Fatty acid composition, colour stability and lipid oxidation of mince produced from fresh and frozen/thawed fallow deer meat

By

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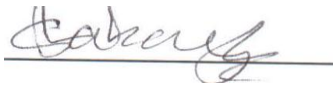
Declaration

I, Chido Chakanya, hereby declare that this dissertation is my original work conducted under the supervision of Prof V. Muchenje, Prof L. C. Hoffman and Dr E. Arnaud and has not been submitted to any university. All assistance towards the production of this and all references contained herein have been duly credited.

Signature  **Date**……..

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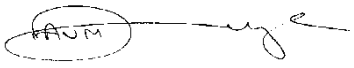
Prof V. Muchenje……  …… **(Supervisor)**

Abstract

Fatty acid composition, colour stability and lipid oxidation of mince produced from fresh and frozen/thawed fallow deer meat

The aim of the study was to determine the fatty acid composition, colour stability and lipid oxidation of fresh mince produced from fallow deer and to evaluate the effect of frozen storage duration on the retail display shelf life of the mince. A total of 31 fallow deer carcasses were used in the study. After cooling for 24hrs, the carcasses were deboned, external fat from the fore and hindquarter muscles removed and individually vacuum packed. For the first trial, seven fallow deer carcasses were used. Meat from the hind and fore-quarters of each carcass was divided into two equal batches per animal. One batch was minced (through a 5 mm die) and packed into oxygen permeable overwraps and refrigerated at 4°C for a period of eight days under retail display conditions. The second batch was vacuum packed and frozen at -20°C for 2 months at the end of which mince was also produced and monitored over an eight day period under the same conditions that were used for the fresh mince. Colour, pH, lipid and myoglobin stability was determined. Proximate and fatty acid composition was also determined. No differences ($P>0.05$) were noted between proximate composition of fresh and frozen/thawed minced meat. The lipid content of fallow deer was 2.4% (±0.04). Total n3 fatty acids differed ($P<0.05$) between treatments and decreased with increased storage and display day. There were significant ($P<0.05$) treatment and time interactions on all measured colour parameters, TBARS and myoglobin forms. Fresh mince was lighter and had higher redness (a^*) and yellowness (b^*) values than mince from two months frozen stored meat. Hue angle for fresh mince remained stable throughout display whereas it increased for frozen/thawed mince. Fresh mince had lower TBARS values than frozen/thawed mince. Minced meat produced from frozen/thawed deer meat

had higher surface met-myoglobin and total met-myoglobin percentages. Surface and total oxy-myoglobin percentage was higher in fresh mince. The first trial clearly showed colour and lipid stability differences between fresh mince and mince from frozen/thawed meat. It also showed that fresh mince has a longer retail display life than mince produced from frozen/thawed meat (six days and four days, respectively).

In the second trial, the effects of frozen storage duration on colour and lipid stability were investigated. Twenty-four fallow deer were used. Twelve were harvested in June (6male 6female) and the other twelve in August (6 male 6female) of the same year.Twenty four hours after harvesting, the fore and hindquarter muscles of the carcasses were deboned, vacuum packed and kept at -20°C until October (i.e. 2months and 4months frozen storage period). Upon thawing, the meat was processed into mince following the same procedure used for the first trialand displayed for a fiveday period under retail display conditions. Frozen duration and gender had no effect ($P>0.05$) on the proximate composition of fallow deer meat. The total amount of saturated fatty acids (SFA) increased and total amount of poly unsaturated fatty acids (PUFA) decreased as frozen duration and display day increased ($P<0.05$). Frozen duration affected ($P<0.01$) lipid oxidation and percentage oxy-myoglobin. Mince pH and all colour parameters (L^*, a^*, b^*,hue and chroma) differed ($P<0.05$) between treatments on day zero and three. Display day was a significant factor ($P<0.05$) on all measured parameters. By day three all parameters except pH showed signs of extended oxidation and discolouration as evidenced by reduced redness, decreased colour intensity and high TBARS values. This study showed that prolonged frozen storage negatively affects the colour and lipid stability of meat and increases oxidation of PUFAs during frozen storage. However, the study also suggests that although

frozen/thawed meat has a shorter retail display shelf life, the proximate composition of the meat remains unchanged.

Keywords: Fresh mince; freezing; frozen duration; fatty acid composition; meat discoloration

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List of abbreviations

α	Alpha
*a**	Colour – redness coordinate
ANOVA	Analysis of variance
ATP	Adenine trio-phosphate
β	Beta
*b**	Colour – yellowness coordinate
BHT	Butylated hydroxytolene
°C	Degree Celsius
Cu^{+}	Copper ion
DFD	Dark firm and dry meat
DMb	De-oxy-myoglobin
Fe^{2+}	Iron ion
FL	Fluorescent lighting
GLM	Generalized linear models
HO_2*	Hydroxyl radical
INC	Incandescent lighting
*L**	Colour – lightness coordinate

LDPE	Low-density polyethylene wrap
MA-TBA	Malonaldehyde thiobarbituric acid complex
MbFe(II)	De-oxy-myoglobin
MbFe(III)	Met-myoglobin
MbFe(IV)	Ferryl-myoglobin
$MbO_2Fe(II)$	Oxy-myoglobin
MH	Metal halide lighting
MMb	Met-myoglobin
MUFA	Mono-unsaturated fatty acids
NADH	Nicotideamide adenine dinucleotide hydride
NO^-	Nitrogen oxide
NO_2^-	Nitrogen dioxide
O_2	Oxygen molecule
$O_2°$	Superoxide anion
°OH	Hydroxyl
OMb	Oxy-myoglobin
$ONOO^-$	Peroxynitrite

PSE	Pale soft and exudative meat
PUFA	poly unsaturated fatty acids
PUFA:SFA	Poly unsaturated fatty acids to saturated fatty acid ratio
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
R°, RO°, ROO°	Free radicals
RH, ROOH, ROOR	Hydro peroxides
SFA	Saturated fatty acids
TBARS	Thiobarbituric reactive substances
μ	Error term
USDA	United States Department of Agriculture
ε	Omega
WBSF	Warner – Bratzer Shear Force
WHC	Water holding capacity

Chapter 1: Background

1.1 Introduction

The role of meat in the human diet as a source of protein cannot be overlooked, as evidenced by an estimated average of 100 g of protein per day being consumed in developed countries; 50% being livestock derived (FAO, 2009). Meat is also a valuable source of essential micronutrients such as unsaturated fatty acids (linoleic acid, omega 3 and 6), vitamins and minerals which are crucial for development and immunity (Serpen *et al*., 2012; Brewer, 2012). For example, the role of omega-3 and 6 (n3 and n6) fatty acids in the involved in the growth of brain and retinal tissues as well as in human disease prevention as been noted (Kallas *et al*., 2014). Conjugated linoleic acid (CLA) reportedly reduces cancer risks, cardiovascular diseases, obesity and diabetes (Nantapo *et al.*, 2015).

On the other hand, great controversy and debate exists regarding the role that meat, especially red meat, plays in development of coronary heart diseases and cancers (McNeill and Van Elswyk, 2012; Dannenburger *et al*., 2013; Polawska *et al*., 2013). This has led to a drop in the consumption of red meat and a demand or search for an alternative red meat or protein sources (Hoffman and Wiklund, 2006). In this regard, game meat is fast gaining popularity with health-conscious consumers (Hoffman *et al*., 2007; Dannenburger *et al*., 2013; Bartôn *et al.*, 2014). Research has shown that in addition to game meat containing a low lipid content, it also has a fatty acid composition that is more favourable than that of traditional domestic species (Hoffman and Cawthorn, 2012; Daszkiewicz *et al*., 2015), and also a desirable mineral composition (VanZyl and Ferreira, 2003; Hoffman *et al*., 2009).

However, the nutritional composition of meat renders its quality susceptible to oxidative processes which can easily result in loss of nutrient, quality and subsequently limit shelf life of meat (Girolami *et al*., 2013). The major limiting factors to meat shelf-life include lipid, protein and myoglobin oxidation processes which often result in huge economic losses (Nassu *et al*., 2012; Rogers *et al*., 2014). Their negative impacts on the flavour, colour and texture of meat result in meat spoilage and consumer rejection of products (Khliji *et al*.,2010). Extrinsic factors such as meat processing (such as mincing), handling and storage and distribution temperatures have a profound impact on the stability of meat and meat products (Mortensen *et al*., 2006; Anese *et al*., 2012; Nassu *et al*., 2012).

It goes without saying that the production of meat and meat products of superior quality is vital in ensuring food security worldwide. Moreover, the preservation of meat in a state of superior quality throughout production, distribution and resale until it reaches the consumer's dinner table cannot be overlooked. Diet manipulation during rearing (Mapiye *et al*., 2010; Nkukwana *et al*., 2013; Ripoll *et al*., 2013), animal welfare during transportation from farm to slaughter houses (Muchenje *et al*., 2009), use of anti-oxidants during further meat processing (Toldra and Reig, 2011), freezing and maintaining a cold chain during distribution (Leygonie *et al*., 2012) and use of improved packaging and storage during retail display in shops (Li *et al*., 2012; Ripoll *et al*., 2013) are some of the many strategies employed by the livestock and meat industry to minimize loss of product due to oxidative processes.

Of all these, the wide use of freezing in the meat industry and has made it to be arguably an indispensable tool in meat preservation (Leygonie *et al*., 2012). However, it is not without its disadvantages, the chief one being ice crystal formation which leads to meat lipid and protein disruption and an instigation of oxidation processes in the meat system (Muella *et al*., 2012). For

this reason, consumers regard frozen/thawed meat and products to be inferior in quality when compared to fresh meat and so fetches little money on the market (Kim *et al*., 2013). Most research done on freezing focuses on the effects of freezing on quality attributes during storage and little work is available on the retail display shelf life of the meat after thawing. Furthermore, there is still little research information available on meat quality parameters of game (Hoffman and Cawthorn, 2012; Dannenburger *et al*., 2013). Thus the study was aimed at investigating the effects of freezing on the quality parameters of fallow deer and on the retail display shelf life of frozen/thawed meat.

1.2 Justification

Freezing is the most popular preservation method used in the meat industry as it has many advantages over other preservation methods; the top most being its having the least adverse effect on meat quality (Castro-Giraldez *et al*., 2014; Kajak-Siemaszko *et al*., 2011; Muela *et al*., 2012). Freezing retards undesirable biochemical reactions in meat, although the formation of ice crystals results in undesirable alteration in cell structure of muscle fibres (Soyer *et al*., 2010). Large quantities of meat are usually kept frozen for specific periods of time at some point along the meat chain (during storage, transportation or in consumers' fridges) before being subsequently sold as chilled products upon thawing (Hansen *et al*., 2004; Pietrasik and Janz, 2009; Muela *et al*., 2012). When it comes to game meat, freezing affords producers greater product control and ease of transportation especially during exports (Leygonie *et al*., 2012).

Currently, worldwide meat exports are estimated to have a value of US$ 13 billion of which freezing plays a vital role in ensuring product quality and safety (Leygonie *et al*., 2012). There is need for a thorough understanding of the effects of freezing on meat quality attributes as this will

go a long way in helping producers ensure quality production of game meat and contribute meaningfully to food safety. With intense socio-economic changes, rising incomes and rapid urbanization in developed countries, it is safe to expect an increase in consumer demand for game meat and as such, there is need to make more information available on the different quality attributes of game meat species.Feral deer populations have been established in South Africa and are growing, opening up opportunities for the expansion of game meat productionin South Africa (Hoffman and Cawthorn, 2012). The exploration of the meat quality characteristics of this meat speciesis desirable as it has the potential of further expanding the game industry and offering a larger variety of options for consumers to choose from.

1.3 Objectives

The broad objectives of this study was to investigate the effects of freezing on the colour and oxidative stability of mince produced from fallow deer meat.

The specific objectives of this study are:

1. To evaluate the colour, pH, lipid and myoglob instability of mince from fresh and frozen/thawed deer, during eight days of retail display
2. To evaluate the effects of frozen duration on the colour, pH, lipid and myoglobin stability of mince from deer, during six days of retail display

1.4 Hypothesis

The null hypothesis being tested was:

1. There are no differences betweenthe colour, pH, lipid and myoglobin stability of fresh and frozen/thawed mince from deer, during eight days of retail display
2. Freezing does not affect the colour, pH, lipid and myoglobin stabilityof mince from deer, during six days of retail display

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Chapter 2: Literature review

2.0 Introduction

Meat shelf-life may be defined as the time that meat and meat products remain satisfactory under specific conditions of distribution, storage and display (Sun and Holley, 2012). Consumers evaluate meat quality according to appearance such as colour and marbling, as well as according to organoleptic attributes such as tenderness, flavor and texture (Muchenje *et al*., 2009). At retail level, consumers consider meat colour to be most indicative of quality before purchasing and to them is synonymous to freshness (Khliji *et al.*, 2010; Nassu *et al*., 2012; Girolami *et al*., 2013). As such, myoglobin and lipid oxidative processes that lead to the discoloration of meat are major limiting factors to meat shelf life.

The physical structure and chemical composition of meat makes it very susceptible to oxidative processes (Falowo *et al*., 2014). Pre-slaughter, animals have strong endogenous anti-oxidant systems such as glutathione, Vitamin C and E which scavenge oxidative species and protect the animal from lipid oxidation (Liu *et al*., 2011). The intrinsic balance between anti-oxidants and pro-oxidants (heme and non-heme iron, cytochromes and ribonucleases); determine the oxidative stability of animal muscle before it is converted to meat *post mortem* (Chaijan, 2008; Luciano *et al*., 2009). Conversely, myoglobin oxidation results in the accumulation of the undesirable met-myoglobin (brown). In living systems, this is prevented by a number of met-myoglobin enzyme reducing systems which reduce met-myoglobin into de-oxy-myoglobin (Bekhit and Faustman, 2005).

Post mortem, this intrinsic balance is disrupted and factors such as storage temperature (freezing or chilling), product packaging (vacuum packing, modified atmosphere packaging and overwraps), mincing and display conditions begin to manipulate oxidation processes and thus the shelf-life of meat (Luciano *et al*., 2009; Estévez, 2011). In industry, large quantities of meat are usually frozen at some point along the meat chain (during storage, transportation and/or in consumers' fridges) before being subsequently sold/eaten following thawing (Hansen *et al*., 2004; Pietrasik and Janz, 2009; Muela *et al*., 2012). In this regard, freezing has become almost indispensable in the meat industry. Furthermore, meat mincing has grown considerably over the years as evidenced by an approximate of 1.3 billion pound (over 589 million kg) of beef sold as ground beef each year in the United Sates (Rogers *et al*., 2014). Consequently, all facets in the meat industry have placed significant efforts in the development of innovative methods and systems which retard oxidation and promote meat colour stability. This chapter reviews some factors that affect the color and oxidative stability of meat.

2.1 Oxidative processes affecting meat shelf-life

2.1.1 Lipid oxidation

The lipid oxidation process involves the saturation of polyunsaturated fatty acids in meat via free radical formation (Estévez and Cava, 2004). Processors and scientists are highly concerned over lipid oxidation as it is the major cause of rancidity and off-flavor in meat and meat products often resulting in loss of desirable colour and flavor (Coronado *et al*., 2002; Kasapidou *et al*., 2012; Muela *et al*., 2014). Furthermore, lipid oxidation has been noted to play significant roles in the pathogenesis of atherosclerosis, aging and carcinogenesis (Shahidi and Zhong, 2010; Girolami *et al*., 2013; Medina-Meza *et al*., 2014). As such there is need for comprehensive

knowledge of mechanisms involved in lipid oxidation and the implication on meat quality so as to improve sustainability of meat and meat products and ensure safe healthy products reach consumers.

The mechanism of lipid oxidation occurs in three stages; initiation, propagation and termination (Chaijan, 2008; Falowo *et al*., 2014). During initiation, a hydrogen atom is lost and a reactive oxygen species (ROS) is formed such as lipid peroxide (ROO°), alkoxyl (RO°), superoxide anion (O_2°) and hydroxyl (°OH) radicals (Min and Ahn, 2005). This step is catalyzed by enzymic and non enzymic interactions with entities such as transition metals (especially iron), heat and light (Renerre *et al*., 1996; Baron and Anderson, 2002). Interstingly, heme-iron and non-heme iron are argued to be the most pivotal catalyzing agents in muscle based food systems (Baron and Anderson, 2002). Excited singlet oxygen that rapidly reacts with meat fatty acids may be formed in a non-radical mechanism, in the presence of light and photo synthesizers (Cardenia *et al*., 2013).

Once free radicals have been formed, they extract protons from neighboring fatty acids and thus propagate the oxidation process. Intermediate hydro peroxide molecules (ROOH) are used to identify the pathway mechanism in oxidation and usually signify primary oxidation (Shahidi and Zhong, 2010). These species are more reactive than normal fatty acids and decompose, causing rancidity in meat (Chaijan, 2008). Depending on the cell or tissue environment, they react to give secondary oxidation products such as hydroperoxyl cycloperoxides and bicycloendoperoxides which are known precursors of malonaldehyde (Min and Ahn, 2005; Qwele *et al*., 2013). Malonaldehyde is a highly reactive three carbon dialdehyde that reacts with 2-thiobarbituric acid to form a pink complex which can be measured spectrophotometrically at the absorbance of 530-535nm (Shahidi and Zhong, 2010). Thus, the amount of this complex in meat tissues is used as a

reflector of the level of lipid oxidation which has occurred in meat. However, since other products of lipid oxidation (alkenals and alkadienals) can also react with 2-thiobarbituric acid forming pink complexes the test has been termed Thiobarbituric Reactive Substances (TBARS). Many researchers have used this technique to measure the extent of lipid oxidation in meat and food systems (Kim *et al*., 2011; Kim *et al*., 2014a; Nkukwana *et al*., 2014) and this same method was used in the current research. Figure 2.1 summarizes the general reaction pathways in lipid oxidation and its effects on meat quality.

Due to increase in demand for lean meat by health conscious consumers and the limit of lipid intake by theUnited States Department of Agriculture(USDA), the industry has made efforts to lower the lipid content in meat. However, low lipid content does not necessarily mean the animal is more nutritive, it is the fatty acid composition that gives this information, more especially the polyunsaturated: saturated fatty acid ratio. Currently meat and meat products have ratios above 15 and nutritionists recommend a ratio below 5 (Nantapo *et al*., 2015). However, studies show that meat produced with such low ratios become more prone to lipid oxidation and will require addition of extra anti-oxidants either through diet manipulation or exogenously after slaughter (Yang *et al*., 2002; Kim *et al*., 2013b; Nkukwana *et al*., 2014; Qwele *et al*., 2013).

