

EVALUATION OF LAMB AND MUTTON QUALITY AT RETAIL LEVEL IN THE TSHWANE METROPOLE

By

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DECLARATION

I hereby declare that the thesis, Evaluation of Lamb and Mutton Quality at Retail Level in the Tshwane Metropole, handed in for the qualification of Magister Scientiae Consumer Science (Food Science) at the University of the Free State, is my own work and that I have not previously submitted the same work for a qualification at another University or faculty. I hereby concede copyright of this thesis to the University of the Free State.

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GLOSSARY OF ABBREVIATIONS

a/A

ALA	Alpha-linolenic acid
ATP	Adenosine triphosphate
a*	Colour coordinate – redness value

b/B

β	Beta
β -AA	Beta-adrenergic agonists
BCFA/s	Branched-chain fatty acid/s
b*	Colour coordinate – yellowness value

c/C

c	<i>Cis</i>
Ca ²⁺	Calcium
CaCl ₂	Calcium chloride
CK	Creatine kinase
CoA	Molecule of coenzyme A
°C	Degrees Celsius

d/D

DAFF	Department of Agriculture, Forestry and Fisheries
DFD	Dark, firm and dry

e/E

e.g.	For example
ELVES	Extra low-voltage electrical stimulation
ES	Electrical stimulation
etc.	Etcetra

f/F

FA	Fatty acid
FADH ₂	Flavin adenine dinucleotide
FAO	Food and Agriculture Organization
FAME	Fatty acid methyl ester/s

Individual FAME:

<i>Abbreviation</i>	<i>Common name</i>	<i>Complete formula</i>	<i>Systematic (IUPAC)</i>
C14:0	Myristic	C14:0	Tetradecanoic
C14:1	Myristoleic	C14:1c9	Tetradecanoic acid
C15:0	Pentadecylic	C15:0	Pentadecanoic
C16:0	Palmitic	C16:0	Hexadecanoic
C16:1	Palmitoleic	C16:1c9	<i>cis</i> -9-Hexadecanoic
C17:0	Margaric	C17:0	Heptadecenoic
C17:1	Heptadenoic	C17:1c10	<i>cis</i> -10-Heptadecenoic
C18:0	Stearic	C18:0	Octadecanoic
C18:1c7	Vaccenic	C18:1c7	<i>cis</i> -7-Octadecenoic
C18:1c9	Oleic	C18:1c9	<i>cis</i> -9-Octadecenoic
C18:1t9	Elaidic	C18:1t9	<i>trans</i> -9-Octadecenoic
C18:2c9,12	Linoleic	C18:2c9,12(n-6)	<i>cis</i> -9,12-Octadecadienoic
C18:2c9t11	CLA	C18:2c9t11	(9c,11t)-octadeca-9,11-

C18:3n-3	α -Linolenic	C18:3c9,12,15(n-3)	dienoic acid cis-9,12,15- Octadecatrienoic
C20:0	Arachidic	C20:0	Eicosanoic
C20:3n-6	Eicosatrienoic	C20:3c8,11,14(n-6)	<i>cis</i> -8,11,14-Eicosatrienoic
C20:4	Arachidonic	C20:4c5,8,11,14(n-6)	<i>cis</i> -5,8,11,14- Eicosatetraenoic
C22:0	Behenic	C22:0	Docosanoic
C22:5	Docosapentaenoic	C22:5c7,10,13,16,19(n-3)	<i>cis</i> -4,7,10,13,16- Docosapentaenoic
C24:0	Lignoceric	C24:0	Tetracosanoic
	Phytanic acid		3.7.11.15-tetramethyl- hexanoic acid
	Pristanic acid		6.10.14- tetramethylpentadecanoic acid

g/G

g/kg	Grams per kilogram
GPx	Glutathione peroxidase

h/H

h	Hour
ha	Hectare
HACCP	Hazard Analysis and Critical Control Points
HVES	High-voltage electrical stimulation

i/I

i.e.	That is
IMF	Intramuscular fat

k/K

kg	Kilogram
kgF	Kilogram-force

I/L

LA	Linoleic acid
LVES	Low-voltage electrical stimulation
L*	Colour coordinate – lightness value

m/M

μ m	Micrometer
μ mol/L	Micromole per litre
MetMb	Metmyoglobin
MFL	Myofibrillar fragment lengths
Mg	Magnesium
mg/day	Milligram per day
MNA/s	4-Methylnonanoic acid/s
MOA/s	4-Methyloctanoic acid/s
MPC	Multicatalytic proteinase complex
MUFA/s	Monounsaturated fatty acid/s

n/N

N	Newtons
NADH	Nicotinamide adenine dinucleotide
nm	Nanometer
n-3	Omega-3
n-6	Omega-6
n-6:n-3	Omega-6 to omega-3 ratio

o/O

OxyMb	Oxymyoglobin
-------	--------------

p/P

pH	Potential of hydrogen
pH ₂₄	pH after 24 hours
pH _μ	Ultimate pH
pI	Isoelectric point
P:S	Polyunsaturated fatty acid to saturated fatty acid ratio
PSE	Pale, soft and exudative
PUFA/s	Polyunsaturated fatty acid/s
PVC-OW	Polyvinyl chloride overwrapped

r/R

RH	Ractopamine hydrochloride
ROS	Reaction of radicals
R ²	Coefficient of determination

s/S

SAMM	South African Mutton Merino
SCF	Subcutaneous fat
SFA/s	Saturated fatty acid/s
SOD	Superoxide dismutase
SPR	Sarcoplasmic reticulum
ST	Somatotropin

t/T

<i>t</i>	<i>Trans</i>
TBARS	Thiobarbituric reactive substances

u/U

UFA/s	Unsaturated fatty acid/s
-------	--------------------------

v/V

V	Volts
---	-------

w/W

WBSF	Warner Bratzler shear force
WHC	Water holding capacity

z/Z

ZAR	South African Rand
-----	--------------------

ZH Zilpaterol hydrochloride

Other

α	Alpha
&	And
Δ	Delta
γ	Gamma
<	Less than
>	More than
%	Percentage

CHAPTER 1

INTRODUCTION

Livestock production is shifting from providing great amounts of high-value proteins that is used for the nourishment of populations, to the production of secure and convenient meats of consistently good eating quality (Hocquette, Gondret, Baéza, Médale, Jurie & Pethick, 2010). For livestock industries to be able to constantly produce good quality meat, they must have an understanding of the influences which causes the variation in quality and the application of management systems to reduce this variation (Warner, Greenwood, Pethick & Ferguson, 2010).

Many regions of the world depend on the farming of ruminants for economic and food security (De Araújo, Pereira, Mizubuti et al., 2017). Higher demand and prices led to producers investing in more permanent infrastructures for the intensive finishing of lambs (Miranda-de la Lama, Villarroel, Olleta, Alierta, Sañudo & Maria, 2009). Intensive lamb production is a high-turnover, low-margin enterprise which requires excellent management skills and an accurate supply of information (Jolly & Wallace, 2007). Therefore, it is important that the supply-chain is of that sort that all the sectors involved can implement best-practice procedures (Pethick, Banks, Hales & Ross, 2006). Variation in meat tenderness and unsatisfied consumers in relation to the toughness of meat are a common concern to the global meat industry (As cited by Hope-Jones, Strydom, Frylinck & Webb, 2010). Therefore, the factors which the production line underscores' is analyzed to improve the quality of meat products to those correlated with consumers' lifestyles and beliefs (Guerrero, Valero, Campo & Sañudo, 2013).

While animal agriculture and the meat industry have lots of challenges that need to be dealt with, the majority of the Western world's population still enjoys a satisfactory amount of meat as part of a balanced diet (Swatland, 1995). The focus of meat production is shifting from quantity to quality, especially to facilitate consumer demands in developing countries (Sosnicki & Newman, 2010). Saturation of food markets owed to efficient modern agriculture contributes to the economic challenge of producing good quality meat products (Geay, Bauchart, Hocquette & Culioli, 2001). Consumers' perception of good quality meat depends greatly on their socio-demographic background, therefore on cultural aspects and health expectations. For this reason livestock animals are not nurtured and fed to the same levels of fatness in different countries (Hocquette et al., 2010). Most sheep breeds are used for wool production, although they are also acknowledged to have meat-production potential (Hoffman, Muller, Cloete & Schmidt, 2003).

The production of sheep is spread across the globe as a result of the capability of sheep to acclimatize to different climates and types of vegetation (De Brito, Ponnampalam & Hopkins, 2017). According to the Food and Agriculture Organization (FAO) figures, from 1993 up until 2013 there were about one billion sheep in the world distributed throughout Asia (41.1%), Africa (23.9%), Europe (13.5%), Oceania (13%) and America (8.5%) (FAO, 2013). As the demand for meat is growing, it is predicted that the world population could reach 9.1 billion humans in 2050 which increases the call for food by 60% (Alexandratos & Bruinsma, 2012). Poultry and pork are developing because their prices allow specialized meat production based on cultivated grains (Boutonnet, 1999). Mutton is a very good source of protein which provides important minerals such as zinc and iron, because of their high bioavailability in meat related to plant sources (Williams, 2007; De Brito et al., 2017). Sheep are easy to handle and suitable to very small farms which makes these small ruminants a very popular small-scale farming enterprise, and also because they can be fed from domestic and farming by-products (Boutonnet, 1999).

Lamb meat is described as an attractive product – it is juicy and tender with a good flavour (Pethick, Banks, Hales & Ross, 2006). If producers want sheep production to continue as a competitive market, lamb and mutton quality should correlate with the demand of the different markets (Rubino, Morand-Fehr, Renieri, Peraza & Sarti, 1999; Sañudo, Santolaria, Maria, Osorio & Sierra, 1996; Sañudo, Nute, Campo, Maria, Baker, Sierra, Enser & Wood, 1998a; Sañudo, Sánchez & Alfonso, 1998b). Significant variations are present in carcass composition as well as the quality of sheep due to the effect of species, age, maturity, sex and interactive effects with production systems and technologies (Webb & Erasmus, 2013). Morgan et al. (1991) indicated that the variation in meat tenderness and thus quality occur under different production and post-mortem handling systems. The predominant assessment is that quality is one of the most important aspects that define a consumer's choice and acceptability amongst meat from different animal species (Homer, Cuthbertson, Homer & McMenamin, 1997; Wood, Enser, Fisher, Nute, Richardson & Sheard, 1999).

Over the years the perception of meat quality has frequently been evolving in response to the growing concerns of consumers in terms of health, origin, ethical aspects, just to name a few (Zervas & Tsiplakou, 2011). Consumers, retailers and producers have different opinions and expectations in terms of carcass and meat quality, as well as the eating experience (Webb, 2015). Consumers of the same country of origin do not automatically prefer the same products, which emphasizes the importance of the provision of a range of products with different physical and sensorial attributes (Oliver, Nute, Font i Furnols, San Julian, Campo, Sañudo, Caneque, Guerrero, Alvarez, Diaz, Branscheid, Wicke & Montossi, 2006). Sheep in South Africa are mostly bred from an assorted range of breeds and crosses (Hoffman et al., 2003). These farms are mostly found in Mpumalanga, the Free State, and the Eastern, Northern and Western Cape provinces (Cloete, Hoffman & Cloete, 2012). In the Eastern Cape, sheep are very important in the communal areas seeing that they are a source of milk, meat and wool and thus

a sustainable income (Mapiliyao, Pepe, Marume and Muchenje, 2012). In 2010, this province had a 30% meat consumption rate, which was the highest percentage of meat consumed locally (DAFF, 2010).

Palatability, especially tenderness, is affected by a number of critical factors that are first introduced with the animal's genotype and concluded with the cooking process (Ferguson, Bruce, Thompson, Egan, Perry & Shorthose, 2001). Therefore, critical control point approaches, similar to those used in food safety programs (e.g. HACCP), help to create proper eating quality assurance schemes (Ferguson et al., 2001). To ensure safe and wholesome meat products for consumers, the Meat Safety Act (Act 40 of 2000) provides guidelines for the conversion of livestock to meat. On the other hand, the Agricultural Product Standards Act (Act 119 of 1990) helps to set and control specific product standards for local and export purposes by means of classification, inspection or grading (by distinctive marks) and sampling for quality control (Webb, 2015). As more consumers are prepared to make lifestyle changes to lessen the risks associated with unhealthy food choices, the focus turned to the science of food (e.g. composition, function, interaction and the complete food matrix) (Schönfeldt & Hall, 2015). As early as 1952 attempts have been made to predict the tenderness of meat in pre-rigor state (Paul, Bratzler, Farwell & Knight, 1952).

Since the lamb/mutton production chain is still hugely fragmented for large parts of the industry, i.e. various role players influence the process independently of the other mostly for their own benefit, the final product quality may firstly vary and secondly the reasons for this variation is difficult to trace to a specific sector or role player. In vertically integrated systems (feedlot, abattoir, meat processor combined), much but not all of this variation is overcome through carefully controlled processes if it is accepted that the manager of such a system has sufficient knowledge. It is well-known that origin of sheep meat in South Africa, as opposed to countries like New Zealand and Australia, is from pasture as well as from feedlots, probably in equal amounts, as opposed to beef that is predominantly from feedlots. This in itself could cause variation with regards to eating quality (flavour and tenderness) and other quality parameters (colour). There are also indications that beta-agonists are used in grain fed sheep meat production that could contribute further to variation in eating quality (Animals Feed Act – Act 36 of 1947).

Research problem and objectives:

The first problem that is identified is the lack of consistent quality meat at the point of purchase (retail) or service (restaurants), and the second is the lack of knowledge about the origin of this variation in quality. Thirdly it is also cumbersome that products are presented with certain claims that imply guaranteed consumer satisfaction while such claims often do not materialise. Due to negative consumer response (active or passive, i.e. reduction in consumption) industries over the world often do national surveys to determine the status of product quality (e.g. in terms of appearance and palatability) (Morgan

et al., 1991; George, Tatum, Belk & Smith, 1999; Maher, Mullen, Keane, Buckley, Kerry & Moloney, 2004) to ascertain the factors involved in the inconsistency and then take certain steps to address this variation.

Various methods could be used to measure quality, such as trained or consumer panels or instrumental methods (e.g. Warner Bratzler shear force, L*a*b* instrumental colour measurements, drip loss, water and fat content etc.). Instrumental measurements are often more practical and cost effective and the outcomes are then related to consumer benchmarks for attributes like visual colour, tenderness and juiciness. It is however accepted that preferences for or against certain quality traits, such as flavour cannot be measured accurately with instruments and in the case of lamb where flavour plays such an important part in the acceptability of the product it is recommended that a trained sensory panel is included. When basic quality measurements have been done (e.g. shear force, colour, water content), additional tests could be used to shed light on the reasons for variation in these primary quality measurements such as muscle fiber detachment by video image analysis (to quantify the structural breakdown of the muscle), sarcomere length (muscle fiber shortening), collagen content and solubility (influenced by age), pH (stress status), oxidative stability of fat and protein (will determine shelf life and acceptability), cooking losses etc. Finally chemical analysis and fatty acid composition could present information on basic nutritional content but also verify health claims that are often made in this regard. All of these methods are extensively used in research to get a better understanding of physical and sensory meat quality and to verify which factors in the process of meat production and processing, have an effect on final quality.

The aims of this study:

- To measure the instrumental/physical quality (shear force tenderness, cooking loss, fat and muscle colour, collagen properties, oxidative status (rancidity), sensory qualities and chemical composition of lamb and mutton loin chops (*M. longissimus dorsi*) from various retail outlets (including brand names and generic products).
- To determine the reasons for variation in quality by chemical, histological, physical and biochemical tests.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Criteria used by consumers to assess lamb quality pinpoint a few main properties. These include the visual appearance i.e. the amount and distribution of fat (there is a preference for leaner meat), lean meat colour, fat colour and appearance. The size of the cuts or portions is also a factor in the purchasing decision, but not a 'quality' factor (Linares, Bornez & Vergera, 2007). The second main property is eating quality or palatability; i.e. tenderness, flavour, juiciness (Eagan, Ferguson & Thompson, 2001). Thirdly nutritional value are also part of this criteria more particularly the risk associated with high intake of saturated fatty acids through red meat (Wood, Enser, Fisher, Nute, Sheard, Richardson, Hughes & Whittington, 2008).

Meats' palatability is determined by a combination of tenderness, juiciness and meat flavour (Koochmaraie, Kent, Shackelford, Veiseth & Wheeler, 2002). Of these, tenderness is the most variable characteristic and it is also rated by consumers as the most important sensory attribute. For these two reasons, inconsistency in tenderness is regarded as a major problem facing the red meat industry (Destefanis, Brugiapaglia, Barge, Dal Molin, 2008). In the recently developed grading system used in Australia to categorise various cuts of the beef carcass according to expected eating experience by the consumer, "tenderness" was indicated as the main attribute, closely followed by "overall liking", while flavour and juiciness contributed less to the final score based on extensive testing for best combinations and options (Watson, Polkinghorne & Thompson, 2008a; Watson, Gee, Polkinghorne, Porter, 2008b). However, based very much on the same attributes, Thompson (2005) recorded slightly higher correlation values between "overall liking" and "like flavour" than between "overall liking" and "tenderness" for lamb using a consumer panel. "Juiciness" recorded lower correlation values with "overall liking". In addition, Huffman, Miller, Hoover, Wu, Brittin and Ramsey (1996) reported that most of the variation in overall palatability of steaks consumed at home could be explained by flavour perceptions ($R^2 = 0.67$).

The influence of various factors from the gate to the final cooked product on tenderness and other quality characteristics is the result of combined efforts by all role players in the industry (Ferguson et al., 2001). Included in these factors are genetics, nutrition, growth promotants, pre-harvest stress, harvest technology (electrical stimulation, chilling), post-harvest conditions (duration of shelf life or aging,

packaging, temperature) and cooking (Miller, Huffman, Gilbert, Hammon & Ramsey, 1995; Wheeler, Savell, Cross, Lunt & Smith, 1990; Koohmaraie, 1996; Tornberg, 1996; Veiseth, Shackelford, Wheeler & Koohmaraie, 2001; Thompson, 2002; Maher, Mullen, Keane, Buckley, Kerry & Moloney, 2004; Dunshea, D'Souza, Pethick, Harper & Warner, 2005; Vestergaard, Madsen, Bligaard, Bredahl, Rasmussen & Andersen, 2007; Linares et al., 2007; Lund, Hviid, Claudi-Magnussen & Skibsted, 2008; Wood et al., 2008; Faria, Bressan, Vieira, Vincente-Neto, Ferrao, Rosa, Monteiro, Cardaso & Gama, 2012; Fernandez & Vieira, 2012; Mortimer, Van der Werf, Jacob, Hopkins, Pannierf, Pearce, Gardnerf, Warner, Geesink, Hocking, Edwards, Ponnampalam, Ball, Gilmour & Pethick, 2014).

2.2. Defining meat quality:

Meat quality can be defined on the basis of its functional or conformational qualities, with functional qualities referring to desirable attributes in a product while conformance qualities encompass producing a product that meets consumers' exact specifications (Adzitey, 2011). Traits the consumer perceives as desirable include visual and sensory traits, traits of safety, as well as health and elusive traits such as 'clean' and 'green' (Becker, 2000). Consumer acceptability is largely determined by the perception of quality, which may or may not lead to the success of any food product (Dransfield, 2001). In order for the lamb/mutton industry to survive in a very competitive market, producers and manufacturers have to meet consumer demands and preferences (Hoffman et al., 2003). Thus consumers have an influence on the whole food chain, agriculture and science (Garnier, Klont & Plastow, 2003).

Seeing that agricultural products cannot be produced to specifications, they need to be classified or graded (Webb, 2015). Carcass and meat quality are seldom measured using objective indices related to nutritional, microbiological or physiological characteristics. Since the 1870's it is said that consumer perception is the best measure of quality (Cardello, 1995). Unfortunately red meat suffers the drawback of too much variation in composition, physical attributes and quality. The response from the meat industry was introducing the Red Meat Scheme of 1964 and 1985–1992, which made provision for a meat grading system that gave the Meat Board control, based on the carcass grade and mass (Webb, 2015). South African Karoo lamb brand name are protected by the Merchandise Marks Act (Act 17 of 1941) where the Karoo Development Foundation (KDF), South Africa, ensures that the rules of the Act are followed and complied with (DTI, 2013; Erasmus, Hoffman, Muller & Van der Rijst, 2016). Carcass classification/grading is based on the description of a carcass by means of defined characteristics that are of most importance to the meat industry and consumers (Webb, 2015). This system was replaced with the South African beef and sheep carcass classification system in 1992 because it did not cater for all the sectors in the meat industry. The new system, which is still in use, classifies carcasses based on physical and compositional attributes (Table 2.2.1) (Webb, 2015). The physico-chemical characteristics

of meat determine its quality, as well as its acceptability by consumers (Martínez-Cerezo, Sañudo, Panea, Medel, Delfa, Sierra, Beltrán, Cepero & Olleta, 2005a).

Table 2.2.1: South African Carcass Classification System for cattle and small stock (Webb, 2015).

South African Red Meat Classification System				
Age category	A	AB	B	C
	0 permanent incisors	1 – 2 permanent incisors	3 – 6 permanent incisors	> 6 permanent incisors
Rollermark code	AAA	ABAB	BBB	CCC
Colour of rollermark	Purple	Green	Brown	Red
Carcass fat codes: 0~no fat; 1~very lean; 2~lean; 3~medium; 4~fat; 5~slightly over fat; 6~excessively over fat				
Conformation scores: 1~very flat to 5~very round				

Factors affecting meat quality can be classified into genetic and environmental factors. Species, sex, breed, strain and genotype are genetic factors (Okeudo & Moss, 2005). The effects on meat quality which are not attributable to genetics are defined as the environmental factors. These include on-farm (type and level of feeding, housing), pre-slaughter, and post-slaughter processing factors (chilling rate, ageing period, etc.) (Okeudo & Moss, 2005; Warner et al., 2010). More than in other livestock species, minor deviations in the weights and types of carcasses that have developed can have significant effects on the price of lamb meat (Sañudo, Santolaria, María, Osorio & Sierra, 1996).

Sensory characteristics are described as the most important quality features in meat and meat products (Bukala & Kedzior, 2001). The intramuscular fat (IMF) content positively influences sensory quality traits, for instance taste and flavour. Therefore, visible IMF is also considered as a positive or negative quality criterion (Hocquette et al., 2010). Textural parameters such as the collagen content, sarcomere length and water holding capacity, instrumental texture etc. are appreciated by consumers and also affect meat tenderness (Martínez-Cerezo et al., 2005a).

2.2.1 Muscle structure:

Skeletal muscle has a very complex organization in that it allows force that is originating from myofibrils to be efficiently transmitted to the entire muscle and finally to the limb that is moved (Huff-Lonergan & Lonergan, 2005). Muscles have a relatively constant chemical composition with about 75% of water, 19-25% of proteins and 1-2% of minerals and glycogen. However, the lipid part is highly variable between muscles and cuts, even between individuals in a given species (Geay et al., 2001). Meat from beef and lamb are generally higher in fat than other types of meat (Hocquette et al., 2010).

Water in muscles is found within the myofibrils, between the myofibrils themselves and between the myofibrils and the cell membrane (sarcolemma), between muscle cells and between muscle bundles (Offer & Cousins, 1992). The ability of fresh meat to preserve its moisture is one of the primary quality characteristics of raw meat products (Huff-Loneragan & Lonergan, 2005).

Every muscle is covered by a thin layer of connective tissue that forms the extension of a tendon, called the epimysium (Figure 2.2.1). Muscle fiber bundles make up every muscle and are covered by another thin layer of connective tissue, the perimysium (Figure 2.2.1) (Feiner, 2006). Muscle fibers are defined as plurinucleus cells which contain contractile proteins, enzymes for storage and utilization of energy and proteolytic enzymes involved in protein metabolism and degradation of proteins during meat ageing (Geay et al., 2001). Furthermore, each fiber bundle is made out of individual muscle fibers which are covered by the endomysium (Figure 2.2.1) (Feiner, 2006). It has been shown that the inter-muscle variations in the amount of perimysium are better linked to the variations in meat tenderness (Lepetit, 2007).

Connective tissues are composed of structures of collagen and elastin fibers set in a matrix of proteoglycan (Lepetit, 2007). Collagen is a family of proteins of about 21 isoforms, with types I, III and IV being the most profound in skeletal muscle (McCormick, 1994). These proteins accounts for 2-15% of the dry matter in connective tissue, depending on the muscle (Geay et al., 2001). In perimysium the size of collagen fibrils is about 65-67 nm and in endomysium 47-48 nm (Fang, Nishimura & Takahashi, 1999). No relationship has been found between the diameter of fibrils and meat tenderness (Lepetit, 2007). The perimysium has a much greater amount of collagen (types I and III) than the endomysium (types I, III and IV) (McCormick, 1999). It was suggested that the contribution of collagen to toughness of meat is muscle dependent, seeing that different muscles have different collagen contents (Rhee, Wheeler, Shackelford & Koohmaraie, 2004). Therefore, muscles like *longissimus* which have low collagen content are influenced by factors such as proteolysis (Starkey et al., 2015). In a study on ovine *semimembranosus* they also found that collagen insolubility increases with animal age (Young & Braggins, 1993). Connective tissue is not influenced by treatments such as chilling conditions, electrical stimulation and ageing and thus described as relatively stable post-mortem (Geay et al., 2001).

Underneath the endomysium is the sarcolemma, a net-like structure directly connected to the actin and myosin filaments, which are the major components of every muscle fiber. The intracellular substance in a muscle fiber is called the sarcoplasm (cytoplasm) and consists of about 80% water as well as proteins, enzymes, lipids, carbohydrates, inorganic salts and metabolic byproducts. The protein part of every muscle can be divided into myofibrillar proteins, sarcoplasmic proteins and structural proteins. The main myofibrillar proteins are myosin, actin, tropomyosin, troponin and actinin. Actin and myosin make up about 7-8% of the total muscle weight and are also known as the myofilaments that are responsible for muscle contraction and relaxation. The special arrangement of actin and myosin give muscle fibers a striated appearance. The spatial arrangement of the myofilaments are altered by

pH changes, screening of anions/cations, presence of divalent cations (Mg^{++} , Ca^{++}), denaturing conditions that alter protein conformation and the presence of plasticizing agents such as adenosine triphosphate (ATP) and enzymes (ATPase) (Feiner, 2006).

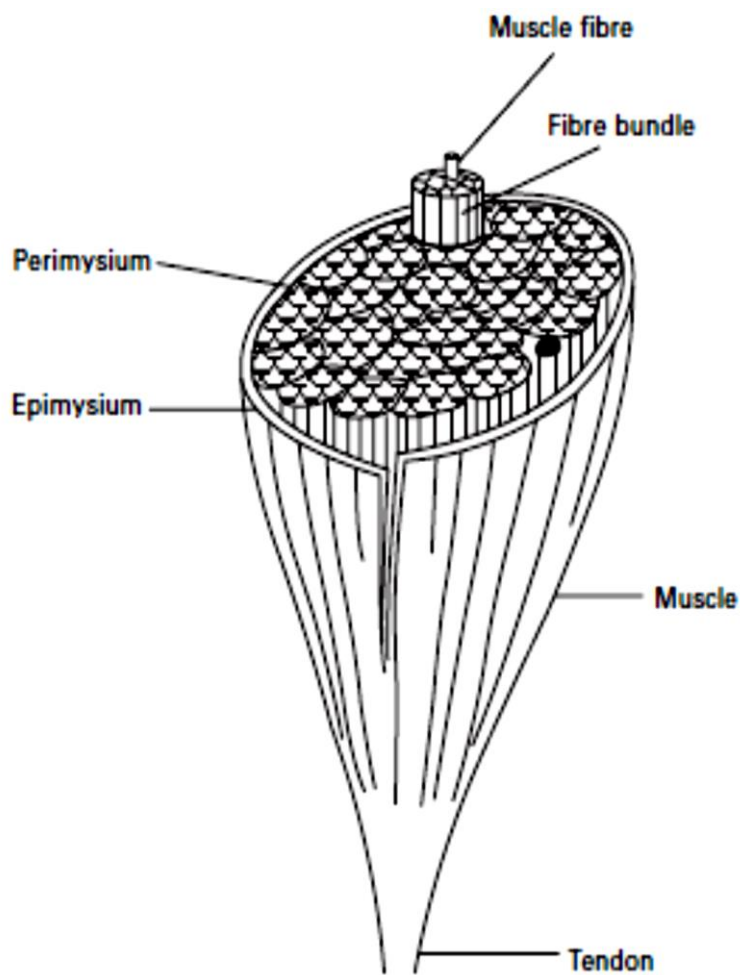


Figure 2.2.1: Structure of a muscle (Feiner, 2006).

It is said that the main determinant of ultimate tenderness is the extent of proteolysis of the key target proteins within muscle fibers (Taylor, Geesink, Thompson, Koohmaraie & Goll, 1995a). According to Koohmaraie and Geesink (2006), the weakening of myofibers is the main event in meat tenderization (Koohmaraie & Geesink, 2006). Numerous studies have shown that specific myofibrillar, myofibril cytoskeleton and costamere proteins are subjected to cleavage (Goll, Thompson, Taylor & Christiansen, 1992; Taylor et al., 1995a; Hopkins & Thompson, 2002; Lametsch, Karlsson, Rosenfeld, Andersen, Roepstoff & Bendixen, 2003; Koohmaraie & Geesink, 2006).

Myofibrils are organelles that produce active force in striated muscles, which contain repeating contractile units called sarcomeres (Figure 2.2.2) (Horowitz, Luo, Zhang & Herrera, 1996). Each

sarcomere is about 2 μm long and lies between two Z-lines (Feiner, 2006). Numerous researchers determined proteolytic systems present in a muscle that participate in postmortem proteolysis and tenderization, which include: the system of calpains, cathepsins and proteasomes (MPC – multicatalytic proteinase complex, called 20S proteasome) (Dransfield, Wakefield & Parkman, 1992a; Dransfield, Etherington & Taylor, 1992b; Koochmaraie, 1996; Koochmaraie & Geesink, 2006; Kemp, Sensky, Bardsley, Buttery & Parr, 2010). Presently, the system of calpains is said to be responsible for postmortem proteolysis of myofibril linkage proteins (e.g., titin and nebulin) (Figure 2.2.2) which lead to the tenderness of meat (Goll, Thompson, Li, Wei & Cong, 2003; Koochmaraie & Geesink, 2006; Neath, Barrio, Lapitan, Herrera, Cruz, Fujihara, Muroya, Chikuni, Hirabayashi & Kanai, 2007). Universally, neutral proteases are activated by calcium (Ca^{2+}) ions (calpains) in animal cells (Xu, Shui, Chen, Shan, Hou & Cheng, 2009).

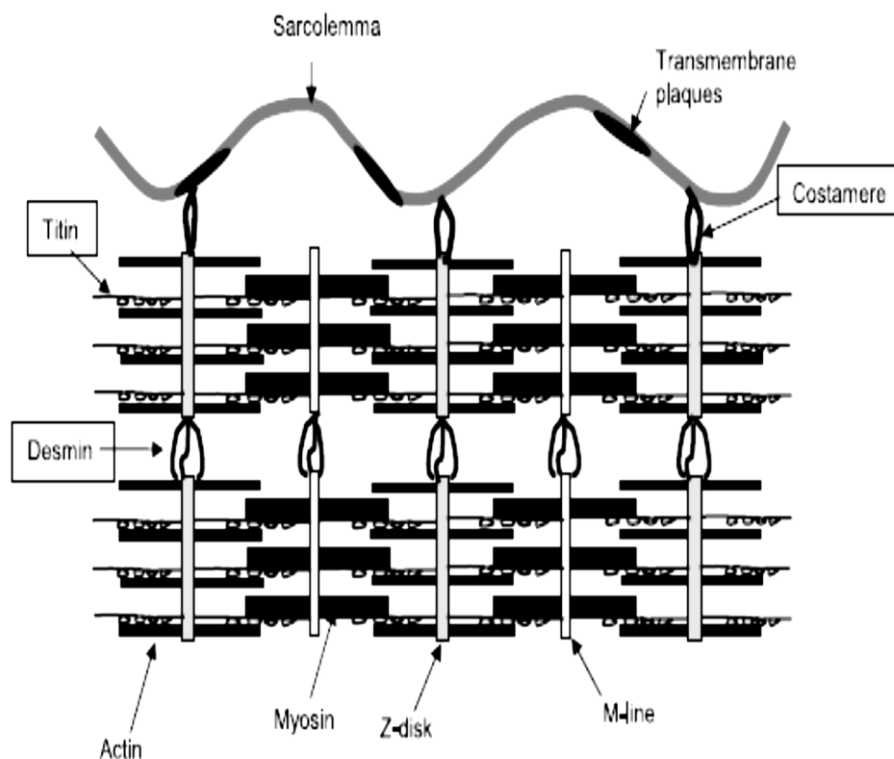


Figure 2.2.2: A sarcomere (Feiner, 2006).

Calpains are super families of 14 non-lysosomal Ca^{2+} -dependent cysteine proteases that cleave proteins in response to Ca^{2+} signals, thus control cellular functions such as cytoskeletal remodeling, cell cycle progression, gene expression and apoptotic cell death (Glading, Lauffenburger & Wells, 2002). In skeletal muscles the calpain system consists of three proteases called calpain I (μ), calpain II (m) and skeletal muscle-specific calpain 3 (p94) as well as calpastatin, a specific endogenous calpain inhibitor (Goll et al., 2003; Hanna, Campbell & Davies, 2008; Moudilou, Mouterfi, Exbrayat & Brun, 2010). In mammals, calpain I and II are ubiquitously expressed (Hanna et al., 2008). The level of calpains and

calpastatin varies significantly between species (beef, pork, mutton), their breed and type of muscle and activity (Northcutt, Pringle, Dickens, Buhr & Young, 1998). In muscles the calpains are located in the cytoplasm and cell membranes, with calpain I being 70% bonded to myofibrils and calpain II in majority located to cytosol (Xu et al., 2009). An earlier investigation by Koohmaraie (1994) showed calpain I is 66% situated on the Z-line and the rest in the I-band (20%) and A-band (14%), whereas calpain II is 52% situated on the Z-line, 27% in the I-band and 21% in the A-band. Calpain 3 is mostly found in a sarcomere near the Z- and M-line (Ilian, Bickerstaffe & Greaser, 2004). Calpain I which is encoded by the CAPN1 gene and calpain II encoded by the CAPN2 gene, are activated *in vitro* by micromolar and millimolar Ca^{2+} concentrations (Goll et al., 2003; Moudilou et al., 2010). These calpains are negatively regulated by the calpastatin, which contains four inhibitory domains that can inhibit calpain activity (Nowak, 2011). Calpain 3 is encoded by the CAPN3 gene (Moudilou et al., 2010). Some researchers stated that calpain 3 plays an important role in meat tenderization, but Geesink, Taylor and Koohmaraie (2005) denied the participation of this calpain in postmortem meat tenderization. Associations proved that different tenderization rates between species (beef<lamb<pork) relate inversely to the ratio of calpastatin:calpain (beef>lamb>pork). They also noted that the calpastatin:calpain ratio increases with ATPase activity and is equal to 1/4, 1/2.5 and 1/1.5 for beef, lamb and pork (Koohmaraie, Whipple, Kretchmar, Crouse & Mersmann, 1991).

Evidence indicated that calpain I plays an important role in the degradation of myofibrillar proteins and tenderization of muscle during refrigerated storage (Geesink et al., 2006). During muscle fiber degradation, myofibrillar and cytoskeletal proteins are degraded which include troponin-I, troponin-T, desmin, vinculin, *meta*-vinculin, dystrophin, nebulin and titin (Koohmaraie & Geesink, 2006). Furthermore, three major cytoskeletal structures are also degraded when meat is tender. That is: Z- to Z-line attachments by intermediate filaments, Z- and M-line attachments to the sarcolemma by costameric proteins and the elastic filament protein titin (Taylor et al., 1995a). Thus the system of calpains contribute to meat tenderization in that they cut along the Z-lines and cut the long fibers into smaller units (Feiner, 2006). These micro and ultrastructure changes are also observed during the ageing process and lead to obtaining the final tenderness of meat (Nowak, 2011). The degree of the cytoskeletal degradation determines the degree of tenderness of meat (Geesink, Taylor, Bekhit & Bickerstaffe, 2001). This correlation is proved by the example of callipyge sheep meat, which is tough and thus characterized by a high level of calpastatin and a slow degradation of proteins (Geesink & Koohmaraie, 1999; Neath et al., 2007).

A troponin complex is made out of troponin I, C and T, which exhibits a high affinity towards Ca^{2+} ions (Feiner, 2006). Troponin-T is a regulatory muscle protein that constitutes the Ca^{2+} -sensitivity switch which regulates the contraction of striated muscle fibers (Geesink & Koohmaraie, 1999). Desmin is an intermediate filament protein which is composed of four subunits. These filaments encircle the Z-disks, forming part of the postmortem degradation that is parallel with tenderization (Geesink & Koohmaraie,

1999). Vinculin links myofibrils bordering the sarcolemma to the costameres which, according to many studies, are degraded during postmortem storage (Koochmaraie, Shackelford, Wheeler, Lonergan & Doumit, 1995; Taylor et al., 1995). Dystrophin is a protein product of the Duchenne muscular dystrophe gene (Hoffman, Brown & Kunkel, 1987). Dystrophin is also part of the costameres which link myofibrils to the sarcolemma and is composed of two subunits (Geesink & Koochmaraie, 1999). As observed in postmortem muscles, the degradation of dystrophin contributes to sarcolemma detachment (Taylor & Koochmaraie, 1998). Nebulin is a high molecular weight protein that is part of the thin filaments and very susceptible to postmortem proteolysis, which means that the degradation of nebulin contributes to postmortem tenderization (Huff-Lonergan, Parrish & Robson, 1995; Koochmaraie et al., 1995; Wang, Knipfer, Huang, Van Heerden, Hsu, Gutierrez, Quian & Stedman, 1996). Titin, also a high molecular weight protein, is half the length of a sarcomere and connects M-lines and Z-lines in the sarcomere of striated muscles (Figure 2.2.2) (Trinick, 1994). Because of the structural role of titin in the myofibril, it may be instrumental for postmortem tenderization (Geesink & Koochmaraie, 1999). Degradation of titin cause the release of alpha(α)-actinin, a cross-linking protein found in both muscle and non-muscle cells (Selliah, Brooks & Roszman, 1996). The α -actinin is slowly released from the Z-disk during postmortem storage, indicating that one or more of the proteins it is attached to, is degraded (Sorimachi, Freiburg, Kolmerer, Ishiura, Labeit, Linke, Suzuki & Labeit, 1997).

When Ca^{2+} binds to calpain, it causes changes in the molecule which enables it to become active and allow calpastatin to interact with the enzyme (Nowak, 2011). Numerous investigations showed that most of the activated calpain is bonded by calpastatin. Respectively, 40 $\mu\text{mol/L}$ and 250-500 $\mu\text{mol/L}$ concentrations of Ca^{2+} ions are required for the inhibition of calpain I and II by calpastatin (Hanna et al., 2008; Moldoveanu, Gehring & Green, 2008). Calpains work effectively at higher pH values such as 6.2-7.0 (Feiner, 2006).

Creatine kinase (CK) is found in the skeletal muscles of animals and is responsible for maintaining homeostasis at the site of high adenosine triphosphate (ATP) (Dieni & Storey, 2009). The rupture of muscles causes the release of CK which is deposited into the blood, thus the presence of CK in the blood plasma indicates muscle damages mostly due to adverse conditions and poor sheep welfare during the pre-slaughter period (Vojtic, 2000; Tadicha, Gallob, Bustamantea, Schwertera, & Van Schaik, 2005; Chulayo & Muchenje, 2013). Recurrent interactions of myosin with actin produce active force that is energized by the hydrolysis of ATP at the nucleotide binding site of myosin (Horowitz et al., 1996).

2.2.2 Determination of meat tenderness:

Unbiased evaluation of meat tenderness can be done by the measurement of shear force and using trained panelists or consumers can be used to evaluate liking of tenderness (Hopkins, Toohey, Warner, Kerr & Van de Ven, 2010). Each method detects subtle differences concluding that differences between genotypes may vary with the method used (Hopkins & Mortimer, 2014). Instrumental meat tenderness is mostly evaluated using the Warner-Bratzler shear force (WBSF) method. According to Perry and others (2001) the Australian market considers lamb meat with a shear force of 40 N to be tender (Perry, Thompson, Hwang, Butchers & Egan, 2001). However, another study concluded that mutton with shear force values less than 49 N can be considered as tender (Hopkins, Hegarty, Walker & Pethick, 2006). These measurements can be variable due to muscle temperature, species, animal management regime, muscle pH and ageing (Schönfeldt & Strydom, 2011). Several authors reported on different strategies to minimize the influence of processing on tenderness which include conditioning (holding at temperatures above chilling for a certain period) after slaughter and ageing (Dransfield, Nute, MacDougall & Rhodes, 1979), ageing for 7 days (Hopkins & Fogarty, 1998) and electrical stimulation and ageing (Hopkins, Walker, Thompson & Pethick, 2005).

