6) and pork (n=6) droëwors were prepared. They consisted of 2% salt, and 0.5% pepper combined with meat / fat mixture made from 80% lean beef and 20% beef fat for beef batches and 85% lean pork and 15% pork fat for pork batches. No antioxidants were used in the preparation of the droëwors. For each batch, the lean meat, fat, salt and pepper were mixed by hand. The mixes were then minced through a 5 mm grinder. Casings were filled with the minced mixtures and dried in an environmentally controlled chamber (Airmaster, Reich, Schechingen, Germany) set at 30 °C and 40% relative humidity for 48 h. The batches were equally represented on each tier in the drier. The weight of each batch was recorded before and after drying to monitor the weight loss.

4.2.3 Droëwors storage

Uncovered and unpackaged, the droëwors were stored in an environmentally controlled Stagionelli chamber (Stagionelli, Italy) set at 25 °C and 50% relative humidity for a period of 26 days. Weight of each batch was also recorded during storage.

4.2.4 Droëwors sampling

A representative 50 g sample was taken from each bath of droëwors at day 0 (before drying) and day 5 (after drying and 3 days of storage) for analysis of moisture, protein and salt content, a_w and pH. A representative 35 g sample was taken from each batch on days 12, 21 and 28 and analysed for moisture content, a_w and pH. Lipid oxidation was determined on all samples. Samples were cut into small cubes, grinded using a Knifetec™ 1095 Mill (FOSS, Höganäs, Sweden) and stored under vacuum at -20 °C for proximate composition, salt content, a_w and pH; and -80 °C for lipid oxidation.

4.2.5 Physico-chemical analysis

Moisture and salt content and fat content of raw materials were determined according to the procedures outlined in Section 3.2.2, 3.2.3 and 3.2.4 respectively. Protein content was determined as described in Section 3.2.2 except that the nitrogen content was determined on whole samples and not on dried and un-defatted ones. Due to the low repeatability of the fat content measurement on pork fat and beef fat and droëwors at day 0, fat content of droëwors was calculated by subtracting moisture, protein and ash contents from 100. All analysis were done in duplicate.

4.2.6 Lipid oxidation

Lipid oxidation was analysed in duplicate by measuring the TBARS by a modified acid-precipitation method. Two grams of each sample were weighed in triplicate into 50 mL tubes, 6.25 mL trichloroacetic acid (TCA, 0.001M) and 6.25 mL distilled water (dH_2O) were added and samples were homogenised (Ultraturax) for 20 sec. Slurry was left to filter through a Wattman n^o1 filter paper. From a stock solution of 1,1,3,3-Tetramethoxypropan (TMP, 0.001M), a standard curve was prepared in duplicate by adding 0, 5, 10 and 20 µL TMP in 1 mL of dH_2O. Three tubes were allocated to each sample and 1 mL of filtered slurry was added to each tube. One millilitre of TBA was added to each standard and to 2 tubes for each

sample, while 1 mL of dH_2O was added to the third sample tube to act as a turbidity blank. All tubes were capped, vortexed and incubated in a water bath at 70 °C for 1 h. Thereafter, samples were allowed to cool, 200 µL was pipetted into a microwell plate and the absorbance was read at 530 nm. TBARS were expressed in mg of malonaldehyde (MDA)/kg dry matter (DM).

4.2.5 Statistical analysis

Data on physico-chemical properties and TBARS of beef and pork droëwors were analysed using PROC GLM procedures of SAS (2003) and pair wise comparisons of least square means were done using PDIFF. Differences were significant at $P < 0.05$. The statistical models used were:

$$Y_{ij=} \mu + \alpha_{i+} e_{ij}$$

Where Y_{ij} = dependant variable (moisture, fat)

μ = overall mean

α_i= i^{th} effect of raw material (lean beef, beef fat, lean pork, pork fat)

e_{ijkl} = random error.

$$Y_{ijkl=} \mu + \alpha_i + \beta_{j+} \alpha\beta_{k+} e_{ijkl}$$

Where Y_{ijkl} = dependant variable (weight loss, moisture, fat, protein, ash, salt, a_w, pH, TBARS)

μ = overall mean

α_i= i^{th} effect of meat type (beef, pork)

β_j = j^{th} effect of day (0, 2(for weight loss), 5, 12, 21, 26)

$\alpha\beta_k$ = kth effect of meat type by day

e_{ijkl} = random error.

4.3 Results and discussion

The moisture and fat content of the raw materials used to produce beef and pork droëwors (lean beef, beef fat, lean pork and pork fat) are presented in Table 4.1. Lean beef and lean pork had the same ($P > 0.05$) moisture content and similar ($P > 0.05$) fat content. The moisture and fat content of the pork fat and beef fat was significantly different, with high variations of fat content. These variations were of the same order than the variations between duplicates analyses. Fat contents of duplicates of droëwors before drying were also not repeatable. Inaccuracies in the chloroform methanol extraction could be attributed to evaporation of chloroform during homogenisation, causing an over estimation of fat content in the final value. Hence, this method of fat extraction may not be suitable for fat tissues. According to Habeck *et al*. (2013), differences can arise in the determination of total fat; dependant on the level of fat in the meat and whether it is cooked or uncooked. Pork fat contained a higher percentage of fat (77.1 g/100 g) and lower percentage of moisture (12.7 g/100 g) than beef fat (51.4 g/100 g fat and 19.4 g/100 g moisture). On the basis of these findings, a lower ratio lean meat to fat was used in pork droëwors (85:15) than in beef droëwors (80:20) in order to attain similar fat content in the beef and pork droëwors.

Beef and pork droëwors showed similar ($P > 0.05$) percentage weight loss during drying (50.4% and 48.2% respectively) (Figure 4.1.). Further loss of weight was recorded during storage at a similar ($P > 0.05$) rate in both droëwors types with a weight loss reaching 67.5 and 68.5% respectively for beef and pork droëwors at the end of storage (day 26). Jones *et al*. (2015a) reported similar weight loss percentages (45-50 %) during drying in blesbok, springbok and fallow deer droëwors, using close drying conditions (30 °C and 30% relative humidity for 48 h). These findings are an indication that the loss of moisture during drying and storage is not influenced by the type of meat. On day 0 (before drying) and day 5 (after drying and 3 days of storage), beef and pork droëwors had similar ($P > 0.05$) proximate

Table 4.1 Fat and moisture content of raw materials (ls mean ± standard error)

Raw material	Moisture (g/100 g)	Fat (g/100 g)
Lean beef (n=3)	$70.2^{a} \pm 1.25$	$4.9^{c} \pm 0.51$
Lean pork (n=3)	$70.2^{a} \pm 0.96$	$5.9^{c} \pm 1.03$
Beef fat (n=3)	$19.4^{b} \pm 0.64$	$51.4^{b} \pm 11.24$
Pork fat (n=3)	$12.7^{c} \pm 0.73$	$77.1^{a} \pm 2.60$

^{abc} Means in the same column with different superscripts are significantly different ($P < 0.05$)

composition salt content and a_w (Table 4.2). This result is consistent with the methodology followed. Proximate composition, salt content and a_w of the droëwors at day 5 were comparable to that of the commercial droëwors analysed in Chapter 3. The protein, fat, ash and salt content were higher on day 5 than day 0 due to the reduction in moisture and are consistent with the percentage of weight lost during drying and 3 days of storage (55.2% and 53.9% in beef and pork droëwors respectively). Moisture loss during drying automatically results in the concentration of dry solids (Santchurn *et al.*, 2012).

Protein, fat, ash and salt content were not measured on day 12, 21 and 26, because they were expected to only change in concentration due to changes in moisture content. The moisture content and the a_w of all droëwors significantly decreased from day 0 to day 21. From day 12, the moisture content and the a_w were lower than the minimal values recorded in Chapter 3. Nortjé et al. (2005) reported that in biltong, a similar and more widely studied South African dried meat, preferences have been noted for "moist" biltong, which is more tender and less dehydrated. While the continued reduction of water promotes inhibition of microbial spoilage during storage, excessive loss of moisture compromises consumer acceptability and satisfaction of the product. It could be recommended that when prolonged storage (> 12 days) is required, protective packaging material that prevents moisture loss should be used. The pH of pork droëwors was significantly higher ($P < 0.05$) than beef droëwors from day 0 to day 26. This could be attributed to the fact that pigs are more susceptible to stress at slaughter than cattle, hence pork is more likely to have higher ultimate pH values (Gajana *et al.*, 2013). The pH of beef droëwors was fairly stable throughout the storage period, while higher on days 12 and 26 for beef and lower at day 21 for pork.

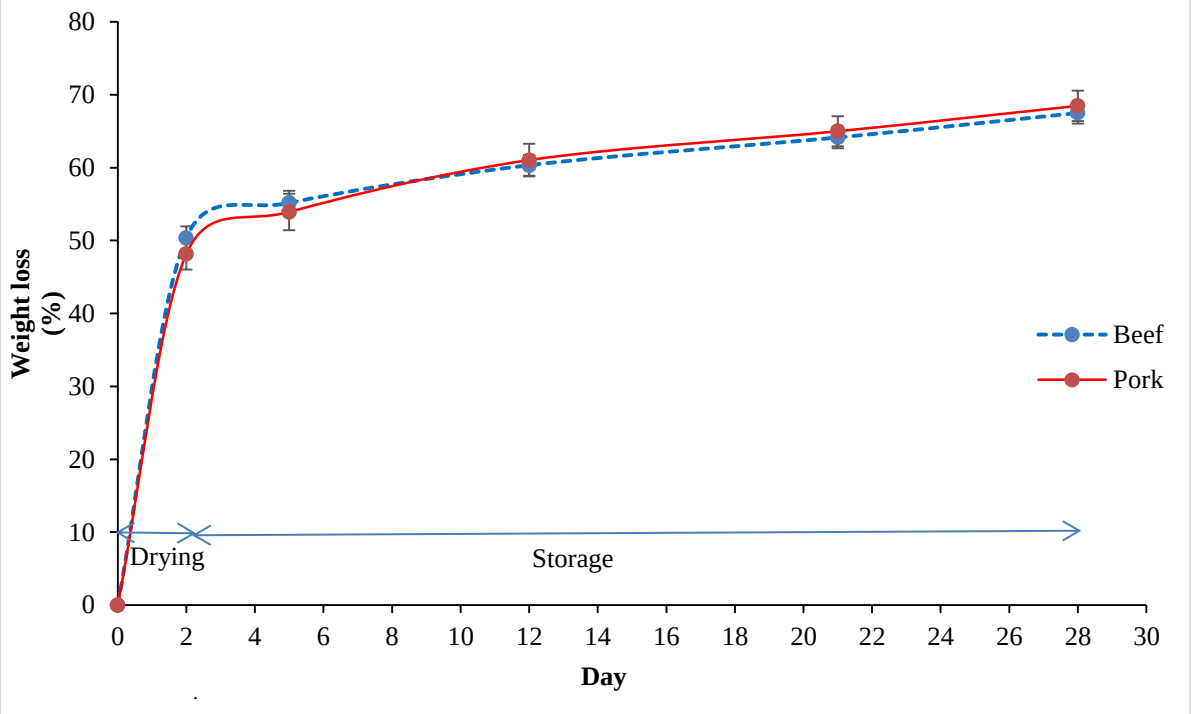


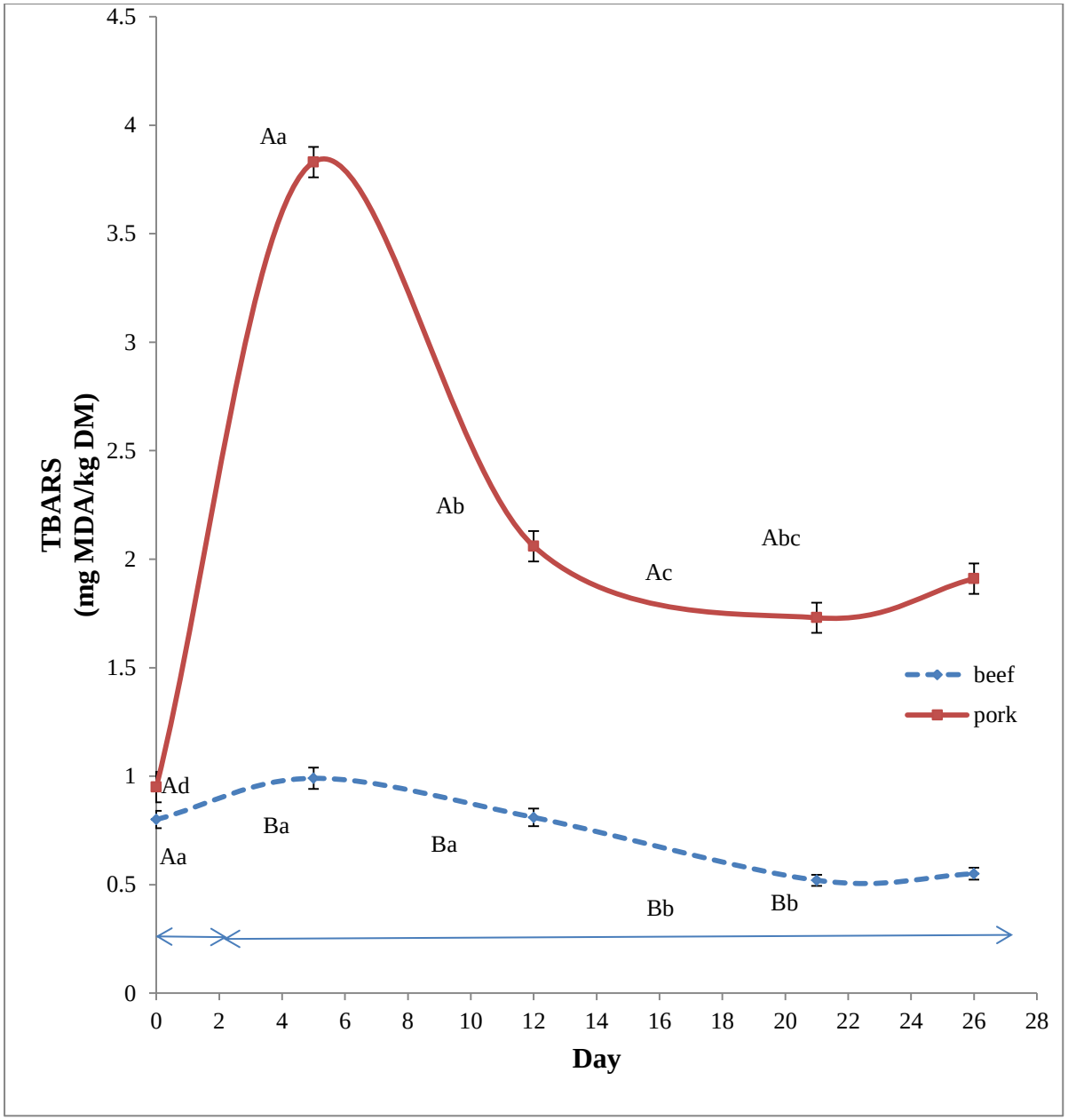
Figure 4.1 Weight loss of beef and pork droëwors during processing and storage

Table 4.2 Physico-chemical characteristics of beef and pork droëwors during processing and storage (ls means ± standard error)