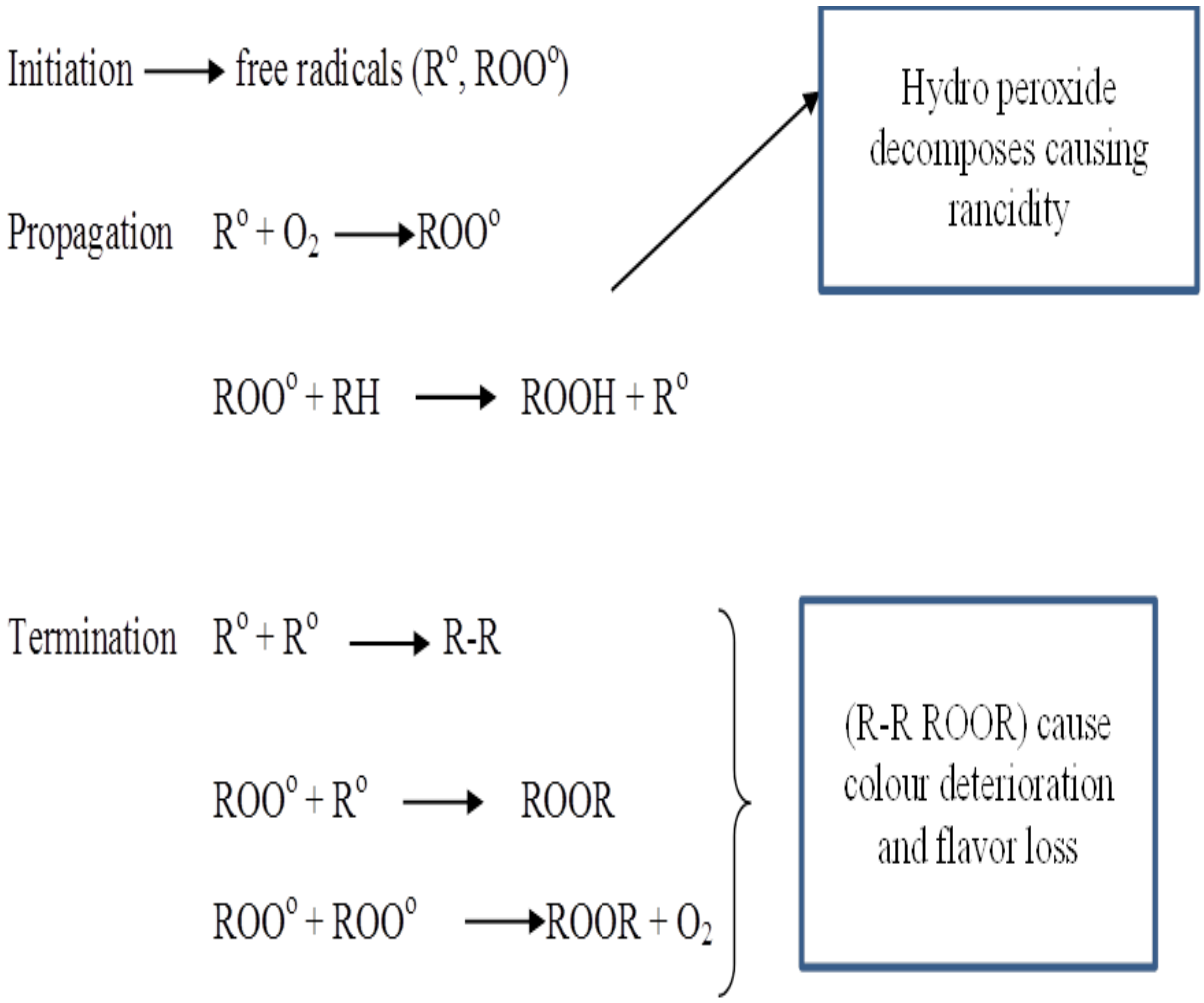

Figure 2.1 General reaction paths in lipid oxidation and effects on meat quality

Adapted from Nawar, 1996 and Chaijan 2008

2.1.2 Myoglobin oxidation

The color of meat is mainly attributed to myoglobin; a protein molecule which can exist in different chemical forms in the meat system as shown in Figure 2.2 (Abril *et al*., 2001; Girolami *et al*., 2013). It consists of a globin protein which is attached to an iron-heme prosthetic group. Its chemical structure is directly related to its biological function; it reversibly binds oxygen and acts as a reservoir until oxygen is needed by tissue cells (Brewer, 2004; Bekhit and Faustman, 2005). Deoxy-myoglobin (MbFe(II)) is purple in colour and exists under very low oxygen partial pressure (<1.4mm Hg) (Mancini and Hunt, 2005). When oxygen partial pressure increases around 70-80mm Hg, it becomes oxygenated into oxy-myoglobin ($MbO_2Fe(II)$) which forms a bright red colour often desired by consumers (Luciano *et al*., 2009). Occasionally when there is no oxygen and carbon monoxide is available, a stable bright red ferryl-myoglobin (MbFe(IV)) complex will form.

As a result of the high reactivity of the ferrous (Fe^{2+}) state of myoglobin, spontaneous oxidation into the ferric (Fe^{3+}) state may occur forming met-myoglobin (MbFe(III)) (Bekhit and Faustman, 2005; Chaijan, 2008). Met-myoglobin cannot bind oxygen and is undesirable physiologically. It is also responsible for the brown colour observed on meat surfaces (Brewer, 2004). The reduction of MbFe(III) into MbFe(II) in meat systems is carried out by met-myoglobin enzyme reducing systems, maintaining the delicate balance between the three forms of myoglobin (Faustman *et al*., 2010). Thus, met-myoglobin is kept in low quantities in the cells and oxy and de-oxy-myoglobin are predominant. Intrinsic factors affecting the rate of myoglobin oxidation include sex, breed, endogenous anti-oxidants, rate of pH decline and ultimate pH (Carlez *et al*., 1995; Faustman *et al*., 2010).

Contrary to the balance which exists between de-oxy/oxy-forms and met-forms found in living muscles, *post mortem* processes continuously inactivate met-myoglobin enzyme reducing systems (Baron and Anderson, 2002). This stimulates acid-catalysed autoxidation of ferrous iron to ferric iron which results in the accumulation of MbFe(III) (Chaijan, 2008; Quevedo *et al*., 2013). Other extrinsic factors also come into play such as oxygen partial pressure, rate of oxygen consumption by tissue, light type exposed to, storage temperature, meat micro flora and packaging (Bekhit and Faustman, 2005).

Myoglobin represents about 70% of the total concentration of heme proteins found in beef, pork and dark muscles broilers (Baron and Anderson, 2002). In game meat however, it comprises the bigger fraction of the total heme proteins (Onyango *et al*., 1998; Hoffman *et al*., 2005; Hoffman *et al*., 2009). This comes about as a result of highly active nature of game species compared to domestic species, resulting in a higher build-up of myoglobin in muscle tissue in order to increase oxygen carrying capacity (Hoffman *et al*., 2005). Furthermore, myoglobin from different species can differ in their primary structure, resulting in different reactivity and reaction mechanisms (Baron and Anderson, 2002). This has a significant impact on the rate of myoglobin oxidation and subsequently shelf-life and colour stability. Figure 2.2 summarizes the conversion and reactivity pathways of myoglobin.

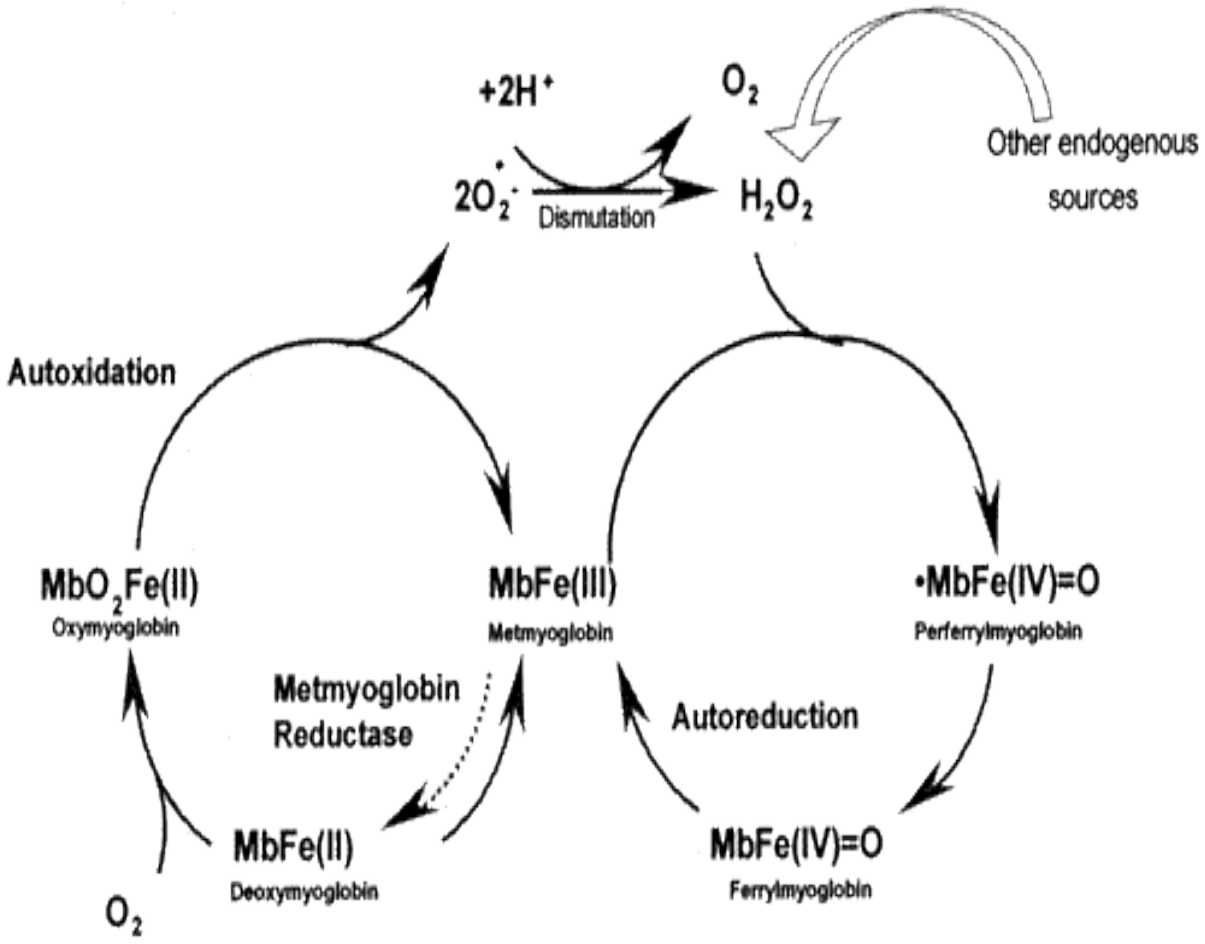

Figure 2.2 Myoglobin conversion and reactivity pathways

From: Baron and Anderson, 2002.

2.1.3 Protein oxidation

The oxidation of proteins plays a major role in meat concerning sensory, nutritional and physical aspects (Falowo *et al*., 2014). For long, this role has been mostly ignored (Lund *et al*., 2011; Xue *et al.,* 2012). This is possibly due to the complex nature of the chemistry behind the oxidation process and lack of suitable and specific assessment methods (Estévez, 2011). According to Lund *et al*.(2011), protein oxidation occurs generally the same way as lipid oxidation, except in the former, more complex interactions and a variety of end products result. Formation of species such as protein radicals, amino acid derivatives, protein breakdown and polymerization is suggested to contribute significantly to protein degradation by proteases and negatively affect digestibility and nutritional value of meat (Xue *et al*., 2012). Furthermore, free radicals such as singlet oxygen and reactive nitrogen species (RNS) such as peroxynitrite ($ONOO^-$), nitrogen dioxide (NO_2^-) and nitric oxide (NO^-) encourage disruptive autoimmune responses which will cause oxidative and nitrosative stress (Falowo *et al*., 2014).

The main oxidative modifications of proteins; thiol oxidation, aromatic hydroxylation and carbonyl group formation, occur on the side chains of amino acids. The most susceptible side chains include methionine and cysteine side chains as they have highly reactive sulfide anions (Zhang *et al*., 2013). Sulfide anions are very rich in electrons creating a very powerful nucleophile which easily loses a hydrogen atom, leaving a protein free radical (Estévez, 2011). This free radical then reacts with oxygen forming a peroxyl radical which further reacts by removing another hydrogen atom from other susceptible molecules (Zhang *et al*., 2013). Other subsequent reactions are summarized in reactions 4-7 in Figure 2.3 and involve the reaction of radicals (HO_2*) with reduced forms of transitional metals (Fe^{2+}, Cu^+) resulting in the formation of alkoxyl radicals and hydroxyl derivatives.

These end products of protein oxidation have been described to enhance quality deterioration although the exact roles are not fully understood. Protein oxidation is thought to negatively affect water holding capacity of meats (Lund *et al.*,2011) which then negatively affects juiciness of meat (Muchenje *et al*., 2009). A summary of the reaction pathways in protein oxidation is given in Figure 2.3.

$$PH + HO_2^* \longrightarrow H_2O + P^* \quad (1)$$

$$P^* + O_2 \longrightarrow POO^* \quad (2)$$

$$POO^* + PH \longrightarrow POOH + P^* \quad (3)$$

$$POOH + M^{n+} \longrightarrow PO^* + HO^- + M^{(n+1)+} \quad (4)$$

$$POOH + HO_2^* \longrightarrow PO^* + O_2 + H_2O \quad (5)$$

$$PO^* + HO_2^* \longrightarrow POH + O_2 \quad (6)$$

$$PO^* + H^+ + M^{n+} \longrightarrow POH + M^{(n+1)+} \quad (7)$$

Figure 2.3 Summary of reaction pathways of protein oxidation

From: Estévez, 2011

2.1.4 Lipid, myoglobin and protein oxidation interactions

The reactive nature of the primary and secondary products derived from lipid oxidation is thought to promote myoglobin and protein oxidation (Faustman *et al*., 2010; Qwele *et al*., 2013). For example, 4-Hydroxynonenal and unsaturated aldehydes have been reported to increase the rate of met-myoglobin formation in vivo (Lynch and Faustman, 2000) and interfere with protein oxidation (Sakai *et al*., 1995). On the other hand, greater myoglobin concentrations are linked to greater lipid oxidation rates (Faustman *et al*., 2010). Oxidation of $MbO_2Fe(II)$ to MbFe(III) produces reactive intermediates that enhance oxidation of unsaturated fatty acids (Baron and Anderson, 2002). For example the intermediate superoxide anion rapidly disrupts into hydrogen peroxide which then reacts with the MbFe(III) simultaneously produced to a MbFe(III) complex which further enhances lipid oxidation (Faustman *et al*., 2010).

Furthermore, in the presence of unsaturated lipids, MbFe(III) is denatured due to formation of a non-catalytic hemi chrome pigment. This denaturing process results in further exposure of heme groups to surrounding lipids, thus propagating lipid peroxidation (Baron and Anderson, 2002). The same redox and spin characteristics displayed by myoglobin are also displayed by cytochromes and ribonucleases in biological tissues and thus the latter are believed to play a role as well in lipid oxidation (Li *et al*., 2012).

Amino acid residues interact with lipid oxidation products forming cross-linkages between proteins thereby regulating their structure and function (Lund *et al*., 2011). Feeding animals with different levels of unsaturated fatty acids has been shown to promote protein oxidation (Nute *et al*., 2007). Zhang *et al*. (2010) reported that the consumption of oxidized oil correlated positively with increased levels of protein carbonyls in the breast meat of broiler chickens.

2.1.5 Interaction between microbial contamination and oxidation

The biological components of meat encourages microbial growth (mainly bacteria, yeast and moulds) which cause meat spoilage by instigating meat discolouration, off odours and changes in texture and flavour (Nychas *et al*., 2008). Additionally, bacterial growth reduces product safety and the presence of pathogenic bacteria raises consumer concern (Papadopoulou *et al*., 2012). The presence of micro-organisms become detectable through off odours and slime when populations 10^7 to 10^8 cfu/cm^2 (Gill, 2007). Predominant bacteria related to meat spoilage under refrigerated conditions include *Brochotrix thermosphacta*, *Carnobacterium* spp, *Enterobacteriaccea*, *Lactobacillus* spp, *Lueconostoc* spp, *Pseudomonas* spp and *Shewanella putrefacians* (Nychas *et al*., 2008).

Microbial quality of fresh meat will depend on the physiological status of the animal at slaughter, cross-contamination during the slaughter process, ultimate pH and the temperature and storage conditions of the carcass/meat (Borch *et al*., 1996; Papadopoulou *et al*., 2012). Of these, temperature can be termed the principle factor affecting microbial growth and thus meat shelf life. Micro-organisms are classified under three categories based on their optimal temperature range. *Psycrophiles* have their optimum temperature below 20°C, *thermophiles* thriveat temperatures above 45°C and *mesophiles* have a temperature range in between the other two (Kennedy *et al*., 2004). As temperatures rise to the microbes' optimal temperature, rate of microbial growth also rises and decreases as the temperature deviates from the optimal.

Literature suggests that micro-organisms interfere with the rate of lipid and myoglobin oxidation in meat. Borch *et al*. (1996) suggested specific lactic bacteria inhibited the growth of spoilage bacteria by producing antibacterial substances. On the other hand, Fik and Leszczynska-Fik

(2007) reported that while growing, *Yersinia enterocolitica* and *Listeria mono-cytogenes* generates enzymes which catalyze protein and lipid oxidation reactions. This will result in the release of decomposition products such as peptides and fatty acids which cause undesirable changes in meat color, taste and odor (Papadopoulou *et al.*, 2012). Bacteria also produce hydrogen sulphide under low glucose and oxygen availability, converting myoglobin to green sulphmyoglobin (Fik and Leszczynska-Fik, 2007). However, sulphmyoglobin is not commonly found in normal pH meat and is associated with high pH (dark firm and dry) meat.

2.2 Post-slaughter processes affecting colour and oxidative stability of frozen/minced meat

2.2.2 Mincing

Commonly, minced meat is produced from the trimmings of joints and cuts or from tough inferior parts of the carcass (e.g forequarter) for which there is insufficient consumer demand (Carlez *et al*., 1995). The processing of such cuts into mince plays a vital role in reducing losses and providing a source of protein to consumers. However, the processing also brings about undesirable chemical reactions which affect the shelf-life of the product.

Minced meat is generally known to have a shorter shelf-life as compared to whole meat cuts (Fik and Leszczynska-Fik, 2007). This is mainly because mincing increases the surface area of meat exposed to oxygen and disrupts and exposes phospholipids to pro-oxidants such as iron and copper (Crowley *et al*., 2010). Mincing also results in iron being released from myoglobin and ferritin, which then react in oxidative chain reactions and thus increasing the rate of lipid and myoglobin oxidation (Fik and Leszczynska-Fik, 2007). During the mincing process, heat is

produced and this increases the overall temperature of the meat, making it more prone to spoilage by microbial growth and oxidative processes (Crowley *et al*., 2010).

The production and storage conditions of meat before and after mincing, affects the subsequent shelf-life duration of the mince. According to EC Regulation 853 ⁄ 2004, meat to be used for mincing should not be over seven days old, or vacuum packed for longer than 15 days and should be frozen at -18°C for an unspecified 'limited time' (Anonymous, 2004). There is argument however, for the validation of this legislation as there is no scientific evidence suggesting that aged meat affects shelf-life of the mince produced (Crowley *et al.*, 2010).

2.2.3 Freezing

Cooling meat below 0 °C causes water to move out of the cells and occupy the intracellular spaces forming ice crystals (Hegernreder *et al*., 2013). These crystals will subsequently draw more water from the intracellular spaces. This phenomenon is responsible for the excessive loss of moisture during thawing as not all the water will be able to return into the intracellular spaces (Leygonie *et al*., 2012). Ice crystals cause structural changes in the cell membrane, resulting in release of substances that trigger oxidative processes (Anese *et al*., 2012). As water moves out of intracellular spaces, the concentration of solutes surrounding the sensitive protein structure increases, subsequently leading to protein aggregation and denaturing (Li and Sun, 2002; Kajak-Siemaszko *et al*., 2011). The process of freezing can be separated into three phases as follows:

1.) a pre-cooling phase when meat is losing heat energy up until it reaches freezing point;

2.) a latent heat phase in which liquid water changes into a solid phase, i.e ice crystal formation;

3.) a slight gradual decrease in temperature whereby the final temperature of the meat is attained (Kasper and Friess, 2011; Kiani and Sun 2011; Castro-Giráldez *et al*., 2014).

The temperature changes which occur during the different freezing phases are depicted in Figure 2.4. The formation, amount and size of ice crystals will depend on the freezing rate, freezing temperature, storage temperature as well as the freezing method used (Pietrasik and Janz, 2009; Soyer *et al*., 2010; Muela *et al*., 2012). Fast rates of freezing generally result in small evenly distributed ice crystals, which minimize the extent of damage caused by freezing (Kim *et al*., 2013a). Most commonly used fast rate freezing methods in the meat industry are air blast, plate contact and cryogenic freezing (Anese *et al*., 2012). These methods usually freeze product within 10-24 hours depending on the size and thermal conductivity of the meat. However, some products require the formation of large crystals such as in freeze drying and freeze concentration (Kiani and Sun, 2011).

Initial freezing point temperature in meat products will determine size of crystals formed (Zhou *et al*., 2010;Farouk *et al*., 2009). This will depend on the nature and concentration of solutes within the meat as well as the particle size, microstructure, porosity and biological aspects such as age and species (Castro-Giráldez *et al*., 2014). Products with higher initial freezing temperatures result in faster freezing rates and small ice crystal formation (Farouk *et al*., 2009).

The temperature attained after freezing highly affects the extent to which cellular damage occurs in meat systems. Literature agrees that a portion of water remains unfrozen and thus acts as a medium for biochemical reactions to occur (Leygonie *et al*., 2012; Anese *et al*., 2012). Temperatures of -20°C do not inhibit oxidative processes but rather slow them down. Temperatures of -80°C are thought to completely freeze out water and thus prevent further deteriorative processes from occurring (Kiani and Sun, 2011; Utrera *et al*., 2014). However, fluctuations in frozen storage temperature greatly affect shelf-life by bringing into play the phenomena of ice crystal redistribution. Ice crystal redistribution entails the growth of larger ice

crystals in place of smaller crystals which would have formed at the initial freezing. Prolonged freezing (longer than 3months) is also thought to result in re-crystallization of smaller crystals into bigger crystals (Mortensen *et al*., 2006).