The main categories that define tenderness include mechanical (hardness, cohesiveness, elasticity), particulate (grittiness, fibrousness) and chemical characteristics such as juiciness and oiliness (Bourne, 1992). Tenderness of meat depends on the muscle(s) of the animal from which it is derived (Feiner, 2006). The amount and solubility of connective tissue, sarcomere shortening during rigor development and postmortem proteolysis of myofibrillar and myofibrillar-associated proteins, are the main factors which determine meat tenderness and the variation thereof (Koochmaraie & Geesink, 2006). A large study in New Zealand indicated that toughness problems are not limited to beef but also occur in sheep (Bickerstaffe, Bekhit, Robertson, Roberts & Geesink, 2001).

The reasons for the importance of meat tenderness include:

- 1) Consumers consider tenderness to be the individual most important element of meat quality (Miller, 1992);
- 2) Consumers can distinguish between tenderness levels and are willing to pay a first-rate for tender meat (Boleman, Boleman, Miller, Taylor, Cross, Wheeler, Koochmaraie, Shackelford, Miller, West, Johnson & Savell, 1997);
- 3) Sensory tenderness have twice the variation coefficient of juiciness and flavour (Shackelford, Wheeler & Koochmaraie, 1995);
- 4) Meat cuts are priced according to their anticipated tenderness (Savell & Shackelford, 1992).

2.3 Factors affecting lamb and mutton quality:

The quality of lamb and mutton are influenced by the factors essential to the animals from which the meat is derived, such as breed, chronological age, sex and slaughter weight (Hoffman et al., 2003). Post-slaughter transformations which include rigor mortis and ageing also have a crucial influence on meat tenderness (Destefanis et al., 2008). For the production of lamb with maximum consumer appeal, producers and processors need to recognize the importance of the interactions between these factors in the utilization of genetics and nutritional regimes (Hoffman et al., 2003). Aspects of meat quality such as pale, soft, exudative (PSE) and dark, firm, dry (DFD) conditions are determined by the initial glycogen levels, stress activation of phosphorylase, state of calcium-release channels, effectiveness of the stunning operation, postmortem reflex activity of muscles and refrigeration protocols (Swatland, 1995). Sañudo and others (1998b) listed the stages and factors that affect lamb quality in Table 2.3.1, some of which a more detailed discussion follows.

2.3.1 Breed:

The most popular sheep breeds found in South Africa include Merino, Dohne Merino, South African Mutton Merino (SAMM), Dorper, the Black-headed Persian and Dorper (DAFF, 2008). Merino sheep are predominantly used for wool production and originated from Europe (Cloete et al., 2012). Dohne Merino's are used for both wool and meat producing purposes and were developed by the South African Department of Agriculture by breeding Merino ewes with German Mutton Merino sires (Kotzé, 1951). These two Merinos comprise about 55% of the South African sheep population (Cloete et al., 2012). The SAMM is also a multi-purpose breed that was bred from the German Merino, with a high growth rate that produces lambs fit for slaughter with the preferred meat characteristics (Neser, Erasmus & Van Wyk, 2000). The Dorper breed was originally bred on the Elsenburg experimental farm (Western Cape) to provide a terminal sire breed for crossbreeding on Merino ewes (Van der Merwe, 1976). The Black-headed Persian is one of the oldest sheep breeds and was used to breed the Dorper. This breed does well under dry conditions and its fat tail is popular amongst some consumers (DAFF, 2008). The Dorper breed is a very successful South African-bred mutton breed which is specifically developed for the more arid areas as it is a strong and non-selective grazer (DAFF, 2015). This breed was originally selected for areas with a rainfall of less than 250 mm/year, but its excellent adaptability characteristics led to the distribution throughout South Africa and the rest of the world (Brand, 2000). The Dorper breed also has exceptional carcass qualities in terms of conformation and fat distribution (DAFF, 2015). Different breeds differ in carcass morphology related to fat quantity and meat quality (Guerrero et al., 2013). Therefore, different sheep breeds can be divided into groups of early-maturing breeds (Dorper), intermediate-maturing breeds (Merino, Dohne Merino) and late-maturing breeds (SAMM)

Table 2.3.1: Factors that affect lamb quality (Sañudo et al., 1998b).

Stage:	Factors:
Animal related	Species
	Breed
	Individual
	Age or dairy capacity
	Sire size
	Type of birth
	Sex
	Age and slaughter weight
	Specific genes
Joint or muscle	Joint
	Muscle and location within muscle
Animal management	Exercise
	Environmental conditions
	Stressors
Diet	Type and bedding quality
	Lactation type
	Age and weaning
	Ingredients
	Physical characteristics of the ration
	Chemical characteristics of the ration
	Water quality and availability
	Additives
Multi-casual factors	Time of birth
	Flock
	Husbandry system
Pre-slaughter, slaughter and post-slaughter procedures	Stunning method
	Rigor mortis and chilling condition
	Ageing: duration and conditions
	Preservative type: atmosphere, vacuum, freezing
	Technological agents: calcium, zinc, etc.
Marketing and consumption	Joint and preparation
	Packaging and presentation
	Cooking: temperature, length and method
	Consumption: environment, temperature, presentation
	Consumption: custom and fashion

(Cloete et al., 2012). Subsequently it can be expected that Dorper sheep will put on fat at an earlier age than SAMM sheep (Cloete, Hoffman, Cloete & Fourie, 2004a). Breeds with low concentrations of total lipid in muscle, of which phospholipids make out a big part, will have bigger proportions of polyunsaturated fatty acids (PUFA's) in the total lipid fraction (Wood et al., 2008). The effect of breed on meat quality (pH, amount of pigments, physical colour, water-holding capacity, instrumental hardness and sensorial characteristics) does not seem significant, with the most significant differences in WHC, colour and texture, but can be justified by differences in precociousness and degree of muscularity (Dransfield, Nute, Hogg & Walters, 1990; Ellis, Webster, Merrell & Brown, 1997; Rousset-Akrim, Young & Berdagué, 1997; Sañudo et al., 1998b). In general, breed is considered as a factor worth considering in product quality studies and production and marketing systems, but less important than factors such as the feeding system (Notter, Kelly & McClaugherty, 1991; Kabbali, Johnson, Johnson, Goodrich & Allen, 1992b).

2.3.2 Chronological age:

It is sometimes difficult to separate the effects of age on meat quality traits from the effect of factors such as carcass weight and slaughter day (Purchas, 2007). The age of livestock especially influences the tenderness of meat, which is why it forms the basis of the current South African carcass classification system (Table 2.2.1) (Webb, 2015). As the animal gets older the number of cross-links in the triple helices (hydrogen- and covalent bonds) of collagen increases, which makes it more heat stable and therefore does not break down during cooking (Feiner, 2006). Generally animal ageing is associated with tougher meat, caused by the amount and cross-bridges of connective tissue (collagen and elastin) (Dreyer, Naude, Henning & Rossouw, 1977; Lawrie, 1998). Younger livestock has meat with collagen that is more soluble and immature and subsequently more tender (Issanchou, 1996; Boleman et al., 1997).

Tenderness depends primarily on the content and state of connective tissue and myofibrils (Dutson, Hostetler & Carpenter, 1976). The complex interactions between the intrinsic and extrinsic factors require careful management. However, as the age become more similar between animals from different feeding and management systems, the use of age as a categorisation factor will become less important (Webb, 2015). Age is usually analyzed with slaughter weight, as a greater weight implies a higher age, except when feed is manipulated (Guerrero et al., 2013). In a study by Zervas et al. (1999) they found a lower amount of abdominal fat in grazing lambs compared to concentrate fed lambs. This was attributed to the different ages and live weights of the lambs used in the experiments seeing that the development of carcass depots follows this order: mesenteric, intramuscular, omental, pelvic and renal, and subcutaneous.

A clear advantage has been reported in the ability of sucker lambs (4 months old and still on their mothers) to withstand stress and reduce the depletion of glycogen before slaughter, found by pH

measurements of the *semitendinosus* muscle (Hopkins, Stanley, Martin, Toohey & Gilmour, 2007). Given the use of antibodies against myosin heavy chains did not indicate a significant change in the ratio of oxidative to glycolytic fibre types as the animals aged, which supported the theory that there was a reduction in the depletion of glycogen (Greenwood, Harden & Hopkins, 2007). In the same study by Hopkins et al. (2007) they also noted a colour change in the meat of older animals. As established by consumer evaluation, using a threshold value of 34 for lightness (Khlijji, Van de Ven, Lamb, Lanza & Hopkins, 2010), it was found that meat colour become less satisfactory for consumers when sheep reach the age of 12-13 months (Hopkins & Mortimer, 2014). After this age meat is considered too dark and red for acceptance at retail (Hopkins et al., 2007).

2.3.3 Gender:

Different carcass characteristics are produced from lambs of different sexes (De Araújo et al., 2017). The gender (male, female, castrated) of ruminants mainly effects the quantity of fat deposited, deposition site of fat, growth rate and carcass yield (Guerrero et al., 2013). Even though males offer production advantages (leaner carcasses, faster growth), it is not clear what effect does gender have on traits like tenderness, as some studies did not found any difference (Hopkins & Mortimer, 2014). However, different authors did found that meat from males and castrates tend to be tougher than those from females (Johnson, Purchas, McEwan & Blair, 2005; Hopkins et al., 2007). A probable explanation for this effect is that collagen accretion may be stimulated by testosterone (Miller, Judge & Schanbacher, 1990; Beerman, Robinson & Hogue, 1995).

When 20 months old rams were compared with ewes of the same age, a higher pH in the m. *longissimus* were found in the rams (Cloete et al., 2012). It was noted that this can be due to the mixing of the animals prior to slaughter. This could also contribute to higher shear force values (tougher meat) in meat of the rams which was verified by including pH as a covariate. In a study by Hopkins and others (2007) it was reported that meat from wether lambs was lighter in colour than that from the ewe lambs but the difference in colour was unlikely to be detected by consumers. Also, no other studies found such difference. In a 1973 study on relatively young lambs no gender effect was found on the shear force measured in the M. *longissimus* and M. *semimembranosus*. Later on Johnson and others (2005) reported on a study about lambs 8 months and younger, and found that the M. *semimembranosus* of rams had significantly higher shear force values than ewes. It was found that across a number of genotypes, with lambs ranging from 4 to 22 months, wethers produced significantly tougher M. *longissimus* than ewes (Hopkins et al., 2007). Subsequently, Cloete and others (2014) also reported a 9% increase in the M. *longissimus* shear force values of rams compared to ewes. Given the fact that rams produce tougher meat, this often does not reflect in the eating quality that can be detected by panelists (Hopkins & Mortimer, 2014). In a large study that was based on the consumer assessment of M. *longissimus* and M. *semimembranosus* they found that within terminal sired lambs the females had

better sensory scores. These untrained consumers scored assessed the samples for tenderness, overall liking, juiciness, liking of flavor and liking of odour (Pannier, Gardner, Pearce, McDonagh, Ball, Jacob & Pethick, 2014).

Not many studies have been done on the effect of gender on the IMF of lambs. It was found that female animals deposit more fat than males and that castration increases fat deposition (Zervas & Tsiplakou, 2011), and that the fatty acid profile of the females is less favourable according to the consumers' health requirements (De Araújo et al., 2017). In 2014 it was found that ewes had significantly higher IMF levels (0.10%) than rams but was suspected that those results only reflected the large number of lambs sampled, seeing that no such difference have been reported in other studies (Pannier et al., 2014). Then Anderson et al. (2015) also reported that ewes have higher IMF percentage and this was consistent across all the muscle types. The fatty acid profile and concentrations may be of more importance to the consumer with work from Solomon and others (1990) that showed lower levels of PUFA's in the *M. longissimus* of the wethers compared to rams. However, later in another study a gender effect was found on the content of the important n-3 fatty acids, eicosapentaenoic and docosahexanoic acid (EPA and DHA), with female *m. longissimus* having greater levels than that of males (Ponnampalam, Butler, Pearce, Mortimer, Pethick, Ball & Hopkins, 2014). Research proposed the possible explanation that as female lambs approaches their reproductive stage, they synthesize long chain omega-3 fatty acids for the production of series-3 eicosanoids that are associated with ovulation, conception and pregnancy (Mattos, Staples & Thatcher, 2000).

2.3.4 Production system/feeding regime:

The basis of livestock production is a system where cattle and sheep are mostly farm-bred and raised (Webb, 2015). It is a common fact that animals' dietary regimen affects growth rate, carcass weight, dressing percentage, fat : muscle ratio, lipid profile and oxidative stability, sensory characteristics (i.e. flavour, tenderness, aroma), fat thickness, meat and fat colour as well as the fatty acid composition (Zervas & Tsiplakou, 2011). Diet contributes to meat quality directly (compounds from the feed source deposit in the meat) or indirectly (primarily by increasing fatness) (Feiner, 2006). Consumers have a low acceptance towards the unique odour of cooked meat originated from older sheep, therefore it is important to have knowledge of the pre-slaughter nutrition which causes this odour through branched chain fatty acids (BCFA's) (Watkins, Rose, Salvatore, Allen, Tucman, Warner, Dunshea & Pethick, 2010).

Most studies focus on carcass fatness rather than the meat. It has been concluded that dietary energy availability is related to carcass fat, which means diets of higher energy density produce fatter carcasses (Chestnutt, 1994). Iso-energetic diets produce a protein level that is too low to contribute significant modifications to carcass fatness (Jason & Mantecon, 1993). Therefore, more tender meat and less problematic pH levels can be expected of high energy diets due to the higher IMF content

(Devine, Graafhuis, Muir & Chrystall, 1993; Speck, Davidson, Dobbie, Singh & Clarke, 1995). On the contrary, reduced food intake increases the amount and efficiency of lean production (Murphy, Loerch, Mc Clure & Solomon, 1994).

Small ruminants such as sheep are said to be the most efficient transformers of low quality forage into high quality animal products (Lombardi, 2005). The improvement of meat production efficiency and its environmental impact remains the main priority for the feed industries, therefore sensorial and nutritional quality manipulation of meat through nutrition strategies is gaining much importance (Garnier et al., 2003). A major approach to supply sheep meat according to new consumer demands is the development of feeding systems which will improve the meat characteristics of sheep (De Brito et al., 2017). The introduction of new plant varieties and species, some genetically modified for a targeted chemical composition, may alter the composition of the macro-elements within feed (Garnier et al., 2003). In South Africa large regions are classified as desert or semi-desert areas which are mainly suitable for sheep farming (Brand, 2000).

Grass:

Globally, sheep production is dominated by cheap pastoral feeding systems which include a mix of grasses, green legumes and other broadleaf plants (Young, Lane, Priolo & Fraser, 2003). Animals raised on this type of diet produce meat with a characteristic flavour called “pastoral” flavour (Keen & Wilson, 1993). In grazing animals the presence of some volatiles are affected by environmental factors such as season, botanical composition of the pasture, its geographical location and the time spent grazing (Fernández-García, Serrano & Nuñez, 2002). Furthermore, ruminants fed on grass produce meat with a better oxidative stability due to the higher concentration of natural antioxidants found in grass (Gatellier et al., 2004). Grass is rich in antioxidants such as vitamins from groups A, C and E as well as phytochemicals such as carotenoids and flavonoids (Daly, Young, Graafhuis & Moorhead, 1999). Numerous studies have been done on the uniqueness of lamb flavour and in 1991 Ha and Lindsay reported that higher levels of alkylphenols can contribute to a more intense flavour, and also noted that this compound would be more established in pasture-finished ruminants (Ha & Lindsay, 1991).

The importance of on-farm nutrition is emphasized as more evidence proves that muscle glycogen levels increase with the metabolisable energy intake (Pethick, Cummins, Gardner, Jacobs, Knee, McDowell, McIntyre, Tudor, Walker & Warner, 2000). Reserves of the glycogen stores act as a buffer in the pre-slaughter period. Some lambs are raised without any supplements, and when feeding is supplemented, it is only at a maintenance level (Lowe, Peachy & Devine, 2002). In the same study it was indicated that pasture production systems provide adequate glycogen levels and low stress levels and are thus not the limiting factor in producing tender meat (Lowe et al., 2002).

Numerous studies have proven that lambs under a grazing system without any supplementation presented a slightly lower fatness degree due to the low energy intake from pasture (Murphy et al.,

1994; Zervas, Hadjigeorgiou, Zabeli, Koutsotolis & Tsiala, 1999a; Priolo, Micol, Agabriel, Prache & Dransfield, 2002). Lambs reared under grazing conditions have greater muscle/total fat ratio and higher omega-3 PUFA (n-3 PUFA) content. Nonetheless, the saturated fatty acid (SFA) content is also higher leading to a less healthy PUFA/SFA ratio (Joy, Alvarez-Rodriguez, Revilla, Delfa & Ripoll, 2008b). The major fatty acid in grass is alpha-linolenic acid (ALA, C_{18:3}), an unsaturated n-3 FA, therefore the omega-6 : omega-3 (n-6 : n-3) PUFA value is closer to the recommended value than that in pigs (Zervas & Tsiplakou, 2011). Grain fed animals consume and deposit more linoleic acid (LA, C_{18:2}), a saturated n-6 FA, and have lower intensity of meat flavours compared to grass fed animals due to the higher amount of ALA (Wood, Enser, Fisher, Nute, Richardson & Sheard, 1999). Even though n-3-enriched meat has superior health benefits, care should be taken because of their susceptibility to oxidation (Zervas & Tsiplakou, 2011). Meat from ruminants grazing grass is said to have a FA composition that is more beneficial to human health than animals fed concentrate diets (Priolo, Cornu, Prache, Krogmann, Kondjayan, Micol & Berdagué, 2004). Diet affects subcutaneous fat colour, with specific reference to the presence of carotenoids in grass-fed lambs (Prache & Theriez, 1999).

While carcass conformation scores and fatness degrees are generally lower in pasture-fed lambs than in concentrate-fed lambs, consumers favor lambs reared on pasture in terms of overall carcass quality (Joy, Alvarez-Rodriguez, Revilla, Delfa & Ripoll, 2008b). It was also found that meat from pasture-fed lambs have α -tocopherol levels similar to meat from lambs fed concentrate diets with 150 IU supplemental vitamin E/kg (Turner et al., 2002). Priolo and others (2002) noted that lower carcass fatness and conformation scores in grass fed lambs can be linked to their physical activity.

Karoo:

The Karoo covers about 30% of South African land, with meat derived from lambs grazing in this region being called “Karoo lamb” (Bramley, Bienabe & Kirsten, 2009). Karoo lamb is known for its excellent quality and exceptional flavour (Weissnar & Du Rand, 2012). This semi-arid region consist of a low grazing capacity that varies from grassy, dwarf shrublands to dwarf, succulent shrubs (Cloete & Olivier, 2010). The norm of Karoo farm areas is estimated at 35 hectare (ha) per large stock unit (Kirsten, Troskie, Vermeulen, Schönfeldt & Bramley, 2008). It is stated that the fragrant Karoo bossies (indigenous plants) is responsible for the unique flavour of Karoo lamb and mutton (Estler, Milton & Dean, 2006). The unique flavour can also be ascribed to the free-ranging grazing conditions of the animals (Weissnar & Du Rand, 2012). Another contributing factor may also be the unique aromatic oils of the Karoo bossies (Erasmus, Hoffman, Muller & Van der Rijst, 2016).

Karoo bossies can endure heat, wind, cold and hail as opposed to grass that can only be found in the rainy season (Leighton, Schonfeldt, Van Zyl, Van Heerden, Van Niekerk & Morey, 2007). The typical rainfall for this region is about 401-600 mm per annum and even lower (<200 mm or 201-400 mm) in

some parts (Palmer & Ainslie, 2005). These bossies are edible and meet the nutritional needs of the animals throughout the year (Le Roux, Kotze, Nel & Glen, 1994; Erasmus et al., 2016).

Grain-fed/Concentrate:

Meat from animals fed concentrates differ from those raised on grass in terms of subcutaneous fat colour, carcass fatness and meat flavour (Prache & Theriez, 1999). Different studies proved that meat from cattle raised on grass were darker in colour than those raised on concentrates (Dufrasne, Gielen, Limbourg, van Eenaeme & Istasse, 1995; Priolo et al., 2001). Lambs that are fed grain or concentrate diets prior to slaughter contain higher concentrations of BCFA's (Duckett & Kuber, 2001). This is ascribed to the increased propionate formation in the rumen consequential of great soluble carbohydrate content in the diet (Young, Lane, Priolo & Fraser, 2003). Young and Braggins (1999) also noted an increased concentration of BCFA's in animals finished on lucerne compared to those finished on ryegrass. It is said that grain feeding (high-energy) increases carcass weight and intramuscular fat (IMF) content, resulting in red meat with more intense flavour than with low-energy forage and grass diets (Melton, 1990). The phospholipid concentration the muscle lipid fraction increases with the time the animal spent in the feedlot (Feiner, 2006).

2.3.5 Growth promotants:

The livestock industry is continuously looking for alternative methods to encourage rapid growth along with improved carcass characteristics (Beermann, 2009). This way the efficiency and economic return of any livestock industry will be improved (Hope-Jones et al., 2010). Therefore numerous researchers have focused on the effects of compounds that modify the growth rate and carcass quality of livestock (Sillence, 2004). Metabolic modifiers are well-defined as compounds that are either implanted, injected or fed to animals to improve their rate of gain, feed efficiency, increase dressing and carcass yield percentage, extend shelf-life and improve visual meat quality, meat's nutritional profile and meat palatability (Dikeman, 2007). Generally these compounds are described as metabolic modifiers which include anabolic steroids, somatotropin (ST) and β -adrenergic agonists (β -AA) (Bell, Bauman, Beermann & Harrell, 1998). In contrast to ST, the effects of β -AA are more noticeable in ruminants with the positive responses in lean tissue protein accretion largely confined to skeletal muscle (Bell et al., 1998).

Beta-adrenergic agonists or phenethanolamines stimulate the β -receptors (β -1- or β -2-adrenergic receptors) that are present on the surface of nearly every type of mammalian cell (Mersmann, 1998). This group of synthetic phenethanolamine derivatives chemically and pharmacologically resembles the natural catecholamines (Bell et al., 1998). β -adrenergic agonist compounds differ in effectiveness within different types β -AA, dose and length of treatment, species treated and rate of absorption (López-Carlos, Ramírez, Aguilera-Soto, Aréchiga, Méndez-Llorente, Rodríguez & Silva, 2010). Therefore

responses between different species have been inconsistent (Estrada-Angulo, Barreras-Serrano, Contreras, Obregon, Robles-Estrada, Plascencia & Zinn, 2008). These compounds increase muscle mass and reduce adipose tissue deposition in domestic animals such as beef cattle (Allen, Ahola, Chahine, Szasz, Hunt, Schneider, Murdoch & Hill, 2009), pigs (Poletto, Rostagno, Richert & Marchant-Forde, 2009) and poultry (Schiaivone, Tarantola, Perona, Pagliasso, Badino, Odore, Cuniberti & Lussiana, 2004). Several different β -AA have been used in sheep including cimaterol, L644-969, clenbuterol, terbutaline and metaproterenol (Nourozi, Abazari, Raisianzadeh, Mohammadi & ZareShahne, 2008; López-Carlos et al., 2010). Ractopamine hydrochloride (RH) and Zilpaterol hydrochloride (ZH) are registered β -AA in Mexico, United States of America and South Africa. These compounds are approved for use in animal feed because they are rapidly eliminated and safe when used adequately (López-Carlos et al., 2010).

In a study by Kretchmar and others (1990), the shear force indicated that the feeding of β -AAs to lambs may result in a significant reduction in meat tenderness (Kretchmar, Hathaway, Epley & Dayton, 1990; Koohmaraie, Shackelford, Muggli-Cockett & Stone, 1991). Avendaño-Reyes and others (2006) pinpointed that the supplementation of β -AAs improves the *longissimus dorsi* area in steers without substantively compromising quality. It has also been reported that dietary β -AA decreased calpain I activity in lamb *longissimus* muscle by 10%, decreased calpain II activity by 34-42% and increased calpastatin activity by 59-75% (Wang & Beermann, 1988; Koohmaraie & Shackelford, 1991). However, it is a common fact that β -AA supplementation produces tougher meat, especially in ruminants, due to increased calpastatin activity and reduced calpain activity (Wheeler & Koohmaraie, 1997).

Zilpaterol hydrochloride is an orally active type 2 β -AA (Estrada-Angulo et al., 2008). When administered at 60 mg/day during the last 30-42 days of the feeding period, ZH improved average daily gain, gain efficiency, carcass yield grade, hot carcass weight and dressing percentage in feedlot cattle (Plascencia, Torrentera & Zinn, 2008). Similar results have been noted in lambs with an increased average daily gain, gain efficiency and hot carcass weight (Pringle, Calkins, Koohmaraie & Jones, 1993; Nourozi et al., 2008). In a study by López-Carlos et al. (2010) both ZH and RH were administered. Zilpaterol Hydrochloride produced greater positive effects than RH for almost all carcass characteristics without compromising digestibility or serum metabolites.

Estrogenic and androgenic implants have been used in the cattle industry for almost 50 years. These compounds can be used individually or in combination and include steroids such as 17-oestradiol, progesterone and testosterone, along with their synthetic counterparts zeranol, melengestrol acetate and trenbolone acetate (Dunshea et al., 2005). Anabolic agents such as these implants increase the rate of muscle protein synthesis and deposition and/or decrease protein degradation and the amount of fat. Even though implants increase the amount of feed intake by 5-10 %, the amount of energy needed for maintenance is decreased (Dikeman, 2007).

In addition to other supplements for growth promotion, magnesium (Mg) also brings improvements in meat quality and reduced the incidence of PSE pork (D'Souza, Warner, Dunshea & Leury, 1999). Pethick and others (2000) found similar results in lamb meat, although another study also proved the toughening of meat caused by Mg (Apple, Watson, Coffey, Kegley & Rakes, 2000; Pethick et al., 2000).

2.3.6 Calcium chloride and vitamin D infusion:

Calcium chloride:

Several methods of post-mortem quality improvement have been tested which include temperature conditioning, lactic acid injection etc. (Berge, Ertbjerg, Lone, Therry, Xavier & Anders, 2001; Rees, Graham & Taid Robyn, 2002). The use of calcium chloride (CaCl_2) for the reduction in beef and lamb toughness also gained some prominence (Geesink, 1993). Since 1989 researchers have reported acceleration in post-mortem tenderization and increased tenderness in lamb primals that is injected with CaCl_2 after slaughter (Koochmaraie, Crouse & Mersmann, 1989; Boleman et al., 2004). This tenderizing effect is attributed to the Ca^{2+} ion dependent protease involved in ageing and intracellular ionic strength inducing protein solubilization (Takahashi, 1992; Koochmaraie, 1994). Landsdell and others reported that alterations to the colour and flavour are dependent on the CaCl_2 concentration (Landsdell, Miller, Wheeler, Koochmaraie & Ramsey, 1995). In 1998 it was also found that the use of high concentration calcium salts led to products with altered tastes and bitter flavour (Perez, Escalona & Guerro, 1998). Later on in 2001 another study proved that marination with CaCl_2 longer than 24 hours resulted in bitter flavour, an undesirable texture and colour changes (Gonzalez, Valeria, Fernando, Adriana & Jorge, 2001). Muscle contraction is heavily regulated by Ca^{2+} ions that is released from the sarcoplasmic reticulum (SPR) based on a nervous impulse (Feiner, 2006).

As mentioned earlier, the skeletal muscle contains two calcium-dependent neutral proteinases, calpain I and II, a specific inhibitor, calpastatin, and a number of other calpains, including the muscle-specific calpain 3 (Geesink, Kuchay, Chishti & Koochmaraie, 2006). Calpains are Ca^{2+} -activated proteases and have an optimum activity at a neutral pH. Calpain I and II undergo autolysis in the presence of Ca^{2+} , which reduces the Ca^{2+} -requirement for half maximal activity (Koochmaraie & Geesink, 2006). The calpains system activity depends on factors such as pH, temperature and most of all the concentration of Ca^{2+} ions (Goll et al., 2003). In relaxed muscles the Ca^{2+} ions level is very low and the myosin head are inhibited by troponin to bind to the actin (Feiner, 2006).

According to Dransfield and others (1992a) the low Ca^{2+} ion concentration in meat after slaughter causes the inactiveness of calpain I (Dransfield, Wakefield & Parkman, 1992a). Some investigations proved that calpain I needs 3-50 $\mu\text{mol/L}$ calcium ions to be activated and calpain II 0.4-0.8 mmol of calcium to reach half of its maximum activity (Dransfield, 1994a). In a living muscle the Ca^{2+} ion concentration only reaches about 0.2 $\mu\text{mol/L}$, much lower than the required level (Goll et al., 2003).

After slaughter this concentration of free Ca^{2+} increases up to 100 $\mu\text{mol/L}$ (Jeacocke, 1993), and Hopkins and Thompson (2001) proved that the concentration of Ca^{2+} ions in *longissimus lumborum* and *longissimus thoracis* muscles of sheep reaches 110 $\mu\text{mol/L}$ at pH 5.5. According to Karges and others higher blood and muscle Ca^{2+} leads to an increased activation level of the calpain system and thus improved tenderness (Karges, Brooks, Morgan, Gill, Breazile & Owens, 2001; Montgomery, Carr, Kerth, Hilton, Price & Galyean, 2002; Montgomery, King, Gentry, Barham, Barham & Hilton, 2004). Numerous investigators used CaCl_2 injections in pre- and post-rigor meat cuts to activate the intra-cellular calpain I and II (Koochmaraie & Shackelford, 1991; Goll, Thompson, Taylor & Zalewska, 1992). As a result of the increased Ca^{2+} ion concentration in the sarcoplasm, the activation of calpain I occurs at about 6 hours after slaughter at a pH of 6.1-6.3 (Dransfield, 1994b). However, when determined *in vitro* the optimum conditions for calpain activation are a pH of 7.2-7.8 and 25 °C (Kanawa, Ji & Takahashi, 2002). It was also proven that the injection of CaCl_2 can overcome the toughness in meat caused by β -agonists (Koochmaraie & Shackelford, 1991).

During ageing, meat is kept at a much lower temperature than 25 °C and the pH reaches 5.5-5.7 (Nowak, 2011). Calpain II swiftly loses activity during ageing when the carcass is infused with CaCl_2 (Koochmaraie, Crouse & Mersmann, 1989). At 4 °C and pH 5.6 calpain I degrade cleaned myofibrils in the presence of 100 $\mu\text{mol/L}$ CaCl_2 (Huff-Lonergan, Mitsuhashi, Beekman, Parrish, Olson & Robson, 1996).

Vitamin D:

Since the 1970's it has been reported that vitamin D_3 functions as a regulator of Ca^{2+} and is required for Ca^{2+} absorption (DeLuca & Schoes, 1976). When the free Ca^{2+} in a muscle is increased, it is possible for the calpain activity and subsequently proteolysis and tenderness to be accelerated (Wiegand, Parrish, Morrical & Huff-Lonergan, 2001). One way to increase circulating Ca^{2+} in animal muscle is by feeding vitamin D_3 in excess of the nutritional requirements (Jones, Strugnell & Deluca, 1998). The biologically active form of vitamin D liberate Ca^{2+} stores from the skeleton and promote intestinal absorption of dietary Ca^{2+} , thus increasing the serum Ca^{2+} concentrations in the body (Wiegand et al., 2001).

However, in a study by Boleman and others (2004) it was found that feeding high levels of vitamin D_3 to lambs did not improve the tenderness or ageing characteristics. It was indicated that factors such as the hormone calcitonin may be regulating Ca^{2+} concentrations and is limiting its deposits in muscle tissues.

2.3.7 Stress ante-mortem:

The major reasons for sub-optimal glycogen levels at slaughter are the excessive glycogen losses from stress during transport and lairage and a poor on-farm nutritional plane (Lowe et al., 2002).

Handling of the animal immediately before slaughter and the carcass processing conditions that are applied during the first 24 hours after slaughter, are by far the most critical factors in the context of palatability (Ferguson et al., 2001). It is important to understand the biological basis for the variation in meat tenderness. Stress previous to slaughter is of greatest interest among numerous factors due to the influence on product image and the effect on meat quality (Sañudo et al., 1998). Pre-slaughter handling includes all the activities and processes the animals undergo prior to sticking (Adzitey, 2011). Pre-slaughter stress is the result of these activities that include the period of collecting the animals in preparation for loading at the farm, loading and off-loading, transportation of animals to the abattoir and lairage period at the abattoir (Table 2.3.2) (Adzitey, 2011).

Table 2.3.2: Effects of pre-slaughter handling on carcass and meat quality (Adzitey, 2011).

Pre-slaughter handling:	Carcass and meat quality effect:
Increased journey time	Decrease in live weight
Poor handling	3.7% carcass condemnation; 7.8% carcass condemnation in lambs/yearlings
Marketing	0.007% mortality for sheep sold directly from the farm to the abattoir; 0.031% mortality for sheep marketed through auctions prior to the abattoir; bruises
Drivers pushing sheep to move faster	25% bruising
Long journey	15% DFD
Handling during transport	Bruises, broken bones, condemnation and death

This is mainly influenced by the animal's previous experiences and specific features observed at the farm (Muchenje, Dzama, Chimonyo, Strydom & Raats, 2009; Adzitey, 2011). It has a negative impact on the eating quality of meat in terms of pH, colour, thawing loss, cooking loss and Warner-Bratzler shear force (indicating the toughness of the meat) (Chulayo & Muchenje, 2013; Vimiso & Muchenje, 2013). Thus apart from being inhumane, high stress levels leads to poor meat quality (Lawrie & Ledward, 2006).

Stress reactions help the animal to cope with the novelty of the environment by inducing physiological and behavioral changes in the animal (Terlouw, 2005; Gregory, 2010). Some pre-slaughter factors lead to fatigue (Warriss, Brown, Knowles, Edward, Kettlewell & Guise, 1998) and cause animals to release certain enzymes and hormones (creatine kinase, cortisol and catecholamines) into the blood stream (De Haan, Koudijs, Wevers & Wieringa, 1995; Terlouw, 2005; Muchenje et al., 2009). This leads to a series of secondary processes that involve energy metabolism, respiratory function, immune

function and osmotic regulation (Hoffman, Spire, Schwenke & Unruh, 1998; Ali, Al-Qarawi & Mousa, 2006). Fatigue in animals is induced by the repeat short contractions of muscles which causes internal muscle damage. Due to the long soft wool, these internal damages are difficult to identify in sheep (Kannan, Terrill, Kouakou, Gazal, Gelaye, Amoah & Samaké, 2000; Ali et al., 2006). Fatigue also leads to the depletion of glycogen reserves in the muscles which cause an increase in the post-mortem lactic acid production, decreasing the meat pH (pH₂₄) (Terlouw & Rybarczyk, 2008; Veiseth-Kent, Grove, Faergestad & Fjaera, 2010). It is said that sheep are less susceptible to stress than pigs and cattle (Warris, Brown, Bevis, Kestin & Young, 1987). Sheep also have different physiological mechanisms as muscle contraction is not a prerequisite of the DFD process in sheep, opposing to cattle (Apple, Dikeman, Minton, McMurphy, Fedde, Leith & Unruh, 1995).

2.3.8 Slaughter weight:

Different diets have different impacts on carcass weight. Animals raised on concentrate diets have higher daily gains than animals raised on grass. Therefore, if animals are slaughtered at constant age they have different slaughter weights and those slaughtered at constant weight differ in age (Priolo et al., 2002). Reducing the feed level of animals before slaughter has a negative influence on the sensory qualities, in particular tenderness, of meat (Vestergaard, Therkildsen, Henckel, Jensen, Andersen & Sejrsen, 2000). This restriction also causes a decreased amount of white glycolytic fibers and an increased amount of red oxydoglycolytic fibers (Geay et al., 2001). Furthermore, a lower IMF content can also be expected due to a decrease in carcass fatness (Bruce, Ball & Mowat, 1991). Research by Martínez-Cerezo and others (2005b) proved that slaughter weight modifies the intensity of lamb odour in an increasing manner, without having any influence on off-odour intensity. In sheep production each country or region has a predetermined carcass type and weight, according to its own production peculiarities (Sañudo et al., 1998).

It was noted that for satisfactory eating quality, which provides juicier tender meat, a certain threshold level of carcass fat, subcutaneous and/or intramuscular fat should be maintained (Wood, 1990). Therefore, reduced carcass fatness along with increased muscle production may affect meat tenderness (Ponnampalam, Hosking & Egan, 2003), even though IMF give reason to only 3-10% variation in samples (Geay et al., 2001). When carcasses are stored under the same conditions, differences in the carcass fatness could lead to differences in the rate of rigor development, which can influence meat colour, tenderness etc. (Priolo, Micol & Agabriel, 2001). Muscle fibers contract when it is chilled to below 12° C before the inception of rigor mortis and lean carcasses chill more rapidly, therefore lean carcasses yield tougher meat (Food Science Australia, 2004). Between early life and the time of slaughter the fat content of the animal and meat increases, which also means a change in the proportion of fatty acids (FA) (Wood et al., 2008).

2.3.9 Alternative carcass suspension methods:

Numerous studies have noted on the link between improved tenderness and sarcomere length, as well as the influencing intrinsic and extrinsic factors (Jeremiah, Dugan, Aalhus & Gibson, 2003). In general, sheep carcasses are suspended from the Achilles tendon, but an alternative method is pelvic suspension, also known as hip suspension, aitch-bone hanging and Tenderstretch (MLA, 2015). With suspension from the Achilles tendon the hind leg is pulled backward in a position that is unlike the normal muscle configuration of a standing animal. This way less skeletal restraint is put along the vertebral column and on the muscles of the hind limb, subsequently curving the vertebral column (Ahnström, Hunt & Lundström, 2012). Suspension from the Achilles tendon cannot restrict myofibril shortening of the *longissimus dorsi* during rigor, while it can be reduced by the Tenderstretch method (Hou, Liang, Mao, Zhang, Niu, Wang, Liu, Liu & Luo, 2014).

With Tenderstretch carcasses are hung from the *obturator foramen* of the pelvic bone or ligament which increases the tension on the hind limb and loin muscles, meaning that no matter what the conditions at rigor are, the muscle fibers are not able to contract and toughen (Bouton, Fisher, Harris & Baxter, 1973). This method improves the tenderness of the affected muscle by 15 to 40% (Ahnström et al., 2012). Therefore, Tenderstretch is considered with renewed interest by the global meat industry (Ahnström, Enfält, Hansso & Lundström, 2006). Even though this method requires extra labour to re-hang the carcass and vary with gender, age and the area of the carcass, the improvement in tenderness is remarkable (Ahnström et al., 2012).

It has been found that Tenderstretch muscle has a slower ageing rate compared to normally hung (by Achilles tendon) carcasses, after the preliminary post-rigor improvements in eating quality (Cited by Thompson et al., 2005). Thompson et al. (2005) noted on advantages they found in the muscles of Tenderstretch carcasses when compared to carcasses hung from the Achilles tendon. This included a general improvement in sensory scores for *longissimus* and *biceps femoris* muscles and at the extremes of 21° C at pH 6, higher sensory scores for the muscle from tenderstretched carcasses. They also found that the tenderstretched samples had lower shear force values than those that were normally hung, meaning the tenderstretched samples were more tender.

2.3.10 Electrical stimulation:

Electrical stimulation is a standard meat processing technology that is primarily used in the beef and lamb industries (Simmons, Daly, Cummings, Morgan, Johnson & Lombard, 2008). In the meat industry, carcass electrical stimulation (ES) is one of the major interferences adopted for enhancing meat quality traits post-mortem (Adeyemi & Sazili, 2014). Electrical stimulation involves the passing of an electric current through the carcass of freshly slaughtered animals, which causes the muscles to contract and increases the rate of glycolysis resulting in an immediate fall in pH (Hwang, Devine &

Hopkins, 2003). High inconsistency exists in the amperage, impulse frequency, duration, type and position of the electrode, pathways and delay time between slaughtering and stimulation. This variation can be ascribed to the dissimilarities in species, breed, animal age, handling and management (Adeyemi & Sazili, 2014). The need for the enhancement of the sensory properties of meat and to curb the variance in the quality traits, necessitate the application of ES (Adeyemi & Sazili, 2014). Although it is mainly used for the tenderization of meat, it also affects meat colour, colour stability and water binding properties (Simmons et al., 2008).

In the late 1970's ES was originally developed in New Zealand to manage the toughening of lambs that were frozen rapidly after slaughter (Simmons et al., 2008). This accelerates post-mortem glycolysis so that when the muscles enter rigor it is prevented from shortening excessively (Hwang et al., 2003). Rigor mortis is the term used for individual muscle fibers becoming depleted of adenosine triphosphate (ATP) (Devine & Graafhuis, 1995). The beneficial effects of ES can only be accomplished if there is ample muscle glycogen before the animal is exsanguinated (Adeyemi & Sazili, 2014). It has been shown that ES hastens rigor which cause tenderization to start earlier at a higher temperature, thus reducing the ageing time (Dransfield et al., 1992b). Thereby ES also improve meat tenderness by the early activation of the Ca²⁺-dependent proteases of the calpain system (Hwang & Thompson, 2001).