Physico-chemical characteristics	Day 0*	Day 5**	Day 12**	Day 21**	Day 26**
Moisture					
Beef (n=6)	62.6^{Aa}± 0.19	18.3^{Ab}± 0.83	9.2^{Ac}± 0.30	6.9^{Ad}± 0.12	6.8^{Ad}± 0.05
Pork (n=6)	63.2^{Aa}± 0.56	23.6^{Ab}± 0.56	10.5^{Ac}± 0.32	7.7^{Ad}± 0.33	7.1^{Bd}± 0.09
Protein					
Beef (n=6)	18.7^{Ab}± 0.33	42.4^{Aa}± 0.42	-	-	-
Pork (n=6)	18.3^{Ab}± 0.28	41.0^{Aa}± 1.22	-	-	-
Fat[$]					
Beef (n=6)	15.9^{Ab}± 0.39	32.8^{Aa}± 1.02	-	-	-
Pork (n=6)	15.7^{Ab}± 0.78	29.5^{Aa}± 1.31	-	-	-
Ash					
Beef (n=6)	2.8^{Ab}± 0.03	6.5^{Aa}± 0.28	-	-	-
Pork (n=6)	3.0^{Ab}± 0.06	5.9^{Aa}± 0.18	-	-	-
Salt					
Beef (n=6)	2.0^{Ab} ± 0.01	4.4^{Aa} ± 0.10	-	-	-
Pork (n=6)	2.0^{Ab} ± 0.05	4.2^{Aa} ± 0.08	-	-	-
aW					
Beef (n=6)	0.98^{Aa}± 0.001	0.72^{Ab}± 0.014	0.55^{Ac}± 0.007	0.49^{Ad}± 0.005	0.48^{Ad}± 0.003
Pork (n=6)	0.98^{Aa}± 0.001	0.81^{Bb}± 0.009	0.60^{Bc}± 0.005	0.54^{Bd} ± 0.009	0.50^{Be}± 0.004
pH					
Beef (n=6)	5.2^{Ab}± 0.04	5.2^{Ab}± 0.01	5.5^{Aa}± 0.04	5.2^{Ab}± 0.01	5.4^{Aa}± 0.01
Pork (n=6)	5.7^{Ba}± 0.03	5.6^{Bab}± 0.05	5.6^{Ba}± 0.04	5.5^{Bb}± 0.04	5.7^{Ba}± 0.04

Moisture, protein, fat, ash and salt contents are expressed in g/100 g;
[AB] For each characteristic, means in the same column with different superscripts are significantly different ($P < 0.05$);
[abcde] Means in the same row with different superscripts are significantly different ($P < 0.05$);
- Not measured;
* Fresh droëwors;
** Droëwors was dried for 2 days before storage;
[$] Calculated from 100- (moisture + protein + ash contents)

The TBARS of beef and pork droëwors during processing and storage are presented in Figure 4.2., expressed on the basis of dry matter content due to continual loss of moisture during drying and storage. Before drying, the TBARS were similar ($P > 0.05$) in both beef and pork droëwors. In pork droëwors, TBARS increased ($P < 0.05$) from day 0 to day 5 (after drying and 3 days of storage) and then decreased. The TBARS in beef droëwors did not increase ($P > 0.05$) from day 0 to day 5 and decreased to be lower ($P < 0.05$) on days 21 and 26 compared to day 0 and 5. Although there is no comparable data in literature on TBARS of beef droëwors, droëwors are expected to undergo oxidation during drying (Jones *et al.*, 2015a). In pork droëwors, the initial increase in TBARS value with time can be explained by the depletion of endogenous antioxidants and the continued exposure to aerobic conditions (Mungure *et al*., 2016). Thereafter TBARS seemed to react with other compounds to form complexes; which rendered a lower total TBARS number on days 12, 21 and 26. Other studies have attributed the decline in TBARS to the degradation of MDA; or to further reactions between the aldehydes and/or free amino acids released from muscle proteins during processing (Antequera *et al.*, 1992; Wu *et al.*, 2016). At day 5, TBARS were higher ($P < 0.05$) in pork droëwors (3.83 mg MDA/kg dm) than in beef droëwors (0.99 mg MDA/kg dm) and continued to be consistently higher up to day 26. This occurred in spite of the fact that the beef and pork droëwors had a similar fat content. The significantly lower TBARS in beef droëwors compared to pork droëwors may be attributed to the use of beef fat, which may have a more saturated fat profile making it less susceptible to oxidation. The higher level of oxidation in pork is consistent with reports that pork is susceptible to rancidity because of its polyunsaturated fat profile (Wood *et al.*, 2003). Increasing the concentration of n-3 PUFA in muscles can result in a significant increase in TBARS (Pouzo *et al.*, 2016).



[AB] For each sampling point, means with different superscripts are significantly different ($P < 0.05$)
[abcd] Along each series, means with different superscripts are significantly different ($P < 0.05$)

Figure 4.2 TBARS of beef and pork droëwors during processing and storage

Jones *et al*. (2015a) reported TBARS ranging from 1.1 to 2.4 mg/kg on dry mass basis in game droëwors made with sheep fat. In another study in which beef fat was used, TBARS ranged from 1.5 to 2.1 mg/kg dm after drying and 1.6 to 3.8 after 2 weeks of storage (Jones *et al*., 2015b). Hoffman *et al.* (2014) reported values up to 7.99 mg/kg meat in ostrich droëwors made with pork fat and dried for 2 weeks of drying. The TBARS in this study did not reach that extent, which may be attributed to the shorter drying period used in this study. Literature on lipid oxidation of other beef salted/dried meat products include kilishi with TBARS increasing from 1.5 to 2 mg MDA/kg of meat during 60 weeks of storage (Igene *et al.*, 1990), beef charqui reaching 4.5 mg MDA/kg of meat after drying (Torres *et al*., 1994). On pork dry sausages, which have a closer fat content to droëwors, TBARS values often less than 1 mg/kg meat, sometimes up to 2 mg/kg, are reported (Liaros *et al.*, 2009; Baka *et al.*, 2011; Gómez *et al.*, 2013; Lorenzo *et al.*, 2013). These lower values could be explained by the use of nitrites / nitrates and/or lower drying temperatures than in droëwors processing. According to Campo *et al*. (2006) and Suman *et al.* (2010), rancidity is detectable when TBARS values are higher than or equal to 2 mg MDA/kg on fresh meat. As the TBARS values of pork droëwors were higher than this, it verifies the unpublished claims that pork is not usually used in droëwors production because it is prone to rancidity. On the basis of these results, further research on the efficacy of antioxidants to inhibit the rate of lipid oxidation in pork droëwors is warranted.

4.4 Conclusion

The results showed pork droëwors underwent significant oxidation during drying. Lipid oxidation was significantly higher in pork droëwors than beef droëwors after drying and throughout the 26 days of storage. Since proximate analysis revealed that beef and pork droëwors contained the same level of fat before and after drying, the higher level of oxidation in pork is consistent with reports that the higher PUFA content of pork makes it more susceptible to rancidity. As no antioxidants were added to the droëwors in this study, further research on the use of antioxidants to reduce the extent of oxidative deterioration in pork droëwors is recommended.

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Chapter 5 Antioxidant activity of *Moringa oleifera* leaf powder *in vitro* and in pork droëwors

Abstract

The study assessed the effect of different levels of *Moringa oleifera* leaf powder (MLP) on antioxidant activity and lipid oxidation in the processing and short term storage of pork droëwors. The total phenolic compounds (TPC), *α*-tocopherol and *β*-carotene content of MLP were determined. Four batches of pork droëwors, containing 0 (M0), 0.5 (M0.5), 1 (M1) and 2 (M2) g/100 g of MLP were produced (dried at 30 °C and 40% relative humidity for 72 h) and sampled during drying (0, 1.5, 5.75, 27.25, 72 h) and after 7 days (168 h) of storage at ambient conditions. Antioxidant activity of the MLP enriched droëwors was determined by the ferric reducing antioxidant power (FRAP) assay, lipid oxidation was measured by analysis of thiobarbuturic acid reactive substances (TBARS), and tocopherol (*α*- and *γ*-) content and *β*-carotene content of the product were quantified by high performance liquid chromatography (HPLC). *Moringa oleifera* leaf powder was found to contain high levels of TPC (7.5 ± 0.2 mg gallic acid eq/g) and substantial levels of, *α*-tocopherol (76.7 ± 1.9 mg/100 g) and *β*-carotene (23.2 ± 2.8 mg/100 g). The FRAP, *α*-tocopherol and *β*-carotene content of pork droëwors increased proportionally with increasing levels of MLP inclusion; while γ-tocopherol content was not significantly affected, indicating that this isomer was not provided by MLP The TBARS values were significantly higher in M0 after 27.25 h of drying until the end of the 7 day storage period. There were no significant differences between TBARS of M0.5, M1 and M2 during drying and storage. Inclusion of MLP significantly increased antioxidant activity and content of antioxidant vitamins in pork droëwors. Lipid oxidation occurred more rapidly when MLP was not added and was similar in all MLP

treatments despite their concentrations indicating that MLP inclusion at 0.5% is sufficient to inhibit lipid oxidation.

5.1 Introduction

Lipid oxidation is the main non-microbial cause of quality deterioration in muscle foods; especially in meat and meat products containing high levels of fat, or more specifically, high levels of polyunsaturated fatty acids (PUFAs) (Aalhus and Dugan, 2004). Hydroperoxides, the primary products of PUFA oxidation, undergo further oxidation to produce secondary oxidation products such as aldehydes (e.g malonaldehyde and hexanal); which characteristically have strong flavours and odours (Papastergiadis *et al.*, 2012). Consequently, lipid oxidation can result in the development of off odours, off flavours and deterioration of colour, texture and nutritive value of meat and meat products (Kanner, 1994).

In muscle tissues, when the generation of reactive oxygen species is in excess of endogenous antioxidant barriers, oxidation has a deteriorative effect at molecular/cellular level (Falowo *et al.*, 2014). As a result, meat lipids are susceptible to oxidative damage because endogenous antioxidants within the muscles are rapidly depleted at slaughter (Xiao *et al.*, 2013). In order to counter this, formulations containing synthetic antioxidants (such as butylated hydroxyanisole, butylated hydroxyl toluene, tertiary butlylhydroquinone) are commonly used in meat processing to reduce the detrimental effects of lipid oxidation on product quality (Shahidi and Wanasundara, 1992; Jones *et al.*, 2015). However, synthetic additives used in meat processing have been implicated with human health risks and may have carcinogenic effects (Clayson *et al.*, 1986 in Estévez *et al.*, 2006). The recent International Agency for Research on Cancer (IARC) report on the carcinogenicity of processed meat (Bouvard *et al.*, 2015) has increased consumer concern about the safety of synthetic additives (Jayawadarna *et al.*, 2015); validating research for suitable natural alternatives.

Moringa oleifera leaves are reportedly a rich source of natural antioxidants (Siddhuraju and Bekker, 2003); and studies on its use as a functional additive in livestock feed has been

effective in reducing lipid oxidation in meat (Qwele *et al.*, 2013; Moyo *et al.*, 2012; Nkukwana *et al.*, 2014). In pigs, however, the dietary inclusion of *Moringa oleifera* leaves has been reported to negatively affect feed conversion efficiency due to the presence of anti-nutritional factors (Mukumbo *et al.*, 2014). *Moringa oleifera* leaves are highly nutritious and safe for human consumption, raising the question of whether direct incorporation of *Moringa oleifera* leaf powder (MLP) into pork droëwors during processing could effectively decrease lipid oxidation in the product. This study aimed to quantify the antioxidant compounds in MLP and to determine the effect different MLP levels on antioxidant activity and lipid oxidation during the processing and short term storage of pork droëwors.

5.2 Materials and methods

5.2.1 Antioxidant activity of *Moringa oleifera* leaf powder

Moringa oleifera leaf powder (MLP) of Senegalese origin (Racines, France), processed by naturally air drying fresh Moringa leaves under shade before grinding through a 2 mm sieve screen, was purchased for the study. All chemicals used in the study were of analytical grade (Sigma-Aldrich, St. Louis, USA). The moisture and dry matter content of the MLP was determined according to the procedures outlined in Section 3.2.2.

5.2.1.1 Total phenolic compounds

Extraction and analysis of total phenolic compounds (TPC) was conducted according to procedures detailed in ISO 14502-1:2005(E), with modifications. The standard curve was prepared using galic acid and results were reported as mg gallic acid equivalent per gram of DM. Two hundred mg of MLP (in triplicate) was vortexed with 5 mL of pre-heated aqueous methanol (70%), incubated in a water bath at 70°C for 10 min, allowed to cool to room temperature and centrifuged at 4000 rpm for 5 min. The supernatant from each extraction tube was carefully decanted and the extraction procedure was repeated on the residues. The 2 extracts were combined and extraction solution was added to bring the volume up to 10 mL. Extracts were diluted (1 mL extract in 20 mL distilled H_2O) and 1 mL of diluted extract was added to 1 mL distilled H_2O, 5 mL Folin-Ciocalteu phenol reagent and 4 mL sodium carbonate solution, vortexed and allowed to stand for 60 min at room temperature before the absorbance was read at 760 nm.

5.2.1.2 α-tocopherol analysis

To 100 mg of MLP (in triplicate), 1 ml NaCl 0.9 % was added and vortexed. Ethanol (with 1% pyrogallol) and saturated KOH was added, samples were placed at 70 °C for 30 min, and then extracted twice with n-hexane. The organic phases were collected, evaporated under

nitrogen and diluted in ethanol for HPLC analysis. Separation was performed with a C-18 reverse phase column and mobile phase ethanol:methanol (60:40), at a flow rate of 1 ml/min Fluorescent detection was done at 296-330 nm excitation and emission respectively. Standard curves were performed using commercial standards.

5.2.1.3 β-carotene analysis

To 100 mg of MLP (in triplicate), 3 mL of distilled water was added and stirred with a magnetic stirrer for 2 min. Twenty five mL of a mixture of ethanol and hexane (40:30) with 0.1% BHT was added and agitated for 4 min before filtering through a sintered filter. The residue was re-extracted twice using the same procedure. The totality of the extract was transferred to a separatory funnel and 50 mL of NaCL 10% was added. The mix was agitated and the organic phase washed with 40 mL of water. The aqueous phase was removed and the yellow organic phase was evaporated to dryness and dissolved in dichloromethane:methanol: MTBE and injected in an HPLC system with a C-30 reverse column. Detection was performed at 450 nm.

5.2.2 Droëwors production

Two kg of lean pork meat and pork fat in the ratio of 80:20 were cut into cubes (2 x 2 cm) and minced together through a 5 mm screen. The mixture was re-weighed and salt (2%) and pepper (0.5%) were thoroughly incorporated to mixture. The mince was separated into 4 batches of 500 g, to which no (M0), 0.5 (M0.5), 1 (M1) and 2 (M2) g/100 g MLP was added. The treatments were minced separately through a 2 mm screen into natural sheep casings (22 mm diameter, Freddy Hirsh) (Figure 5.1) cut into individual portions from each batch, and hung vertically in a drying chamber at 30°C and 40% relative humidity for 72 hrs.