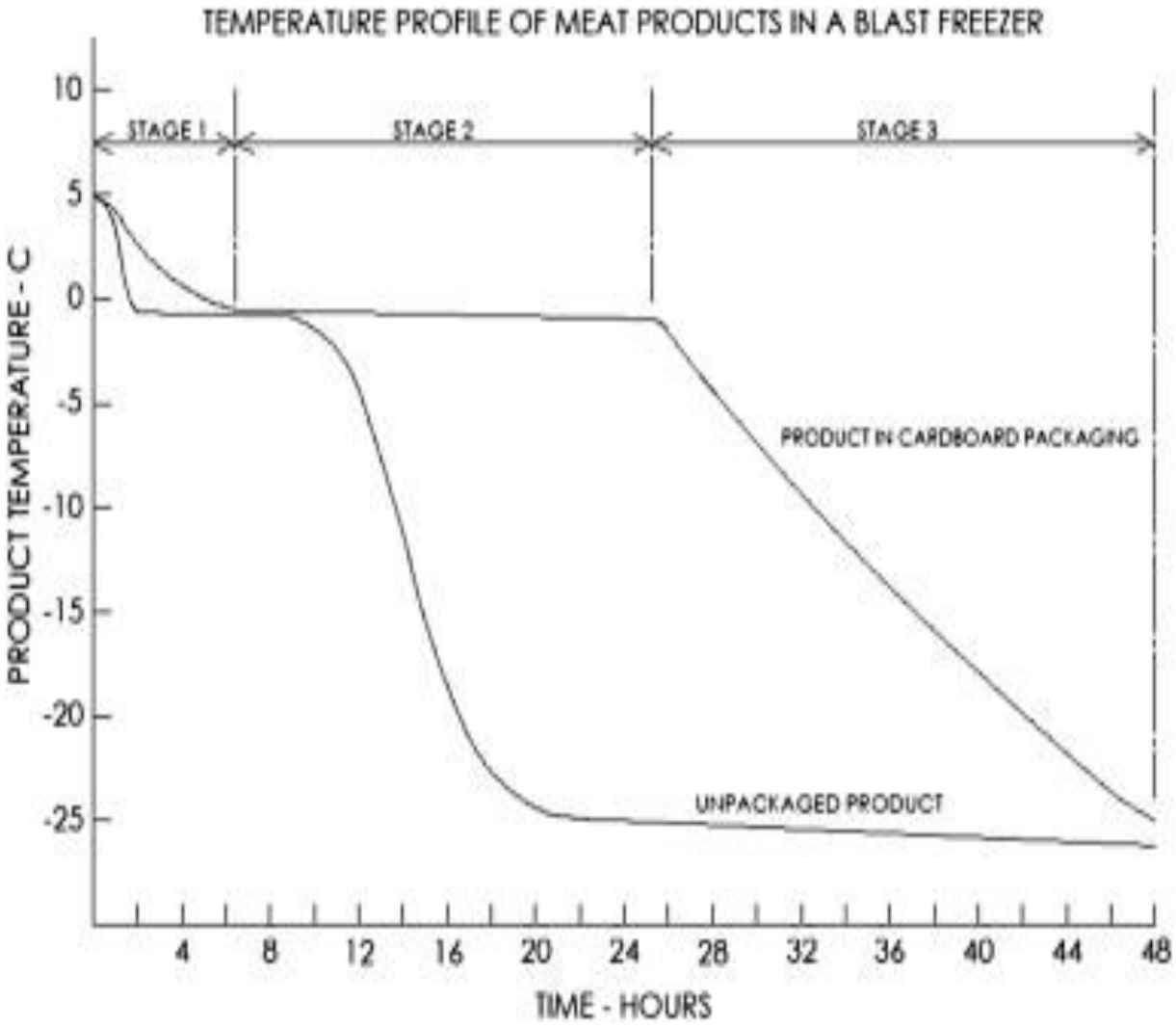

Figure 2.4 Freezing curve of meat systems

From: Dempsey and Bansal, 2012

2.2.4 Retail packaging

Meat packaging serves to protect the product from further deteriorative processes (lipid and myoglobin oxidation and microbial contamination) as well as to contain and present the product to consumers in a convenient way (Kerry *et al*., 2006; Nassu *et al*., 2012). Many packaging systems are available and these range from overwraps for short term display to broad modified atmosphere packaging systems for longer display (Kerry *et al*., 2006). The choice to use any of these systems borders on diversity of product characteristics, convenience to producers and consumers and ability to function economically (Rogers *et al*., 2014).

For long, fresh meat was often displayed in overwrap material with oxygen permeable films which allowed quick myoglobin oxygenation and the development of desirable red colour (Ripoll *et al*., 2013; Rogers *et al*., 2014). This packaging method has proved to be cost effective and readily acceptable to consumer as it allows easy inspection (McMillin, 2008). However, the uncontrolled oxygen supply to the product allows for oxidative processes to accelerate without hindrance and products have been reported to show signs of discoloration after just one day of display (Jeremiah and Gibson, 2001; Kim *et al.*, 2013a).

Modified atmosphere packaging is becoming more popular for use in extending shelf-life (McMillim, 2008). It entails removing and replacing the atmosphere surrounding meat before sealing it off in vapor impermeable variables (Arvanitoyannis and Stratakos, 2012). The replaced atmosphere has different compositions of oxygen and carbon dioxide. Modified atmosphere packaging systems which use controlled amounts of different gas composition are now popular (Kim *et al*., 2013b; Rogers *et al*., 2014). Vacuum packing excludes oxygen from meat, promoting the maintenance of myoglobin in the deoxygenated form. However, vacuum packing gives a purplish colour to products which is unfamiliar with consumers and not readily

acceptable. Additionally, vacuum packing of fresh meat has been noted to cause exudate to accumulate.

2.2.5 Retail display conditions of meat

To attract consumers, retailers take into account display conditions such as display units, display temperatures, lighting source and lighting intensity and wave length (Barbut, 2001). Display temperatures play a vital role in shelf-life stability. Literature suggests the optimum retail display temperature for packaged meat to be 2°C and considers anything above 5°C to be abusive (Mills *et al.*, 2014). Fluctuations in display temperature result in significant loss of shelf-life due to microbial growth and oxidation. For example, Barbut (2001) modelled that for every 1°C increase from 1.5°C up to 5°C, loss of shelf-life is predicted to be 15, 35 and 65%.

Light sources (fluorescent (FL), incandescent (INC) and metal halides (MH)) used in retail display units may be in the form of overhead fixtures or may be positioned inside the display case (Barbut, 2001). Fluorescent lighting is more popular than INC and MH because it produces relatively low heat and thus minimizes microbial growth (Barbut, 2001). However, lighting is known to accelerate oxidative reactions (Martínez *et al*., 2007) and even though FL releases a small amount of radiation, if used in cabinets it should be carefully positioned to avoid any undesirable effect on the meat display life.

2.3 Meat quality attributes affected by freezing and thawing

2.3.1 Moisture

Free water in muscles is found in the myofibrils, between the thick and thin filaments. A small proportion of this water (4-5%) is bound by electrostatic attractions to proteins (Cheng and Sun,

2008). Post slaughter there is rapid decline in pH, loss of ATP and onset of rigor mortis resulting in moisture release from cells into interfibrillar spaces (Leygonie *et al*., 2012). About 1-3% of this moisture is lost during normal meat conversion processes but freezing accentuates moisture loss up to 10-18% (Kim *et al*., 2013a). Many reports confirm the increase in exudate formation when meat is frozen for long periods of time (Hansen *et al*., 2004, Kim *et al*., 2013a; Muela *et al*., 2014). Low freezing temperatures are reported to result in high thaw loss than high freezing temperatures (Mortensen *et al*., 2006).

During thawing, water previously frozen in the intercellular spaces is reabsorbed back into the cells. Depending on the rate of thawing, not all of the water is reabsorbed and some is lost as exudate (Zhang *et al*., 2005; Muchenje *et al*., 2009). Slower rates of thawing favor more water reabsorption and less exudate formation. Contrary to this, Hegernreder *et al*. (2013) showed that fast thawed meat had less exudate than slow thawed meat. However, the same author noted that thaw loss of fast thawed meat accumulated faster than slow thawed meat during display.

The loss of moisture as exudate not only affects the final weight of a product thereby having an effect on yield, but it also affects eating quality of meat in terms of juiciness (Cheng and Sun, 2008). Moreover, exudate formation represents loss of important minerals such as amino acids and vitamins. Myoglobin has been found by electrophoresis to be in exudate; accounting, in part, for colour loss in frozen/thawed products (Leygonie *et al*., 2012).

2.3.2 Meat pH

Decline in meat pH is normal *post mortem* as blood flow stops and H^+ ions accumulation due to anaerobic glycolysis (Kim *et al*., 2014b). The ultimate pH (pH_u) is correlated to the ability of meat to disperse light and ultimately affects the color of meat (Muchenje *et al*., 2009). A pH_u of

5.4–5.5 causes less water to be bound by proteins in the muscle leading to exudation formation on the meat surface. This water makes the surface wet and enables meat to reflect light more easily, giving meat a characteristic bright red colour (Abril *et al*., 2001; Hughes *et al*., 2014). If A pH_u >6.0 causes proteins to associate more with water and the fibres to be tightly packed. This reduces the ability of meat to scatter light and the color appears darker (dark firm and dry meat; DFD) (Hughes *et al*., 2014). Conversly, a rapid fall in pH results pale soft and exudative (PSE) meat. This latter phenomenon has been well displayed in pork meat and studied extensively (O'Neill *et al*., 2003; Barbut *et al*., 2008; Gajana *et al*., 2013). Freezing causes a general decline in meat pH (Leygonie *et al*., 2011; Muela *et al*., 2014). A possible reason for this could be the release of hydrogen ions by denatured proteins during thawing and a possible increase in the concentration of solutes during thawing caused by exudate (Leygonie *et al*., 2012).

2.3.3 Tenderness

A person's perception of meats organoleptic qualities such as softness on tongue and resistance to pressure, contribute to the tenderness of meat. Meat tenderness varies and is mainly determined by myofribillar protein structure and the changes which occur to this structure during slaughter up until it is consumed (Muchenje *et al*., 2009). For example, refrigerating a carcass soon after slaughter will result in a phenomenon called cold shortening whereby muscles rapidly and severely contract. This contraction will require much more shear force to separate the muscles. Tenderness is measured by an instron machine in Newtons (N) using Warner-Bratzer Shear Force (WBSF). This machine records the amount of force required to break myofribillar proteins in meat. Therefore, the higher the WBSF values, the less tender the meat.

Loss of muscle fiber integrity and weakening of muscle due to freezing is expected to increase tenderness. Many researchers agree with this statement (Muela *et al*., 2014; Utrera *et al*., 2014). Some authors suggest that freezing results in the loss of the calcium dependant caplain system inhibitors, slowing down enzyme activity but once thawed, enzyme activity and proteolysis would be improved (Crouse and Koohmaraie, 1990). Kim *et al*. (2013a) reported an initial increase in toughness (high WBSF values) in freeze-thawed pork compared to fresh pork. Low WBSF values were recorded later on during days of display. However, literature is inconclusive on the effects of freezing on tenderness (Vieira *et al*., 2009). Differences in results may be explained by the different freezing rates, methods and final freezing temperature attained or due to different ageing regimes before freezing. Conversely, Veira *et al*. (2009) notes that the tenderizing effects of freezing become insignificant when meat is properly aged before freezing.

2.3.4 Colour

Huge losses (about 4-5% of the wholesale price annually) have been reported in Canada and the United States as a result of product rejection by consumers due to meat discoloration (Nassu *et al*., 2012). Meat color is commonly quantified by the CIE-L^* (black and white), a^* (red-green) and b^* (blue-yellow) values. Meat lightness is represented by L^* which ranges from 0 to 100 whilst a^* and b^* represent the chromatic components of meat and range from -120 to +120 (Priolo *et al*., 2001; Girolami *et al*., 2013). Freezing seems to affect the colour parameters of meat differently.

Lightness is the least affected of the colour parameters by storage and display. The reason could be because of the lack of link between lightness and myoglobin oxidation (Utrera *et al*., 2014). Generally, fresh meat is lighter than frozen/thawed meat (Muela *et al*., 2012; Kim *et al*., 2013a).

Literature widely reports that frozen storage reduces redness (Farouk *et al*., 2009; Muela *et al*., 2012). This reduction in redness is directly linked to myoglobin denaturing due to the cold (Thiansilakul *et al*., 2012). During freezing and frozen storage, met-myoglobin enzyme reducing systems are denatured and upon thawing lose their ability to reduce met-myoglobin, resulting in the accumulation of met-myoglobin. Farouk *et al*., (2009) reported that ageing meat prior to freezing could greatly improve the colour stability of frozen/thawed meat. The reason for this is not clear although speculation is that ageing meat may allow maintenance of endogenous reducing co-factors such as NADH within meat thus impeding oxidative processes during retail display (Kim *et al*., 2011).

2.6 Fallow deer (*Dama dama*)

The deer is a ruminant mammal which belongs to the cervidae family. Several deer species are extensively and intensively farmed internationally such as the red deer (*Cervus elaphus)*, white-tailed deer (*Odocoileus Virginianus*), roe deer and fallow deer (*Dama dama*) (Volpelli *et al*., 2003). Fallow deer are intermediate-sized ruminants with males and females weighing 70 kg and 40 kg respectively. They are classified as intermediate selective foragers and will thrive in many areas.

There has been a rapid increase of fallow deer farming over the years. This is mainly attributable to the increased demand from consumers due to its specific sensory properties and healthy qualities such as low fat and cholesterol content (Ramanzin *et al*., 2010; Hoffman and Cawthorn, 2012; Daszkiewicz *et al*., 2015), fallow deer farming has increased over the years. The estimated global population of farmed deer is 5 million, of which more than half is produced in New Zealand (Daszkiewicz *et al*., 2015). Conversely, the fallow deer is not as popular in South Africa

and with South African game consumers as springbok and blesbok (Hoffman and Cawthorn, 2012). The species is mostly harvested through hunting from feral populations that were introduced in the early 1900's. A recent study by Daszkiewicz *et al*. (2015) suggests that meat from wild populations is significantly different from farmed populations to be considered as different meat products.

Feral deer populations have been established and growing considerably in South Africa and are increasingly becoming more accessible. Little research has been done to determine the meat quality traits of the fallow deer species in South Africa and there is little knowledge by local consumers of this species thus its performance on the market remains questionable. It would be worthwhile to consider the species for meat consumption. Table 2.1 shows the nutrient composition of fallow deer farmed intensively which compares favorably with other common game species as well as common domesticated animals.

The lipid content of fallow deer, like most game species, is not only lower than domestic meat species but also has a favorable fatty acid composition (Hoffman and Wiklund, 2006). Bartoň *et al*. (2014) reported significant differences between the total intramuscular lipid content and the fatty acid profile of eland and beef. Many studies confirm that game meat has high amounts of poly-unsaturated fatty acids and favourable PUFA : SFA ratios (Volpelli *et al*., 2003; Polak *et al*., 2008 ;Sales and Kotrba, 2013). Fallow deer is no exception. However, these attributes are thought to affect shelf life and colour stability of game meat.

Table 2.1 Proximate composition (means ± standard error) of fallow deer and selected game and domestic species

Component	Wild fallow deer[5]	Farmed fallow deer[1]	Springbok[2]	Blesbok[3]	Beef[4]
Moisture %	-	76.02±0.54	73.14±0.53	75.09±1.22	71.6±0.45
Protein %	22.79±0.35	21.67±0.58	20.71±0.36	22.32±1.19	20.94±0.35
Intramuscular fat %	0.50±0.14	0.64±0.14	1.21±0.26	0.78±0.23	6.33±0.21
Ash %	1.10±0.09	1.13±0.04	1.28±0.13	1.29±0.20	1.03±0.05

Source: [1]Volpelli *et al.*, 2003; [2] Hoffman *et al.*, 2008; [3] Hoffman *et al.*, 2009; [4] USDA, 2011; [5]Daszkiewicz *et al*., 2015

2.7Summary

With increasing world population and the need to supply consumers with safe, healthy and appealing products, it is of paramount importance that meat quality attributes be preserved and shelf-life prolonged. Colour stability of meat products is a major determinant of product acceptability by consumers and is affected by *post mortem* processing conditions such as freezing and mincing. From literature it is clear that these conditions need to be thoroughly understood and controlled such that the industry produces meat products of consistently high quality. Continual use of freezing in the meat industry to mitigate lipid and myoglobin oxidation is inevitable. Although a lot of research has been dedicated to fully understanding meat shelf-life, gaps still exist especially where venison and game meat is concerned. With market demand for game rising over domestic species, it would be of great benefit for the industry to invest more in researching meat quality attributes of game and venison, especially in South Africa. Moreover, it would be advantageous to conduct research on the meat quality attributes of fallow deer as there is a growing interest in South Africa of using the species for meat production.

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Chapter 3

Colour, myoglobin and oxidative stability of mince produced from fresh and frozen/thawed fallow deer, during eight days of display storage

Abstract

The colour and lipid stability of minced meat made from fresh fallow deer meat and from two months frozen/thawed fallow deer meat was investigated over eight days of display. Proximate and fatty acid composition was also determined. Seven (7) mature fallow deer were harvested, carcasses cooled for a period of 24 hours, meat from the fore and hindquarters deboned, all external fat removed and half of the meat minced (through a 5 mm die) and packed into oxygen permeable overwraps. The mince was then refrigerated under retail display conditions for eight days at 4°C. The rest of the fore and hindquarter meat was vacuum packed per animal and frozen at -20°C for 2 months at the end of which mince was also produced, packaged and displayed under the same conditions as fresh mince. No differences ($P>0.05$) were observed between proximate composition of fresh and frozen/thawed minced meat. The lipid content of fallow deer was 2.4% (±0.04). Total n3 fatty acids differed ($P<0.05$) between treatments and decreased with increased storage and display day. There were significant ($P<0.05$) treatment and time interactions on all measured color parameters, TBARS and myoglobin redox forms. Fresh mince was lighter and had higher redness (a^*) and yellowness (b^*) values than mince from frozen/thawed meat. Hue angle for fresh mince remained stable throughout display whereas it increased for frozen/thawed mince. Fresh mince had lower TBARS values than frozen/thawed mince. Although myoglobin content remained constant in both fresh and frozen/thawed mince, it was higher in fresh mince than in frozen/thawed mince (4.3mg/g and 2.6mg/g). Surface met-myoglobin of fresh mince increased throughout display whereas the total met-myoglobin in fresh

mince remained rather constant. However, in frozen/thawed mince, both surface and total met-myoglobin increased throughout display. Surface and total oxy-myoglobin percentage was higher in fresh mince. It decreased during display but total oxy-myoglobin in fresh mince remained rather stable. The results showed that fresh mince had more colour and lipid stability than frozen/thawed mince and that fresh meat had a longer display shelf life as compared to frozen/thawed mince.

Keywords: Fallow deer meat; retail display; colour stability; oxidative stability; freezing

Introduction

During retail display, the bright cherry red colour of meat tends to change to an unattractive brown colour (Liu *et al*., 2011; Calnan *et al*., 2014). Consumers begin to question the freshness of meat as soon as deviations from the cherry red colour become visible (Girolami *et al*., 2013). Hence retailers begin to discount products if they do not sell within the first 48 hrs and once discolouration is visible to avoid losses (Behkit and Faustman, 2005). Arguably colour remains the most indicative quality trait used by consumers and retailers to assess freshness and quality, thus colour limits the retail display shelf life of all meat and meat products (Luciano *et al*., 2009; Li *et al*., 2012).

Researchers have accounted on the many factors that affect meat colour and colour stability (Kannan *et al*., 2001; Nute *et al*., 2007; Esmer *et al*., 2011; Ripoll *et al*., 2013). Leygonie *et al*. (2012b) reported reduced redness in ostrich meat after frozen storage and attributed it to the denaturing of the myoglobin moiety which occurs during freezing, frozen storage and thawing. Jacob *et al*. (2014) reported a negative relationship between redness and met-myoglobin accumulation. Ponnampalam *et al*. (2012) went further to quantify the relationship between lipid oxidation, myoglobin oxidation and anti-oxidant and meat colour. However, there is limited research to investigating the colour stability of meat from game species.

Game meat consumption has gained popularity owing to its low intramuscular fat and high amounts of poly-unsaturated fatty acids (Hoffman *et al*., 2009; Filgueras *et al*., 2010). This has given way to increased interest in identifying potential African ungulates for use in meat production. An example is the consumption of fallow deer in South Africa which is relatively new but gaining popularity in game meat production (Hoffman and Cawthorn, 2012). The species is successfully used in venison production in Europe (Volpelli *et al*., 2003). Conversely,

little is known about the South African species. Daszkiewicz *et al*. (2015) suggests that the meat quality of feral populations may significantly differ from farmed populations to be identified as a separate species. Therefore, more research needs to be done to qualify this.

