ENHANCEMENT OF BEEF BY MEANS OF INFUSING A PHOSPHATE AND LACTATE BLEND

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree

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ABSTRACT

Consumers demand beef products of consistent and satisfactory tenderness, acceptable meat colour, the necessary nutritional value, as well as being microbiologically safe. However, inconsistency in meat quality, and particularly tenderness, is a problem that has continuously plagued the meat industry

Firstly, an investigation was undertaken to determine the muscle variation, particularly in the tenderness of South African beef muscles. Beef muscles were removed from the right-side of beef carcasses and the physical, chemical and sensory characteristics determined 72 h post-mortem. As the beef muscles differed significantly ($P \leq 0.05$) in these attributes, the need for increased attention to enhanced processing and meat ageing is accentuated. Therefore, two further investigations were executed, including an enhancement study and a shelf-life study.

The enhancement study determined the time effect of a blend of sodium and potassium salts, various phosphates and lactates on the physical quality, chemical composition and sensory quality of South African beef muscles. The corresponding muscles were removed from the left-side of the same beef carcasses and infused 3 d post-mortem. The changes in beef quality over 19 d and the initial proximate and mineral composition were determined. The infused beef increased in tenderness, while maintaining an acceptable red colour. The infused muscles had higher moisture and ash contents, and lower lipid and protein contents. The mineral content of the treated muscles increased due to the minerals contained in the infusion blend. For the sensory analysis, beef muscles were stored under vacuum for 24 h (4°C) until the physical data had been collected. Thereafter, the muscles were stored at −18°C until the descriptive sensory analysis could be performed. The infusion of beef muscles successfully enhanced the sensory attributes, resulting in significantly ($P \leq 0.05$) more juicy and tender beef. When used in a consumer preference test, the infused samples illustrated a significantly ($P \leq 0.05$) higher degree of liking.

In the shelf-life study the effect of the blend on the physical attributes, proximate composition and microbial growth of South African beef muscle was determined. One muscle from the left-side of beef carcasses was infused 4 d post-mortem. The purge loss, colour changes and the microbial growth on the muscle steaks over 10 d (overwrap storage) was determined. The infused muscle showed greater colour loss (lower a*, b* and chroma values) during the 10-d period. Physical analysis (purge loss) indicated a

significant difference ($P \leq 0.05$), with the treated sample losing 2.78% more purge. The infusion extended the microbiological shelf-life of beef by 1 d.

Improvement of beef quality and its consistency is essential for the survival of the beef industry. The infusion of beef muscles has the potential to improve the current status of low meat consumption and inconsistent tenderness of fresh beef in South Africa. Despite decreased colour stability in the infused steaks, negatively affecting the purchasing decision of the consumer, the blend could still be applied successfully in the South African meat industry to extend the shelf-life of fresh beef and improve meat quality attributes, providing the consumer with a more acceptable beef product.

UITTREKSEL

Die verbruiker verwag sekere eienskappe van vars beesvleis: ‘n sagte tekstuur, aanvaarbare kleur, goeie voedingswaarde en mikrobiologiese veiligheid. Die kwaliteit en veral sagtheid van vars beesvleis varieër egter betekenisvol en is problematies vir die vleisbedryf.

Eerstens, is die mate van variasie in veral die sagtheid van Suid-Afrikaanse vars beesvleis ondersoek. Monsters (spiere) is geneem van die linkerkant van beeskarkasse en die fisiese, chemiese en sensoriese eienskappe 72 h nadoods bepaal. Die beesvleismonsters se eienskappe het betekenisvol verskil ($P \leq 0.05$) en beklemtoon die behoefte vir toenemende aandag om prosessering en vleisveroudering te verbeter. Vervolgens is twee verdere ondersoeke onderneem, naamlik ’n verbeteringstudie en ’n rakleeftydstudie.

Die verbeteringstudie het die tydseffek van ’n oplossing bestaande uit natrium en kalium soute, verskeie fosfate en laktate op die fisiese kwaliteit, nutriëntsamestelling en sensoriese kwaliteit van Suid Afrikaanse beesvleis bepaal. Dieselfde spiere is verwyder van die linkerkant van dieselfde beeskarkasse en 3 d nadoods ingespuit. Die veranderinge in die beesvleiskwaliteit oor 19 d en die aanvanklike proksimale en minerale samestelling van die beessnitte is bepaal. Die ingespuite beesvleissnitte (vakuum verpak) het toegeneem in sagtheid en het ’n aanvaarbare rooi beesvleis-kleur behou. Die ingespuite spiere het toegeneem in vog- en asinhoud en afgeneem in lipied- en proteïeninhoud. Die minerale-inhoud van die behandelde spiere het toegeneem in die minerale wat in die ingespuite oplossing teenwoordig was. Vir die sensoriese analises, is die beesvleismonsters vir 24 h (4°C) vakuum verpak tot die fisiese analises uitgevoer is. Daarna is die monsters by −18°C geberg tot die beskrywende sensoriese analise uitgevoer kon word. Die behandeling van beesvleis met ‘n versagtingsoplossing het die sensoriese kwaliteitseienskappe verbeter – die beesvleis was betekenisvol sagter en sappiger ($P \leq 0.05$). ‘n Verbruikerspaneel het ook aangedui dat die behandelde beesvleismonsters betekenisvol ($P \leq 0.05$) meer aanvaarbaar was.

In die rakleeftydstudie is die effek van die oplossing op die fisiese eienskappe, proksimale samestelling en mikrobiese groei van Suid Afrikaanse beesspier bepaal. ’n Spier is verwyder van die linkerkant van beeskarkasse en 4 d nadoods ingespuit. Die vloeistofverlies, kleurveranderinge en mikrobiese groei op beesvleis oor 10 d (suurstof-deurlaatbare verpakkingsmateriaal) is bepaal. Die ingespuite monsters het meer kleurverlies (laer a*, b* en chroma waardes) ondergaan tydens die 10 d. Die

vloeistofverlies van die behandelde monsters was ook betekenisvol ($P \leq 0.05$) meer (2.78% meer vloeistofverlies) as die onbehandelde monsters. Die inspuiting het die mikrobiologiese rakleeftyd van beesvleis met 1 d verleng.

Die verbetering van beesvleiskwaliteit, asook die volhoubaarheid daarvan is van kardinale belang vir die Suid-Afrikaanse vleisbedryf. Die behandeling van beesvleis met ‘n vloeistof ten einde die sagtheid van die finale produk te verbeter het die potensiaal om die inname van beesvleis in Suid Afrika te verhoog. Ten spyte van die feit dat die gemelde behandeling ‘n afname in kleurstabiliteit tot gevolg het en dus die verbruiker se aankoopbesluit negatief sal beinvloed, kan die behandeling steeds suksesvol aangewend word in die Suid Afrikaanse vleisbedryf om die rakleeftyd te verleng en die vleiskwaliteit te verbeter om aan die verbruiker ‘n meer aanvaarbare beesvleis produk te verskaf.

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Language and style used in this thesis are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

CHAPTER 1

INTRODUCTION AND MOTIVATION

The decreasing consumption of red meat in established Western markets (Becker *et al*., 1998; Dransfield, 2003; Jeremiah & Gibson, 2003) is a key issue which the red meat industry should address globally. Beef consumption is also declining in South Africa (Anon., 2004) and it is the goal of red meat producers to increase productivity and red meat consumption (Anon., 2003). In South Africa there is both a developing and developed consumer sector (Strydom, 2003) and, as such, two areas can be identified as contributing towards decreased meat consumption in Southern Africa. Many households in South Africa are restricted in their choice (variety) of dietary intake due to low expendable income. Poverty and high costs of living therefore constrain people in low-income households from purchasing fruits, vegetables and foods of animal origin (Van Heerden & Schönfeldt, 2003). Contrary to the above-mentioned situation, the source of the declining meat sector can also be sought at the higher income households. The level of inconsistent meat quality and the individual consumer who is increasingly becoming concerned about the eating quality (Dransfield, 2003), nutritional value, healthiness (Hoffman *et al*., 2005) and safety of the meat products they consume thus lead to the declining consumption of beef (Strydom, 2003).

Before the problem of unreliable meat quality can be resolved, a clear understanding of what the consumer wants, expects and needs in terms of quality is required (Bryhni *et al*., 2002). According to Webb (2003), producers, retailers and consumers have different perceptions and expectations of meat quality. However, it is clear that consumer demand for leaner beef will influence the way that producers feed their cattle in the 21st century (Spears, 1996). Producers and manufacturers therefore have to meet consumer demands, trends and preferences in order to survive in an increasingly competitive market (McGee *et al*., 2003).

Furthermore, consumers need to have the assurance of consistent quality of the beef products they purchase and that they are guaranteed a pleasant eating experience. The acceptability of any food product is largely determined by the consumers' perception of quality and will ultimately determine the success of the food products (Dransfield, 2001). The consumer's decision to purchase beef is guided by the perception of healthiness and a variety of sensory attributes (Carpenter *et al*., 2001) including colour, tenderness,

juiciness and flavour (Verbeke & Viaene, 1999). The rapid changes in scientific knowledge, analytical methods and consumer expectations, perceptions and preferences in beef quality (Webb, 2004; Bernabéu & Tendero, 2005) within the meat-production industry have led to the continuously evolving concept of quality (Nardone & Valfré, 1999). The concept of meat quality includes many factors and numerous scientists have tried to define meat quality (Dransfield, 2003; Torrescano *et al*., 2003; Webb, 2004). Warkup & Marie (1995) defined beef quality as comprising four main categories:

- Visual quality: Factors evaluated in carcass classification and/or factors that affect consumers' decisions when purchasing meat (e.g. meat and fat cover, bone content, subcutaneous fat cover);
- Eating quality: Tenderness, juiciness, odour and flavour intensity of the cooked product;
- Nutritional quality: Proportions of proteins, vitamins and minerals relative to energy density;
- Safety: Negligible risk from food-borne illness or poisoning and absence of drug, chemical, antibiotic or hormone residues.

Despite various definitions of quality beef as well as substantial and constant research in meat science the aim of consistent eating quality continues to be an elusive goal (Tarrant, 1998). The beef industry considers solving the problem of inconsistency of the utmost importance (Koohmaraie, 1996; Koohmaraie *et al*., 2002; Robbins *et al*., 2003). The problem of inconsistent meat quality is linked mostly to appearance and palatability characteristics, in particular tenderness (Jeremiah *et al*., 2003; Koohmaraie *et al*., 2003).

Meat research over the years has indicated the degree of variation within various aspects of meat quality. Early meat scientists defined the physical and sensory properties of the major muscles found in the beef carcass (Ramsbottom *et al*., 1945; Ramsbottom & Strandine, 1948; Strandine *et al*., 1949) and research clearly demonstrated that variation occurred between carcasses and even within the same breed. Paul *et al*. (1970), McKeith *et al*. (1985) and Polidori *et al*. (2000) also reported that variation occurred between muscles within a cut and even between parts of the same muscle (Smith *et al*., 1969; Denoyelle & Lebihan, 2003), further confirming the problem of inconsistency. The extent of meat quality variation between muscles in cattle produced in South Africa is not clear, due to a lack of research on South African animals. However, international reports indicate a distinct tendency of muscle variation (Rhee *et al*., 2004).

Variation in beef quality is extensive and exists from conception to consumption, i.e. the whole food-production chain (Andersen *et al*., 2004). Several factors or a combination

of factors such as production and biological factors contribute to the large variation in beef quality. Differences in genetic background, sex, age, management, nutrition (Webb, 2004; Delgado *et al*., 2005) and the anatomical location of the muscle (Jeremiah *et al*., 1991), different methods of pre-slaughter handling and different post-mortem storage and processing conditions (Koohmaraie, 1996; Dikeman, 2002; Denoyelle & Lebihan, 2003) all have an influence on the quality of the meat (Koohmaraie, 1994; Warriss, 2000; Koohmaraie *et al*., 2003). The effect of different ageing (post-mortem storage) periods has been researched numerous times (Dransfield, 1994), indicating that beef muscles differ in their response to conditioning (Olson *et al*., 1976; Koohmaraie *et al*., 1988). This phenomenon affects the final meat quality attained. The lack of adequate guidance in processing techniques to achieve the most palatable product is another contributing factor to this variation (Jeremiah & Gibson, 2003).

It is therefore clear that there is substantial variation in the physical properties, nutritional value and sensory traits of individual beef muscles (Koohmaraie, 1994; Jeremiah *et al*., 2003). Such wide variation has a considerable impact on the appearance and palatability attributes, as well as the consumer acceptance of the product (Raes *et al*., 2003).

The beef industry has to focus on total quality management to ensure a final product consistent in high quality and value (Strydom, 2003). According to several authors, it is becoming increasingly necessary for the food industry to provide high-quality, convenient food products at moderate prices (Jeremiah & Philips, 2000; Desmond & Troy, 2001). In the meat industry, where the high cost of meat production has led to the development of ways to further utilise the whole carcass and maximise the yield of saleable product (Wilson, 1993), the high-quality requirement is of particular importance. This has led to the development of various methods and technologies of restructuring and extending low-value cuts to enhance their appearance and textural properties and hence increase their market value (Wheeler *et al*., 1993; Robbins *et al*., 2003). It is important to recognise that variation and inconsistency occur regardless of the value of the cut and that a high-value cut is also in need of managing and reduction in muscle variation.

Several processes can be implemented to manage and reduce variation and improve consumer satisfaction (Koohmaraie *et al*., 2003). It has long been known that beef quality, in particular the tenderness of beef, could be enhanced by means of blade or needle tenderisation. Enhanced meat is becoming increasingly popular in countries abroad (Anon., 2005). However, it is still a fairly new concept in the South African meat industry. The physical disruption of the tissue by numerous penetrating needles and the

solution injected into the meat product results in improved tenderness (Wheeler *et al*., 1997) and does not have detrimental effects on other beef-quality attributes (Lawrence *et al*., 2003; McGee *et al*., 2003).).

Therefore, in the present study, a basting (Freddy Hirsch Tenderbite # 802539), consisting of sodium and potassium salts, various phosphates and lactates was injected into selected beef muscles to determine the effect of the enhancing solution on the sensory, physical and chemical properties and the shelf-life of the beef muscles (Figure 1).

The objective is to improve individual muscle palatability and reduce the effect of muscle variation on meat quality, hence providing a more uniform and consistent product to the consumer, as well as aid in meeting consumer demands for a higher-quality product (Kerth *et al*., 1995). This enhancement of beef quality and attaining consistency is crucial for the survival of the beef industry (Jeremiah and Philips, 2000).

This study was therefore undertaken in collaboration with the Department of Food Science and Animal Sciences at the University of Stellenbosch and the Freddy Hirsch Group. In Figure 1 the overall research aims and three experiments conducted is shown.

- The aim of Experiment 1 was to determine the muscle variation of selected South African beef muscles pertaining to the physical, chemical and sensory quality. In Figure 2 the research design and chapter aims of the muscle variation study are illustrated.

- The aim of Experiment 2 was to determine the effect of a phosphate and lactate blend on the physical, chemical and sensory quality and consumer preference of South African beef muscles during a 19-d ageing period. In Figure 3 the research design and chapter aims of the enhancement study are illustrated.

- The aim of Experiment 3 was to determine the effect of a phosphate and lactate blend on the physical and chemical quality and shelf-life (microbiological growth) of South African beef muscle (*Longissimus thoracis et lumborum*) during a 10-d storage period. In Figure 4 the research design and chapter aims of the shelf-life study are illustrated.

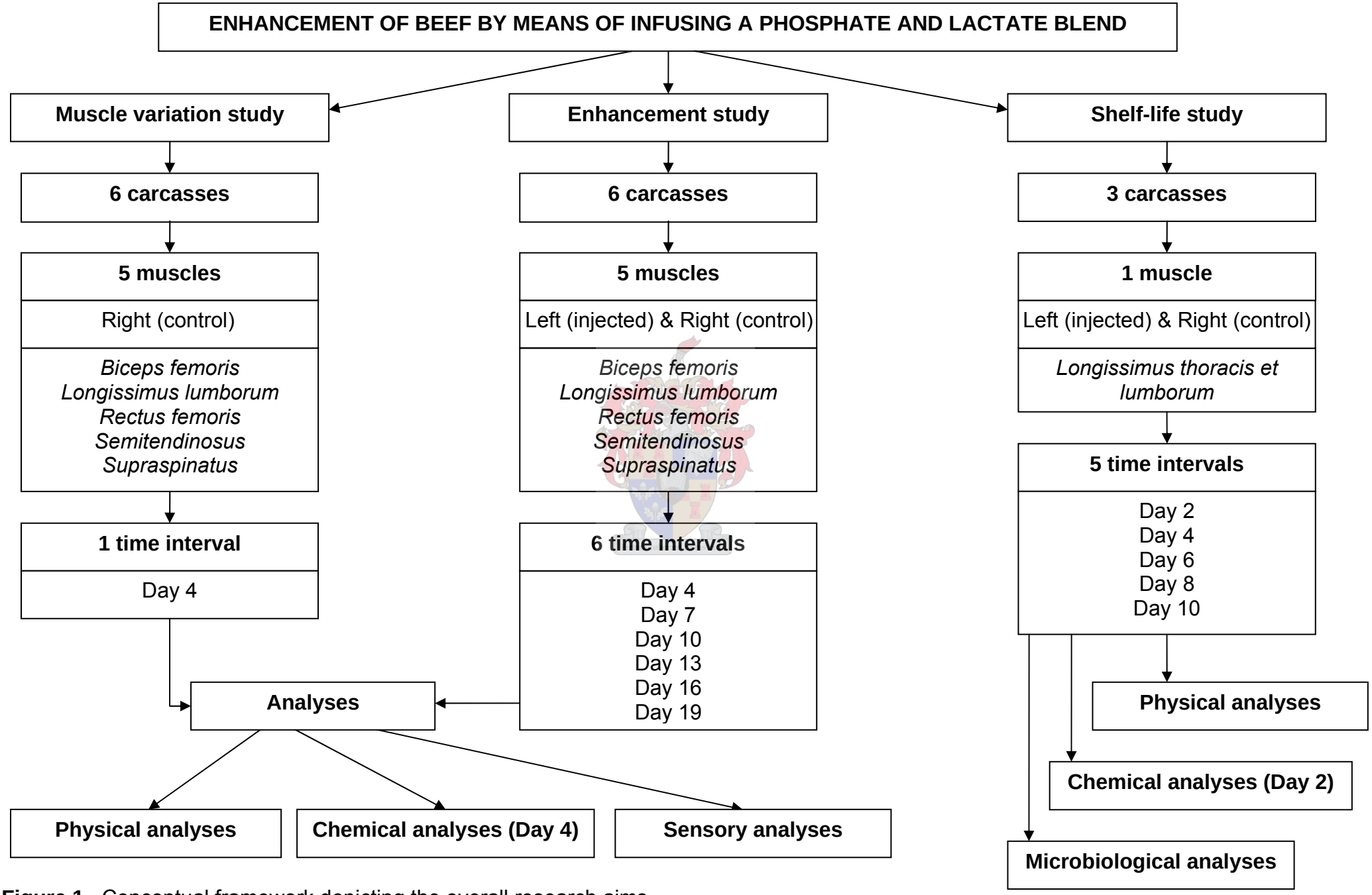

Figure 1. Conceptual framework depicting the overall research aims.

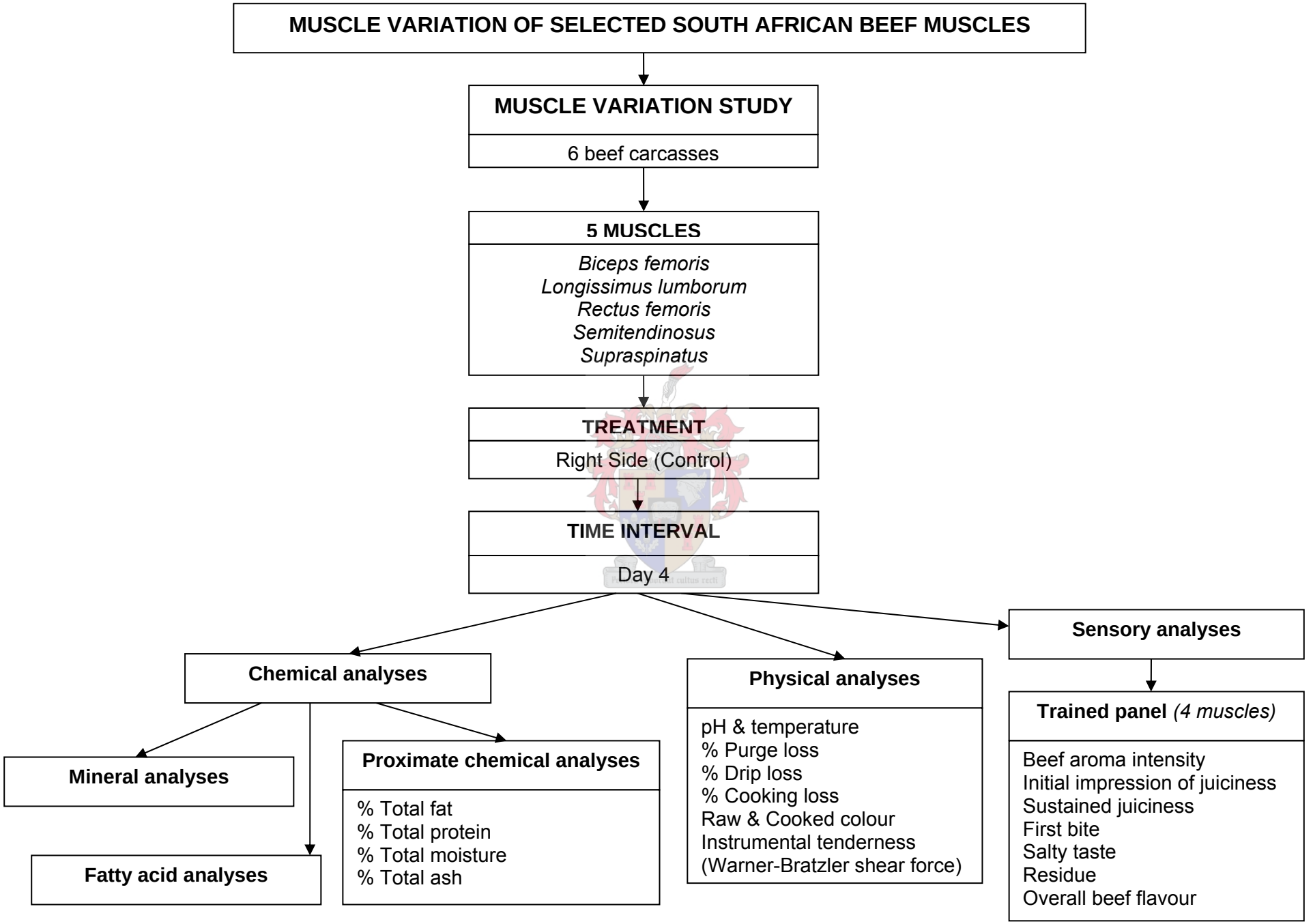

Figure 2. Conceptual framework depicting the muscle variation study.