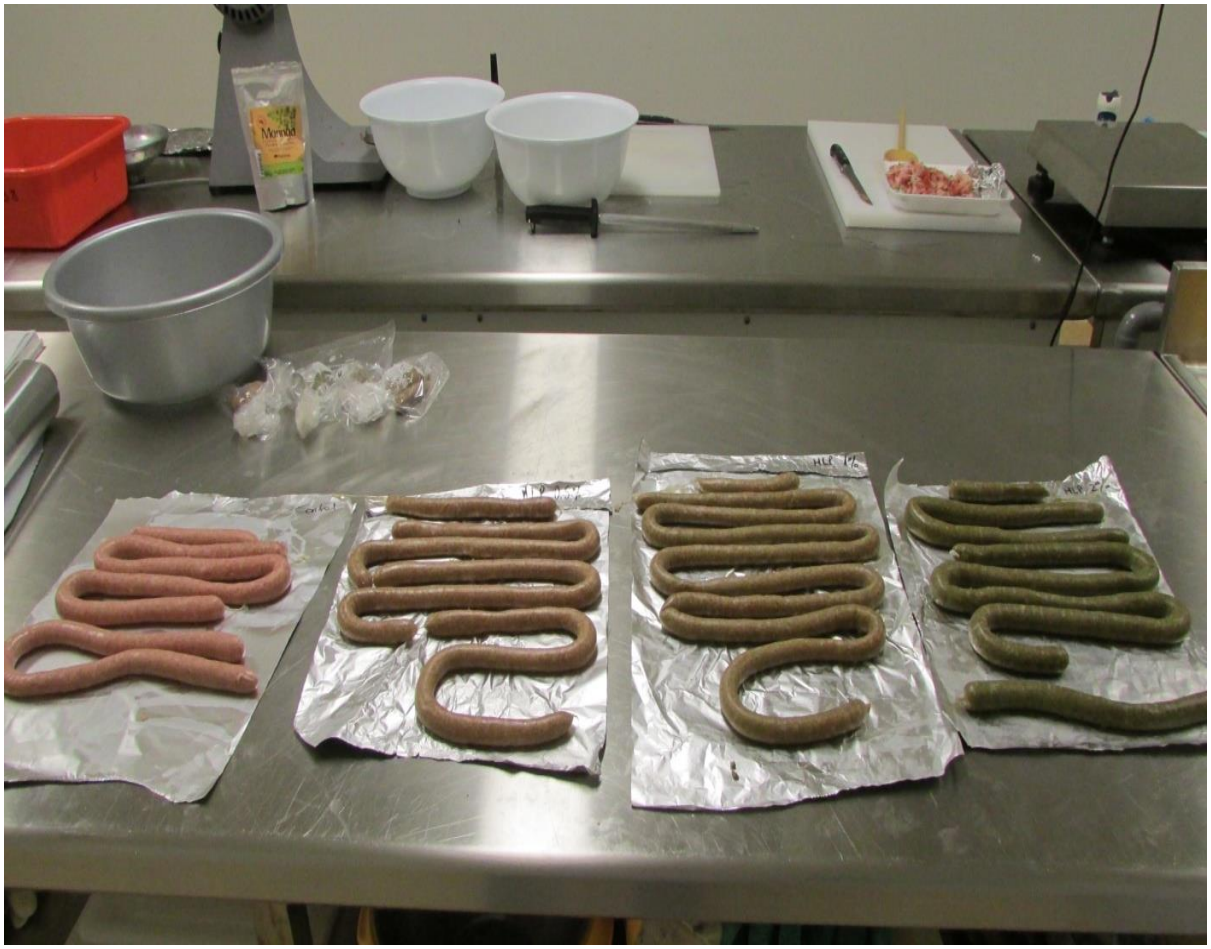


Figure 5.1 Pork droëwors (crude, before drying) containing 0, 0.5, 1 and 2 g/100 g MLP (from left to right)

5.2.3 Sampling and storage

Triplicate samples (± 50 g each) were taken from each batch at 0, 1.5, 5.75, 27.25, and 72 h during drying. The droëwors were stored, unpackaged and uncovered, in a ventilated room under ambient conditions and sampled after 7 days (168 h).

5.2.4 Lipid oxidation

The content of thiobarbituric acid reactive substances (TBARS) was determined by acid precipitation using the technique described by Descalzo *et al.* (2005). Briefly, samples were homogenized with 12.5 mL of TCA [20% trichloroacetic acid in 1.6% metaphosporic acid (HPO_3)] and 12.5 mL distilled water for 180 sec using a Stomacher 400 Laboratory Blender (Seward Medical, London, UK). Slurries were filtered (0.45 µm) and duplicate samples of filtrate (3 mL) were added to an equal volume of 0.02M 2-thiobarbituric acid. An equal volume of distilled water was added to the third replicate to act as a turbidity blank for each sample. Samples were vortexted for 10 sec, incubated in a water bath at 70°C for 1 hrs until pink colour development, allowed to cool for 30 min and the absorbance was read at 532 nm. TBARS were calculated using 1,1,3,3-tetraethoxypropane (TEP) as a standard. Results were expressed as mg of malonaldehyde (MDA) equivalents/kg DM.

5.2.5 Ferric iron reducing power

Analysis of FRAP was analysed at each sampling point. Analysis was done according to the procedure of Pouzo *et al.* (2016), with modifications. Triplicate 1 g ground samples taken from each batch of droëwors were extracted in 5 mL KOH buffer (pH 7.2) homogenised (Ultraturrax, IKA, Germany) for 1 min in. Homogenates were centrifuged at 10 000 rpm, for 5 min. The supernatant was collected and 50 µL was diluted in 150 µL distilled water and 1.8 mL FRAP reagent, made by mixing an equivalent volume of 300 mM acetate buffer (pH 3.6), 20 mM $FeCL3.6H_2O$ with 10 mM TPTZ in 40 mM HCl, and absorbance read at 593 nm. The

FRAP activity of the samples was measured against a calibration curve made with ferrous sulphate (Fe2SO4·7H2O) within the range from 100 to 1000 μM and results were expressed as Fe^{2+} equivalent in μM.

5.2.6 Tocopherols and *β*-carotene content of pork droëwors

Tocopherols (α- and γ-) and *β*-carotene were analysed on day 0 and day 7. For tocopherol analysis, the extraction procedure was adapted from Descalzo *et al.* (2005). Triplicate 2 g ground droëwors samples from each batch were added to 4 mL NaCl 0.9% in a conical tube and homogenized for 30 s at 3000 rpm with an Ultraturrax T25 (IKA, Germany). Aliquots of 1 g homogenate were placed into a screwcap test tube with 3 mL of ethanol with 1% pyrogallol to prevent oxidation during the extraction. Thereafter, 3 mL of 10 N KOH in water was added to each tube for saponification. The tube contents were mixed by vortexing for 2 min, and placed in a water bath at 70 °C for 30 min, with agitation. After cooling, 5 mL NaCl (1 N) was added to each tube. Following the addition of 10 mL n-hexane, the samples were mixed by vortexing for 2 min; the upper hexane layer was then transferred into a new screw cap tube and the aqueous phase was re-extracted with 10 mL of hexane. The combined extracts were taken to dryness under a dry nitrogen gas stream, and the residue was dissolved in 500 μL of absolute ethanol. Calibration curves were performed with DL-α-tocopherol (Merck, Darmstadt, Germany), γ-tocopherol, *β*-carotene. All samples and standards were analyzed by reverse phase high performance liquid chromatography (HPLC). For tocopherols, separation was performed with a C-18 reverse phase column and mobile phase ethanol:methanol (60:40). Fluorescent detection was done at 296-330 nm excitation and emission respectively. For *β*-carotene, a C-30 reverse column was used and detection was performed at 450 nm.

5.2.7 Statistical analysis

Data were analysed using PROC GLM procedures of SAS (2003). Pair wise comparisons of least square means were done using t-tests (PDIFF option). Differences were significant at $P < 0.05$. For TPC, α-tocopherol and β-carotene content of MLP; the model used was:

$$Y_{i=} \mu + e_i$$

Where Y_i = dependant variable (TPC, α-tocopherol, β-carotene content)

μ = overall mean

e_i= random error.

For FRAP, TBARS, α-tocopherol, γ-tocopherol and β-carotene content of MLP enriched pork droëwors, the main effects of MLP level, time and their interaction were included in the model:

$$Y_{ijkl=} \mu + \alpha_i + \beta_j + \alpha\beta_k + e_{ijkl}$$

Where Y_{ijkl} = dependant variable (TPC, α-tocopherol, γ-tocopherol, β-carotene, FRAP, TBARS)

μ = overall mean

α_i= i^{th} effect of MLP level (0, 0.5, 1 and 2 g/100 g)

β_j = j^{th} effect of time (0, 1.5, 5.75, 27.75, 72, 168 h)

$\alpha\beta_k$ = k^{th} MLP level by time effect

e_{ijkl} = random error

5.3 Results and discussion

The TPC, α-tocopherol and β-carotene content of MLP is presented in Table 5.1. While several studies have reported on antioxidant compounds in MLP, it should be noted that variations in the quantities of antioxidant compounds often occur and can be attributed to several factors; including plant variety, stage of maturity, climatic conditions, post-harvest handling, processing, storage, and differences in the agrological zones in which it is grown (Siddharuja and Bekker, 2003; Sreelatha and Padma, 2009). For this reason, it was important to quantify the antioxidant compounds in the MLP being used in this study, as it was of Senegalese origin and there is presently no literature available on the antioxidant compounds in *Moringa oleifera* of Senegalese origin. The TPC of the aqueous methanol extracts of MLP in this study (7.5 mg gallic acid eq/g) is higher than the TPC reported in ethanolic extracts (4.6 – 5.6 mg gallic acid eq/g) and lower than TPC reported in aqueous extracts (8.6 – 9.7 mg gallic acid eq/g) of MLP (Wangcharoen and Gomolmanee, 2011). This is expected, as differences in the TPC can arise from the use of different extraction solutions. The mean α-tocopherol (76.7 mg/ 100 g) and β-carotene (23.2 mg/100 g) content of MLP this study was comparable to the vitamin E (77 mg/100 g) and higher than the β-carotene (18.5 mg/100 g) content reported by Moyo *et al.* (2011). *Moringa oleifera* leaves are frequently reported to contain high levels of α-tocopherol and β-carotene and this is consistent with the results found in this study.

The evolution of TBARS on a dry matter basis for each treatment are presented in Figure 5.2. TBARS values of all treatments were similar ($P > 0.05$) in the initial phase of drying from 0 to 5.75 hrs. An unusual peak was recorded for M0 at 1.5 h, which may be attributed to sampling error; as the TBARS decreased again 5.75 h. An increase in TBARS at 27.25 h in M0 (0.42 mg MDA/kg dm) signified the onset of lipid oxidation, while TBARS remained lower

Table 5.1 Total phenolic content, α-tocopherol and β-carotene content of aqueous methanol of *Moringa oleifera* leaf powder*

Antioxidant component	**Unit**	**means ± standard error**
***As is* basis**		
TPC	(mg gallic acid eq/g)	7.5 ± 0.2
α-tocopherol	(mg/100 g)	76.7 ± 1.9
β-carotene	(mg/100 g)	23.2 ± 2.8
DM ** basis		
TPC	(mg gallic acid eq/g DM)	7.9 ± 0.2
α-tocopherol	(mg/100 g DM)	80.9 ± 1.9
β-carotene	(mg/100 g DM)	24.5 ± 2.8

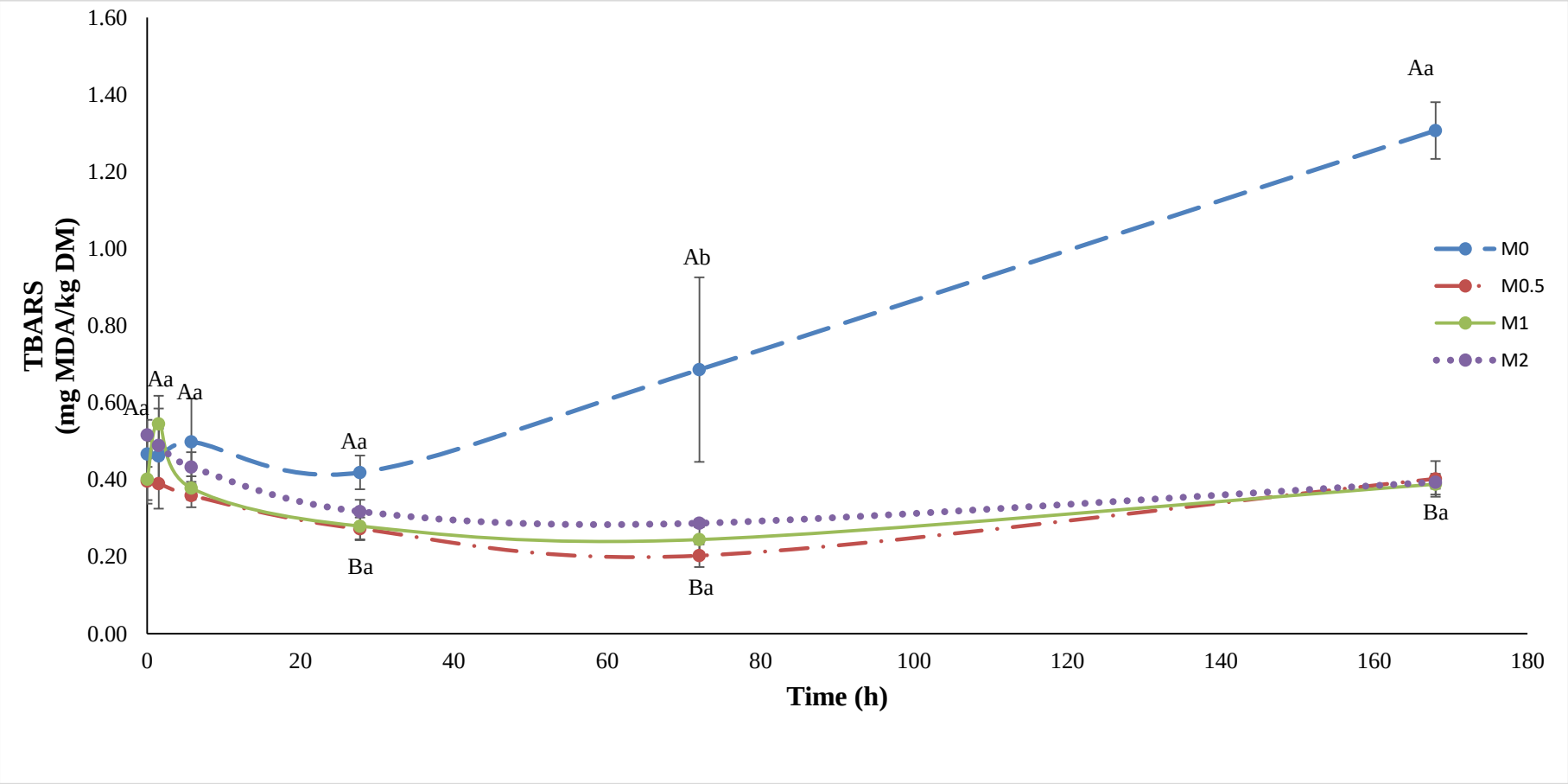
*: Grown and processed in Senegal
TPC: total phenolic compounds, DM: dry matter, **: DM content = 94.86 ± 0.26 g/ 100 g

(P < 0.05) in M0.5 (0.27 mg MDA/kg dm), M1 (0.28 mg MDA/kg dm) and M2 (0.32 mg MDA/kg dm). Throughout the remainder of the drying and storage period, TBARS of M0.5, M1 and M2 at each sampling point were similar (P > 0.05) and consistently lower (P < 0.05) than M0. TBARS values in M0.5, M1 and M2 were similar did not increase significantly across the sampling points and were more than 3 times lower (P<0.05) than that of M0 after 72 h (0.20, 0.24, and 0.29 mg eq MDA/kg DM respectively, vs 0.69 mg eq MDA/kg DM in M0) respectively) of drying and 168 h of storage (0.40, 0.39, and 0.39 mg MDA/kg DM respectively, vs 1.31 mg MDA/kg DM in M0). The lower TBARS values in MLP enriched droëwors may be attributed to the inhibition of lipid oxidation by polyphenols and antioxidant vitamins in MLP. Jayawardana *et al.* (2015) similarly found significantly lower TBARS values in chicken sausages with 0.5, 0.75 and 1% MLP added compared to the control treatment, with no significant differences between the MLP treatments after 1 week of storage. Since no differences were detected in the TBARS of MLP treatments, it may be preferable to use lower levels in order to reduce cost and minimize potential changes in the product appearance (Figure 5.1) and flavour; which will be assessed in further chapters of this study.