There are three major types of ES which include: extra low-voltage electrical stimulation (ELVES), low-voltage electrical stimulation (LVES) and high-voltage electrical stimulation (HVES). Extra low-voltage electrical stimulation is carried out at <100 volts (V), LVES is carried out at 100-110 V and HVES at >110 V. Low voltage electrical stimulation is mostly applied when there is a 10-20 minute delay between bleeding and stimulation.

2.3.11 Post-mortem muscle biochemistry:

The selection of specific animals for muscling can result in changes leading to less aerobic muscle, less intramuscular fat and therefore sometimes a reduction in tenderness (Pethick et al., 2006). As muscle is transformed to meat, several changes occurs which include:

- (i) gradual depletion of energy;
- (ii) shifting from aerobic to anaerobic metabolism producing lactic acid, resulting in pH declining to 5.4-5.8;
- (iii) increased ionic strength due to inability of ATP-dependant calcium, sodium and potassium pumps to function and
- (iv) increased inability of the cell to maintain reducing conditions (Huff-Lonergan & Lonergan, 2005).

Biochemical changes that occurs postmortem dramatically affect the flavour and tenderness of meat (Figure 2.3.1). These changes that occur in muscle tissue postmortem due to the absence of oxygen (anaerobic), bound actin and myosin in the actomyosin complex (Feiner, 2006).

It is estimated that tenderization will begin as soon as 3 h after slaughter, but is slightly variable among individual carcasses (Veiseth et al., 2001). A study by Koohmaraie and others in 2002 indicated that immediately after slaughter, lamb *longissimus* has intermediate shear force values, toughens during the first 24 hours, and becomes tender during the following postmortem storage at 4 °C. Directly after slaughter inter- (desmin and vinculin) and intra- (titin, nebulin and troponin T) myofibrillar proteins are degraded, followed by ruptures in the sarcomere structure after which tenderization proceeds (Geay et al., 2001).

Once insufficient energy is rebuilt and the present energy in muscle tissue is primarily decomposed as lactic acid, rigor mortis eventually subsides to the relaxation of muscle tissue (Feiner, 2006). Seeing that no oxygen is available for aerobic glycolysis, no acetyl-CoA (a coenzyme in processing citric acid and fatty acids) enters the citric acid cycle to reduce NADH and FADH₂, also no oxidative phosphorylation takes place (Figure 2.3.1). Pyruvate is reduced to lactic acid and catalyzed by lactate dehydrogenase. Prior to slaughter muscle tissue consist of 7-11 g/kg glycogen (Feiner, 2006). As cell metabolism continues under anaerobic conditions, the cell consumes the creatine phosphate and glycogen stores to maintain homeostasis (Geay et al., 2001). The sarcoplasmic reticulum pump cytosolic calcium until energy and ATP levels becomes too low, followed by the formation of a non-reversible actomyosin complex that induce meat toughening which is called “rigor mortis” (Lawrie, 1992). As the pH decreases osmotic pressure increases until the onset of rigor mortis is completed (Ouali, 1990). Increased osmotic pressure adds to the modification of myofibrillar integrity and dissociation of contractile proteins and differs according to muscles (Geay et al., 2001).

The factors that determine meat tenderness during post-mortem storage are background toughness, the toughening phase and the tenderization phase (Koohmaraie & Geesink, 2006). Meat processors are responsible for the latter two phases and can be managed with different ageing periods, electrical stimulation, temperature control methods and tender-stretching (Hwang, Devine & Hopkins, 2003; Martínez-Cerezo et al., 2005). All of these methods are aimed at the prevention of myofibril shortening by the acceleration of ATP depletion via glycolysis, which is linked to increased carcass pH (Ferguson & Gerrard, 2014). While the toughening and tenderization phases take place, background toughness exists at the same time of slaughter and does not change during the storage period (Koohmaraie & Geesink, 2006). According to Marsh & Leet (1966), background toughness of meat is defined as “the resistance to shearing of the unshortened muscle”, of which variation is due to the connective tissue component of muscle (Koohmaraie & Geesink, 2006). During rigor development this toughening phase is caused by sarcomere shortening (Wheeler & Koohmaraie, 1994; Koohmaraie, Doumit & Wheeler, 1996). Although the toughening phase is similar in all carcasses under similar processing conditions, the tenderization phase varies in both the rate and extent (Koohmaraie & Geesink, 2006). Thus is the assumption correct that postrigor *longissimus* is tough and that differences in the degree of postmortem tenderization are the cause of variation in tenderness of *longissimus* after

storage (Koochmaraie et al., 1996). However, the toughening phase can be lessened by controlling the extent of muscle shortening during rigor development (Hopkins & Thompson, 2001).

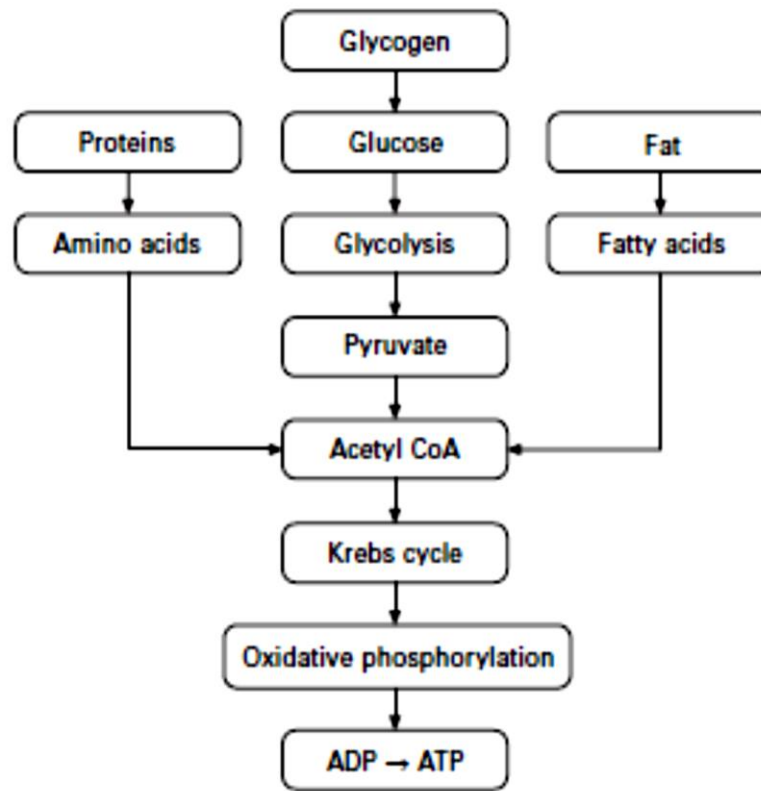


Figure 2.3.1: The process of obtaining ATP under aerobic conditions (Feiner, 2006).

Myoglobin is the heme protein that is responsible for meat colour, thus meat discolouration is a result of the conversion of oxymyoglobin (OxyMb, red) to metmyoglobin (MetMb, brown) (Faustman et al., 2010). This protein is situated in a bond to the external membrane of the mitochondria and sarcoplasmic reticulum (Geay et al., 2001). Consumers will reject meat with 40% metmyoglobin on its surface (Kim, Frandsen & Rosenvold, 2011). Alpha(α)-tocopherol slows the conversion of oxymyoglobin to metmyoglobin by this means slowing the development of an unattractive brown colour in displayed meat products (Lanari, Cassens, Schaefer & Scheller, 1994). This change in colour is due to the oxidation of the central iron atom within the heme group, changing to its ferric form and replace oxygen with a water molecule (Faustman et al., 2010). Fresh forage leads to high α -tocopherol concentrations in the muscle of lambs resulting in improved colour and oxidative stability without any supplementation (Turner, McClure, Weiss, Borton & Foster, 2002). Furthermore, muscle structure influence meat colour in terms of the amount of light absorbed or reflected and oxygen that is penetrated (Geay et al., 2001).

2.3.12 Postmortem ageing:

In suspended carcasses muscles will shorten and toughen during rigor but can be reduced with ageing under refrigerated storage (Starkey, Geesink, Oddy & Hopkins, 2015). Ageing of meat is described as a post-mortem process where a set of complex reactions tenderize meat through the actions of endogenous proteinases (Geesink et al., 2001; Hopkins & Thompson, 2001). Later in another study by Geesink and others (2006) it was noted that calpain I (μ) in particular is responsible for myofibrillar protein degradation during ageing (Geesink, Kuchay, Chishti & Koochmaraie, 2006). Subsequently myofibrils are weakened and therefore tenderization follows (Starkey et al., 2015). Degradation of desmin, a substrate of the calpains, determines the extent of proteolysis during ageing (Huff-Lonergan & Lonergan, 2005).

Ageing does not change the visual appearance of meat, as the breakdown of muscle fibers takes place on a microscopic level (Feiner, 2006). It is stated as a general rule that an increased ageing time, increases tenderness (Devine & Graafhuis, 1995). As proven by many studies, of all the post-slaughter factors, ageing time is the main factor associated with textural parameters (Bouton & Harris, 1972; Shorthose, Powell & Harris, 1986; Wheeler & Koochmaraie, 1994; Hopkins & Thompson, 2001). Lamb meat ages quickly but it can vary in terms of breed and slaughter weight in this species (Ilian, El-Din Bekhit & Bickerstaffe, 2004). As the amount of some flavour compounds change, consumer perceptions can be more positive (more tender meat) or negative (off-flavours) when longer ageing times are applied (Wasserman, 1979; Martínez-Cerezo et al., 2005b). Research by Jaime and others in 1992 proved that an increased ageing time from 1 to 7 days made lamb meat more tender (Jaime, Beltrán, Ceña, López-Lorenzo & Roncalés, 1992). In a study by Martínez-Cerezo et al. (2005a) they found that in lambs with a slaughter weight of 10-12, 20-22 and 30-32 kg, 4 days of ageing were enough to obtain high quality meat. They also noted that 16 days of ageing does not decrease meat quality in the light (20-22 kg) and early fattening (30-32 kg) lambs.

The improvement in meat tenderness with ageing varies between animals within a breed and the muscles within the animal (Wicklund, Homco-Ryan, Ryan, McKeith, McFarlane & Brewer, 2005). In a study by Boleman et al. (2004) it was proven that the aging time required to maximize tenderness is muscle dependent. They found that the Warner Bratzler shear values for *M. longissimus lumborum* and *M. semimembranosus* declined with increased aging time whereas the *M. semitendinosus* showed no decrease in Warner Bratzler shear values before 10 days of aging. Wheeler and Koochmaraie (1999) also noted that Warner Bratzler shear values for lamb *longissimus* muscle declined quicker during ageing than they did for *M. psoas major*. In Starkey et al. (2015) found higher shear force values on day 1 (21.7-57.6) than on day 14 (16.3-37.4) ($p < 0.001$) of ageing.

Ageing also tends to cause a change in the colour of meat as it become lighter (Warner, Ferguson, Cottrell & Knee, 2007). Shorter myofibrillar fragment lengths are usually an indication of degradation

that occurs with the ageing of meat (Polak, Gašperlin & Žlender, 2007). In a study by Kolczak et al. (2003) degradation was found in both the M- and Z-lines after only 6 days of storage, while Hughes et al. (2014) later noted cracks in the I-band after 12 days of storage. Evidence of changes in actin and/or cytoskeletal proteins is provided by an increase in the sarcomere length which is connected to the enlargement of the I-band. This collection of changes can lead to more light scattering and reflection (Hughes et al., 2014).

The tenderness of meat is caused by a combination of breakdown within muscle fibers due to enzymatic activity and loosening of connective tissue such as collagen (Feiner, 2006). Tender meat is obtained through a certain temperature-pH relationship that should be adhered to post-slaughter (Feiner, 2006). A more detailed discussion follows in the next section.

2.3.13 Temperature and pH:

During the process of converting muscle to meat, lactic acid is building up in the muscle leading to a decline of pH of the meat (Huff-Lonergan & Lonergan, 2005). High carcass temperatures and low pH can lead to meat tenderness through enhancing the release of cathepsins by the disruption of the lysosomal membranes, allowing access to myofibril proteins (Hopkins & Taylor, 2002). It is often interpreted that high pH meat comes from highly stressed animals but is mostly inferred from post-slaughter ultimate pH (pH_u) values (Lowe et al., 2002). A high mean pH affect flavour and aroma leading to reduced quality (Young, Reid & Scales, 1993). When meat pH levels exceed 5.8, shelf life is reduced and lead to darker meat as pH increases, which affect purchase decisions (Fogarty, Hopkins & Van de Ven, 2000). As the concentration of lactic acid within the muscle tissue of meat increases, the pH declines to approximately 5.4 which is also known as the isoelectric point (pI) (Huff-Lonergan & Lonergan, 2005; Feiner, 2006). The pH_u value depends on the glycogen content (available energy stores) which in turn depends on the nutritional status of the animal (Geay et al., 2001).

Carcass fatness play a role in the cooling process as the fat protects the muscle from rapid refrigeration (Jones & Tatum, 1994). The tenderness of meat improves during cooler storage due to the post-mortem proteolysis of myofibrillar and myofibrillar-associated proteins (Koochmaraie & Geesink, 2006). The problem with meat tenderness concern the time spent in cooling conditions until final tenderness are obtained. With beef the required time is at least 14 days, for pork 5-7 days and lamb 7-10 days (Nowak, 2011). Once the meat reaches a temperature of 7 °C, the pH value must be kept at 5.7 or lower. At the temperatures below 7 °C the risk of cold shortening are presented when the pH reaches 5.7 (Feiner, 2006). This leads to tougher meat, particularly the loin muscle (Muela et al., 2010). In domestic meat producing animals, muscle glycogen levels must be sufficient at slaughter for muscle pH to decrease to 5.5, which leads to optimal tenderization during ageing (Warriss, 1990; Purchas & Yan, 1997). A decreased pH level is in favour of the storage of meat as it slackens the development of microflora (Geay et al., 2001).

2.3.14 Lipid fraction:

The lipid fraction of meat serves as a solvent for volatile compounds that develop during production, handling and thermal processing (Calkins & Hodgen, 2007). A main determining factor of the eating quality of red meat is the percentage IMF because of its positive influence on flavour, juiciness and therefore tenderness (Pannier, Pethick, Boyce, Ball, Jacob & Gardner, 2014b). Hopkins and others (2006) concluded that in lamb 4-5% IMF is required for consumer satisfaction. Muscles that are composed of more oxidative fibres naturally contain more triglycerides and thus more IMF (Hocquette et al., 2010). Furthermore muscles that are responsible for upkeep of posture are especially more oxidative with a higher IMF percentage (Anderson et al., 2015). It has been shown that variations in IMF percentage are related to species, breed type, gender and muscle fibre type (Pannier, Pethick, Geesink & Ball, 2014a). Minimum requirements for extractable fat that will lead to consumer satisfaction when grilling red meat cuts is 3-4% for beef, 5% for sheep and 2-2.5% for pork (McPhee, Hopkins & Pethick, 2008). In more recent work by Pannier et al. (2014) it was suggested that 3.9% extractable fat for sheep is sufficient for a good mouthfeel. Moreover, fat levels above 7.3% result in a negative effect on the flavour and overall acceptability of meat (Calkins & Hodgen, 2007). Fat colour is influenced by the diet and globally yellow fat is not appreciated by consumers (Priolo et al., 2002).

2.3.14.1 Marbling:

Marbling is defined as the last adipose tissue to be deposited in finishing animals, although adipose tissue starts to accumulate in the early weaning periods (Hauser, Mourot, De Clercq, Genart & Remacle, 1997; Harper & Pethick, 2004). Lipids in ruminants are mainly accumulated as triacylglycerols in adipocytes which is located in subcutaneous, inter- and intramuscular adipose tissue, and abdominal, i.e. perirenal and omental fat depots (Carrasco, Ripoll, Sanz, Alvarez-Rodriguez, Panea, Revilla & Joy, 2009). As the animal's live weight increases, the quantity of subcutaneous fat increases more than that of intramuscular tissue (Diaz, Valesco, Cañeque, Lauzurica, De Huidobro, Perez, Gonzalez & Manzanares, 2002). The marbling score (intramuscular fat) are used in many carcass grading systems as a factor in forecasting eating quality (McPhee et al., 2008). Earlier it was concluded that only 10-15% of the variance in palatability can be ascribed to marbling (Dikeman, 1987), but later Thompson (2004) concluded that due to the contribution of marbling to juiciness and flavour it will become a more important determinant of palatability.

Intramuscular fat can be increased with a high plane of nutrition during the finishing phase, depending on species, breed, animal age etc. This can be used as an indicator of meat quality, as highly marbled meat has traditionally been seen as ideal because of the effects of fat on flavour and tenderness (Feiner, 2006). Aging leads to increased internal fat depots, mainly through an increased cell size, and

a decreased water content and connective tissue in the tissue as well as a colour change from grey to white (Zervas & Tsiplakou, 2011).

In a study by Angold et al. (2008) they concluded that juiciness is the most affected by higher levels of marbling fat and is also associated with more water retention in meat during cooking. Marbling fat allow muscle to be more easily broken down in the mouth, through its location in the perimysial connective tissue between muscle fiber bundles, by “opening” the structure of the muscles (Wood et al., 2008).

2.3.14.2 Fatty acid composition:

Fatty acids influence the quality of meat in terms of tissue firmness (hardness), shelf life (lipid and pigment oxidation) and flavour (Wood, Richardson, Nute, Fisher, Campo, Kasapidou, Sheard & Enser, 2003). Phospholipids found in muscle membranes contain about 45-55% PUFA (Geay et al., 2001). Due to the health risks involved such as cancers and coronary heart disease it is recommended that the ratio of PUFA to saturated fatty acids (SFA) (P:S) of meat should be above 0.4 (Wood et al., 2003). A balance between omega-3 polyunsaturated fatty acids (n-3 PUFA) formed from alpha(α)-linolenic acid (C18:3) and omega-6 polyunsaturated fatty acids (n-6 PUFA) formed from linoleic acid (C18:2) is also important seeing that the ratio of n-6:n-3 PUFA is a risk factor in the formation of blood clots that leads to a heart attack (Williams, 2000). The recommendation for this ratio is less than 4 (Wood et al., 2003). For human consumption the recommended value is 2, with ruminant meat (beef and lamb) being superior over pork in this regard with n-6:n-3 PUFA values of 1-2 versus 7 for pork (Wood et al., 1999). This is ascribed to the fact that linolenic acid, ample in fresh forages, is stored in great volumes in ruminant tissues (Enser, Scollan, Choi, Kurt, Hallett & Wood, 1999).

Edible fats of ruminants does not seem healthy due to the high SFA content, n-6 PUFA content and variable trans fatty acid (*trans*-FA) content (Bessa, Alves & Santos-Silva, 2015). Fatty acids are degraded to monounsaturated fatty acids (MUFA's) and SFA's by microbial bio-hydrogenation in the rumen, of which only about 10% of linoleic acid is incorporated into tissue lipids (Wood, Enser, Fisher, Nute, Sheard, Richardson, Hughes & Whittington, 2008). Lamb and goat fat contains much higher concentrations of branched-chain fatty acids (BCFA's) than other ruminants (Duckett & Kuber, 2001). Lipid metabolism in rumens includes the presence of saturated fatty acids which lead to the negative health image regarding ruminant products (Zervas & Tsiplakou, 2011). Fatty acid compositions in the muscles and adipose tissue of ruminants are less influenced by nutritional factors than those of monogastric animals, due to the low lipid content of their diet and hydrogenation of dietary lipids in the rumen (Nürnberg, 1998). In sheep some FA levels are higher in muscle than in adipose tissue (Wood et al., 2008).

Different FA's have different melting points which means that the fatty acids composition affects the firmness of adipose tissue (Wood et al., 2008). Lamb subcutaneous fat has great amounts (4%) of

methyl BCFA's of medium to long length, particularly 4-methyloctanoic acids (MOA's) and 4-methylnonanoic acids (MNA's) (Young, Berdagué, Viallon, Rousset-Akrim & Theriez, 1997). It is also said that these two BCFA's are responsible for the unique odour associated with cooked meat of older sheep (Young et al., 2003). As the animal grow older the concentration of these compounds in sheep fat increases (Young, Lane, Podmore, Fraser, Agnew, Cummings & Cox, 2006). Sheep that have consumed high grain diets, especially rams, contain high levels of FA's such as propionic acid and therefore have soft oily fat (Enser & Wood, 1993). These authors also found that the melting points vary within the year, with the lowest being in Summer and Spring. The presence of a rumen in cattle and sheep ensure that the manipulation of their fatty acid compositions is more difficult through diet (Wood et al., 2003).

2.3.14.3 Oxidative stability:

Oxidative damage can be caused at storage all the way through to the cooking process (Papas, 1999). This is one of the principal mechanisms behind non-microbial degradation of meat (Guyon, et al., 2016). A variety of factors influence the degree of oxidative damage which include temperature, exposure to light and air, amount and saturation of the lipid component, presence of oxidation promoting metals etc. (Halliwell, Murcia, Chirico & Aruoma, 1995). All meat and fish products are prone to oxidation, with poultry meat to be considered more prone to the development of oxidative rancidity than red meat (Kemin, 2009). Oxidation in meat leads to the loss of quality in terms of texture, colour, flavour and nutritional value (Gatellier, Mercier & Renerre, 2004).

Lipid oxidation:

Different muscles contain different amounts and proportions of storage lipids (triacylglycerols) and structural lipids (phospholipids) (Guyon, Meynier & de Lamballerie, 2016). The big emphasis on lean meat has resulted in the production of carcasses with fat codes ranging between 2 and 3. Carcass fat enhances the juiciness and certain flavour components in meat, thus contribute to the organoleptic properties (Webb & O'Neill, 2008).

The oxidative stability of meat depends greatly on the balance between anti- and pro-oxidative substances which include the composition and concentrations of PUFA's, cholesterol, proteins and pigments in the duration of storage (Zervas & Tsiplakou, 2011). Lipids which contain PUFA's are especially prone to be attacked by free radicals (Gatellier et al., 2004). During fat oxidation polyunsaturated fatty acids are degraded to volatile short-chain oxidation products, which then lead to an off-flavour and off-odour formation, and also may affect the ability of membranes to hold water (Kemin, 2009). Oxidation products include aromatic compounds such as aldehydes which react readily with proteins, leading to modifications of their organoleptic and nutritional properties (Guyon et al., 2016).

Oxidation of unsaturated fatty acids (UFA's) in meat are decreased by endogenous protective systems such as small peptides (glutathione), carnosine and anserine or proteins that are also implicated (Gatellier et al., 2004). Superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) are the principal mechanisms that is protecting cells from oxidative damage in vivo (Halliwell, 1997). In a study by Pradhan and others (2000) it was reported that catalase contribute to the antioxidative process during storage and distribution of raw meats (Pradhan, Rhee and Hernandez, 2000). Alpha(α)-tocopherol, a lipid-soluble antioxidant, also delay lipid oxidation in meat from various livestock species (Faustman, Sun, Mancini & Suman, 2010).

Protein oxidation:

Muscle contains high amounts of proteins which play an important role in the quality of meat in terms of the sensory, physic-chemical and nutritional properties (Falowo, Fayemi & Muchenje, 2014). Protein oxidation takes place through a chain reaction of free radicals like in the event of lipid oxidation (Lund, Heinonen, Baron & Estevez, 2011). This is described as the covalent modification of a reaction of radicals (ROS) induced by proteins or by a reaction with secondary by-products of oxidative stress (Shacter, 2000).

Oxidation of proteins also causes deterioration of colour and texture as well as the loss of nutrients such as essential amino acids and decrease protein digestibility (Guyon, 2016). The positions of amino acids on the chain determine their extent of exposure to oxidation. Furthermore, cysteine, tyrosine, tryptophan, phenylalanine, proline, histidine, methionine, lysine and arginine are easily converted to carbonyl derivatives because they are more susceptible to reactive oxygen species (Fu, Liu, Zhang, Li, Wang & Zhou, 2015).

2.3.15 Impact of freezing and thawing:

In the case of lamb, a seasonal product due to species physical restrictions, freezing plays a main role especially when prices increase due to lower availability (Muela, Sañudo, Campo, Medel & Beltrán, 2010). This is in contradiction to the fact that consumers will not buy or consume thawed meat in restaurants, indicating the lack of knowledge about the freezing process in general by consumers (Damen & Steenbekkers, 2007, Bueno, Resconi, Campo, Cacho, Ferreira & Escudero, 2013). Shelf-life of meat has always been a major concern seeing that meat is very perishable (Muela et al., 2010). Freezing is the most efficient long-term preservation practice due to the inhibition of microbial proliferation (Fernandes et al., 2013). These same authors noted that most producers established a shelf life of 12 months for frozen lamb cuts. Frozen storage of meat allows the supply of meat to be spread out all over the world (Leygonie, Britz & Hoffman, 2012), leading to more stabilized markets with greater flexibility (Muela et al., 2010). Moreover, consumers are also willing to pay a premium for prolonged freshness (Bailey, Jayas, Holley, Jeremiah & Gill, 1997).

During frozen storage meat has lower water retention capacity due to the denaturation of proteins (Fernandes et al., 2013). Denaturation of proteins under freezing conditions occurs as water crystallizes and stops hydrating proteins, favouring intermolecular interactions and subsequently the loss of the solubility of meat proteins (Pereda, Rodríguez, Álvarez, Sanz, Minguillón, Perales & Cortecero, 2005). Increased storage temperatures from -1.5° C to -1° C and from -1.5° C to 2° C, lead to reduced storage life of 10% and 50% (Muela et al., 2010). However, during storage frozen-thawed meat demonstrates a slower rate of oxygenation and more rapid discolouration than fresh meat cuts (Ben Abdallah, Marchello & Ahmad, 1999).

In a more recent (2016) lamb quality study in Spain the leg chops were evaluated after 1, 9 and 15 months in frozen storage followed by modified atmosphere packaging once thawed. They found that neither trained nor untrained sensory panels could distinguish between frozen and thawed meats. Although the visual appearance was affected by frozen storage, thawed meats still showed acceptable colour after 2-3 days of display (Muela et al., 2016).

2.3.16 Modern retail packaging:

The retail acceptability of pre-packaged fresh lamb depends greatly on its colour and colour stability. Modified atmosphere packaging (MAP) are packaging with an oxygen concentration of greater than 50% inside the packaging (Channon, Baud & Walker, 2005). The gas mixtures used in MAP usually consists of 70-80% CO₂ and 20-30% O₂ (Gill, 1996). The high level of CO₂ helps to maintain the attractive red colour through the oxymyoglobin bloom.

The surface of vacuum-packed lamb comprises of a mixture of adipose and muscle tissue which can lead to bacteria and spoilage due to a high pH (Kiermeier, Tamplin, May, Holds, Williams & Dann, 2013). The growth of aerobic spoilage bacteria is prevented through the depletion of the oxygen present in the atmosphere of the meat and release of CO₂, as a result of the ongoing respiration of the meat tissue (Reis, Reis, Mills, Ross & Brightwell, 2016).

Unlike fruits and vegetables, meat is a perishable product with a short shelf-life that needs to be presented with a 'use by' date. Products like meat, that are likely to support the growth of pathogenic and/or spoilage bacteria, must be kept at cold temperatures. It is of great importance that this cold chain should not be interrupted (EFSA, 2016). Even though the various packaging solutions such as MAP and vacuum-packaging can help to extend the shelf-life of meat, inadequate storage, distribution and retail temperatures will lead to a great reduction (Kiermeier et al., 2013). Modern technology allows us to chill meat directly after slaughter to reach an internal temperature of 3 °C to < 7 °C within 16-24 hours in small carcasses (lamb) and within less than 48 hours in large carcasses (beef, pork) (Nychas, Skandamis, Tassou & Koutsoumanis, 2008).

2.4 What the consumer wants in terms of lamb and mutton quality:

Meat is said to be a nutritionally significant element of the human diet (Keskin, Kor & Karaca, 2012). Excessive consumption of meat, high cooking temperatures, processing and salt addition led to a negative perception amongst consumers towards health properties of meat, resulting in decreased consumption (Young, Therkildsen, Ekstrand, Che, Larsen, Oksbjerg & Stagsted, 2013). Also the association of fat with high cholesterol levels and a higher risk for heart disease, cause for the ever changing market demand for leaner meat (Van der Westhuizen et al., 2010). This quest for healthy food also leads to the desire for better nutritional value and sensory properties of meat (De Brito et al., 2017). Consumer preferences have beneficial influences on the improvement of meat quality (Keskin et al., 2012). Manufacturing industries have responded to comparable customer expectations by adopting strict quality-control and management programmes, which is based on extensive analysis measurements being performed on both starting materials and finished products (Swatland, 1995).

Factors such as family income, prices, culture and traditions, individual preferences and beliefs, as well as geography, social status and the environment and economy interact to determine dietary consumption (Grunert, 2006). Consumer preferences vary among the different regions in a country with local products being well known and liked (Muela, Monge, Sañudo, Campo & Beltrán, 2016). Therefore, origin of lamb can also be described a main factor influencing consumer decisions (Bernués, Ripoll & Panea, 2012). This factor is ascribed to 'typicality' and indicates quality and/or guarantee for consumers (Rubino et al., 1999). A study by Bernabéu and Tendero (2005) showed that price is the secondary element at the point of purchase, while other factors such as the image, food safety and final quality of the product have a greater influence on consumer choices. Even though consumer studies are difficult, the preferences of consumers are unquestionably one of the main factors that determine their purchase tendencies (Martínez-Cerezo et al., 2005b). Consumer expectations and perceptions can be a very useful tool to improve meat quality, as future lifestyles, predicted ageing profiles and the demand for convenience promote more innovative thinking (Issanchou, 1996; Troy & Kerry, 2010).

2.4.1 Point of sale:

At the point of sale consumers is mostly driven by what they see in the supermarket or butchery. Most important visual traits include colour and texture (tenderness and juiciness) of the meat, fat colour, the amount and distribution of fat and the absence of excess water (Glitsch, 2000). The colour of meat is reckoned as a measure of freshness and quality at the point of sale (Khliji, Van de Ven, Lamb, Lanza & Hopkins, 2010). Hood and Mead (1993) indicated that an attractive bright red colour is associated with a long shelf-life and good eating quality. However, in reality the colour of fresh meat is not well correlated with the eating quality. Discolouration of meat limits its shelf life after preparation for retail, leading to a big economic concern for the meat industry (Jeyamkondan, Jayas & Holley, 2000). In

addition to the bright red colour, a good WHC is also important at this point, seeing that increased drip loss in the packaging is unattractive to consumers, more susceptible to bacterial growth and overall leads to deterioration of eating quality (Lagerstedt, Ahnström & Lundström, 2011). Furthermore, factors such as convenience determine the way meat products are cooked and consumed. In some countries lamb is perceived as a product that requires long and elaborate cooking preparations, in which case convenience will influence especially young people who do not consist of proper cooking skills (Muela et al., 2016).

2.4.2 Point of consumption:

For consumers, eating satisfaction is the result of the interaction between juiciness, flavor and tenderness (Koochmaraie et al., 2002). Acceptance of meat by consumers is mainly determined by the texture which may vary according to the changes in the structure of myofibrillar proteins (Wood et al., 1999). Meat tenderness has been identified as the primary determinant of eating quality at supermarkets, which also determine if consumers will buy the product repeatedly (Destefanis et al., 2008). Only 0.1% of unhappy customers will complain about poor quality retail meat (Bickerstaffe et al., 2001). Good eating quality can only be guaranteed if the factors that mostly affect tenderness are controlled along the production chain (Thompson, 2002). Customers want meat to be lean (with all the flavour and tenderness of fat meat), consistent (reliably tender) and competitively priced (Swatland, 1995). According to Huffman et al. (1996), consumers can distinguish between tough and tender meat. Thus, are they willing to pay a premium for guaranteed tender meat (Boleman et al., 1997; Lusk, Fox, Schroeder, Mintert & Koochmaraie, 2001; Shackelford, Wheeler, Meade, Reagan, Byrnes & Koochmaraie, 2001).

In domestic and international markets consumers have an increasing desire for low fat, muscled lamb retail cuts that present value for money and a healthy meal (Pethick, Banks, Hales & Ross, 2006). Even though health characteristics remain important (Pannier et al., 2014b), eating quality has also shown much importance among consumers (Harper & Pethick, 2004). The satisfaction derived from the consumption of a food product is seen as the ultimate consumer satisfaction (Jeremiah, Tong & Gibson, 1998). Once the meat is cooked, consumer satisfaction is mainly determined by the tenderness as well as the flavour/odour and juiciness (Glitsch, 2000).

While tenderness is regarded as the most important factor that determines consumer acceptability in species such as beef, flavour is most important for lamb, followed by tenderness (Boleman et al., 1997; Martínez-Cerezo et al., 2005b). The flavour related to a specific species is associated with species-dependent adipose tissues (Guerrero et al., 2013). Cooked meat, especially lamb, is defined by its aroma (Matsuishi, Igeta, Takeda & Okitani, 2004). Thompson and others (2005) concluded that tenderness is well correlated with the overall liking of cooked lamb meat. In Australia, consumers of lamb place the highest weighting on flavour/odour, followed by tenderness and then juiciness (Pethick,

Pleasants, Gee, Hopkins & Ross, 2006). Consistent tender lamb which meets consumer demands will result in repeat purchase of the product (Grunert, 2005).

Over the time period of 2012-2017 the sheep meat consumption in South Africa reached a peak in 2014/15 with a total of 193 000 tons. Since then it has been declining over the following two years with a total of 186 000 tons in 2016/17 (Figure 2.4.1). In the same year (2016/17) the pork consumption was 255 000 tons while the beef and poultry consumption was 1 032 000 and 2 167 000 tons. The average price paid to farmers/feedlots per kg for sheep, beef, pork and poultry varies as follows: R61.67; R40.15; R24.99 and R24.33. It is clear that sheep meat is the most expensive with almost three times the price as per kg of poultry (DAFF, 2018). We can assume that due to economic reasons more consumers would start purchasing cheaper meat products. Therefore, it is important that consumers who still prefer the more expensive products like lamb or mutton get good value for their money, especially in terms of tenderness.

2.5 Other international lamb and mutton quality audits

All around the world inconsistencies in the quality of retail meat have been recognized. Therefore meat industries have designed quality assurance programs, like the 5-star quality grading system in Australia and the 'blue print' and 'label rouge' guidelines in the UK and France (Bickerstaffe et al., 2001). In New Zealand, two programs were initiated to improve the quality of domestic meat during 1997. The first program was an ongoing survey by a major supermarket group of the tenderness of retail beef, lamb and pork. The second was to set standards for animal welfare, meat eating quality, microbiological quality and storage life. For the product to qualify for the 'Beef and Lamb Quality Mark', it must be derived from animals of New Zealand origin, be within certain age categories and not treated with growth promotants.

In Australia a few studies were conducted by the lamb industry to understand consumers' attitudes towards lamb eating quality. Research initiated by consumer taste panels indicated that tenderness is the main factor that consumers use to determine lamb quality. This accounted for 85% of the variation after visual characteristics was excluded (SMART, 1994). Yann et al. (1994) found that 75% of consumers would buy more lamb if they had access to consistently tender and tasty meat. According to Food Standards Australia and New Zealand (2004) meat needs to have a fat content of less than 3% to be claimed as 'low fat', but the choice of less fat will have a negative influence on the juiciness, flavour and cooking properties of lamb.

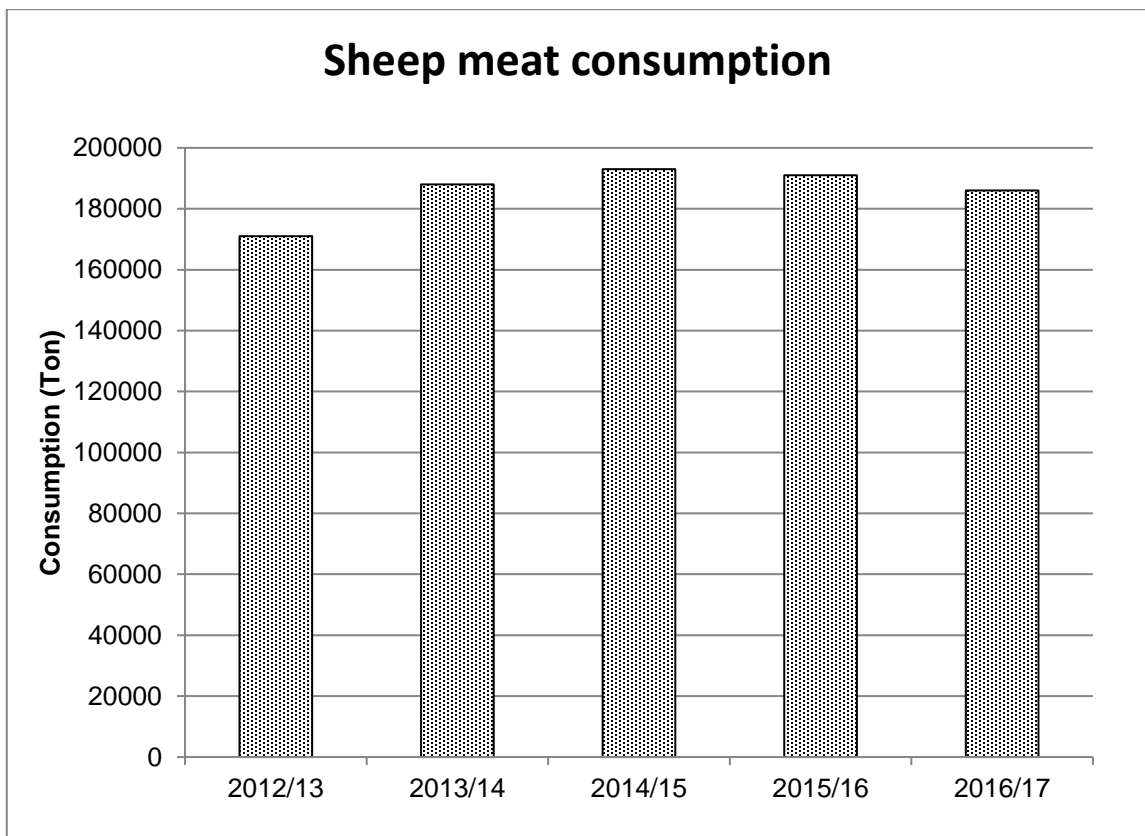


Figure 2.4.1: Total sheep meat consumption in South Africa from 2012 to 2017 (DAFF, 2018).

2.6 Conclusions

It is clear from literature that both genetic and environmental factors do have an influence on the quality of meat, especially tenderness. Therefore, it is important to know what these factors are as well as how they affect the quality of meat and meat products. Seeing that family income and prices also play a significant role in the purchasing decision of consumers, it is important that consistent good quality lamb/mutton must be produced to ensure consumers get what they pay for. As technology improves and newer lamb production methods are discovered, it is however important that regular lamb/mutton quality audits needs to be done. It is also necessary that selected muscles or cuts are identified for the production of products from meat from older animals.

Using the limitations set by these influencing factors as guidelines, proper improvements can be made to the red meat industry of South Africa. This should also be reflected in the food standards that are set for South Africa. From this literature review in can be deduced that many lamb quality audits has been done internationally but not in South Africa. There is clearly a gap in the knowledge of quality and especially consistency of quality with regards to South African lamb meat. It was therefore time to conduct such a quality audit.

CHAPTER 3

MATERIALS AND METHODS

3.1 Sourcing of samples

The purpose of this survey was to evaluate lamb and mutton quality at retail level. The survey was limited to the loin chop (loin: *M longissimus dorsi*), since this cut responds the most to various intrinsic and environmental effects and consists largely of a single muscle. Seventeen prominent butcheries/retailers with different suppliers have been identified and were combined with 2 outlets each of the 4 major retail food chains. Furthermore, persons in charge of meat distribution of the 4 large retail outlets was contacted to verify the sourcing of meat and to select the most relevant outlets. In the further survey and final choice of outlets the following factors were considered and described (Table 3.1.1):

- size and type of retail outlet – butchery, food market, size;
- variety of products (restricted to loin chops) in terms of branding, generic etc;
- information on packaging/processing dates to verify aging days;
- origin of meat so that some sort of traceability could be used to assist in explaining variation recorded in quality.

Over a period of three months samples was purchased from each outlet (to accommodate variation over time). The final number of samples depended on how many different samples were selected per store, but at least one generic and where available one or more branded samples were selected during each round. A branded sample is identified as one where a specific claim in terms of diet/production system and/or aging period or similar claim is included and no unverified brand names will be considered. Sufficient sample ($\pm 1000-1200$ g) was purchased to perform various tests as described further on in this section. A total of 14 visits to each store were done representing the same number of repetitions per outlet, which statistically is the minimum to determine a reliable mean value and allow for possible variation for each quality characteristics. This was true for all the products except for two, namely Kings Meat Deli Karoo and Pick'nPay Free Range. These products were not always available due to the national drought which occurred during this trial. Six repetitions were however obtained which we felt was adequate for statistical analyses under these conditions. More than one product was purchased from some butcheries/retailers where more than one product was available and certain butcheries/retailers also sold Karoo or Free Range lamb. A total of 23 products were purchased from the 17 butcheries/retailers with six of these products being either Karoo or Free Range. Different

packaging methods were also included namely Polyvinyl Chloride (PVC) overwrap, Modified Atmospheric Packaging (MAP), fresh cut samples and samples on display with no packaging (Table 3.1.1).