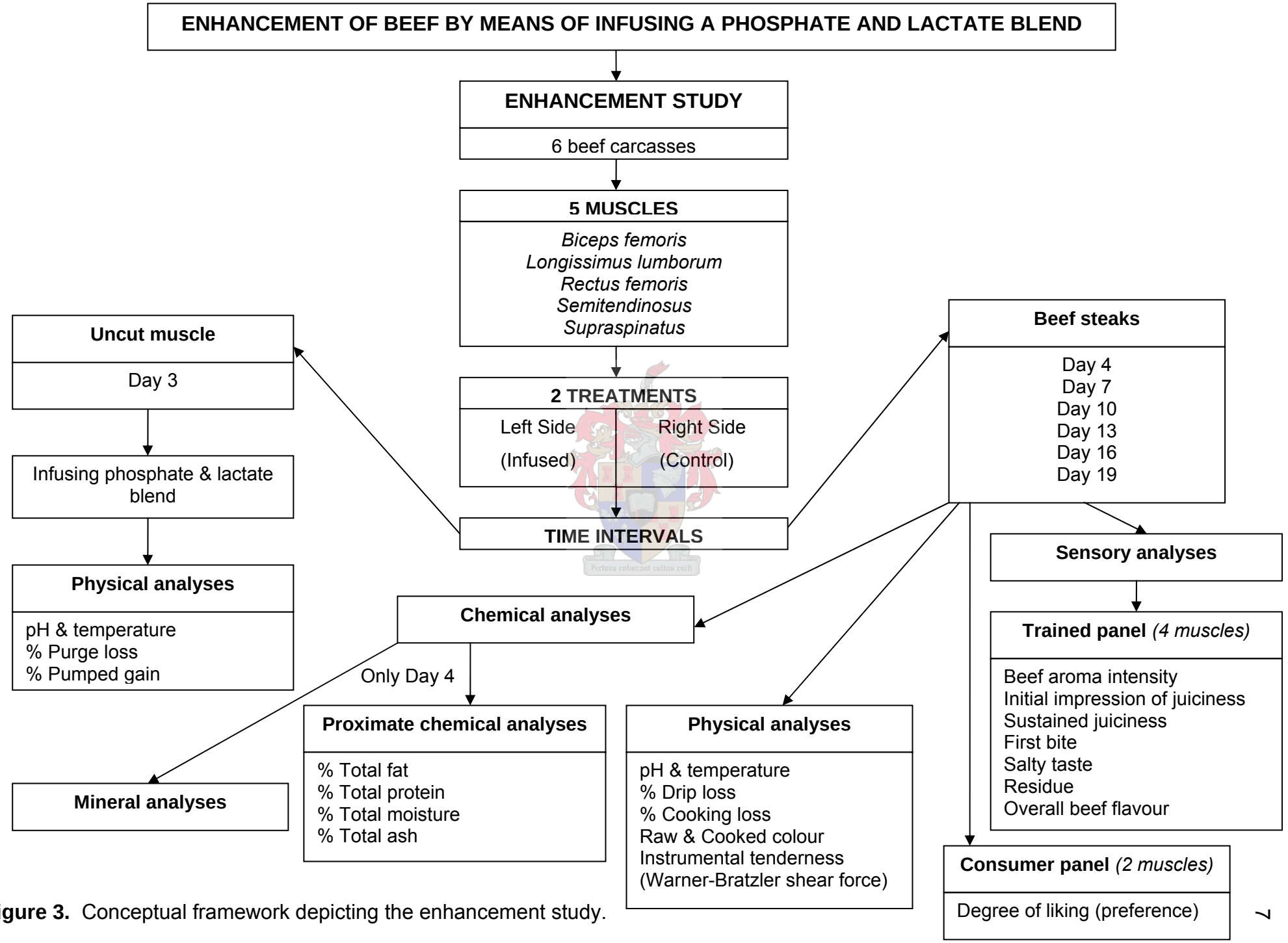

Figure 3. Conceptual framework depicting the enhancement study.

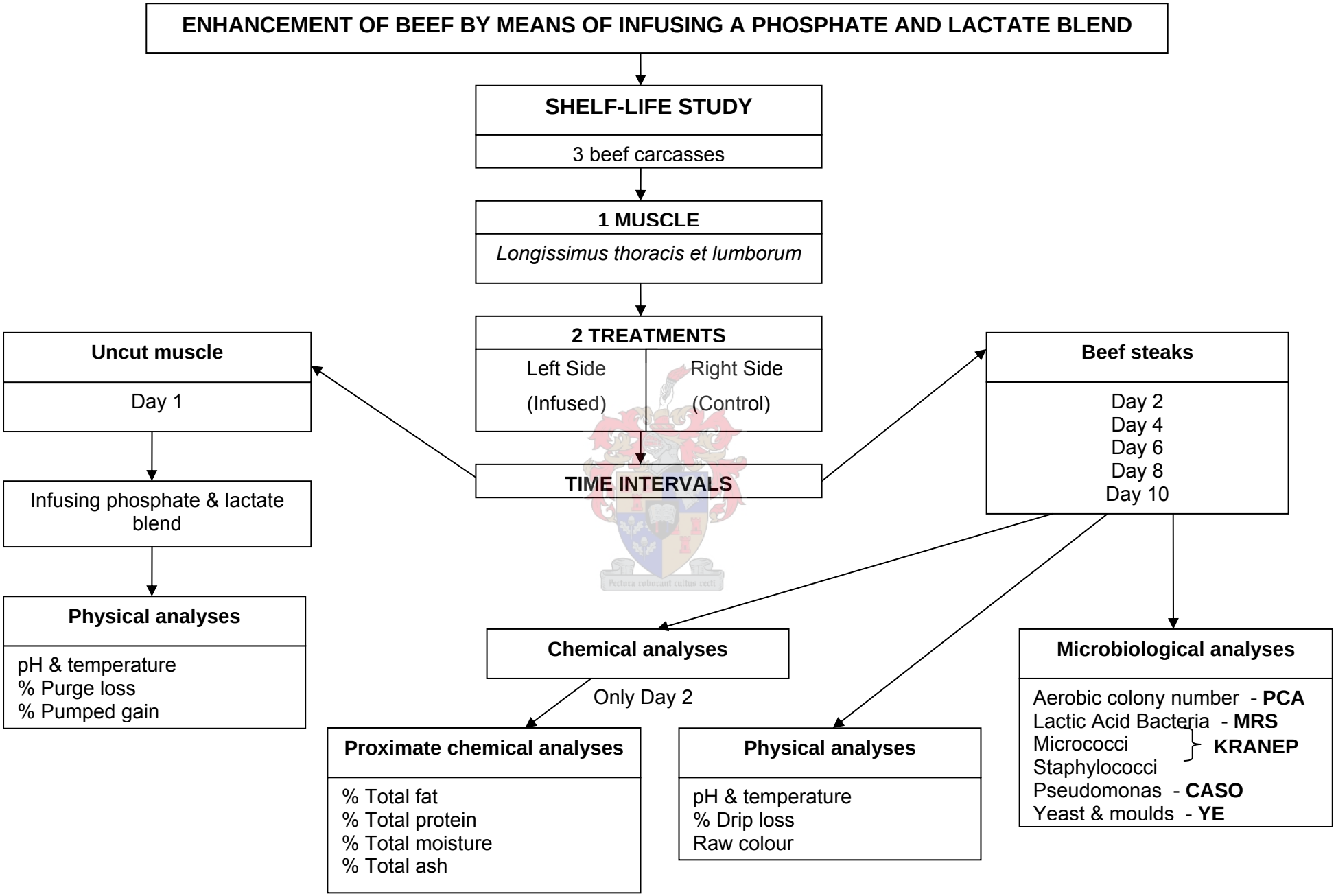

Figure 4. Conceptual framework depicting the shelf-life study.

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CHAPTER 2

LITERATURE REVIEW

2.1. BACKGROUND

The prospect of achieving success (efficient technologies applied, increased market share and increased beef consumption) in the international meat industry looks very promising, when the expected large increase in meat consumption in the developing countries is considered. However, in developed countries meat consumption has shown limited variation after an initial increase. Meat consumption in these countries is impaired as a result of issues such as meat safety crises and inconsistent meat quality, which inevitably led to new consumer demands (Valin, 2000). As far as the safety crises are concerned, the meat outbreak which transformed the consumers' awareness of and concern about safety was the *Escherichia coli* O157:H7 outbreak. This was attributed to undercooked hamburgers by a fast-food chain in the United States of America (USA) in late 1992 and early 1993, which resulted in 501 documented cases, of which four people died (Fung *et al*., 2001). An example of inconsistent meat quality is the variation in meat tenderness as a result of variations during slaughter and post-mortem storage, and between muscles (Koohmaraie *et al*., 2003). To overcome these problems basic research and integrated multidisciplinary approaches should be given priority (Valin, 2000). Since both the developing and developed consumer sectors are present in Southern Africa, the meat industry should respond to the above-mentioned aspects ideally by focusing on efficiency of production (quantity) to provide for the growing demand for meat and simultaneously attend to consumer concerns such as food safety, nutritional demands and unreliable eating quality (Strydom, 2003). Hence the need for a solution which will address the problems (declining beef consumption, quality variation) in the South African meat industry and provide a product that fulfils the consumers' expectations.

Before the above-mentioned issues can be addressed, several aspects of the muscle itself should be understood in regard to the conversion of the muscle to the meat product. Muscle is a highly organised biological tissue with an intricate, complex structure, a unique composition and a very active biochemical capability. The meat scientist should have a comprehensive knowledge of the muscle anatomy, composition, rigor mortis development and functional properties of the muscle in order to understand and improve meat processing (Anon., 2001). The quality of meat is dependent on considerable inter-

and intra-animal variation. Significant variation also exists between different muscles on a particular carcass as well as within a particular muscle with reference to the structural, compositional and biochemical properties of the muscle. This discrepancy between and within muscles relates to the type of action each muscle performs in the body and to induced effects arising from pre-slaughter and post-slaughter handling procedures (Jones, 1985).

It is essential to bear in mind that meat remains highly variable as a raw material, so that a proper understanding of the different properties will help achieve the best finished products, as animal variation due to species, age, sex, environment and pre-slaughter handling affect the properties of its meat (Lawrie, 1985). This, in turn, affects the basic characteristics inherent in muscle, biochemical changes that occur in converting muscle to meat and the chemical properties of the most prevalent meat components (Anon., 2001).

This variation again gives rise to discrepancies in the meat quality of the product and so, in order to diminish this variation, several methods have been developed to improve the appearance and more importantly the textural properties of beef, thereby enhancing its market value (Desmond & Troy, 2001). This aspect of enhancing the quality of beef by using several methods and the natural ageing of meat, as well as enhancing the various quality aspects such as sensory, chemical and shelf-life of the meat product, will be considered in this review.

In conclusion, the process of muscle conversion to meat and all the factors that influence consumer choice and product acceptability, sensory properties of meat, and the way in which these are quantified by the use of different physical and chemical methods, as well as health issues and safety associated with the consumption of meat, will be discussed in this literature review.

2.2. THE CONVERSION OF MUSCLE TO MEAT

Natural ageing

Meat is derived from skeletal muscle, whose function is to provide movement by its ability to expand and contract in length. At the death of the animal a series of biochemical and biophysical changes are initiated which change muscle into meat, as we know it. The nature of these changes and the rate at which they occur affect meat quality in terms of tenderness, juiciness, colour, keeping quality, etc. and may be expected to differ between the various muscles (Wilson, 1981).

Although this process of change is physiologically fairly complex, the main requirements for optimum quality entail sufficient reserves of muscle glycogen at slaughter and a controlled process when glycogen converts to lactic acid to achieve a final muscle pH of c.a. 5.5 once rigor mortis has been completed (Strydom, 2003). This will provide optimum quality in terms of ageing ability, basic tenderness, colour and limited drip loss.

The process of muscle changing to meat

In the process of the muscle converting to meat, several post-mortem events take place, namely acidification of the muscles, the development of rigor mortis and, lastly, the resolution of the muscles and tenderisation of the meat (Jiang, 1998; Warriss, 2000). These concepts will be discussed in the following section.

Post-mortem glycolysis and acidification

When an animal dies, oxygen, glucose and free fatty acids no longer reach the muscles due to the failure of the blood circulatory system. Therefore, any post-mortem metabolism takes place via the anaerobic pathway and adenosine-triphosphate (ATP) can only be regenerated through the breakdown of glycogen by glycolysis, since oxidative decarboxylation and phosphorylation will no longer operate (Warriss, 2000). This reaction provides enough energy to maintain structural and functional integrity in the muscle (Varnam & Sutherland, 1995). As glycogen is broken down through the Embden-Meyerhof pathway, lactic acid starts to accumulate in the muscle. As a result of the cessation of the blood system, this lactic acid cannot be removed and the muscle gradually becomes more acidic. In the muscle of a relaxed and well-fed animal, the pH value falls from about 7.2 to around 5.5. The ultimate pH (pHu) finally reached depends on the muscle and animal species. This process of acidification (reaching the ultimate pH) normally takes 4 to 8 h in pigs, 12 to 24 h in sheep, 15 to 36 h in cattle (Dransfield, 1994) and is relatively rapid in poultry (10 to 15 min) (Pearson & Young, 1989), although 2 h has been reported by Dransfield (1994).

The ultimate pH is inversely proportional to the lactate concentration of lactate and the initial glycogen concentration becomes limiting below about 10 mg/g muscle. If glycogen is unlimited, the production of lactic acid ceases when the enzymes start to denature and will no longer function at the low pH of 5.5 (Warriss, 2000).

As the muscle proteins denature with the fall of pH, they start to lose their ability to bind water, thus reaching their isoelectric point (pH 5.4-5.5), which is also the point of minimum water-holding capacity. The myofibrillar proteins, myosin and actin, also reach

their isoelectric point. The isoelectric point is the pH at which the protein molecules have no net electrical charge and tend to lose the water that is normally bound to them. The consequence of this reaction is exudation of fluid from the muscle fibres. Eventually this exudate produces drip that will lead to weight loss. The light scattering properties of the contractile elements of the muscle fibre are increased because of the protein change. The outcome is an opaque and paler meat colour, in contrast with the dark and translucent appearance of the muscle in the live animal (Warriss, 2000; Swatland, 2004). Figure 1 illustrates the reaction described.

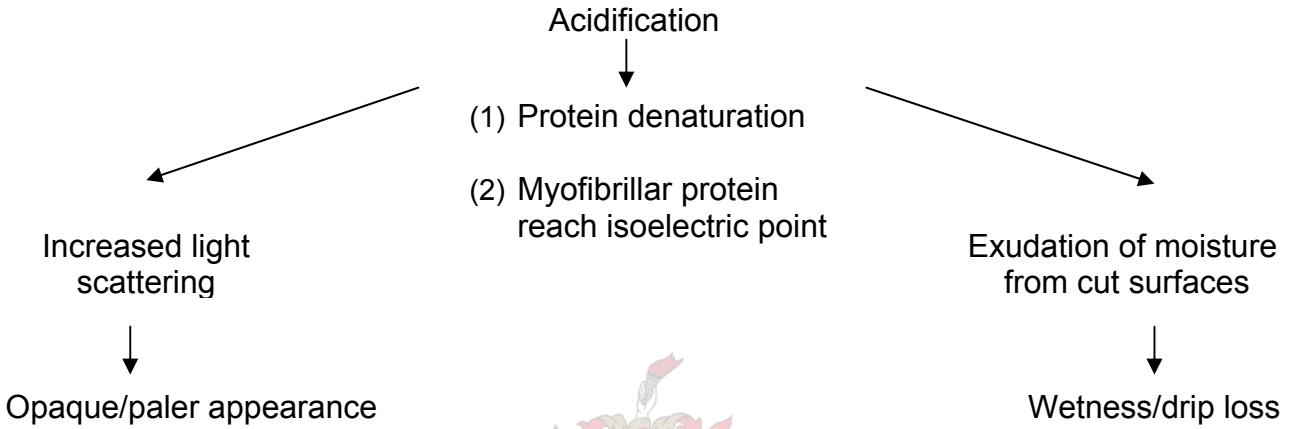

Figure 1. The consequences of muscle acidification as pertaining to the appearance and water-holding capacity of meat (Warriss, 2000).

The development of rigor mortis

In the resting muscle ATP keeps the muscle in a relaxed state by preventing the formation of actomyosin. Contraction occurs only when ATP is hydrolysed to adenosine-diphosphate (ADP). The ATP concentration is maintained by the breakdown of glycogen until lack of substrate or unsuitable conditions, particularly pH, for the enzymes, inhibits glycolysis. The level of creatine phosphate then falls, as it is used to regenerate ATP from ADP. Eventually none of the mechanisms will be able to maintain ATP levels and the supply of regenerated ATP fails (Varnam & Sutherland, 1995).

A dramatic consequence of the cessation of post-mortem glycolysis and the fall in ATP levels is the onset of rigor mortis. Rigor mortis occurs when the ATP concentration falls below the very low level (~5 mmol/kg) required to maintain relaxation (Warriss, 2000). At this point the actin and myosin molecules of the thin and thick filaments interact to form inextensible actomyosin, and extensibility of the muscle is lost. Cross-bridges are permanently formed and there is in effect a very weak contraction. Once muscle ATP reserves are exhausted, each muscle fibre goes into rigor very quickly (Bendell, 1973), but

individual fibres do vary and this leads to a more gradual development of stiffness in the whole muscle as more and more fibres become inextensible (Pearson & Young, 1989).

The time passed after slaughter for rigor to start will be determined by factors that influence the level of glycogen and creatine phosphate (Cp) at death and the rate of post-mortem muscle metabolism. If an animal has undergone stress or exhaustion at death, or the glycogen has been depleted by longer-term pre-slaughter stress, rigor occurs faster (Jiang, 1998). If the carcass is cooled quicker, the rate of rigor development will be reduced. Care should, however, be taken when cooling the carcass with respect to the effect on quality. If the carcass is cooled too quickly or too slowly, it could have a negative impact on meat tenderness, colour and juiciness (Strydom, 2003).

Rigor differs in time of onset in different species, ranging from about 4 h in chicken to over 24 h in excised beef muscle (Etherington, 1984). As more muscles enter rigor mortis the whole carcass becomes increasingly stiff and “sets” (Warriss, 2000). The rigor mortis process is completed in approximately 6 to 12 h in beef and 1 to 6 h in pork (Epley, 1992).

The tenderisation of meat

The muscles soften after a variable period of time, when there is a progressive ‘resolution’ of rigor (Warriss, 2000). Although the rate of tenderisation varies considerably with temperature and among the different animal species; a similar mechanisms seems to be involved in each case (Varnam & Sutherland, 1995).

The process occurs faster at higher temperatures, making it necessary to ensure that the temperature is controlled at a maximum of 5°C to obtain optimum ageing of the muscles (Varnam & Sutherland, 1995). The final tenderness achieved in a certain time more than doubles with every temperature increase of 10°C. About 8 h after death chicken meat achieves 80% of its maximum tenderness, whereas beef takes 10 d to reach the same level of tenderness (Warriss, 2000).

These differences in the rate of tenderisation lead to different recommended ‘ageing’ times prior to cooking the meat. Therefore, to maximise the benefits of post-mortem storage on meat tenderness, these ‘ageing’ times should be applied. Beef should be stored for 10 to 14 d, lamb for 7 to 10 d and pork for 5 d at 1°C (Koohmaraie, 1996; Jiang, 1998). However, the cost of storing the meat at refrigerated temperatures is high. These expenses include the storage space, refrigeration and the inevitable weight loss from the surface of the carcass due to the evaporation of water, or the loss of exudate from butchered joints (Warriss, 2000). Therefore, several methods have been developed

to minimise the time in storage, but not impair the meat quality, especially tenderness. These post-mortem tenderisation technologies for the improvement of meat quality will be discussed later in the section on enhanced ageing in this literature review.

The post-mortem process of storing meat post-rigor for various lengths of time under adequate conditions of temperature to improve meat quality is known as conditioning or ageing (Boakye & Mittal, 1993). This is the natural process of tenderisation and could be attributable to two types of processes, namely changes in the connective tissue components of the meat or weakening of the myofibrils (Warriss, 2000). Nishimura *et al*. (1998) suggested two phases in which conditioning takes place. The first phase occurs at a rapid rate and is the denaturation and proteolysis of key myofibrillar and associated proteins (Jiang, 1998). The slower second phase involves the structural weakening of the intramuscular connective tissue (Nishimura *et al*., 1998). Research shows that the more important changes occur in the myofibrillar component, whereas the major connective tissue components such as collagen undergo only very small changes (Lawrie, 1998b; Warriss, 2000). Nevertheless, there is some cleavage of collagen cross-links and small structural changes can be seen under the electron microscope after extended ageing times. These structural changes in the connective tissue are possibly due to lysosomal enzymes (Lawrie, 1998a).

As mentioned above, larger changes in the myofibrillar component can be seen. The attachments of the thin (actin) filaments to the Z disks show some breakdown and there is an increase in the amount of water-soluble nitrogen compounds. However, the muscle does not become more extensible during ageing and the tenderisation process is not associated with any dissociation of actomyosin (Koohmaraie, 1994). The thick (myosin) and thin (actin) filaments remain locked together by the myosin cross-bridges. Tenderisation is not caused by the filaments regaining the ability to slide over one another, i.e. the release of the cross-bridges (Etherington, 1984).

The mechanisms involved in the meat tenderising process are considered to be the results of the activities of endogenous proteolytic enzymes present in the muscles. Their normal role is in the breakdown and recycling of proteins, which occurs continuously in all living tissues (Ouali, 1990). Although several proteolytic systems related to the tenderisation process are described in the literature, i.e. calpains-calpastatin, cathepsins-cystatin, and proteasome or macropain, only two of them have attracted attention from meat scientists. They are μ- and m-calpains and lysosomal proteinases, namely, cathepsins D, B, H and L (Goll *et al*., 1989).