Antioxidant activity of pork droëwors increased (P < 0.05) with increasing levels of MLP, as shown in Figure 5.3. During drying, the FRAP of M0 was significantly lower than M0.5 (from 0 – 5.75 h); M1 (from 0 – 27.25 h) and M2 (from 0 h – 72 h). The FRAP decreased over time during drying and storage, showing an inverse tendency with respect to TBARS progression; indicating that the consumption of antioxidants during drying and storage is necessary to prevent lipid oxidation. The increase in antioxidant activity with MLP enrichment can be attributed to increasing levels antioxidant compounds including tocopherols, as presented in Figure 5.4 and Figure 5.5, and phenolic compounds. The α-tocopherol content was significantly higher with increasing levels of MLP inclusion on day 0

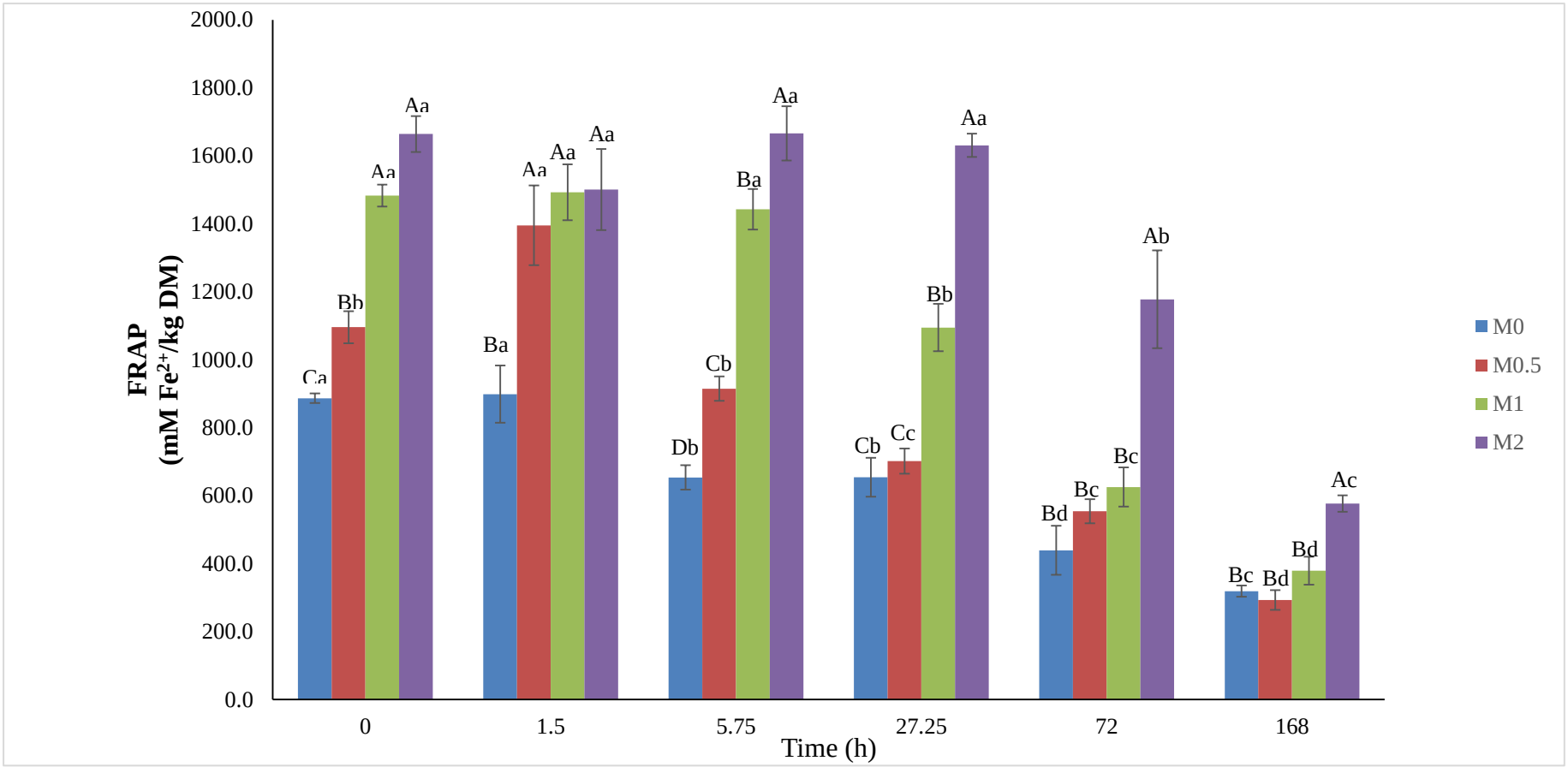
and on day 7. This was expected, as α-tocopherol content would increase proportionally to increasing the content a substance containing α-tocopherol. The reduction in content of α-tocopherol and β-carotene on day 7 can be attributed to their antioxidant action leading to the consumption. Pouzo *et al.* (2016) reported a drastic reduction in tocopherol concentration in beef after aerobic exposure for 5 days as a result of counteractive action towards lipid oxidation. The slope of the linear trend lines show an increasing rate of consumption with increasing levels of MLP inclusion.

There was a difference in behaviour between tocopherol isomers in droëwors. The addition of MLP into the droëwors was directly proportional to the levels of *α*-tocopherol in the samples. However it didn't influence the amounts of *γ*-tocopherol in the same way, as there was no difference (P >0.05) in *γ*-tocopherol between treatments on day 0 and 7. This means that *γ*-tocopherol came rather from the meat through dietary delivery (Descalzo and Sancho, 2008), while *α*-tocopherol came from MLP. There was consumption of *γ*- tocopherol from day 0 to 7, demonstrating that it contributed to the inhibition of lipid oxidation. The gradient of the linear trend lines show that the consumption rate was less than that of α-tocopherol. Both isomers have antioxidant properties. However, from a nutritional point of view, the *α*-tocopherol isomer is the most biologically active form which can be incorporated in mammals when consumed (Chun *et al.*, 2006). *β*-carotene was not detected in M0, while the increase in *β*-carotene content in M0.5, M1 and M2 was proportion to increasing MLP levels. This was expected, as *β*-carotene and other pro-vitamin A carotenoids are ingested with the diet, mainly found in plant sources (Carvalho *et al.*, 2012). Consumption of *β*-carotene was inversely proportional to TBARS values, although at a slower rate than α-tocopherol.



[AB] Means of different treatments with different superscripts at the sample sampling point are significantly different (P < 0.05)
[ab] Means of the same treatment with different superscripts across the sampling points are significantly different (P < 0.05)

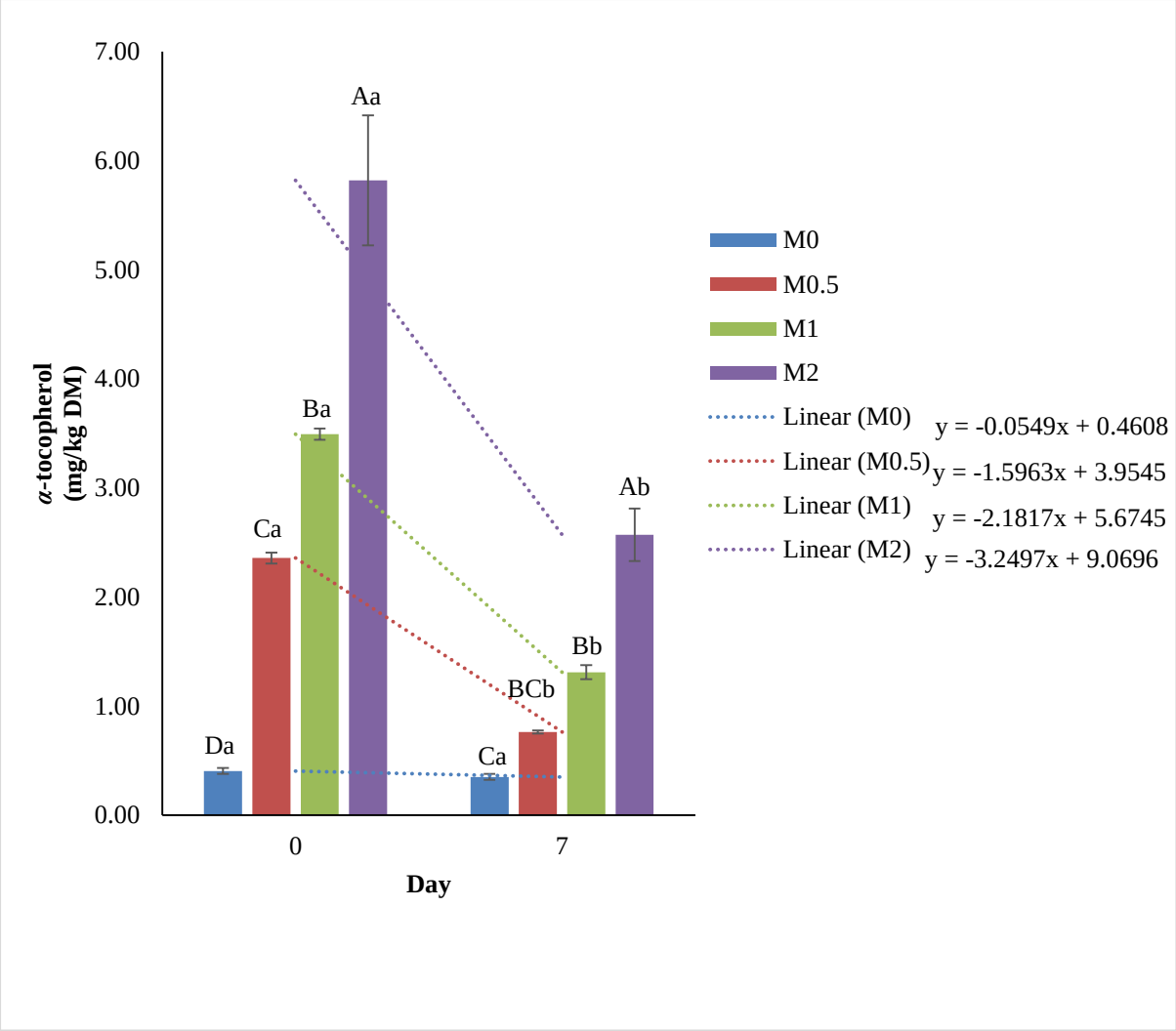
Figure 5.2 TBARS of MLP enriched pork droëwors (n=12) during 72 h of drying and 7 days (168 h) of storage



ABCD Means of different treatments with different superscripts at the sample sampling point are significantly different ($P < 0.05$)

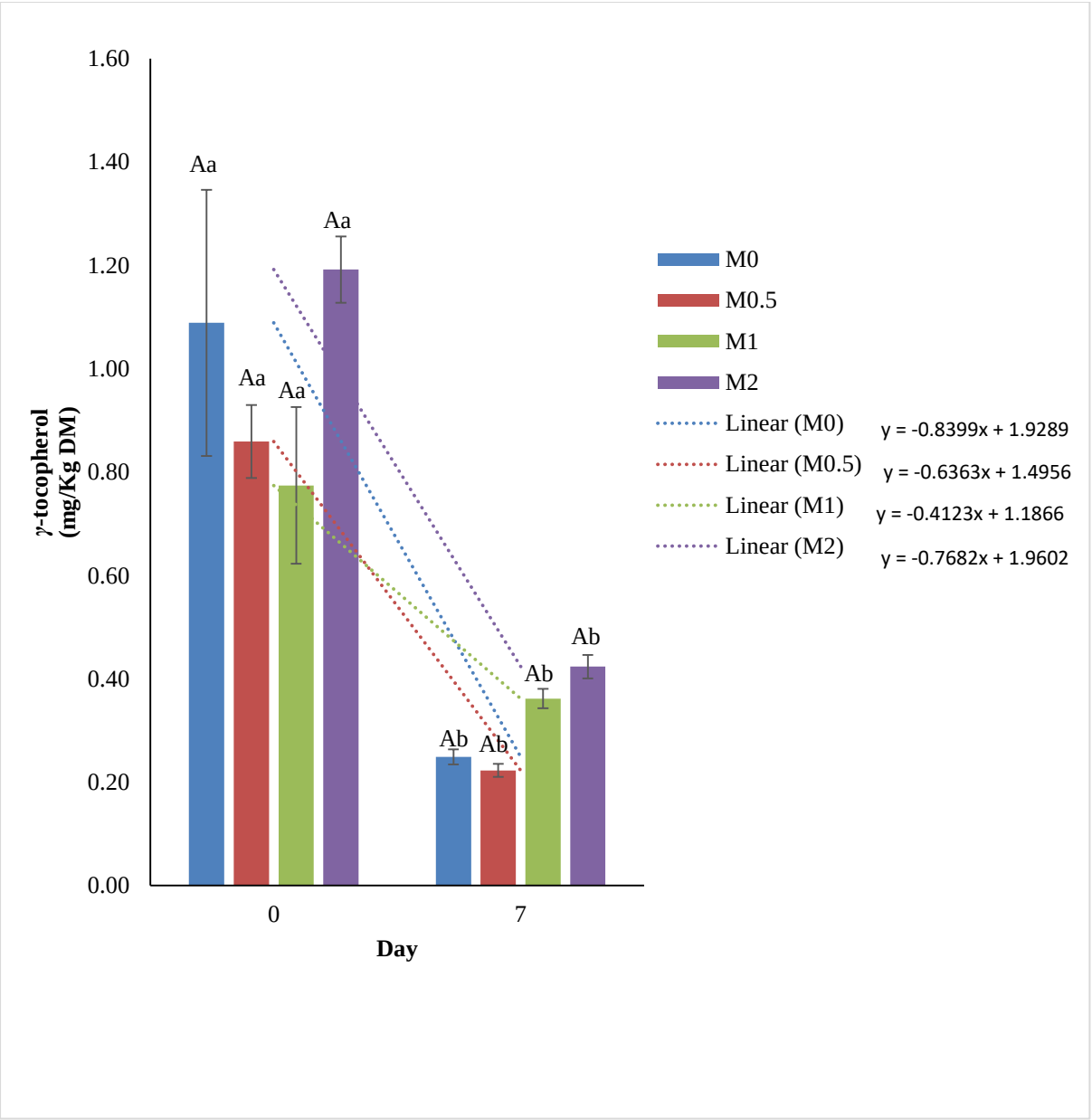
abcd Means of the same treatment with different superscripts across the sampling points are significantly different ($P < 0.05$)

Figure 5.3 Ferric reducing antioxidant power (FRAP) of pork droëwors enriched with 0, 0.5 1 and 2 g/100 g *Moringa oleifera* leaf powder (MLP)



[ABCD]Means of different treatments with different superscripts at the sample sampling point are significantly different (P < 0.05)
[ab]Means of the same treatment with different superscripts across the sampling points are significantly different (P < 0.05)