The fact that meat in general is distributed all over the country from various production and processing plants, and considering that much of those operations are in Gauteng and distributed to Pretoria outlets, this study is limited to proper sampling and testing within the Pretoria metropolis. All the participating outlets of this study receive meat from different operations, thereby assuring a reliable sample of the industry.

Table 3.1.1: List of products from the different production systems (Karoo / Grain-fed / Grass-fed).

Retailer/Butcher name	Retailer/Butcher code	Type*	Packaging	Replications
Checkers	R1	Grain-fed	PVC-OW	1 – 14
Makro	R2	Grain-fed	PVC-OW	1 – 14
Makro Karoo	R2K	Karoo	PVC-OW	1 – 14
PicknPay Free Range	R3FR	Grass-fed	PVC-OW	1 – 6
PicknPay MAP packaging	R3MAP	Grain-fed	MAP	1 – 14
PicknPay PVC overwrap	R3OW	Grain-fed	PVC-OW	1 – 14
Superspar	R4	Grain-fed	PVC-OW	1 – 14
Woolworths	R5	Grass-fed	PVC-OW	1 – 14
Woolworths Free Range	R5FR	Grass-fed	MAP	1 – 14
Boma	B1	Grain-fed	PVC-OW	1 – 14
Boma Karoo	B1K	Karoo	PVC-OW	7 – 12
Food Lovers Market	B2	Grain-fed	PVC-OW	1 – 14
Groenkloof East	B3	Grain-fed	PVC-OW	1 – 14
Hokaai	B4	Grain-fed	PVC-OW	1 – 14
Jandres Neat Meats	B5	Grain-fed	Fresh Cut	1 – 14
Kings	B6	Grain-fed	PVC-OW	1 – 14
Kings Karoo	B6K	Karoo	PVC-OW	1 – 14
Maders	B7	Grain-fed	On Display	1 – 14
Meat World	B8	Grain-fed	PVC-OW	1 – 14
UitKyk	B9	Grain-fed	Fresh Cut	1 – 14
Waverly Slaghuis	B10	Grain-fed	Fresh Cut	1 – 14
Weirde Slaghuis	B11	Grain-fed	PVC-OW	1 – 14
Toits	B12	Grain-fed	PVC-OW	1 – 14

* Assumed that lamb not marked as Karoo or Free-Range is from grain-fed origin. However, there were no label claims to confirm this. (B: butchery; R: retailer; K: karoo; FR: free-range).

3.2 Quality measurements

For all the tests conducted in this study, the cold chain was maintained from sampling to freezing or testing. All the tests were conducted on frozen samples that have been thawed overnight in a cold room at 4 °C, excluding colour analysis.

3.2.1 Fat : meat ratio:

Two loin chops from each sample were dissected into fat and meat and bone. The ratios were calculated by the weight of the individual tissues as a percentage of the weight of the whole chop. The meat part was subdivided for the measurement of chemical composition and collagen properties.

3.2.2 Meat colour:

One chop of each sample was used to determine instrumental colour of the fresh meat the day after purchasing with a Minolta colour meter (Konica Minolta 600d spectrophotometer with Spectramagic NXPro Software). Recordings were done on 6 chops to get an accurate colour measurement and an average value was calculated from the 6 measurements. Colour measurements followed the CIE colour convention (CIE L*a*b*; Commission Internationale de l'Eclairage, 1978), where the three fundamental outputs are L*, a* and b*. L* represent lightness on a scale of 0 (all light absorbed) to 100 (all light reflected); a* represent red to green which spans from +60 (red) to -60 (green) and b* represent yellow to blue spanning from +60 (yellow) to -60 (blue). The hue angle is defined as $\tan^{-1}(b/a)$ and describes the fundamental colour of a substance and chroma (saturation index), defined as the square root of $a^{*2}+b^{*2}$, describes the vividness (MacDougall, 1977). Values for chroma (S) higher than 20 relate to the bright red colour of bloomed meat and S=18, S=14 and S<12, as dull, distinctly brown and brown to gray-greenish brown. Reflectance was measured from 400 to 740 nm in increments of 10 nm. The myoglobin fractions MetMb, deoxymyoglobin (DeOxyMb) and oxymyoglobin (OxyMb) were calculated according to Krzywicki (1982) using the reflex attenuation ($\log 1/R$) at the isobestic points 572, 525 and 473 nm (calculated by linear interpolation), and at 730 nm. The instrument was calibrated beforehand against a white tile (C: $Y=94.4\pm 0.05$, $x=0.313\pm 0.07$, $y=0.320\pm 0.07$). Mean values were used for statistical analysis.

3.2.3 Cooking loss:

Chops were oven-grilled at 200 °C to an internal temperature of 70 °C (AMSA, 1995). For cooking losses: total cooking loss, drip loss, evaporation loss and thawing loss were all calculated from weights collected at the time of cooking. Cooking losses were determined by calculating the differences between the raw and cooked weight of the sample.

3.2.4 Proximate analysis:

Proximate analysis of protein, moisture and ash was measured according to the methods described by the Association of Official Analytical Chemists (1990). The fat determined the amount of marbling, while protein was used in collagen measurements.

3.2.5 Collagen:

Collagen content and solubility were determined according to the method described by Bergman and Loxley (1963), Hill (1966) and Weber (1973).

3.2.6 Intramuscular Fat Content and Fatty Acid Analysis:

Lipids for fatty acid analysis were extracted from each muscle sample (± 5.0 g) quantitatively according to the Folch method (Folch, Lees & Sloane-Stanley, 1957), using chloroform and methanol in a 2:1 ratio. A 0.001% concentration of butylated hydroxytoluene (BHT) was added as an antioxidant. After drying the extracts under vacuum in a rotary evaporator, it was further dried overnight in a vacuum oven at 50 °C with a moisture absorbent, phosphorus pentoxide. The total intramuscular fat content (IMF) was weighed and determined as % intramuscular fat (w/w) per 100 g tissue. The fat free dry matter (FFDM) content was determined by weighing the residue on the Whatmann no. 1 filter paper which was used for the Folch extraction, after drying in the vacuum oven. The FFDM was expressed as % FFDM (w/w) per 100 g tissue, by determining the difference in weight. The moisture content of each sample was determined by 100% - % lipid - % FFDM and expressed as % moisture (w/w) per 100 g tissue. Extracted lipids were stored in glass polytopes under a blanket of nitrogen (N₂) and frozen at -20 °C awaiting fatty acid analysis.

Lipid samples of ± 15 mg were converted to fatty acid methyl esters (FAME) with base-catalysed transesterification (Park, Albright, Cai & Pariza, 2001). The methyl esters were quantified using a Varian 430 flame ionization GC, with a fused silica capillary column (Chrompack, CPSIL 88, 100 m length, 0.25 mm ID, 0.2 μ m film thicknesses). Analysis was performed using an initial iso-thermic period of 40 °C for 2 min. Subsequently the temperature was increased at a rate of 4 °C/minute to 230 °C, followed by an iso-thermic period of 230 °C for 10 minutes. Fatty acid methyl esters in 1 μ l *n*-hexane were injected into the column using a Varian CP-8400 Autosampler. Both the injection port and detector were maintained at 250 °C. Hydrogen functioned as the carrier gas and N₂ was employed as the makeup gas. Data was recorded in the form of chromatograms by Galaxy Chromatography Data System Software. Identification of each sample's FAME was made by comparing the relative retention times of FAME peaks with those of standards obtained from Supelco (Supelco 37 Component FAME Mix 47885-U, Sigma-Aldrich Aston Manor, Pretoria, South Africa). Conjugated linoleic acid standards were obtained from Matreya Inc. (Pleasant Gap, United States), which included: *cis*-9, *trans*-11 and *trans*-10, *cis*-12 C18:2 isomers.

Fatty acid data was used to calculate the following ratios of fatty acids: total MUFA; total SFA; total PUFA; total UFA; MUFA/SFA; PUFA/SFA; desaturase index (C18:1c9/C18:0) and the omega-6 to omega-3 (n-6)/(n-3) fatty acids ratios. Atherogenicity index (AI) was calculated as: $AI = (C12:0 + 4 \times C14:0 + C16:0)/(MUFA + PUFA)$ (Chilliard, Ferlay, Rouel & Lambere, 2003). Desaturase index (DI) was calculated as: $DI = (C18:1/C18:0)$.

3.2.7 Warner Bratzler shear force:

Seven loin chops per retail outlet were vacuum packed and frozen at -20 °C from the day after purchase until analyses could be completed. Loin chops were thawed at 2 ± 1 °C for 24 h and cooked using an oven-broiling method (Mielé, model H217, Mielé and Cie, Gütersloh, Germany) with direct radiant heat (American Meat Science Association, AMSA, 2016). The steaks were broiled at 200 °C (pre-set) to 70 °C internal temperature and cooled down to 18° C. Six round cores (12.7 mm diameter) were removed from each steak parallel to the muscle fibres (American Meat Science Association, AMSA, 2016). Each core was sheared once through the center, perpendicular to the fibre direction, by a Warner Bratzler shear device with 1 kN loadcell mounted on an Universal Instron apparatus (Model 4301, Instron Ltd, Buckinghamshire, England; cross head speed = 200 mm/min) and the mean value of the 6 recordings used as a shear value (kg).

3.2.8 Myofibrillar Fragment Lengths (MFL's):

A 3 g frozen muscle sample per chop was placed in a 50 ml Bühler glass containing 30 ml myofibrillar fragment length (MFL) extraction buffer [0.02 M Potassium phosphate buffer containing 100 mM KCl, 1 mM MgCl₂, 1 mM EDTA and 1 mM NaN₃ (pH 7.0) (4 °C)]. After a 60 second thawing period samples were homogenised in a Bühler HO 4/A homogeniser for 30 s, at 2000 rpm (blade turned in order to fragment myofibrils rather than to cut them). Centrifugation followed at 3000 rpm, 4 °C for 15 min. The pellet (after discarding the supernatant) was suspended in 4 °C 30 ml MFL extraction buffer and once more centrifuged at 3000 rpm, 4 °C for 15 minutes. The pellet (after discarding the rest of the supernatant) was suspended in another 4 °C 10 ml MFL extraction buffer. This suspension was filtered under vacuum through a 1000 µm polyethylene strainer, with an additional 4 °C 5 ml MFL extraction buffer to facilitate the passing of myofibrils through the strainer. Another filtering process followed, where the filtrate was filtered through a 250 µm polyethylene strainer. This filtrate was used to measure the MFL with the VIA. Myofibrils were extracted (Culler, Parrish, Smith & Cross, 1978) with some modifications suggested by Heinze & Brueggermann (1994). The extracted myofibril fragments were examined with an Olympus BX40 system microscope at a 400x magnification. AnalySIS Life Science software package were used to measure 100 myofibril fragments of each sample.

3.2.9 Thiobarbituric acid reactive substances (TBARS):

Lipid oxidation was evaluated using 2-Thiobarbituric acid (TBA; 4,6-dihydroxy-2-mercaptopyrimidin) according to the method described by Raharjo, Sofos and Schmidt (1992). A brief description follows. A meat sample of 5.0 g was homogenized in 20 ml 5.0 % (w/v) aqueous solution of trichloroacetic acid (TCA) for 1 min using an Ultra Turrax homogenizer (T25, IKA Labortechnik, Janke & Kunkel, Staufen, Germany). The meat slurry was centrifuged at 9000 rpm for 5 min followed by a filtering process through Whatman 1 filter papers into 25 ml volumetric flasks. The filtrate was made up to 25 ml by adding TCA, from which 4.0 ml was mixed with 4.0 ml 40 mM TBA and incubated at 94 °C in a waterbath for 15 min. The absorbance of the red pigment formed was scanned with a spectrophotometer at 532 nm (DU 7500 Beckman, Beckman Instruments Incorporated, Fullerton, California, USA). Results are expressed as 2-thiobarbituric acid reactive substances (TBARS) in mg malondialdehyde per kg measured using a standard curve prepared from 1,1,3,3-tetraethoxypropane (malonaldehyde). In the final calculations a 78% recovery value of malondialdehyde-TBA complex was used. Mean values of two independent determinations was used for statistical analysis.

3.2.10 Sensory analysis:

Loin chops were cooked according to an oven broiling method (Mielé, Model H217, Mielé and Cie, Gütersloh, Germany) with direct radiant heat (American Meat Science Association, AMSA, 2016). The steaks were broiled at 200 °C (pre-set) to 70 °C internal temperature. Ten cubed samples (10 mm × 10 mm × 10 mm) were cut from the middle of the muscle half and immediately wrapped individually in pre-coded (with 3-digit random numbers) aluminium foil squares (9 cm × 9 cm). The samples were served warm (\pm 40 °C) on pre-warmed plates to a sensory panel within 20 minutes from the time the cut was removed from the oven. An external experienced trained sensory panel consisting of ten members (10 ladies, mainly housewives because they were available in the morning to taste) was used. During the four-day training sessions (two hours per day, 8 hours in total), panelists received representative samples of each of the different treatments of the loin one at a time. They were trained in order to increase their sensitivity and ability to discriminate between specific samples and the sensory attributes of each sample. A clear definition of each attribute was developed, based on ASTM to describe the specific product attribute (lexicon) to be evaluated. The panelists had to score the first bite, overall tenderness, residual as well as the following attributes, also described in Table 3.2.1: typical lamb flavour, sweet (caramelized browned fat flavour tones), metallic/liver/bloody, barnyard (musty, earthy, farmyard, wet animal), sour (lactic acid as experienced in extendedly aged products) and Karoo “bossie” (grassy or shrubby flavour tones). An 8 score was used for high intensities and 1 for extreme blandness or the absence of an attribute.

3.3 Statistical Analysis

The results presented in tables are mean values \pm standard deviations. The bars in graphs represent average values whereas error bars represent standard deviations. One-way Analysis of Variance (ANOVA) was performed to determine significant differences between the 23 products as well as between the three production systems (Karoo, Grain-fed and Grass-fed) (NCSS 11 Statistical Software, 2016). The Tukey-Kramer multiple comparison test ($\alpha = 0.05$) was carried out to identify significant differences between the treatment means (NCSS 11 Statistical Software, 2016). Pearson's correlation analysis was performed to determine the relationship between different quality characteristics (NCSS 11 Statistical Software, 2016). Fatty acid and sensory data were visualized in a 2-dimensional space by principle component analysis (PCA) (XLSTAT, 2018). Discriminant analyses (DA) was used to identify the drivers (variables) that played the most important role in distinguishing the difference between the 23 products (306 samples in total) (XLSTAT, 2018).

Table 3.2.1: Sensory analysis attributes and descriptions.

Attribute	Description
Aroma	Typical Lamb Sour (Lactic acid) Sweet (Caramelized) Metallic/tin/bloody/liver Barnyard (Farm yard/Wet animal) Shrub/Grassy (Karoo Bossie)
Impression of juiciness	The impression of juiciness that you form as you start chewing
Overall tenderness	Chew sample with a light chewing action
Flavour	Combination of taste while chewing and swallowing: Typical Lamb Sour (Lactic acid) Sweet (Caramlized) Metalic/tin/bloody/liver Barnyard (Farm yard/Wet animal) Shrub/Grassy (Karoo Bossie)
Mouthfeel	Mouthfeel

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Background to survey

While most new meat research projects are designed to address quality challenges, very little is known about the quality of red meat offered to the consumer at retail level. The purpose of this survey was to evaluate lamb and mutton quality at retail level. The survey was limited to the loin chop (loin: *M longissimus dorsi*), since this cut responds the most to various intrinsic and environmental effects and consists largely of a single muscle. It is also noted that the loin musculature is the most valuable cut in the carcass (Pannier, Pethick, Geesink & Ball, 2014a). The attributes of lamb/mutton quality identified comprise the properties found at retail, consumption and the general consideration of products.

The properties found at retail include the visual appearance (amount and distribution of fat), lean meat colour and fat colour and appearance (Linares et al., 2007). The most important properties at consumption are tenderness, juiciness and flavour, with tenderness being the most variable quality factor (Eagan et al., 2001). In general with nutritional value, consumers are most concerned with the risks involved with a high intake of saturated fatty acids (Wood et al., 2008). Tenderness of meat is determined by the solubility and amount of connective tissue, postmortem sarcomere shortening, ageing, cooking processes and especially myofibrillar breakdown by proteolytic enzymes (Koochmaraie & Geesink, 2006). Therefore, in this study physical, histological and biochemical measurements were performed in an attempt to measure and explain any variation in consumer related properties.

The following keys were used to identify the origin of the products in figures and tables in the next section:

Grain-fed:



Grass-fed:



Karoo:



4.2 Price

Price may not be a quality factor but does play an important role in the purchasing decision of meat. Consumers want the best value for their money, which would not necessarily mean that the cheapest product would be the best choice. The grain-fed products had the best/lowest price of R155.27/kg (Table 4.1; $p < 0.001$). Attributes such as % meat, % fat, % bone and WBSF values can

help determine which product is the best value for money, and will be discussed in the sections that follow.

Table 4.1: Difference in price (ZAR/kg) between the products of different production systems (Karoo / Grass-fed / Grain-fed).

	Karoo	Grass-fed	Grain-fed	p-value
Price (ZAR/kg)	182.06 ^b ±34.74	194.28 ^c ±19.96	155.27 ^a ±17.89	p < 0.001

Means with different superscripts in the same row differ significantly.

The average price of the Karoo products (R182.06/kg) and grass-fed products (R194.28/kg) were well above the average price of lamb (R163.98/kg) (Table 4.1; p < 0.001). This makes the difference between the price of the grain-fed and grass-fed products about R40/kg, and between grain-fed and Karoo products about R28/kg. However, retailer 2 (R2) sold their Karoo (R2K) and grain-fed (R2) products for the same price, which was also lower than the average price of lamb, making this Karoo product extremely affordable (Figure 4.1). According to the quality traits which will still be discussed, the Karoo product that was offered at a lower price by the one retailer performed the same compared to Karoo products that were offered at a more expensive price by other retailers. This product was from a major retailer who sold much more product than the smaller specialized butcheries. However, this was the only difference in price detected between retailers (R) and butcheries (B). It was expected that the Karoo and grass-fed products would be more expensive as they are sold as a specialized 'healthier' products compared to the grain-fed products. Packaging also had no effect on the price. For instance, samples R3MAP (MAP; R152.85/kg), R3OW (PVC-OW; R152.85/kg) and B10 (fresh-cut; R155.99/kg) were all from the grain-fed production system, with no significant differences between them. Furthermore, two of the grass-fed products with different packaging, R5 (PVC-OW) and R5FR (MAP) were also sold for the same price (R206.42) (Figure 4.1; p < 0.001).

4.3 Tissue distribution

4.3.1 Meat/Fat/Bone ratio:

In Figures 4.2 to 4.4 the variation in the meat/fat/bone ratio as a percentage of the weight of the whole loin chop is indicated. There were significant differences between the percentage meat (p < 0.001), fat (p < 0.001) and bone (p = 0.003). The percentage meat (Figure 4.2) and percentage bone (Figure 4.4) had similar groupings with the Karoo (B1K, R2K and B6K) and grass-fed products (R5, R3FR and R5FR) both being in the upper range, having a higher meat and bone percentage than the grain-fed products. As could be expected, the percentage fat (Figure 4.3) showed the opposite results with the Karoo and grass-fed products being in the lower range, having less fat than the grain-fed products. It is a well-know fact that muscle and fat have a negative relationship as was confirmed by the study by Strydom et al. (2009), which indicated that the meat and bone proportions declined as the

subcutaneous fat increased. During the period of fattening, bone and muscle grow at a slower rate than the fatty tissue, meaning both tissues decrease as fat increase (Thompson, Atkins & Gilmour, 1979).

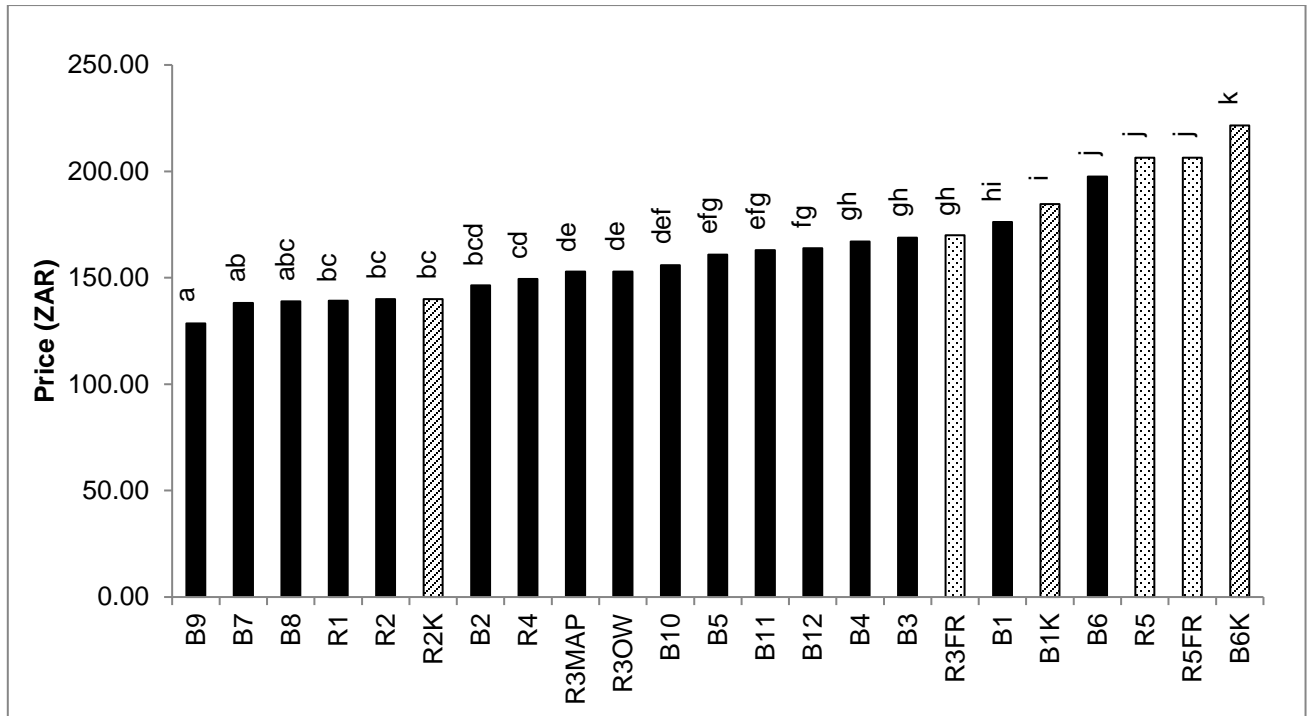


Figure 4.1: Variation in price over a range of products sampled across various retailers and butcheries (R: retailer, B: butchery, K: Karoo, FR: grass-fed). ($p < 0.001$). Bars with different letters differ significantly.

The use of beta-adrenergic agonists in sheep feedlot systems may lead to the expectation that the grain-fed products would have less fat, as beta-adrenergic agonist supplementation improves weight gain and carcass yield (Shook, Van Overbeke, Kinman, Krehbiel, Holland, Streeter, Yates & Hilton, 2009). However, because we have no information of the carcass weight and did not measure the loin muscle area, we could not verify if the lean (loin) yield relative to carcass weight (at the same fatness) was different for grain-fed vs. free-range products. Furthermore, it could be expected that despite the use of beta-adrenergic agonists, the feeders in general keep lambs in the pens until the maximum fat code is reached within the market specifications and preferences. Despite the fact that meat of free-range animals in South Africa is normally leaner than meat of grain-fed animals, the drought during the time of sampling could also have contributed to the significantly ($p < 0.001$) lower fat content of the free-range products.

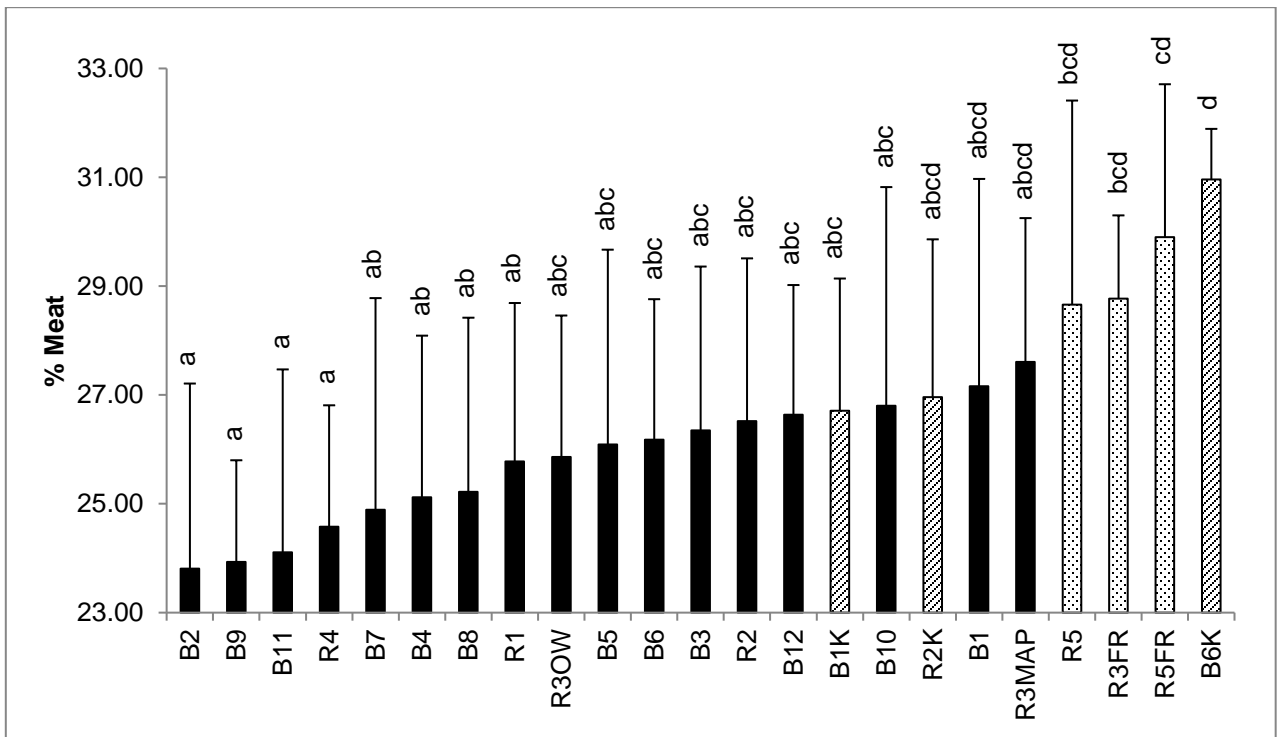


Figure 4.2: Variation in percentage meat (loin muscle only) over a range of products sampled across various retailers and butcheries (R: retailer, B: butchery, K: Karoo, FR: grass-fed). ($p < 0.001$). Bars with different letters differ significantly.

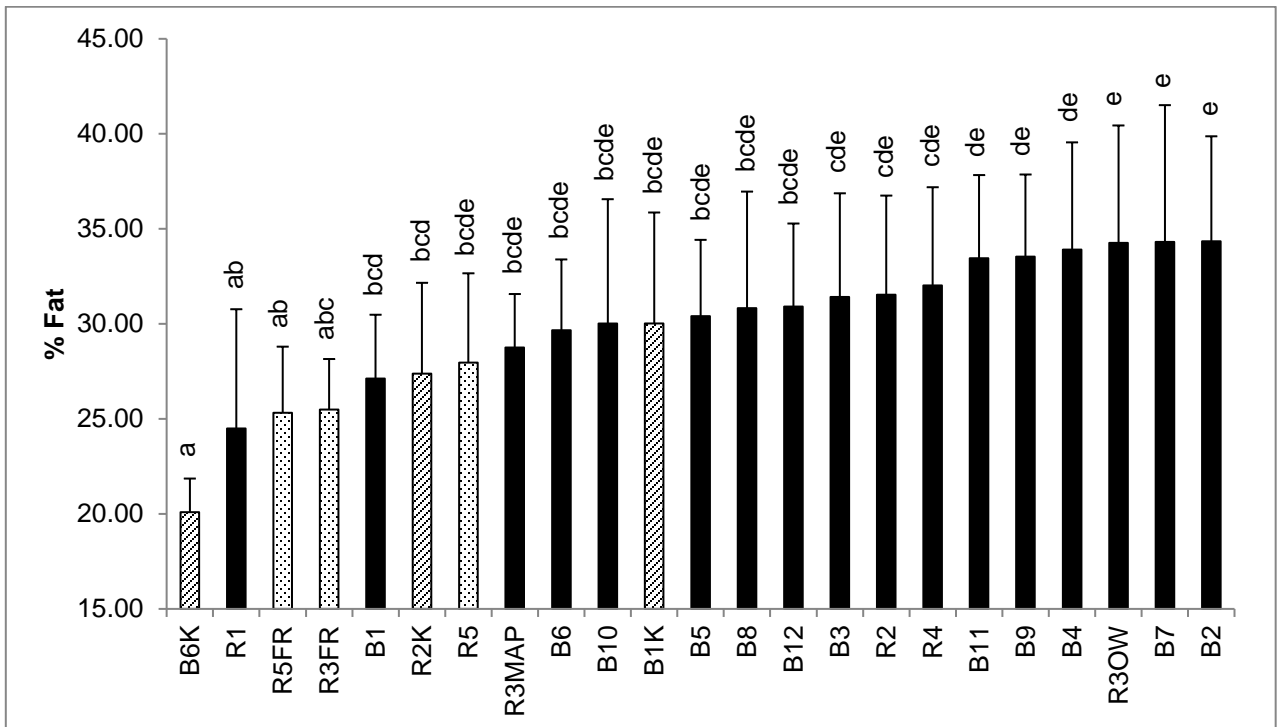


Figure 4.3: Variation in percentage fat over a range of products sampled across various retailers and butcheries (R: retailer, B: butchery, K: Karoo, FR: grass-fed). ($p < 0.001$). Bars with different letters differ significantly.

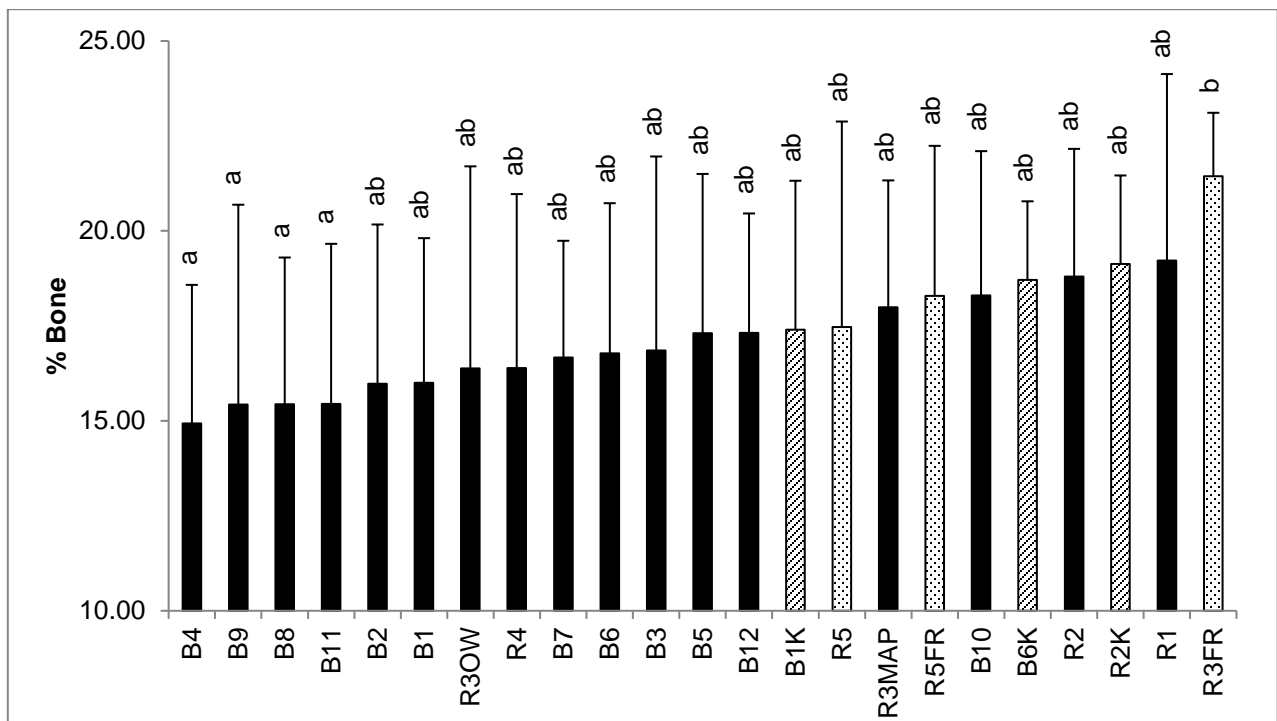


Figure 4.4: Variation in percentage bone over a range of products sampled across various retailers and butcheries (R: retailer, B: butchery, K: Karoo, FR: grass-fed). ($p = 0.003$). Bars with different letters differ significantly.

The increased meat yield in Karoo and grass-fed products (Figure 4.2) is a positive finding because as mentioned previously these products are sold at a higher price. However, it must be noted that total meat (percentage of meat) amounted to approximately 50% of the whole loin chop. This is interesting as the product in general is an expensive product (at an average price of R163.98/kg; Figure 4.1) and half of the product is fat and bone. There was however a correlation ($r = 0.3783$; $p = 0.001$) between price (Figure 4.1) and meat % (Figure 4.2), with an increase in price resulting in a larger percentage of meat (Appendix 1). There was also a good relationship between meat% and between fat% and fat% and bone%. This showed that as meat% increases fat% decreases and as fat% increases so does bone% decrease.

4.4 Proximate composition

4.4.1 Muscle protein and moisture content:

Red meat contains high value proteins and macronutrients that are needed for good health (Williams, Droulez, Levy & Stobaus, 2007). While breed, production systems, season and meat cut will cause variation, lean red meat in general has a low fat content, is moderate in cholesterol and rich in protein as well as essential vitamins and minerals. Raw red meat contains about 20-25 g protein/100 g

meat, while cooked red meat contains 28-36 g protein/100 g. This is attributed to the fact that as the water content decreases during cooking the nutrients become more concentrated (Williams, 2007).

There was no real pattern in the percentage protein for the Karoo, grass-fed or grain-fed products, with some samples appearing in the lower range and some in the upper range (Figure 4.5). One of the grass-fed products, sample R3FR, differed significantly ($p < 0.001$) from the other two grass-fed products as well as the Karoo products. This sample had the highest percentage protein of all 23 products and also presented the lowest percentage of moisture (Figure 4.6; $p = 0.004$) compared to the other free-range products. In research by North & Lovatt (2012) they noted on the effect of freezing on the protein content of meat. They explained that as meat are frozen the solutes move away from the crystals which will result in a high amount of drip loss and meat with a higher protein content. However, as all the Karoo and grass-fed samples were on the lower side for fat content (Figure 4.3; $p < 0.001$), a higher protein content is expected.

The mean protein contents of the different production systems indicated that the Karoo products had a significantly ($p = 0.001$) lower protein content than the grain- and grass-fed production systems (Table 4.2; $p = 0.001$). With regard to the mean moisture contents only the grass-fed and grain-fed production systems differed significantly ($p = 0.001$) from each other (Table 4.2). The grain-fed production system had the least amount of moisture, followed by the Karoo production system and the grass-fed production system having the highest percentage. The moisture content (Figure 4.6) relates to the percentage meat (Figure 4.2) discussed in the previous section, meaning samples with a higher percentage meat also had higher moisture contents. Due to the drought conditions some of the samples from our study were purchased frozen. This is mainly true for the Karoo products. Frozen meats' water holding capacity are reduced due to the distortion of the muscle fibers by ice crystals (Feiner, 2006), which can be explanatory for the high moisture content of the free-range products that will be lost during the cooking process.

Table 4.2: Variation between protein (%) and moisture (%) contents of the products from three different production systems (Karoo / Grass-fed / Grain-fed).

	Karoo	Grass-fed	Grain-fed	p-value
Protein content (%)	20.28 ^a ±0.52	20.82 ^b ±0.65	20.62 ^b ±0.68	$p = 0.001$
Moisture content (%)	75.42 ^{ab} ±1.07	75.64 ^b ±1.25	74.91 ^a ±1.36	$p = 0.001$

Means with different superscripts in the same row differ significantly.

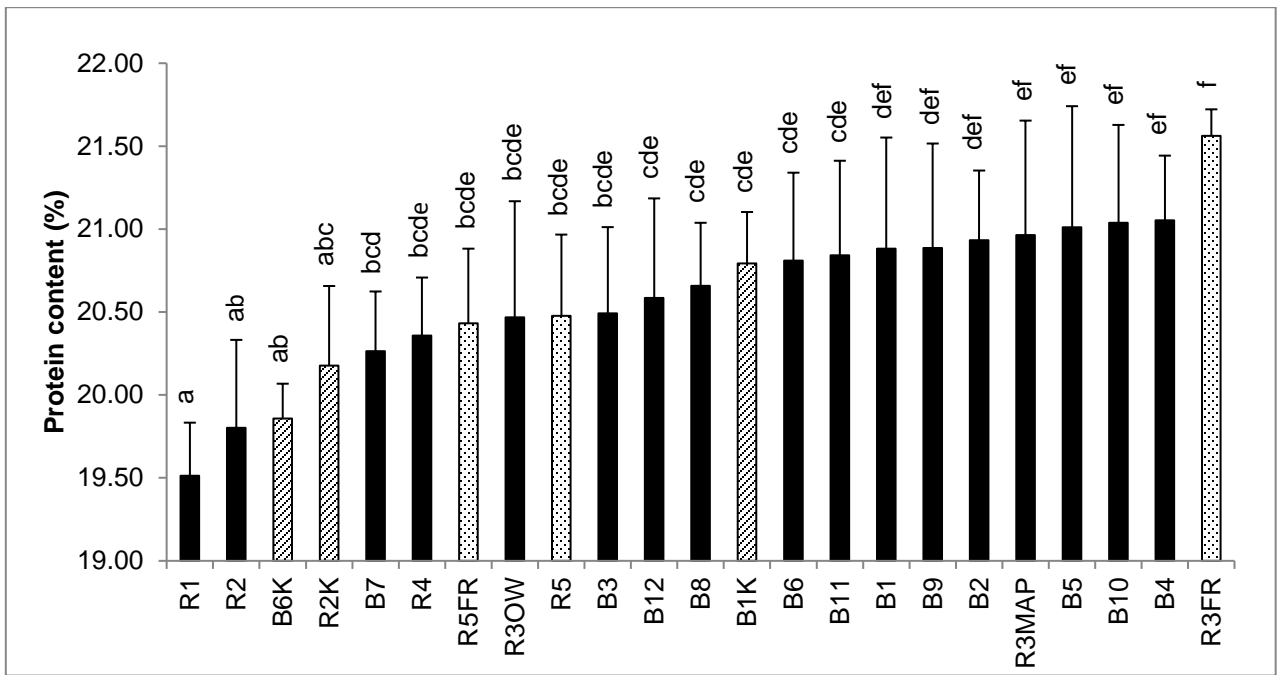


Figure 4.5: Variation in protein content over a range of products sampled across various retailers and butcheries (R: retailer, B: butchery, K: Karoo, FR: grass-fed). ($p < 0.001$). Bars with different letters differ significantly.