There are four phenomena which reveal the involvement of calpain in post-mortem muscle tenderness:

- The ultra-structural degradation of post-mortem myofibrils is quite similar to that of myofibrils treated with calpain;
- Post-mortem myofibrillar proteins, untreated or treated with calpain have similar electrophoretical degradation patterns;
- The Z-disk, where the calpains are localized, is extremely susceptible to calpain-catalysed hydrolysis.
- The higher the level of calpain in muscle, the faster the rate of post-mortem tenderisation (Dayton *et al*., 1976).

There has been considerable debate about the specific proteases responsible for post-mortem changes. A protease must meet certain criteria to be considered a possible candidate for involvement in post-mortem tenderisation (Koohmaraie, 1996). Firstly, the protease must be endogenous to skeletal muscle cells. Secondly, the protease must have the ability to reproduce post-mortem changes in myofibrils *in vitro*. Finally, the protease must have access to myofibrils in tissue (Goll *et al*., 1983). If a protease does not have these characteristics, it cannot be considered as a candidate in the post-mortem tenderisation process. Of all the potential candidates, calpains are the proteases that meet all of the above criteria. Based on the results of numerous experiments reported by different laboratories, it can be concluded that proteolysis of key myofibrillar proteins by calpains is the underlying mechanism of meat tenderisation that occurs during storage of meat at refrigerated temperatures (Koohmaraie, 1994).

Differing from the calpains, which are considered to specifically attack certain proteins of the Z-line, such as desmin, filamin, nebulin, and to a lesser extent connectin (King, 1984), cathepsins preferentially attack myosin and actin (Jiang, 1998). Furthermore, they can attack contractile proteins at various strategic points. Cathepsin B can rapidly degrade myosin heavy chains, while cathepsin L degrades the troponins T and I and the C-protein rapidly, and myosin, actin, tropomyosin, nebulin, titin and α-actinin slowly (Jiang, 1998). Although calpains have an optimum pH range for activity near neutrality, cathepsins, especially B and L, have pH optima, which are more closely associated with the pH range (5.5 ~ 6.5) found in many post-mortem skeletal muscles (Etherington, 1984). Nevertheless, Koohmaraie (1996) did not consider the cathepsins to be responsible for the tenderisation of meats. A possible explanation would be that lysosomal proteases might still be located in lysosomes and have no access to myofibrils or cytosol. However, the fall in pH during post-mortem glycolysis weakens the walls of

organelles such as lysosomes (Etherington, 1984), which consequently causes the release of lysosomal proteinases, such as cathepsins B, H and L, which have pH optima at around 5.5~6.5. Furthermore, when aged muscle is extended, fractures mainly appear close to the Z-lines and less frequently at the junctions of the A-bands and I-bands. Fragilisation of these regions in stored meat can perhaps be ascribed to the action of lysosomal proteinases (Ouali, 1990), which can contribute to the post-mortem tenderisation of meat (Jiang, 1998).

Meat tenderisation is a very complex multifactorial process, which is controlled by a number of various endogenous proteinases, and has as yet poorly understood biological parameters. A more detailed investigation is needed to clarify which parameters have the greatest impact on the tenderness of meat at temperatures which cause minimal cold-shortening (Jiang, 1998). However, a detailed analysis of these parameters is not included in the objectives outlined for this thesis and the literature review will focus more on methods used in enhancing meat quality, predominantly tenderness, rather than on a detailed investigation of the mechanisms involved in tenderisation of meat.

However, the general findings discussed in this section indicate that the modifications established at the myofibrillar structure level in aged meat can be explained by the proteolytic action of muscle proteinases (Jiang, 1998). Therefore, the proteinases such as the calpains and lysosomal proteinases contribute to post-mortem tenderisation of meats (Koohmaraie, 1996).

Additional changes occurring in the conversion of muscle to meat

In the previous section several major changes which occur in the muscles after the death of the animal were discussed, such as acidification, rigor development and its resolution. These, however, are not the only changes that take place in the muscle. Meat-quality characteristics such as colour and water-holding capacity are influenced by the acidification (pH rate and decline), whereas tenderisation is the outcome of rigor resolution. As the process continues, the eating qualities, juiciness and flavour also improve and this is of importance, especially in cooked meat. These attributes of eating quality, namely colour, water-holding capacity and juiciness, flavour and tenderness, will be the subject of discussion in a later section.

Cessation of the circulation of blood at death initiates a complex series of changes in the muscular tissue, converting muscle into meat and in the end producing a meat product with a certain quality, either of a good or low standard. These changes should occur under controlled conditions to diminish the effect of variability among different

muscles and ensure an optimum meat product. However, despite controlled conditions, there are several ways to pursue and to secure, to some extent, improved or enhanced meat quality. This is the subject of the following section, where methodologies to improve meat quality are considered in more detail.

Enhanced ageing

As stated earlier, there are unmistakable variations between carcasses, muscle groups and muscles, and sometimes within a muscle, leading to inconsistent and unreliable meat quality. Koohmaraie *et al*. (2003) identified inconsistent meat quality and more specifically toughness as one of the major problems facing the meat industry today. According to Strydom (2003) tenderness is the most important factor in determining the continued support of red meat (purchasing) after consumption. Numerous studies indicate that the consumer considers tenderness the most important eating attribute of fresh meat, with juiciness and flavour further supporting a good eating experience (Koohmaraie, 1988; Ouali, 1990; Dransfield, 1994).

To minimise the risk of a consumer having a poor eating experience, the cause of variation in the rate and extent of post-mortem tenderisation must be identified, in order to manipulate the tenderisation process and develop potential approaches for managing this variation and improve consumer satisfaction (Koohmaraie *et al*., 2003). This issue will be considered in the tenderness subdivision under the eating quality section of this chapter.

There are several possible approaches to be taken to improve meat quality. Enzymatic, mechanical and chemical methods are just a few of these treatments applied to produce a product with the desired meat-quality attributes (Desmond & Troy, 2001).

Methods for enhancing meat quality

Artificially enhancing of the qualities of meat can take place in the living animal or during the post-mortem period of ageing. The latter is more frequently applied in the industry.

Ante-mortem treatment

An ante-mortem treatment was developed in which proteolytic enzymes of plant origin such as papain, ficin and bromelin are administered (injected) to animals just before slaughter. The ante-mortem treatment is applied where the enzyme is injected into the jugular vein of the animal 30 min before slaughter to be circulated in the blood. This allows for even distribution of the enzymes in the muscles. The enzyme is injected in an inactive but stable form, but is activated during the acidification of the muscles post-

mortem and also when the meat is cooked (Varnam & Sutherland, 1995). If the animal is not slaughtered, the inactivated enzyme will be excreted through the kidneys. If the animal is slaughtered, the muscle will become depleted of oxygen and, as a result, free thiols and other reducing agents produced will reactivate the enzyme.

Active enzymes can also be injected into animals but only in small doses. If a large dose of active enzyme is injected, the serum complement pathway in the animal will be activated, causing a "fatal shock" reaction and death of the animal (Anon., 2003a).

This treatment of ante-mortem injection proved to be the most effective method of introducing proteolytic enzymes into meat so that they penetrate uniformly into the furthest interstices/crevices of the tissue. However, other researchers reported potential practical problems with the use of these infusions, such as uneven distribution throughout the body and negative effects on organs such as the liver. In addition, some animal welfare concerns have been raised with the use of this procedure (Varnam & Sutherland, 1995).

Post-mortem treatments

After slaughtering, several post-mortem treatments can be applied to reduce cold-shortening and subsequent toughness of meat and improve the quality. Various examples of these procedures applied in the meat industry are slow or delayed pre-rigor chilling, electrical stimulation, hot processing, novel carcass suspension methods (physically stretching or restricting the muscle from contracting) (Sørheim & Hildrum, 2002) and tenderising the meat by marinating and injection / infusion with salts and acids as well as the use of pressure (Anon., 2003b). The tenderising treatments to be discussed in this review involve the physical, chemical and enzymatic disruption of the muscle tissue.

- Carcass handling

Electrical stimulation: Electrically stimulating the carcass at certain times after slaughter is an innovation commonly used in the meat industry to increase tenderness. Electrical stimulation will speed up the normal post-mortem processes (Epley, 1992) by causing intense muscle contractions (tearing and stretching) (Tornberg, 1996; Koohmaraie *et al*., 2003). The contractions exhaust the glycogen and creatine phosphate stores, thus accelerating glycolysis, resulting in a rapid pH fall and early development of rigor mortis (Lawrie, 1998c). When stimulation stops, the carcass temperature is still high and the sarcoplasmic reticulum can absorb the previously released calcium, resulting in the muscle becoming relaxed once more. If the pre-rigor muscle is then exposed to low temperatures during rapid chilling, the muscle no longer responds to the cold stimulus and there is no

danger of toughening (Etherington, 1984). As mentioned, electrical stimulation dramatically shortens the rate of pH fall. For example, in beef carcasses the decrease in pH to below 6.0 could take 10 to 12 h under normal conditions, but with electrical stimulation, this time may be reduced to only 1 to 2 h (Warriss, 2000). Electrical stimulation not only prevents cold-shortening, but also appears to tenderise the meat and improves appearance and possibly flavour. The reasons for these effects are still not fully understood. It has been suggested that tenderisation could be caused by several mechanisms, such as intense contractions that might physically disrupt and so weaken the muscle structure (Savell *et al*., 1978). During the contractions calcium is released, which stimulates the calpains at a time when the muscle temperature and pH are still high, causing greater proteolytic breakdown. According to Dutson *et al*. (1980), the release of the lysosomal enzymes due to lysosome disruption also causes proteolytic breakdown and improves tenderness. The colour of beef from electrically stimulated carcasses is also brighter, possibly due to the rapid muscle acidification that leads to some protein denaturation, increasing light reflectance from the meat surface. The flavour may also be improved by the stimulation affecting the concentrations of flavour precursors and enhancers in the muscles (Warriss, 2000). Even though electrical stimulation seems to be a positive solution for preventing toughness in meat, it was not applied in this study. The intention was to determine only the effect of the solution used in the experimental study on the meat quality and to exclude those variables which may influence or mask the effect of the treatment.

Hot-boning / hot-processing: Conventionally, the whole carcass is chilled to a temperature of 7°C or lower before subsequent cutting into smaller pieces and further processing. This is wasteful of chiller capacity and energy consumption as most of the bone and fat is removed and discarded and has therefore been cooled unnecessarily. In addition, intact carcasses are irregular in size and shape and thus cool unevenly (Warriss, 2000). A solution is to process the carcass when it is still hot, before chilling, i.e. removal of the muscles from the carcass in the pre-rigor state shortly after slaughter while they are still extensible (Sørheim & Hildrum, 2002). This processing is often referred to as *hot-boning*, *hot-deboning* or *hot-cutting*. *Hot-boning* is a technique with several benefits such as reduced weight loss, lower chilling costs, higher binding capacity of the meat (Pisula & Tuburcy, 1996), cost-effectiveness in terms of refrigeration space and energy, less labour required and a reduction in the time needed to produce marketable meat. A negative aspect of hot-boning is that muscles disconnected to the framework of the skeleton while

being exposed to low-temperature cold-shortening conditions are more prone to contract and become tough than muscles on a carcass. To make hot-boned muscles as tender as possible, they must be prevented from contracting, or the chilling rate must be carefully controlled until rigor mortis is passed (Sørheim & Hildrum, 2002).

Tenderstretch^{TM}: The conventional way to suspend carcasses during chilling is by the hind legs using a hook passed behind the Achilles tendon (Thompson, 2002). The carcass weight puts many muscles into tension by stretching them when they pass into rigor. This stretching produces more tender meat as a result of the increase sarcomere lengths (Herring *et al*., 1965). However, because of the antagonistic way in which groups of muscles work, some muscles are in tension while others are free to contract and this leads to inconsistent tenderness (Geesink *et al*., 1995; Warriss, 2000). Therefore, if an S-shaped hook is placed into the obturator foramen (the hole in the skeleton of the pelvic girdle (Epley, 1992) between the ilium, ischium and pubis bones) or the eye of the aitch bone, then the valuable *Longissimus dorsi* and the muscles on the outside of the hip, such as the *Semimembranosus* and Se*mitendinosus*, are stretched when they enter rigor, increasing their tenderness. This process is called pelvic suspension, hip-free suspension or the *Tenderstretch*^{TM}. A disadvantage is that, although some muscles become more tender, others toughen (Sørheim & Hildrum, 2002). However, these are usually either the less valuable ones, or muscles like the *psoas major*, which is inherently very tender anyway, so the slight toughening is of little importance. Another problem is that the carcasses are unconventional in shape after pelvic hanging and take up more space in the chillers (Epley, 1992; Sørheim & Hildrum, 2002).

Tendercut: The procedure for the *Tendercut* involves making cuts in the skeleton of the pre-rigor carcass shortly after slaughter, while maintaining the Achilles tendon in suspension. The weight of the carcass below the point of cutting stretches many of the major loin and round muscles. Bones in the skeleton, connective tissue, adipose tissue and some minor muscles are severed in two selected positions of beef carcass sides (Sørheim & Hildrum, 2002). One cut is placed between the 12th and 13th thoracic vertebrae, at or near the normal position for separating the fore- and hindquarter of the side. The other cut is positioned in the junction between the sirloin and the round. This cut severs the ischium of the pelvic bone, the junction between the 4th and the 5th sacral vertebrae and adjacent connective tissue (Sørheim & Hildrum, 2002). Several studies confirm the benefits of *Tendercut* in increasing the tenderness of beef loin and round

muscles (Wang *et al*., 1994) and reducing the variation in sensory tenderness of beef muscles.

- Handling of meat cuts or joints

Several methods have also been developed in which a meat cut is subjected to a certain technology/methodology with the purpose of enhancing the tenderness of the meat product.

Blade tenderisation: The *Bladetender* is a machine which has multiple blades or needles that penetrate meat as it passes through on a conveyor. The first machine used was known as a *Jaccard* and most people refer to the process as "Jaccarding" (Anon., 2003c). It has long been known that blade or needle tenderisation could be used to improve the tenderness of meat. The physical disruption of the tissue from numerous penetrating needles results in improved tenderness (Benito-Delgado *et al*., 1994; Koohmaraie *et al*., 2003). The myofibrils and connective tissue, specifically the stromal proteins, can be severed by a blade or needle tenderiser (Davis *et al*., 1977). Although this is a convenient and effective method of tenderisation, it can still cause substantial drip losses during storage and may reduce storage life as a result of higher microbial counts due to contaminated blades of needles (Davis *et al*., 1977).

Marinating and injection / infusion: Meat can be tenderised by the action of salt solutions or organic acids. Acid marinating has long been recognised as a traditional culinary technique used to enhance the tenderness and flavour of meat prior to cooking (Rao & Gault, 1990). The effect of marination (acid) on the meat is to break the muscle structure down by promoting the action of collagenase and cathepsins, which perform best at a low pH (Offer & Trinick, 1983, Livisay *et al*., 1996). This causes the myofibrils to swell and retain the water better, thereby increasing tenderness and juiciness (Warriss, 2000). An extension of marinating is to infuse or inject these solutions into whole cuts of meat, a technique used particularly to tenderise and improve the so-called low-value meat cuts (Desmond & Troy, 2001). The process consists of injecting cuts of meat (either pre-rigor or post-rigor) with a solution. Following the infusion, cuts are vacuum packed and stored under refrigerated conditions for 7 d prior to consumption. For optimum results, the use of commercial, automatic pickle injectors is recommended to ensure uniform distribution of the solution throughout the cut of the meat. If possible, one should avoid the use of hand-held injectors to prevent contaminating the meat and to ensure even distribution of the

solution (Koohmaraie *et al*., 2003). The process is more effective in pre-rigor (first 3 h after slaughter) meat, but can be used up to 14 d post-mortem. The injected solution will not affect meat that is already tender; therefore tender meat will not turn “mushy”. The process is also effective in cuts of meat expected to be unusually tough, for example, meat from old cows (Hoffman, 2006). This treatment has been tested successfully under commercial conditions in a large beef processing facility (Koohmaraie *et al*., 2003). There are numerous infusion or marination solutions on the market that can be used to enhance the quality of the meat product. In the following section some of the more frequently used types such as calcium salts, sodium/potassium lactate and phosphates will be discussed.

o Calcium salts: Conditions in post-mortem muscle are not always optimum for calcium to be available to activate the calpains. However, exogenous calcium can be added to meat, thus activating the calpains and inducing more rapid and extensive tenderisation. This process, known as calcium-activated tenderisation (CAT) (Koohmaraie *et al*., 2003), consists of injecting cuts of meat (either pre-rigor or post-rigor) with 5% (by weight) of a 2.2% solution of food-grade calcium chloride ($CaCl_2$). The use of $CaCl_2$, at the recommended level, mentioned above, does not significantly influence other meat-quality traits, such as flavour. However, when higher concentrations of $CaCl_2$ were used for tenderisation, several undesirable effects on the flavour were found. Research shows that calcium chloride injection induces bitter, metallic and livery off-flavours, unacceptable colour deterioration and increased aerobic plate counts. Furthermore, it promotes faster oxidation of the haem pigments, so that the surface of the meat turns brown sooner and this consequently shortens the shelf-life (Wheeler *et al*., 1993). However, this effect can be overcome by combining the calcium chloride with an anti-oxidant such as ascorbic acid (Vitamin C) to inhibit pigment oxidation and discolouration (Warriss, 2000).

In contrast to the above approach, where calcium chloride injection causes tenderisation by increased calpain activity, there is a possibility that the mechanism may be a direct effect of the salt on the muscle proteins, since sodium chloride seems to work as well and calcium chloride also seems to work even if the calpain system is inhibited (Warriss, 2000). Calcium ascorbate has also been used in the research on calcium marination technology. This calcium salt has several advantages and disadvantages, i.e. inhibition of lipid oxidation and tenderness enhancement in selected meat samples. However, less colour shelf-life was obtained due to muscles becoming darker more quickly, the loss of purge was higher and off-flavours were found (Lawrence *et al*., 2003). When calcium lactate is used, the tenderness is increased as well as the other palatability

traits, and the colour shelf-life and microbial inhibition were acceptable. The only disadvantage with the use of calcium lactate is the enhanced lipid oxidation, seeing that the salt seems to be a pro-oxidant of lipid oxidation (Lawrence *et al*., 2003).

o Sodium/potassium lactate blend: The acidic environment that develops with post-mortem glycolysis favours activation of muscle lysosomal hydrolases and subsequent hydrolysis of cellular and extracellular components, thereby bringing about resolution of rigor mortis (i.e. conditioning). A more rapid decrease in pH, brought about by post-slaughter injection of lactic acid, should speed the activation of muscle cathepsins and possibly enhance collagen degradation in the muscle connective tissue during the subsequent conditioning period, i.e. accelerate conditioning. Fresh, unconditioned meat colour and general appearance were unaltered following injection of 0.1 M lactic acid (and lower concentrations) to a level of 10% of the original weight of the muscles. However, higher (0.2 M) injected concentrations led to highly unacceptable meat colour changes (Stanton & Light, 1990). Purac, the world's largest and most experienced producer of natural lactic acid and lactates, developed a cost-effective solution to enable the meat industry to provide safer and enhanced quality meat products. PURUSAL, a sodium/potassium lactate solution is a natural ingredient produced from natural L (+)-lactic acid, an organic acid naturally present in meat products (Anon., 2003d). This solution is based on two principles: firstly it is a hygroscopic salt and binds water and thus reduces water activity, and secondly it has a specific lactate effect, in that it influences the enzyme metabolism in the bacteria cell, which reduces and slows the growth of the bacteria (Anon., 2003e). This solution offers the meat industry and meat products extended shelf-life, enhanced intrinsic product safety by controlling and inhibiting the growth of a wide range of bacteria such as spoilage bacteria (i.e. lactic acid bacteria*, Brochotrix*) and pathogenic bacteria (i.e. *Salmonella, C. botulinum, L. monocytogenes, E. coli*), and the solution is cost effective and enhances and protects meat flavour. PURASAL is generally recognised as safe (GRAS), according to the Food and Drug Administration (FDA), and can be used in European countries and the USA (Anon., 2003d). The maximum level of use of the lactates is 4.8% lactate (dry solids), which means 5% PURASAL in cooked and uncooked items. To guarantee shelf-life of meat during the summer season, a concentration of 2.5-3% PURASAL (based on end-product weight) is normally sufficient, while for inhibiting growth of pathogenic bacteria at least 3% PURASAL is required (Anon., 2003e). The benefit of marinating products with PURASAL becomes clear if the product is overcooked. The injection solution keeps the meat from drying out quickly, giving it an

overall tender mouth feel. The solution also increases water retention, decreases cooking loss and preserves the colour (Anon., 2003d).

o Phosphates: Many investigators have reported the effect of the addition of phosphates on fresh and cured meats. Several studies have shown the importance of phosphates in their role of improving meat characteristics such as water retention, water binding (Koohmaraie *et al*., 2003), pH and meat swelling (Shults *et al*., 1972). Sodium phosphate is commonly used in meat processing and has been documented to increase protein solubility and the water-binding ability of meat (Smith *et al*., 1984). The injection of brine containing sodium tripolyphosphate into pork *Longissimus* increased juiciness and reduced Warner-Bratzler shear values, and also increased juiciness when injected into beef S*emimembranosus*. Addition of sodium phosphate (as either pyrophosphate or hexametaphosphate) has also been reported to prevent rigor mortis and increase tenderness of freshly slaughtered beef (Scanga *et al*., 2000).

Pressure treatment: A process has been described (Solomon *et al*., 1997) in which meat is subjected to a shock wave generated while the meat is submerged under water. The *Hydrodyne* process is very effective in tenderising beef muscles, including those muscles which are tough as a result of cold-shortening, and it could be a potentially useful way of processing meat to make it more palatable (Warriss, 2000). When used correctly, the *Hydrodyne* does not discolour or damage the meat. The *Hydrodyne* is a sealed stainless steel tank in which a tough cut of meat is submerged in water. A small explosive charge is suspended in the water a short distance from the meat. The explosion sends supersonic shock waves pulsing through the water and subsequently through the meat. A microscopic view of the meat shows the effect of the shock waves on the meat structure, where the matrix of interconnecting protein filaments in the muscle is radically broken (Anon., 2003b). These tight interconnections are the cause of toughness in a poor cut of meat and when they are broken apart the toughness largely disappears. Tests conducted improved beef tenderness by 50 to 72%. Research is being done to determine whether *Hydrodyne* can be used to kill pathogens and organisms that limit the shelf-life of meat products (MacInnis, 1999).