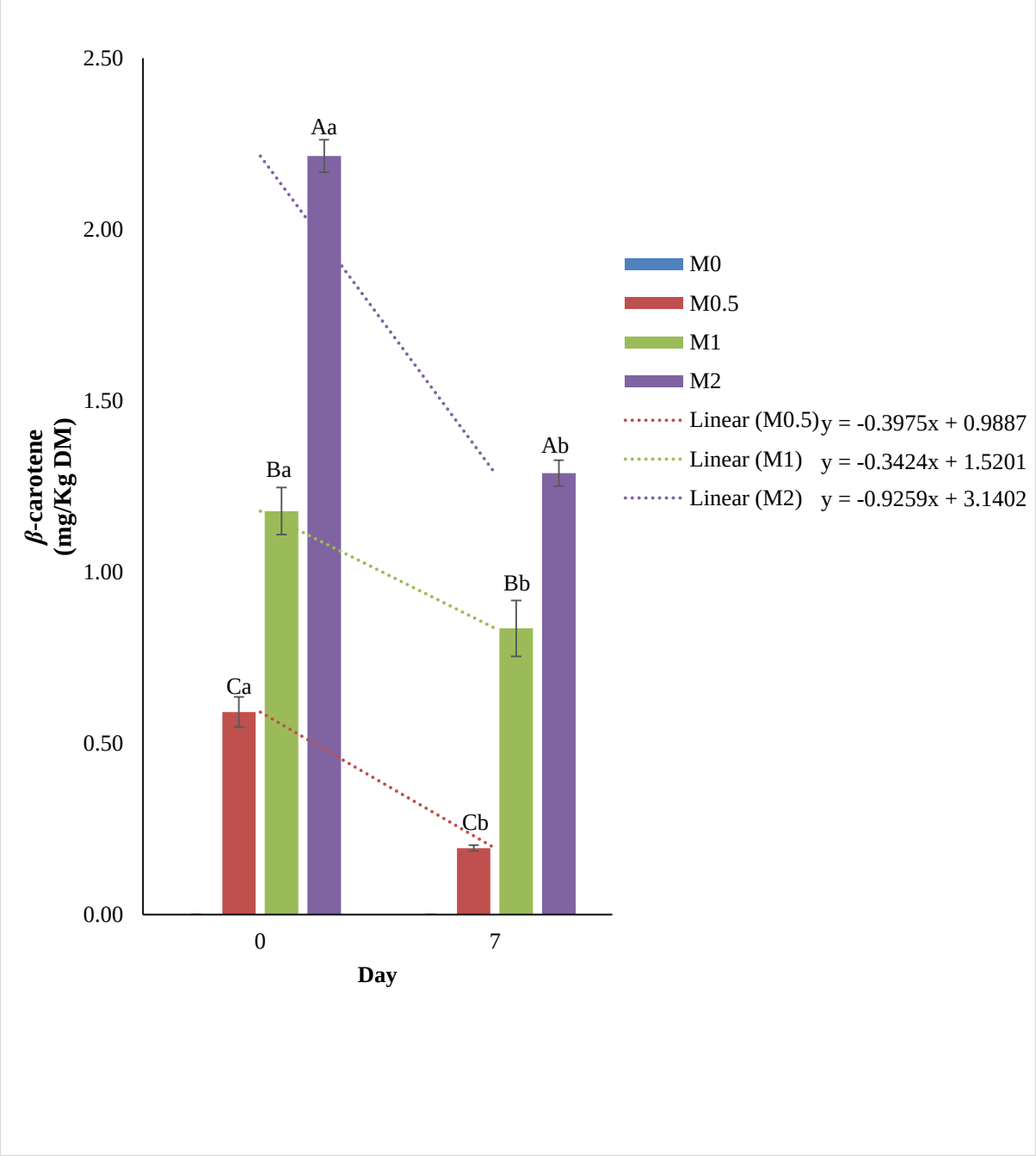
Figure 5.4 *α*-tocopherol content of pork droëwors (n=12) enriched with 0, 0.5 1 and 2 g/100 g *Moringa oleifera* leaf powder (MLP)



[AB]Means of different treatments with different superscripts at the sample sampling point are significantly different (P < 0.05)

[ab]Means of the same treatment with different superscripts across the sampling points are significantly different (P < 0.05)

Figure 5.5 *γ*-tocopherol content of pork droëwors (n=12) enriched with 0, 0.5 1 and 2 g/100 g *Moringa oleifera* leaf powder (MLP)



[AB]Means of different treatments with different superscripts at the sample sampling point are significantly different (P < 0.05)
[ab]Means of the same treatment with different superscripts across the sampling points are significantly different (P < 0.05)

Figure 5.6 *β*-carotene content of pork droëwors (n=12) enriched with 0, 0.5 1 and 2 g/100 g *Moringa oleifera* leaf powder (MLP)

5.4 Conclusion

Moringa oleifera leaf powder was found to contain significant levels of antioxidant compounds, making it a potentially suitable plant source of antioxidants for use in food processing. MLP significantly inhibited lipid oxidation in the processing and short term storage of pork droëwors. All of the antioxidant compounds quantified in MLP enriched droëwors exhibited consumption rates inversely proportional to TBARS values; evidence that they contributed to the increased antioxidant activity of pork droëwors as was reflected in increasing FRAP values with increasing MLP content. Since there was no significant difference in the TBARS values between the MLP treatments, MLP levels of 0.5 % could be used for inhibition of lipid oxidation in pork droëwors.

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Chapter 6. Effect of *Moringa oleifera* leaf powder on drying kinetics, physico-chemical properties, α-tocopherol content and lipid oxidation of pork droëwors during long term storage

Abstract

The study was conducted to determine the effect of *Moringa oleifera* leaf powder (MLP) on drying kinetics, physico-chemical properties, lipid oxidation and *α*-tocopherol in pork droëwors. Three treatments were prepared: no antioxidant added (negative control, C), 0.75 g/ 100 g MLP added (treatment M) and 15 mg/kg vitamin E oil (positive control, VE). Droëwors were dried at 30 °C and 40% relative humidity for 72 h and sampled before (day 0) and after (day 3) to measure dying kinetics, *α*-tocopherol content, physico-chemical properties (moisture, salt, water activity ($a_{w)}$, pH) and TBARS. Droëwors were stored at 25 °C and 40 % RH for 112 days, and physico-chemical properties and TBARS were measured during storage (sampled on day 7, 28, 56, 84 and 112). There were no significant differences in the drying curves and physico-chemical properties of the three treatments. Moisture content and a_w decreased ($P < 0.05$) in all treatments after drying and progressively through storage; while the salt content increased in concentration. After drying, TBARS were significantly lower in M (1.0 mg MDA/kg DM) than in VE (1.4 mg MDA/kg DM) and C (3.0 mg MDA/kg DM), remained significantly lower until day 56 and there were no significant differences between treatments on day 84 and 112. The *α*-tocopherol content of M before (2.8 mg/kg DM) and after (1.7 mg/kg DM) drying was significantly higher than VE (1.0 and 0.6 mg/kg DM) and C (0.5 and 0.2 mg/kg DM). The results indicate that there is potential to use MLP to inhibit lipid oxidation and increase *α*-tocopherol content in pork droëwors.

6.1 Introduction

Droëwors are processed by drying sausages made from a mixture of ground meat and animal fat (Burnham *et al.*, 2009). They are an intermediate-dried product (a_w of 0.7-0.75) and during drying, moisture loss of up to 50 % is targeted (Jones *et al.*, 2015b). Traditionally, droëwors are dried by hanging sausages in a well ventilated area for 1-2 weeks. However, it has become common to utilise drying chambers for large scale production; which allows for shortening of the drying period to a few day (Van der Riet, 1982; Santchurn *et al.*, 2012), better control over hygiene, food safety and product uniformity. Drying is one of the oldest and most diverse food preservation methods; hence there are over 400 different kinds of dryers reported in literature and over 100 that are commonly used (Mujumdar and Devahastin, 2004), as various food products dry differently. Drying curves are used to illustrate the transfer phenomena that occur during meat drying, and drying kinetics are illustrations of the decrease in moisture content plotted as a function of drying time (Santchurn *et al.*, 2012). Data on the drying kinetics of food products are useful for the design of full scale dryers for industrial use.

In addition to physical changes resultant of moisture loss (e.g. shrinkage), the environmental conditions in the dryer predispose the product to unfavourable biochemical reactions, such as lipid oxidation (Mujumdar and Devahastin, 2004). Lipid oxidation results in the development of off odours, off flavours and deterioration of colour, texture and nutritive value of meat and meat products (Kanner, 1994). The addition of exogenous antioxidant substances is necessary to limit the negative effects of lipid oxidation. The use of synthetic antioxidants (e.g butylated hydroxyanisole, butylated hydroxyl toluene, tertiary butlylhydroquinone) in meat products to delay lipid oxidation has been implicated with toxicity and carcinogenic effects on human health (Falowo *et al.*, 2014). Consequently there has been an increase in restrictions on what

substances can be used as antioxidants because consumers are concerned about the safety of synthetic additives (Troy and Kerry, 2010).

The current demands that modern consumers have for meat products revolve around convenience and safety; and consumers are increasingly interested in food products with functional health benefits. In the development of functional meat products, either the presence of natural antioxidants (Insani *et al.,* 2008) or the addition of exogenous bioactive compounds can be used as a strategy to impart or improve the health beneficial properties of meat (Jiménez-Colmenero *et al.*, 2012). Vitamin-E/tocopherol, in all its isomer tocopherol-forms (α-, β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol) possesses natural antioxidant properties and *α*-tocopherol in particular is the most bioavailable isomer for humans (Higdon, 2000, Azzi and Strocker, 2000, Azzi, 2007). It functions as a chain-breaking antioxidant, preventing the propagation of free radicals in membranes and plasma-lipoproteins and it is also likely involved in strengthening certain aspects of cell-mediated immunity (Traber and Packer 1995) and reviewed in Falowo *et al.* (2014). The recommended daily dietary intake level of vitamin E is 14.2 mg and it has been reported that meat consumption on average contributes only 0.14 mg vitamin E; which is around 1% of the daily recommended value (MARM, 2008 in Jiménez-Colmenero *et al.,* 2012). Research has shown that *Moringa oleifera* leaves possess strong antioxidant properties and a high vitamin E content (Verma *et al.,* 1999; Moyo *et al.,* 2012). It was hypothesised that the addition of MLP into pork droëwors during processing, at a level between 0.5 and 1 g/ 100 g, would not affect drying or physico-chemical properties; would inhibit lipid oxidation during drying long term and storage, and would increase the *α*-tocopherol content. This study was conducted to determine the effect of MLP on drying kinetics, α-tocopherol content and lipid oxidation during drying and long term storage.

6.2 Materials and methods

6.2.1 Raw materials

Moringa oleifera leaf powder (MLP) of Senegalese origin (Racines, France) was purchased for the study. Leaves were processed by naturally air drying fresh *Moringa oleifera* leaves under shade before grinding through a 2 mm sieve screen. The TBARS data obtained in Chapter 5 was used to determine the MLP inclusion level for this study. A level between 0.5 and 1 g/100 g was targeted, in order to minimise product colour change and maximise α-tocopherol content. The mean TBARS value of M0 represented 100% and the percentage inhibition of M0.5, M1 and M2 were calculated, plotted on a graph with a polynomial trend line (Appendix 1) and the intersection of the curve and the trend line was selected as the MLP inclusion level (i.e, 0.75 g/100 g of MLP). Lean pork (6 kg), pork fat (2 kg), dehydrated natural sheep casings (stored in salt and rehydrated in water prior to use) (22 mm diameter, Freddy Hirsch), salt and ground black pepper were purchased for droëwors preparation.

6.2.2 Droëwors preparation

Lean pork meat and pork fat in the ratio of 80:20 was cut into cubes (2 x 2 cm) a separated into 3 treatment batches: no antioxidant added (C), 0.75 g/ 100 g MLP added (M) and 15 mg/kg vitamin E oil. Each treatment batch was separated into triplicate sub-batches which were were minced separately through a 5 mm screen before the incorporation of salt (2%), pepper (0.5%) and MLP/vitamin E oil. After thorough incorporation, each sub batch was minced again through a 2 mm screen into the casings and hung vertically in a drying tunnel at 30°C and 40% relative humidity for 72 hrs.

6.2.3 Drying kinetics

Droëwors were dried in a pilot dryer developed by Cirad (Figure 6.1). This research equipment allows the measurement of drying kinetics under well-controlled conditions of

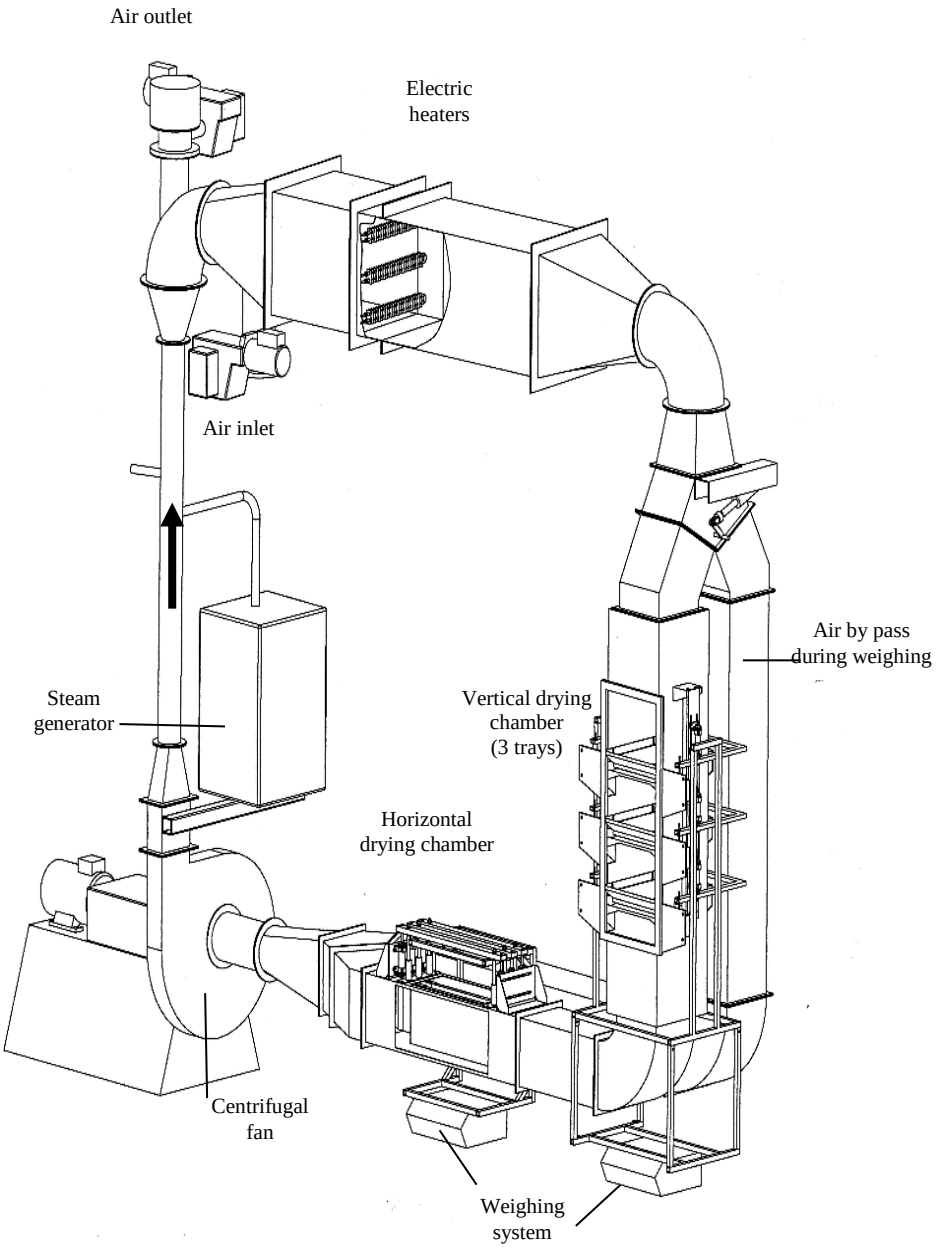


Figure 6.1: Cirad pilot dryer

temperature, relative humidity and air flow. It is a re-circulating hot air dryer. Temperature is controlled with electric heaters and a cooling system composed of a heat exchanger with a high exchange area. Relative humidity is controlled by a system with a steam generator at atmospheric pressure and 2 valves for air renewal. Temperature and relative humidity are measured with a capacitive hygrometer Rotronic (converter HTS11X and probe HYGROCLIP IC-3) annually certified and recorded every 30 s. Droëwors were hung in the vertical drying chamber. It's proportions (0.25 m long × 0.25 m wide × 0.92 m high) allow a good homogeneity of air flow. Hot air (30 °C and 40 % RH) was circulated at 3 ms^{-1}. Each treatment was mounted on a separate tray and the weight of each tray was recorded intermittently (every 15 min). During weighing, air flow is by passed in order to avoid interference. Regulation, start stop and weighing are controlled by a computer. Each treatment was mounted on a separate platform in the drying tunnel. Moisture content was determined before drying and the evolution of moisture content (dry matter basis) was plotted as a function of drying time.