Furthermore, during game harvesting, trimmings or tough cuts are usually used for mince production or frozen stored until demand (Crowley *et al*., 2010; Esmer *et al*., 2011; Rogers *et al*., 2014). Frozen storage is believed to significantly alter the quality of meat and subsequently, frozen/thawed meat and meat products are considered to be of an inferior quality than fresh meat (Mortensen *et al*., 2006). This is attributed to the ice crystals which form during freezing which alter the physical and chemical composition of the meat proteins and lipids (Faurouk *et al*., 2009; Muela *et al*., 2015). Subsequently the question arises as to whether ice crystal formation during freezing and frozen storage results in differences in the quality of mince produced from fresh or frozen thawed trimmings. Therefore, this study aimed at investigating the colour and oxidative stability of mince produced from fresh and frozen/thawed fallow deer meat.

Materials and methods

3.1 Harvesting of animals

Seven fallow deer were harvested in February 2015 on Brakkekuil farm (34◦ 18’ 24.0” S and 20◦ 49’ 3.9” E; 93 m above sea level), near Witsand in the Western Cape Province, South Africa. The harvesting period is part of the general management strategies of the farms and no preference was given to the selection of male or female deer. The study area is classified as the Coastal Renosterveld and receives 300–500mm of rainfall throughout the year, although higher amounts of precipitation generally occur in February and March (autumn) and again in

September to November (spring). Harvesting was done at night and animals were shot in the head or the high neck area with a 0.308 caliber rifle. Subsequently exsanguination occurred within 2 min, while in the field and no unnecessary *ante mortem* stress was experienced by the animals. Ethical clearance was obtained (SU-ACUM 14-00044 and SU-ACUM13-00011-SOP).

3.2 Sample preparation

After harvesting, carcasses were cooled (0–5°C) shortly after dressing (45 min *post mortem*). After 24 hours of cooling, the forequarter and hindquarter of each animal was deboned, individually vacuum packed and transported back to Stellenbosch University. All external fat was removed from the fore and hind quarter of each animal before mixing the lean meat per animal and separating into two equal batches. The first batch was minced using a 5mm grinder (at room temperature) and packed into low-density polyethylene wrap (LDPE) (moisture vapor transfer rate of 585 gm^{-2} 24 h^{-1} 1 atm^{-1}, O_2 permeability of 25 000 cm^{-3} m–2 24 h^{-1} 1 atm^{-1} and a CO_2 permeability of 180 000 cm^{-3} m^{-2} 24 h^{-1} 1 atm^{-1}). It was then refrigerated at 4°C for a period of eightdays; analyses were done on samples taken on day 0 (immediately after mince production), 1, 2, 4, 6 and 8. The second batch of meat was vacuum packed and frozen at -20°C for 2 months after which it was thawed for 45 hours at 4°C,made into mince and refrigerated for an eight period under the same conditions as fresh mince.

3.3 Physico-chemical analysis

3.3.1. Proximate composition

The samples were analyzed to determine the moisture (Method 934.01) and ash (Method 942.05) content according to the AOAC (2002). The protein content used AOAC (1992) procedure

992.15, whereas the fat was determined using the chloroform/methanol (2:1) fat extraction method according to Lee *et al*. (1996). All analyses were performed in duplicate.

3.3.2 Fatty acid composition

Two grammes of each sample were homogenized for 30 seconds in 50 ml chloroform: methanol (2:1; v/v) solution containing 0.01% butylated hydroxytoluene (BHT) as antioxidant by use of a polytron mixer (WiggenHauser, D-500 Homogenizer).Heptadecanoic acid (C17:0) was used as an internal standard to enable quantification of the individual fatty acids in the original muscle sample. A sub-sample was taken from the extracted fats and transmethylated for 2 h at 70 °C with a methanol: sulphuric acid (19:1; v/v) solution. The sub-sample was cooled to room temperature after which the resulting fatty acid methyl esters (FAME) were extracted with the use of water and hexane. The top hexane phase was transferred to a spotting tube and dried under nitrogen. Fifty microlitres of hexane was added to the dried sample of which 1 µl was injected. The FAME were analysed by gas–liquid chromatography (Varian Model 3300 equipped with a flame ionization detector) using a 60 m BPX70 capillary column of 0.25 mm internal diameter (SGE International Pty Ltd, 7 Argent Place, Ringwood, Victoria 3134, Australia). The hydrogen gas flow rate was 25 ml/min and the hydrogen carrier gas flow rate was 2–4 ml/min. Temperature programming was linear at 3.4 °C/min with the following temperature settings: initial temperature of 60 °C; final temperature of 160 °C. Injector temperature was 220 °C and detector temperature was 260 °C. The run time was ≈45 min. The FAME in the total lipids of each sample (mg/g sample) were identified by comparing the retention times with those of a standard FAME mixture (Supelco™ 37 Component FAME Mix, 10 mg/ml in CH2Cl2, Catalogue Number 47885-U. Supelco™, North Harrison Road, Bellefonte, PA 16823-0048,

USA). The fatty acid profile was calculated and compared as a proportion of the total amount of fatty acids present in each sample.

3.3.3 Meat pH

Meat pH was determined using the iodoacetate method; 1g sample was homogenized in 10 ml iodoacetate/KCl reagent. The reagent was adjusted to pH 7.0 with 0.01 M KOH/0.1 M HCl. pH values of the homogenate were measured at 0°C temperature using a desktop pH meter (Jenway 3510 pH meter; Lasec SA, Cape Town, South Africa; calibrated using pH 4.0 and pH 7.0 standard buffers). Samples were kept on ice to keep them cold and at a constant temperature. Readings were done in duplicate per sample.

3.3.4 Colour

Colour was carried out using a spectro-guide D65/10° (daylight illumination, aperture opening) 45°/0° colorimeter (BYK Gardner GmbH, Gerestried, Germany). Colour measurements were done onto the overwrap packaging material on a flat portion of the meat and an average of 5 readings taken from different portions of the mince was used in the analysis. The colour-guide was standardized before each day's reading to minimize bias and errors. The green standard was used to check if calibration was needed and calibration was done using the black and white standards (L^*=95.13, a^*= -0.89, b^*=0.66). Colour was described as coordinates: Lightness (L*), redness (a*, ±red–green), and yellowness (b*, ±yellow–blue). From these coordinates, hue and chroma (saturation index referring to how vivid or dull the color is) were calculated as follows:

$$\text{Hue} = \tan^{-1} b^*/a^*$$

$$\text{Chroma} = (a^{*2} + b^{*2})^{1/2}$$

Spectral data was downloaded onto excel spread sheet from the colour guide and used to estimate different myoglobin redox forms. Surface oxy-myoglobin (OMb), de-oxy-myoglobin (DMb) as well as met-myoglobin (MMb) were determined using the formulae:

$$\%OMb = \frac{\frac{K/S\ 610}{K/S\ 525 \text{ for } 100\%\ MMb} - \frac{K/S\ 610}{K/S\ 525 \text{ for sample}}}{\frac{K/S\ 610}{K/S\ 525 \text{ for } 100\%\ MMb} - \frac{K/S\ 610}{K/S\ 525 \text{ for } 100\%\ OMb}} \times 100$$

$$\%\ MMb = \frac{\frac{K/S\ 572}{K/S\ 525 \text{ for } 100\%\ DMb} - \frac{K/S\ 572}{K/S\ 525 \text{ for sample}}}{\frac{K/S\ 572}{K/S\ 525 \text{ for } 100\%\ DMb} - \frac{K/S\ 572}{K/S\ 525 \text{ for } 100\%\ MMb}} \times 100$$

$$\%DMb = \frac{\frac{K/S\ 474}{K/S\ 525 \text{ for } 100\%\ OMb} - \frac{K/S\ 474}{K/S\ 525 \text{ for sample}}}{\frac{K/S\ 474}{K/S\ 525 \text{ for } 100\%\ OMb} - \frac{K/S\ 474}{K/S\ 525 \text{ for } 100\%\ DMb}} \times 100$$

Where K is the absorbent coefficient and S is the scatteringcoefficient

3.4 Lipid oxidation

The lipid oxidation process was followed by measuring the thiobarbituric acid reactive substances (TBARS) using the spectrophotometric method described by Rosmini *et al*. (1996).A 1g sample was taken and homogenized in a blender with 10 ml of 0.15M potassium chloride buffer for 20 s. The absorbance was measured at 532nm using a Cecil CE2021 2000 Series spectrophotometer (Lasec SA (Pty) Ltd). The TBARS were expressed as mg malonaldehydes (MDA) per kg product. Analysis was done in duplicate per sample.

3.5 Total myoglobin and myoglobin forms

Potassium phosphate buffer was made by adding 4.87 g of KH2PO4 and 2.48 g of K2HPO4 to 1000 ml deionised water and adjusted to pH 6.8 using 0.1 M HCl/ 0.5 M NaOH. Ten grammes of minced meat sample was taken and 100 ml cold potassium phosphate buffer added. The sample and the buffer were homogenised and left for 1 hour at 4°C for extraction. The extract was centrifuged at 4000 rpm for 30 min at 4°C then filtered. 200 µl was pipetted into separate wells of a microplate.The absorbance was measured from 400nm – 800nm using a Cecil CE2021 2000 Series spectrophotometer (Lasec SA (Pty) Ltd).

Total myoglobin content (mg/g meat) was calculated using the formula:

A433 x (1M Mb/114 000) x [(1mol/L)/M] x (17 000gMb/mol Mb) x (1000mg/g) x dilution factor of 0.10L/10g meat

Relevant wavelengths (A503, A525, A557 and A582) were used to calculate the myoglobin redox ratios (Tang *et al*., 2004). The equations and wavelengths used to calculate are as follows:

$[DMb] = [DMb] / [Mb] = -0.543R_1 + 1.594R_2 + 0.552R_3 – 1.329$

$[OMb] = [OMb] / [Mb] = 0.772R_1 - 1.432R_2 – 1.659R_3 + 2.599$

$[MMb] = [MMb] / [Mb] = -0.159R_1 – 0.085R_2 + 1.262R_3 – 0.520$

Where R_1 = A582 / A525, R_2 = A557 / A525, R_3 = A503 / A525

3.6 Statistical analysis

The experimental design was a 2x6 factorial in a completely randomized design with 2 treatments (fresh or 2 months frozen storage) and display time (0, 1, 2, 4, 6, 8 days) as main

effects. The GLM model of STATISTICA (version 8) statistical software was used to compare LS Means. Ho was rejected at $P<0.05$. Fisher's LSD was used for post hoc testing. Normal probability plots were continuously checked for deviations from normality and possible outliers. The statistical model was represented by:

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ij}$$

Where Y_{ij} is the response variable (pH, color, TBARS, total myoglobin, total myoglobin, % met-myoglobin, % de-oxy-myoglobin, % oxy-myoglobin)

μ is the overall mean

α_i is effect due to treatment (fresh and 2 months frozen/thawed)

β_j is effect due to display time (0, 1, 2, 4, 6, 8 days)

$(\alpha\beta)_{ij}$ is the interaction

And e_{ij} is the error

3.7 Results

3.7.1 Proximate composition

The proximate composition of fresh and frozen mince produced from fallow deer meat is summarized in Table 3.1 below. There were no significant differences between fresh and frozen/thawed mince in moisture, protein, lipid and ash.

Table 3.1 Proximate composition (means and standard errors) of fresh fallow deer minced meat and minced meat produced from fallow deer meat frozen for two months

	Treatment		P value	Significance
	Fresh (n = 7)	**Two months frozen storage (n = 7)**		
Moisture	75.6 ± 0.35	75.2 ± 0.22	0.47703	NS
Protein	21.7 ± 0.32	21.6± 0.24	0.324	NS
Lipid	2.3 ± 0.04	2.5 ± 0.6	0.3545	NS
Ash	1.1 ± 0.01	1.0 ± 0.01	0.8165	NS

NS, means not significantly different ($P > 0.05$)

3.7.2 Fatty acid composition

The results for the fatty acid composition of fresh mince and mince produced from 2months frozen stored fallow deer meat are shown in Table 3.2. No differences ($P>0.05$) were recorded between fresh and frozen samples. However, total n3 fatty acids differed ($P<0.05$) between treatments and decreased with increased storage and display day. Stearic and palmitic acid were the most dominant saturated fatty acid (SFA) in the samples (26% and 23.5%, respectively). Linoleadic acid and oleic acid were the dominant poly-unsaturated fatty acids (PUFAS) in the samples (9.2% and 8.6%, respectively). The ratios for PUFAS: SFA and n3:n6 were not affected by storage or display day.

Table 3.2 Fatty acid composition (mean and standard errors) of fresh fallow deer minced meat and minced meat produced from fallow deer meat frozen for two months

Storage	**Fresh (n = 7)**	**Fresh (n = 7)**	**2months frozen (n = 7)**	**2months frozen (n = 7)**
Display day	0	8	0	8
C14:0 (Myristic acid)	1.1^{a} ±0.41	1.8^{a} ±0.41	1.4^{a} ±0.41	2.0^{a} ±0.41
C16:0 (Palmitic acid)	24.0^{a}±2.96	25.8^{a}±2.96	23.5^{a}±2.96	25.8^{a}±2.96
C18:0 (Stearic acid)	26.0^{a}±1.91	25.7^{a}±1.91	27.3^{a}±1.91	26.9^{a}±1.91
C15:1 (Pentadecenoic acid)	7.5^{a}±0.79	9.0^{a}±0.79	9.2^{a}±0.79	8.5^{a}±0.79
C16:1 (Palmitoleic acid)	1.7^{a} ±0.13	1.5^{a} ±0.13	1.5^{a} ±0.13	1.6^{a} ±0.13
C18:1n9c (Oleic acid)	10.5^{a} ±2.14	7.9^{a} ±2.14	6.2^{a} ±2.14	10.3^{a} ±2.14
C18:2n6t (Linoleadic acid)	11.4^{a} ±2.32	8.6^{a} ±2.32	7.6^{a} ±2.32	8.0^{a} ±2.32
C22:2n6 (Docosadienoic acid)	0.6^{a} ±0.16	0.5^{ab} ±0.09	0.7^{a} ±0.11	0.4^{b} ±0.03
C20:3n6 (Eicosatrionoic acid)	1.0^{a} ±0.09	0.9^{a} ±0.09	1.0^{a} ±0.09	0.7^{b} ±0.09
C20:4n6 (Arachidonic acid)	7.0^{a} ±1.31	6.8^{a} ±1.31	6.3^{a} ±1.31	5.7^{a} ±1.31
C18:3n3 (α-linolenic acid)	2.5^{a} ±0.39	2.5^{a} ±0.39	2.1^{a} ±0.39	2.3^{a} ±0.39
C20:3n3 (Eichosatrienoic acid)	0.9^{a} ±0.11	0.9^{a} ±0.11	0.9^{a} ±0.11	0.6^{b} ±0.11
C20:5n3 (Eicosapentanoic acid)	2.0^{a} ±0.33	2.0^{a} ±0.33	1.7^{a} ±0.33	1.6^{a} ±0.33
C22:6n3 (Docosahexaenoic acid)	1.3^{a} ±0.09	0.8^{b} ±0.09	0.7^{bc} ±0.09	0.6^{c} ±0.09
Total SFA	51.0^{a} ±5.43	53.3^{a} ±6.67	52.2^{a} ±3.93	57.4^{a} ±5.03
Total MUFA	19.7^{a} ±1.49	18.4^{a} ±2.35	16.9^{a} ±1.11	20.4^{a} ±1.60
Total PUFA	26.7^{a} ±4.3	22.9^{a} ±4.9	21.0^{a} ±3.3	19.9^{a} ±4.3
Total n6	20.1^{a} ±3.43	16.8^{a}±4.05	15.8^{a}±2.83	14.8^{a}±3.48
Total n3	6.7^{a}±0.98	6.1^{b}±0.94	5.4^{c}±0.53	5.1^{d}±0.82
PUFA:SFA	0.5^{a} ±0.13	0.4^{a} ±0.13	0.4^{a} ±0.13	0.4^{a} ±0.13
n6/n3	2.9^{a}±0.33	2.8^{a}±0.41	2.9^{a}±0.33	2.9^{a}±0.35

Means with different superscripts in the same row are significantly different ($P < 0.01$).SFA – saturated fatty acids; MUFA – mono unsaturated fatty acids; PUFA – poly unsaturated fatty acids; n3 – omega 3 fatty acids(C18:3n3, C20:3n3, C20:5n3, C22:6n3); n6 – omega 6 fatty acids (C18:2n6t, C22:2n6, C20:3n6, C20:4n6); PUFA:SFA – poly unsaturated fatty acid: saturated fatty acid ratio; n6/n3 –omega 6/ omega 3 fatty acid ratio

3.7.3 pH

The pH of fresh and frozen mince over an eight day display period is shown in Figure 3.1. There were treatment and time interactions ($P<0.05$) for TBARS and pH.The pH of fresh mince was consistently higher than that of frozen/thawed mince.

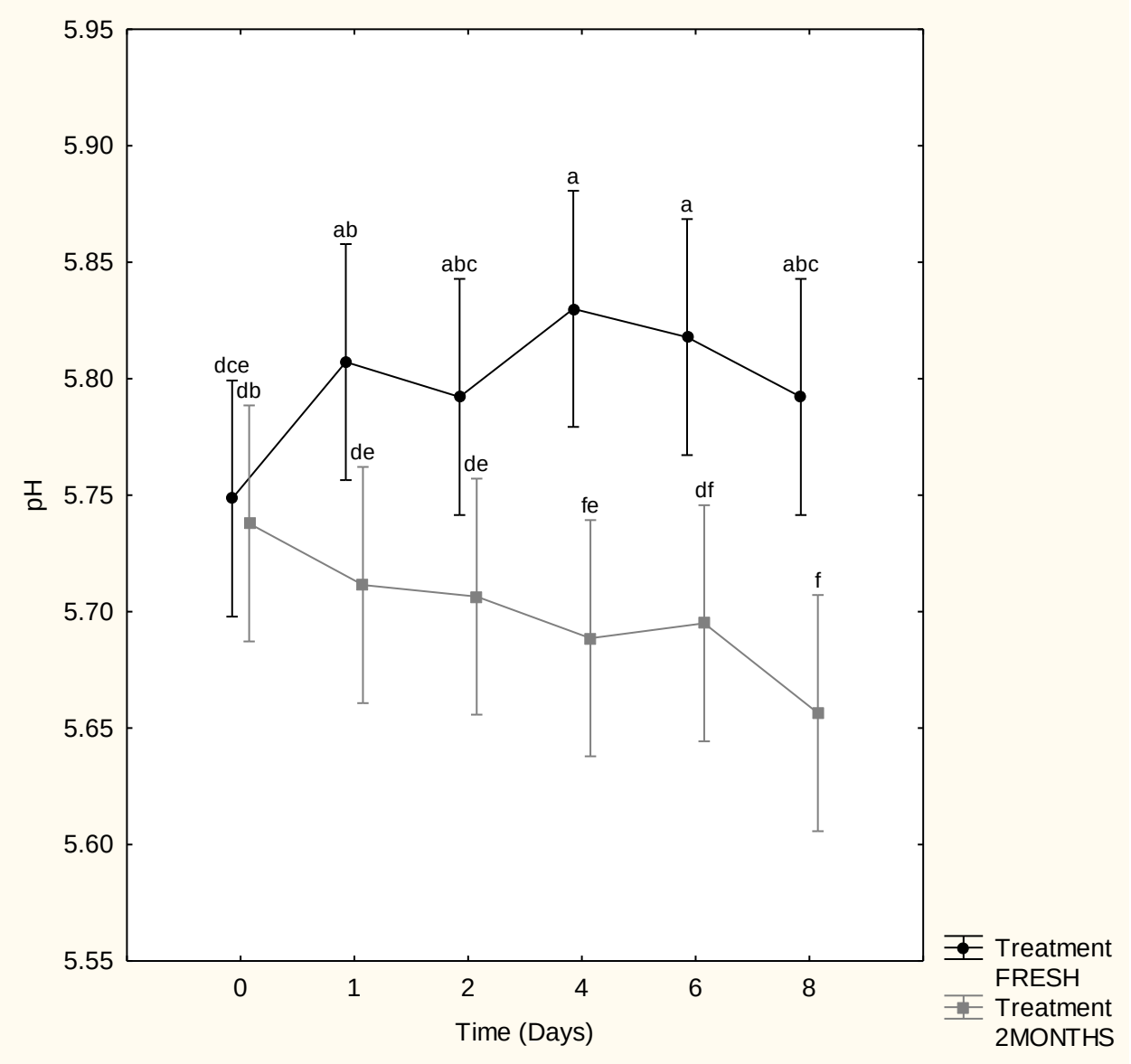

Figure 3.1 Effects of freezing on pH of mince produced from fallow deer meat over eight days of display. Least square means with different superscript letters are significantly different ($P<0.05$)

3.7.4 Colour

The results showing colour differences between fresh and frozen/thawed mince are shown in Figure 3.2 and Figure 3.3. There were significant ($P<0.01$) Treatment and Time interactions on all measured colour parameters. Fresh mince was lighter and had higher redness (a^*) and yellowness (b^*) values. Hue angle for fresh mince remained stable throughout display whereas hue angle for frozen/thawed mince increased over time. Chroma for fresh mince decreased over time whereas chroma for frozen/thawed mince decreased rapidly from day 0 but became stable from day 4 to day 8.