Artificial enzyme: Many years ago meat was wrapped in papaya leaves during cooking or dipped in papaya or pineapple juice as a means of tenderising the meat. This method has now developed into a modernised tenderising treatment. Researchers found that certain

plants, fungi and bacteria (Foegeding & Larick, 1986) produced non-toxic proteolytic enzymes and started to use these exogenous enzymes as commercial meat tenderisers (Lawrie, 1998b), i.e. to disrupt the integrity of the myofibrils and the connective tissue (Anon., 2003c). The enzymes used in these formulae are papain (papaya), bromelin (pineapple) and ficin (fig). The connective tissue can also be disrupted by enzymes such as Rhozyme, obtained from a fungus *Aspergillus oryzae* (Anon., 2003c). Papain is a cysteinyl-proteinase enzyme found in papaya and degrades the connective tissues in meat. It is only activated by heat, therefore proteolysis is active when the meat is cooked (Anon., 2003a). In the processing plants the meat was initially sprayed or dipped into a solution containing the enzyme. However, the results were unsatisfactory, due to the meat surface becoming over-tenderised and forming a soft "mushy" texture with occasionally an undesirable flavour. Also, the enzymes were only effective on the meat surface, because they were unable to penetrate the meat and hence the meat was still tough and unaffected in the centre. In addition, these enzymes have an effect on minced meat and not on whole meat, as the collagen must be exposed in order for the enzyme to act (Desmond & Troy, 2001). As a result of the difficulty of the enzymes penetrating the meat, a new technique was developed based on injecting the tenderiser either before slaughter or post-mortem (Lawrie, 1998b). Post-mortem injection consists of injecting large thick meat cuts with aqueous or gaseous carriers such as nitrogen. Gaseous carriers injected under pressure will separate the muscle fibres, providing a uniform enzyme distribution. Enzymes should be injected before onset of rigor mortis as it is believed that the "hot" carcass will distribute the enzyme more successfully, resulting in a more tender meat (Anon., 2003a). In addition to plant enzymes, other enzymes from bacterial and fungal origin – for example, protease 15; rhozyme; fungal amylase and hydralase D – have also been utilised as meat tenderisers. The bacterial and fungal proteolytic enzymes act only on the proteins of the muscle fibre. They first digest the sarcolemma, causing disappearance of nuclei, and then degrade the muscle fibre, eventually causing loss of cross-striations. The action of the proteolytic enzymes of plant origin is preferentially against connective tissue fibres. They first break up the mucopolysaccharide of the ground substance matrix, and then progressively reduce the connective tissues fibres to an amorphous mass. Unlike the tenderising changes during conditioning, the enzymes used in artificial tenderising break down connective tissue proteins to soluble, hydroxy-proline-containing molecules (Lawrie, 1998b).

However, disadvantages of enzymatic tenderisation of meats with exogenous enzymes are the preferential hydrolysis of myofibrillar and sarcoplasmic proteins over stromal tissue proteins (Fogle *et al*., 1982; Brooks *et al*., 1985).

2.3. BIOCHEMICAL PROPERTIES AND NUTRITIONAL VALUE OF BEEF

A change in the relative consumption of the different types of meat has been observed, particularly in the European countries and the USA, with beef consumption progressively decreasing (Geay *et al*., 2001). In South Africa the same situation is reflected, where the per capita consumption of beef declined from 18.68 kg in 1990/91 to 13.72 kg in 2001/02. The most noticeable trend in the South African meat market from 1960 to 2002 is the change in the proportion of the red to white meat consumed. The production share of white meat in 1960 was only 5.7% of the total production of meat, but increased to 41.8% in 2002. The share of red meat, however, dropped from 94.3 in 1960 to 58.2% of the total production of meat in 2002. The total per capita consumption of all types of meat remained at 43 kg (RMRDT, 2004; Anon., 2005).

The sources of the declining red meat sector have been sought at the level of the individual consumer, who appears to be concerned about safety and quality of foods, and also about the environment and ethical considerations in meat production (Dransfield, 2003). The decrease in red meat consumption can partly be explained by the fierce competition with white meats, which are marketed at a relatively low price. An additional reason could be attributed to consumers and their concerns about their health. Good nutrition entails the importance of maintaining wellness and the prevention of diseases. Therefore, consumers prefer foods with positive health benefits, i.e. foods that will add the important minerals, vitamins and high-value proteins, and possibly essential fatty acids to their diet. Health concerns are largely due to statements from medical circles and mass media that red meat such as beef and lamb/mutton contain a substantial amount of saturated fatty acids (SFA) and cholesterol. These two factors are key contributors to the development of coronary heart disease (CHD), which is one of the foremost killers in adults (Wood *et al*., 2003). The risk of developing coronary heart disease is increased by mainly two factors: high blood pressure and high blood cholesterol levels. Both these factors are adversely influenced by diets high in fat, particularly SFA, although there also seems to be an important genetic component to a person's susceptibility (Department of Health, 1994). However, a limited consumption of unsaturated fatty acids (UFA) and SFA is recommended by the National Cholesterol Educational Programme (1988). In addition,

meat with low levels of polyunsaturated fatty acids (PUFA), especially those of the n-3 series, may be beneficial for human health by lowering blood cholesterol concentrations.

Other factors which may be responsible for the decline in red meat consumption are the recent media reports on, for example, the use of hormones, illicit trading and animal health concerns such as bovine spongiform encephalopathy (BSE) (Tarrant, 1998). Despite these negative reports, muscle foods remain a valuable primary dietary component and form a very important part of a balanced, varied diet (Morris, 1991). The B-vitamins in the diet, particularly vitamin B_{12}, are significantly enhanced by inclusion of meat in the diet, since these are not present in plant foods (Kim, 2001).

Livestock breeders and animal scientists face the task of researching and explaining the mechanisms affecting the quality of meat and determining the possibility of enhancing the health-promoting properties of meat and meat products (Tarrant, 1998).

In the living animal the skin, bones, muscles and organs exert high levels of metabolic activity, in contrast to the adipose tissue. These metabolic tissues are regarded as nutrient-dense foods, due to the large quantities of very important nutrients, e.g. essential amino acids, fatty acids, vitamins, minerals and some carbohydrates (mostly glycogen) they contain as energy source (Simonsen *et al*., 1988). The protein content and amino acid composition, collagen content, lipid content and fatty acid composition as well as the mineral and vitamin content of meat in general will now be discussed.

Protein and amino acid composition

One sixth of the proteins consumed by humans is supplied by meat products (Warriss, 2000). In terms of nutrition, meat is considered as a highly concentrated source of protein, with a high biological value and rich in essential amino acids, vital for human health. Although these proteins are slightly deficient in sulphur amino acids, they are rich in lysine (Geay *et al*., 2001).

Amino acids form the basis of the protein structure. They can be grouped into two classes, namely essential and non-essential amino acids. Some of the essential amino acids can be synthesised by the body, but in insufficient quantities to meet our metabolic needs. It is therefore necessary for the essential amino acids to be supplied as part of the diet. The essential amino acids are threonine, tryptophane, histidine, lysine, leucine, iso-lysine, methionine, valine, phenyl-alanine and arginine. With respect to the essential amino acids, beef would appear to have a higher content of leucine, lysine and valine than pork and lamb, as well as a lower content of threonine (Lawrie, 1998d). The non-essential amino acids are alanine, aspartic acid, asparagine, glutamic acid, glutamine, glycine,

proline and serine. Non-essential amino acids can either be synthesised from the essential amino acids or from nitrogen and carbon precursors (Mahan & Escott-Stump, 1996). Tryptophane is an amino acid that functions as the precursor of niacin in the body and can be found in all meats (Bennion & Scheule, 2000).

The protein contents of meats change according to species; for example, beef contains 20.1 mg protein/100 g meat, whilst both mutton and pork have a protein value of 13.9 mg; and ostrich has 22.2 mg/100 g (Paleari *et al*., 1998; Sayed *et al*., 1999). Although minor differences exist between species pertaining to amino acid composition, more significant differences may possibly exist between muscles, and between animals of different ages and breeds. Processing such as heat and sterilisation may influence the amino acid content of meat, though the effect would be minimal, if sufficient precautions are taken to ensure that the processing conditions are not severe and prolonged (Lawrie, 1998d). With the addition of a solution containing water and salt, the protein content would decrease as a result of the increased water and ash (mineral) content (Hoffman, 2006)

Lipid content and fatty acid composition

The nutritive value, appearance, processability, shelf-life and palatability of meat are affected by the amount and quality of fat in the meat. Fat is therefore an important determinant of meat quality and the degree of fat saturation contributes substantially to the sensory properties of meat (Webb *et al*., 1994). In terms of nutrition, dietary fat is one of the three main energy-providing macronutrient groups and is considered to be a very concentrated source of energy. Factors such as animal species, breed, sex, nutritional status and the location of the cut in the carcass generate the variation in fat percentage (Nürnberg *et al*., 1998). The fat percentage would also change when water and salt solutions are added to the meat product. The increased moisture and ash content in effect leads to a decreased lipid content (Hoffman, 2006).

Most neutral lipids consist of ±95% triglycerides. The remaining 5% include monoglycerides, diglycerides, phospholipids, sterols and fatty acids (Mahan & Escott-Stump, 1996).

The structure of fatty acids consists of unbranched hydroxycarbon chains terminating in a methyl group on the one end and a carboxyl group at the other end. There are 24 different fatty acids, which are distinguished by the degree and nature of saturation and chain length. The classification of fatty acids is based on the number of carbons, position of the first double bond and the number of double bonds. The monounsaturated fatty acids (MUFA) have only one double bond and the polyunsaturated

fatty acids (PUFA) have two or more double bonds, whereas the SFA contain the maximum amount of hydrogen that the chain can hold (Mahan & Escott-Stump, 1996).

Unsaturated oleic acid (C18:1 *n*-9), with one double bond, is one of the most abundant fatty acids in meat fat (Forrest *et al*., 1975). Other naturally occurring fatty acids also commonly found are palmitic (C16:0) and stearic acid (C18:0), both saturated fatty acids.

Certain fatty acids should be a fundamental part of the diet as they cannot be synthesised in the body. These essential dietary fatty acids include linoleic acid (C18:2 *n*-6), α-linolenic acid (C18:3 *n*-3) and arachidonic acid (C20:4 *n*-6) and are known to prevent certain deficiency symptoms (Mahan & Escott-Stump, 1996). Arachidonic acid serves as an active biological compound and occurs primarily in animal fat (AMIF, 1960).

The fatty acid composition of the lipids consumed significantly influences the diet/health relationship, as a result of the different effect each fatty acid has on the plasmatic lipids. Beef lipids contain 45% saturated fatty acids (SFA) and 55% unsaturated fatty acids (UFA), resulting in a ratio of 0.8 SFA:UFA. According to Jiménez-Colmerro *et al*. (2001), the level of plasma low-density lipoproteins can be reduced by including MUFA and PUFA in the diet. The general description of meat as a highly saturated food can therefore be discarded.

The eating quality and shelf-life of meat are influenced by the composition of the fatty acids in lipids. SFA increase the hardness of fat and influence meat palatability by being easily solidified upon cooling. Unsaturated fatty acids, on the other hand, increase the possibility of lipid oxidation in meat, which affects its keeping quality (Banskalievaa *et al*., 2000). As mentioned earlier, the lipid content is decreased by the addition of moisture and salt solutions. However, pertaining to the fatty acid composition, no detailed research could be found reporting the effect of such solutions on the fatty-acid composition of meat. Some reports mention that salt has an accelerating effect on the oxidation of fat, as for example in cured meats (Lawrie, 1998e). However, in the case of a phosphate-containing salt solution, a possible effect of it on the fatty acids in meat could only be a matter of speculation. At high pH levels the cytochrome activity in muscles is enhanced, which results in increased metmyoglobin-reducing activity. Thus the high ultimate pH minimises the pro-oxidant conditions and offsets the inherently greater tendency of the lipids of the infused muscles to oxidise (Lawrie, 1998c). This speculation is highly debatable and it is clear that a considerable number of factors must be known before an accurate prediction of the behaviour of the infused muscles can be made and the subsequent effect of the blend on the oxidation status of the product.

The concentration of the unsaturated fatty acids (UFA) varies between the muscles within a given species and also between fractions within any single muscle (Lawrie, 1998d). Within different meat species the characteristics of the fatty acid tissue also varies due to differences in degree of saturation and chain length (Penfield & Campbell, 1998). One of the distinctions between the ruminant gut and that of the monogastric animal is the hydrogenation of dietary fatty acids in the rumen. This reaction produces intramuscular fatty acids that are far more saturated in ruminants than in pigs and poultry (Geay *et al*., 2001).

The major plant fatty acid, linoleic acid (C18:2 *n*-6), is found in much lower concentrations in ruminant tissue than in pork. This low concentration in ruminant meat creates a polyunsaturated:saturated (PUFA:SFA) fatty acid ratio (an important nutritional index) below the recommended value of 0.45. In contrast, the *n*-6:*n*-3 value for beef is closer to the recommended value of 4.0 than that of pork (Warriss, 2000). The fact that linolenic acid (LNA) (C18:3 *n*-3) – the major fatty acid in grass – is relatively high in ruminant animals causes this difference. Although a high proportion of LNA is broken down to stearic acid (C18:0) in the rumen, a considerable amount still passes through the rumen to be absorbed in the small intestine (Wood *et al*., 1999). Conversely, in the muscle of animals supplied with concentrated feed, there are higher concentrations of linoleic acid (C18:2 *n*-6). This is the major fatty acid in cereal-based concentrates (Enser *et al*., 1998).

As mentioned above, there are two important indexes for the nutritional evaluation of fat, namely the ratio between polyunsaturated and saturated fatty acids (P:S), and the ratio between *n*-6 and *n*-3 fatty acids (Department of Health, British, 1994). The recommended figure for the P:S ratio is 0.45 for the British diet as a whole (Department of Health, British, 1994). Although ruminant meats normally have a low ratio of polyunsaturated fatty acids (PUFA) compared to saturated fatty acids (SFA), the muscle contains a series of C20 and C22 PUFA of both the *n*-6 and *n*-3 series, which may be of potential significance in human health and nutrition (Enser *et al.,* 1998). Humans have the capacity to syntheses the C20 PUFA, eicosapentaenoic acid (C20:5 *n*-3; EPA) and, to a lesser extent, docosahexaenoic acid (C22:6 *n*-3; DHA), from C18:3 *n*-3 (LNA).

A comparison of the P:S and *n*-6:*n*-3 ratios of beef, mutton and pork are presented in Table 1.

Table 1. A comparison of the P:S and *n*-6:*n*-3 ratio of beef, mutton and pork.

Species	P:S	*n*-6:*n*-3
Beef	0.11	2.11
Mutton	0.15	1.32
Pork	0.58	7.22

Data obtained from Warriss (2000).

Mineral content

Due to its high bioavailability, meat is considered an excellent source of iron (Fe), zinc (Zn), phosphorus (P) potassium (K) and magnesium (Mg) in the human diet (Lin *et al*., 1989).

The most common nutritional deficiency disease in the world is iron deficiency anaemia. Iron forms an integral part of the human diet because of its ability to participate in oxidation and reduction reactions (Mahan & Escott-Stump, 2000). Iron in meat has a high bioavailability, the main reservoir being the component of the haem protein, myoglobin. Around 40% of the iron in meat is haem iron, which is more easily absorbed by the human gut than non-haem iron, found in plant materials (Simonsen *et al*., 1988). Most research papers on the mineral content of meat show that calcium (Ca), phosphorus (P), iron (Fe) and potassium (K) are investigated the most (AMIF,1960). In meat potassium (K) is quantitatively the most important mineral, followed by phosphorus (P) and iron (Fe). Conversely, the calcium (Ca) content of meat is relatively low. Some copper (Cu) and other trace minerals are also supplied by meat (Bennion & Scheule, 2000). Approximately 25% of the daily requirements of selenium (Se) are provided by meat, which is about 10 µg selenium (Se) per 100 g of meat. Recent data illustrate that meat, raw and cooked, provides Se with a greater bioavailability that that from plant foods (Higgs, 2000).

Species, age, meat cut, diet, breed, season and geographical differences are some of the aspects that contribute to the difference in the mineral content of meat. Under normal circumstances the potassium content of beef lessens with increased animal age, while the sodium values are not significantly influenced by the age of the animal. The magnesium and calcium content of lean tissue does not increase with age (Doornenbal & Murray, 1981), whereas the iron content, as well as the zinc levels do increase with the ageing of the animal (Kotula & Lusby, 1982).

Several studies reported that different muscles vary in their mineral concentrations. These differences are due to the variation in muscle fibre type and physical activity of the

different muscles (Doornenbal *et al*., 1981; Lin *et al*., 1989). When the mineral content of both the red and white fibres were compared (Beecher *et al*., 1968), a higher level of iron, copper, sodium and zinc, in addition to a lower potassium content was reported for the red fibres.

In the case of infusing meat muscle with a solution containing minerals such as potassium and sodium, the mineral content of the meat would increase, as expected. Despite the higher sodium content in infused meats, previous research studies showed that with the infusion of a similar solution the sodium content was within the requirements of the South African Heart Foundation. The Heart Foundation requires the sodium concentration of a foodstuff to be lower than 120 mg/100 g sample that is to be consumed (Anon., 1972).

Vitamin content

A diverse range of vitamins is found in all meat types. The quantity of any particular vitamin, however, depends on the type of meat and whether the meat is cooked or in the raw state.

The principal vitamins in all animal tissues are the B-vitamins. Lean meat is a rich source of especially vitamin A (retinol) and thiamine (B_1), nicotinic acid (niacin), riboflavin (B_2), B_6 and cyanocobalamin (B_{12} - lacking in plant foods). Despite the relative similarity in the content of the B-vitamins from different cuts within species, large differences are noted between species (AMIF, 1960). Pork has a significantly higher content of vitamin B_1 than other meat types, whereas beef has a relatively high concentration of folic acid when compared to meat from other species (Lawrie, 1998d).

Lean meat contains only a small amount of fat-soluble vitamins A and D, whereas the liver is particularly rich in vitamin A (Simonsen *et al*., 1988). The liver also is a particularly rich source of nicotinic acid, vitamin B_6, pantothenic acid, biotin and vitamin B_{12} (AMIF, 1960). High ascorbic acid content (Vitamin C) is found in the organs, especially the liver, whereas in lean meat this vitamin is detected in only small amounts (Simonsen *et al*., 1988).

The vitamin content of meat obtained from ruminants is not influenced directly by the feed intake of the animal, as in the case of monogastric animals (for example, fresh pork). Some nutrient substances and vitamin B complex vitamins may be absent in the ingested feed, but are synthesised by rumen micro-organisms. These micro-organisms may also make use of a significant proportion of such nutrients when the feed contains

sufficient amounts. Thus the variations in the nutrient content of the feed are regulated and balanced (equalised) by the overall operation of the rumen (Lawrie, 1991).

Again with reference to infusing a meat muscle with a moisture and salt solution, no relevant reports on the effects of this on the vitamin concentrations could be found.

2.4. THE EATING QUALITY OF MEAT

The consumer is guided in his decision to purchase beef by the perception of healthiness and a variety of sensory traits including colour, tenderness, juiciness and flavour (Verbeke & Viaene, 1999). After the decision to purchase a meat product, the acceptability of the meat will be determined by ascertaining the satisfaction experienced after consumption (Jeremiah *et al*., 1990) together with the fulfilment of their nutritional requirements.

The qualities of meat products can be evaluated by using both the human senses and instrumental applications. With respect to the human senses, the sensory properties can be measured through the use of a trained taste panel and, when relevant, a consumer panel. Instruments are again applied to quantify the physical and chemical properties of meat (Warriss, 2000).

Sensory analysis can be defined as a scientific method used to evoke, measure, analyse and interpret the responses to products as perceived through the senses of sight, smell, touch, taste and hearing (Stone & Sidel, 1993). According to Lawless and Heymann (1998), sensory analysis consists of a set of techniques applied to measure the human responses to foods accurately and attempts to isolate the sensory properties of food for further enhancement. Sensory measurements offer the advantage of approximating the actual measurement of different meat characteristics as encountered under normal conditions of eating (Pearson, 1963). The ‘instrument’ used for this type of analytical testing is a group of trained panellists. This panel is trained to assess differences in the three major palatability attributes of meat, i.e. tenderness, juiciness and flavour (Charley & Weaver, 1998).

The colour, water-holding capacity and some of the odour of meat can be perceived before and after cooking, whereas juiciness, tenderness, taste and most of the odour are primarily detected on mastication (Lawrie, 1998b). These attributes of eating quality will be considered in this part of the literature review

Colour

The appearance of the meat is the first important criteria that the consumer takes into account when judging meat quality (Barton-Gade *et al*., 1988). The colour of the lean and fat are important quality attributes which influence the consumer's initial decision to purchase meat (Hood & Riordan, 1973) and determine the acceptability of the meat by the consumer (Stevenson *et al*., 1992; Demos *et al*., 1996). Colour is the visual characteristic of the meat that can be measured by using an instrument or a descriptive panel.

Three sources are identified to be the cause of meat colour variation. Firstly, the red pigment, myoglobin, will influence the colour of the meat. Secondly, the colour is affected by the pre-slaughter period, the slaughter process and also the rate and extent of pH and temperature decline. Thirdly, the colour and colour changes occurring during processing, handling, storage, distribution and display are influenced by the processes of oxygenation and oxidation of myoglobin (Honikel, 1998).

Muscle tissue gets its unique colour from the presence of the haem pigment, namely myoglobin. The pigments in red meat are three fourths myoglobin and the remainder is due to the presence of the blood pigment haemoglobin (Charley & Weaver, 1998). In the blood vessels both pigments combine reversibly with oxygen, whereas myoglobin retains it in the tissue. This protein is bound to the external membrane of the mitochondria and sarcoplasmic reticulum (Geay *et al.,* 2001). Several factors determine the quantity of myoglobin in muscle such as species, breed, sex, age, nutritional status, exercise and type of muscle (Lawrie, 1991). Beef contains more myoglobin than meat from other traditionally farmed species, which is the reason why beef is generally darker.