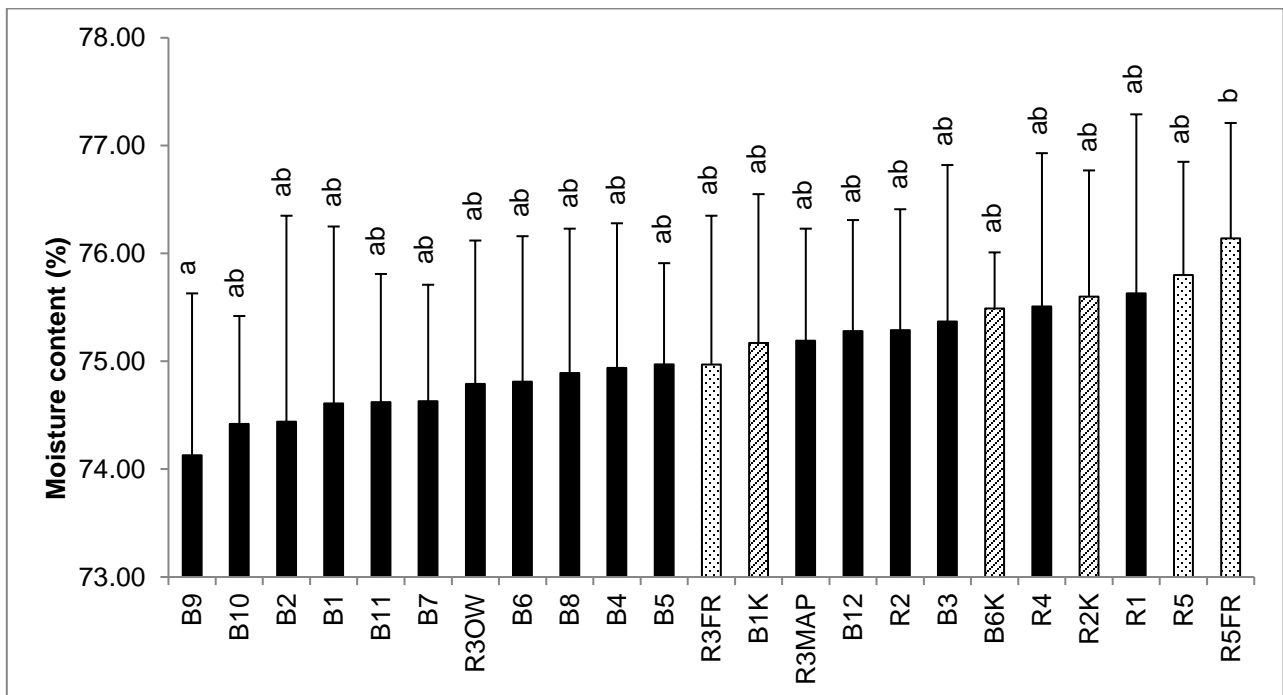


Figure 4.6: Variation in moisture content over a range of products sampled across various retailers and butcheries (R: retailer, B: butchery, K: Karoo, FR: grass-fed). ($p = 0.004$). Bars with different letters differ significantly.

4.4.2 Dissectable- and intramuscular fat:

Different species of meat producing animals vary in the amount of separable fat with the total for lamb loin chops being about 37% compared to the 1% for veal steak (Cobiac, Droulez, Leppard & Lewis, 2003). In a study by Wood and Enser (1997) they found that dissectible fat contents resulted in 156, 302 and 211 g/kg for beef, lamb and pork respectively. Intramuscular fat content is very influential on the sensory aspects of meat such as flavour. Furthermore, IMF encourages connective tissue remodeling which in turn reduces the amount of collagen cross-links (Nishimura, Hattori & Takahashi, 1999). Therefore, the quantity and distribution of IMF affects meat quality (Hocquette et al., 2010). Compared to lean meat deposition, fat deposition represents an increase in cost in terms of feed energy (Ponnampalam et al., 2003). Concerns have been raised by the Australian lamb industry about the fact that low IMF could lead to dry and tasteless meat (McPhee et al., 2008), seeing that such results have already been found in young, highly muscled cattle and modern pig genotypes (Channon, Reynolds & Baud, 2001). The data found by MCPhee et al. (2008) did suggest that in sheep IMF is early maturing in relation to the total fat of the carcass. Animals' intramuscular fat percentage is affected by breed, age, diet, weight and degree of fatness (Zervas & Tsiplakou, 2011).

To prevent meat from being dry and tough a certain amount of fat is recommended but too much fat is regarded as unhealthy by consumers (Strydom, Van Heerden, Schönfeldt, Kruger & Smith, 2009). Therefore, all over the world leanness is an important purchasing criterion (Sañudo et al., 2000). In the South African market lamb and mutton are produced and sold relatively lean, however according to nutritional guidelines even cuts in low fat classes are too fatty (Van Heerden, Schönfeldt, Kruger & Smith, 2007). The Agricultural Product Standards ACT No. 119 of 1990 established seven fat classes into which carcasses are classified according to their subcutaneous fat (SCF) cover. Carcasses with fat score 1 have 0 mm back fat thickness (less than 1% SCF) while carcasses with fat score 6 have more than 11 mm back fat thickness (that is more than 17.6% SCF).

The dissectible fat contents from the samples in our study are indicated in Figure 4.3 under the meat/fat/bone ratio. In Figure 4.7 the IMF percentages are indicated. More than half of the upper range of the IMF percentage consists of samples from animals raised in feedlots with higher values. The lower range consisted mostly of the Karoo (R2K, B1K and B6K) and grass-fed (R3FR, R5 and R5FR) products, with no significant difference between them. The only significant ($p < 0.001$) differences identified in the IMF content were between the two samples with the lowest IMF content (R1 and R5FR) and the two samples with the highest IMF content (B2 and B9), with the differences between them being only about 1.3%. The fact that the Karoo and grass-fed products had lower IMF contents was expected due to free-range animals being less fat. Zervas et al. (1999b) also noted that stall fed lambs have lower acetate/propionate ratios in their rumen which might explain their higher IMF deposition. There was a fairly strong correlation (0.4502; $p = 0.001$) between dissected fat and the percentage of fat found in the

muscle with an increase in dissected fat correlating with an increase in muscle fat percentage (Appendix 1). In Pethick et al. (2005) found that lamb loin contains 4-5% IMF and suggested that this level must be obtained. Later on, as mentioned in the literature review, it was found that 3.9% extractable fat for sheep is still acceptable for good eating quality (Pannier et al., 2014).

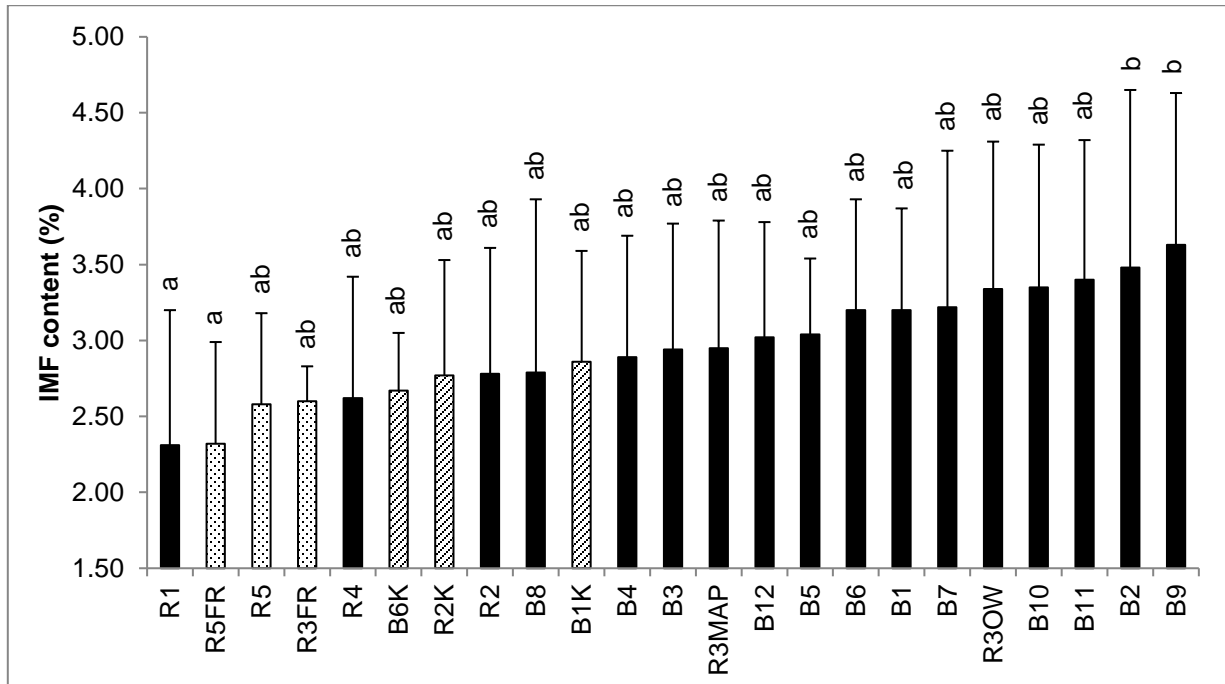


Figure 4.7: Variation in IMF content over a range of products sampled across various retailers and butcheries (R: retailer, B: butchery, K: Karoo, FR: grass-fed). ($p < 0.001$). Bars with different letters differ significantly.

Priolo et al. (2002) found that carcass fatness did correlate positively with tenderness (0.44; $p = 0.01$). The influence of fatness on tenderness can occur directly through fat that is softer than lean meat or indirectly through reduced muscle shortening. On average the Karoo products from our study had an IMF value of 2.76% and 2.50% for the grass-fed products, while the grain-fed products had a significantly ($p < 0.001$) higher percentage of 3.07% (Table 4.3). All of these values were well below the threshold mentioned by Pannier et al. (2014). However, all the products were regarded as tender with a more detailed discussion on tenderness and juiciness to follow.

Table 4.3: Variation between dissectible fat content (%) and intramuscular fat content (%) of the products from three different production systems (Karoo / Grass-fed / Grain-fed).

Ratios	Karoo	Grass-fed	Grain-fed	Sign. level
Dissectible fat (%)	25.83 ^a ±6.09	26.26 ^a ±3.82	31.24 ^b ±5.69	$p < 0.001$
IMF (%)	2.76 ^{ab} ±0.63	2.50 ^a ±0.54	3.07 ^b ±0.92	$p < 0.001$

Means with different superscripts in the same row differ significantly.

4.5 Composition of the lipid fraction

4.5.1 Saturated fatty acids (SFA's):

Different production systems (concentrate vs forage/pastures) lead to different FA's in the meat of animals (Wood & Enser, 1997). The main difference between ruminant fat and that of single-stomached species is the high SFA content and low proportion of PUFA in the former. On average, SFA makes out 40% of the total FA in the lean component and 48% in the fat component of ruminant meat (Williams, 2007). This is the result of rumen bacteria converting PUFA's extracted from forage or concentrate diets into SFA by the action of hydrogenation (Wood & Enser, 1997). A diet containing foods with low levels of SFA's, high levels of PUFA's and a low n-6 : n:3 ratio are considered favorable for human health (Wood et al., 2003). Consequently, due to the high SFA content in ruminant meat it is not considered as part of a healthy human diet (Scollan, Hocquette, Nuernberg, Dannenberger, Richardson & Moloney, 2006). Therefore, there is considerable interest in the SFA content of red meat.

In lamb and mutton, palmitic acid (C16:0) and stearic acid (C18:0) are in the majority when considering the SFA component, and also found in similar proportions (Williams, 2007). Even though stearic acid (C18:0) is part of the SFA group, a few studies have noted on its positive contribution of lowering low-density lipoprotein (LDL) cholesterol levels as well as lowering the ratio of the total and high-density lipoprotein (HDL) cholesterol (Li, Siriamornpun, Wahlqvist, Mann & Sinclair, 2005; Hunter, Zhang & Kris-Etherton, 2010). In our study no significant differences were found between the palmitic acid contents of the three production systems (Table 4.4; $p = 0.749$). With regards to the stearic acid content, the Karoo and grass-fed production systems delivered significantly ($p = 0.002$) higher values than the grain-fed production system (Table 4.4). For myristic acid (C14:0) the Karoo production system had a significantly ($p < 0.001$) higher content (Table 4.4). It was noted in some studies that the lauric acid (C12:0) and myristic acid (C14:0) contents was in a similar way affected by the diet (Demirel, Ozpinar, Nazli & Keser, 2006; Scerra, Caparra, Foti, Galofaro, Sinatra & Scerra, 2007) while in other studies no effect was found (Hajji, Joy, Ripoll, Smeti, Mekki, Molino Gahete, Mahouachi & Atti, 2016). Myristic acid (C14:0) and palmitic acid (C16:0) are not recommended for human health due to their association with obesity, hypercholesterolaemia and some cancers (Wood et al., 2003).

Phytanic acid (C20:0,3,7,11,15-tetramethyl hexanoic acid) is one of the characteristic FA of ruminant meat (Young et al., 2013). This FA is synthesized from phytol cleaved from chlorophyll and turned into phytanic acid by the actions of oxidation and hydrogenation (Ackman & Hansen, 1967). Lampen et al. (2001) noted that as docosahexanoic acid (DHA), phytanic acid also increase the metabolism of all-trans-retinoic in intestinal cells. Very little data on phytanic acid is available, however Brown et al. (1993) found that lean organic beef contains about 4 mg phytanic acid per 100 g meat. The differences in the phytanic acid contents of the products from our study were quite small, but still

significant (Figure 4.8; $p < 0.001$). The product with the highest content (B6K) differed with 0.03% from the product with the lowest content (R4). As the primary substrate is chlorophyll, it would be expected that the Karoo and grass-fed products would have the highest mean values. In this regard the Karoo production systems differed significantly ($p < 0.001$) from both the grass-fed and grain-fed production system with more than double the value (Table 4.4). The drought during the time of sampling might be responsible for this occurrence as the Karoo 'bossies' would more likely have survived these conditions. This leads to an overall conclusion that the products from the grain-fed production system had a significantly ($p < 0.001$) lower total SFA content than the other two production systems (Table 4.4). This lower saturated fatty acid content may be due to less biohydrogenation due to lower rumen pH in grain-fed animals (Cited by Carvalho et al., 2015).

Table 4.4: Variation between IMF saturated fatty acid contents (%) of the products from three different production systems (Karoo / Grass-fed / Grain-fed).

	Karoo	Grass-fed	Grain-fed	p-value
Myristic (C14:0)	4.13 ^b ±1.06	3.42 ^a ±1.01	3.02 ^a ±1.06	$p < 0.001$
Palmitic (C16:0)	28.55±2.05	28.22±1.95	28.33±2.08	$p = 0.749$
Margaric (C17:0)	1.30±0.15	1.24±0.17	1.27±0.27	$p = 0.486$
Stearic acid (C18:0)	17.49 ^b ±2.10	17.38 ^b ±1.99	16.44 ^a ±2.22	$p = 0.002$
Phytanic acid (C20:0,3,7,11,15-tetramet)	0.03 ^b ±0.02	0.01 ^a ±0.02	0.01 ^a ±0.02	$p < 0.001$
Total SFA	52.55 ^b ±2.43	51.08 ^b ±3.34	49.76 ^a ±3.25	$p < 0.001$

Means with different superscripts in the same row differ significantly.

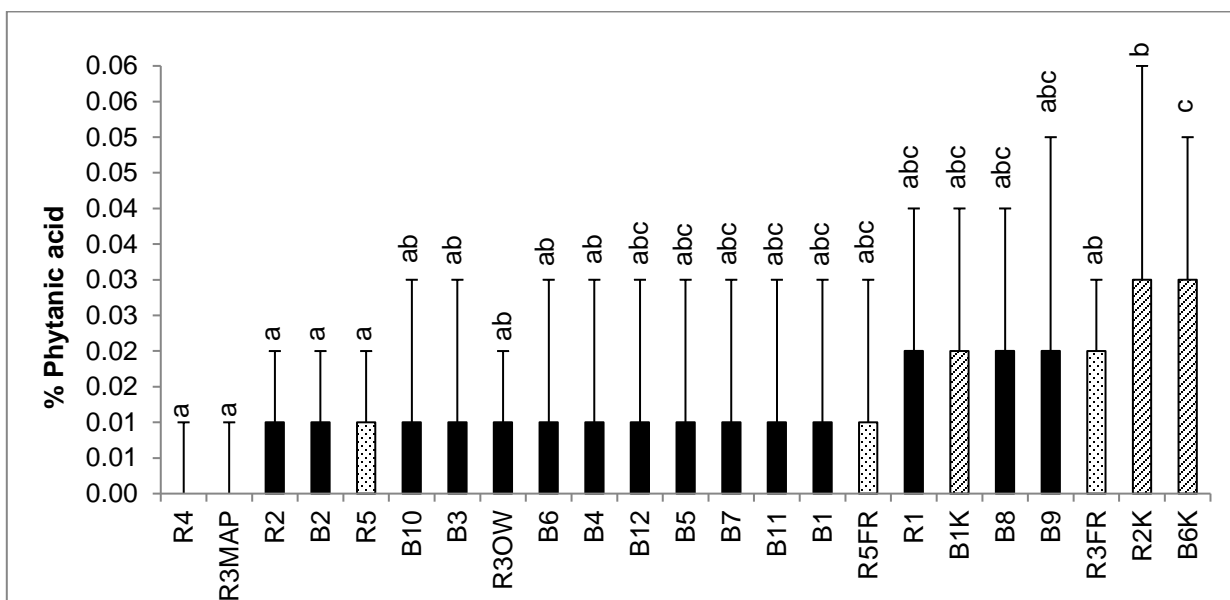


Figure 4.8: Variation in percentage phytanic acid over a range of products sampled across various retailers and butcheries (R: retailer, B: butchery, K: Karoo, FR: grass-fed). ($p < 0.001$). Bars with different letters differ significantly.

4.5.2 Unsaturated fatty acids (UFA's):

4.5.2.1 Monounsaturated fatty acids (MUFA's):

The UFA portion in meat consists of MUFA and PUFA. Lamb/mutton contain a significant amount of MUFA, which oxidize considerably slower than PUFA's. As a result of biohydrogenation in the rumen a great amount of the double bonds are the *trans*-type, meaning the hydrogen atoms point in different directions. This structure gives these FA a particularly low melting point. With regards to the MUFA portion, the *trans*-type FA include elaidic acid (C18:1t9) and vaccenic acid (C18:1t11) (Webb & O'Neill, 2008). The predominant MUFA's (Table 4.5) found in the products from our study included palmitoleic acid (C16:1c9), elaidic acid (C18:1t9), oleic acid (C18:1c9) and vaccenic acid (C18:1t11).

Overall in this study the products from the grain-fed production system had a significantly ($p < 0.001$) higher total MUFA content than the grass-fed and Karoo production systems, especially with reference to oleic acid (C18:1c9) ($p < 0.001$) (Table 4.5). In previous studies oleic acid was also the major FA of the MUFA group for our lamb products (Hopkins, Clayton, Lamb, Van de Ven, Refshauge, Kerr & Ponnampalam, 2014; Yagoubi, Joy, Ripoll, Machouachi, Bertolín & Atti, 2018). Oleic acid is formed from the SFA, stearic acid (C18:0) by a major lipogenic enzyme, stearoyl Co-A desaturase. This result can be seen as the grain-fed production system presented products with the lowest stearic acid content (Table 4.4; $p = 0.002$).

Vaccenic acid is also a very good example of a biohydrogenation product of linoleic acid (C18:2n-6). Both oleic and vaccenic acid occur at higher levels in neutral lipids than in phospholipids and also occur at higher levels in adipose tissue than in muscle (Wood et al., 2008). This means that the edible fat of ruminants is the main natural dietary source of vaccenic acid (Bessa et al., 2015). With regard to vaccenic acid (Table 4.5) the Karoo products had a significantly ($p = 0.002$) lower content than the grass-fed and grain-fed products. Palmitoleic acid is a marker of endogenous lipid production as it is a product of the conversion of acetyl Co-A to FA (Paillard, Catheline, Duff, Bouriel, Deugnier, Pouchard, Daubert & Legrand, 2008; Biancarosa, Liland, Day, Belghit, Amlund, Lock & Gilburn, 2018). Even though palmitoleic acid belongs to the MUFA group, it can also act as a SFA by increasing LDL and decreasing HDL-cholesterol concentrations (De Fabiani, 2011). In terms of the palmitoleic acid, only the Karoo and grain-fed production systems differed significantly ($p = 0.005$) from each other. The Karoo production system delivered the highest content (1.72 %) and the grain-fed production system the lowest (1.55 %).

4.5.2.2 Polyunsaturated fatty acids (PUFA's):

Muscle contains large amounts of long chain PUFA's (C20-22) formed from linoleic acid (C18:2c9,12 n-6) and alpha-linolenic acid (ALA; C18:3c9,12,15 n-3) by the action of $\Delta 5$ and $\Delta 6$ desaturase and elongase enzymes (Wood et al., 2008). Williams (2007) noted that the PUFA in ruminant

meat contributes about 11-29 % of the total FAs. However in our study the maximum contribution was 10%. Diets of humans as well as other monogastric mammals require n-6 and n-3 FA as they cannot be synthesized *de novo*. These FA are carriers for the fat soluble vitamins A, D, E and K which subsequently play a crucial role in the immune response of both man and animal (Webb & O'Neill, 2008). The nutritional value of the meat of ruminants is changed to correlate with human health by attempting to increase the n-3 PUFA content (Zervas & Tsiplakou, 2011). The consumption of red meat to achieve the recommended levels of n-3 PUFAs have long been used as a marketing tool (Pethick, Hopkins, D'Souza, Thompson & Walker, 2005). In McAfee et al. (2010) noted that about 43% of the total dietary intake of n-3 PUFAs is contributed by the consumption of meat and meat products whereas 48% are contributed by the consumption of oily fish.

Table 4.5: Variation between IMF unsaturated fatty acid contents (%) of the products from three different production systems (Karoo / Grass-fed / Grain-fed).

	Karoo	Grass-fed	Grain-fed	p-value
Elaidic (C18:1t9)	0.27 ^a ±0.72	0.17 ^a ±0.46	0.80 ^b ±1.21	p < 0.001
Heptadecanoic (C17:1c10)	0.11±0.16	0.11±0.14	0.08±0.15	p = 0.290
Myristoleic (C14:1c9)	0.10 ^b ±0.04	0.07 ^a ±0.04	0.06 ^a ±0.04	p < 0.001
Palmitoleic (C16:1c9)	1.72 ^b ±0.31	1.61 ^{ab} ±0.26	1.55 ^a ±0.31	p = 0.005
Oleic acid (C18:1c9)	34.98 ^a ±3.21	35.60 ^a ±2.42	37.53 ^b ±3.13	p < 0.001
Vaccenic (C18:1t11)	0.09 ^a ±0.45	0.82 ^b ±1.35	0.77 ^b ±1.18	p = 0.002
Arachidonic (C20:4c5,8,11,14)	2.04 ^b ±0.69	1.94 ^b ±0.57	1.59 ^a ±0.78	p < 0.001
Conjugated linoleic acid (C18:2c9t11 n-6)	0.35 ^b ±0.15	0.29 ^{ab} ±0.09	0.27 ^a ±0.13	p < 0.001
Docosahexanoic (C22:6 n-3)	0.08 ^b ±0.05	0.08 ^b ±0.05	0.05 ^a ±0.06	p < 0.001
Docosapentaenoic (C22:5c7,10,13,16,19 n-3)	0.55 ^b ±0.16	0.53 ^b ±0.17	0.33 ^a ±0.22	p < 0.001
Eicosapentaenoic (C20:5 n-3)	0.44 ^b ±0.16	0.46 ^b ±0.18	0.27 ^a ±0.22	p < 0.001
Linoleic (C18:2c9,12 n-6)	5.10 ^a ±1.39	5.91 ^{ab} ±1.98	5.93 ^b ±2.10	p = 0.047
γ-Linolenic (C18:3c6,9,12 n-6)	0.005 ^a ±0.01	0.013 ^{ab} ±0.02	0.017 ^b ±0.02	p = 0.002
α-Linolenic (C18:3c9,12,15 n-3)	1.33 ^c ±0.28	1.10 ^b ±0.26	0.79 ^a ±0.43	p < 0.001
Total n-3	2.41 ^b ±0.55	2.18 ^b ±0.56	1.44 ^a ±0.84	p < 0.001
Total n-6	7.64±1.97	8.24±2.26	7.90±2.64	p = 0.545
Total MUFA	37.41 ^a ±3.18	38.50 ^a ±2.85	40.90 ^b ±3.11	p < 0.001
Total PUFA	10.05±2.45	10.42±2.42	9.34±2.96	p = 0.039

Means with different superscripts in the same row differ significantly.

Regarding the n-3 FA group, the most important FA's are ALA, eicosapentaenoic acid (EPA; C20:5c5,8,11,14,17 n-3), docosahexanoic acid (DHA; C22:6c4,7,10,13,16,19 n-3) and docosapentaenoic acid (DPA; C22:5c7,10,13,16,19 n-3) (Corino, Rossi, Cannata & Ratti, 2014). Omega-3 PUFAs have anti-inflammatory and cardiovascular benefits, which particularly point to EPA

and DHA when discussing meat (Hopkins & Mortimer, 2014). A few studies have proven that lambs grazing on pastures have a higher n-3 concentration than feedlot animals, which in turn decreases the SFA concentration and improves the PUFA : SFA and n-6 : n-3 ratios (Wood & Enser, 1997; Aurousseau, Bauchart, Calichon, Micol & Priolo, 2004; Gatellier, Mercier, Juin & Renerre, 2005). In our study it was rather the total MUFA content that was reduced. As mentioned the PUFA that animals ingest through their feeding system are progressively saturated in the rumen by a process called biohydrogenation. Subsequently, feeding systems that reduce ruminal biohydrogenation will ultimately increase PUFA deposition in ruminant tissue (Buccioni, Decandia, Minieri, Molle & Cabiddu, 2012). However, elevated levels of PUFA in cell membranes make meat susceptible to oxidation (Luciano, Moloney, Priolo, Röhrle, Vasta, Biondi, López-Andrés, Grasso & Monahan, 2011). In our study the mean values indicated that the Karoo and grass-fed production systems had significantly higher DHA ($p < 0.001$), DPA ($p < 0.001$) and EPA ($p < 0.001$) contents than the grain-fed production system (Table 4.5). As mentioned, this could be expected. Table 4.6 indicates how two of the grain-fed products, R1 and B8, stood out with values higher than some of the Karoo and grass-fed products. As specific label claims were not always present on the samples, we assumed that the products that were not labeled as grass-fed or Karoo were of grain-fed origin. However, we had no way of knowing this for sure and might samples R1 and B8 actually be part of one of the free-range production systems.

Linoleic acid (C18:2c9,12 n-6) was the most abundant PUFA found in the meat of all the production systems. Once again the only significant ($p = 0.047$) difference was between the Karoo and grain-fed production systems, with the Karoo production system having the lowest content (5.10 %) and the grain-fed production system having the highest content (5.93 %) (Table 4.5). Conjugated linoleic acid (CLA; C18:2c9t11 n-6) is a biologically active substance and a product of the biohydrogenation of linoleic acid (Pariza, Park & Cook, 2001). Therefore, ruminant fats are among the richest sources of CLA (Ha, Storkson & Pariza, 1990). Conjugated linoleic acid is considered as the most important n-6 FA as it has demonstrated positive anti-diabetic, anti-cancer and anti-adipogenic effects (Bas, Berthelot, Pottier & Normand, 2007). It is mostly found in the fat component of red meat with about 1 g/100 g, although some are also in the muscle part with 10-46 mg/100 g in raw red meat and 30-100 mg/100 g in cooked red meat (Williams, 2007). The overall conclusion for the CLA content (Table 4.5; $p < 0.001$) is that the Karoo and grass-fed products were superior in this regard, although two grain-fed products (R1 and B8) also had high values (Table 4.6; $p < 0.001$). In general, when consuming lamb the n-6 FA contribute about 0.5-1.0% of the energy intake that is recommended for humans. However, when the meat is consumed with other foods the contribution falls to a safe level (Chikwanha, Vahmani, Muchenje, Dugan & Mapiye, 2018). There were no significant ($p = 0.177$) differences in the total n-6 contents of the Karoo, grass-fed and grain-fed production systems, with the lowest value being 6.69 for sample B5 and the highest value 9.54 for sample B3, both being grain-fed products (Table 4.6). Due to health risks such as blood clots high amounts n-6 PUFA's is not preferred (Williams, 2000). For total n-3 content the

Karoo and grass-fed production systems had significantly ($p < 0.001$) higher contents than the grain-fed production system (Table 4.5). Once again the two grain-fed products (R1 and B8) stood out in this regard with values higher than some of the Karoo and grass-fed products (Table 4.6; $p < 0.001$). With regards to the total PUFA content there were no significant differences between the individual products (Table 4.8; $p = 0.394$) nor between the production systems (Table 4.5; $p = 0.039$).

Table 4.6: Variation in CLA, total n-6 PUFA and total n-3 PUFA content over a range of products sampled across various retailers and butcheries (R: retailer, B: butchery, K: Karoo, FR: grass-fed).

Common name Abbreviation	Conjugated linoleic acid (CLA) C18:2c9t11 n-6	Total n-6	Total n-3
R1	0.33 ^{ab} ±0.12	7.91±2.87	2.47 ^{fg} ±0.75
R2	0.27 ^a ±0.11	7.14±2.20	1.91 ^{bcdefg} ±0.73
R2K	0.36 ^{ab} ±0.16	7.34±2.10	2.37 ^{efg} ±0.66
R3FR	0.29 ^{ab} ±0.07	7.55±0.71	2.50 ^{fg} ±0.46
R3MAP	0.30 ^{ab} ±0.18	8.13±2.14	1.47 ^{abcde} ±0.80
R3OW	0.21 ^a ±0.12	7.88±2.72	1.07 ^{ab} ±0.71
R4	0.23 ^a ±0.11	8.49±2.65	1.09 ^{ab} ±0.60
R5	0.32 ^{ab} ±0.11	8.27±2.66	2.22 ^{cdefg} ±0.61
R5FR	0.25 ^a ±0.08	8.89±2.76	1.83 ^{bcdefg} ±0.40
B1	0.26 ^a ±0.15	7.37±1.79	1.32 ^{abc} ±0.61
B1K	0.25 ^a ±0.10	7.36±2.43	2.29 ^{defg} ±0.64
B2	0.23 ^a ±0.09	8.03±3.70	0.78 ^a ±0.59
B3	0.26 ^a ±0.15	9.54±2.73	1.33 ^{abc} ±0.50
B4	0.29 ^{ab} ±0.14	7.48±2.72	1.75 ^{bcdefg} ±0.93
B5	0.28 ^{ab} ±0.13	6.69±1.87	1.63 ^{abcdef} ±0.65
B6	0.27 ^a ±0.16	9.05±2.40	0.80 ^a ±0.42
B6K	0.45 ^b ±0.12	8.21±1.17	2.58 ^g ±0.27
B7	0.28 ^{ab} ±0.08	7.81±3.12	1.40 ^{abcd} ±0.64
B8	0.36 ^{ab} ±0.14	6.73±2.82	2.20 ^{cdefg} ±0.99
B9	0.21 ^a ±0.07	7.80±2.76	1.01 ^{ab} ±0.90
B10	0.33 ^a ±0.12	8.41±1.82	1.41 ^{abcd} ±0.73
B11	0.28 ^{ab} ±0.16	6.93±2.43	1.36 ^{abcd} ±0.80
B12	0.23 ^a ±0.14	8.93±2.83	1.44 ^{abcde} ±0.87
Sign. level	p < 0.001	p = 0.177	p < 0.001

Means with different superscripts in the same row differ significantly.

4.5.3 Fatty acid ratios:

4.5.3.1 PUFA : SFA

The PUFA : SFA and n-6 : n-3 ratios are important tools that can be used to evaluate the nutritional value of meat for human consumption (McAfee, McSorley, Cuskelly, Moss, Wallace & Bonham, 2010). The PUFA : SFA ratio of meat is also a factor in the development of cancers and coronary heart disease, especially with reference to the formation of blood clots that can lead to a heart attack (Hunter et al., 2010). A recommended value of 0.4 or above have been set, meaning the higher the ratio the healthier the product (WHO, 2003). Due to the biohydrogenation of the UFA's, red meat generally has a low

PUFA : SFA ratio that is about 0.1-0.2 (Ponnampalam, Hopkins, Butler, Dunshea, Sinclair & Warner, 2009).

There were no significant ($p = 0.325$) differences in the PUFA : SFA ratios between the production systems (Table 4.7), and also not between the individual products with values ranging from 0.17 for sample B11 to 0.23 for sample B3 (Figure 4.9; $p = 0.613$). Both of these samples were of grain-fed origin with the same packaging. The Karoo and grass-fed products were also scattered across the spectrum with no significant conclusion. Even though the Karoo and grass-fed production systems had significantly ($p < 0.001$) higher total SFA contents (Table 4.4) than the grain-fed production system, the high amount of total n-3 FA's (Table 4.5; $p < 0.001$) improved their PUFA : SFA ratios. As in our study, Mitchell et al. (1991) also noted that this is evidence that diets based on forage or grass will lead to a higher n-3 PUFA content in meat, while diets based on concentrate will lead to a higher n-6 PUFA content.

Table 4.7: Variation between FA indexes of the products from three different production systems (Karoo / Grass-fed / Grain-fed).

Ratios	Karoo	Grass-fed	Grain-fed	p-value
PUFA : SFA	0.19±0.05	0.21±0.06	0.19±0.07	$p = 0.325$
n-6 : n-3	3.17 ^a ±0.47	3.99 ^a ±1.43	7.73 ^b ±5.98	$p < 0.001$
Atherogenicity index	0.96 ^b ±0.15	0.87 ^a ±0.16	0.82 ^a ±0.16	$p < 0.001$
Desaturase index	2.03 ^a ±0.32	2.07 ^a ±0.28	2.32 ^b ±0.36	$p < 0.001$

Means with different superscripts in the same row differ significantly.

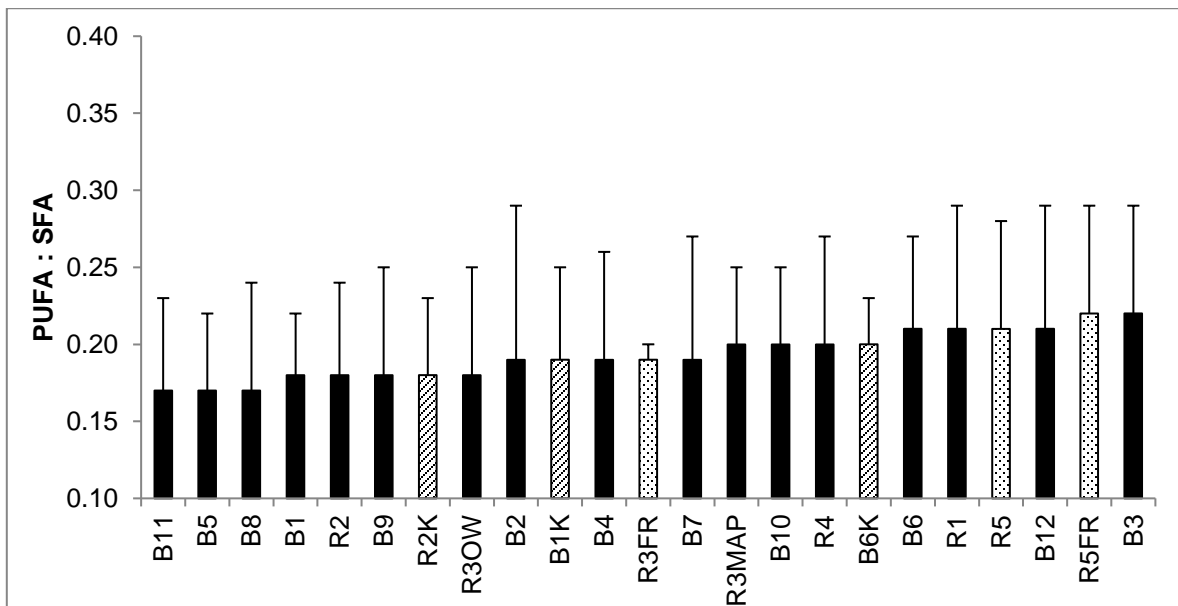


Figure 4.9: Variation in PUFA : SFA ratios over a range of products sampled across various retailers and butcheries (R: retailer, B: butchery, K: Karoo, FR: grass-fed). ($p = 0.613$).

Table 4.8: Variation in PUFA content over a range of products sampled across various retailers and butcheries (R: retailer, B: butchery, K: Karoo, FR: grass-fed).

Common name	Linoleic	α -Linolenic	Arachidonic	Eicosapentaenoic (EPA)	Docosapentaenoic (DPA)	Docosahexanoic (DHA)	Total PUFA
Abbreviation	C18:2c9,12 n-6	C18:3c9,12,15 n-3	C20:4c5,8,11,14	C20:5 n-3	C22:5c7,10,13,16,19 n-3	C22:6 n-3	
R1	5.80 ^{ab} ±2.22	1.28 ^{efg} ±0.42	1.65±0.76	0.53 ^{fg} ±0.20	0.55 ^{def} ±0.23	0.11 ^{bc} ±0.08	10.38±3.38
R2	4.87 ^{ab} ±1.43	0.99 ^{bcdefg} ±0.34	1.92±0.89	0.36 ^{abcdefg} ±0.19	0.48 ^{bcdef} ±0.25	0.08 ^{abc} ±0.07	9.06±2.70
R2K	4.83 ^{ab} ±1.52	1.29 ^{efg} ±0.32	1.98±0.72	0.44 ^{cdefg} ±0.20	0.55 ^{def} ±0.18	0.08 ^{abc} ±0.05	9.70±2.70
R3FR	5.13 ^{ab} ±0.59	1.21 ^{defg} ±0.23	2.03±0.13	0.58 ^g ±0.12	0.59 ^{ef} ±0.11	0.11 ^c ±0.04	10.05±0.66
R3MAP	6.20 ^{ab} ±1.61	0.71 ^{abc} ±0.46	1.54±0.66	0.33 ^{abcdefg} ±0.25	0.37 ^{abcdef} ±0.21	0.06 ^{abc} ±0.08	9.60±2.17
R3OW	5.75 ^{ab} ±2.14	0.60 ^{ab} ±0.32	1.81±0.69	0.18 ^{abc} ±0.20	0.25 ^{ab} ±0.17	0.03 ^a ±0.05	8.95±2.77
R4	6.31 ^{ab} ±1.84	0.59 ^{ab} ±0.27	1.85±1.00	0.19 ^{abc} ±0.17	0.27 ^{ab} ±0.16	0.05 ^{abc} ±0.06	9.59±3.06
R5	6.02 ^{ab} ±2.18	1.19 ^{cd} ±0.28	1.84±0.67	0.46 ^{defg} ±0.19	0.50 ^{bcdef} ±0.19	0.07 ^{abc} ±0.04	10.48±3.03
R5FR	6.58 ^{ab} ±2.47	0.92 ^{abcdefg} ±0.15	1.95±0.74	0.34 ^{abcdefg} ±0.12	0.50 ^{bcdef} ±0.21	0.07 ^{abc} ±0.04	10.73±2.94
B1	5.82 ^{ab} ±1.41	0.85 ^{abcdef} ±0.36	1.18±0.59	0.23 ^{abcd} ±0.20	0.20 ^a ±0.15	0.04 ^{ab} ±0.06	8.70±2.00
B1K	5.00 ^{ab} ±1.64	1.33 ^{fg} ±0.34	1.97±0.90	0.38 ^{bcdefg} ±0.16	0.50 ^{bcdef} ±0.20	0.08 ^{abc} ±0.06	9.65±2.97
B2	6.35 ^{ab} ±3.04	0.48 ^a ±0.25	1.34±0.83	0.11 ^a ±0.15	0.16 ^a ±0.17	0.03 ^a ±0.05	8.81±4.08
B3	7.23 ^b ±2.22	0.72 ^a ±0.24	1.94±0.81	0.24 ^{abcd} ±0.12	0.32 ^{abcd} ±0.15	0.05 ^{abc} ±0.05	10.87±3.00
B4	5.69 ^{ab} ±2.28	1.02 ^{bcdefg} ±0.51	1.38±0.66	0.32 ^{abcdefg} ±0.23	0.35 ^{abcdef} ±0.20	0.05 ^{abc} ±0.06	9.23±3.20
B5	4.97 ^{ab} ±1.56	0.92 ^{abcdefg} ±0.39	1.33±0.45	0.34 ^{abcdefg} ±0.22	0.34 ^{abcde} ±0.18	0.03 ^a ±0.03	8.32±1.96
B6	6.78 ^{ab} ±1.83	0.44 ^a ±0.20	1.93±0.70	0.12 ^{ab} ±0.09	0.22 ^a ±0.13	0.02 ^a ±0.03	9.85±2.67
B6K	5.47 ^{ab} ±0.92	1.37 ^g ±0.13	2.18±0.36	0.51 ^{efg} ±0.10	0.61 ^f ±0.08	0.09 ^{abc} ±0.04	10.79±1.39
B7	6.00 ^{ab} ±2.67	0.84 ^{abcde} ±0.32	1.40±0.67	0.24 ^{abcd} ±0.18	0.28 ^{abc} ±0.17	0.04 ^{ab} ±0.06	9.21±3.44
B8	4.50 ^a ±1.92	1.15 ^{cd} ±0.47	1.76±1.03	0.43 ^{cdefg} ±0.27	0.53 ^{cdef} ±0.26	0.09 ^{abc} ±0.06	8.93±3.59
B9	6.01 ^{ab} ±2.06	0.60 ^{ab} ±0.41	1.48±0.84	0.16 ^{ab} ±0.24	0.21 ^a ±0.22	0.04 ^{ab} ±0.06	8.81±3.43
B10	6.24 ^{ab} ±1.51	0.77 ^{abcd} ±0.36	1.72±0.67	0.26 ^{abcde} ±0.19	0.34 ^{abcde} ±0.18	0.05 ^{abc} ±0.06	9.82±2.20
B11	5.18 ^{ab} ±2.00	0.77 ^{abcd} ±0.52	1.37±0.71	0.25 ^{abcde} ±0.20	0.29 ^{abc} ±0.18	0.05 ^{abc} ±0.05	8.29±2.59
B12	7.09 ^{ab} ±2.21	0.75 ^{abcd} ±0.44	1.50±0.84	0.28 ^{abcdef} ±0.28	0.35 ^{abcdef} ±0.24	0.04 ^{abc} ±0.06	10.37±3.28
p-value	p = 0.005	p < 0.0001	p = 0.005	p < 0.0001	p < 0.0001	p < 0.0001	p = 0.394

Means with different superscripts differ significantly.