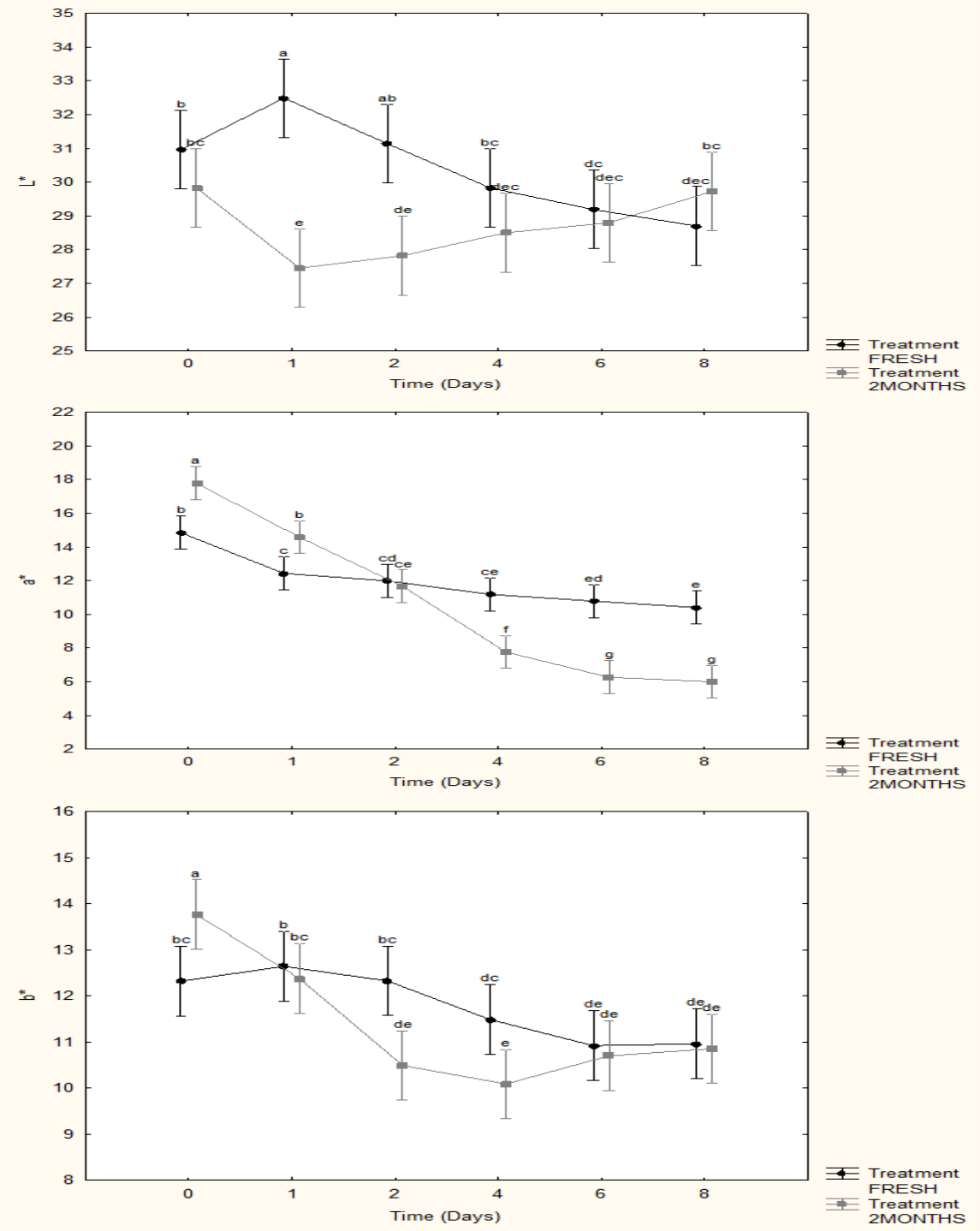

Figure 3.2 Effects of freezing on colour parameters of mince produced from fallow deer over eight days of display. Least square means with different superscript letters are significantly different (P<0.05).

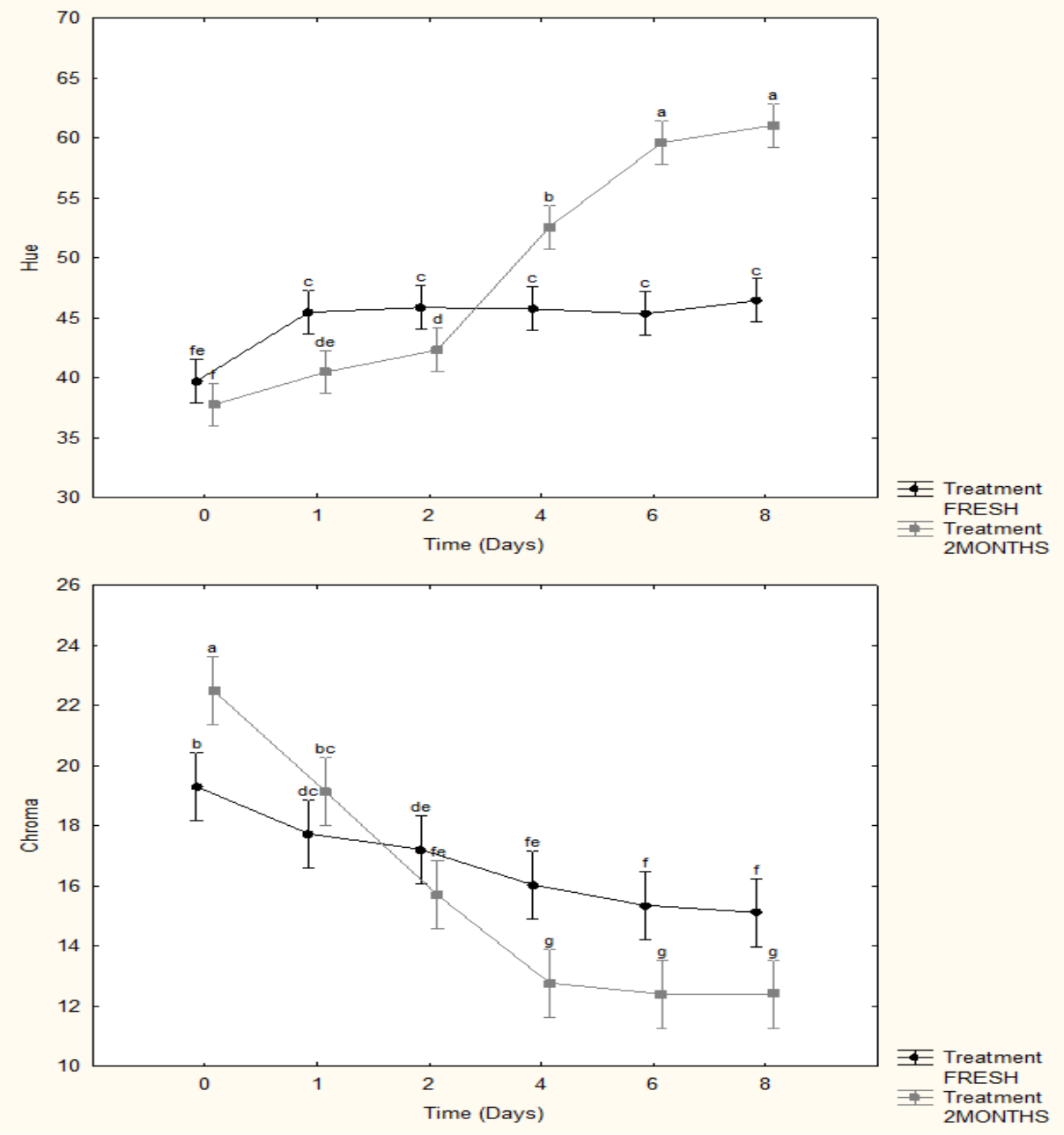

Figure 3.3 Effect of freezing on hue and chroma of minced meat produced from fallow deer over eight days of display. Least square means with different superscripts are significantly different ($P<0.05$)

3.7.5 Total myoglobin and myoglobin forms

Total myoglobin content of fresh and frozen mince over an eight day display period is shown in Table 3.3. No significant treatment and time interactions were observed for myoglobin content. Myoglobin content was higher in fresh mince than in frozen/thawed mince. The myoglobin content remained constant in both fresh and frozen/thawed mince throughout display period.

The results showing the different percentages of myoglobin forms on the surface and in the whole mince in fresh and frozen/thawed mince are shown in Figure 3.4 and Figure 3.5. Significant treatment and time interactions were noted in all measured myoglobin forms. Surface met-myoglobin, surface de-oxy-myoglobin and surface oxy-myoglobin refer to the measured amounts of myoglobin forms on the surface of meatonly. Total met-myoglobin, total de-oxy-myoglobin and total oxy-myoglobin refer to myoglobin forms measured in the whole minced meat. Minced meat produced from frozen/thawed deer meat had higher surface met-myoglobin and met-myoglobin percentages. Surface met-myoglobin of fresh mince increased throughout display whereas the total met-myoglobin in fresh mince remained rather constant. However, in frozen/thawed mince, both surface and total met-myoglobin increased throughout display. Fresh mince had higher percentages of surface and total de-oxy-myoglobin than mince produced from frozen/thawed meat. Surface and total oxy-myoglobin percentage was higher in fresh mince. It decreased during display but total oxy-myoglobin in fresh mince remained rather stable.

Table 3.3 Myoglobin content (means and standard errors) (mg/g) of fresh fallow deer mince and minced meat produced from fallow deer meat frozen for two months

Treatment	**Display day**					
	0	1	2	4	6	8
Fresh mince (n=7)	3.8^{c}±0.44	4.5^{ab}±0.33	4.5^{ab}±0.41	4.1^{b}±0.17	4.1^{b}±0.14	4.5^{a}±0.16
2months frozen (n=7)	2.1^{ab}±0.35	1.9^{b}±0.31	2.2^{ab}±0.41	2.6^{a}±0.51	2.4^{a}±0.31	2.8^{a}±0.41
	P values					
Treatment	Time		Treatment x time effect			
***	NS		NS			
0.00001	0.4536		0.5487			

Means with different superscripts in the same row are significantly different (P< 0.05). n=sample size. NS = not significant. *** = P<0.0001

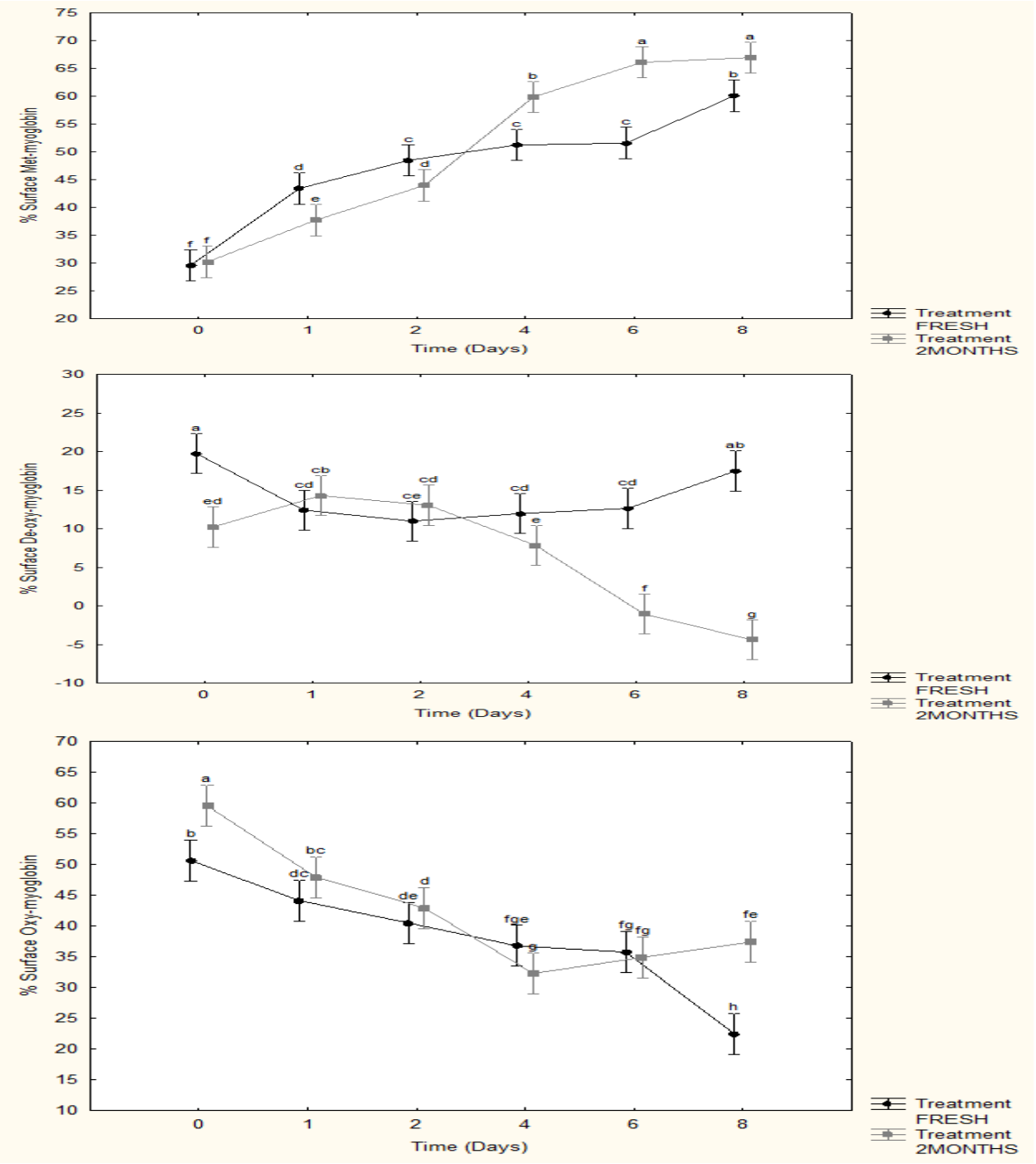

Figure 3.4 Effectsof freezing on surface myoglobin forms of minced meat produced from fallow deer over eight days of display. Least square means with different superscripts are significantly different (P<0.05).

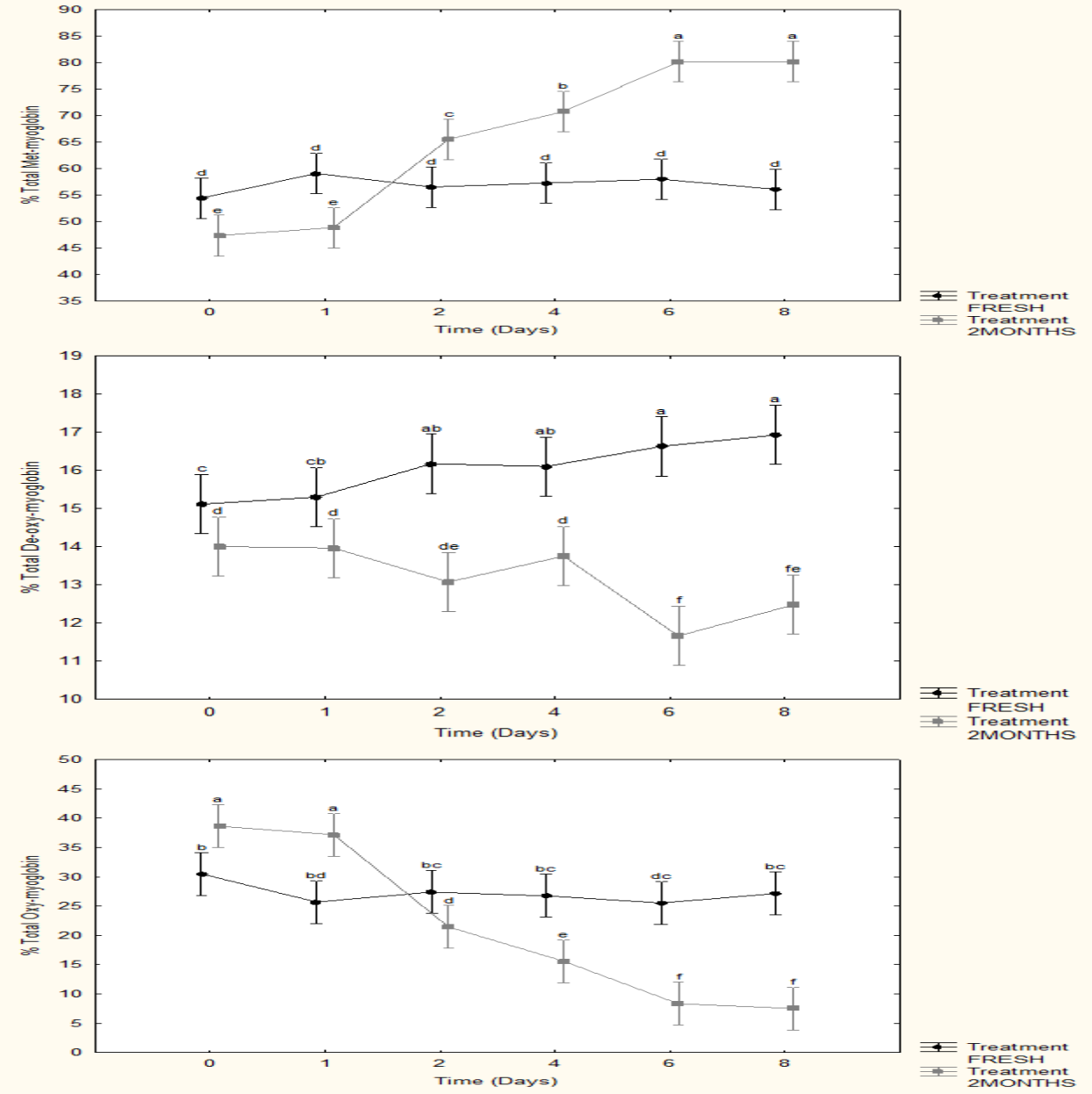

Figure 3.5Effects of freezing on total myoglobin forms of minced meat produced from fallow deer over eight days of display. Least square means with different superscripts are significantly different (P<0.05).

3.7.6Lipid oxidation

The TBARS of fresh and frozen mince over an eight day display period is shown in Figure3.6. There were treatment and time interactions ($P<0.05$) for TBARS. Fresh mince had lower TBARs values than frozen/thawed mince.

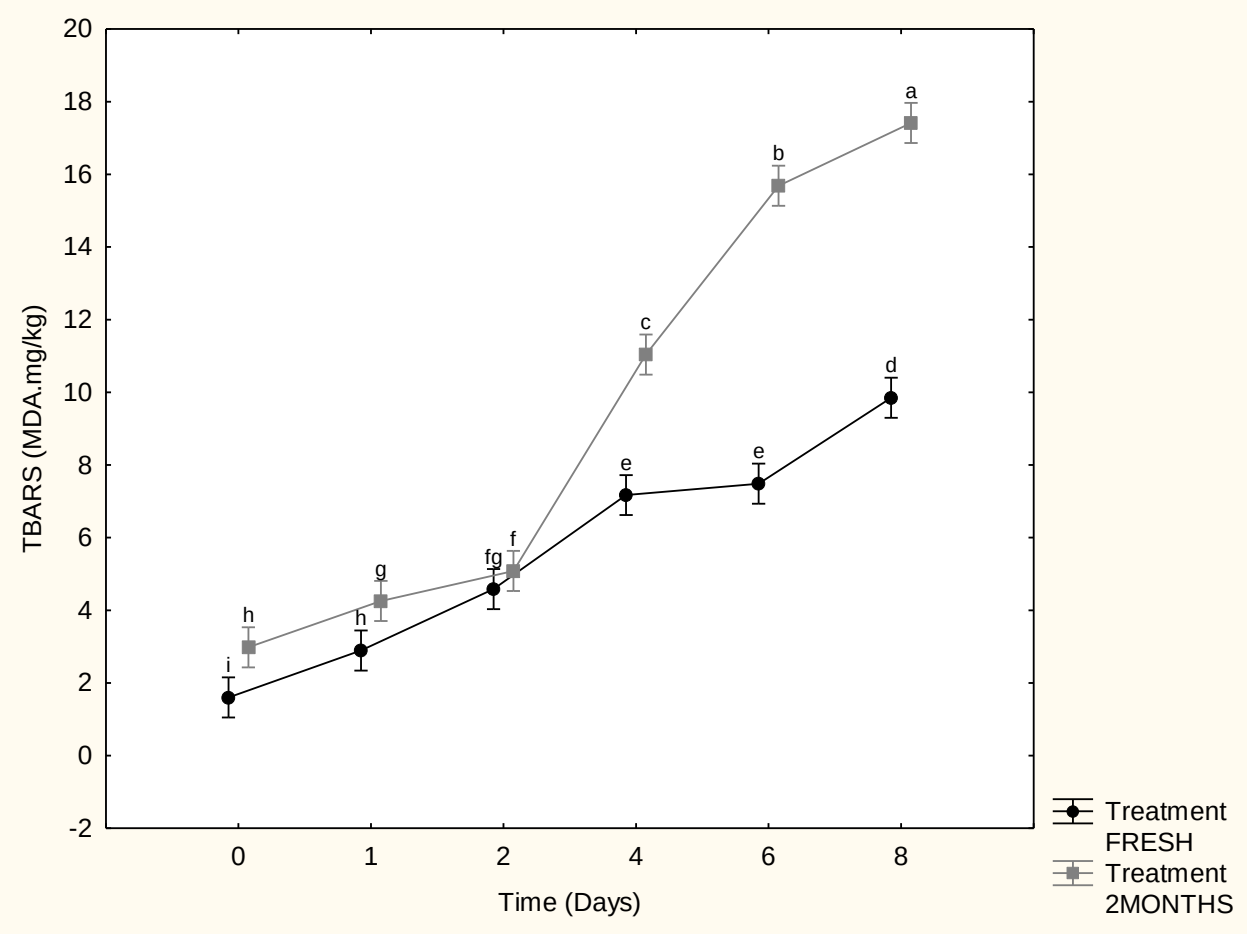

Figure 3.6 Effects of frozen duration on lipid oxidation of minced meat produced from fallow deer over eight days of display. Least square means with different superscripts are significantly different ($P<0.05$).