The colour of meat, however, depends not only on the quantity of the myoglobin present, but also on the type of myoglobin molecule and also the chemical state of myoglobin present as well as on the chemical and physical conditions of the other components in meat (Lawrie, 1998b).

The myoglobin molecule consists of a haematin nucleus attached to a globulin-type protein to form a conjugated protein myoglobin. The haematin portion consists of four pyrrole groups and a central iron atom linked covalently to the nitrogen of the four pyrrole groups (Barton-Gade *et al*., 1988).

Myoglobin is a pigment with a purplish-red colour and in living tissue exists in equilibrium with its red oxygenated form, namely oxymyoglobin. After slaughter, blood circulation stops and no more oxygen is carried to the tissue. In the tissue the oxygen is depleted rapidly by muscle glycolysis and the pigment exists almost entirely in the purplish ferrous state. When the meat is cut, the oxygen combines with myoglobin and forms

oxymyoglobin, leaving a bright red meat surface. As long as the oxygen levels remain high, the pigment is maintained in the oxygenated form. The reaction is reversed when oxygen is excluded, which results in a purple red colour as the pigment is deoxygenated to myoglobin (Penfield & Campbell, 1998). The brownish red pigment, metmyoglobin, develops when the iron in myoglobin is oxidised from the ferrous to the ferric state. Metmyoglobin is changed back to myoglobin with the presence of reducing substances in the meat. However, when the reducing power of the muscle is lost the colour of the meat becomes brownish.

The colour of fresh meat is limited to the surface accumulation of the brown-coloured pigment metmyoglobin (Madhavi & Carpenter, 1993). The metmyoglobin pigment initially forms a layer underneath the meat surface and spreads towards the surface, in time affecting the overall meat appearance (Madhavi & Carpenter, 1993). Metmyoglobin formation is accelerated by environmental factors such as high temperatures, relative humidity, oxygen partial pressure, aerobic micro-organisms and the presence of fluorescent and incandescent light.

Other intrinsic factors including pH, lipid oxidation (in particular fatty acids) and oxygen consumption rates also play an important role in pigment development. A significant relationship is found between meat colour and pH, where a high pH alters both the colour and texture of the meat (Simonsen *et al.,* 1988). A low ultimate pH is associated with a brighter meat colour (Swatland, 1985), which means that a high ultimate pH causes the meat to appear dark (Swatland, 1990). Colour stability is defined by the oxygen consumption rates in the early post-mortem period. However, once the oxygen consumption is reduced, metmyoglobin-reductase will dominate the ageing activity.

Measurement of meat colour

As mentioned, the meat colour can be measured physically with a colour meter or as a sensory attribute. Although appearance and meat colour are important sensory attributes to consumers, beef colour can be evaluated by an objective method using the CIE Lab values instead of a trained panel. This three-component equation consist of L* values indicating lightness, a* values representing the red-green range and b* values indicative of the blue-yellow range. The chroma value and hue angle (colour intensity) are calculated by using the a* and b* values in the following equation (CIE, 1978):

Chroma: $C^* = \sqrt{(a^*)^2 + (b^*)^2}$ Hue angle: $h_{ab} = \tan^{-1}\left\{\frac{b^*}{a^*}\right\}$

As b* rotates towards a*, there is an increase in the hue angle, which results in more redness. High a* and b* values indicate a higher colour saturation (chroma), which causes muscle to appear bright with better, more desirable colour purity (Onyango *et al*., 1998).

It is recommended that the colour of the meat should not be measured until the ultimate pH is reached (Boccard *et al*., 1981). Therefore, colour readings should be taken after rigor mortis and after the meat has bloomed, thus ensuring that the surface myoglobin is fully oxygenated. Three to five measurements are recommended, to be made on different sites of the exposed surface of the sample (Honikel, 1998).

Colour changes during cooking

Pigment changes that occur during cooking determine the final colour of the cooked meat. The brown pigmentation in cooked meats is normally a desirable attribute of meat quality. The pigment changes are determined by the cooking method, duration and temperature of the cooking process (AMIF, 1960). The pigments in muscle undergo denaturation when heated, where the protein molecules unfold and increasingly expose the central iron atom. Between a temperature range of 40 to 50°C the globin of oxymyoglobin is denatured and the ferrous iron is oxidised to the ferric state. Meat cooked just to the point of pigment denaturation is reversed and reoxygenation occurs (Penfield & Campbell, 1998).

During cooking the colour of the fat portion of meat undergoes very little change. The only significant colour change is on the meat surface, which turns brown in the case of dry-heat cooking. This surface browning is the result of fat decomposition and polymerisation with carbohydrate and protein decomposition products. The surface change contributes greatly to the attractive appearance of meat cooked by dry heat (AMIF, 1960).

Water-holding capacity and juiciness

Possibly the most significant manifestation of the post-mortem denaturation of the muscle proteins, in terms of meat quality, is their loss of water-holding capacity (Lawrie, 1998b). The water-holding capacity is an attribute of great importance primarily for three reasons. Firstly, as a result of poor water-holding capacity, exudate or drip forms and detracts from the appearance of the meat. This is of relevance in modern retail packs where drip tends to collect, rather than draining away, despite the inclusion of absorbent pads to soak up the liquid. Secondly, increased weight loss in fresh meat due to drip loss, and reduced water retention and product yield in processed meats are a consequence of poor water-holding capacity. The last reason is the influence of water-holding capacity on the

perceived juiciness of fresh meat after cooking. Meat with a low water-holding capacity loses more fluid during cooking and may taste dry and lack succulence (Warriss, 2000).

About 70% of lean tissue consists of water, making it the largest component of the muscle tissue. In living muscle approximately 10% of the water is bound, with another 5% to 10% located in the small channels between the muscle fibres in the extracellular space. Most of the water in muscle is located in the spaces between the myosin and actin/tropomyosin. Only about 5% of the total water in muscle is directly bound to the hydrophilic groups of the proteins, whereas the rest of the water molecules are held by capillary forces between the thick and thin filaments. The latter water molecules are recognised as “free water”. When the ratio of “bound water” to “free water” is high, less moisture will be lost during cooking, giving a more juicy impression (Lawrie, 1985; Huff-Lonergan & Lonergan, 2005).

Drip loss

When a carcass passes into rigor, some of the water is lost in the form of drip, which occurs mainly from the cut end of the meat (Offer & Trinick, 1983). Fluid leaks out from the extracellular space, forming drip. As the pH decline decreases, the amount of fluid formed decreases substantially (Honikel, 1987; Swan & Boles, 2002), indicating that the degree of drip loss is more dependent on the rate of pH decline than the ultimate pH (Guignot *et al*., 1994). As the pH declines, denaturation of the muscle proteins occurs. Consequently, as the myofibrillar proteins reach their isoelectric point (this is the state in which the protein molecules have no net electrical charge and have a propensity to lose the water that is normally bound to them), they tend to loose some of their water-binding capacity. Consequently, this results in the fluid being exudated from the muscle fibres and forming a moisture layer (Warriss, 2000). This eventually causes the water being exudated to drip when hung for 24 h at 4°C, and hence the method for physical analysis of drip loss (Honikel, 1998).

Cooking loss

A high water-binding capacity results in less moisture being lost during the cooking of the meat and hence a favourable impression of juiciness during mastication. The different meat proteins will denaturate at varying temperatures (37°C to 75°C) during the cooking process (Honikel, 1998). The protein structure is altered by the denaturation, which result in cooking loss. The expansion of the filament lattice causes the myofibrils to shrink and as a result water is lost during cooking.

Conditions where a pH value of 5.5 and temperatures of 40°C and above prevail rapidly increase the appearance of shrinkage (Offer & Trinick, 1983). With increased pH, the water retention will cause a decrease in cooking loss (Bouton, *et al*., 1973). A high cooking loss causes low water-holding capacity, and the water-holding capacity is at its minimum at low ultimate temperatures (Onyango *et al.,* 1998). The structural changes caused by myofibrillar denaturation include the destruction of cell membranes, transverse and longitudinal shrinkage of muscle fibres, the aggregation of sarcoplasmic proteins and shrinkage of the connective tissue (Honikel, 1998). All these changes, particularly the connective tissue changes, are responsible for the cooking losses found in meat. According to Honikel (1998), the physical analysis of cooking loss is the difference in weight between the freshly cut meat sample and the sample after being cooked at 80°C for 1 h.

Juiciness

The juiciness of meat is another valuable meat-quality attribute that is of great concern to the consumer. Meat juiciness plays an important role in conveying an overall impression of palatability to the consumer. Despite the fact that many of the important flavour components are contained in the meat juices, it also assists in the process of fragmenting and softening of meat during chewing. Regardless of the other virtues of meat, an absence of juiciness severely limits its acceptability and impairs its unique palatability characteristics (Forrest *et al.,* 1975).

Juiciness depends on two aspects, namely, the inherent water-binding capacity (the ability of meat to retain its natural water content) (Offer & Trinick, 1983), or in other terms, the capacity of muscle to release its constitutive water (initial juiciness) and also the infiltration fat content (sustained juiciness) (Dryden & Marchello, 1970). In terms of descriptive sensory analysis, juiciness is described by two sensory components. Firstly, the initial impression of juiciness is the amount of fluid forming on the cut surface when pressed between the thumb and forefinger. Secondly, the sustained juiciness is the impression that is formed after the first two to three chews between the molar teeth (AMSA, 1995). After cooking the combination of water and melted lipid constitutes a broth that, when retained in the meat, is released upon chewing. The apparent juiciness of the meat is enhanced when this broth stimulates the flow of saliva (Forrest *et al*., 1975). Thus, a certain quantity of free water together with the lubricant effect of fat favours meat palatability (Dryden & Marchello, 1970). The juiciness can further be enhanced indirectly by the presence of marbling (intramuscular fat) in meat. During cooking the melted fat

becomes translocated along the bands of perimysial connective tissue. This uniform distribution of lipid throughout the muscle may act as a barrier to moisture loss during cooking. Less shrinkage during cooking and a juicier meat are associated with marbling. Subcutaneous fat also minimises drying and moisture loss during dry-heat roasting (Forrest *et al*., 1975).

In general, initial juiciness seems to lessen with an increase in animal age, whereas sustained juiciness appears to increase as the animal gets older. The decrease in initial juiciness, with an increase in animal age, could be attributed to the fact that this ability of the muscle to retain water decreases with age. Cooking losses as a result will be higher in cuts from mature animals. With higher cooking losses, a drier end-product is expected. As a result, this meat would be without the rapid release of meat fluid during the first few chews as is likely in meat from young animals. As mentioned, the sustained juiciness improves with an increase in age. A possible explanation is that samples from older animals require additional mastication, due to more cross-linking of the collagen as age increases. Consequently more saliva would be released to increase the perceived sustained juiciness (Schönfeldt, 1989).

Another explanation could be the higher marbling content found in older animals. As previously mentioned, the juiciness in meat is also directly related to the intramuscular fat content (Lawrie, 1985). The sustained juiciness is the fluid that remains in the meat after cooking. The function of the lipids in meat is that of lubrication and to guarantee juiciness (Warriss, 2000). Usually, the water content is about 3.5 to 7.7 times the amount of protein present. In living tissue, for every kilogram of protein the body synthesises, 3.5 to 3.7 kilograms of water is required to surround the proteins. Fat tissue normally contains 7 to 8% water. Meat with a high fat content will have lower amounts of protein and water; therefore, water content varies inversely with the fat content of the meat. Huff and Parish (1993) also concluded that samples from beef carcasses of older animals are juicier than samples from carcasses of young bulls and steers.

Tenderness

Tenderness is considered the most important palatability attribute of meat (Lawrie, 1985). The positive relationship shown between the price of meat and its relative tenderness confirms this statement (Savell & Shackelford, 1992). According to many authors, tenderness is viewed as the most important sensory attribute determining meat-eating satisfaction (Ouali, 1990) as well as the predominant quality determinant and probably the most important sensory characteristic of red meat (Koohmaraie, 1988).

Meat tenderness can be described as the force needed to shear, compress and ground the meat during mastication and consumption, thus referring to the ease with which the consumer disorganises the meat structure (Lepetit & Culioli, 1994). The impression of tenderness is an intricate physical process, given that chewing involves not only cutting and grinding, but also squeezing, shearing and tearing. The chewing motions involve both vertical and lateral movements of the jaw, making it extremely difficult to measure tenderness objectively. All these activities together produce the tenderness sensation (Pearson, 1963).

Measurement of meat tenderness

The methods and interpretation of meat tenderness measurements are extremely variable (Honikel, 1998). Both instrumental and sensory techniques can be used in the analysis of meat tenderness. The instrumental method most widely (78%) used to determine meat tenderness mechanically is the Warner-Bratzler shear test. The Warner-Bratzler Shear Device is attached to an instrument such as the Instron Universal Testing Machine. This shear force test has an 80% satisfaction rate and remains the main reference (Lepetit & Culioli, 1994) for comparative studies and correlations between sensory analysis and determining meat tenderness instrumentally. By shearing the meat perpendicular to the muscles (Lepetit & Culioli, 1994) the configuration and myofibrillar component of the cooked meat can be closely related (Cross *et al*., 1973). Salè (1971) found that when myofibrils are rigid, simultaneous shearing of muscles and connective fibres would take place in cooked meat.

The accuracy of these instruments has been found to be affected by factors such as doneness of the cooked meat, uniformity of cylindrical sample size, direction of the muscle fibres, amount of connective tissue, fat deposits present, temperature of the sample and the speed at which the sample is sheared (Simonsen *et al*., 1988).

Higher shear force values are obtained with meat from older animals, since the force required to shear across the collagen grain rises as the collagen becomes less soluble (Young & Braggins, 1993).

Tornberg (1996) stated that the ultimate test for analysing meat tenderness is its evaluation by the human senses. The overall impression of tenderness to the palate involves penetration of the meat by teeth, the ease of fragmentation, and the amount of connective residue left in the mouth after chewing (Jeremiah & Phillips, 2001).

A relationship between taste panel scores for tenderness and meat quality characteristics, i.e. juiciness, flavour, residue, and collagen solubility and total collagen has

been reported. Bruwer *et al*. (1987) found that in tender meat the juices are released more rapidly by chewing, less residue remains in the mouth after chewing, solubility is higher and collagen content is lower.

Factors affecting meat tenderness

Meat tenderness originates in the structural and biochemical properties of muscle fibres, especially myofibrils and intermediate filaments, and of the intramuscular connective tissue (the endomysium and the perimysium), which are composed of collagen fibrils and fibres (Takahashi, 1996).

Meat tenderness or toughness is an extremely multifaceted attribute, since it is not caused by a single factor, but an acquisition and integration of many factors that influence, or are associated with, meat tenderness. Numerous variables have been correlated to tenderness, for example, environmental factors, animal age and sex, the amount of intermuscular fat, collagen content, as well as electrical stimulation and post-mortem ageing (Koohmaraie, 1994; Koohmaraie, 1996). Other variables included by Davis *et al*. (1979) to predict and explain 68% of the variation in meat tenderness were a percentage of expressible juice, fragmentation index, sarcomere length, cooking loss, and the percentage of soluble collagen of beef steaks. In a report by Hawkins *et al*. (1987) 51% of the variation in meat tenderness was ascribed to muscle traits, such as sarcomere length and the percentage of moisture and fat.

Several ante-mortem factors such as species, age, sex and nutritional status have all been identified as influencing meat tenderness. In addition to the above, post-mortem factors such as slaughtering methods, pre-slaughtering stress, handling, processing and cooking temperatures have been linked to tenderness. Other factors that also play a significant role in the overall tenderness of meat include the collagen and glycogen content, and the level of enzymes (especially calpains). With this background it becomes important to discuss the factors that might have an effect on the overall tenderness of beef.

Ante-mortem factors

The first fundamental variation in discussing the tenderness of meat is the relationship between species, age, sex, muscle type, collagen content and nutritional status. These ante-mortem factors influence the choice the consumer will make, as they determine the commercial value and the manner in which the meat will be treated during post-mortem

tenderisation and especially during the processing and preparation (Lepetit & Culioli, 1994).

The species, age, sex, muscle type and nutritional status of the animal also influence multiple causes of toughness, for instance, the rate of ageing is dependent on the live mass, while the live weight, in turn, varies considerably within all the latter factors (Ouali, 1990).

Much of the muscle variation in tenderness that exists within an animal is due to differences in the amount and nature of the connective tissue. Estimates of the amount of collagen in meat cuts generally indicate that low connective tissue content is associated with tenderness. Most of the connective tissue fibres in the muscles are collagenous, however, elastin and reticulin fibres are also present and may add to toughness in meat. The decrease in tenderness associated with increased animal age is thought to be a result of the connective tissue changes. It is probable that the additional exercise that mature animals experience causes the structure of the connective tissue fibre to become stronger. Even though there appears to be very little change in the quantity of connective tissue after maturity, the number of intermolecular crosslinks in the collagen fibrils probably increases. Consequently the solubility of the collagen decreases and a resistance to shearing or chewing action increases (Forrest *et al*., 1975).

Tender meat is generally associated with a higher soluble collagen content. Boccard *et al*. (1979) reported that the collagen content has an effect on the meat toughness. However, the solubility rather than the content of the muscle collagen seem to influence the shear force values to a larger extent (Bailey & Light, 1989)

A wide range of tenderness occurs among muscles in any one animal. Generally, those muscles containing the most connective tissue are the least tender, while the muscles with the least amount of connective tissue, such as *Psoas major*, are the most tender (Carmack *et al*., 1995). Within muscles there is also variation in tenderness. Tenderness decreases rapidly from the pelvic end in the *Semimembranosus*, is almost uniform in *Biceps femoris* and *Semitendinosus*, and increases from the centre to both ends of the *Longissimus thoracis et lumborum* (AMIF, 1960). The *Semimembranosus* is the most representative muscle for overall carcass tenderness (Schmidt, 2002).

Post-mortem factors

- Post-mortem glycolysis

In the period between animal slaughter and meat consumption several changes to the myofibrillar protein structure of muscle take place, which gives rise to the variation in

tenderness. For example, the rapid refrigeration of the carcass after slaughter causes severe muscle contraction and results in cold-shortening. These muscles would require a significantly greater force to shear the fibres of the cooked sample. This problem can, however, be prevented in the tender muscles of the hind leg and back by applying different carcass suspension methodologies (which were discussed above). On the other hand, the application of high-voltage electrical stimulation to the carcass depletes the energy stores in the muscles so that there is none available for contraction (Wood *et al.,* 1999). Manipulating meat tenderness by the use of longer conditioning times is also a realistic option and has been acknowledged for many years (Dransfield, 1994).

Several significant changes occur in muscle before, during and immediately after slaughter. One of the changes in the muscle is acidification and pH fall, as previously discussed. It must, however, be borne in mind that the pH of the meat sample itself is temperature dependent. Average pH-temperature dependence ($\Delta pH/\Delta T$) of about –0.011 pH units/°C is suggested by Jansen (2001). This can, however, be influenced by both the animal species and the type of tissue sampled, resulting in a considerable variation. Though not much research appears to have been done on the subject of the dependence of pH on temperature in post-rigor tissue, one can rationally expect a difference between the pH-temperature interactions, since differences exist between functioning cells and chilled dead tissue (Jansen, 2001).

The meat quality of the muscles after slaughter is affected by the ultimate pH and the rate of pH decline, which are both important biochemical parameters (Byrne *et al*., 2000). Jansen (2001) states that tissue pH is widely used as a correlate in meat studies and frequently as a means of monitoring meat quality.

- Conditioning

The chemical properties of living muscle or the post-mortem chemical and physical changes which it undergoes during its phenomenal transformation to meat and subsequent ageing are responsible, either singularly or jointly, for much of the variation in meat tenderness (Briskey, 1963). It is well known that tenderness can be enhanced by ageing, however, it involves an extremely variable process. Several biological factors including age, sex, muscle type, anabolic and repartitioning agents, electrical stimulation, temperature and the storage duration affect this complex development (Ouali, 1990). Dransfield (1994) defined ageing as a means of tenderising meat by storing the meat for longer than the normal time taken for setting and cooling. Given that the meat normally takes 24 h to set and cool, ageing usually occur 24 h post-mortem. The ageing time can

be reduced by increasing the rate of rigor development and temperature, especially during the early stages after slaughter. At a temperature between 0°C and 40°C the rates of ageing increase about 2.5-fold for every 10°C rise in temperature (Dransfield *et al*., 1981).

Cooling: Tenderness is also significantly influenced by the handling and processing conditions, in particular the temperature at which the meat is aged (Byrne *et al.*, 2000). A high muscle temperature accelerates the rate of pH decline post-mortem (Busch *et al*., 1967), as these conditions permit enzymatic activity to continue. The indirect relationship between ultimate pH and toughness is related to differences in myofibrillar shortening and occurs in those muscles which are not restrained from shortening whilst cooling (Bouton *et al*., 1982). Cooling of meat is crucial in preventing the growth of bacteria that leads to meat spoilage. Initial cooling of the carcass from a temperature of 37°C to 4°C within the first 24 h post-mortem is particularly important (Dransfield, 1994). If the carcass temperature is too low the enzymes will be inactivated, resulting in less tender meat due to slower rigor development (Wheeler *et al*., 1990). Dransfield (1994) indicated that when effective and appropriate cooling is applied, 50% of toughening could be overcome.

- Cooking meat

To obtain an acceptable product, the processing and cooking methods are important aspects to control. In managing the processing and cooking, a palatable, tender product from meat that might normally be expected to be less tender may well be produced (Vail, 1963). The nature of the relationship between the ultimate pH and meat tenderness is influenced by the temperature applied during the cooking process (Dransfield *et al.,* 1981). The pH greatly affects the water content, which is an important factor affecting tenderness of cooked meat. Dransfield (1994) states that slow cooking enhances the tenderness of meat. Cooking meat at a temperature of 80°C to 90°C results in tougher meat than meat cooked at 50°C to 60°C, which indicates that the cooking conditions also have an effect on the tenderness (Bryce-Jones, 1969). Meat with a higher water-holding capacity and high pH results in a more tender meat product (Bouton *et al.,* 1973). This shows that the cooking loss and juiciness can be correlated with tenderness (Silva *et al*., 1999).

Changes in meat during cooking: The effect of cooking on the tenderness of muscle depends on the time and temperature of heating, the internal temperature reached and the particular muscle being cooked. The general changes that occur when meat is cooked are the conversion of collagen to gelatine, resulting in the connective tissue becoming tender.