4.5.3.2 n-6 : n-3

Nowadays consumers are well aware of the importance of red meat in the human diet as it is a rich source of iron and zinc. Therefore, to achieve the recommended levels of n-3 and n-6 FA's, it has been suggested that the level of these health-claimable FA's can be used to market meat (Pethick et al., 2006). A 2010 lamb consumer survey supports this fact as 65% of the participants considered n-3 FA as an important part of their diet (Lamb, Van de Ven & Hopkins, 2010).

Physicians and dieticians have pointed out that meat should contain a small amount of fat with an appropriate FA profile, especially focusing on the n-6 : n-3 ratio (Janiszewski, Grześkowiak, Lisiak, Borys, Borzuta, Pospiech & Poławska, 2016). The ratio n-6 : n-3 PUFA is also a risk factor in cancers and coronary heart disease (Enser, 2001), and is recommended to be as close as possible to 1:1. Diets with ratios of less than 1:1 inhibit the conversion of linoleic acid to long chain fatty acids (Bezerra et al., 2016). The 1:1 ratio is not realistic for the modern Western diet that is characterized by a n-3 deficiency and excessive n-6, thus are values of above 4 considered acceptable (WHO, 2003). Wood et al. (2003) also recommended a ratio of 5:1.

The Karoo and grass-fed production systems had significantly ($p < 0.001$) lower n-6 : n-3 ratios than the grain-fed production systems (Table 4.7). This was consistent with a study by Wood et al. (2003) who found particularly low n-6 : n-3 ratio in grass fed animals. Our ratios were much higher than 1:1 (more pork like according to literature) and similar to those found by Campo et al. (2016) who reported a n-6 : n-3 ratio close to 10:1 for grain-fed lamb. However, the n-6 : n-3 ratios of the Karoo and grass-fed production systems were still below 4 with the grain-fed production systems having an average ratio of 7.73. Enser et al. (1998) also noted that the finishing of lamb on pastures will lead to a low ratio with a value of about 2 while finishing on concentrates will lead to a ratio of about 6-10.

4.5.3.3 *Atherogenicity index:*

Knowledge about the FA profile of meat allows indexes such as the atherogenicity and desaturase indexes to be calculated for the use in human nutrition (Leticia, Paola, Jordi, Julio & Sancho, 2017). The atherogenicity index (AI) is used as an indicator of the level of atherogenicity of promotion of cardiovascular disease. This is accomplished using the method introduced by Ulbricht and Southgate (1991) which indicates the sum of the SFA's and the sum of the UFA's that are anti-atherogenic.

The Karoo production system had a significantly ($p < 0.001$) higher AI than the grass- and grain-fed production systems (Table 4.7). In this regard the grain-fed products had the lowest value (0.82). In our study the values ranged between 0.73 and 1.00 (R2K) (Figure 4.10; $p < 0.001$). In a study by Carvalho et al. (2015) it was noted that a higher UFA's concentration, lower palmitic acid concentration and decreasing myristic acid concentrations led to a lower AI. This same finding was made for sample B6 in our study which had the lowest AI.

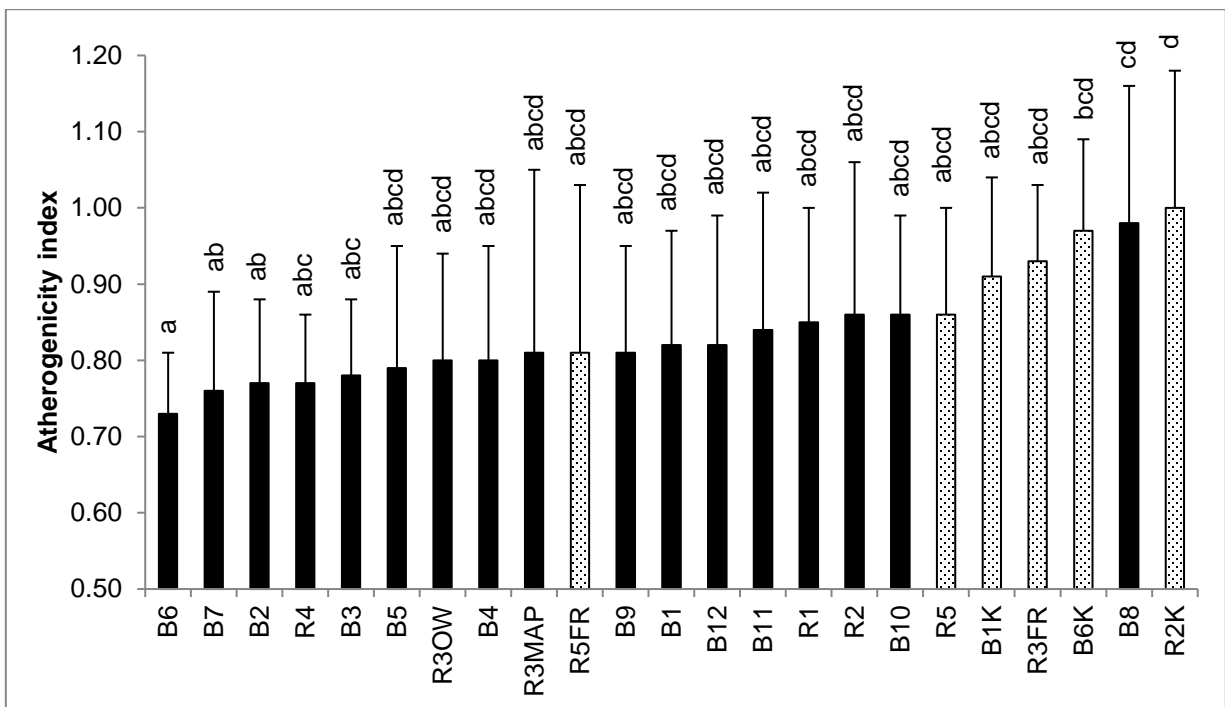


Figure 4.10: Variation in Atherogenicity index over a range of products sampled across various retailers and butcheries (R: retailer, B: butchery, K: Karoo, FR: grass-fed). ($p < 0.001$). Bars with different superscripts differ significantly.

4.5.3.4 Desaturase index:

The FA composition of meat is in part the consequence of endogenous FA desaturation. This desaturation is catalyzed by enzymes introducing a double bond in the FA chains. Stearoyl-CoA desaturase-1 (SCD-1) synthesizes MUFA from SFA such as $\Delta 9$ -desaturase which converts SFA into *cis*-9 MUFA, whereas $\Delta 4$ -, $\Delta 5$ - and $\Delta 6$ -desaturases which convert C18 PUFA's into C20-22 PUFA's and elongase which converts C16:0 into C18:0 (Vessby, Gustafsson, Tengblad, Boberg & Andersson, 2006; Bressan, Rossato, Rodrigues, Alves, Bessa, Ramos & Gama, 2011). This alteration of the FA composition is associated with insulin resistance and CVD with inflammation being a possible mediator, however only limited data are available on the effect of specific fatty acids on insulin resistance (Pettersson, Basu, Cederholm & Risérus, 2008).

The opposite results from the atherogenicity index were found for the desaturase index with the grain-fed production system having a significantly ($p < 0.001$) higher value than the Karoo and grass-fed production systems (Table 4.7). In this regard values between 1.71 (R1) and 3.60 (B9) were recorded in our study (Figure 4.11; $p < 0.001$). The main reason for this finding may be due to the high $\Delta 9$ -desaturase activity which led to the significantly ($p < 0.001$) higher total MUFA (Table 4.5) and significantly ($p < 0.001$) lower total SFA (Table 4.4) contents of the grain-fed products. We also assume that the key contributor was the significantly ($p < 0.001$) higher oleic acid (C18:1c9) content of these products (Table 4.5).

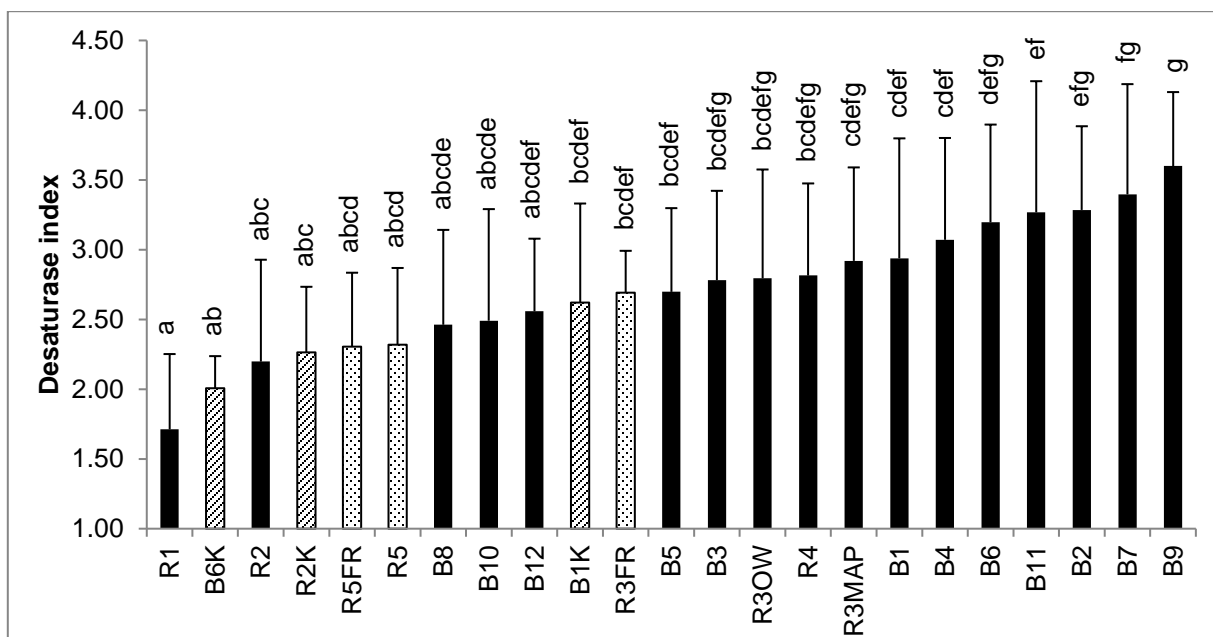


Figure 4.11: Variation in Desaturase index over a range of products sampled across various retailers and butcheries (R: retailer, B: butchery, K: Karoo, FR: grass-fed). ($p < 0.001$). Bars with different letters differ significantly.

Principle components analysis (PCA) is a multivariate technique that describes the relationship between response variables, and also explains the total variation in the data. ‘Components’ refer to the equations that are constructed from the original variables. This is a very useful tool, especially for research, where the variables under study are highly correlated or when a large number of independent variables are present (Alkarkhi & Alqaraghuli, 2018).

The clear significant differences in certain fatty acids and fatty acid ratios between grain-fed and Karoo lamb found by Analyses of Variance (Tables 4.4, 4.5 and 4.7), were confirmed with principle component analyses where grain-fed and Karoo clustered on opposite sides of dimension 1. Fatty acids from the grass-fed meat that were sometimes in agreement with grain-fed and other times in agreement with Karoo, clustered inbetween grain-fed and Karoo of dimension 1. Dimension 1 of the PCA bi-plot explain 75.6 % of the variation, whereas dimension 2 explain 24.4%. On the PCA bi-plot (Figure 4.12) it is very clear that the free-range products are associated with healthier FA’s such as phytanic acid (C20:0,3,7,11,15-tetramethyl hexanoic acid), CLA (18:2c9t11 n-6), total n-3 PUFA and PUFA:SFA ratio. Subsequently the grain-fed products had a healthy oleic acid (C18:1c9) and MUFA content as well as a better n-6 : n-3 ratio. Martínez et al. (2018) noted on the impact of the IMF content on the FA composition, and stated that a high IMF are associated with a higher total MUFA’s and lower n-6 PUFA, n-3 PUFA and total PUFA’s contents. The results from our study were consistent with this finding as the grain-fed production system did present the highest IMF content (Table 4.3). As phytanic acid is

synthesized from phytol cleaved from chlorophyll a higher amount is expected in the free-range products as these animals mostly grazed on greens.

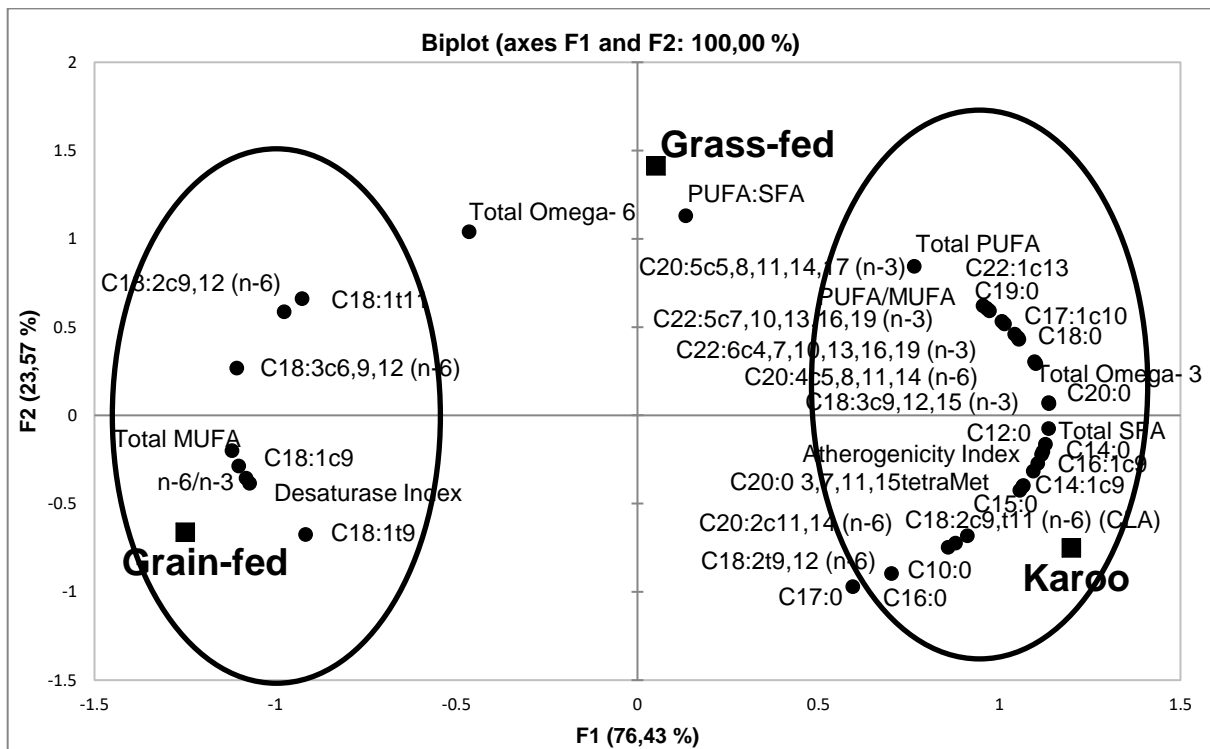


Figure 4.12: Principle component analysis bi-plot of the variation between fatty acid compositions of the products from three different production systems (Karoo / Grass-fed / Grain-fed).

During biohydrogenation linoleic acid (C18:2) and linolenic acid (C18:3) are converted to CLA. Subsequently, CLA is converted to vaccenic acid (C18:1t11) which turns out as stearic acid (C18:0) in the end product (Cited by Carvalho et al., 2015). Oleic acid is formed by $\Delta 9$ desaturase from stearic acid (C18:0) (Díaz, Álvarez, De La Fuente, Sañudo, Campo, Oliver, Font i Furnols, Montossi, San Julián, Nute & Cañeque, 2005; Wood et al., 2008). With the high $\Delta 9$ -desaturase activity in the products from the grain-fed production system (Table 4.7), the results with regards to the CLA and oleic acid contents can also be anticipated.

4.6 Physical characteristics

4.6.1 Meat colour:

The colour of meat is perceived as an important parameter that has an influence on the purchasing choice of consumers (Zervas & Tsiplakou, 2011). Therefore, the colour of meat is also regarded as an important trait for economic reasons as an undesirable colour can cause the industry to lose money (Hughes, Kearney & Warner, 2014). Changes in the colour of meat are connected to protein

denaturation because of the relation with either globin denaturation or heme group displacement (Carlez, Veciana-Nogues & Cheftel, 1995).

Aggregation of the myofibrillar and sarcoplasmic proteins also has a connection to the loss in colour and cause changes in the opaqueness of meat (Guyon et al., 2016). Due to meats' translucent nature the light that is measured at the surface is reflected from the surface and different depths within the meat (Jacob et al., 2014). Jacob and Thomson (2012) speculated that chilling conditions during processing can potentially influence the reflectance of light. The colour of meat can be managed through manipulation of non-genetic factors which operate at different times in the supply chain (Jacob, D'Antuono, Smith, Pethick & Warner, 2007). In fatter carcasses a slow cooling rate of muscles leads to rigor being attained at higher temperatures. The slow cooling rate is connected to a rapid pH decline which might be responsible for the differences in the colour of meat (Farouk & Lovatt, 2000). Traditionally, CIE-L* (black-white), a* (red-green) and b* (blue-yellow) values are used to quantify the colour of meat. The ratio of reflected light between the wavelengths 580 nm and 630 nm, known as "oxy/met", are used to determine the chemical changes in meat due to oxygenation or oxidation of myoglobin (Jacob et al., 2014). This ratio has also been connected with consumer preference for the colour of meat (Khlijji et al., 2010). Khlijji et al. (2010) noted on the acceptable L* and a* thresholds for lamb which is 34-35 and above and below 19.

At the point of sale consumers still demand lamb meat to be a brick red colour (Troy & Kerry, 2010). All of the samples had lightness (L*) values in the range of 37-41 with all of the grass-fed products having values well above 40 (Table 4.9; $p < 0.001$). According to Hopkins (1996) with values above 34 the meat will be light in colour and acceptable to consumers. However, there were no significant differences between the Karoo, grass-fed and grain-fed groupings (Table 4.10; $p = 0.137$). All of the redness (a*) values (Table 4.9; $p < 0.001$) were below 19, with values ranging from 14 to 18, meaning all the samples were regarded as acceptable (as prescribed by Khlijji et al., 2010) and the colour are more to the red end of the spectrum as to the opposing green end. Priolo et al. (2002) found that meat from stall-raised lambs was lighter in colour in the first 24h, and could be ascribed to the difference in ultimate pH seeing that high pH meats are darker in colour. Overall it was expected that the Karoo and grass-fed samples would be darker in colour compared to the grain-fed samples, as the grain-fed samples are most likely from animals that were supplemented with beta-adrenergic agonists. This is supported by the study of Dávila-Ramírez et al. (2013) who found lower L*, a* and b* values in lambs supplemented with zilpaterol hydrochloride. However, limited information is available on the effect of beta-adrenergic agonists on the colour of lamb meat. Some studies have also reported that meat from lambs fed forage-based rations have lower glycogen content in the muscle tissue, and subsequently a higher ultimate pH and darker colour meat when compared to lambs fed concentrate-based rations (Young, Daly, Graafhuis & Moorhead, 1997). Ponnampalam et al. (2015b) ascribed these findings to the lower energy intake due to the loss of dietary energy caused by factors such as gas associated with

degradation of high-fibrous roughage materials. Furthermore, animals fed forage-based diets spend more energy when walking long distances to harvest their food (Aguayo-Ulloa, Miranda-de la Lama, Pascual-Alonso, Fuchs, Olleta, Campo, Alierta, Villaroel & María, 2013), which leads to the production of less fat and less reflectance of light (Díaz et al., 2002). With regards to the yellowness (b^*), the difference between the products from the different production systems varied with values ranging from 10.20 (B6K) to 13.33 (R3FR) (Table 4.9). Our results are consistent with those of Thompson et al. (2005) who found similar b^* values for their lamb samples, with the average b^* value for their mutton samples being 9.90 respectively. Earlier in a study by Díaz et al. (2002) they found that the production systems only had an effect on the lightness (L^*) of the *longissimus dorsi* muscle, with the lambs fed on pastures having darker meat. Vestergaard et al. (2000) ascribed the small meat colour differences between the lambs of different production systems to the different physical activity undertaken, as more walking would lead to more red fibre types and haemic pigments in the muscle.

Research has shown that the A-illumination measurements are the more suitable to report on meat colour than D-65. Chroma_A ($p = 0.002$) show that more than half the samples have a value above 20 which means they display the bright red colour of bloomed meat where the rest are slightly dull but do not fall into the distinctly brown category yet. This means that the colour of lamb is quite acceptable across the full range of products (Table 4.9).

Once again the possible effects on colour from beta-adrenergic agonists commonly used in the feedlot regime would lead to the assumption that the Karoo and grass-fed samples will group together to have a higher chroma value (Hope-Jones et al., 2012). Mersmann (2002) noted that pale meat caused by beta-adrenergic agonists is probably caused by reduced heme pigmentation and a larger proportion of fast twitch glycolytic fibers. However, no pattern was found between grain-fed and free-range production systems (Table 4.10). It was also expected that either packaging or whether a sample was cut fresh (B5, B9 and B10) or was on display (B7) would have made a difference to the colour of the meat but there were no distinct patterns (Table 4.9). This is evidence that sheep meat is quite tolerant in terms of colour exposure.

The Hue angle indicates the proportions of red and yellow (Velasco, Cañeque, Lauzurica, Pérez & Huidobro, 2004). In our study the grass-fed production systems had a significantly ($p < 0.001$) higher Hue angle value than the Karoo and grain-fed production systems (Table 4.10). This was also true for the study by Priolo et al. (2002) who found higher values from the grass-fed animals and also noted that the wider the Hue angle, the more the colour corresponds to yellow. Therefore, the main reason for the higher Hue angle value for the products from the grass-fed production system was due to a numerically higher b^* (yellowness) component for these products. This will most probably be detected by consumers who will find these products unattractive. The Hue angle is in agreement with the brown colour of metmyoglobin meaning that these types of products were in storage for a longer period (Luciano et al., 2012).

Table 4.9: Average lightness (L*), redness (a*), yellowness (b*), chroma and hue angle values of a range of products sampled across various retailers and butcheries (R: retailer, B: butchery, K: Karoo, FR: grass-fed).

Sample ID:	L*	a*	b*	Chroma	Hue
R1	40.80 ^{bc} ±2.07	15.59 ^{abcd} ±3.21	10.49 ^a ±2.90	18.87 ^{ab} ±4.15	32.71 ^{ab} ±5.03
R2	40.37 ^{abc} ±2.43	16.11 ^{abcd} ±2.72	11.04 ^a ±1.97	19.63 ^{ab} ±2.92	34.33 ^{abc} ±5.14
R2K	38.57 ^{ab} ±2.06	15.18 ^{ab} ±2.95	10.84 ^a ±3.00	19.23 ^{ab} ±4.60	33.99 ^{abc} ±4.09
R3FR	40.49 ^{abc} ±2.08	16.87 ^{abcd} ±2.55	13.33 ^a ±1.43	21.57 ^{ab} ±2.77	38.77 ^c ±2.50
R3MAP	39.22 ^{abc} ±2.47	16.46 ^{abcd} ±2.56	11.29 ^a ±1.48	19.37 ^{ab} ±2.16	36.51 ^{bc} ±5.27
R3OW	39.29 ^{abc} ±2.45	15.36 ^{abc} ±2.26	11.52 ^a ±2.29	19.03 ^{ab} ±3.11	35.07 ^{abc} ±6.00
R4	39.57 ^{abc} ±3.31	17.17 ^{abcd} ±2.95	11.54 ^a ±2.72	20.81 ^{ab} ±3.74	34.06 ^{abc} ±2.49
R5	41.60 ^{bc} ±1.68	14.10 ^a ±1.59	10.68 ^a ±2.19	17.77 ^a ±2.30	36.72 ^{bc} ±4.73
R5FR	40.52 ^{abc} ±2.44	17.46 ^{bcd} ±2.40	12.04 ^a ±1.89	21.27 ^{ab} ±2.70	34.41 ^{abc} ±4.07
B1	41.25 ^{bc} ±2.67	16.81 ^{abcd} ±2.28	11.44 ^a ±2.46	20.39 ^{ab} ±3.11	33.81 ^{abc} ±3.65
B1K	39.92 ^{abc} ±2.90	17.20 ^{abcd} ±2.43	11.35 ^a ±2.72	20.67 ^{ab} ±3.38	32.84 ^{ab} ±3.89
B2	41.33 ^{bc} ±2.31	15.21 ^{abc} ±1.40	11.13 ^a ±2.06	18.91 ^{ab} ±2.05	35.89 ^{bc} ±4.43
B3	39.92 ^{abc} ±2.07	17.91 ^{bcd} ±2.88	11.89 ^a ±3.18	21.28 ^{ab} ±4.38	32.20 ^{ab} ±4.37
B4	41.49 ^{bc} ±2.42	15.41 ^{abc} ±2.31	11.07 ^a ±1.86	19.03 ^{ab} ±2.70	35.42 ^{abc} ±3.53
B5	41.00 ^{bc} ±2.49	17.28 ^{bcd} ±2.01	11.05 ^a ±2.63	20.58 ^{ab} ±2.99	32.10 ^{ab} ±3.96
B6	41.40 ^{bc} ±1.83	15.98 ^{abcd} ±2.23	11.77 ^a ±2.28	19.89 ^{ab} ±2.97	36.10 ^{bc} ±3.47
B6K	40.98 ^{bc} ±1.59	15.81 ^{abcd} ±1.73	10.20 ^a ±2.02	18.87 ^{ab} ±2.51	32.12 ^{ab} ±2.46
B7	41.08 ^{bc} ±1.61	17.54 ^{bcd} ±1.20	10.72 ^a ±2.32	20.69 ^{ab} ±2.13	31.35 ^{ab} ±4.39
B8	37.50 ^a ±1.98	18.67 ^d ±1.78	10.93 ^a ±2.27	21.71 ^{ab} ±2.43	30.06 ^a ±4.06
B9	42.33 ^c ±2.47	17.81 ^{bcd} ±1.43	10.96 ^a ±2.33	20.99 ^{ab} ±2.28	31.28 ^{ab} ±4.26
B10	41.05 ^{bc} ±2.84	17.83 ^{bcd} ±2.17	10.82 ^a ±3.36	20.69 ^{ab} ±3.16	29.88 ^a ±4.64
B11	39.10 ^{ab} ±1.73	17.65 ^{bcd} ±1.70	10.95 ^a ±1.53	20.86 ^{ab} ±2.02	31.77 ^{ab} ±2.68
B12	41.37 ^{bc} ±1.90	18.32 ^{cd} ±2.02	13.18 ^a ±1.81	22.61 ^b ±2.50	35.53 ^{abc} ±2.64
p-value	p < 0.001	p < 0.001	p = 0.092	p = 0.002	p < 0.001

Means with different superscripts in the same column differ significantly.

Meats' ability to remain red during display is called colour stability. In this regard lamb meat is said to be less stable than beef (Gutzke & Trout, 2002). Faustman and others (2010) ascribed this in part to differences in the sequence of amino acids in the globin moiety of myoglobin, which during oxidation interact with the aldehyde compounds that are produced. Due to oxygenation or oxidation of myoglobin colour changes are found in meat over time (Jacob, D'Antuono, Gilmour & Warner, 2014). During this process myoglobin becomes red (oxymyoglobin) due to oxygenation whilst deoxygenated myoglobin is purple in colour, which turns brown (metmyoglobin) due to oxidation (Faustman, 1990). After meat is

sliced “blooming” takes place where the purple surface of the meat turns red which extends to a few millimeters below the surface (Krzywicki, 1979).

In meat, a decrease in redness and saturation (C^*) and an increase of the hue angle relates to the oxidation of myoglobin over time (Khliji et al., 2010). The product sold on display (B7) that was probably cut in the morning, as well as the three freshly cut products (B5, B9 and B10) all showed very low metmyoglobin (MMb) levels (Figure 4.13; $p < 0.001$) as would be expected. Modified atmosphere packaged (MAP) samples, with their increased available oxygen, showed desirable Chroma_A values for R5FR but not for R3MAP although this was not significant. Oxymyoglobin (OxyMb) levels (Figure 4.13; $p < 0.001$) that were consistent with the Chroma_A values were higher for R5FR than for R3MAP. Similar patterns, or lack thereof, were however observed for all myoglobin levels (Figure 4.13; Table 4.10). Overall, the grain-fed production system had significantly ($p < 0.001$) lower metmyoglobin values than the other two production systems. For the deoxymyoglobin levels it was the grass-fed production system that scored significantly ($p < 0.001$) lower. No significant ($p < 0.001$) difference was detected in the oxymyoglobin levels of the three production systems (Table 4.10).

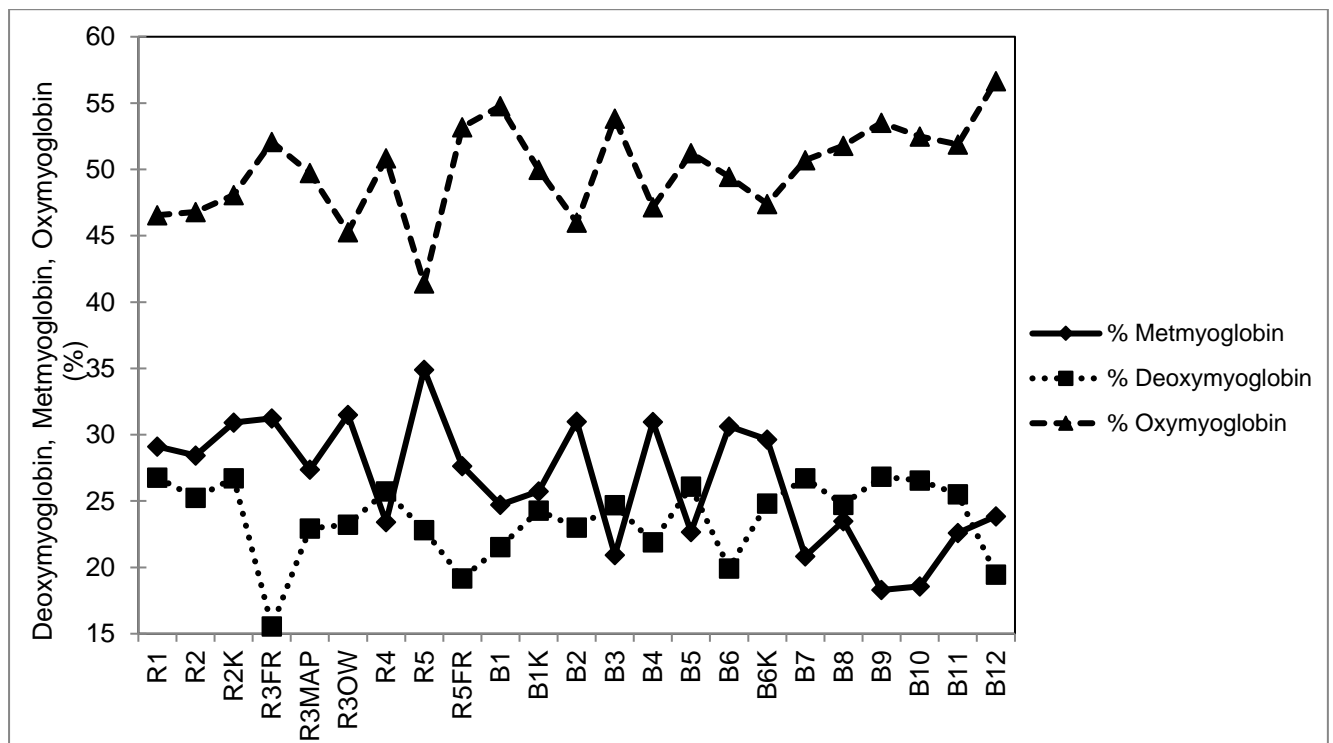


Figure 4.13: Variation in percentage metmyoglobin, deoxymyoglobin and oxymyoglobin over a range of products sampled across various retailers and butchereries (R: retailer, B: butchery, K: Karoo, FR: grass-fed). ($p < 0.001$).

Table 4.10: Variation between colour parameters of the products from three different production systems (Karoo / Grass-fed / Grain-fed).

	Karoo	Grass-fed	Grain-fed	p-value
L*	39.82±2.41	40.87±2.10	40.50±2.55	p = 0.137
a*	16.06±2.51	16.14±2.62	16.89±2.43	p = 0.053
b*	10.79±2.59	12.01±2.12	11.28±2.37	p = 0.059
Chroma	19.59±3.60	20.21±3.08	20.31±3.04	p = 0.384
Hue	32.98 ^a ±3.55	36.63 ^b ±4.19	33.42 ^a ±4.59	p < 0.001
Metmyoglobin (%)	28.77 ^b ±5.63	31.26 ^b ±6.17	25.20 ^a ±6.89	p < 0.001
Deoxymyoglobin (%)	25.28 ^b ±6.66	19.19 ^a ±5.02	24.17 ^b ±6.44	p < 0.001
Oxymyoglobin (%)	48.48±6.49	48.89±8.57	50.51±8.01	p = 0.186

Means with different superscripts in the same row differ significantly.

4.6.2 Cooking loss:

Cooking loss consists of about 80% evaporation loss and 20% drip loss (fat and meat juices). The main structural elements which impact the loss of water from muscles is myofibrillar lattice spacing, membrane permeability, extracellular space and drip channel formation (Offer & Cousins, 1992). Hughes et al. (2014) found that water loss has an inverse relationship to juiciness which leads to a positive correlation between sensory juiciness and tenderness. Cooking loss is relevant to the consumer as it determines the final yield of the cooked product and affects eating quality, e.g. juiciness and tenderness perceptions. Factors influencing the cooking loss of meat include amount of purge before cooking, thickness of the cut, cooking temperature and amount of fat (Huff-Lonergan & Lonergan, 2005).

Table 4.11 indicates the mean cooking losses found between the different production systems in our study which include % drip loss, % thawing loss, % cooking loss and % evaporation loss. With regards to the % cooking loss (p = 0.000), % evaporation loss (p < 0.001) and % thawing loss (p < 0.001) the grass-fed products differed significantly from the Karoo and grain-fed products, with the highest percentages. Cooking losses are the losses that occur when meat is prepared for consumption and are affected by the diet, slaughter weight, genetics, water-holding capacity and fat (Sañudo, Campo, Sierra, María, Olleta & Santolaria, 1997). Several studies have noted that concentrate-finished lambs have better WHC than lambs finished on pastures (Santos-Silva, Mendes & Bessa, 2002; Karaca, Erdoğan, Kor & Kor, 2016). However, in a more recent study by Bezerra et al. (2016) the diet did not affect the cooking losses. It must be noted that the actual average thawing loss was very low (± 0.2 %). The fact that the grass-fed products had the highest percentages of cooking loss, evaporation loss and thawing loss is a bit concerning as it is sold as a superior product with a significantly higher price than the Karoo and grain-fed products. It should however be taken into account that these products also had the least amount of IMF which influence water retention as mentioned earlier.

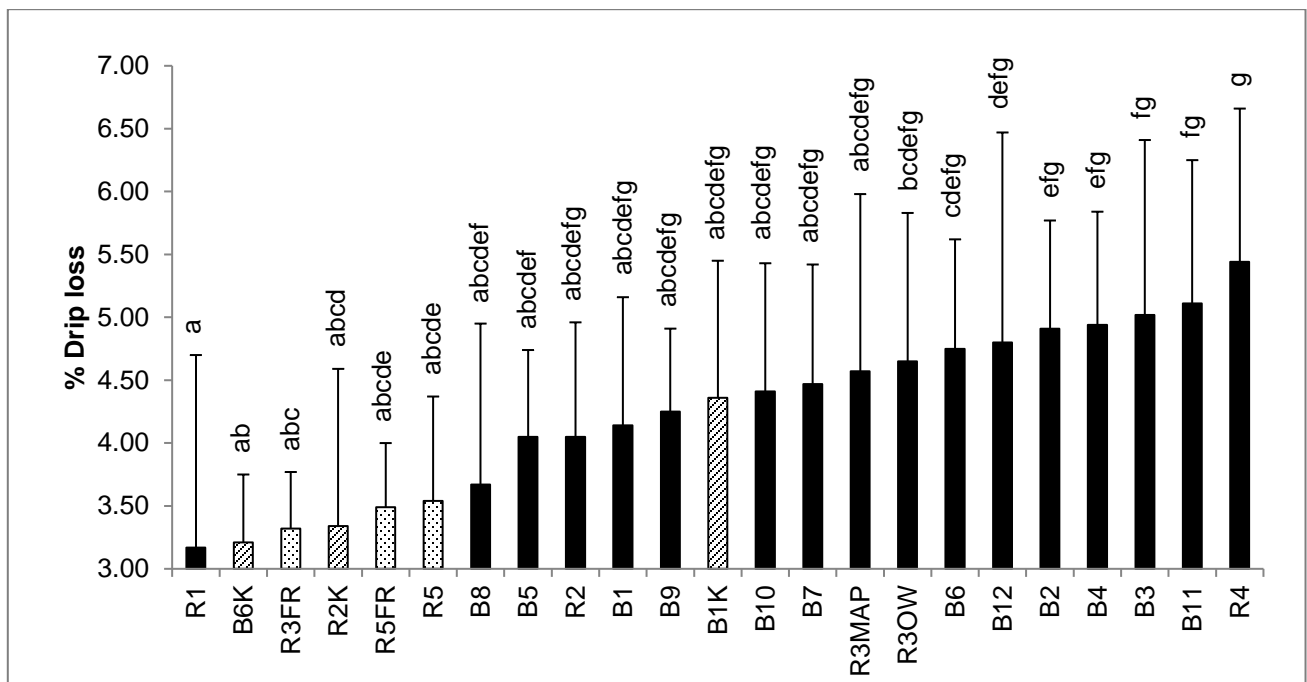


Figure 4.14: Variation in percentage drip loss over a range of products sampled across various retailers and butcheries (R: retailer, B: butchery, K: Karoo, FR: grass-fed). ($p < 0.001$). Bars with different letters differ significantly.

The overwrapped (PVC-OW) and modified atmosphere packaged (MAP) products from R3 were very similar in drip loss with the MAP (4.57%) product being only numerically better than the OW (4.65%) product (Figure 4.14; $p < 0.001$). There were similarities between the percentages drip loss and dissectible fat with samples R1 and B6K also having the lowest percentages fat and sample B1K the highest of the free-range products.

The drip loss discussed in this section is not purge found in the drip tray of the products but drip loss during the cooking process. In terms of the % drip loss, the grain-fed products had a significantly ($p < 0.001$) higher value than the Karoo and grass-fed products (Table 4.11). It is clear that the % fat did not have an effect in this instance as the grain-fed products also had a significantly higher amount of fat.

Table 4.11: Variation between cooking loss (%), evaporation loss (%), thawing loss (%) and drip loss (%) of the products from three different production systems (Karoo / Grass-fed / Grain-fed).

	Karoo	Grass-fed	Grain-fed	p-value
Cooking loss (%)	18.20 ^a ±1.68	19.92 ^b ±1.70	18.85 ^a ±1.92	$p < 0.001$
Evaporation loss (%)	14.31 ^a ±1.60	16.26 ^b ±1.51	14.25 ^a ±1.81	$p < 0.001$
Thawing loss (%)	0.18 ^a ±0.15	0.53 ^b ±0.27	0.21 ^a ±0.21	$p < 0.001$
Drip loss (%)	3.64 ^a ±1.11	3.45 ^a ±0.61	4.49 ^b ±1.23	$p < 0.001$

Means with different superscripts in the same row differ significantly.

Some of the samples were purchased frozen due to the drought conditions (the butchery therefore trying to “save” Karoo lamb so that it could be available over the drought period when no more could be obtained). The freezing could therefore reduce the WHC which resulted in drip in the form of meat stock.

4.7 Tenderness characteristics

4.7.1 Warner Bratzler shear force and myofibrillar fragment lengths:

The Warner Bratzler shear force (WBSF) method is used to mechanically determine the tenderness of meat, with higher values indicating tougher meat. This is the maximum shear force that is employed to a cut of a cooked subsample, using a cutting plane across the myofibrils (Holman, Alvarenga, van de Ven & Hopkins, 2015). Warner Bratzler shear force can be affected by many factors such as handling prior and at slaughter, age, breed, sarcomere length, type of muscle and cooking manipulation (Abdullah & Qudsieh, 2009). Starkey et al. (2016) explained how IMF would also influence WBSF, for instance, a 3 to 4% increase in IMF would lead to a 0.40 kg decrease in shear force. Over the years lots of research has led to the development of a shear force threshold, describing the tenderness that would be acceptable to consumers (Holman, Fowler & Hopkins, 2016). In a study by Hopkins et al. (2006) they noted that 2.75 kg (used a Model LRX with attached Warned Bratzler blade) is the upper limit for lamb tenderness to be regarded as satisfactory by consumers. Likewise, Aalhus et al. (2004) reported that 4.90 kg is a satisfactory threshold for beef tenderness.