6.2.4 Droëwors storage

Uncovered and unpackaged, the droëwors were stored in an environmentally controlled chamber set at 25 °C and 50% relative humidity for a period of 112 days.

6.2.5 Droëwors sampling

A representative 50 g sample was taken from each bath of droëwors at for duplicate analysis of moisture, protein and salt content, a_w and pH. A representative 50 g sample was taken from each batch on days day 0 (before drying), day 3 (after drying); and after 7, 28, 56, 84 and 112 days of storage for analysis of moisture content, a_w, pH, lipid oxidation and α-tocopherol. Samples were cut into small cubes, grinded and stored under vacuum at -20 °C for proximate composition, salt content, a_w and pH; and -80 °C for lipid oxidation and α-tocopherol.

6.2.6 Physico-chemical analysis

Moisture and salt content were determined according to the procedures outlined in Section 3.2.2 and 3.2.3 respectively. Water activity and pH were determined according to the procedures outlined in Section 3.2.4 and 3.2.5 respectively.

6.2.7 Lipid oxidation

The content of thiobarbituric acid reactive substances (TBARS) was determined by acid precipitation using the technique described by Descalzo *et al.* (2005), detailed in Section 5.2.4.

6.2.8 α-tocopherol

A-tocopherol content was analysed by HPLC, as detailed in Section 5.2.1.2.

6.2.9 Statistical analysis

Data on moisture, a_w, pH, TBARS and α-tocopherol content were analysed using PROC GLM procedures of SAS (2003) and pair wise comparisons of least square means were done using t-tests(PDIFF option). Differences were significant at $P < 0.05$. The main effects of MLP level, time and their interaction were included in the model:

$$Y_{ijkl=}\ \mu + \alpha_i + \beta_j + \alpha\beta_k + e_{ijkl}$$

Where Y_{ijkl} = dependant variable (moisture, a_w, pH, TBARS and α-tocopherol)

μ = overall mean

α_i= i^{th} effect of treatment (C, M, VE)

β_j = j^{th} effect of time (0,3, 7, 28, 56, 84, 112 days)

$\alpha\beta_k$ = k^{th} treatment by time effect

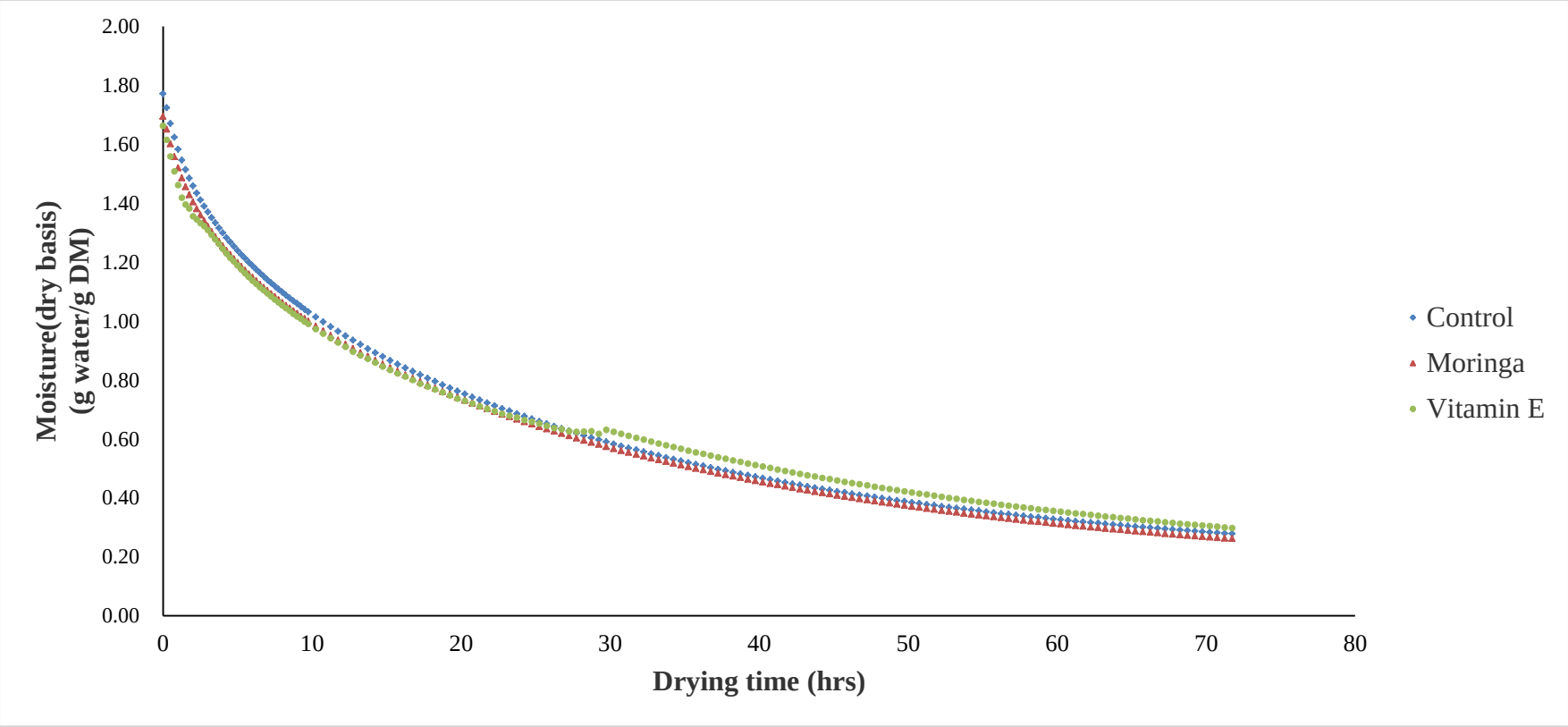
e_{ijkl} = random error

Pearson’s correlation coefficients were analysed between the physico-chemical traits, TBARS and α-tocopherol content.

6.3 Results and discussion

Drying kinetics of pork droëwors are presented in Figure 6.2. The drying curves were consistent with that of a wet solid under fixed drying conditions; initially exhibiting a linear decrease in moisture content with time, followed by a non-linear decrease moving towards equilibrium moisture content (Mujumdar and Devahastin, 2004). The rate of moisture loss over time was similar ($P > 0.05$) for all three treatments. The percentage weight loss was 46.1%, 46.8% and 48.8% for C, M and VE respectively. Increasing the drying period to 3 days resulted in a similar percentage weight loss compared to pork droëwors in Chapter 3 (48.2%), which were dried for 2 days under the same conditions. This may be attributed to the higher fat content (20%) used in this study because fat tends to have a barrier effect on moisture loss; resulting in a slower drying rate (Santchurn *et al.*, 2012). In line with the objective of Chapter 3, the percentage of fat added to pork droëwors was reduced to 15% in order to make it comparable to the fat content of beef droëwors. In the present study, the maximum recommended level of fat inclusion in droëwors recipes was used. The results indicate that the addition of 0.7 g/100 g MLP and 15 mg/kg vitamin E oil did not affect the drying rate.

The addition of 0.75 g/100 g of MLP and 15 mg/kg vitamin oil had no significant effect on the physico-chemical properties of all three treatments (Table 6.1); as the moisture, salt, a_w and pH values were similar ($P > 0.05$) at each sampling point. On day 3, there was a significant loss of moisture, increase in salt concentration and reduction of a_w y as a result of the drying process, consistent with reports from other studies (Hoffamn *et al.*, 204; Jones *et al.*, 2015a; Jones *et al.*, 2015b). The average physico-chemical properties of pork droëwors (after drying) in this study were 22.7 g/100 g moisture, 4.4 g/100 g salt, a_w of 0.78 and pH of 5.4 are comparable to pork droëwors produced in Chapter 4



C (no antioxidant added), M (0.75 g/100 gMLP), VE (15 mg/kg vitamin E oil)

Figure 6.2 Kinetics of moisture content over time in pork droëwors (n=9) during drying (30 °C, 40% Relative humidity)

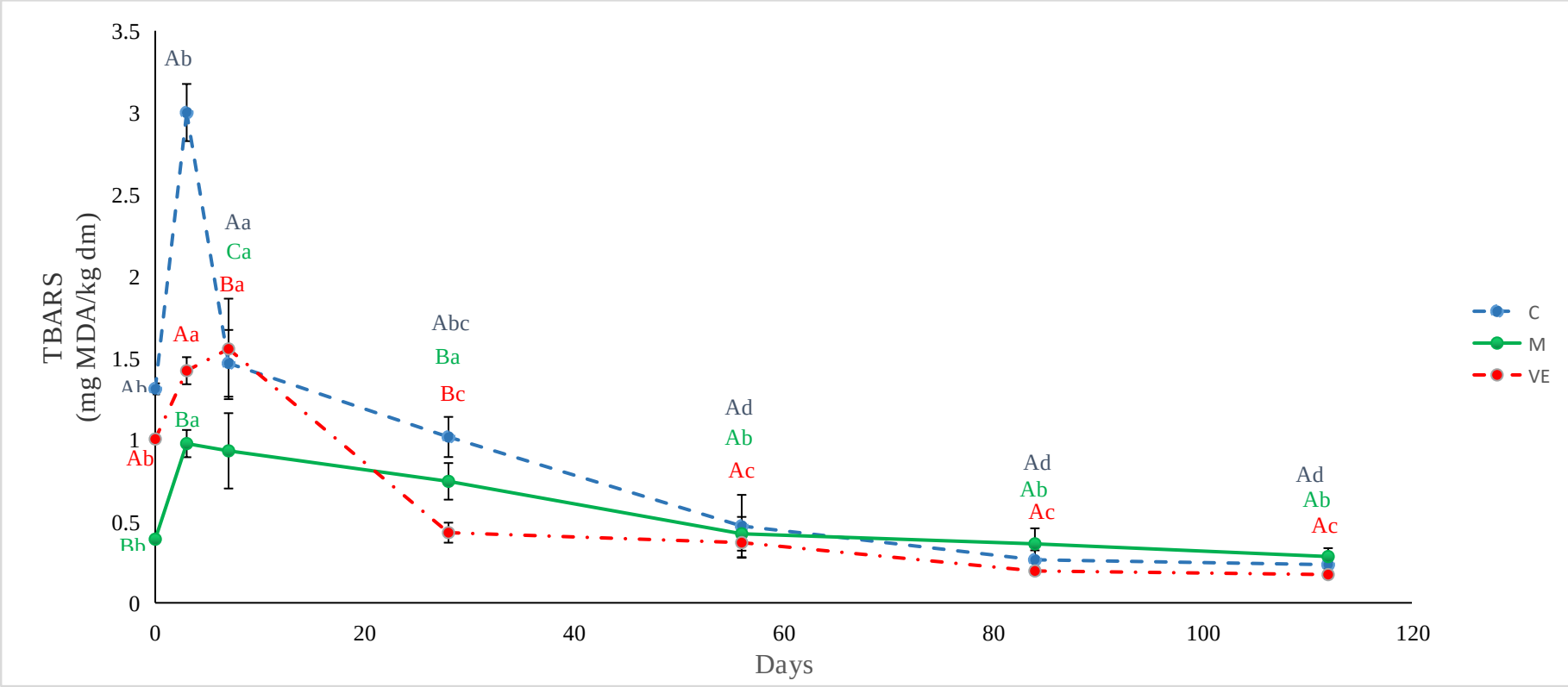
Table 6.1 Physico-chemical properties of pork droëwors during drying and 112 days storage

	Day						
	0*	3**	7**	24**	56**	84**	112**
Moisture							
C (n=3)	$63.9^{Aa} \pm 0.08$	$21.6^{Ab} \pm 0.27$	$13.5^{Ac} \pm 0.15$	$9.8^{Ac} \pm 0.25$	$10.1^{Ac} \pm 0.12$	$8.4^{Ad} \pm 0.35$	$6.4^{Ae} \pm 0.10$
M (n=3)	$62.9^{Aa} \pm 0.10$	$22.3^{Ab} \pm 1.15$	$13.0^{Ac} \pm 0.10$	$10.7^{Ac} \pm 0.08$	$10.5^{Ac} \pm 0.23$	$9.1^{Ad} \pm 0.26$	$7.2^{Ae} \pm 0.10$
VE (n=3)	$62.4^{Aa} \pm 0.19$	$24.1^{Ab} \pm 0.63$	$13.6^{Ac} \pm 0.60$	$10.5^{Ac} \pm 0.19$	$10.1^{Ac} \pm 0.54$	$8.9^{Ad} \pm 0.50$	$7.6^{Ae} \pm 0.18$
Salt							
C (n=3)	$2.5^{Ae} \pm 0.03$	$4.6^{Ad} \pm 0.03$	$5.2^{Ac} \pm 0.17$	$5.5^{Abc} \pm 0.12$	$5.0^{Ac} \pm 0.16$	$^{\$}5.8^{Ab} \pm 0.16$	$^{\$}6.2^{Aa} \pm 0.16$
M (n=3)	$2.2^{Ae} \pm 0.11$	$4.2^{Ad} \pm 0.11$	$4.8^{Ac} \pm 0.07$	$4.9^{Ac} \pm 0.02$	$4.7^{Ac} \pm 0.02$	$^{\$}5.3^{Ab} \pm 0.02$	$^{\$}5.7^{Aa} \pm 0.02$
VE (n=3)	$2.3^{Ae} \pm 0.09$	$4.4^{Ad} \pm 0.09$	$5.1^{Ac} \pm 0.09$	$4.7^{Ac} \pm 0.03$	$4.9^{Ac} \pm 0.07$	$^{\$}5.5^{Ab} \pm 0.07$	$^{\$}5.6^{Aa} \pm 0.07$
a_w							
C (n=3)	$0.97^{Aa} \pm 0.00$	$0.77^{Ab} \pm 0.01$	$0.63^{Ac} \pm 0.01$	$0.57^{Ad} \pm 0.00$	$0.56^{Ade} \pm 0.01$	$0.54^{Ade} \pm 0.01$	$0.54^{Ae} \pm 0.01$
M (n=3)	$0.97^{Aa} \pm 0.00$	$0.78^{Ab} \pm 0.02$	$0.62^{Ac} \pm 0.01$	$0.59^{Ad} \pm 0.00$	$0.57^{Ade} \pm 0.00$	$0.55^{Ade} \pm 0.01$	$0.56^{Ae} \pm 0.01$
VE (n=3)	$0.97^{Aa} \pm 0.00$	$0.79^{Ab} \pm 0.01$	$0.62^{Ac} \pm 0.01$	$0.58^{Ad} \pm 0.00$	$0.56^{Ade} \pm 0.00$	$0.55^{Ade} \pm 0.02$	$0.54^{Ae} \pm 0.00$
pH							
C (n=3)	$5.8^{Aa} \pm 0.05$	$5.3^{Ab} \pm 0.00$	$6.0^{Aa} \pm 0.06$	$6.0^{Aa} \pm 0.00$	$5.7^{Aa} \pm 0.03$	$6.0^{Aa} \pm 0.04$	$6.0^{Aa} \pm 0.02$
M (n=3)	$5.9^{Aa} \pm 0.02$	$5.4^{Ab} \pm 0.16$	$6.0^{Aa} \pm 0.10$	$5.9^{Aa} \pm 0.06$	$5.7^{Aa} \pm 0.01$	$6.0^{Aa} \pm 0.04$	$5.9^{Aa} \pm 0.01$
VE (n=3)	$6.0^{Aa} \pm 0.06$	$5.6^{Ab} \pm 0.10$	$5.8^{Aa} \pm 0.00$	$6.0^{Aa} \pm 0.03$	$5.7^{Aa} \pm 0.04$	$6.0^{Aa} \pm 0.03$	$6.0^{Aa} \pm 0.04$

Moisture and salt contents are expressed in g/100 g; C (no antioxidant added), M (0.75 MLP), VE (15 mg/kg vitamin E oil);
^{AB} For each characteristic, means in the same column with different superscripts are significantly different ($P < 0.05$);
^{abcde} Means in the same row with different superscripts are significantly different ($P < 0.05$);
- Not measured; N/A Not applicable; a_w Water activity; MLP *Moringa oleifera* leaf powder;
*Fresh droëwors;
**Droëwors was dried for 3 days (30 °C, 40% RH) before storage (25 °C, 50% RH);
$^{\$}$Calculated on the basis of percentage moisture loss

(23.6 g/100 g moisture, 4.2 g/100 g salt, 0.81 a_w, 5.6 pH). Droëwors continued to lose moisture during storage, with moisture content reducing from day 3 to day 112. The exact shelf life of droëwors is not reported in literature. It has been reported that storage of droëwors at ambient temperatures over a period (several weeks) is characterised by further drying due to air circulation, and that this facilitates preservation and prevents fungal growth (Heinz and Hautzinger, 2010). The increase ($P < 0.05$) in salt content with continual moisture loss in this study and in others (Hoffamn *et al.*, 2014; Jones *et al.*, 2015a; Jones *et al.*, 2015b); and a strong negative correlation ($r^2 = -0.7$, $P < 0.0001$) (Table 4.2). Due to a technical fault with the chloride analyser which could not be resolved in the duration of the study, the salt contents on days 84 and 112 were calculated on the basis of moisture loss. Water activity reduced during storage in proportion with reduction of moisture content, as expected; and a strong positive correlation was found between the two parameters ($r^2 = 0.96$, $P < 0.0001$). The pH was higher ($P < 0.05$) on day 3 and stable ($P > 0.05$) during storage. The decrease in pH after drying may be an indication of some fermentation during drying. This could possibly be due to acid production from natural microflora on the meat and it has been reported that dry sausages can undergo changes during production from microbial activity (Santchurn *et al.*, 2012). This would explain the stabilisation of pH after drying, as the reduced moisture content, a_w and concentration of salt content is unconducive for the continued proliferation of microorganisms. pH was correlated with TBARS ($r^2 = 0.5$, $P < 0.0001$) and negatively correlated to a_w ($r^2 = -0.26$, $P = 0.04$).