In contrast, the myofibrillar proteins coagulate when heated and become less tender. The most suitable cooking method is determined by the composition of the cut of meat (Moelich, 1999).

Two separate phases of toughness are associated with an increasing cooking temperature. In the first phase there is a three- to fourfold toughening of muscle proteins as the cooking temperature rises from 40°C to 50°C. This increase in temperature causes the myofibrillar structure to denaturate. During the second phase, as the cooking temperature rises from 60°C to 75°C, the toughness is doubled. The second-phase toughening is associated with collagen shrinkage, specifically the epi- and perimysium causing compression of muscle bundles and water loss (Davey & Gilbert, 1972). Moist heat cooking methods are linked to a higher correlation between collagen solubility and tenderness, in comparison to dry-heat cooking methods (Boccard, 1973).

Collagen: On exposing collagen fibres to a high temperature of about 65°C, they will contract to about one quarter of their original length and become rubber-like (Bailey & Sims, 1977). This thermal contraction causes a change in tension and generates more fluid to be exuded from the muscle, resulting in closer hydrophobic and ionic interactions of the denaturised myofilaments. This increases the toughness of the meat (Bailey & Sims, 1977). With increased collagen cross-linkages, the heat-dependent solubility decreases and causes the perimysial collagen to remain as a resistant framework in the cooked meat (Bailey & Light, 1989). The extent of this is determined by the thermal stability of the intermolecular cross-links (Bailey & Sims, 1977). Muscles that are rich in collagen therefore tend to be tougher (Dransfield, 1977).

The quality of collagen is responsible for the progressive toughening of meat as animals grow older (Bailey, 1990). The increase in toughness in mature animals is due to the greater thermal stability of the bonds, resulting from a conversion of the labile reducible cross-links to stable non-reducible bonds (Bailey & Sims, 1977). This causes collagen solubility to decrease with increased animal age.

A positive correlation exists between total collagen concentration and collagen insolubility and the toughness of meat (Young & Braggins, 1993). The qualification of the total collagen will increase the understanding of factors influencing the toughness, although collagen solubility is generally regarded as the factor affecting meat tenderness (Light *et al*., 1985). However, it has been suggested that the contribution of total collagen is more important in different muscles types than its content (Dransfield, 1994).

As stated above, the processing methods can increase the toughness of meat. For this reason it is important to establish guidelines to inform and educate consumers about effective cooking and preparation methods to enhance meat tenderness.

Flavour

Flavour is an essential element of the eating quality of all foods, including meat. Pietersen (1988) defines flavour as a complex sensation involving odour, taste, temperature and pH. The meat characteristics overall, flavour and aroma are very complex with reference to their analysis and description. It is difficult to separate these two qualities, since many of the flavour properties are the result of odour sensations. Odour and taste are most challenging to define objectively and can be influenced by the appearance, texture and even sound made upon mastication (Amerine *et al*., 1965). This meat quality characteristic is a key aspect in the sensory analysis that involves the overall acceptability of the meat.

Raw meat has a bland, salty and blood-like flavour, whilst the true “meaty” flavour develops during the cooking process (AMIF, 1960). It is during cooking that the desirable attributes of flavour develop, hence its significance. The nature and intensity of meat flavour are partially determined by the cooking method used and the time taken and temperature of cooking. Cooking methods, where dry heat is applied, develop certain flavours, especially at the exposed surfaces where the temperature becomes very high (Schönfeldt, 1989). On the other hand, moist cooking methods results in distinctive and unique flavour changes to development in the deeper tissues of meat cuts (Schönfeldt, 1989).

The volatile flavour compounds found in cooked meat can be categorised into two classes, namely those compounds that develop from the Maillard reaction and those formed from lipid oxidation (Elmore *et al*., 2000). During the Maillard reaction the carbonyl group of a reducing sugar, such as glucose, reacts with the amino group of an amino acid or peptide. This reaction is known as the “browning” reaction and the carbonyl-amines condensation is vital in the developing of meat flavours (Penfield & Campbell, 1998). Carbonyl compounds contribute a major portion of the odour obtained upon heating. During cooking these carbonyls undergo a condensation reaction (browning reaction) with the amino acids from cyclic carbonyls. The degradation of S-containing amino acids in meat protein leads to the release of sulphur which increases with cooking time and temperature. Sugar and S-containing amino acids compounds form thiols, which contribute to meaty flavours (Schönfeldt *et al*., 1997). These flavour compounds, or their

precursors, are apparently present in only trace amounts in the fat. Heating the bulk of the triglycerides, after these trace components are removed, does not generate the characteristic beef aroma.

Variability in flavour

Flavour and aroma are inherent qualities of a particular sample of meat, determined by both intrinsic and extrinsic factors (Lawrie, 1985). Intrinsic factors include fatty acid composition, mineral content and pre-slaughter factors such as species, age, gender and diet (Sink, 1979). The extrinsic factors mostly include the post-mortem conditions such as slaughter conditions, handling and extent of ageing and the cooking method used (Lawrie, 1998b).

Intrinsic factors

There appears to be little doubt that the flavour of muscle foods is highly species dependent (Sink, 1979). According to Shahidi and Rubin (1986), the meaty flavour of red meats develops during cooking through degradation and reactions of water-soluble compounds. The species flavour originates in the fatty tissue and both lipids and water-soluble components are necessary to develop a species' characteristic flavour and aroma (Wasserman & Spinelli, 1972). An increase in animal age is generally associated with an increase in flavour intensity; therefore the natural flavour of meat is not fully developed until the animal reaches maturity (Lawrie, 1985).

Fatty acid composition plays an important role in the definition of meat quality and is related to differences in sensory properties of the meat, especially flavour (Wood & Enser, 1997). Meat flavour is affected by the fatty acid composition through the volatile, odorous, lipid oxidation products produced during cooking, as well as the involvement of these products with Maillard reaction products to form other volatiles which contribute to odour and flavour (Wood *et al*., 2003).

Fatness itself affects the flavour and aroma, since the triacylglycerols in the fatty acid composition of the total lipid is more saturated than phospholipids in the muscle membranes (Marmer *et al*., 1984), and with increased fatness the triacylglycerols will increase (Enser *et al*., 1998). The role of fat has been studied intensively, suggesting that the basic meat aroma is derived from a water-soluble fraction of muscle, while fatty acids provide the characteristic species aroma and flavour (Mottram & Edwards, 1983). Therefore, both lipids and water-soluble components are crucial for the development of aroma (Wasserman & Spinelli, 1972). An important precursor of meat aroma is thiamine,

since the thermal degradation of thiamine produces at least eight volatile compounds that have been identified as cooked meat aroma (Lawrie, 1985).

Extrinsic factors

Variations in flavour are caused by several extrinsic factors, which include diet (Lawrie, 1985), post-slaughter handling and storage, as well as processing methods. Variability in flavour caused by diet refers to relatively undesirable features derived from specific components rather than to the level of intensity of feeding. Little evidence exists to support flavour differences between grass-fed animals or those intensively reared on concentrates other than those expectable from animal age (Lawrie, 1998b). Contrary to the previous statement, Fisher *et al*. (1999) and Elmore *et al*. (2000) found that the basic flavour characteristics of beef can be influenced by animal diet. The effect of diet on the sensory properties of meat is accomplished through the deposition of unique components in the fat, while the extent of the influence is dependent on the animal species. It is accepted that dietary factors do not alter the body fat composition in ruminants to the degree that they do in monogastric animals. Beef aroma is influenced by both long-chain fatty acids and volatile fatty acids, other than the dietary influences on the eating quality of beef, which are limited to the long-chain fatty acids (Webb *et al.,* 1994). Variations in the different fatty acids and the absolute concentration will affect the flavour profile.

Most processing operations, although designed to preserve sensory quality, as well as to prevent microbial spoilage, tend to cause flavour deterioration (Lawrie, 1998b).

In mentioning the pre-slaughter and post-slaughter variables, it is important to also discuss the pH changes as pertaining to the flavour before, during and after slaughtering. The desirability of flavour declines as muscle pH increases, causing the meat to have lower flavour intensity with a higher ultimate pH (Bouton *et al*., 1957). Ford and Park (1980) also confirm that the meaty character of cooked beef diminishes progressively as the ultimate pH increases, causing the cooked meat to have a different flavour. The reason for this effect is the swollen structure that interferes with substances' access to the palate (Lawrie, 1985). Silva *et al.* (1999) reports that meat with a high ultimate pH is dark and is consequently more susceptible to bacterial growth, which causes meat spoilage and reduced flavour. Therefore, attempts should be made to control such factors and improve pre-slaughter and post-slaughter conditions to optimise the flavour and aroma.

Meat flavours are generated by numerous types of heat-induced reactions (Lawrie, 1985), while the volatile compounds of cooked meat determine the aroma attributes and contribute most to the characteristic flavours of meat (Shahidi, 1998). A wide range/variety

of these volatile components have been isolated and identified, which shows that both lipids and the water-soluble components of the depot adipose tissue are necessary to develop a characteristic aroma to distinguish between species (Wasserman & Spinelli, 1972). Mottram and Edwards (1983) found that lipids contribute to the desirable flavour of cooked meat, either through changes in the thermal oxidative-producing compounds, which can also react with components from lean tissue to form other flavour compounds or can act as a solvent of aroma for the compounds accumulated during cooking, or even in the processing and production stages.

2.5. CONSUMER CONCERNS AND MEAT SAFETY

The demand for meat is at risk of being adversely affected as a result of consumer concerns about several aspects of meat consumption and production (Harrington, 1994). Consumers are increasingly becoming concerned about healthy and safe products and the demand for these products is escalating. Consumers expect the meat products on the market to have the required nutritional value, be wholesome, fresh and lean, and have adequate juiciness, flavour and tenderness (Dransfield, 2003).

Meat quality comprises several important aspects, i.e. nutritional properties like protein, fat, minerals and essential sub-constituents; the appropriate proportions of the bioactive compounds; sensory characteristics such as tenderness, juiciness and flavour; healthiness of the meat, such as fatty acid composition; and the views or perceptions about the production conditions and food safety (Zotte, 2002; Strydom, 2003). Meat is an excellent source of nutrients, energy and building materials, and numerous substances that are essential for normal human growth, development and survival. These aspects of the nutritional value of meat have been discussed above. Despite the excellent dietary value of meat, the risks associated with meat consumption are possibly the most commonly acknowledged reason for declining meat consumption (Richardson *et al*., 1994).

According to Zotte (2002) the quality and safety of the food that consumers consume are increasingly becoming a concern. Food safety is a major issue for consumers and the public demands high-quality, safe, wholesome and convenient meat products, with a natural flavour, a relatively long shelf-life and which has been minimally processed. In addition, the meat should be produced under hygienic and animal-friendly conditions (Snijders & Van Knapen, 2002).

Although most food-borne diseases are transmitted through poultry, eggs, shellfish and milk, rather than through meat, meat products can still act as a vector for food-borne diseases (Sanders, 1998). Meat contains an abundance of all nutrients required for the growth of bacteria, yeasts and moulds, and an adequate quantity of these constituents exists in fresh meats in a readily available form. It is for this reason that meat is very perishable. The very high protein and moisture content of meat products make them susceptible to possible contamination and proliferation with pathogenic micro-organisms, such as *Salmonella* and *Campylobacter* (Mulder, 1996; Warriss, 2000).

In an attempt to ensure safe meat the temperature and time of meat storage should be managed correctly, thus aiming to extend the shelf-life of the meat product. The shelf-life of red meat at refrigerated temperatures (0° - 4°C) is, however, very limited. According to Warriss (2000), the shelf-life of certain species is as follows: approximately 10 to 14 d for beef, between 7 and 10 d for lamb and about 4 d for pork.

Spoilage of chilled beef at refrigeration temperatures is due to the proliferation of various bacteria, yeasts and moulds on the meat surface (Jensen, 1954; Borch *et al*., 1996; Merck, undated). The shelf-life of the meat is determined by the numbers and types of micro-organisms initially present and their subsequent growth (Borch *et al.*, 1996). The dressing process is responsible for causing more than 99% of the initial contamination (Warriss, 2000). Merely 10% of the initial microbes present have the ability to grow at refrigerated temperatures, and an even smaller percentage of microbes are able to produce spoilage. Environmental factors such as temperature, gaseous atmosphere and salt content will select specific microbes and will consequently affect their growth rate and activity (Borch *et al*., 1996).

Several micro-organisms predominate in spoiled meats, as a result of the pH value of meat, which is within the growth rate of most organisms. Micro-organisms such as *Enterobacteriaceae, Shewanella putrefaciens, Micrococcus, Achromobacter*, *Aeromonas, Pseudomonas, Acinetobacter, Moraxella, Psychrobacter, Brochothrix thermosphacta, Staphylococcus,* coryneforme bacteria*, Lactobacillus*, *Leuconostoc* and *Weissella* are the primary source of meat spoilage and are able to thrive at refrigerated temperatures (Jensen, 1954; Borch *et al*., 1996; Merck, undated).

Borch *et al*. (1996) define spoilage as a specified maximum microbial level or an off-flavour/off-odour or appearance which is undesirable. Fortunately, there are legally set microbiological standards to monitor the safety of most food products, including meat. The maximum acceptable level of bacteria that can be reached during storage is 10^7 - 10^9 CFU.cm^{-2} (Borch *et al*., 1996). According to Merck (undated), spoilage generally occurs at

about 1×10^6 CFU.cm^{-2}. The International Commission for the Microbiological Specifications of Foods (ICMSF) (1986) recommended a general viable count of less than 1×10^7 CFU.g^{-1}.

Meat packaging is becoming increasingly more significant, mainly for three reasons. Firstly, it protects the meat from contamination and inhibits microbial growth, consequently delaying spoilage and prolonging shelf-life. Secondly, it reduces weight loss and surface drying, and finally it may enhance the appearance of the meat (Warriss, 2000). Three different types of packaging are currently used for red meat, namely air, vacuum and modified atmosphere packaging (MAP) (different levels of oxygen and carbon dioxide, balanced with inert nitrogen). In aerobically stored meat, the dominant organisms may be *Pseudomonas* spp and because of their rapid growth rate, the shelf-life is only a matter of days (Borch *et al*., 1996). In studies on vacuum-packed chill-stored beef, the most predominant spoilage bacteria were lactic acid bacteria (*Lactobacillus curvatus*, *Lb. sakei*, *Carnobacterium piscicola* and *C. divergens*) (Sakala *et al*., 2002).

Various negative changes develop as a result of spoilage and pathogenic micro-organisms, which include off-odours, off-flavours, discolouration, slime and gas production and a pH decline. Certain spoilage micro-organisms are associated with certain types of off-odours and off-flavours produced during meat spoilage. Some examples of these include, *Pseudomonas* spp. that produces ethyl esters causing sweet and fruity odours during the early stages of spoilage. Sulphur and putrid odours are generally associated with sulphur-containing compounds, including hydrogen sulphide (*Enterobacteriaceae*) and dimethyl sulphide (*Pseudomonas* spp.). Acetion/diacetyl and 3-methylbutanol formed by *Enterobacteriaceae, Brochothrix thermosphacta* and homofermentative *Lactobacillus* spp. are mainly responsible for cheesy odours (Borch *et al*., 1996). Furthermore, *Enterobacteriaceae, Pseudomonas* and *Brochothrix thermosphacta* are mainly responsible for deterioration, *Lactobacillus* and *Brochothrix* for acidification and *Shewanella putrefaciens* and *Aeromonas hydrophila* for greening (Merck, undated).

There are many techniques that can be applied to reduce or eliminate bacterial growth and preserve meat longer, such as drying, curing and smoking, irradiation, refrigeration, cooking, canning and the mentioned packaging alternatives. Another route to pursue and minimise this risk of meat exposure to potential human pathogens and spoilage micro-organisms is to install food safety systems with specific requirements. An example of one of these systems recommended for controlling food-borne pathogens is the implementation of hazard analysis of critical control points (HACCP) (Kennedy *et al*., 2000). HACCP is defined as an integrated approach, assuring microbial safety and

preventing the possible contamination of food products. Measures integrated in the HACCP approach are inspection systems and specific personnel hygiene regimens as well as control of processes such as heating, cooling, cleaning and sterilisation. In an integrated quality control system, the importance of having information about the facility is crucial in ensuring safe meat and proper inspection and management of the slaughterhouse (Snijders & Van Knapen, 2002). This information is necessary since hazards and their potentially negative health impact vary within and between production systems, regions and countries. In an attempt to guarantee the safety of beef, from any source, species or region, the application of appropriate safety procedures are required in order to maintain hygiene and safety in all the activities and processing methods.

Along with the improvement of the microbiological quality of food, good hygiene practice (GHP), good manufacturing practice (GMP) and HACCP, it is also important to prevent recontamination of food (Panisello *et al*., 2000). Unfortunately the implementation of HACCP alone is not enough for the extension of the shelf-life of products. The National Food Processor Association (NFPA) in the USA recommended the incorporation of a suitable food preservative into the product (Kennedy *et al*., 2000). The criteria for a preservative are that it has to be effective in small quantities and have a wide spectrum of anti-microbial activity, and it must not diminish the quality of the food nor be detrimental to the consumer's health (Kennedy *et al*., 2000).

The use of chemicals to prevent or delay the spoilage of foods derives in part from the fact that such compounds have been used with great success in the treatment of diseases of humans, animals and plants. A large number of chemicals have been described that show potential as food preservatives, however, only a relatively small number are allowed in food products (Jay, 1978).

The Foodstuffs, Cosmetics and Disinfectants Act and Regulations 54/1972, define a preservative as any substance which inhibits, retards or arrests fermentation, acidification or other decomposition of foodstuffs, but does not include preservatives such as common salt (NaCl), sugar (sucrose), lactic acid, vinegar, alcohol or portable spirits, herbs, hop extract, spices and essential oils (Anon., 1972). Existing preservatives include sulphites, sulphur dioxide, sodium chloride, phosphates, hydrogen peroxide, nitrates (NO_3), nitrites (NO_2), Na-diacetate, β-propiolactone, benzoic acid and benzoates, sorbic acid and sorbates, acetic acid and acetate salts, lactic acid, propionic acid, fumaric acid, citric acid, parabens and therapeutic antibiotics (Magnison, 1997; Kennedy *et al*., 2000).

One of the chemical compounds most widely used in red meat is sodium chloride (NaCl). The salt exerts a drying effect upon both food and micro-organisms, where a

higher concentration leads to a greater preserving effect. The growth of many food spoilage bacteria and potential pathogens on meat is inhibited, or at least delayed, by the addition of salt, as it decreases water activity. The most widely employed organic acids used to preserve meat are acetic and lactic acid. The antimicrobial effect of organic acids is due both to the depression of pH below the growth range and metabolic inhibition by the non-dissociated acid molecules (Jay, 1978).

Researchers have examined naturally occurring metabolites produced by lactic acid bacteria that inhibit the growth of undesirable micro-organisms (Abee *et al*., 1995; Kennedy *et al*., 2000; Ross *et al*., 2002). These metabolic inhibitors include organic acids and anti-microbial peptides or bacteriocins. These inhibitors suppress the growth and survival of undesirable food spoilage and pathogenic micro-organisms in the foodstuffs.

2.6. CONCLUSIONS

The variation in beef quality is considerable as a result of many factors, such as differences in genetic background, sex, age, nutrition and meat processing. Both the chemical composition and the cooking properties of muscle exert important influences on beef palatability and consumer acceptance (Jeremiah, 1978). Numerous reports confirmed that muscles in the beef carcass were extremely variable in their cooking, chemical and connective tissue properties. This variation certainly influences both their palatability attributes and consumer acceptance (Jeremiah *et al*., 2003a).

For food products to have optimum consumer appeal, they must not only provide a high degree of eating satisfaction, but also be consistent in their palatability attributes and nutritional value, as well as be safe to eat. A problem which has plagued the beef industry for several decades is the lack of consistency in palatability.

Without a doubt variation and inconsistency of quality must be controlled to improve customer satisfaction with meat products. The consumer's decision to purchase beef is guided by the perception of healthiness and a variety of sensory traits including colour, tenderness, juiciness and flavour (Verbeke & Viaene, 1999). Consumers are willing to pay more for meat products with a higher or guaranteed tenderness. It is therefore worthwhile considering discrepancies in meat quality at the consumer level with respect to both sensory traits and health aspects (Raes *et al*., 2003).

Several processes can be implemented to limit this inconsistency. There are several critical control points that must be managed in order to obtain the desired meat quality and tenderness. These areas implicated include: pre-slaughter handling

procedures, electrical stimulation, chilling, aging and post-mortem tenderisation technologies (e.g. injection / infusion, blade tenderisation) (Koohmaraie *et al*., 2003). Effective post-mortem intervention techniques and/or alternative cooking methods will have to be developed and applied to improve the palatability of beef muscles (Jeremiah *et al*., 2003b)

Natural and enhanced (for example, the infusion of beef muscle with a moisture and salt solution) tenderisation of meat should be optimised to improve traditionally less tender meat cuts and aid in meeting consumer demands for a higher quality and more convenient product that is consistent in meat quality, especially in tenderness (Koohmaraie *et al*., 2003).

Despite improved quality of meat gained from conditioning, the cost of holding carcasses for an extended period of time can be very high. Therefore, the producer is searching for ways to reduce this holding time without incurring a forfeit on quality (Etherington, 1984). The approach of injecting a meat cut with a phosphate and lactate blend may provide some benefits by accelerating the ageing process and enhancing qualities such as palatability and extending shelf-life, yet not having detrimental effects on the nutritional value of the meat.

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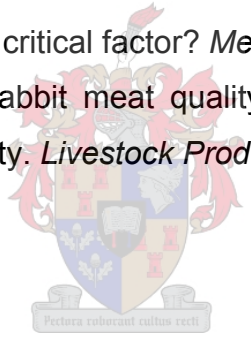

CHAPTER 3

PHYSICAL, CHEMICAL AND SENSORY PROPERTIES OF SELECTED SOUTH AFRICAN BEEF MUSCLES

ABSTRACT

In fresh meat the sensory characteristics and nutritional properties are the key drivers for degree of liking and purchasing intent. If meat qualities such as colour and drip loss vary, consumers refrain from purchasing the products. Inconsistency in meat quality is a problem that has plagued the meat industry for many years. One of the main contributors to this problem is muscle variation. In the present investigation several beef muscles, i.e. *Biceps femoris* (BF, silverside), *Longissimus lumborum* (LL, striploin), *Rectus femoris* (RF), *Supraspinatus* (SS, scotch fillet) and *Semitendinosus* (ST, eye of the silverside) were selected and removed from the right side of six beef carcasses. Seventy-two hours post-mortem the physical, chemical and sensory characteristics were determined. The selected beef muscles differed significantly ($P \leq 0.05$) with regard to most of the physical, chemical and sensory attributes. The impact of variation in the quality of beef muscle is of great concern to the meat industry and should not be neglected. Improvement of beef quality and its consistency are essential for the survival of the beef industry. These results demonstrate the need for more attention to processing, preparation and ageing of meat.