In Figure 4.15 a box-and-whisker plot is used to illustrate the shear force tenderness that was recorded by each of the outlets over the 14 weeks of sampling. A box-and-whisker plot is ideal for representation as it shows the distribution of the dataset at a glance, and as WBSF is such an important attribute in this study it is important to show more detail. The top of the whiskers shows the maximum (greatest value excluding the outliers) and the bottom part the minimum (smallest value excluding the outliers). The dots on the graph represent the outliers and the box the upper and lower quartile. Inter-quartile range is used to describe the range of scores from the lower to upper quartile. The middle 50% of scores fall within the inter-quartile range. The + is the average and the line in the box is the median (Flowingdata, 2008).

Differences between the WBSF values (Figure 4.15) of the products from the different production systems were not big however, still significant ($p = 0.003$). The product with the highest value (least tender; R1; 2.71 kg) only differed by 0.67 kg from the product with the lowest value (most tender; R2K; 2.04 kg). The least tender product was from the grain-fed production system while the most tender product was a Karoo product. This confirms that the tenderness of meat is a multi-factorial aspect. The fact that some of the samples were frozen had no effect on the shear force values. In fact, previous research has shown improved tenderness in lamb meat that was chilled then frozen (Kim et al., 2011).

In contrast, it was noted that lamb kept under frozen storage of 9 months to 2 years showed minimal effect on eating quality (Fernandes et al., 2013).

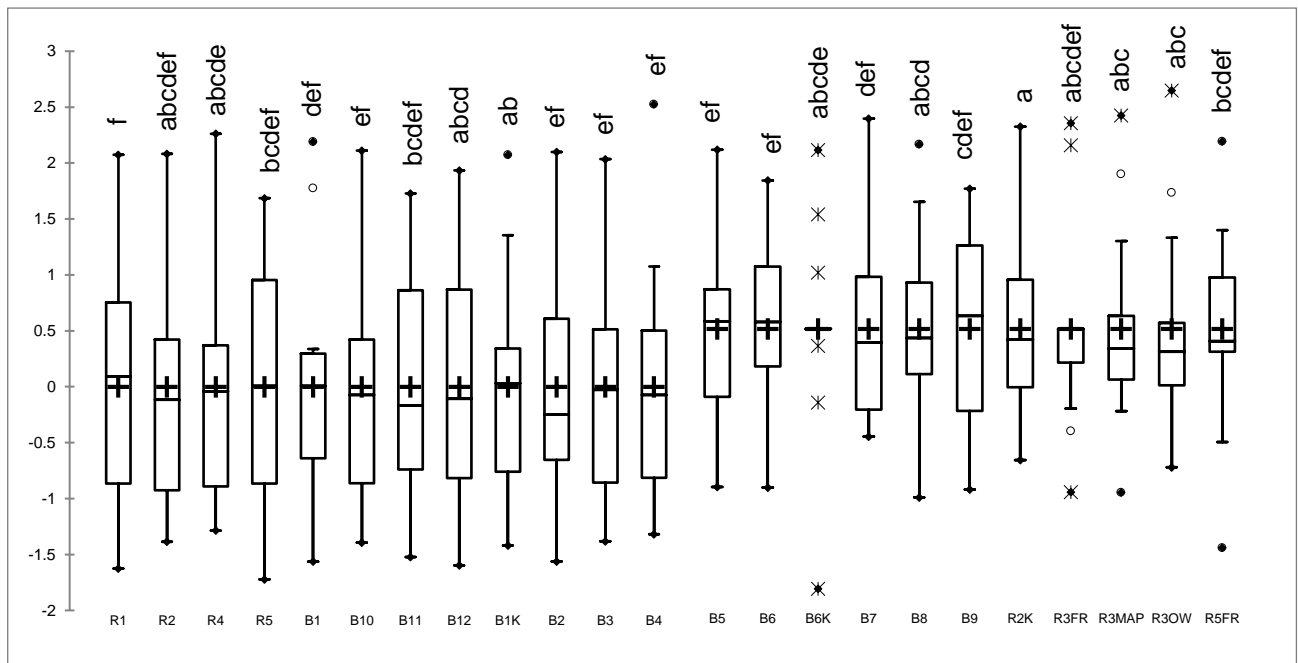


Figure 4.15: Box-and-whisker plot graph of the WBSF values recorded by each of the outlets over the 14 weeks (R: retailer, B: butchery, K: Karoo, FR: grass-fed). ($p = 0.003$).

The differences in MFL ($p = 0.737$) and shear force ($p = 0.092$) between the different packaging (fresh cut / MAP / PVC-OW) are presented in Table 4.12. As indicated the fresh cut samples (B5, B9 and B10) had the longest MFL's as well as the highest shear force values (Table 4.12; Figure 4.17). However, it should also be noted that these samples were all from the grain-fed production system and as already mentioned the grain-fed products were the least tender. Only 2 of the 23 products had MAP packaging, one product was a grain-fed product (R3MAP) and the other a grass-fed product (R5FR). These products had the shortest MFL's and lowest shear force values. When the results of Table 4.12 are compared to Table 4.13, the same number of variations was recorded for all the fresh-cut products (5 out of 14) with the MAP packaging giving variations of 2 and 3 out of 14. This means that in terms of the packaging the products can be regarded as consistently tender.

As mentioned under the price section, attributes such as % meat, % fat, % bone and WBSF values can help to determine which product will be the best value for money. It is clear that the Karoo products were significantly ($p < 0.001$) more tender than the grass-fed and grain-fed products. Even though the price of the Karoo products was significantly ($p < 0.001$) higher than that of the grain-fed products, the good WBSF values along with the meat percentage and lowest fat content makes the Karoo products better value for money (Table 4.14).

Table 4.12: Difference in MFL and Shear Force between products with different packaging (Fresh cut / MAP / PVC-OW).

Packaging method	MFL	Shear force
Fresh cut	27.15 ± 4.92	2.59 ± 0.61
MAP	26.25 ± 5.25	2.31 ± 0.48
PVC-OW	26.87 ± 4.90	2.45 ± 0.56
p-value	p = 0.737	p = 0.092

Inconsistencies in the WBSF of the 23 products over the 14 weeks are demonstrated in Figure 4.16. This illustration is based on Walter A. Shewhart's control chart which was developed in the 1920's (Mohammed, Cheng, Rouse & Marshall, 2011). The chart has been adapted to accommodate the Hopkins et al. (2006) threshold (2.75 kg) to which our products was measured to determine satisfactory tenderness. Usually the mean value would be used as a norm with the outer limits being calculated as 2 to 3 times the standard deviation of recording during a process.

In Figure 4.16 the total mean value, Hopkins et al. (2006) upper limit (2.75 kg), values of the most tender product (R2K) as well as values of the least tender product (R1) are indicated. The mean value (2.50 kg) represents the average WBSF value of all 306 products. As mentioned, when compared to the Hopkins et al. (2006) threshold all of the products that were part of this study can be classified as tender. It is interesting to see that the most tender product (R2K) was consistently tender over the 14 week sampling period while the least tender product (R1) varied over this time. Over the 14 weeks, sample R2K never had a value above the 2.75 kg threshold with the lowest value being below 2.00 kg and the highest value just above the mean value of 2.50 kg. The event of a value being above 2.50 kg happened only on one of the 14 occasions. On the contrary, sample R1 had values above 2.50 kg and the 2.75 kg threshold on numerous occasions.

In Table 4.13 the variation in tenderness over the 14 week sampling period was also demonstrated. Each sample, with the number of times that a WBSF value above the Hopkins et al. threshold (2.75 kg) was recorded and their mean WBSF value are indicated. When compared to the results of a beef audit in 2015 (Van Wyngaard, 2015), South African retail lamb is more consistently tender than the beef sold at retail. In our lamb audit the highest number of variations was 7 out of 14, for only one of the samples, whereas the beef audit presented 13 samples with 10 out of 20 and above variations (total of 21 samples; retail threshold of 4.6 kg). Furthermore, only two of the Karoo products in our study (R2K and B6K) had 0 values above the 2.75 kg threshold. The other Karoo product (B1K) had one value above the threshold but also presented the second lowest mean WBSF value (2.11 kg). The one sample with 7 values above the threshold was R1, a grain-fed product. This sample was also regarded as the least tender although, a mean WBSF value of 2.71 kg was recorded for this product which is still below the 2.75 kg threshold. The Karoo products had the least variations (1 out of 14) with

the grass-fed products having slightly more with up to 5 out of 14. However, an overall conclusion is that the free-range products (Karoo and grass-fed) were more consistently tender than the feedlot products.

Table 4.13: Number (out of 14) of incidents where the WBSF measured above the Hopkins threshold and the mean WBSF (Hopkins et al., 2006).

Sample Code	Production system	Hopkins threshold >2.75 kg	Mean WBSF(kg)
B6K	Karoo	0	2.25 ^{abcde}
R2K	Karoo	0	2.04 ^a
B12	Grain fed	1	2.20 ^{abcd}
B1K	Karoo	1	2.11 ^{ab}
B8	Grain fed	1	2.19 ^{abcd}
R3FR	Grass fed	2	2.42 ^{abcdef}
R3MAP	Grain fed	2	2.13 ^{abc}
R3OW	Grain fed	2	2.16 ^{abc}
R2	Grain fed	3	2.38 ^{abcdef}
R4	Grain fed	3	2.28 ^{abcde}
R5FR	Grass fed	3	2.49 ^{bcdef}
B4	Grain fed	4	2.62 ^{ef}
B7	Grain fed	4	2.57 ^{def}
R5	Grass fed	5	2.49 ^{bcdef}
B1	Grain fed	5	2.57 ^{def}
B10	Grain fed	5	2.63 ^{ef}
B3	Grain fed	5	2.63 ^{ef}
B5	Grain fed	5	2.63 ^{ef}
B9	Grain fed	5	2.52 ^{cdef}
B11	Grain fed	6	2.45 ^{bcdef}
B2	Grain fed	6	2.62 ^{ef}
B6	Grain fed	6	2.62 ^{ef}
R1	Grain fed	7	2.71 ^f
		p-value	p = 0.003

Table 4.13 also indicates that the Karoo (R2K, B1K and B6K) products were more tender ($p = 0.003$) than the grass-fed (R3FR, R5 and R5FR) and grain-fed products, with lower shear force values. All the grass-fed products had shear force values ranging from 2.42-2.46 kg. Previous literature (Priolo et al., 2002; Perlo, Bonato, Teira, Tisocco, Vicentin, Pueyo & Mansilla, 2008) found that meat from feedlot lambs was more tender than meat from grass-fed lambs. In South Africa however, most feedlots make use of beta-adrenergic agonists which could explain this difference in tenderness between grain-fed and Karoo products. In fact for WBSF the Karoo products from a particular store were almost on the opposite end of the spectrum to the feedlot counterparts from the same store (B6 and to a certain extent R2 and B1) (Table 4.13). The average WBSF for the Karoo products was 2.13 kg and 2.47 kg for both the grass- and grain-fed products (Table 4.14; $p = 0.001$).

According to the threshold set by Hopkins et al. (2006) all of the products were considered as satisfactory by the consumers. There was a slight correlation (0.3467; $p = 0.001$) between WBSF and myofibrillar fragment lengths (MFL) (Appendix 1) but this will be discussed in the next section.

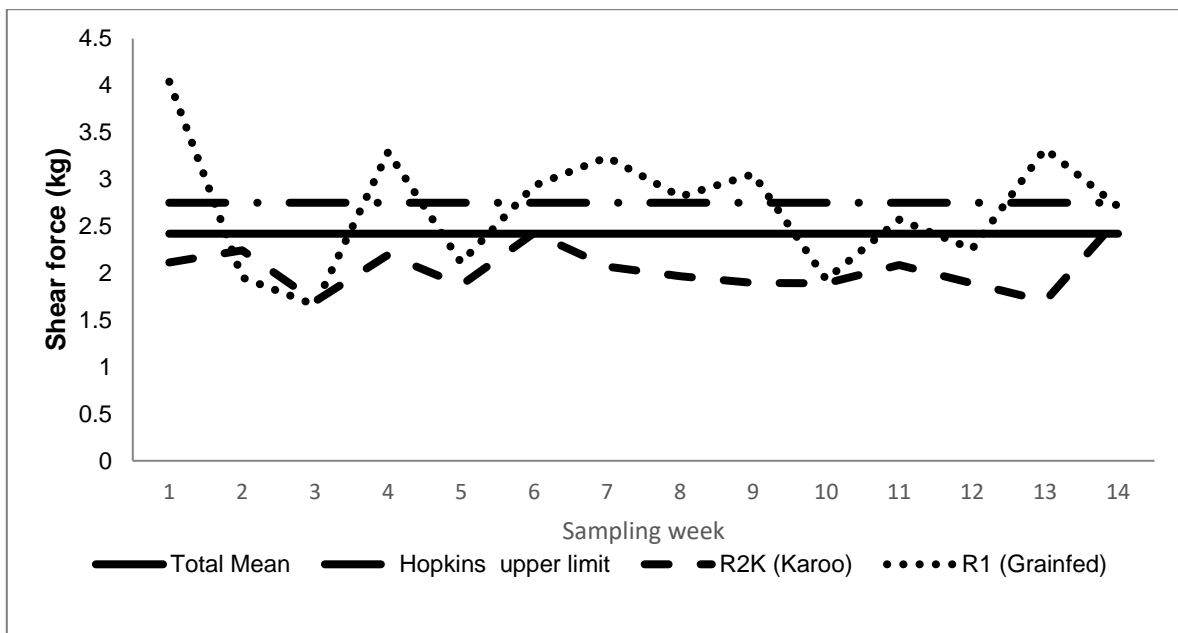


Figure 4.16: Variation in WBSF of R2K (most tender) and R1 (toughest) over a range of products sampled across various retailers and butchereries over a 14 week period (R: retailer, B: butchery, K: Karoo, FR: grass-fed).

The extent of the muscle structural and associated proteins alteration determine the final tenderness of meat (Hopkins & Taylor, 2002). Alteration would include the cleavage of major myofibrillar proteins such as actin and myosin, which is responsible for maintaining structural integrity, by the proteolytic activity of the calpain system (Goll et al., 1992). The impact of processing conditions would also affect the tenderness of meat through changes in the myofibrillar fragment lengths (MFL). This is accomplished by increasing/decreasing shortening or the stretching of the muscle pre-rigor (Thompson et al. 2005). As mentioned, the determination of meat tenderness with the WBSF includes the cutting of a cooked subsample across the myofibrils (Holman et al., 2015). This mimics the chewing action of the consumer, hence also the tenderness that they will experience. Therefore, the length of the myofibrils plays an important role due to its direct effect on meat tenderness. The shorter the myofibrils, the more tender the meat.

In Figure 4.17 ($p = 0.005$) a trend that the Karoo products had shorter MFL's than the grass-fed and grain-fed products can be seen. It was confirmed by the mean MFL values, with the products from the Karoo production system presenting significantly ($p < 0.001$) shorter MFL's and significantly ($p = 0.001$) lower WBSF than that of the other two production systems (Table 4.14). The MFL values also correlates weakly but significantly (0.3467; $p = 0.001$) with the WBSF tenderness ($p = 0.003$) with the Karoo products, R2K and B1K, being the most tender (Appendix 1). The grass-fed products were once again in the middle of the range with their counterparts, R3OW and R3MAP, being in the lower range with lower MFL values. The average MFL value for the Karoo products differed with 4.3 microns from

the grain-fed products and with 4.1 microns from the grass-fed products. Even though the differences in the MFL's were not big, it was still significant ($p < 0.001$) and concurrent with the WBSF values (Table 4.14).

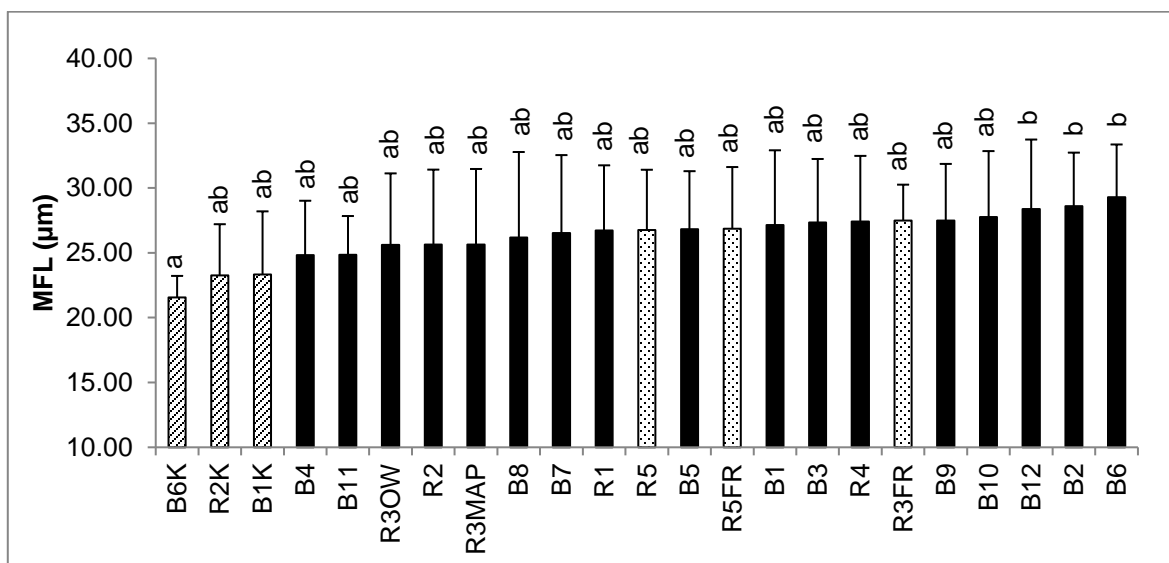


Figure 4.17: Variation in MFL over a range of products sampled across various retailers and butcheries (R: retailer, B: butchery, K: Karoo, FR: grass-fed). ($p = 0.005$). Bars with different letters differ significantly.

4.7.2 Total collagen and solubility:

As mentioned in literature, collagen content and solubility are classified as background toughness that might cause variation in meat tenderness (Safari, Channon, Hopkins, Hall & Van den Ven, 2002). Collagen solubility is affected by factors such as animal age (Young & Braggins, 1993), gender and muscle type (Wheeler, Shackelford & Koohmaraie, 2000). Warner et al. (2010) noted that for the *longissimus*, total collagen content is of limited value when predicting meat tenderness, whereas the collagen solubility would be expected to affect meat tenderness. As animals age, the amount of cross-links between collagen fibrils and molecules increases and subsequently increases the heat stability of collagen (Reiser, Me Cormick & Rucker, 1992). This increase explains the occurrence of tougher meat in older animals (Bailey, 1985). Even though Starkey et al. (2015) did find different collagen contents over different ageing periods in lamb *longissimus*, the overall variation in shear force was only 8% and was explained by the soluble collagen content. Similar findings were made in a study of 1 day aged porcine *longissimus* (Wheeler et al., 2000). Bovine *longissimus* was studied by Rhee et al. (2004) who found only 1% variation in shear at day 14 of ageing.

For collagen content (Figure 4.18) one of the Karoo products (B1K) grouped with two of the grass-fed products (R5 and R5FR) in the lower range. Subsequently, the other grass-fed product (R3FR) grouped with the other two Karoo products (R2K and B6K) in the upper range. However, no significant

($p = 0.805$) differences were found between any of the products with values ranging from 1.23 mg col/g (B4) and 1.74 mg col/g (R3FR). The mean collagen content values of the three production systems (Karoo / Grass-fed / Grain-fed) (Table 4.16) did however indicate that the Karoo and grass-fed products had significantly ($p < 0.001$) higher collagen contents than the grain-fed products (Table 4.14).

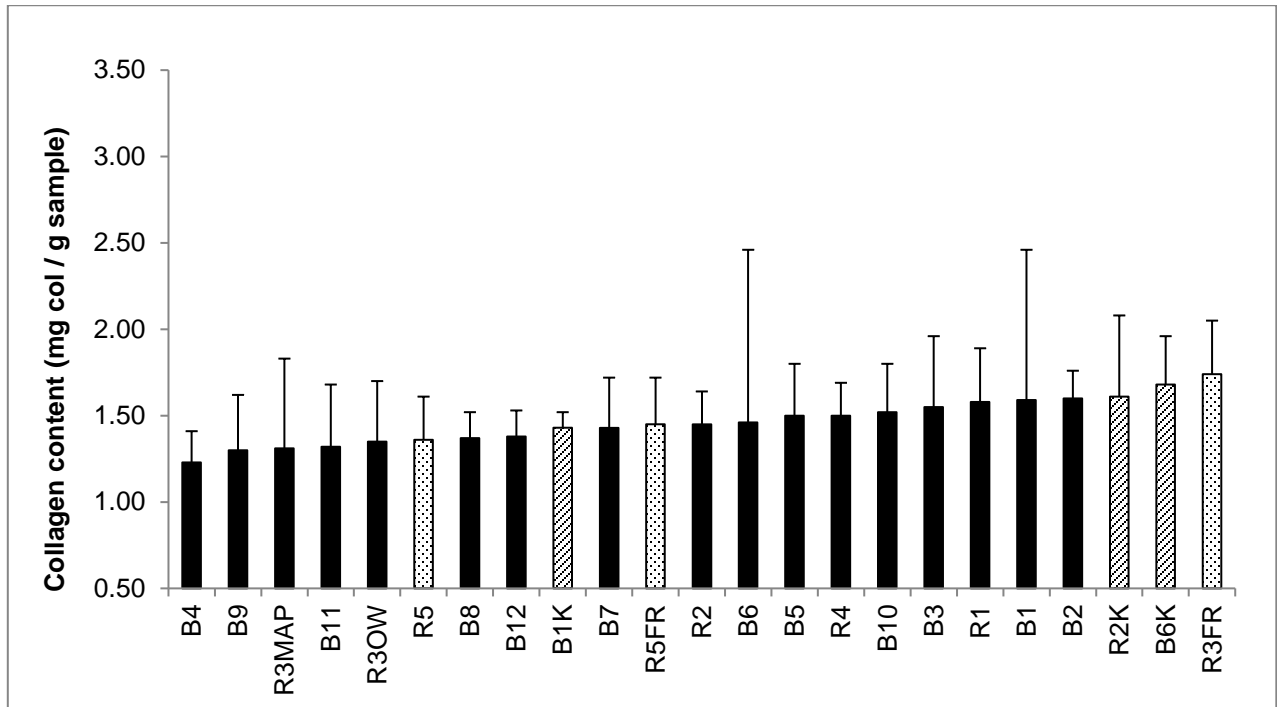


Figure 4.18: Variation in collagen content over a range of products sampled across various retailers and butcheries (R: retailer, B: butchery, K: Karoo, FR: grass-fed). ($p = 0.805$).

There was also no significant ($p = 0.815$) differences between the percentage collagen solubility (Figure 4.19) between any of the products (Figure 4.19). Once again the mean values (Table 4.14) indicate significant ($p < 0.001$) differences between the production systems with the Karoo products having much higher collagen solubility than the grass-fed and grain-fed products. However, overall the collagen content and solubility were at acceptable levels due to all animals being young and the *longissimus* is known as a low connective tissue muscle where collagen properties will play a lesser role in tenderness variation (Rhee et al., 2004).

When considered collectively (Table 4.14), it is clear that MFL does positively correlate (0.3467; $p = 0.001$) with the WBSF, in other words the tenderness of meat (Appendix 1). The correlation (0.0018) between collagen solubility and WBSF was not significant. Opposite results were found for the average collagen solubility when compared to the average WBSF and MFL, with the Karoo products having significantly lower WBSF ($p = 0.001$) and MFL ($p < 0.001$) values and significantly higher collagen solubility ($p < 0.001$) than the grass- and grain-fed products. The small effect of the collagen content is also illustrated. The difference between the Karoo and grass-fed production systems were only 0.06 mg

col/g ($p < 0.001$), while in terms of the collagen solubility these two systems differed significantly ($p < 0.001$) with 1.34%.

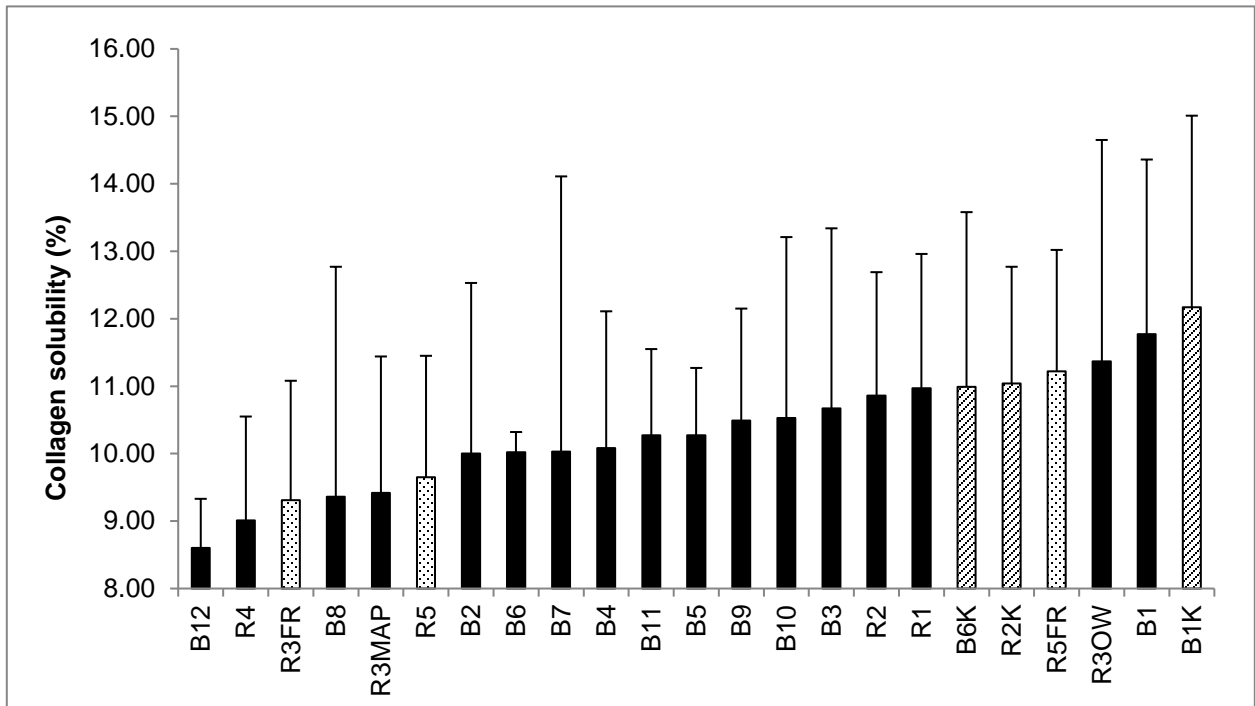


Figure 4.19: Variation in percentage collagen solubility over a range of products sampled across various retailers and butcheries (R: retailer, B: butchery, K: Karoo, FR: grass-fed). ($p = 0.815$).

Table 4.14: Variation between Price (ZAR), % meat, % fat, % bone, WBSF values, MFL's, collagen content and collagen solubility of the products from three different production systems (Karoo / Grass-fed / Grain-fed).

	Karoo	Grass-fed	Grain-fed	p-value
Price (ZAR/kg)	182.06 ^b ±34.74	194.28 ^c ±19.96	155.27 ^a ±17.89	$p < 0.001$
% Meat	55.71 ^a ±2.95	54.08 ^b ±2.83	51.91 ^a ±3.18	$p < 0.001$
% Fat	25.83 ^a ±6.09	26.26 ^a ±3.82	31.24 ^b ±5.69	$p < 0.001$
% Bone	18.41 ^b ±2.92	19.07 ^b ±4.26	16.78 ^a ±4.20	$p < 0.001$
WBSF (kg)	2.13 ^a ±0.29	2.47 ^b ±0.47	2.47 ^b ±0.58	$p = 0.001$
MFL (µm)	22.71 ^a ±3.74	27.04 ^b ±4.07	26.84 ^b ±5.07	$p < 0.001$
Collagen content (mg col/g)	3.49 ^b ±0.47	3.43 ^b ±0.58	3.18 ^a ±0.41	$p < 0.001$
Collagen solubility (%)	11.40 ^b ±1.43	10.06 ^a ±1.28	10.22 ^a ±1.53	$p < 0.001$

Means with different superscripts in the same row differ significantly.

4.8 Lipid stability

4.8.1 Fat oxidation (TBARS):

During processing and retail display, the fatty acid composition of muscle affects its oxidative stability, through the PUFAs in phospholipids that are liable to oxidative breakdown at this stage (Wood

et al., 2008). Meat's oxidative stability is very important as it might lead to rejection by consumers due to factors such as lipid oxidation, off-flavour development and deterioration of colour (Faustman et al., 2010). As mentioned in Chapter 3, the thiobarbituric acid reactive substances (TBARS) test was used to measure the oxidative stability. A value of 0.5 (mEq MDA / kg meat) is considered as the critical point, as products with values above this point produce a rancid odour and taste that are detected by consumers (Wood et al., 2008).

Meat from lambs raised on pastures generally has lower TBARS values than the meat from lambs raised on concentrates due to the lower natural antioxidant concentration in feedlot diets (Gatellier et al., 2004). This was supported by a few studies (Luciano, Biondi, Pagano, Scerra, Vasta, López-Andrés, Valenti, Lanza, Priolo & Avondo, 2012; Ponnampalam, Hopkins & Bekit, 2014b) where it was also noted that the meat from lambs fed on pasture showed better oxidative stability of lipids during storage than the meat from lambs fed concentrate diets. Sheep muscle often has very low levels of vitamin E which are improved by grass feeding (Zervas & Tsiplakou, 2011) and as mentioned earlier, vitamin E can improve the oxidative stability of meat.

The TBARS values for the individual samples from our study are indicated in Figure 4.20 ($p > 0.001$). Although significant differences were observed between the different products, no clear trend in terms of retailer type or production systems were observed. With regard to the Karoo products, samples R2K and B6K had the second and third lowest values of the range with sample B1K being in the middle of the spectrum. The grass-fed products (R5, R3FR and R5FR) were scattered all over the range with no significant trend. It is interesting that samples from the grain-fed production system that were fresh cut (B5, B7, B9 and B10) had lower TBARS values than the samples that were PVC-overwrapped. Both of the samples with MAP packaging (R5FR and R3MAP) were located in the upper half of the spectrum. This was consistent with the study by Frank et al. (2017) who noted on the significant effect of packaging on lipid oxidation, and found higher TBARS values in samples with MAP packaging than in samples with vacuum-skin packaging.

The mean values in Table 4.15 did however indicate that no significant ($p = 0.103$) differences were found between the TBARS values of the different production systems. All three production systems presented values that were well below the acceptable threshold of 0.5 with values ranging from 0.14-0.19 mEq MDA/kg, indicating good oxidative stability for all the products.

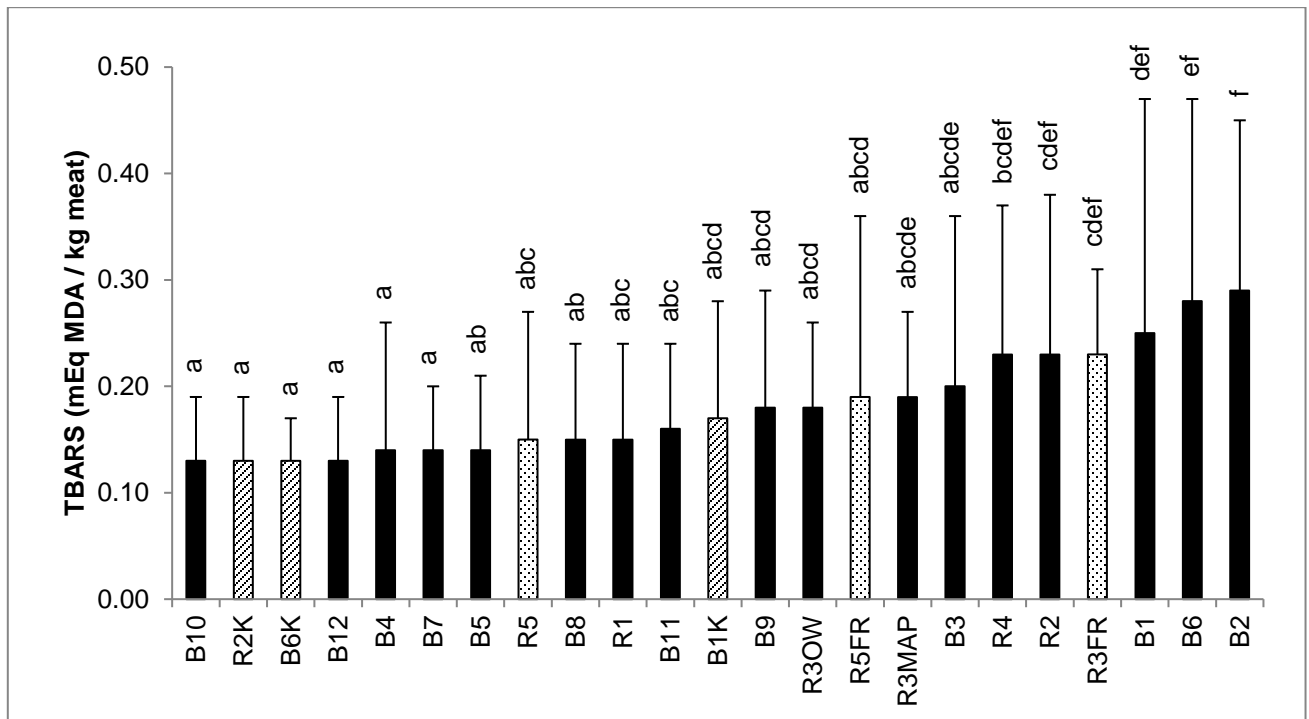


Figure 4.20: Variation in TBARS values over a range of products sampled across various retailers and butcheries (R: retailer, B: butchery, K: Karoo, FR: grass-fed). ($p > 0.001$). Bars with different letters differ significantly.

Table 4.15: Variation between TBARS values of the products from three different production systems (Karoo / Grass-fed / Grain-fed).

	Karoo	Grass-fed	Grain-fed	p-value
TBARS (mEq MDA/kg)	0.14±0.08	0.19±0.13	0.19±0.13	$p = 0.103$

4.9 Sensory analysis

4.9.1 Aroma and Flavour:

Aroma is regarded as one of the most important intrinsic elements which determine meat flavour (Mottram, 1998). In the case of lamb meat, flavour and aroma can play as an important role as tenderness. This is especially true when comparing free-range lamb meat to feedlot lamb meat (grass-fed vs. grain-fed) and even more so with Karoo lamb which has a very specific flavour and aroma due to specific herbs which are grazed on in a particular region. Variation can be found between ruminant tissue fed concentrate and those fed pastures in terms of volatile compounds, such as the carbonyl compound derived from lipid oxidation (Vasta & Priolo, 2006).

Table 4.16 describes the mean variation in the sensory aroma profiles over the range of products from different production systems that were sampled. Results for the 'typical lamb' ($p < 0.001$) and 'sweet' ($p < 0.001$) aroma profiles presented the same results, with the Karoo production system having

significantly lower scores. In terms of the 'sour' ($p < 0.001$), 'metallic' ($p < 0.001$) and 'barnyard' ($p < 0.001$) aroma profiles the opposite results were obtained. Variation in the 'Karoo bossie' overtones however delivered interesting results. It was expected that the Karoo products would have the highest scores but this was not true. The Karoo and grass-fed production systems delivered the same results with the grain-fed production system presenting a 0.10 lower score. These differences were not significant ($p = 0.123$).

Table 4.16: Variation between the sensory aroma profiles of the products from three different production systems (Karoo / Grass-fed / Grain-fed).

	Karoo	Grass-fed	Grain-fed	p-value
Aroma: Typical lamb	4.61 ^a ±0.41	5.05 ^b ±0.54	4.94 ^b ±0.43	$p < 0.001$
Aroma: Sour	2.50 ^b ±0.27	2.19 ^a ±0.24	2.28 ^a ±0.35	$p < 0.001$
Aroma: Sweet	1.69 ^a ±0.22	1.92 ^b ±0.27	2.00 ^b ±0.29	$p < 0.001$
Aroma: Metallic	3.06 ^b ±0.40	2.72 ^a ±0.34	2.73 ^a ±0.40	$p < 0.001$
Aroma: Barnyard	3.49 ^b ±0.81	2.56 ^a ±0.52	2.55 ^a ±0.64	$p < 0.001$
Aroma: Karoo 'bossie'	2.28±0.38	2.28±0.33	2.18±0.40	$p = 0.123$

Means with different superscripts in the same row differ significantly.

According to Tables 4.16 and 4.17 the sensory scores for aroma and flavour follow the same pattern. This can be ascribed to the fact that aroma is actually part of the flavour observation.

When the tenderness of lamb/mutton is constant, flavour is the most important sensory attribute affecting consumer preferences (Thompson et al., 2005). It is well known that sheep meat has a characteristic species flavour due to specific branched chain fatty acids, in particular 4-methyloctanoic and 4-methylnonanoic (Priolo et al., 2001). Flavour is also a very important attribute to be considered when comparing meat from animals fed different diets (De Brito et al., 2017). Sañudo et al. (2000) noted that lambs fed grass or grains exhibit odour and flavour changes due to the n-3 and n-6 FA that are incorporated into their meat. These changes are ascribed to the high PUFA content and Maillard reaction compounds which causes interactions between lipid oxidation products and subsequently altering the concentration of the natural flavour volatiles (Wood et al., 2003). As also proven earlier grass contains high levels of linolenic acid (precursor of n-3 FA) while concentrates contain high levels of linoleic acid (precursor of n-6 FA). These variations give the characteristic taste to the meat of animals fed different diets (Díaz et al., 2002).

Table 4.17 describes the mean variation in the sensory flavour profiles over the range of products from different production systems. The 'typical lamb' ($p = 0.002$) and 'sweet' ($p < 0.001$) flavour overtones presented the same results as the aroma profile, with the Karoo production systems having the lowest scores. Variation in the 'metallic' ($p < 0.001$) and barnyard ($p < 0.001$) overtones showed the opposite results with the Karoo products having the highest scores. In the study by Priolo et al. (2002) grain-fed lambs had a higher 'lamb' and 'fatty' flavour and a lower 'livery' flavour, which could be ascribed

to different concentrations of branched chain fatty acids. It was expected that variations in the 'Karoo bossie' ($p = 0.094$) and 'sour' ($p = 0.072$) overtones would also prove the Karoo products to have superior scores however, this was not true and no significant differences was found between the Karoo, grass-fed and grain-fed production systems. In a study by Jeremiah et al. (1998) they observed that the flavour intensity of lamb increased with age from 3-6 months to 6-9 months but the differences were not significant (Jeremiah, Tong & Gibson, 1998). Martínez-Cerezo et al. (2005) found that lamb flavour become more intense for heavier lambs. As mentioned earlier the increase in the PUFA content of meat can cause changes in the aroma and flavour profiles, as these FA's are susceptible to oxidation (Wood et al., 1999). However, there were no significant differences in the PUFA contents of the three production systems from our study (Tables 4.5 and 4.6). This might be explanatory as to why the differences found between the attributes were so small and sometimes not significant.

Modified atmosphere packaging (MAP) did not seem to affect aroma or flavour drastically, although R3MAP did group more with the Karoo products in being more metallic in flavour (Figure 4.21; $p = 0.005$). Frank et al. (2017) found that high oxygen packaging did not affect overall liking at 5 days compared to vacuum-shrink packaging but decreased significantly from day 5 to 10 days. Two potential confounding reasons were given namely a corresponding decrease in tenderness for MAP packaging and also possible oxidative processes that could have generated off-flavours. It must be noted that for all aroma and flavour overtones, even though there were difference between grain-fed and pasture samples, the scores were quite low indicating that the aroma and flavour overtones were not very intense.

Due to practical reasons all sensory analyses samples were frozen. Various studies (Resconi et al., 2010; Muela et al., 2012 and Muela et al., 2016) showed that freezing and duration of freezing had no effect on aroma and flavour overtone scores. Lamb from these studies was grain-fed with pasture incorporated in the diet.

Table 4.17: Variation between the sensory flavour profiles of the products from three different production systems (Karoo / Grass-fed / Grain-fed).

	Karoo	Grass-fed	Grain-fed	p-value
Flavour: Typical lamb	4.89 ^a ±0.31	5.10 ^b ±0.38	5.12 ^b ±0.39	$p = 0.002$
Flavour: Sour	2.84±0.33	2.71±0.32	2.69±0.39	$p = 0.072$
Flavour: Sweet	1.82 ^a ±0.24	1.90 ^b ±0.26	2.02 ^b ±0.28	$p < 0.001$
Flavour: Metallic	3.56 ^b ±0.30	3.31 ^a ±0.31	3.21 ^a ±0.41	$p < 0.001$
Flavour: Barnyard	3.32 ^b ±0.75	2.56 ^a ±0.49	2.45 ^a ±0.64	$p < 0.001$
Flavour: Karoo 'bossie'	2.63±0.42	2.66±0.45	2.52±0.48	$p = 0.094$

Means with different superscripts in the same row differ significantly.