The TBARS (dry matter basis) before dying, after drying and during storage are presented in Figure 6.3. Before drying, TBARS were lower ($P < 0.05$) in M (0.4 mg MDA/kg DM) than VE (1.0 mg MDA/kg DM) and C (1.3 mg MDA/kg DM). After drying (Day 3), TBARS were significantly higher in all treatments. The highest was C (3.0 mg MDA/kg DM), VE was significantly lower (1.42 mg MDA/kg DM) and M was significantly lower than both (1.0 mg



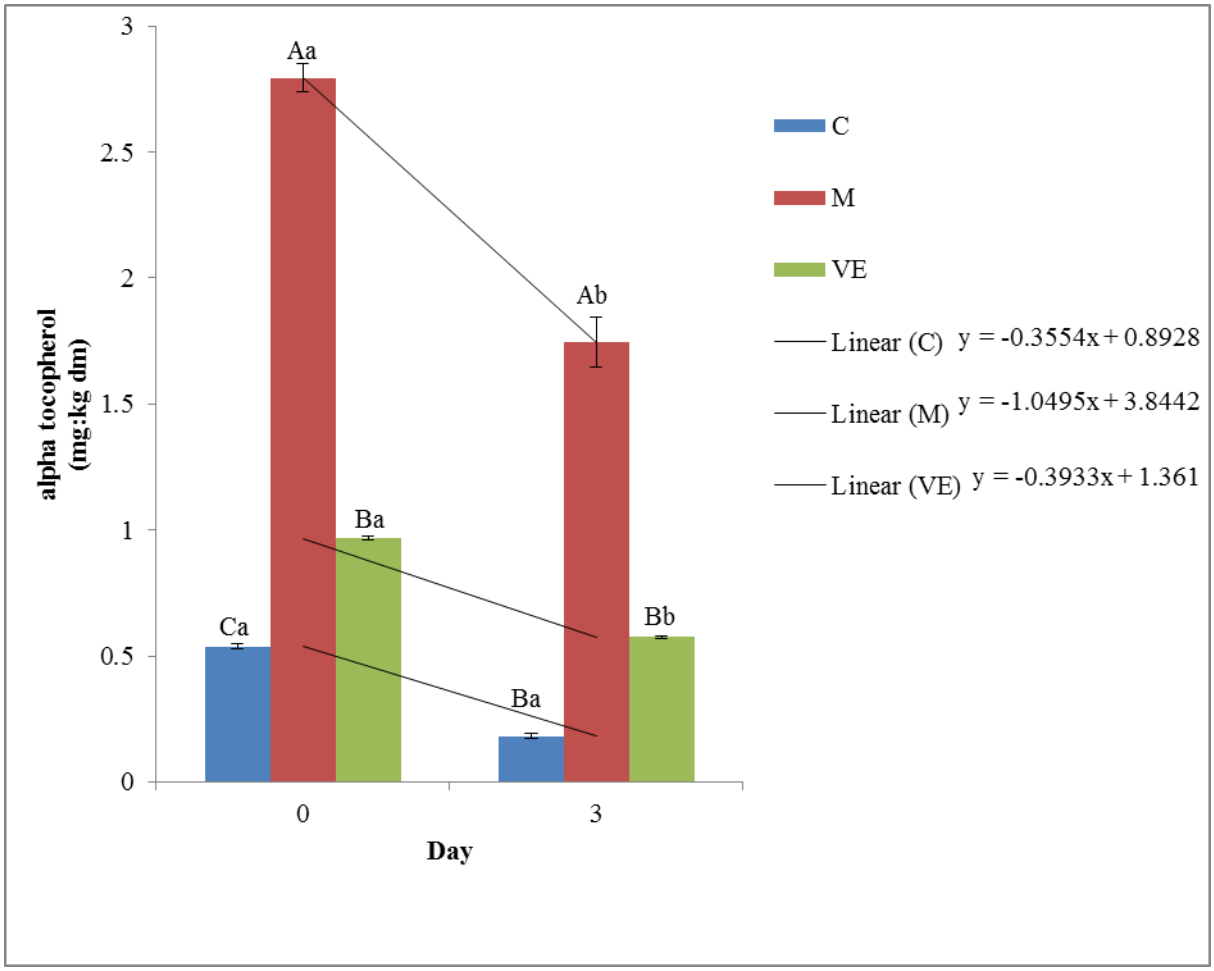
C (no antioxidant added), M (0.75 g/100 gMLP), VE (15 mg/kg vitamin E oil);
[AB] Means of different treatments with different superscripts at the sample sampling point are significantly different (P < 0.05);
[abc] Means of the same treatment with different superscripts across the sampling points are significantly different (P <0.05).

Figure 6.3 TBARS of pork droëwors (n=9) during drying (30 °C, 40 % RH) and 112 days storage (25 °C, 50% RH)

MDA/kg meat); remaining significantly lower in M on day 7. The increase in TBARS during drying was expected in all treatments as a result of the pro-oxidant influences of processing methods (mincing and drying). According to Muthukumar *et al.* (2012), mincing and thermal processing disrupt the integrity of muscle membranes, exposing lipid membranes to metal ions and facilitating the interaction of pro-oxidants with unsaturated fatty acids resulting in generation of free radicals and propagation of oxidative reaction. After day 7, TBARS in all treatments followed a decreasing trend, consistent with reports that over time, MDA is degraded by reactions with other compounds (Antequera *et al.*, 1992; Wu *et al.*, 2016). On day 28, TBARS in M and VE were significantly lower than C and from day 56 – 112, TBARS were similar ($P > 0.05$) in all three treatments. Lower TBARS in VE and M throughout drying and storage are consistent with reports on the antioxidant activity of α-tocopherol and *Moringa oleifera* leaves (Verma *et al.*, 1999; Sidharuju and Bekker, 2005; Moyo *et al.*, 2012) and the results of Chapter 5. Jayawardana *et al.* (2015) similarly reported that the addition of 0.75% *Moringa oleifera* leaves to chicken sausages resulted in significantly lower TBARS during storage. Lower TBARS in M than VE can be attributed to the presence of both higher tocopherols levels and other additional antioxidant compounds in MLP, including phenolic compounds, flavonoids, carotenoids and ascorbic acid (Sidharuju and Bekker, 2005; Sreelatha and PaDMa, 2009). As detailed in Chapter 5, the MLP used in this study had a TPC of 7.5 mg galic acid eg/g, 76.7 mg/100 g α-tocopherol and 23.2 mg/100 g β-carotene. Antioxidant compounds inhibit lipid oxidation by donating electrons to terminate propagation of the oxidation cycle, and/or by scavenging free radical oxidation catalysts (reactive oxygen species) thereby preventing initiation (reviewed in Falowo *et al.*, 2014).

The α-tocopherol content of the three treatments before and after drying is presented in Figure 6.4. On day 0, a significantly higher concentration of α-tocopherol was found in M

(2.8 mg/kg DM) and VE (0.97 mg/kg DM) C (0.5 mg/kg DM). After drying (day 3), the *α*-tocopherol content reduced significantly in all treatments. This is an indication of α-tocopherol consumption during drying. The decrease in *α*-tocopherol is proportional to TBARS inhibition and a negative correlation (Table 6.2) was found between α-tocopherol and TBARS ($r^{2\ =}$ -0.34, P = 0.006); an indication that it was acting as an antioxidant. The amount of *α*-tocopherol was significantly higher in M than VE, and this also explains the significantly lower TBARS in M than VE from day 0 to 28. Inclusion of 0.75 g/100 g MLP produced α-tocopherol enriched droëwors, with 1.75 mg/kg DM, in contrast ($p < 0.05$) with 15 mg/kg α-tocopherol oil (0.57 mg/kg DM) and C (0.18 mg/kg DM). The results indicate that the addition of 7.5 g/100 g MLP to pork droëwors has the potential to significantly increase its' α-tocopherol content.



C (no antioxidant added), M (0.75 g/100 gMLP), VE (15 mg/kg vitamin E oil)
[ABC] For each day, means with different superscripts significantly different ($P < 0.05$)
[ab] Across days, means of the same treatment with different superscripts are significantly different ($P < 0.05$)

Figure 6.4 α-tocopherol content of pork droëwors (n=12) before and after drying

Table 6.2 Correlation between physico-chemical properties, TBARS and α-tocopherol content of pork droëwors

	Salt	a_w	pH	TBARS	α-tocopherol
Moisture	-0.71***	0.96***	-0.09^{NS}	-0.06^{NS}	0.35^{*}
Salt		-0.71***	0.55***	-0.10^{NS}	-0.41*
aW			-0.26*	0.40**	0.32*
pH				-0.50***	0.03^{NS}
TBARS					-0.34**

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ^{NS} Not significant ($P > 0.05$); a_w: Water activity

6.4 Conclusion

The addition of MLP and α-tocopherol oil did not significantly affect drying kinetics and produced pork droëwors with similar physico-chemical properties. Significant changes that occurred in all treatments during storage were the continued reduction of moisture content and a_w, the concentration of salt content due to moisture loss and the increase in TBARS during drying. Inhibition of TBARS in was highest in M, followed by VE and lest in C. Inclusion of 0.75 g/mg MLP significantly increased the α-tocopherol content of pork droëwors and could therefore shows potential for use as a functional natural antioxidant additive.

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Chapter 7. General Discussion

The study reported here was designed to determine the effect of using *Moringa oleifera* leaf powder as an antioxidant and functional additive in pork droëwors. It was hypothesised that MLP addition would increase antioxidant activity, inhibit lipid oxidation during processing and storage and increase the α-tocpherol content of the product, without adversely affecting the physico-chemical properties of the product. Other objectives were to characterise the physico-chemical properties of commercial droëwors produced from different kinds of meat; to conduct a comparative analysis of lipid oxidation in beef and pork droëwors; and to quantify the levels of some antioxidant compounds in MLP. Increasing the content of α-tocopherol was a priority, for it's antioxidant potential nutritional value and associated health benefits.

Droëwors are popular amongst South African consumers and have become increasingly popular on the international market (Jones *et al.*, 2015a). However, little has been reported on it's composition and physico-chemical characteristics. In Chapter 3, samples of droëwors that are typically available in the South African market were analysed for proximate composition and physico-chemical properties related to product stability at ambient temperatures; relevant data owing to the fact that droëwors are frequently stored at ambient temperatures for long periods (Jones *et al.*, 2015b). The average salt content of droëwors, never reported before in the literature, was found to be high although in the range of many dry cured meat products. The average salt consumption in South Africa (8.1 g/day) is more than double the daily recommended intake level (4-6 g/day) (Pretorius and Schönfeldt, 2016). It would be beneficial for consumers to be made aware of the average salt content of droëwors, to enable them to regulate consumption in line with dietary guidelines. As droëwors are often packaged

into un-labelled packaging at the point of sale, which carries no nutritional information; consumer access to information on the product composition is limited (although more formalised packaging materials are being used more frequently).

Furthermore, in Chapter 4, the concentration of salt increased during prolonged storage, as the product continued to lose moisture. This indicates that dietary intake of salt from droëwors increases the longer it is stored and again, this information would be beneficial for consumers. As with all processed meats, moderation of consumption is advisable in order to minimise the risk of negative health implications from excessive salt consumption. Concerning lipid stability, it was confirmed in Chapter 4 that droëwors made from pork are more susceptible to oxidative deterioration than droëwors made from beef. Quantitative data on lipid oxidation in beef and pork droëwors have never been reported in literature. Since consumers have shown keen interest in droëwors made from meat types other than beef (Jones *et al.*, 2015b), research on effective ways to safely inhibit oxidative deterioration in pork droëwors can be justified.

In chapter 5, the antioxidant potential of MLP was determined, and compared favourably with values reported in literature. No published works analysing the progression of lipid oxidation during droëwors processing have been done previously. It effectively reduced TBARS compared to the control treatment. As there was no significant difference between treatments, inclusion levels between 0.5 g/ 100 g and 2 g/ 100 g can be utilised to produce a similar inhibitory effect. Higher levels, however, would be recommendable if the aim is also to increase the α-tocopherol content. The *α*-tocopherol content of droëwors was increased by MLP addition. This could prove to be functionally beneficial, as the *α*-tocopherol isomer is the most biologically active form which can be incorporated in mammals when consumed (Chun *et al.*, 2006). Consumption of plant derivatives containing antioxidant components including *α*-tocopherol, carotenoids and polyphenols may decrease the risk of degenerative

diseases (Hygreeva *et al.*, 2014). Hung *et al.* (2016) propose that meat could be a valuable resource for functional food development its rich nutritional value, versatility to a wide range of processing methods and strong consumer appeal.

In Chapter 6, the effect of MLP on drying kinetics, content of antioxidant compounds in the product, physico-chemical characteristics and oxidative stability during storage was determined. The MLP inhibited lipid oxidation during prolonged storage and increased α-tocopherol and β-carotene. The drying kinetics were also determined here, which MLP had no effect on. This Chapter presents the first drying curves of droëwors production in literature, as the drying kinetics of droëwors have not previously been studied.