Keywords: Beef, muscle variation, physical attributes, proximate composition, minerals, fatty acids, sensory properties.

INTRODUCTION

It is well known that consumers demand consistency when purchasing food and that food products should provide them with the assurance of a satisfactory eating experience (Jeremiah *et al*., 2003c). However, the beef industry is still unable to resolve the problem of inconsistent meat quality (Morgan *et al*., 1991; Boleman *et al*., 1998; Sørheim & Hildrum, 2002) pertaining to appearance and palatability characteristics (Jeremiah *et al.*, 2003c), in particular tenderness (Dransfield, 1994; Lusk *et al*., 1999; Brooks *et al*., 2000). The demand, acceptability and repurchase of meat diminish drastically with the appearance of undesirable colour and poor physical characteristics such as visible drip

(Livisay *et al*., 1996). In addition, the consumer feels uncertain about the expected meat quality, so the possibility of experiencing a tough steak adds to the declining demand and consumption of beef (Lusk *et al*., 1999).

Some of the first work on muscle variation was done by Ramsbottom *et al*. (1945), Ramsbottom and Strandine (1948), Strandine *et al*. (1949) and Paul *et al*. (1970). These researchers defined the physical and sensory properties of the major beef muscles and established that variation existed between carcasses even within the same breed. These and other studies stated that variation occurred in a carcass (Shorthose & Harris, 1990) between muscles within a cut and within a single muscle (Ramsbottom *et al*., 1945; Paul *et al*., 1970; McKeith *et al*., 1985). Extensive research has been done on the subject of specific muscle quality. Reuter *et al*. (2002) reported that intramuscular tenderness variation exists within the beef round such as the *Biceps femoris*. Other studies found that the *Rectus femoris* (Bratcher *et al*., 2005) and *Semitendinosus* (Denoyelle & Lebihan; 2003) had a location effect giving rise to tenderness variation. Gariepy *et al*. (1990) reported that location along the *Longissimus thoracis et lumborum* influenced the muscle pH, colour, shear force and drip. Research on beef muscle indicated that beef muscles differed in size, weight, final pH, composition and ultrastructure (Ramsbottom & Strandine, 1948; Strandine *et al*., 1949), and sensory (Carmack *et al*., 1995), physical (Jeremiah *et al*., 2003b), cooking (Crouse *et al*., 1984) and chemical properties (Jeremiah *et al.*, 2003a, b).

Physical methods as well as sensory analysis are used to assess the difference in meat quality and to understand the sources of variation (Lepetit *et al*., 2002). Muscle variation is caused by various known and unknown intrinsic (genetic/productive) and extrinsic (environmental/technological) factors (Webb, 2003; Monsón *et al*., 2005). These factors interact and determine the outcome of metabolic processes in the live animal, as well as the period after death (Klont *et al.*, 1998). Some of the factors that contribute to the variation in quality are breed, sex, age, nutrition (Raes *et al*., 2003; Webb, 2003), pre-slaughter handling, post-mortem cooling, electrical stimulation and different post-mortem storage and processing techniques (Ouali, 1990; Dransfield, 1994; Varnam & Sutherland, 1995; Koohmaraie, 1996; Warriss, 2000; Koohmaraie *et al.*, 2003).

The anatomical location of muscles (Jeremiah *et al.*, 1991), as well as myofibrillar properties (Koohmaraie, 1992; 1994), sarcomere length, collagen content (MacKeith *et al*., 1985; Valin, 1995; Wheeler *et al*., 2000) and muscle fibre characteristics (type and size) (Totland *et al*., 1988) account for variation in meat quality (Crouse *et al*., 1991; Valin, 1995). The anatomical location of muscles is categorised into two groups, i.e. locomotion

and support muscles. Muscles from the front shoulder (*Supraspinatus*) and hind leg (*Biceps femoris*, *Rectus femoris*, *Semitendinosus*) are used for working (locomotion) and have higher percentages of connective tissue and hence collagen. These muscles are tougher because of their function and composition. Support muscles such as the loin muscles (*Longissimus lumborum*) from the back have less connective tissue, which results in a more tender beef cut (Anon., 2005a; Belew *et al*., 2003). These generalities are not always the best indicators of meat quality, because some muscles in close proximity have shown marked differences in tenderness (Belew *et al*., 2003).

Beef muscles differ in their response to conditioning because of muscle fibre composition (Olson *et al.*, 1976; Koohmaraie *et al.*, 1988). Fibre type and fibre areas of particular muscles greatly influence pre- and post-mortem biochemical processes and therefore meat quality, especially tenderness (Ouali, 1990; Klont *et al*., 1998). Beef muscles differ largely in their rates of physiological reaction, reflecting the proportion of each fibre type (Anon., 2005b). The fibre types are categorised into four major functionally specialised groups, i.e. slow-twitch-oxidative (Type I); fast-twitch-oxidative-glycolytic (Type IIA), fast-twitch-glycolytic (Type IIB), and intermediate (Type IIC) (Varnam & Sutherland, 1995; Jiang, 1998). The fibres contain different amounts of myoglobin, oxidative and glycolytic enzymes and differ quantitatively in contractile proteins, have different proteinase and inhibitor systems. Smulders *et al*. (1990) concluded that the tenderness of slow-glycolysing muscles is highly dependent on sarcomere shortening, while the tenderness of fast-glycolysing muscles is due to more rapid ageing (rapid pH decline). The tenderness of slow-glycolysing muscles is completely independent of more rapid pH. Vestergaard *et al*. (2000a) reported that ST had the lowest frequency of Type I fibres (19.5%), followed by LD with 27.6% and then SS with the highest frequency of 58.7%, however, with the Type IIB fibres the situation was reversed.

From this brief summary on muscle variation, it is clear that muscle variation does exist and that inconsistency in quality contributes to the increasing economic concern regarding rising meat prices and declining meat consumption.

Limited research is found on the variation in South African beef muscles as pertaining to the physical attributes, chemical composition and sensory attributes of beef. This lack of knowledge in the meat quality of South African beef adds to the incidence of unreliable beef quality at retail level. South African beef is different from beef imported from other countries, because of the low average fat content and the classification system used, based on age classification by dentition (Webb, 2003; Strydom, 2003). Research on South African beef cuts by Schönfeldt included the nutritional composition and sensory

properties of primal cuts (Schönfeldt & Welgemoed, 1996; Schönfeldt *et al.*, 1997a, b). Research abroad shows more extensive and detailed papers on muscle variation (Koohmaraie *et al*., 1988; Belew *et al*., 2003; Rhee *et al*., 2004) and an American website based on bovine myology and muscle profiling (Anon., 2005c) adds to the research database overseas.

The present study was therefore undertaken to determine whether selected beef muscles from South African beef carcasses varied in their physical, chemical and sensory properties, thereby providing the South African beef industry with a better knowledge of the degree of variation between different beef muscles.

MATERIALS AND METHODS

Animals and sampling

Beef carcasses representing South African beef breeds [Brahman × Simmentaler cross; n = 3, average mass = 300.73 kg and Charolais × Hereford cross; n = 3, average mass = 297.87 kg] finished off in a feedlot, were sourced from a commercial abattoir in Paarl, Western Cape, South Africa. At the abattoir, the animals were slaughtered, dressed and thereafter processed according to standard South African techniques and conditions. No electrical stimulation was applied to the carcasses. The animals were selected to represent culled steers from a typical commercial scenario, representative of the South African market.

The carcasses were all classified as A2 according to the South African classification system (Government Notice No R. 1748, 26 June 1992). An A2 animal is a young animal of the A age group (no permanent incisors) with a fat code of 2, representing a lean fat cover (1-3 mm thick subcutaneous fat depth measured between the 10^{th} and 11^{th} rib, 50 mm from the midline of the cold unquartered carcass). The whole, intact carcasses were chilled at *ca.* 2°C for 24 h in a cooling chamber before being weighed and quartered at the abattoir (Day 1). Twenty-four hours (Day 2) post-mortem (pm) the beef quarters were moved into a mobile cooling unit (set at 4°C) and transported to the Meat Science Laboratory at Stellenbosch University, where the carcasses were stored in the cooling facility at 4°C. On the same day (Day 2; 24 h pm) the right side *Biceps femoris* (BF, silverside), *Longissimus lumborum* (LL, striploin), *Rectus femoris* (RF), *Supraspinatus* (SS, scotch fillet) and *Semitendinosus* (ST, eye of the silverside) were removed from the carcasses. The muscles were trimmed of all visible subcutaneous fat and superficial

collagen, weighed, labelled, vacuum packed and stored in a cooler at *ca.* 4°C until further processing.

Sample preparation

Forty-eight hours after storage (Day 4, 72 h pm), the samples were removed from the cooler and packaged for analyses. The samples were divided into two portions, the first for physical and chemical analyses and the second for sensory analysis. The latter samples were labelled, vacuum packed once again and frozen at −18°C, until tested for sensory attributes using a descriptive panel.

Upon analysing the physical characteristics of the muscle, the samples were dried by means of absorbent paper and reweighed for calculation of purge loss (exudate collected in the vacuum bag). Meat slices of approximately 1.5 cm thick were cut cross-sectional to the muscle fibre for the determination of instrumental colour (CIE Lab) of the raw and cooked muscles, drip loss, cooking loss and instrumental tenderness of the cooked muscles.

Throughout the trial, an attempt was made to ensure that all activities were similar to a typical commercial scenario. Care was taken throughout the investigation to ensure that the handling procedures were similar for all the samples.

Physical analyses

pH measurement

The pH measurements were conducted by using a penetrating glass electrode on a hand-held Crison pH/mV-507 meter with an automatic temperature compensator. The pH measurements were taken approximately in the middle of the muscle cut. The pH meter was re-calibrated after every fourth reading with pH 4.01 and pH 7.02 standard buffers and the electrode rinsed with distilled water between each measurement.

Purge loss

For the determination of purge loss of the muscles, the sub-samples, were weighed on Day 2 prior to packaging and then re-weighed on the designated sampling date (Day 4). The purge loss was calculated as weight loss expressed as a percentage of the initial weight of the sample (Honikel, 1998). The purge loss was the amount of exudate that collected in the vacuum bags after a storage period of 48 h.

Instrumental meat colour

Colour was evaluated according to the method described by Honikel (1998) to determine L*, a* and b* values. The colour (CIE Lab) of the fresh and cooked samples was measured at ambient temperature using a Colour-guide 45°/0° colorimeter (Catalog No 6805; BYK-Gardner, USA). The colour measurements for each sample were taken in triplicate at randomly selected positions. The readings were taken after a blooming period of 30 min, thereby ensuring that sufficient blooming (the time in which a freshly cut surface of meat is exposed to air to allow colour stabilisation) (Wulf & Wise, 1999) had occurred. The meat samples used for the determination of the cooking loss were used to evaluate the colour of the cooked meat. Both the fresh and cooked meat colour analyses were done on a white surface at an ambient temperature of 8°C.

Colour was expressed by the coordinates L*, a*, b* of the CIE Lab colorimetric space (CIE, 1978). The L*, a* and b* values are an indication of the lightness (black-white axis), redness (red-green spectrum) and yellowness (blue-yellow spectrum) of colour respectively. The hue-angle and the a* and b* chroma are psychometric correlates of perceived hue and chroma (Honikel, 1998) and were determined using the following equation (CIE, 1978):

Chroma: $C^* = \sqrt{(a^*)^2 + (b^*)^2}$ Hue angle: $h_{ab} = \tan^{-1}\left\{\frac{b^*}{a^*}\right\}$

Drip loss

For the determination of the drip loss, 15 mm thick muscles samples, cut perpendicular to the longitudinal axis of the muscle, were weighed immediately after being cut. Drip loss was determined according to the procedure described by Honikel (1998). The samples were weighed individually (approximately 100 g) and suspended with a wire (under atmospheric pressure) into an inflated polythene bag in such a manner that the exudate did not come into contact with the sample, but collected in the bag. After a storage period of 24 h at *ca.* 4°C, the samples were blotted with absorbent paper and re-weighed. The drip loss was calculated as weight loss expressed as a percentage of the initial weight of the sample (Honikel, 1998).

The remaining meat samples were homogenised, placed in polyethylene bags, vacuum-sealed and stored in a freezer at −18°C, until the chemical analysis could be conducted. The samples for the chemical analysis were homogenised within 2 d after deboning.

Cooking loss

For cooking loss determination, samples of 15 mm thick were cut from the muscle and the initial weight determined. Individual slices of approximately 100 g were placed in thin-walled plastic bags in a water-bath at *ca.* 80°C. The samples were removed from the water-bath after 1 h and cooled under cold water. The meat was removed from the bag, blotted dry and weighed. Cooking loss was calculated as the difference in sample weight before and after cooking, expressed as a percentage of the initial sample weight (Honikel, 1998).

Shear force resistance

After measurement of the cooking loss, the samples were stored overnight (24 h) in a refrigerator (4°C) and used for instrumental determination of tenderness. The shear force values of the cooked meat samples were obtained with a Warner-Bratzler shear (WBS) attachment (Voisey, 1976), fitted to an Instron Universal Testing Machine (Model 4444). The number of samples taken was limited in the SS because of the small sample area and visible connective tissue found in this muscle. Three randomly sampled cylindrical cores were cut from the centre of each cooked meat sample using a 1.27 cm diameter bore. The tenderness was measured as the maximum force (Newton) required to shear a 1.27cm diameter cylindrical core of cooked meat (perpendicular to the grain) at a crosshead speed of 200 mm/min. The mean was calculated for each muscle. A larger value indicated greater shear force and therefore tougher meat (Honikel, 1998).

Chemical analyses

The analyses pertaining to the chemical composition comprised of proximate chemical analysis (total percentage fat, moisture, ash and protein), as well as the mineral composition and fatty acid profile of the five homogenised muscles, *Biceps femoris* (BF, silverside), *Longissimus lumborum* (LL, striploin), *Rectus femoris* (RF), *Supraspinatus* (SS, scotch fillet) and *Semitendinosus* (ST, eye of the silverside).

Proximate chemical analyses

The total percentage of moisture, protein, fat and ash of the raw beef muscle samples were determined according to AOAC Methods (AOAC, 2002). The moisture content was analysed by drying a 2.5 g sample at 100°C for a period of 24 h (Method 934.01, AOAC, 2002) and ashing was done by cremating the samples at 500°C for a period of 6 h. The protein content was determined by the Dumas combustion method (Method 968.06,

AOAC, 2002) on the defatted samples using a FP528 Nitrogen Analyser. The total lipid content was determined by extracting the fat with a 1:2 mixture of chloroform: methanol according to the method of Lee *et al*. (1996).

Mineral analyses

The mineral composition of the meat was determined after ashing the defatted meat samples. The defatted meat samples (1-3 g) were air-dried and ground to pass through a 0.5 to 1.0 mm sieve. Thereafter the samples were ashed overnight in a muffle furnace at 550°C. A 6 N hydrochloric acid (HCl) solution was prepared by diluting 500 ml of a 36% (m/m) HCl solution to 1 liter. After ashing, 5 ml of a 6 M HCl was added to dissolve the cooled sample. Thereafter, the samples were dried in a water bath. After cooling, a 5 ml 6 N nitric acid (HNO_3) solution was added to the samples. The 6 N HNO_3 solution was prepared by diluting 429 ml of a 65% (m/m) solution to 1 liter. After adding the latter solution, the samples were heated in a water bath and removed after the boiling point was reached. The solution was subsequently filtered through filter paper into a 100 ml volumetric flask and diluted to volume with deionised water (Giron, 1973).

The concentrations of calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), sodium (Na), phosphorus (P), lead (Pb) and zinc (Zn) of the digestates were determined by using the Inductively Coupled Plasma Spectrometry (ICP) detection method (Method No AgriLASA 6.1.1) (Handbook of Feed & Plant Analysis, Volume 2).

Fatty acid analyses

The fatty acid composition was determined using the same method described by Tichelaar *et al.* (1998). After thawing the meat, a 2 g sample was extracted with chloroform/methanol (CM 2:1; v/v) according to a modified method of Folch *et al.* (1957). All the extraction solvents contained 0.01% butylated hydroxytoluene (BHT) as an antioxidant. A polytron mixer (Kinematica, type PT 10-35, Switzerland) was used to homogenise the sample within the extraction solvent. Heptadecanoic acid (C17:0) was used as an internal standard (Sigma H3500-5G, Batch No 072K2642) to quantify the individual fatty acids. A sub-sample of the extracted lipids was transmethylated for 2 h at 70°C using methanol/sulphuric acid (19:1; v/v) as transmethylating agent. After cooling, the resulting fatty acid methyl esters (FAME) were extracted with water and hexane. The top hexane phase was transferred to a spotting tube and dried under nitrogen.

The FAME were purified by TLC (silica gel 60 plates) and analysed by GLC (Thermo Finnigan Focus GC equipped with flame ionisation detection) using 60 mm

BPX70 capillary columns of 0.25 mm internal diameter, 0.25µm film (SGE, Australia). Gas flow rates were: hydrogen, 25 ml/min; and hydrogen carrier gas 2-4 ml/min. Temperature programming was linear at 4°C/min, with an initial temperature of 140°C, a final temperature of 240°C, an injector temperature of 220°C and a detector temperature of 260°C. The FAME were identified by comparison of the retention times to those of a standard FAME mixture (Supelco^{TM} 37 Component FAME, 100 g Neat, F.A.M.E. mix C4-C24, Sigma Catalog No 18919-1AMP, Lot No LB-16064) (Nu-Chek-Prep Inc., Elysian, Minnesota).

Descriptive sensory analysis

Sample preparation

The vacuum-packed meat samples taken from the right side of the carcass were defrosted at a temperature of 2-4°C for a period of 48 h prior to cooking on their pre-assigned sensory analysis dates. Per carcass, four samples of meat (four muscles) were used for each sensory analysis session. The right *Biceps femoris* (BF, silverside), *Longissimus lumborum* (LL, striploin), *Rectus femoris* (RF) and *Semitendinosus* (ST, eye of the silverside) were analysed. The meat samples were cut to uniform size and placed on aluminium foiled-covered metal racks. Each metal rack was placed in a coded oven bag and a temperature probe was inserted into the centre of the meat sample. The temperature changes were monitored with the thermocouples connected to hand-held digital recorders. Samples were oven-roasted at 160°C in two conventional electric Defy 835 ovens connected to a computerised electronic temperature control system (Viljoen *et al.*, 2001) to an internal temperature of 68°C. After cooking, the meat was allowed to rest for 5 min, in which time an endpoint temperature of 72°C was reached.

Cubed samples (1.5 x 1.5 cm) were taken from the middle of each sample and wrapped individually in aluminium foil. The samples were placed in preheated glass ramekins, which were marked with random three-digit codes and positioned in a preheated oven of 100°C. The samples were evaluated within 10 min by the panel (AMSA, 1995).

Sensory analysis

An analytical sensory analysis panel consisting of eight members was used to analyse the sensory attributes of the selected muscles. The panellists were selected and trained according to the guidelines for the sensory analysis of meat of the American Meat Science Associations (AMSA, 1995) and the generic descriptive analysis technique (Lawless & Heymann, 1998). The panellists were trained in two sessions on the attributes to be

evaluated. The meat was analysed in 5 sessions over a period of 3 d, with four samples (muscles) from one carcass per session.

The meat was analysed using the standard questionnaire of the American Meat Science Association (AMSA, 1995). This is a numerical, eight-point structured scale from low in intensity (1) to extremely high in intensity (8). One attribute was added to the standard questionnaire, namely salty taste. The descriptive sensory analysis performed on the meat included beef aroma, overall beef flavour, initial impression of juiciness, sustained juiciness, first bite, residue and salty taste. Table 1 depicts the definitions of the attributes used in the sensory analysis of the beef.

Table 1. Definitions of the sensory attributes used for descriptive sensory analysis of beef.

Attribute and Scale	Definition
Beef aroma 1=Extremely untypical; 8=Extremely typical	Characteristic aroma associated with the meat of the animal species
Overall beef flavour 1=Extremely untypical; 8=Extremely typical	Characteristic flavour associated with the meat of the animal species
Initial juiciness 1=Extremely dry; 8=Extremely juicy	Amount of fluid exuded on the cut surface when pressed between the thumb and forefinger
Sustained juiciness 1=Extremely dry; 8=Extremely juicy	Amount of water perceived during mastication
First bite 1=Extremely tough; 8=Extremely tender	Force needed to compress the meat sample between molar teeth on the first bite
Residue 1=Abundant; 8=None	Amount of connective tissue remaining after most of the sample has been masticated
Salty taste 1=No salty taste; 8=Extremely salty	Taste on the tongue associated with sodium ions

The panellists were seated in individual booths in a light-controlled and temperature-controlled room. Each judge received four samples from each carcass per session. Every sample was marked with a different, randomly chosen, three-digit code and the serving order of the samples was completely randomised for each session and each panellist. The panel used crackers, apple slices and distilled water to cleanse the palate in-between samples (AMSA, 1995).

Experimental design and statistical analysis

A randomised complete block design experiment was performed with five treatments (muscle) and six block (animals/carcasses) replications. The means of the variables purge loss, pH, colour, cooking loss, drip loss, shear force, proximate composition, minerals and fatty acids were calculated. The sensory characteristics were measured by using a 9-point hedonic scale and the score data analysed as such.

Analysis of variance was performed on the data using SAS statistical software Version 8.2 (SAS, 2003). The Shapiro-Wilk test was performed to test for non-normality (Shapiro & Wilk, 1965). In some cases deviations from normality were the cause of one or two outliers, which were removed before the final analysis. Where there was still significant evidence of non-normality, this could be ascribed to kurtosis rather than skewness. Interpretation of results was thus continued (Glass *et al.*, 1972). Student's t-Least Significant Difference (LSD) was calculated at the 5% confidence level to compare treatment means (Ott, 1998). The relationships between variables were assessed by a Pearson simple correlation.