3.8 Discussion

3.8.1 Proximate and fatty acid composition

The proximate composition of fallow deer minced meat was not affected by frozen storage (Table 3.1). In this study, South African wild fallow deer showed a fat content of 2.4% (± 0.04). This is higher than the fat content of both wild and farmed fallow deer reported by Daszkiewicz *et al*. (2015). Volpelli *et al*. (2003) and Ramanzin *et al*. (2010) also reported lower fat content in fallow deer raised on farm. Differences can be attributed to differences in diet as well as location of populations. However, the lipid content of the fallow deer in this study is within the range of other reported game species (Hoffman *et al*., 2009; Hutchison *et al*., 2010; Neethling *et al*., 2014). Moreover, since the values reported in this study for fallow deer are lower than the reported range for domestic red meat species (USDA, 2011), fallow deer meat may be considered as a suitable red meat alternative. The moisture and protein content of fallow deer in this study was in agreement with many studies done on game meat (Volpelli *et al*., 2003; Hutchison *et al*., 2010; Neethling *et al*., 2014).

The fatty acid composition gives a more comprehensive account of the nutritional value of meat. In this study, stearic and palmitic acid dominated and contributed most to the total SFA. This was expected of forage animals as most saturation occurs via hydrogenation in the rumen of the animal (Nantapo *et al*., 2015). Palmitic acid is associated with increased cholesterol levels and atherogenicity (Nantapo *et al*., 2014). Conversely, stearic acid is considered a healthier fatty acid compared to other SFAs and is associated with lowered low density lipoprotein cholesterol (Hunter *et al*., 2009). Linoleadic acid and oleic acidare considered beneficialfatty acids in the diet (Wood *et al*., 2003) and were found in high proportions in this study (Table 3.2). However,

the ratio of these PUFAs, especially the n6:n3 ratio is important nutritionists recommend ratios below 5 (Kouba and Mourot, 2011). The n6: n3 ratios in this study were found to be well below five and similar to the findings of Dannenburger *et al*. (2013) in roe deer.

3.8.2 Colour

Lightness of fresh mince decreased over time whereas it was vice versa for the frozen/thawed mince. On day zero and one, mince from frozen/thawed meat had higher redness values than fresh meat. However, the redness decreased more rapidly than for fresh meat and from day three onwards, fresh mince had higher redness values than mince from frozen/thawed meat. This indicated a higher rate of browning in frozen/thawed mince due to surface met-myoglobin formation, which corresponds well with results in Figure 3.2 and Figure 3.4. Frozen storage disrupts met-myoglobin reducing enzyme systems in cells, resulting in slow conversion of met-myoglobin into de-oxy-myoglobin and subsequently, accumulation of met-myoglobin upon thawing (Leygonie *et al*., 2012a). Redness (*a**) and met-myoglobin accumulation in fresh mince was rather stable indicating that although fresh fallow deer minced meat was less red during the first two days of display, it was able to retain its redness for longer and thus has a longer shelf life than frozen/thawed mince.

Hue gives a more realistic view of meat discoloration and colour changes over time as it is a function of *a** and *b** (Luciano *et al.* 2009). Hue for fresh mince remained constant, indicating that redness was maintained. The hue for mince produced from frozen/thawed fallow deer increased over display time showing that as display days increased, discoloration also increased (Kim *et al*., 2011). Visible meat discoloration (browning) was evident during the study from day two onwards for frozen/thawed mince. These findings support reports of strong positive

correlations between sensory discoloration and hue values (Kim *et al*., 2011). Chroma is an indication of colour intensity. Colour intensity decreased throughout display. However, mince from frozen/thawed fallow deer meat displayed a more rapid loss of intensity compared to fresh mince. Kim *et al*. (2013) also recorded similar findings.

Yellowness generally decreased throughout display for both fresh mince and frozen/thawed minced meat. This is in agreement with other similar studies (Leygonie *et al*., 2012b). Although yellowness does not directly affect appearance of meat colour, it is negatively correlated to lipid oxidation (Seydim *et al*., 2006) and positively related to redness (Esmer *et al*., 2011). This suggests that a decrease in redness and increase in TBARS leads to reduced yellowness which concurs with the results in this study.

3.8.3 Myoglobin content and forms

Myoglobin content in fresh mince was higher than myoglobin content in frozen/thawed mince. The reason for this may be attributed to exudate loss upon thawing. Myoglobin has been found by electrophoresis to be in exudate, accounting in part, for colour loss in frozen/thawed products as well (Leygonie *et al*., 2012a). Although surface met-myoglobin in fresh mince increased rapidly during display time, total met-myoglobin remained rather stable. This indicates that the surface which was exposed to oxygen experienced greater oxidation and the surface below was protected due to low oxygen penetrance (American Meat Science Association, 2012). However, for frozen/thawed mince, both surface met-myoglobin and total met-myoglobin increased rapidly during display time. The reason for this may be attributed to the denaturing effects of freezing on met-myoglobin reducing enzyme systems (Kim *et al*., 2011). Met-myoglobin enzyme reducing

systems convert met-myoglobin to de-oxy-myoglobin. Thus if the systems are disrupted, met-myoglobin will accumulate in the meat system.

3.8.4 Myoglobin content, TBARS and pH

The extent of lipid oxidation in meat systems is usually determined by the amount of TBARS in the system. Mince produced from frozen/thawed fallow deer had consistently higher TBARS compared to fresh mince. This was expected as frozen storage is known to accelerate the rate of oxidation due to cellular lipid structure damage caused by ice crystals. Furthermore, TBARS accumulated faster in frozen/thawed mince, indicating a faster rate of lipid oxidation as compared to fresh mince. The TBARS recorded in this study are higher than those recorded in other meat species and exceeded the threshold detecting level for rancidity in meat (2mgMDA/kg). Detection threshold for rancidity and off flavours differs between species differs and has been determined to be 2.28mgMDA/kg in beef and 1mgMDA/kg in lamb (Campo *et al*., 2006; Ripoll *et al*., 2011). The amounts of TBARS recorded in the study suggest that there are high amounts of PUFAs and haem pigment myoglobin in game meat which makes it more susceptible to oxidation than traditional meat species. However, threshold values for detection of rancidity in fallow deer has not been determined and warrants investigation. Game meat is reported to have higher pH values than traditional domestic species (Bartoň *et al*., 2014). This is due to greater activity experienced by game during harvesting (Hoffman *et al*., 2004). The pH of fallow deer in this study was low due maybe to night cropping which exerts little stress on the animals being harvested. Leygonie *et al*. (2012b) recorded a decrease in ostrich meat pH after frozen storage, which is in agreement with this study. There was little change in pH over display time in both fresh and frozen/thawed mince.

Conclusion and recommendations

Fallow deer has a lipid content that is lower than domestic red species and a fatty acid composition which is favourable. Fresh fallow deer minced meat is capable of maintaining its cherry red colour for the first four days of retail display before discolouration becomes visible. By day fourof retail display storage, frozen/thawed mince was showing extended signs ofoxidation and discolouration. It can be concluded that fresh fallow deer mince has a longer shelf life than mince from frozen/thawed fallow deer meat. This study clearly showed that freezing affects the colour and oxidative stability of minced meat. There is need to therefore to determine if frozen duration will affect the colour and oxidative stability of minced meat since meat is kept under frozen storage for different periods of time before use.

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Chapter 4

Effects of frozen storage on physiochemical attributes and lipid stability of mince from fallow deer meat during fivedays of display storage

Abstract

The lipid, myoglobin and colour stability of mince produced from twenty four frozen/thawed fallow deer fore and hindquarters was investigated. Proximate and fatty acid composition was also determined. Muscles were vacuum packed and frozen at -20°C for two and four months. Upon thawing, all external fat was removed, the muscles mixed and minced (through a 5 mm die) per animal, packed into oxygen permeable overwraps and refrigerated at 4°C for five days. Average lipid content of fallow deer meat was 2.7% and did not differ between treatments ($P>0.05$). The total amount of SFA increased ($P<0.05$) and the total amount of PUFA decreased ($P<0.05$) as frozen duration increased. Lipid oxidation and met-myoglobin accumulation increased as frozen storage increased ($P<0.001$). No differences ($P>0.05$) in CIE L^*,CIE a^* and chroma were recorded between treatments except on day zero of display. By day three all samples showed signs of extended oxidation and discolouration as evidenced by reduced redness, chroma and high TBAR values.

Keywords: Fallow deer; ground meat; freezing; lipid oxidation; oxy-myoglobin; colour stability

Introduction

Game meat consumption as an alternative to domestic red meat species has become popular with the health conscious consumer (Bartoň *et al*., 2014) owing to its low intramuscular fat (Hoffman and Wiklund, 2006). This has led to increased interest in identifying potential African ungulates for use in meat production. An example is the South African fallow deer. Although little information exists on the quality attributes of this species in South Africa, it is successfully reared for meat production in Europe (Volpelli *et al*., 2003). Research on the meat quality attributes of fallow deer have shown the species to contain high amounts of iron and heam iron as well as myoglobin and poly-unsaturated fatty acids (PUFAS) (Cifuni *et al*., 2014; Daszkiewicz *et al*., 2015). This makes it more susceptible to oxidative processes which quickly deteriorate the meat quality.

Freezing as a meat preservation method is quite popular in the meat industry (Castro-Giraldez *et al*., 2014; Kajak-Siemaszko *et al*., 2011; Muela *et al*., 2012). In the case of game meat, it also offers the added advantage of easy transportation during exportation and enables product control since game is a seasonal product (Leygonie *et al*., 2012a). However, frozen/thawed products are regarded to be of an inferior quality as compared to fresh meat products and thus fetch low prices (Mortensen *et al.*, 2006). The reason for this belief is mainly attributable to the disruptive actions of crystals which concentrates pro-oxidant solutes and alters the cell membrane lipids' conformation (Coronado *et al*., 2002). This leads to an increased formation of reactive oxygen species in the frozen meat system and consequently a rapid onset of secondary lipid and myoglobin oxidation upon thawing (Soyer *et al*., 2010; Leygonie *et al*, 2012b).

The duration of frozen storage determines the extent and rate at which oxidationwill occur upon thawing (Muela *et al*., 2012). Long periods of freezing (greater than three months) reportedly

result in greater cellular damage and thus less colour and shelf life stability of product upon thawing (Hansen *et al*., 2004; Soyer *et al*., 2010). However, some researchers have postulated and demonstrated that due to the redistribution of crystals which occurs even after meat has been frozen, the extent of damage is levelled out as freezing is prolonged (Mortensen *et al*., 2006; Leygonie*et al*., 2012a; Muela *et al*., 2012).

Most research has focused on the display shelf life of frozen/thawed muscle cuts and little knowledge is available on frozen/thawed meat processed into mince. In the United States, approximately 1.3 billion pounds of ground (minced) beef is produced for retail each year comprising of more retail space than any other product (Papadoupoulo *et al*., 2012; Rogers *et al*., 2014). As such knowledge on the shelf life stability of frozen/thawed minced products is of utmost importance. The main objective of this study was therefore to determine the effects of frozen duration on the meat quality attributes of fallow deer mince during retail display following thawing.

Materials and methods

4.1 Harvesting of animals

Twenty four fallow deer were harvested on two different occasions on Brakkekuil farm (34° 18' 24.0" S and 20° 49' 3.9" E; 93 m above sea level), near Witsand in the Western Cape Province, South Africa. One set was harvested in June 2014 (6 male and 6 female) and the other set was harvested in August 2014 (6 male and 6 female). The study area is classified as the Coastal Renosterveld and receives 300 – 500mm of rainfall throughout the year, although higher amounts of precipitation generally occur in February and March (autumn) and in September to

November (spring). These harvesting periods form part of the general management strategies of the farms and as such, no preference was given to gender selection. No seasonal differences were expected as well as the times of harvesting fall under the same winter season. Harvesting was done at night and animals were shot once in the head or the high neck area with a 0.308 caliber rifle. Consequently exsanguination occurred within two minutes, while in the field. No unnecessary *ante mortem* stress was experienced by the animals. Ethical clearance was obtained (SU-ACUM 14-00044 and SU-ACUM13-00011-SOP).

4.2 Sample preparation

After harvesting, carcasses were cooled at 0– 5°C shortly after dressing (45 min *post mortem*). After 24 hours of cooling, the forequarter and hindquarter of each animal was deboned, individually vacuum packed and frozen at -20°C. Two months and four months after harvesting the first and second sets respectively, all fore and hindquarter muscles were thawed for 45 hours at 4°C. All external fat was removed and discarded from the fore and hind quarters before mixing and mincingthe lean meat using a 5mm grinder(at room temperature) for each animal. The mince was packed into low-density polyethylene wrap (LDPE) (moisture vapor transfer rate of 585 $g.m^{-2}.24\ h^{-1}.atm^{-1}$, O_2 permeability of 25 000 $cm^{-3}.m^{-2}\ .24\ h^{-1}.atm^{-1}$ and a CO_2 permeability of 180 000 $cm^{-3}.m^{-2}.24\ h^{-1}.atm^{-1}$) and refrigerated at 3.9°C for a period of five days under retail display conditions; analyses were done on samples taken on day 0 (immediately after mince production), 1, 2, 3, 4 and 5.

4.3 Physico-chemical analysis

4.3.1. Proximate composition

The procedure was as described in section 3.3.1

4.3.2 Fatty acid composition

Fatty acid determination was performed as described in section 3.3.2

4.3.3 pH

Mince pH was determined as described in section 3.3.3.

4.3.4Colour

Colour was determined as described in section 3.3.4. However, colour spectral readings were not included when determining colour.

4.4 Lipid oxidation

Lipid oxidation was determined as described in section 3.4.

4.5 Total myoglobin and myoglobin forms

Total myoglobin and myoglobin forms were determined as described in section 3.5.

4.6 Statistical analysis

The experimental design was a 2x2x6 factorial in a completely randomized design with frozen duration (2 or 4 months), gender (male or female) and display time (0, 1, 2, 3, 4, 5days) as main effects. The GLM model of STATISTICA (version 8) statistical software was used to compare

LS Means. Fisher's LSD was used for post hoc testing. Normal probability plots were continuously checked for deviations from normality and possible outliers.

The statistical model was represented by:

$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + e_{ijk}$

Where Y_{ijk} is the response variable (pH, color, TBARS, total myoglobin, % met-myoglobin, % de-oxy-myoglobin, % oxy-myoglobin)

μ is the overall mean

α_i is effect due to frozen duration (2 months and 4 months)

β_j is effect due to gender (male or female)

$\gamma_k$is effect due to display time (0, 1, 2, 3, 4, 5 days)

$(\alpha\beta)_{ij}$ $(\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk}$ are the interactions

And e_{ijk} is the error

4.6 Results

4.6.1 Proximate composition

The effects of gender and frozen duration on the proximate composition of fallow deer mince on day zero are summarized in Table 4.1. There were no interactions between treatments; therefore the main effects are discussed further. No differences ($P > 0.05$) between meat frozen for 2 and 4 months in moisture, protein, lipid and ash were recorded. Gender affected ($P < 0.05$) moisture content with females having a lower moisture content than males (73.7 ± 0.68 and 74.5 ± 0.21,

respectively); however, it can be argued that biologically the two values do not differ particularly as none of the other components of the proximate chemical composition differed between genders.

Table 4.1 Proximate composition (means and standard errors) of minced meat produced from fallow deer meat frozen for two and four months

	Gender		**Frozen duration**	
	Male	Female	2 months	4 months
	n = 12	n = 12	n = 12	n = 12
Moisture	$74.5^{a} \pm 0.68$	$73.7^{b} \pm 0.21$	$74.3^{a} \pm 0.45$	$73.9^{a} \pm 0.80$
Protein	$22.3^{a} \pm 0.74$	$22.7^{a} \pm 0.58$	$22.1^{a} \pm 0.65$	$22.9^{a} \pm 0.66$

Lipid	$2.6^{a} \pm 0.54$	$2.8^{a} \pm 0.43$	$2.8^{a} \pm 0.51$	$2.6^{a} \pm 0.50$
Ash	$1.2^{a} \pm 0.06$	$1.2^{a} \pm 0.06$	$1.2^{a} \pm 0.04$	$1.2^{a} \pm 0.07$

^{a,b} means with different superscripts in the same row are significantly different ($P < 0.05$). n=sample size.

4.6.2 Fatty acid composition

An analysis of the fatty acid composition of mince produced from wild fallow deer meat kept under frozen storage for 2 and 4 months and for different display timesis shown in Table 4.2. Gender did not affect ($P>0.05$) frozen duration and so is not included in the results. Significant time and display day interactions ($P<0.05$) were recorded for total saturated fatty acids (SFAs), mono unsaturated fatty acids (MUFAs) and PUFAs. Significantly higher percentages of total SFA were recorded in mince produced from 4 months frozen stored deer meat compared to mince produced from 2 months frozen stored deer meat on day zero (50.5% and 55.6%, respectively). The total percentage of MUFAs were higher in mince produced from 2 months frozen stored deer meat than in 4 months frozen stored deer meat (25.6% and 18.4%, respectively). Palmitic acid (28.5%), stearic acid (18.0%), linolelaidic acid (14.3%) and γ-linolenic acid (10.4%) were the dominant fatty acids in fallow deer meat on day zero.A principal component analysis showing the correlations among different fatty acids, frozen duration and display day is shown in Figure 4.1. The first two principle components (PCs) explained 67% of the total variability. Poly unsaturated fatty acids such as eichosatrinoic acid, linolelaidic acid and γ-linolenic acid attributed the most effective variables for PC1. Saturated fatty acids such as arachidonic acid and acid were useful for defining PC2.