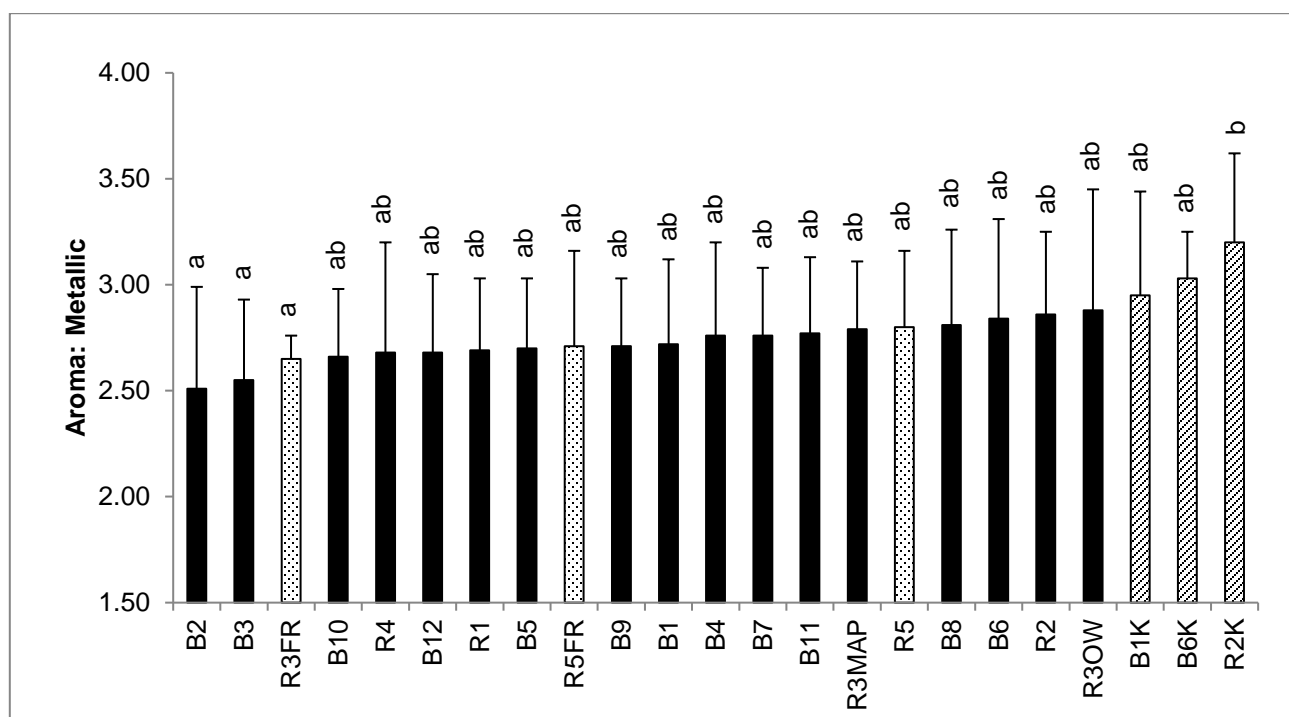


Figure 4.21: Variation in sensory aroma for metallic overtones over a range of products sampled across various retailers and butcheries (R: retailer, B: butchery, K: Karoo, FR: grass-fed). ($p = 0.005$). Bars with different letters differ significantly.

4.9.2 Texture:

In Table 4.18 'first bite' refers to the sensory tenderness of the products that was detected by the trained taste panel. Sensory tenderness is scored in the opposite manner than WBSF, with a higher value indicating a more tender product. Once again the Karoo product, R2K, was the most tender (Figure 4.22; $p < 0.001$). The results for the other Karoo products (B1K and B6K) were not exactly the same for WBSF and sensory tenderness, but collectively, the Karoo products were more tender than the grass-fed and grain-fed products (Table 4.18; $p < 0.001$). One of the grass-fed products, R5FR, performed poorly but also scored lower for juiciness. It is interesting how sample R3MAP scored in the higher side of the range. This is in contrast to Kim et al. (2012) who found that MAP packaging resulted in lower sensory tenderness scores. As mentioned sample R5FR was also a MAP packaged product and did not show the same good attributes having lower sensory scores as well as longer MFL's. This was consistent with the study by Frank et al. (2017) who found that loin samples stored in high oxygen MAP packaging had decreased sensory scores for tenderness and juiciness compared to vacuum-skin packaging.

Increased juiciness can give the perception of a more tender product. As the grain-fed products had the highest score for carcass fatness one would think that they would score the highest for juiciness (Table 4.18), but the opposite results were found.

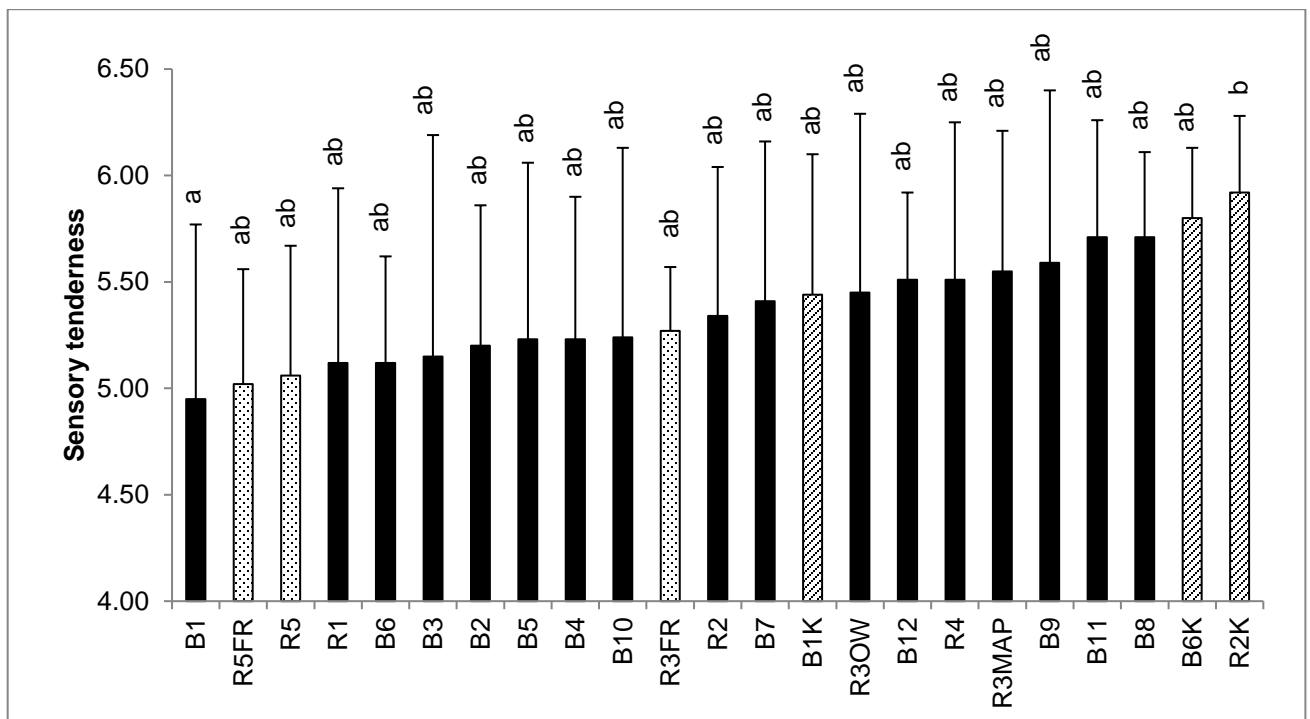


Figure 4.22: Variation in sensory tenderness over a range of products sampled across various retailers and butcheries (R: retailer, B: butchery, K: Karoo, FR: grass-fed). ($p = 0.005$). Bars with different letters differ significantly.

This was contradictory to the results found by Priolo et al. (2002) who found positive correlations between carcass fatness and tenderness along with juiciness. However, there were no significant ($p = 0.050$) differences in juiciness scores between the different types of products in our study. The overall good quality where tenderness was concerned in our study was a surprise. Residual mouthfeel refers to the 'fatty' feeling that is sometimes experienced after chewing meat (Table 4.18). Although the grain-fed products had the highest amount of dissectible- and intramuscular fat this had no effect as there were once again no significant ($p = 0.216$) differences between the products of the different production systems.

Table 4.18: Variation between texture attributes (juiciness, first bite, residual mouthfeel) of the products from three different production systems (Karoo / Grass-fed / Grain-fed).

Attribute	Karoo	Grass-fed	Grain-fed	p-value
Juiciness	4.82	4.83	4.69	$p = 0.050$
First Bite	5.72 ^b	5.12 ^a	5.36 ^a	$p < 0.001$
Residual Mouthfeel	3.03	3.06	3.11	$p = 0.216$

Means with different superscripts in the same row differ significantly.

The clear significant differences in sensory properties between grain-fed and Karoo lamb found by Analyses of Variance (Tables 4.16, 4.17 and 4.18), were confirmed with principle component

analyses where grain-fed and Karoo clustered on opposite sides of dimension 1. Sensory properties from the grass-fed meat that were sometimes in agreement with grain-fed and other times in agreement with Karoo, clustered inbetween grain-fed and Karoo of dimension 1. Dimension 1 of the PCA bi-plot explain 75.6 % of the variation, whereas dimension 2 explain 24.4%. On the PCA bi-plot (Figure 4.23) it is indicated that the intense aromas and flavours (i.e. 'Karoo bossie', 'metallic', 'barnyard' and 'sour') are associated with the grass-fed and Karoo products, while the grain-fed products scored significantly better for the 'typical lamb' and 'sweet' attributes. This same finding was made Larick and Turner (1990) in terms of the sweet and sour attributes. They noted that the grain-fed products in their study had a significantly sweeter flavour and gamey aftertaste when compared to the products from pasture-fed animals. They considered the possibility that this finding is caused by unsaturated lactones formed from the oxidation of linolenic acid. The grain-fed products in our study had significantly ($p = 0.002$) higher amounts of γ -linolenic acid (C18:3c6,9,12 n-6) and significantly ($p < 0.001$) lower amounts of α -linolenic acid (C18:3c9,12,15 n-3).

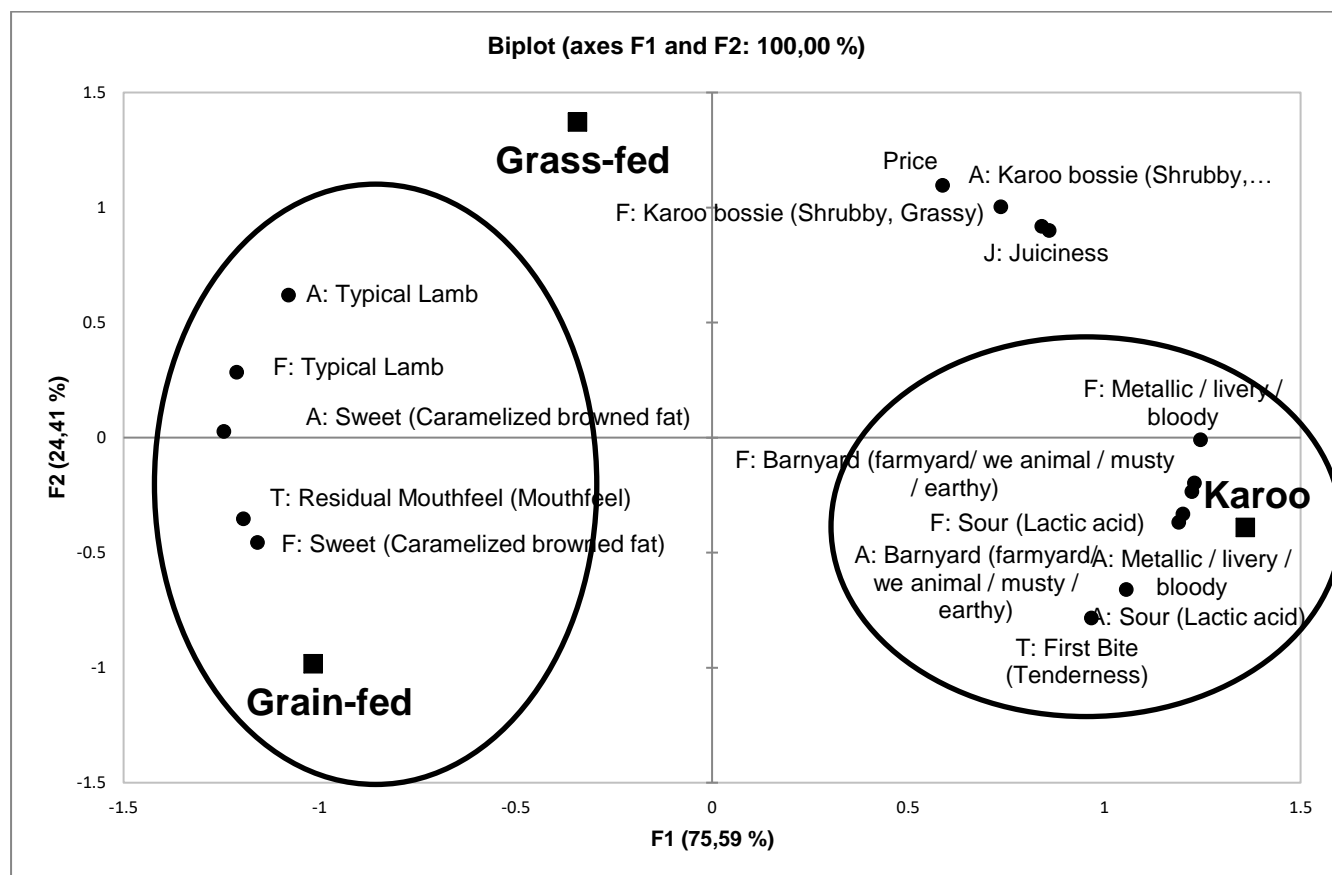


Figure 4.23: Principle Component Analysis bi-plot of the variation between the sensory evaluation scores of the products from three different production systems (Karoo / Grass-fed / Grain-fed) (A = aroma; J = juiciness; T = texture; F = flavour).

The branched chain fatty acids (i.e. 4-methyloctanoic and 4-methylnonanoic acids) are considered responsible for the 'typical lamb' and 'livery' flavours and aromas (Priolo et al. 2001). These FA's are derived from ruminal propionate that is produced in higher amounts in grain-rich diets, hence the higher content in grain-fed animals (Kennedy, Kennedy, Blanchflower, Scott, Weir, Molloy & Young, 1994). Young et al. (1993) also reported that a high ultimate pH can lead to a reduction in sheep meat flavour. In general, meat from pasture-finished animals have a higher ultimate pH than the meat from concentrate-finished animals, due to pastures' high fiber and low starch content which lead to a higher acetate/propionate ratio (Priolo et al., 2001). Even though pH measurement was not part of our study, a higher pH of the meat from pasture-fed animals are confirmed by lower a^* values as a higher pH also leads to darker colour meat. This can be explanatory why the 'typical lamb' flavour and aroma was associated with the grain-fed products. Consumers from different countries/regions give different responses to what they consider as 'typical' therefore, results for 'typical lamb' flavours and aromas should only be considered as indicative (Sañudo et al., 1998).

4.10 Discriminant analysis

Discriminant analysis (DA) is a multivariate technique that uses a linear or quadratic function to predict to which previously defined group a certain product belongs. Products with higher prediction values have consistently the same characteristics whereas products with lower values will prove a lack of consistency (Fralely & Raftery, 2002).

Figures 4.24 and 4.25 illustrate the first round of modeling with discriminant analysis. All the variables (% dissectible fat, % drip loss, % thawing loss, WBSF, hue angle and price) that presented significant differences between the production systems, thus likely to influence consumers' purchasing decisions, were included. The bi-plot in Figure 4.24 shows the variables as well as the two factors, F1 and F2. Factor F1 is presented on the horizontal axes with an 88.97 corresponding % of variance and factor F2 is presented on the vertical axes with a 4.31 corresponding % of variance. At the top a 2-dimensional illustration of the two factors are given with a cumulative % of 93.28. The results should first be interpreted separately in terms of the two factors and then together in the 2-dimensional space. For F1 the main driver was price and for F2 % drip loss, % thawing loss and the hue angle were regarded as the strongest drivers.

In Figure 4.25 the observations of the variables in Figure 4.24 are expressed in terms of product centroids with 95% confidence circles, indicating the products individually. It is clear that sample B6K (far right end) were the most expensive product and sample B9 (far left end) the cheapest product. A difference of R93.08/kg was recorded between these two products. As expected the % thawing loss contrasted with the % drip loss and % dissectible fat. Sample R3FR (close to the bottom of the graph) had clearly the highest percentage of thawing loss. This product was from the grass-fed production

group and also had the second lowest % dissectable fat. Sample R3FR also had the highest hue angle value which indicates a more yellow product, and could be ascribed to the loss of proteins with the thawing loss. As mentioned in our results all of the products from this study can be regarded as tender hence did WBSF not play an important part in separating the products. This is evident by the fact that the most tender product (R2K) is situated closely to the least tender product (R1).

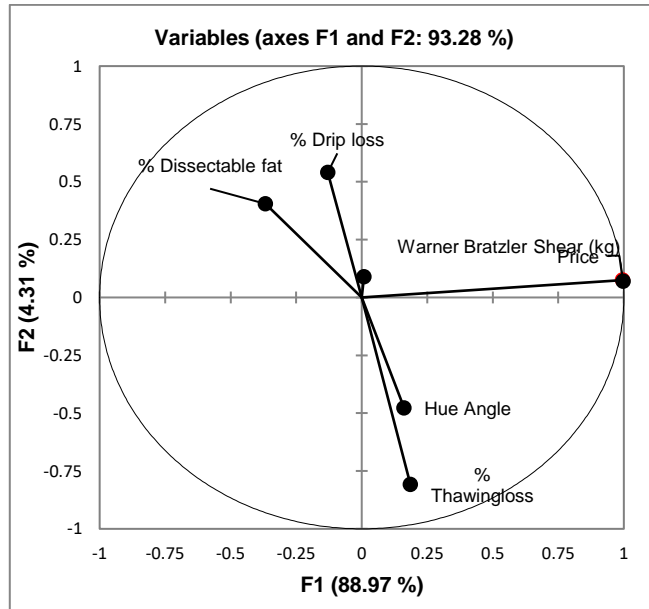


Figure 4.24: Discriminant analysis bi-plot of consumer related drivers (attributes) contributing to 93.28 % of the variation among the different products (Price included).

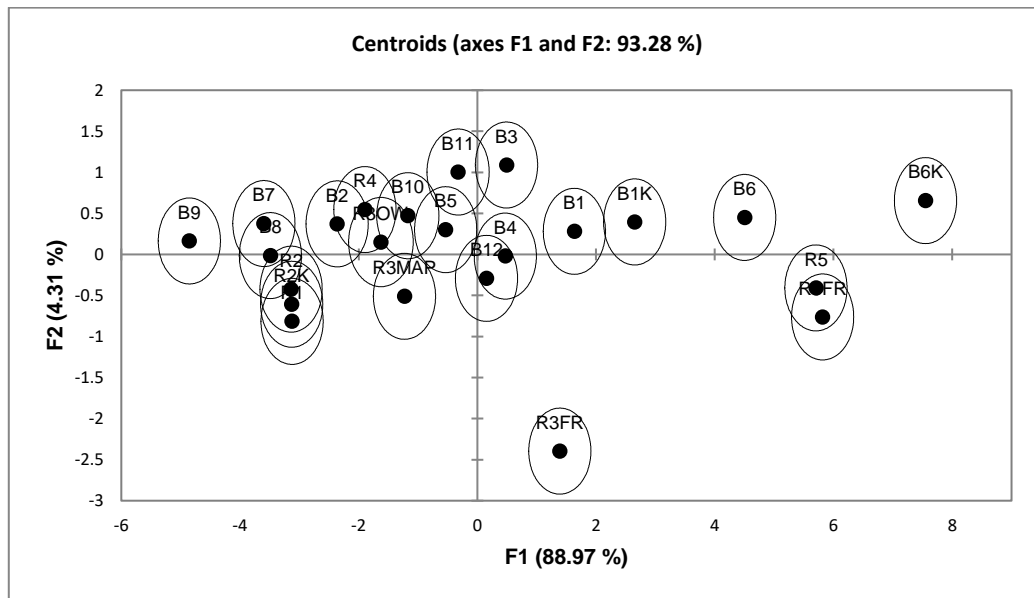


Figure 4.25: Product centroids with (95 %) confidence circle the direction of and magnitude of separation among the 21 products as influenced by the drivers in Figure 4.25 (Price included).

As proven in our results the price factor did not indicate the best value for money in terms of good quality, as the grain-fed products had the best/lowest price but also the lowest meat percentage, highest fat content and scored the same as the grass-fed products in terms of WBSF tenderness. Thus price was removed in the second round of modeling (Figures 4.26 and 4.27). For this model the bi-plot (Figure 4.27) showed factors F1 (43.52%) and F2 (29.04%) with quite different results. The WBSF was the only constant variable while all the others moved to the opposite ends of the axes. It was interesting to see that % dissectable fat, % drip loss and % thawing loss were the main drivers for F1, while the hue angle and % dissectable fat also being the main drivers for F2. However, similar results were found with once again no clear difference in WBSF between the products from the different production systems. Also, sample R3FR was situated away from the other samples in the top left quadrant, indicating a significantly higher thawing loss percentage and a more yellow product.

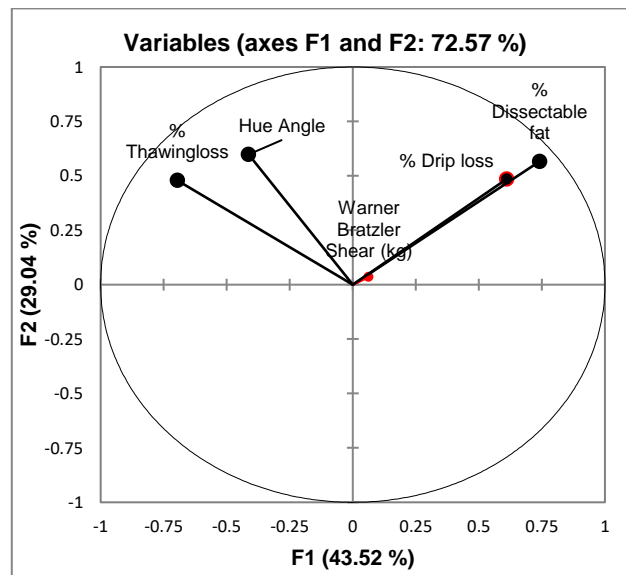


Figure 4.26: Discriminant analysis bi-plot of consumer related drivers (attribute) contributing to 93.28 % of the variation among the different products (Price excluded).

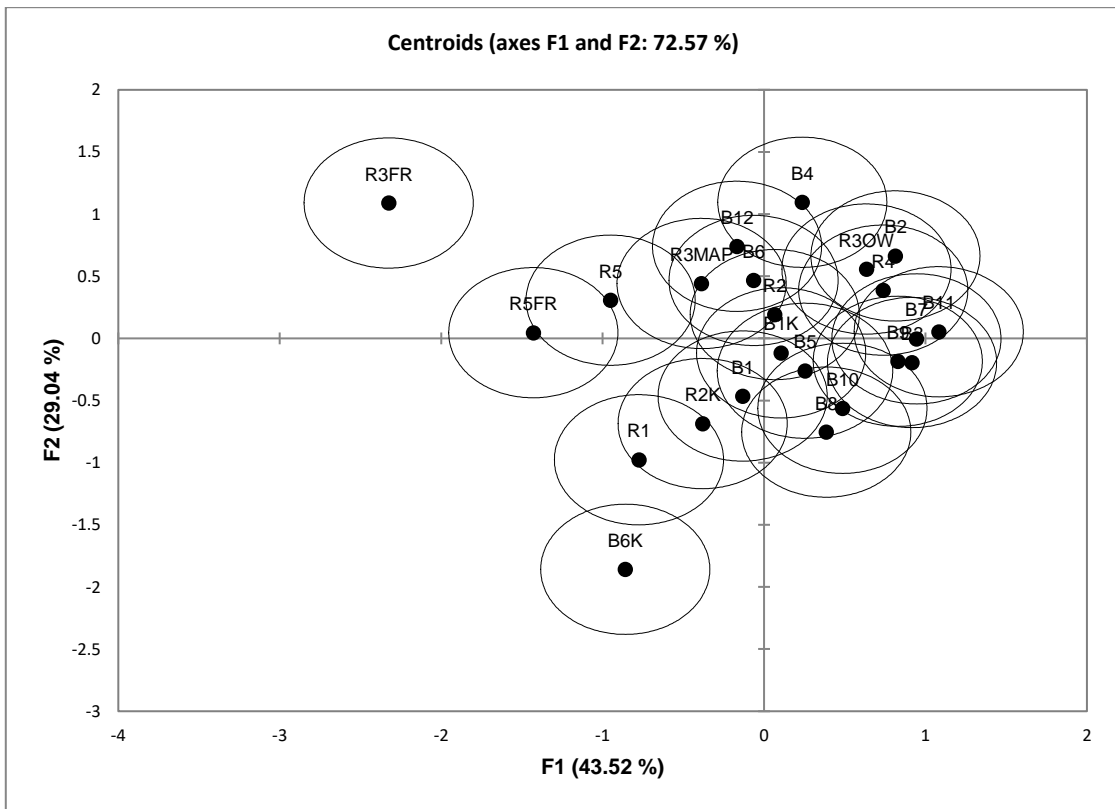


Figure 4.27: Product centroids with (95 %) confidence circles the direction of and magnitude of separation among the 21 products as influenced by the drivers in Figure 4.1 (Price excluded).

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

The three key drivers that influence the purchase and 'willingness to pay' decisions of consumers is lamb that is lean, palatable and still have good nutritional attributes (Pethick et al., 2006). Additionally, consumers' lifestyles have shifted towards convenient food preparation which is provided through freezing until the chosen date of consumption (Kim et al., 2011). Russell et al. (2005) described sheep meats' eating quality as a function of the production, processing, value-adding and cooking methods that is used to prepare the product for consumption. Furthermore, Thompson et al. (2005) referred to these different stages as 'critical control points' (CCP's) and noted that failure at one or more of the CCP's in the supply chain will improve the chances of unsatisfied consumers. Thus is it important to identify the CCP's in the sheep meat supply chain as well as their impact on the eating quality.

Most researchers are in agreement about the one desire that remain constant with all consumers, 'meat must be consistently tender' (Koochmaraie, 1995). The tenderness of meat are influenced by many factors such as fatness levels, age etc. In South Africa younger leaner animals are used for the purpose of producing meat. This follows the rapid growth of the lamb/mutton market to meet the increased need for consumption (Morris, 2009). One positive aspect in this regard is that the meat from younger animals is more tender as there are fewer collagen cross-links than in the meat from older animals (Purslow, 2005). The South African meat market classify carcasses according to age, degree of fatness and conformation with a premium being placed on young carcasses with a reasonable amount of fat (Webb & O'Neill, 2008).

Although a significant amount research has been done on the factors which cause quality challenges in the meat industry, very little is known about the quality of red meat offered to consumers at retail level. This survey was limited to the loin chop (*M. longissimus dorsi*) since this cut responds the most to various intrinsic and environmental effects and consist largely of a single muscle. It is also noted that the loin musculature contains the most valuable cut in the carcass (Pannier et al., 2014a). Twenty three products were collected from the shelves of five major retail outlets and twelve smaller butcheries. A total of 306 samples were collected which varied in type namely Karoo lamb, grass-fed or grain-fed and also in packaging that is modified atmosphere packaging (MAP), polyvinyl chloride overwrapping and (PVC-OW), fresh-cut and on display. Price was recorded and shear force tenderness, sensory analyses, colour of meat, drip loss, cooking losses and meat/fat/bone ratios were measured as properties valued by consumers at or after purchase. Physical, histological and biochemical

measurements (that is proximate and fatty acid analyses, lipid oxidation and collagen) were performed in an attempt to explain variations in consumer related properties.

It is difficult to determine at retail level whether the meat is that of lamb or mutton. This can be confusing as many consumers do not know the difference between the terms. In South Africa, lamb is defined as the meat from sheep that has no permanent incisors and is younger than 1 year, while mutton is the meat of sheep that has 2 or more permanent incisors and is two years or older. Usually carcasses are cut in such a way that the markers are not visible anymore and no indications are made on the packaging. By comparing our results with that of Thompson et al. (2005) who studied differences between lamb and mutton, we found that ours is more similar to the results of the lamb samples in their study, hence the assumption that the samples that we bought are all of lamb carcasses.

Globally consumers desire low fat, muscled lamb retail cuts that present a nutritious meal and is subsequently good value for money (Pethick et al., 2006). In Bernabéu and Tendero (2005) noted that price is the secondary element at the moment of purchase after factors such as image and food safety. However, in the modern world price are becoming a much more important factor at the point of purchase, especially with regard to meat and meat products. In our study the average price of the Karoo products was R182.06/kg and that of the grass-fed products was R194.28/kg. Both these types of products were well above the average price of lamb (R163.98/kg) as they are sold as specialized 'healthier' products. The grain-fed products had the best/lowest price with the difference between grain-fed and grass-fed being about R40/kg and between grain-fed and Karoo about R28/kg. Usually a higher price is associated with better quality nevertheless it does not mean that the grain-fed products from our study presented the best value for money.

The main characteristic of ruminants is the ability of the micro-organisms in their gut to degrade cellulose and subsequently convert forages into products that is useful for human nutrition (i.e. meat, milk, blood) (Priolo et al., 2001). Since propionate is the predominant glucogenic FA in the meat of ruminants, glycogen deposition is influenced which in turn has an effect on meat ultimate pH and colour (Daly et al., 1999). In our study the free-range products (i.e. Karoo and grass-fed) had healthier FA profiles than the products from grain-fed production system. This include FA's such as phytanic acid (C20:0,3,7,11,15-tetramethyl hexanoic acid), CLA (C18:2c9t11 n-6), total n-3 PUFA and PUFA : SFA ratio, while the grain-fed products were associated with a healthy oleic acid (C18:1c9) and MUFA contents. The same finding was made by Popova (2007) who noted that the meat of grass-fed lambs have a more appropriate FA profile for human health as well as a better oxidative stability due to the vitamin E content in grass. A few years later Belo et al. (2009) also studied the effect of feeding systems on meat and noted on the advantage of pasture only diets in terms of better n-6 : n-3 PUFA ratios and higher CLA contents. With regards to the TBARS values which were used to determine the oxidative stability of the samples, the products from the Karoo production system had better values than the products from the grass- and grain-fed production systems although this was not significant. It was

interesting to find that with regards to the grain-fed products, samples that were fresh cut had lower TBARS values than the samples that were PVC-overwrapped or covered with MAP. However, all of the products in our study were well below the acceptable threshold of 0.5 with values ranging from 0.13 to 0.29.

The effect of diet on the colour of meat is considered a rare finding and dependent on a specific effect of the diet on the myoglobin in the muscle (Hopkins & Nicholson, 1999). Priolo et al. (2001) noted that generally no difference in the myoglobin between pastures and concentrates were found in the experiments that they have examined. The results from our study were consistent with these findings with the only significant difference being in the Hue angle, which indicate the proportions of red and yellow. In this regard the grass-fed products had significantly higher values than the Karoo and grain-fed products. The wider the Hue angle, the more the colour corresponds to yellow. The lack of patterns in the myoglobin levels also indicate that the samples we bought were from young animals, as an increase in the myoglobin concentrations occur with physiological maturity (Bezerra, Barbosa, Carvalho, Simionato, Freitas, Araújo, Pereira, Silva, Lacerda & Carvalho, 2016).

The tenderness of the samples was determined by the sensory scores allocated by a trained sensory panel, and also mechanically by means of the Warner Bratzler shear force (WBSF) method. Results gathered from the trained sensory panel showed that the Karoo products were the most tender. With regards to the WBSF we compared our results to the 2.75 kg threshold set by Hopkins et al. in 2006 (Hopkins et al., 2006). Once again the products from the Karoo production system were proven to be the most tender, and more importantly, the most consistently tender. The fact that the grain-fed products were the least tender could be ascribed to the use of beta-adrenergic agonists in feedlots. The influence of myofibrillar fragment lengths (MFL's) on WBSF were also demonstrated by the short MFL's of the Karoo products that contributed to these products being the most tender. Differences in the WBSF and MFL's between the products with different packaging were not big hence the assumption that it had no effect on the tenderness of the products.

Researchers have noted that flavour ratings seem to be related to sensory panelist's preference of lamb as well as previous exposure (Sanudo, Enser, Campo, Nute, Maria, Sierra & Wood, 2000). Oltra et al. (2015) identified 'sweet' and 'roast lamb' as the flavour attributes which drove consumer liking of lamb loin steaks. In general, consumers can distinguish between red meat from animals raised on pastures and animals raised on concentrates, particularly in terms of flavours (Priolo et al., 2001). In our study the Karoo and grass-fed production systems were associated with the intense aroma and flavour attributes (i.e. 'Karoo bossie', 'metallic', 'barnyard' and 'sour') while the grain-fed production system scored significantly better for the 'typical lamb' and 'sweet' attributes. This was consistent with previous research. Larick and Turner (1990) considered the possibility that the 'sweet' attributes found in the grain-fed products could be caused by unsaturated lactones formed from the oxidation of linolenic acid. A high ultimate pH can lead to a reduction in sheep meat flavour (Young et al., 1993). This can be

explanatory why the 'typical lamb' flavour and aroma was associated with the grain-fed products, as meat from pasture-fed animals usually have a higher ultimate pH due to a higher acetate/propionate ratio (Priolo et al., 2001).

Consumers are sceptical about free-range (grass-fed and Karoo) products especially with the price difference however, in this study clear evidence is presented with the FA ratios for instance that free-range products is healthier than grain-fed products. Significant differences between the WBSF values, proximate composition and drip loss of the grain-fed and free-range products indicated that production systems and abattoir practices do influence lamb and mutton quality. The fact that no significant differences were found in the colour parameters, prove that the handling at retail in terms of packaging did not play a significant role.

From this study it can be deduced that if knowledge regarding animal production systems, abattoir practices and handling at retail is applied it can be used as a tool to improve tenderness and quality characteristics in a cost effective manner. In conclusion, South African loin chops are tender therefore consumers can consistently buy tender loin chops at any retailer or butchery.

As one of our biggest concerns is the high amount of cooking losses identified in the grass-fed production system, important future research on this subject can include the improvement of the water holding capacity of the meat from this production system. This might have an influence on other quality characteristics which will make this product a bit more competitive towards other production systems. From another perspective, consumer behaviour can be monitored in terms of packaging that present more information about the production and feeding systems, giving the chance of a more informed purchasing decision, compared to the uninformed. Subsequently, more consumer preferences can also be identified. It is recommended that a further study should be done where lambs from different breeds should be subjected to grain-fed, grass-fed and Karoo production systems followed by similar meat quality evaluation as conducted. This way other factors influencing quality will be eliminated with a stronger focus on the effect of the production systems.

CHAPTER 6

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CHAPTER 7

SUMMARY

Summary

The palatability of meat is determined by tenderness, juiciness and flavour. All over the world consumers consider tenderness as the most important palatability attribute. However, inconsistent quality is one of the biggest problems that have been identified in the meat industry. This ascribed the lack of knowledge about the origin of variation in quality and certain claims regarding consumer satisfaction that do not materialize.

Over a period of three months twenty three products were collected from five major retail outlets and twelve smaller butcheries. A total of 14 rounds of purchasing were done representing 14 repetitions. Overall 306 samples were collected. Products varied in type namely Karoo lamb, grass-fed or grain-fed lamb and also in packaging that is modified atmosphere packaging, polyvinyl chloride overwrapping, fresh-cut and on display. Physical, histological and biochemical measurements were performed in an attempt to explain variations in consumer related properties. Data was analyzed with analyses of variance, Tukey-Kramer multiple comparison test, principle component analyses and discriminant analyses to demonstrate differences in meat quality.

Price is one of the most influential factors on consumers' choice of purchase, although they still want good value for their money. The grain-fed products had the lowest/best price with the products from the grass-fed production system being the most expensive. Nevertheless, when considering the meat/fat/bone ratios the grain-fed products had the least amount of meat and the most fat, leaving the products from the Karoo production system as the product with the best value for money. Cooking losses have pointed out problem areas with regards to the grass-fed products as these products had the highest values while being sold as a superior product with the highest price.

The Karoo and grass-fed products were associated with healthier FA such as phytanic acid, CLA, total n-3 PUFA and PUFA : SFA ratio. Subsequently, the grain-fed products had healthy oleic acid and MUFA contents as well as a good n-6 : n-3 ratio. This means that the free-range products are regarded as the better option for a healthy diet.

No significant differences were found in the TBARS values of the different production systems. It was interesting to find that the samples that were fresh cut had lower TBARS values than the samples with PVC-OW or covered with MAP. Even so all of the products were well below the point where rancidity would start to develop.

All of the products in our study were regarded as tender when comparing the WBSF values to preconceived thresholds. However, significant differences were found between the different production systems with the products from the Karoo production system being the most tender. This was in agreement with the sensory tenderness performed by a trained taste panel.

Flavour and aroma especially play an important role when discussing lamb/mutton. Some consumers are discouraged by the unique flavour of sheep meat while others embrace it, especially from Karoo lamb. The Karoo and grass-fed production systems was associated with all the intense aromas and flavours (i.e. barnyard, metallic, Karoo 'bossie', sour) while the grain-fed production system were superior in terms of the typical lamb and sweet attributes.

The factors influencing tenderness have been described as critical control points (CCP's). From this quality audit it is clear that all the participants do take the CCP's into account as all the products in our study have been classified as tender. However, there are a few aspects where improvement is still needed with regards to all of the production systems. This include the meat/fat/bone ratio of the grain-fed products, the waterholding capacity of the grass-fed products which might also improve the tenderness and the aroma and flavour of the Karoo products, especially in terms of the sweet attributes that might attract more consumers.

Keywords: Good eating quality; production systems; Karoo; grass-fed; grain-fed; tender.

Appendix 1: Correlation analysis (r-values) table of selected meat quality attributes.

	Shear Force	Price/kg	% Meat of cut	% Fat of Cut	MFL	Sensory tenderness	% IMF	% Protein	% Collagen solubility	TBARS	Total SFA	Total MUFA	n-6/n-3
Shear Force	1												
Price/kg	0.0104 ^{NS}	1											
% Meat of cut	-0.0331 ^{NS}	0.3783 ^{***}	1										
% Fat of Cut	0.0332 ^{NS}	-0.3221 ^{***}	-0.5249 ^{***}	1									
MFL	0.3467 ^{***}	-0.0772 ^{NS}	-0.0635 ^{NS}	-0.0404 ^{NS}	1								
Sensory tenderness	-0.5504 ^{***}	-0.0846 ^{NS}	-0.0635 ^{NS}	0.0130 ^{NS}	-0.4511 ^{***}	1							
% IMF	-0.0397 ^{NS}	-0.1429 [*]	-0.2858 ^{***}	0.4502 ^{***}	-0.0359 ^{NS}	0.0269 ^{NS}	1						
% Protein	0.1752 ^{**}	0.0368 ^{NS}	-0.0956 ^{NS}	0.1454 ^{**}	0.1751 ^{**}	-0.1878 ^{***}	0.2517 ^{***}	1					
% Collagen solubility	0.0018 ^{NS}	0.1062 ^{NS}	0.1157 [*]	-0.0872 ^{NS}	-0.0510 ^{NS}	-0.0081 ^{NS}	-0.0509 ^{NS}	-0.1003 ^{NS}	1				
TBARS	0.0119 ^{NS}	-0.0016 ^{NS****}	-0.1321 [*]	0.0594 ^{NS}	-0.0601 ^{NS}	0.0127 ^{NS}	0.1627 ^{**}	0.0904 ^{NS}	0.0102 ^{NS}	1			
Total SFA	-0.1561 ^{**}	0.0282 ^{NS}	0.0780 ^{NS}	-0.1118 [*]	-0.2304 ^{***}	0.1768 ^{**}	-0.1252 [*]	-0.0792 ^{NS}	-0.0126 ^{NS}	-0.1155 [*]	1		
Total MUFA	0.1009 ^{NS}	-0.1313 [*]	-0.2312 ^{***}	0.3475 ^{***}	0.0588 ^{NS}	-0.0897 ^{NS}	0.4435 ^{***}	0.1378 [*]	-0.0497 ^{NS}	0.1299 [*]	-0.6331 ^{***}	1	
n-6/n-3	0.0478 ^{NS}	-0.0683 ^{NS}	-0.2238 ^{***}	0.2803 ^{***}	0.1792 ^{**}	-0.0350 ^{NS}	0.3252 ^{***}	0.1491 ^{**}	-0.0716 ^{NS}	0.2658 ^{***}	-0.3289 ^{***}	0.3781	1

NS = Not significant; * = 0.05; ** = 0.01; *** = 0.001