Recommendations

Moringa oleifera leaf powder exhibited potential for use as a natural antioxidant and functional additive in this study. Further research is recommended on:

- The effect of MLP on the organoleptic properties of pork droëwors/other meat products. It is recommended that sensory evaluation trials be conducted, as this will help in determining appropriate inclusion levels for consumer acceptability.
- The effect of MLP inclusion of the content of other bioactive compounds.
- The microbiological effect of MLP inclusion *Moringa oleifera* leaves have in numerous studies been reported to possess antimicrobial properties
- The effect of lowering salt content with increasing levels of MLP inclusion on product stability and microbiological status.. It could be hypothesised that the inclusion of *Moringa oleifera* leaf powder in droëwors could inhibit microbial growth, and in this way, counteract the detrimental effects on product shelf life associated reducing the salt content. This could potentially enable the addition of less salt in the product

- The effect of MLP on the full nutritional profile, including fibre content, vitamins, minerals, amino acids and fatty acids
- Characterisation of the full nutritional profile of droëwors available in local and international markets
- Determination of the fatty acid profiles of droëwors produced from different meat species in relation to lipid oxidation

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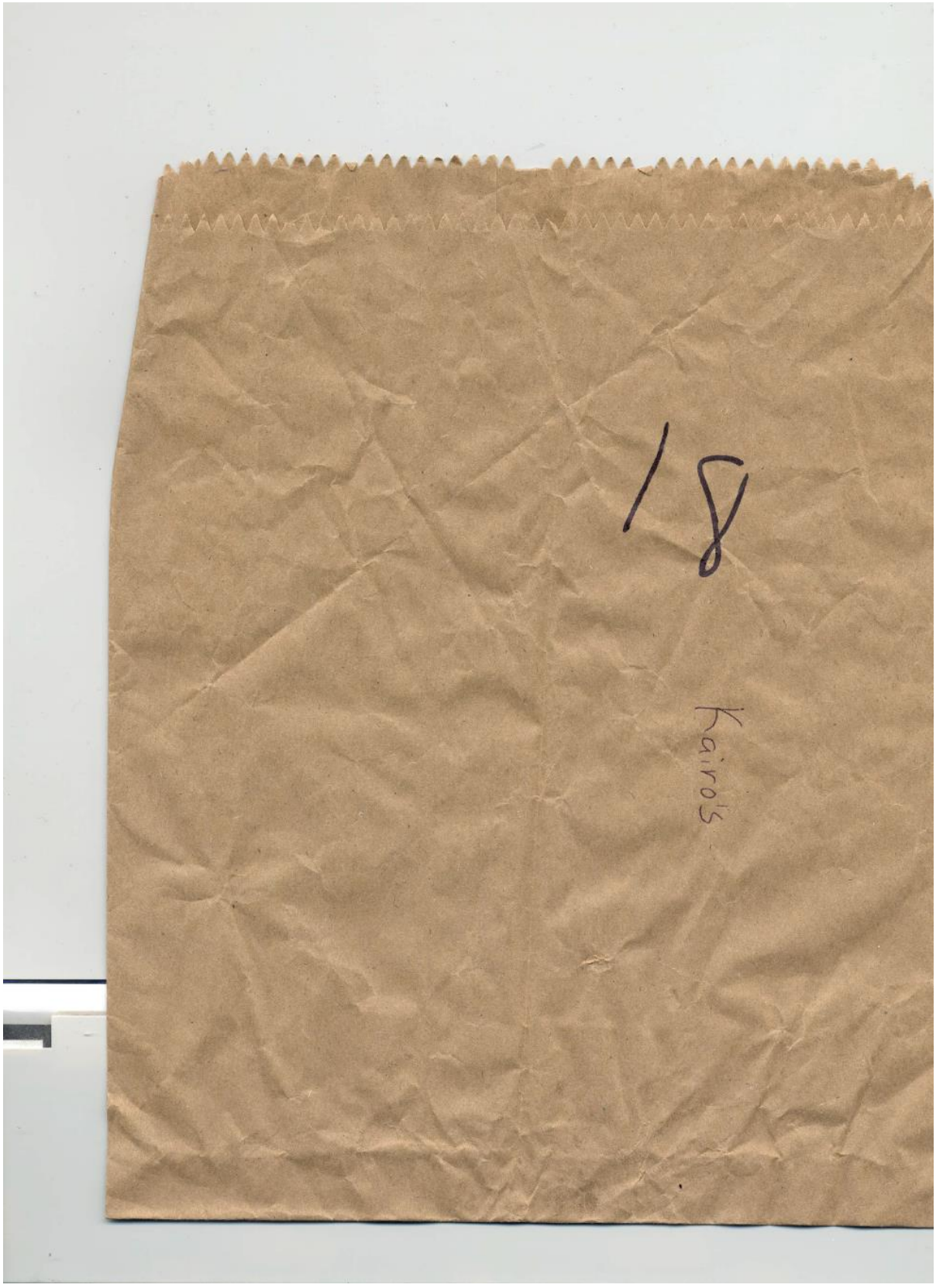
Appendices

Appendix 1 Packaging information/labels of purchased beef, game meat and ostrich droewors


18
Kairos


cape deli
droëwors
PLAIN
lean & tasty
beef, blended
and spiced just right
PACKED IN A PROTECTIVE ATMOSPHERE
STORAGE CONDITIONS: STORE IN A COOL DRY PLACE. ONCE OPENED, REFRIGERATE AND CONSUME WITHIN 3 DAYS
PRODUCT OF SOUTH AFRICA
BEST BEFORE
19
4
3
5a
5b
9
smoked
CHILLI
OSTRICH
KUDU
ORIGINAL BEEF DROËWORS
Ingredients: Beef, Animal Fat [Beef], Seasoning [Salt, Spices (Irradiated), MSG (Flavour Enhancer), Curing Mix (Salt, Curing Agent [Sodium Nitrite], Sodium Carbonate, Colourant), Sucrose, Spice Extract], Water, Vinegar [Vinegar, Preservative (Sulphur Dioxide)]
May contain wheat, gluten, soya, cow's milk and egg.
STORE IN A COOL, DRY PLACE. ONCE OPENED, PLACE IN A SEALED CONTAINER AND REFRIGERATE EAT WITHIN 3 DAYS
6 009687 462600
SATISFACTION GUARANTEED
This product is made for your enjoyment and we take great care to ensure that it reaches you in excellent condition. 100% satisfaction guarantee or we will gladly refund you or replace your product.
Manufactured by: Geofina (Pty) Ltd. t/a Joubert & Monty
Cnr Gunners Circle and Cochrane Ave.
Epping 1, Cape Town
www.jouberttandmonty.co.za
CUSTOMER HELP LINE: 021 555 1717
230g
Batch No. 19/10/2016
Best Before:
PRODUCT OF SOUTH AFRICA
5
JOUBERT & MONTY
J&M
FAMOUS BILTONG
Everybody loves J&M biltong!
Original
Beef Droëwors
8
GAME DROEWORS
TARE 0.008 KG
R/kg 299.99
WEIGHT(kg) 0.134
Paked 28-04-16
Sell By 07-06-16
R40,20
SUPERSPAR
die Boord biltong
Springbok
GAME DROEWORS
TARE 0.008 KG
R/kg 299.99
WEIGHT(kg) 0.084
Paked 28-04-16
Sell By 07-06-16
R25,20
SUPERSPAR
7
Kudu
BEEF DROEWORS
TARE 0.008 KG
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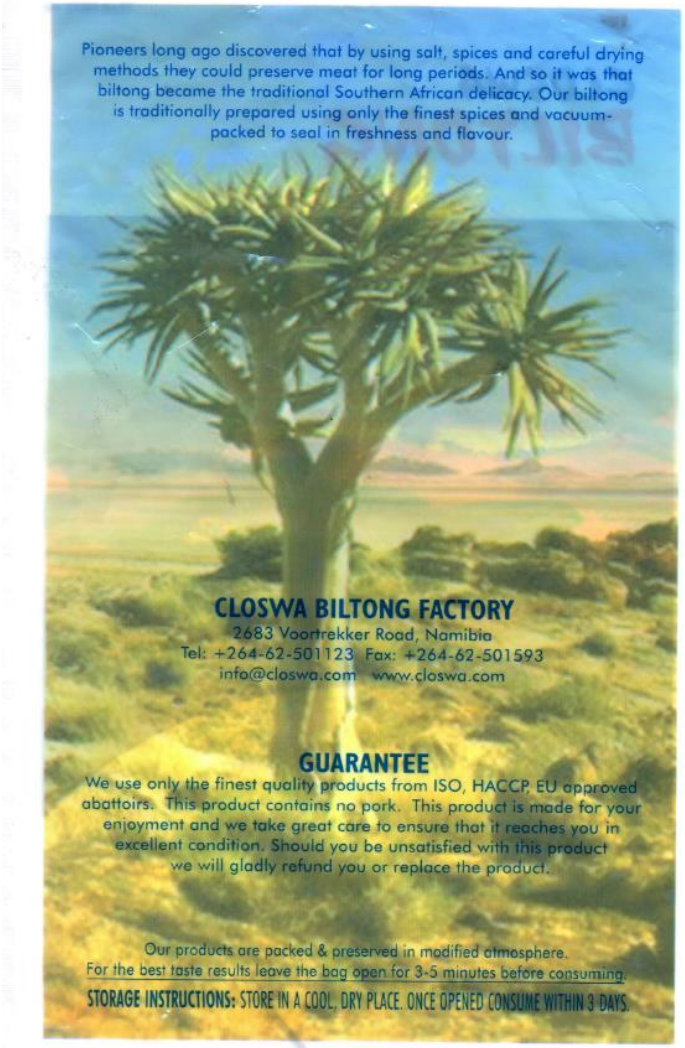
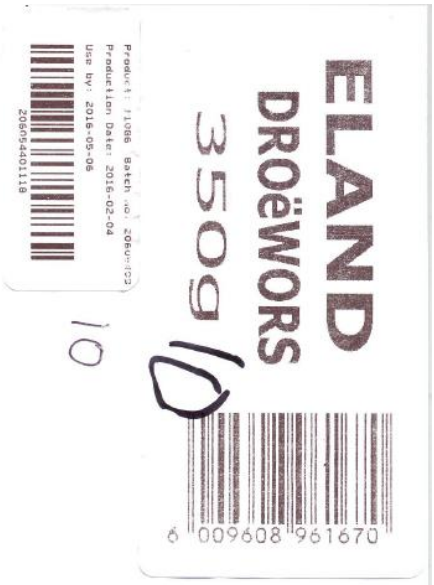
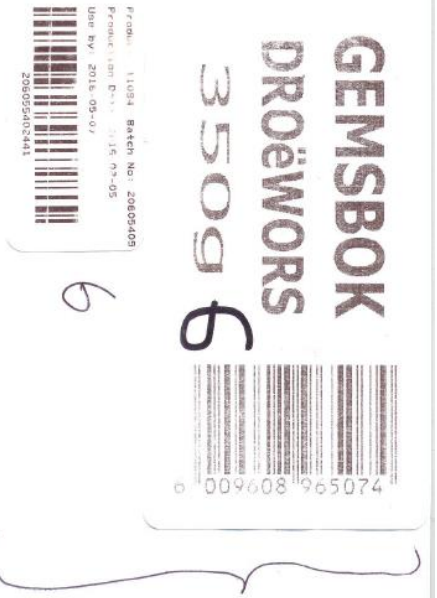
WEIGHT(kg) 0.076
Paked 28-04-16
Sell By 28-05-16
R17.48
SUPERSPAR
1



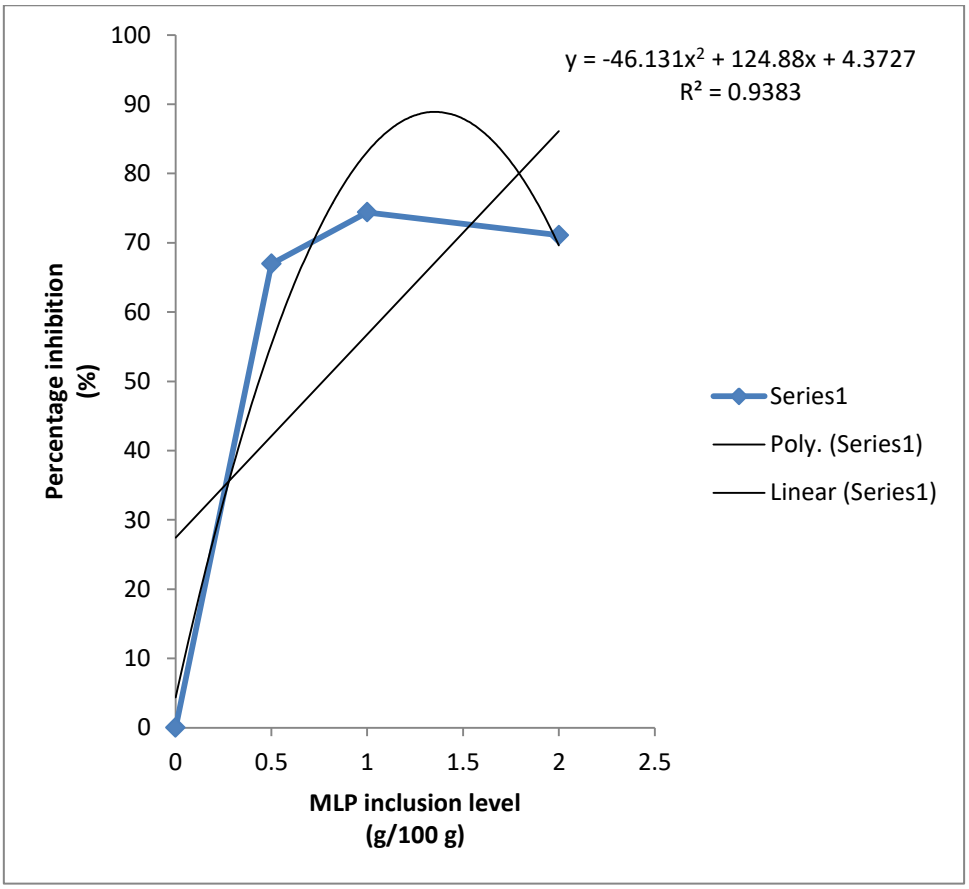




Product of an uncompromising country of splendour and beauty.
Free roaming animals as natural and healthy
as the vastness of the plains they graze.
The taste of true Namibian quality.

CLOSWA BILTONG

Appendix 2 Percentage inhibition of TBARS by *Moringa oleifera* leaf powder in pork droëwors



Appendix 3 Ethical Clearance Certificate

University of Fort Hare
Together in Excellence

ETHICAL CLEARANCE CERTIFICATE
REC-270710-028-RA Level 01

Certificate Reference Number:	MUC381SMUK01
Project title:	**The use of Moringa oleiferas as an antioxidant in pork droewors.**
Nature of Project:	PhD in Livestock and Pasture
Principal Researcher:	Felicitas Esnart Mukumbo
Supervisor:	Prof V Muchenje
Co-supervisor:	N/A

On behalf of the University of Fort Hare's Research Ethics Committee (UREC) I hereby give ethical approval in respect of the undertakings contained in the above-mentioned project and research instrument(s). Should any other instruments be used, these require separate authorization. The Researcher may therefore commence with the research as from the date of this certificate, using the reference number indicated above.

Please note that the UREC must be informed immediately of

- Any material change in the conditions or undertakings mentioned in the document
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research

The Principal Researcher must report to the UREC in the prescribed format, where applicable, annually, and at the end of the project, in respect of ethical compliance.

Special conditions: Research that includes children as per the official regulations of the act must take the following into account:

Note: The UREC is aware of the provisions of s71 of the National Health Act 61 of 2003 and that matters pertaining to obtaining the Minister's consent are under discussion and remain unresolved. Nonetheless, as was decided at a meeting between the National Health Research Ethics Committee and stakeholders on 6 June 2013, university ethics committees may continue to grant ethical clearance for research involving children without the Minister's consent, provided that the prescripts of the previous rules have been met. This certificate is granted in terms of this agreement.

The UREC retains the right to

- Withdraw or amend this Ethical Clearance Certificate if
  - Any unethical principal or practices are revealed or suspected
  - Relevant information has been withheld or misrepresented
  - Regulatory changes of whatsoever nature so require
  - The conditions contained in the Certificate have not been adhered to

- Request access to any information or data at any time during the course or after completion of the project.

- In addition to the need to comply with the highest level of ethical conduct principle investigators must report back annually as an evaluation and monitoring mechanism on the progress being made by the research. Such a report must be sent to the Dean of Research's office

The Ethics Committee wished you well in your research.

Yours sincerely

Professor Wilson Akpan
Acting Dean of Research

26 October 2016