Table 4.2 Fatty acid composition (means and standard errors) of minced meat produced from fallow deer meat frozen for two and four months

Frozen duration	**2months**		**4months**	
	n=12	**n=12**	**n=12**	**n=12**
Display day	0	5	0	5
C14:0 (Myristic acid)	2.7^{a} ±0.46	1.8^{ab} ±0.46	1.1^{b} ±0.46	2.0^{ab} ±0.46
C16:0 (Palmitic acid)	28.5^{a}±2.46	25.7^{ab}±2.46	20.8^{b}±2.46	23.9^{ab}±2.46
C18:0 (Stearic acid)	18.0^{b}±2.90	23.3^{b}±2.90	33.3^{a}±2.90	31.6^{a}±2.90
C20:0 (Arachidic acid)	1.3^{a}±0.25	0.6^{b}±0.25	0.4^{b}±0.25	0.4^{b}±0.25
C15:1 (Pentadecenoic acid)	4.3^{b}±0.88	7.4^{a}±0.88	7.2^{a}±0.88	7.7^{a}±0.88
C16:1 (Palmitoleic acid)	1.8^{a} ±0.13	1.3^{b} ±0.13	1.4^{b} ±0.13	1.4^{b} ±0.13
C18:1n9c (Oleic acid)	6.7^{a} ±1.17	6.3^{a} ±1.17	6.8^{a} ±1.17	6.6^{a} ±1.17
C18:1n9t (Elaidic acid)	6.7^{a} ±0.17	1.2^{b} ±0.17	0.5^{c} ±0.17	0.7^{c} ±0.17
C18:2n6t (Linoleadic acid)	14.3^{a} ±2.18	12.6^{a} ±2.18	13.0^{a} ±2.18	12.0^{a} ±2.18
C22:2n6 (Docosadienoic acid)	1.5^{a} ±0.16	0.9^{b} ±0.16	0.3^{b} ±0.16	0.3^{b} ±0.16
C18:3n6 (γ-linolenic acid)	10.4^{a} ±0.83	9.1^{b} ±0.83	1.1^{b} ±0.83	1.2^{b} ±0.83
C18:3n3 (α-linolenic acid)	2.4^{a} ±0.76	2.9^{a} ±0.76	4.2^{a} ±0.76	3.5^{a} ±0.76
C20:3n3 (Eichosatrienoic acid)	5.2^{a} ±0.38	0.7^{b} ±0.38	0.9^{b} ±0.38	0.9^{b} ±0.38
C22:6n3 (Docosahexaenoic acid)	0.3^{b} ±0.16	0.8^{a} ±0.16	1.1^{a} ±0.16	0.9^{a} ±0.16
Total SFA	50.5^{b} ±5.02	52.8^{ab} ±5.02	55.6^{ab} ±5.02	57.9^{a} ±5.02
Total MUFA	19.5^{a} ±1.62	16.2^{ab} ±1.62	15.9^{b} ±1.62	16.4^{a} ±1.62
Total PUFA	34.1^{a} ±4.6	27.0^{ab} ±4.6	20.6^{b} ±4.6	18.8^{b} ±4.6
Total n6	26.2^{a}±2.71	22.6^{a} ±2.71	14.4^{b}±2.71	13.5^{b}±2.71
Total n3	8.2^{a}±0.97	4.4^{b}±0.97	6.2^{b}±0.97	5.4^{b}±0.97
PUFA:SFA	0.7^{a} ±0.14	0.5^{ab} ±0.14	0.4^{ab} ±0.14	0.3^{a} ±0.14
n6/n3	3.2^{b}±0.40	5.1^{a}±0.40	2.3^{c}±0.40	2.6^{bc}±0.4

Means with different superscripts in the same row are significantly different ($P < 0.05$).

SFA – saturated fatty acids; MUFA – mono unsaturated fatty acids; PUFA – poly unsaturated fatty acids; n3 – omega 3 fatty acids (C18:2n3, C20:2n3, C22:6n3); n6 – omega 6 fatty acids (C18:2n6t, C22:2n6, C18:3n6); PUFA:SFA – poly unsaturated fatty acid: saturated fatty acid ratio; n6/n3 –omega 6/ omega 3 fatty acid ratio

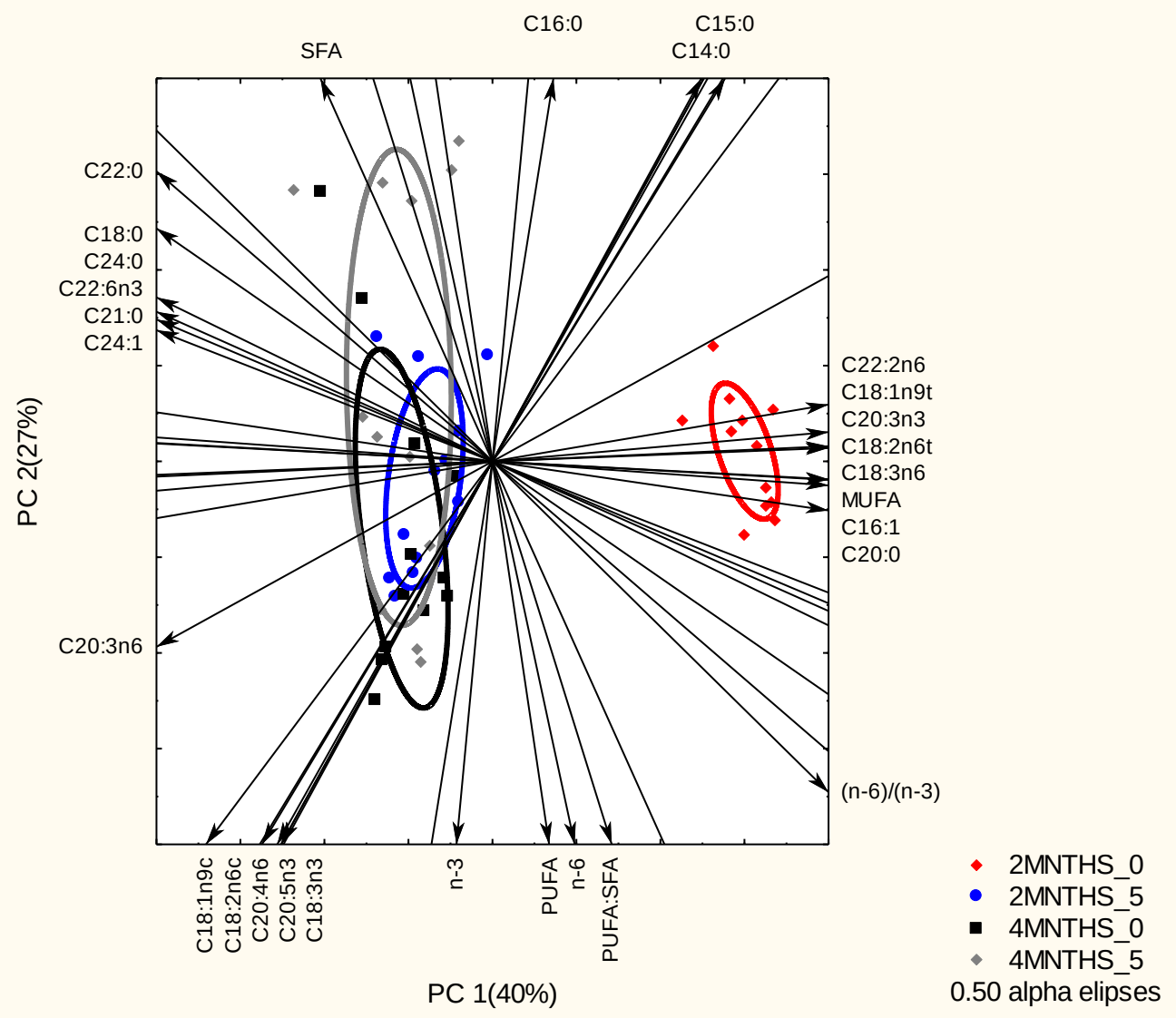

Figure 4.1 Principle Component Analysis showing correlations between fatty acid composition, frozen duration and display day.

4.6.3 pH

The effects of frozen storage and display day on pH are shown in Figure4.2. No significant gender differences were observed and so the table does not show gender effects.Significant treatment and time interactions were observed ($P<0.01$) and although the trend was not linear, a gradual decrease in pH over time was observed for both treatments.

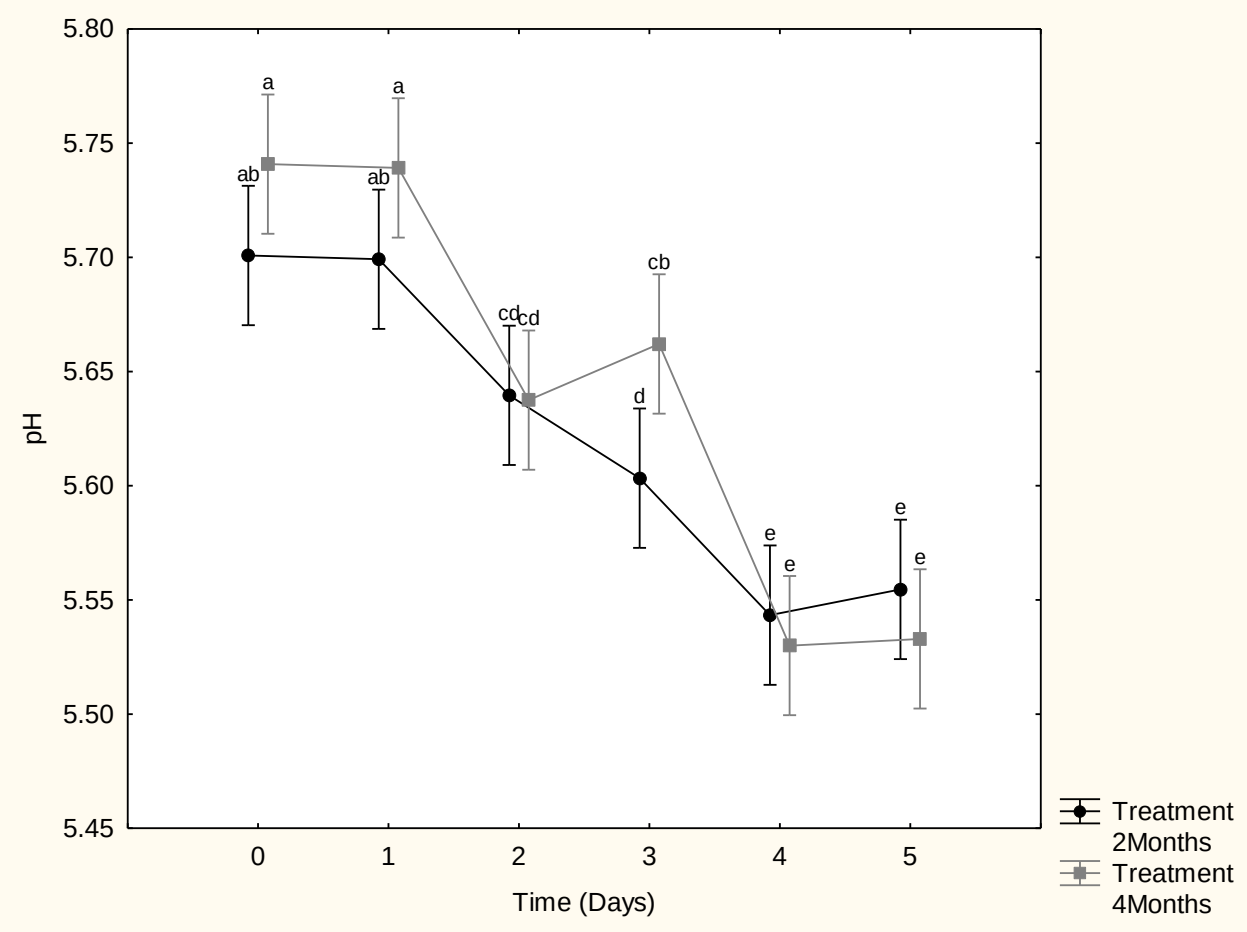

Figure 4.2 Effects of frozen duration (2months and 4months) on pH of minced meat produced from fallow deer over five days of display. Least square means with different superscripts are different ($P<0.05$).

4.6.4 Colour

No significant gender differences (P=0.2344) were observed for any of the colour variables and thus only frozen duration and display time is reported further. The effects of frozen duration and display day on the various colour parameters are summarized in Figure 4.3 and Figure 4.4. Significant time and treatment interactions (P<0.05) were observed on all colour parameters except for meat lightness.There were no significant storage effects on all colour parameters (P>0.05).

Changes (P<0.001) were observed in lightness from day to day throughout the display storage period. Display day significantly affected redness (*a**) (P<0.001) irrespective of treatment. Meat yellowness (*b**) generally decreased throughout display storage although the decrease was not linear. Meat frozen for 2 months had higher *b** values than meat frozen for 4 months on day zeroand four (13.85 and 13.09) although biologically the differences can be said to be insignificant.

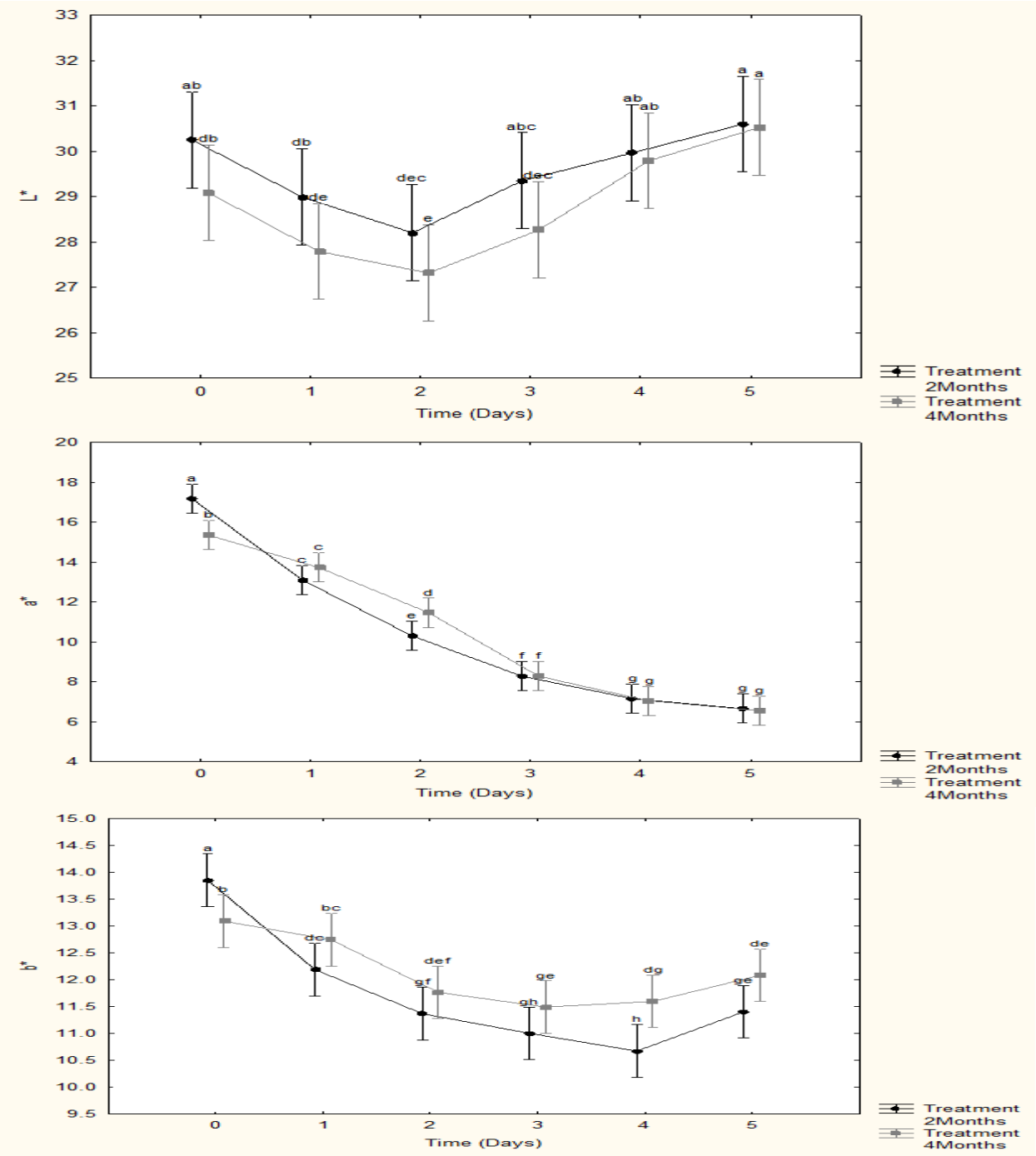

Figure 4.3 Effects of frozen duration (2months and 4months) on colour parameters of minced meat produced from fallow deer meat over five days of display. Least square means with different superscripts are different ($P<0.05$).

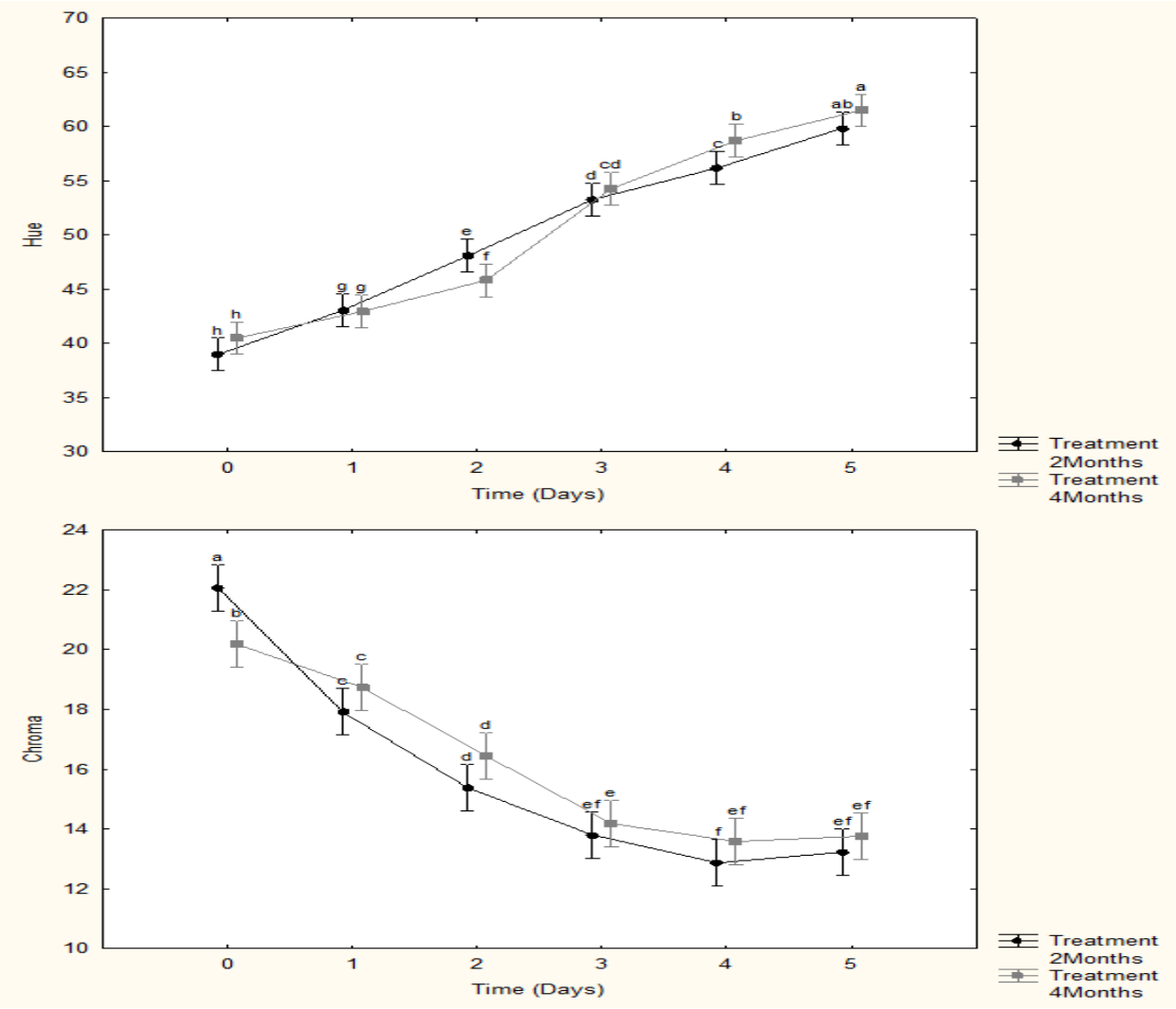

Figure 4.4 Effects of frozen duration (2months and 4months) on hue and chroma of minced meat produced from fallow deer over five days of display. Least square means with different superscripts are significantly different (P<0.05).

4.6.5 Myoglobin content and myoglobin forms

No significant gender differences (P=0.432) were observed for myoglobin content and all myoglobin forms and thus only frozen duration and display time is reported further. The effects of frozen duration and display day on the myoglobin content and myoglobin forms are summarized in Table 4.3. Significant storage and time interactions were recorded for myoglobin content ($P<0.05$). A general decrease was observed throughout display. Mince produced from 4 months frozen meat had more myoglobin content on day 0 compared to mince produced from 2 months frozen meat (6.8mg/g and 5.9mg/g, respectively).

No significant interactions were observed for all myoglobin forms. Treatment and display day were significant factors for all myoglobin forms ($P<0.001$ for both), with mince produced from meat frozen for 2 months having higher percentages of OMb than mince produced from meat frozen for 4 months.

Table 4.3 Effect of frozen duration and display time (days) on myoglobin forms (means and standard errors) of fallow deer meat made into minced meat

Treatment	Total myoglobin (mg/g)	Myoglobin form		
		%Mmb	%Omb	%Dmb
		2months frozen storage		
Day 0	6.0^{a}± 0.19	25.8^{e}±1.46	61.8^{a}±2.08	12.0^{c}±0.88
Day 1	6.1^{a}± 0.17	51.2^{d}±2.62	33.2^{b}±2.36	15.6^{a}±0.56
Day2	6.1^{a}± 0.21	64.4^{c}±1.15	20.4^{c}±0.75	15.1^{a}±0.6
Day3	5.8^{ab}±0.21	65.1^{b}±1.1	19.0^{c}±0.7	15.9^{a}±0.57
Day4	5.7^{b}±0.18	70.0^{a}±0.91	16.1^{d}±0.66	13.7^{b}±0.41
Day5	5.5^{b}±0.17	70.3^{a}± 0.73	15.8^{d}±0.55	13.6^{b}±0.39
		4months frozen storage		
Day 0	6.8^{a}±0.25	32.8^{e}±2.52	55.1^{a}±3.16	12.1^{c}±1.14
Day 1	6.3^{b}±0.22	52.2^{d}±2.62	31.2^{b}±2.64	16.7^{a}±0.39
Day2	5.3^{d}±0.11	66.5^{c}±1.15	18.0^{c}±1.22	15.6^{b}±0.39
Day3	5.7^{c}±0.12	70.5^{b}±1.51	13.6^{d}±1.05	16.1^{ab}±0.51
Day4	5.7^{c}±0.15	71.6^{b}±1.22	12.7^{d}±0.95	15.9^{ab}±0.34
Day5	5.5^{cd}±0.13	74.7^{a}±1.01	9.6^{e}±0.81	15.8^{ab}±0.34
		P - values		
Storage effect	NS	**	**	*
Time effect	***	***	***	**
Storage x time	***	NS	NS	NS

Means with different superscripts in the same column are significantly different (P<0.05). n=sample size. NS = not significant. * = P<0.05 **= P<0.01 ***= P<0.001

4.6.6 Lipid oxidation

The effect of frozen storage and display day on lipid oxidation is shown in Figure 4.5. No significant gender differences were observed and so the table does not show gender effects. Significant treatment and display time interactions ($P<0.05$) were found for TBARS and myoglobin content. There was a rapid increase in TBARS throughout the display period with mince produced from meat frozen for 4 months showing higher TBAR values compared to mince produced from meat frozen for 2 months.

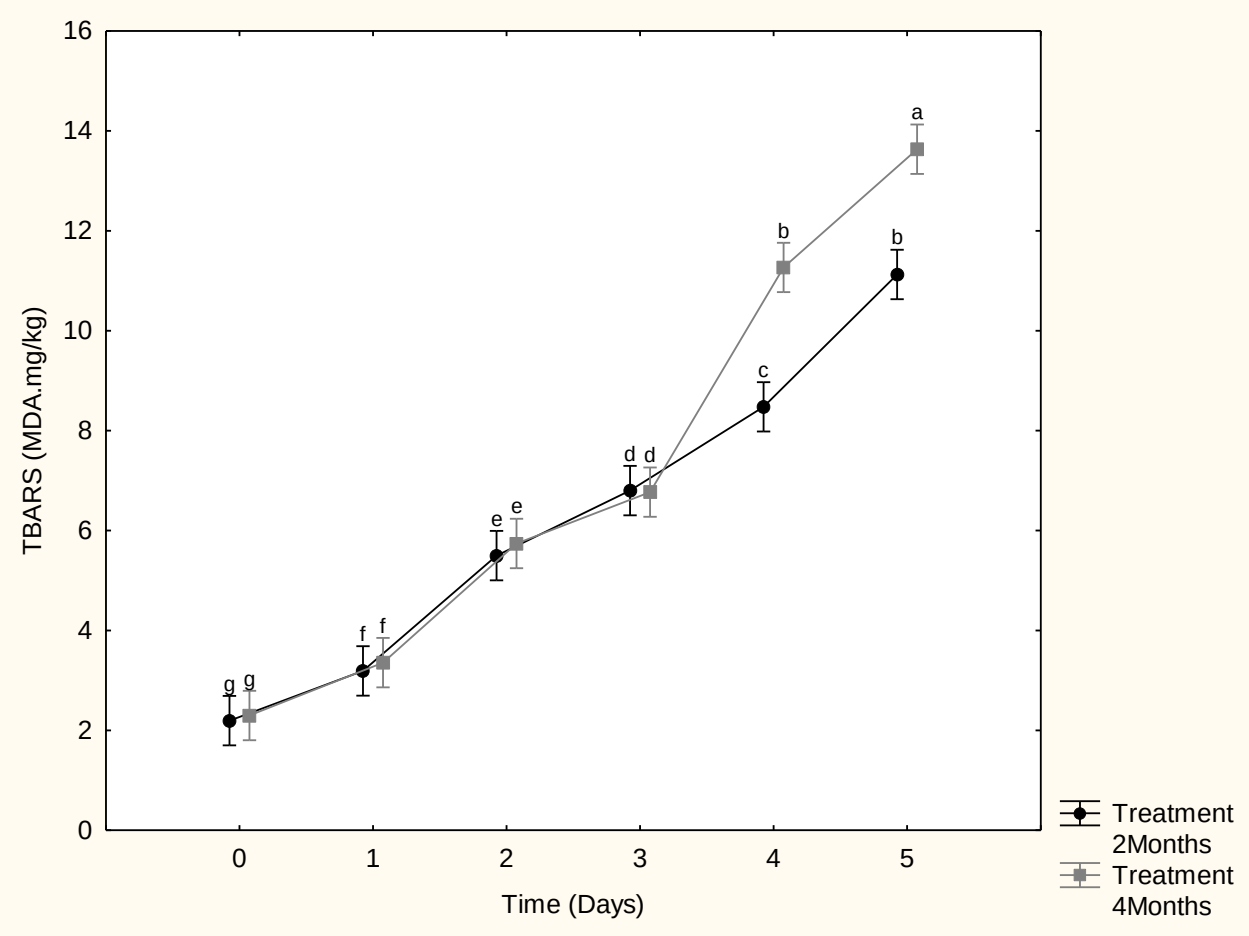

Figure 4.5 Effects of frozen duration (2months and 4months) on lipid oxidation of minced meat produced from fallow deer over five days of display. Least square means with different superscripts are different ($P<0.05$).

4.7 Discussion

4.7.1 Proximate composition

Protein and moisture content of fallow deer was not affected by frozen duration and was found to be in the same range as other reports on game species (23 – 24% for protein and 73 – 75%) (Dannenburger *et al*., 2013). Average fat content of wild fallow deer meat in this study was 2.7 (±0.43) % (Table 4.1). This value is higher than previous reports of farmed fallow deer (Volpelli *et al*., 2003; Ramanzin *et al*., 2010). The difference in fat content are attributable to differences in diet (linked to season) and region. Daszkiewicz *et al.* (2015) also found wild fallow deer to have higher fat content in a study to compare the meat quality of farmed and wild fallow deer in Poland. However, it is difficult to compare these results with the fat content of deer meat from South Africa as this is the first report of the fat content of these feral cervids that are becoming more popular in South Africa. The fat content was lower than that of traditional meat species and within the reported range of most game species (Hoffman and Wiklund, 2006), suggesting that it may be considered as a lean alternative to domestic red meat species.

4.7.2 Fatty acid composition

The fatty acid composition of meat gives a better understanding of the nutritional value of meat as it the different proportions of the fatty acids which determine the health risks or benefits associated with meat (Muchenje *et al*., 2009; Nantapo *et al*., 2015). Saturated fatty acids are expected to be in high quantities in ruminants due to the hydrogenation action of rumen bacteria which results in the conversion of high forage PUFA into SFA (Mapiye *et al*., 2015). Thus the high percentage (52.5% ±5.02) of total SFA in this study was expected (Table 4.2). Palmitic acid, which was the most dominant SFA in the study, was not affected by storage or display day and contributed approximately 47% of the total SFA (Table 4.2). Other studies involving cervid

meat have reported similar findings (Volpelli *et al*., 2003; Wiklund*et al*., 2003; Daszkiewicz *et al*., 2015) whilst other studies reported higher amounts of palmitic acid in blesbok (Hoffman *et al*., 2008), eland and beef (Bartoň*et al*., 2014). This saturated fatty acid is associated with increased cholesterol blood levels which results in increased risk of cardiovascular diseases although no results for its direct involvement have been established (Nantapo *et al*., 2015).

Oleic acid was the dominant MUFA (Table 4.2) contributing 6.7 % of the total fatty acids and 18% of the total MUFA (Table 4.2). This fatty acid is desired as it reduces cholesterol levels and increases membrane stability (Rani *et al*., 2014). The ratio of PUFAs, especially the n6:n3 ratio is important for human health and nutritionists recommend ratios below five (Kouba and Mourot, 2011). The n6:n3 ratios in this study were found to be well below five and similar to the findings of Dannenburger *et al*. (2013) in roe deer.

Prolonged storage of meat results in the oxidation of unsaturated fatty acids, resulting in rancidity and off flavours (Nute *et al*., 2007; Ponnampalam *et al*., 2012). The results regarding fatty acid composition seem to corroborate this as the amounts of PUFA decreased with increased frozen storage and display day. In the first two principle components of the PCA studied, variables exhibited a positive correlation between 2months frozen storage, display day zero and PUFA (Figure 4.1). However, a negative correlation was shown between mince produced from 4months frozen stored meat and n3 PUFAs and oleic acid. This relationship can be explained by the deterioration of PUFA during prolonged frozen storage and display time which result in decrease of PUFA. This confirms that prolonged frozen storage and consequent retail display may affect the fatty acid composition of fallow deer meat negatively.

4.7.3 Colour, pH and oxidative stability

Lightness is generally described as the most stable color parameter as it is not related to myoglobin oxidation reactions (Ripoll *et al*., 2011; Muela *et al*., 2014). As such, it is not considered an appropriate indicator of meat discoloration (Mancini and Hunt, 2005; Luciano *et al*., 2009).

Changes in redness (*a**) on the other hand, have been used to signify discoloration. A general decrease in meat redness indicates myoglobin oxidation and consequent accumulation of met-myoglobin (Quevedo *et al*., 2013). This trend is evident in the study and is in agreement with previous studies on beef (Muchenje *et al*., 2009), lamb (Luciano *et al*., 2009) rhea meat (Filgueras *et al*., 2010). On day 0 meat frozen for 2 months had higher *a** values than meat frozen for 4 months (17.17 and 15.35, respectively). This could be due to greater denaturing of the myoglobin molecule and the loss in activity of the met-myoglobin reducing enzyme systems during freezing and frozen storage (Leygonie *et al*., 2012b) resulting in slower met-myoglobin reducing activity in meat frozen for 4 months. This corresponds well with the results for the percentage of met-myoglobin (Table 4.3) which is higher in meat frozen for 4months on day 0. Moreover, the higher percentage oxy-myoglobin (OMb%) in meat frozen for 2 months on day 0 suggest a higher met-myoglobin reducing activity rate in meat frozen for 2 months and corresponds well with the *a** values recorded for day 0.

Interestingly, on day 2 the 4 months frozen meat had higher *a** values than the 2 months frozen meat (11.46 and 10.29, respectively). The reason is not clear although it can be argued that biologically the differences are not significant as no differences were observed on subsequent days (Figure 4.3).

The *a** values recorded on day 0 are generally higher than those found in other game species (Hoffman*et al.*, 2009) suggesting that fallow deer produces meat that is more red. However, colour stability in terms of redness was relatively poor as there were huge differences (about 10 units) in redness between day 0 and day 5. This huge difference could possibly be because minced meat was used instead of whole muscle or cuts, thereby exposing more surface area to myoglobin oxidation (Crowley*et al.*,2010). The same can be said for chroma, TBARS and met-myoglobin accumulation as the same trend was observed in these parameters as well.

Although some researchers have reported that frozen storage and display time increase yellowness (*b**) due to the accumulation of lipid oxidation products and myoglobin oxidation (Ripoll*et al.*, 2011; Muela*et al.*, 2015), the opposite was observed in this study. Bingol and Ergun (2011) observed the same trend with ostrich meat displayed over 10 days under air and MAP conditions. Leygonie*et al.* (2012b) reported decreased yellowness in ostrich meat frozen for one month. Furthermore, Esmer*et al.* (2011) reported a positive correlation between *a** and *b** (r^2 = 0.908 and P<0.01) whilst Seydim, Acton, Hall & Dawson (2006) reported negative correlations between *b** values and TBARs (r^2 = −0.835 and P<0.001) suggesting that a decrease in redness and increase in TBARS leads to reduced yellowness. The *b** value does not contribute strongly to the appearance of meat and is frequently not discussed (Leygonie*et al.*, 2011).

Hue, being a function of *a** and *b** gives a more realistic perspective of meat discoloration and is a better suited to describe colour changes over time (Luciano *et al.* 2009). Hue increased linearly over display time showing that as display days increased, discoloration also increased (Kim*et al.*, 2011). Visible meat discoloration (browning) was evident during the study which supports reports of strong positive correlations between sensory discoloration and hue values (Kim*et al.*, 2011). Chroma is an indication of colour intensity. On day 0, chroma for 2 months frozen meat

was higher than that of the 4 months frozen meat (22.06 and 20.17, respectively). This corresponds well with the initial redness of the meat showing that meat frozen for 2 months had a more intense redness than meat frozen for 4 months. Chroma decreased throughout display (Table 4.4) indicating that as discoloration (Hue) increased, colour intensity decreased. Kim*et al*. (2013) also observed similar findings. The colour parameters confirm the known characteristics of game and venison which is dark red (L^*<40, high a^* and low b^* values) (Volpelli *et al*., 2003, Hoffman *et al*., 2005).

Percentage met-myoglobin (MMb) rapidly increased from day 0 until day 2 where it became constant for the remaining display days. On day one, it had already exceeded the 40% cut off which is when consumers are reported to reject products for discoloration (Kim*et al*., 2011). Nerimetla*et al*. (2014) reported that low pH conditions favor faster rates of MMb formation due to the low affinity for oxygen at this pH and specific acid catalysis. This could further explain the high MMb percentages observed in the study. Percentage de-oxy-myoglobin (DMb) increased rapidly on day 0 and 1 from 12.4% to around 16% where after it became rather stable throughout display. A possible explanation for the rapid increase and subsequent stable percentage could be the regaining of activity of MMb enzyme reducing systems since DMb is a result of MMb reduction (Bekhit and Faustman, 2005). During frozen storage met-myoglobin reducing enzyme systems cannot function due to cold damage and regain some of their properties upon thawing.

During frozen storage, not all oxidation is retarded; primary oxidation will occur albeit at slow rates resulting in the formation of intermediate hydroperoxide molecules (Muela *et al*., 2014). However upon thawing, these species are more reactive than normal fatty acids and will react to give secondary oxidation products which are known precursors of malonaldehyde TBAR substances (Shahidi and Zhong, 2010). The rapid increase in TBAR values during display days

in this study (Table 4.6) indicates an accelerated rate of secondary oxidation. The use of mince instead of whole muscle could also be a contributing factor to this trend. Mincing increases the surface area exposed to oxygen; disrupts and exposes phospholipids in cell membranes and intramuscular fat to pro-oxidants such as iron and copper (Crowley *et al*., 2010). Higher TBAR values were recorded throughout this study (greater than 2mg MDA/kg meat) as compared to traditional meat species (usually lower than 2mg MDA/kg meat). Similar findings have been reported in previous studies on game species (Seydim *et al*., 2006; Leygonie*et al*., 2011; Cifuni *et al*., 2014). Values from day 0 had already exceeded 2mg MDA/kg meat (Table 4.4), suggesting that the presence of high amounts of PUFAs and haem pigment myoglobin in game meat makes it more susceptible to oxidation than traditional meat species. Detection threshold for rancidity and off flavours differs between species and has been determined to be 2.28mg MDA/kg in beef and 1mg MDA/kg in lamb (Campo *et al*., 2006; Ripoll*et al*., 2011). However, threshold values for detection of rancidity in fallow deer has not been determined and warrants investigation.

It was expected that meat frozen for 2 months would have more myoglobin as is commonly accepted that the longer meat is frozen, the more exudate is formed due to more damage caused on the cell ultra-structure (Kim*et al*., 2011) and thus logically, the more myoglobin is lost in the exudate. The reasons for the reverse findings are not clear but could be an indication of the effects of different freezing rates or fluctuations in temperature during frozen storage of the deer meat as the meat was not frozen during the same time. Throughout display myoglobin content continued to decrease possibly due to continued purge loss that naturally happens to meat during cold storage display (Kim*et al*., 2013).

The decline in meat pH during display period recorded in this study (Table 4.4) is in agreement with Kim *et al*. (2013) who recorded a decrease in pH in freeze/thawed pork displayed over 7 days. Since pH is a measure of hydrogen ions, freezing and successive exudates formation may possibly denature proteins, release hydrogen iond and increase solute concentration, thus lowering pH of the meat (Leygonie *et al*., 2012a). The pH ranged from 5.6 – 5.8 which is acceptable for game meat (Hoffman, *et al.*, 2004).

Conclusions

Frozen storage duration significantly affected lipid oxidation and percentage oxy-myoglobin. Meat frozen for four months had significantly higher TBARS than meat frozen for two months. However, pH and all colour parameters only differed significantly between treatments on day zero. A loss in mince quality was observed generally by the end of the display duration as observed by reduced redness, decreased colour intensity and increased discolouration and high oxidation. This study demonstrated that fallow deer can be stored frozen for up to two months without adverse colour and quality changes when minced and used quickly (within three days). It also demonstrated that mince from frozen/thawed fallow deer meat has a short shelf life when packaged under oxygen permeable material. Frozen/thawed mince from fallow deer meat frozen for two months is generally redder on the first day of display than meat frozen for four months. However, as display days increase, the effects of frozen duration become insignificant. Possible future research areas should look at consumer detection levels of rancidity in fallow deer.

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Chapter 5:General discussions, conclusions and recommendations

5.1 General discussion

Meat quality will always be affected by processing, handlingand storage and transport conditions. Furthermore, meat has a tendency of changing colour from a bright cherry red to an unfavourable brown colour during retail display storage (Rogers *et al*., 2014). Consumer association of bright cherry red meat colour and freshness forces the meat industry to ensure that meat and meat products maintain a colour that is favoured by consumers for long. The broad objective of the study was to determine the colour and lipid stability of fresh fallow deer minced meat and the effects of freezing and frozen duration on the retail display life of mince. It was hypothesised that there were no differences between colour and lipid stability of fresh and frozen/thawed mince from deer during display and that frozen storage duration did not affect the colour and lipid stability of mince produced from fallow deer.

The colour and lipid stability of fresh mince was determined in Chapter 3 and compared with mince produced from two months frozen stored fallow deer. The hypothesis in this chapter was that no differences existed in the colour and lipid stability of fresh and frozen/thawed mince. Significant colour and lipid differences were recorded between fresh and frozen/thawed mince. Fresh mince had low TBARS values indicating low oxidation of lipids. The hue angle and total oxy-myoglobin % of fresh mince was more constant throughout display storage whereas for frozen/thawed mince, hue quickly went down and met-myoglobin % accumulation was rapid. This clearly showed that colour and lipid stability of fallow deer was significantly affected by frozen storage and was attributed to the damaging action of ice crystals on lipid structure and met-myoglobin reducing enzyme systems (Leygonie *et al*., 2012).

In Chapter 4, the objective was to determine the effects of frozen storage duration. Prolonged freezing (three months or more) is thought to worsen the detrimental effects of freezing which were shown in Chapter 3 (Soyer*et al*., 2010).However, other researchers say that the detrimental effects are levelled out as storage duration increases due to ice crystal redistribution in the meat system during frozen storage (Mortensen *et al*., 2006; Muela *et al*., 2012). The hypothesis here was that frozen duration has no effect on the colour and lipid stability of mince. Minced meat was produced from 2months and 4 months frozen stored fallow deer meat. Significant differences were recorded between treatments and by day three both meat samples where showing signs of extended discolouration. The results from chapter four seem to be in support of the findings of Soyer*et al*. (2010).

5.2 Conclusion

Several conclusions were reached through this study. Firstly, it was concluded that colour and lipid stability of fresh and frozen/thawed mince differ significantly and that freezing lowers the retail display life of fallow deer minced meat. It was further concluded that frozen duration has a significant effect on the colour of mince. However, as display days increase, the effect of frozen duration on colour parameters becomes insignificant. The retail display shelf life of mince produced from frozen/thawed fallow deer meat was three days. It was evident from the results freezing and frozen duration affects the lipid stability of mince but does not affect the proximate and fatty acid composition.

5.3 Recommendations and future research

Future research should focus on quantifying the interactions of lipid and myoglobin oxidation and relating these with physical observed colour. It is also important for microbial and sensory

evaluation of fallow deer meat to be done over retail display time so as to determine if discolouration corresponds to unsafe microbial numbers and detectable rancid or off-flavours.

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