RESULTS AND DISCUSSION

Physical properties

The mean values for the physical meat quality parameters of the *Biceps femoris* (BF), *Longissimus lumborum* (LL), *Rectus femoris* (RF), *Supraspinatus* (SS) and *Semitendinosus* (ST) are illustrated in Table 2. Most of the attributes tested showed significant differences ($P \leq 0.05$) between the selected muscles.

pH findings

It is well known that the ultimate pH of the muscle is an important contributing factor to the quality of meat (Greaser, 2001). Several authors have investigated the use of pH to predict meat quality (Bruce *et al*., 2001) and studies indicated an acceptable pH range between 5.3 to 5.7 which could be used to guarantee eating quality (Anon., 2004). According to Thompson (2002), the eating quality of beef improves as the pH declines from the threshold of 5.7 (*ca*. 1 palatability unit).

In the present investigation the pH measured at 72 h post-mortem differed significantly ($P \leq 0.05$) between the muscles (Table 2) and ranged from 5.40 ± 0.026 (LL) to 5.51 ± 0.026 (SS). The pH readings of the muscles were still all within the normal pH range of 5.4-5.5, which was typical of the ultimate pH (pHu) of aged beef muscle (Lawrie,

1998a). Similar results in muscle pH differences are reported by other studies (Vestergaard *et al*., 2000a; Hoffman, 2006). Variation in pH exists between muscles (Ramsbottom & Strandine, 1948) and can be the result of muscle location causing muscles to have different rates of temperature declines and thus different rates of pH declines (Newbold & Harris, 1972).

The significant differences in pH subsequently caused differences between the water-holding capacity (WHC) of the meat (Honikel, 1987; Baublits *et al*., 2005), illustrated through the purge loss, drip loss and cooking loss (Briskey *et al*., 1960; Crouse *et al*., 1984). Fluid-retaining characteristics is one of the eating-quality attributes consumers use to evaluate beef during the purchase decision (Andersen *et al*., 2004) and the drip formed does not appeal to the consumer and represents a portion of the product that cannot be used (Payne *et al*., 1998). The differences in WHC may be attributed to protein denaturation and the effect of the post-mortem pH/temperature ratio (Guignot *et al*., 1994). In other words, as the pH approaches the isoelectric point, the protein net charge decreases and consequently reduces the water binding (Wierbicki *et al*., 1963). Therefore, with the alteration of the muscle cell structure and lowering of the pH, immobilised water is exuded as purge (Offer & Knight, 1988b).

Numerous studies, such as those of Honikel (1987) and Offer and Trinick (1983) on beef, have found that an increase in pH causes an increase in the WHC of the meat. The higher the final pH, the stronger the WHC of the meat and reduced moisture loss will occur, because of fluid being tightly bound within the muscle structure resulting in limited exudate (Swatland, 1995; Warriss, 2000). Contradicting the above findings, low ultimate pH is associated with low WHC development and unacceptably high purge loss (Huff-Lonergan & Lonergan, 2005). In the present study there was a tendency towards an inverse relation between pH and the fluid loss (drip loss and purge loss) of the muscles investigated (Table 2). However, the correlation was not significant (P>0.05).

The high pH in SS (5.51 ± 0.026) was supported by the negative relationship with the fluid loss in terms of the drip loss and purge loss percentage. The drip loss percentage was the lowest in the SS muscle (0.72 ± 0.116%) and SS also had a low purge loss (2.16 ± 0.572%). A similar association between the fluid loss and pH was observed in the LL and RF muscles. Both LL (5.40 ± 0.026) and RF (5.42 ± 0.034) had low pH values and RF had the highest purge loss (3.76 ± 0.964%) and drip loss (1.39 ± 0.290%), whereas LL was high in purge loss (2.90 ± 0.781%) and cooking loss (40.11 ± 1.138%). Therefore, a decrease in pH caused more fluid to be exudated (Hoffman *et al*., 2005).

One of the most important quality characteristics of fresh meat is the ability of the raw product to retain moisture (Huff-Lonergan & Lonergan, 2005). From the percentage fluid exudated by the various muscles (Table 2), the average loss was within reported fluid-loss results. Offer and Knight (1988a) suggested that fresh retail cuts could lose on average 1-3% weight due to purge. The loss of fluid not only results in reduction of saleable weight, but also of valuable proteins. A significant amount of fluid is exuded with the purge (Offer & Knight, 1988a, b). Hoffman (2006) found similar drip loss percentages for the LL and ST muscles, whereas Schönfeldt *et al*. (1997a) reported on primal cuts and indicated significantly higher drip losses for the various muscles.

With cooking loss, Boakye and Mittal (1993) reported a positive correlation between pH and cooking loss. However, this relation was not significant in the present study ($P>0.05$). The cooking losses (Table 2) were within the range of some of the studies reported for beef (Schönfeldt *et al*., 1997a; Hoffman, 2006), however, other reports indicated much lower cooking losses for the selected muscles (Jeremiah *et al*., 2003b; Rhee *et al*., 2004). The muscles used in the present study, showed higher cooking losses, ranging from 34.22 ± 0.769 % (BF) to 40.45 ± 1.087 % (SS) than those reported by Jeremiah *et al*. (2003b), who reported cooking losses ranging from 21.51% to 33.26%.

It is important to remember that water-holding values are extremely method dependent (Hamm, 1986; Boles & Shand, 2001). Therefore, the values obtained with a specific method may not correlate well with values obtained by other methods (Kauffman *et al*., 1986). This aspect makes comparing data with reported findings difficult. When comparing the total cooking losses of muscles from different research studies, it should be recognised that cooking losses are closely associated with the cooking methods applied. It is common for the cooking methods employed to differ greatly among research studies (Jeremiah *et al*., 2003b).

The ranking of the various muscles according to drip and cooking loss (Table 2) revealed that there was a tendency for the drip loss to be inversely related to cooking loss. Honikel (1986) explained that drip loss involves other compartments of bound water than cooking loss and therefore a correlation does not exist between these two properties. In the present study the cooking loss and drip loss variation was significant between muscles ($P\leq0.05$). However, the correlation between cooking loss and drip loss for the present study was not significant ($P>0.05$).

Instrumental tenderness

Meat tenderness (Dikeman, 1987; Miller *et al*., 1995; Vestergaard *et al*., 2000b) is the single most important factor in consumer acceptance (Taylor *et al*., 1995). Complaints by consumers regarding tough meat and tenderness variation are a primary concern for the meat industry (Pearson, 1994; Sørheim & Hildrum, 2002). The variable process of post-mortem tenderisation is dependent on several biological factors, such as age, fibre (muscle) type and technological (external) factors (Dransfield *et al*., 1980-1981).

Tenderness variation occurs at slaughter or develops during post-mortem storage or a combination of both. Other contributing factors that explain the variation in tenderness of aged meat is sarcomere length, connective tissue content and proteolysis of myofibrillar proteins. These factors are extremely muscle dependent. For example, the major determinant of *Longissimus* tenderness is proteolysis and in *Biceps femoris* it is the connective tissue content (Koohmaraie *et al*., 2002). The meat preparation by the consumer also plays a key role in the source of tenderness variation (Jiang, 1998).

The shear force values of the muscles (Table 2) were generally higher than those reported by other workers (Belew *et al*., 2003; Schönfeldt *et al*., 1997a; Hoffman, 2006). The muscles also differed significantly ($P \leq 0.05$) in their Warner-Bratzler shear force (WBSF) resistance values (Table 2). The BF (46.26 ± 5.536N) had the lowest shear force, followed by RF, SS, LL and ST (86.23 ± 11.88N) with the highest shear force and hence the lowest instrumental tenderness. The high shear force value of ST is supported by its content of connective tissue (Sørheim & Hildrum, 2002). Reports showed that ST contains heavy deposits of elastin fibres, which contributes to meat toughness (Bendall, 1967; Lawrie 1998a).

The results from this investigation are not in agreement with other findings in the literature (McKeith *et al*., 1985; Belew *et al*., 2003). Some reports indicated that shear force values were lowest in the *Longissimus* muscle (rib eye (*Longissimus thoracis* - LT) and striploin (LL) (Kinsman, 1961) and that the rib and loin muscles were more tender that the muscle from the chuck (SS) and round (BF, ST, RF) (McKeith *et al*., 1985). McKeith *et al.* (1985) found the *Longissimus lumborum* (loin muscle) followed by *Semitendinosus* and *Rectus femoris*, in that order, to be the most tender muscles. The *Biceps femoris* followed by *Supraspinatus* were among the toughest muscles. Other studies also found the BF muscle to have the highest WBSF values (Brooks *et al*., 2000; Rhee *et al*., 2004). However, Lawrence *et al*. (2003) found no differences in shear force values for *Longissimus* and *Semitendinosus* steaks. This supports the present data, where these two muscles (LL and ST) were not significantly different in WBSF values ($P > 0.05$).

Koohmaraie (1988) supported the present result of a tougher LL compared to BF. The LL was the toughest muscle 1 d after slaughter and the BF had an intermediate toughness after 1 d. After 14 d all the muscles had similar shear force values. In the latter study the difference in tenderness after 1 d of ageing could not be explained by collagen content. Although BF had a higher collagen content than LL, BF indicated a lower shear force value than LL after 1 d of ageing and could be considered to be more tender than LL. The most plausible explanation for the latter could be sarcomere length and fibre size. The BF has an intermediate sarcomere length and fibre size, whereas LL has the shortest sarcomere length and the largest fibre size. A shorter sarcomere length and larger fibre size suggest a tougher muscle, which was the case with the Koohmaraie (1988) report. The same situation was found in the present study (Table 2), where LL was significantly tougher than the usually tough BF. According to Boles and Shand (2001), WBSF values for beef muscles 3 d post-mortem were 65.5 N for BF muscle, followed by RF, with a value of 74.3 N and then ST, with the highest value of 86.3 N. Rhee *et al*. (2004) also found BF to be more tender (lower WBSF value) than LL, ST and SS, where BF and RF were rated as the more tender muscles. Schönfeldt *et al*. (1997a) reported ST to be the toughest muscle, a result supporting the present investigation (Table 2), where ST showed the highest WBSF value.

A study done by Frylink and Heinze (2003) on the tenderness of indigenous South African and other beef breeds supports the high WBSF value for LL in the present investigation. The tenderness of LL from the Brahman, Hereford and Simmental breeds was 143 N, 113 N and 157 N, respectively. These WBSF values for LL after 3 d of ageing are much higher than those of reported European studies (McKeith *et al*., 1985) on LL tenderness. A possible explanation is that the *M. longissimus* of European breeds such as the *Bos Taurus* tends to be more tender than that of typical South African breeds as reported by Frylink and Heinze (2003).

Both instrumental and sensory tenderness of meat are significantly influenced by the ultimate pH (Dutson, 1983) and the variation in ultimate pH is a possible source of tenderness variation (Anon., 2005b). The relationship between pH and tenderness, however, is controversial and has been reported in many research studies. Some reports indicated a linear relationship between these two parameters (Bouton *et al.*, 1972; Guinot *et al.*, 1994). Devine *et al.* (1993), for example, found an increase in shear force (SF) with higher pH values in the range of 5.4 to 6.0, whereas other authors (Jeremiah *et al.*, 1991; Purchas, 1990; Purchas & Aungsupakorn, 1993; Young *et al.*, 1993) found a curvilinear relationship, with minimum tenderness between pH values of 5.8 and 6.3. This curvilinear

relationship could be explained by differential proteolysis activity, where meat is tenderised by the activity of neutral proteases, i.e. calpains at an ultimate pH of 6.3 and more. In the case of a low pH of below 5.8, the meat is tenderised by the enhanced cathepsins activity. With a pH of between 5.8 and 6.3, i.e. an intermediate pH, the lowest degree of tenderisation during ageing occurs (Yu & Lee, 1986). Some others also suggested non-enzymatic tenderisation by the direct effect of calcium on the myofibrillar proteins (Takahashi, 1996). The influences of sarcomere length and cooking temperature on the relationship between ultimate pH and tenderness have also beef investigated (Purchas, 1990). For example, when meat is cooked at 70°C the main determinant of tenderness is the myofibrillar component, especially in young animals (Bouton *et al*., 1981). Some research also indicated no relationship between pH and shear force values (Safari *et al.*, 2001). In the present study there was no significant association ($P>0.05$) between the pH, measured 72 post-mortem, and instrumental tenderness.

Cooking loss also correlates significantly with tenderness (Silva *et al*., 1999). Bouton *et al*. (1973) suggested that a higher WHC would contribute to a higher tenderness score and lower shear force value. In the present investigation the cooking loss had a highly significant association (r=0.680, $P<.0001$) with the shear force of the meat samples.

The most apparent difference between the present investigation and reported studies, pertaining to the instrumental tenderness, is the ageing time allowed. Most studies employ approximately 6 to 14 d post-mortem storage at 4°C (Schönfeldt *et al*., 1997a; Rhee *et al*., 2004; Hoffman, 2006). According several studies (O'Halloran *et al*., 1997; French *et al*., 2000; Chambaz *et al*., 2002; Koohmaraie *et al*., 2003), beef should be aged for 14 d before sale to prevent inconsistency of tenderness at the consumer level. For the present study instrumental tenderness was measured 4 d post-mortem, thus the ageing period was limited. This resulted in relatively high shear force values and therefore a degree of toughness.

The cause of the significant differences in shear force is not clear. Additional more detailed research is required to determine if the variation is due to collagen content or sarcomere length. Other techniques such as determining the myofibrillar fragmentation index (MFI) can also be applied to predict tenderness in the cooked product by using raw muscle for the analysis (Olson & Parrish, 1977; Davis *et al*., 1979; Calkins & Davis, 1980; Calkins *et al*., 1980). The MFI is positively related to instrumental as well as to sensory tenderness and proved to be a suitable indicator of tenderness (Vestergaard *et al*., 2000b; Chambaz *et al*., 2002).

Table 2. Means (±SD)[#] for physical attributes of selected beef muscles.

Attribute	Muscle					LSD (*P*=0.05)
	***Biceps femoris* (BF)**	***Longissimus lumborum* (LL)**	***Rectus femoris* (RF)**	***Supraspinatus* (SS)**	***Semitendinosus* (ST)**	
$pH_{72\ h}$	5.45 ± 0.039^{b}	5.40 ± 0.026^{c}	5.42 ± 0.034^{bc}	5.51 ± 0.026^{a}	5.45 ± 0.030^{b}	0.034
Purge loss (%)	1.53 ± 0.487^{c}	2.90 ± 0.781^{b}	3.76 ± 0.964^{a}	2.16 ± 0.572^{bc}	2.67 ± 1.100^{b}	0.797
Drip loss (%)	1.25 ± 0.676^{a}	1.19 ± 0.171^{ab}	1.39 ± 0.290^{a}	0.72 ± 0.116^{b}	0.98 ± 0.507^{ab}	0.500
Cooking loss (%)	34.22 ± 0.796^{c}	40.11 ± 1.138^{a}	37.32 ± 2.274^{b}	40.45 ± 1.087^{a}	39.02 ± 1.330^{ab}	1.746
WBSF (N)	46.26 ± 5.536^{d}	79.04 ± 16.673^{ab}	58.68 ± 11.825^{dc}	69.71 ± 8.950^{bc}	86.23 ± 11.880^{a}	13.25
Raw L*	39.29 ± 1.758^{c}	38.72 ± 1.417^{c}	49.74 ± 4.205^{a}	39.99 ± 0.989^{c}	44.06 ± 3.166^{b}	2.692
Raw a*	15.33 ± 1.470^{a}	13.82 ± 1.048^{bc}	13.19 ± 1.131^{c}	14.76 ± 0.556^{ab}	15.06 ± 1.434^{ab}	1.349
Raw b*	13.57 ± 1.585^{b}	12.32 ± 0.900^{b}	15.43 ± 1.392^{a}	12.88 ± 0.811^{b}	15.25 ± 1.107^{a}	1.352
Raw Chroma	20.50 ± 2.053^{ab}	18.57 ± 1.103^{c}	20.33 ± 1.502^{ab}	19.65 ± 0.793^{bc}	21.46 ± 1.612^{a}	1.728
Raw Hue_{ab}	41.46 ± 1.940^{c}	41.62 ± 2.409^{c}	49.44 ± 2.767^{a}	40.97 ± 1.895^{c}	45.46 ± 2.172^{b}	2.291
Cooked L*	38.88 ± 2.800^{c}	49.39 ± 3.395^{a}	50.52 ± 2.709^{a}	39.87 ± 2.658^{bc}	42.42 ± 3.653^{b}	3.514
Cooked a*	5.74 ± 0.896^{b}	5.33 ± 1.296^{b}	4.15 ± 0.531^{c}	6.90 ± 0.754^{a}	7.06 ± 0.764^{a}	1.035
Cooked b*	13.11 ± 0.785^{d}	17.56 ± 1.235^{a}	16.47 ± 0.505^{b}	15.30 ± 0.525^{c}	15.67 ± 0.773^{bc}	0.914
Cooked Chroma	14.34 ± 0.629^{c}	18.42 ± 1.382^{a}	17.01 ± 0.536^{b}	16.82 ± 0.639^{b}	17.23 ± 0.504^{b}	0.966
Cooked Hue_{ab}	66.30 ± 3.998^{b}	73.31 ± 3.482^{a}	75.77 ± 1.727^{a}	65.69 ± 2.121^{b}	65.72 ± 3.246^{b}	3.323

[#] SD: Standard deviation.

[a, b, c, d] Means in the same row with different superscripts are significantly different (*P*≤0.05).

LSD: Least significant difference (*P*=0.05).

WBSF: Warner-Bratzler Shear Force.

Instrumental meat colour

Colour is almost certainly the single most important appearance factor used for evaluating meat quality (Andersen *et al*., 2004) and ultimately determines the purchase of a cut of meat (MacKinney *et al*., 1966; Kropf, 1980). Despite the strong effect that the visual appearance of meat has on retail sales, the visual properties of meat are still not fully understood (Swatland, 2004). Research has shown that meat colour is a poor guide to eating quality, however, most consumers still make purchase decisions based on colour display (Young *et al*., 1999). Carpenter *et al*. (2001) noted a strong association between colour preference and purchase intent, with consumers discriminating against purple or brown meat. Consumers prefer a bright red meat colour when buying fresh meat and perceive the red colour as an indication of freshness, wholesomeness and eating quality (Faustman *et al*., 1989; Mancini & Hunt, 2005). If the surface of the fresh beef product has reached 30-40% brown metmyoglobin (Metmb) of the total pigments on the surface, the consumer is likely to make a no-purchase decision (Greene *et al*., 1971).

The colour of red meat is influenced by various factors such as muscle pigment, pH, pre and post-slaughter handling and cut variability (Brody, 2000). Muscle type accounts for approximately half of the variances in colour stability and is an important controlling factor in the discolouration rate of beef muscle, when exposed to oxygen (Hood, 1980; Varnam & Sutherland, 1995).

The colour-related parameters of the raw and cooked muscles showed significant differences ($P \leq 0.05$) between all the muscles for the L*, a*, b* and chroma values and the hue angle (Table 2). Greater a* values indicate a more red beef sample, whereas if a* and b* values were more similar, then a more purple meat colour is described. Greater b* values indicate a greater contribution of yellow and a visual description of brown is reported (Carpenter *et al*., 2001).

Torrescano *et al*. (2003) report similar results for the colour values of the raw meat. In Table 2 the RF and ST were the muscles with the highest colour parameter values, and appeared pale and light pink. A lower a* value with a higher L* value reflects a paler meat colour (Raes *et al*., 2003). Livisay *et al*. (1996) report beef colour values of L* = 22.6; a* = 6.5 and b* = 2.7 for beef of a normal pH between 5.4 and 5.6. Kim and Lee (2003) report similar results as in the present investigation for both the L* and a* values, however, the b* values results obtained from the muscles used in the present study were much higher.

Studies on beef muscle colour indicated that the *Longissimus* muscle is a very colour stable muscle (Hood, 1980; Klont *et al*., 1998). Benito-Delgado *et al*. (1994) also found *Longissimus* colour values that concur with the present data regarding the L* and b*

values. However, the a* value of the LL in the present investigation (Table 2) was lower than that of Benito-Delgado *et al*. (1994), indicating a less red lean colour for the LL muscle from the present investigation. Vestergaard *et al*. (2000a) studied three muscles from animals (±360 kg) fed concentrates (ad libitum) and reported similar L* colour values for the ST, LL and SS muscles. The a* values in the Vestergaard study were higher and therefore more red than those of the present investigation. With regard to the b* values, Vestergaard *et al*. (2000a) reported lower b* and thus less yellow values. The colour hue angle in Table 2 had much higher values than Vestergaard *et al*. (2000a) found, indicating higher intensity in colour of the samples from the present investigation (Table 2). The chroma values, on the other hand (Table 2), did agree with the mentioned study.

Several studies demonstrated a relationship between ultimate muscle pH (Purchas, 1990; Watanabe *et al*., 1995) and muscle colour (Jeremiah *et al*., 1991; Wulf *et al*., 1997; Mancini & Hunt, 2005). When the ultimate pH is lower, the meat colour is brighter, suggesting that a high ultimate pH causes meat to appear darker (Swatland, 1984). The pH of meat showed an inverse association with the lightness (L*) of the meat (Vestergaard, *et al*., 2000a) and Swatland (2004) found that meat with a low pH is usually paler than meat with a high pH. For the present study this relation between final pH and L* values was not significant ($P>0.05$).

Swan and Boles (2002) reported a significant effect of pH on the Hunter L*, a* and b* values of raw beef. High pH had lower L*, a* and b* values, than pH within the normal range. The darker meat colour found with meat with a high pH results from a combination of effects, i.e. high pH meat has a more compact muscle structure, which limits the oxygen diffusion and light absorption (Swatland, 2004). In addition, when pH is increased, less myoglobin is denaturated and the a* values increase, indicating a more red sample (Brewer & Novakofski, 1999).

The fact that colour intensity (hue angle) is not only dependent upon ultimate pH, but also on the myoglobin concentration could be the reason for BF having the highest a* value and high colour saturation (chroma) (Kim *et al*., 2003).

The association of meat colour and beef toughness is still unclear, however, a relation with sarcomere and paleness has been researched. Short sarcomeres cause beef toughness and the shorter the sarcomeres the more light is scattered than in the long sarcomeres. Hence, the light scattering is negatively correlated with sarcomere length (Swatland, 2003). The conflict exists with the post-mortem glycolysis, where high scattering indicates rapid post-mortem glycolysis and thus tender meat. This contradicts the situation where high scattering indicates short sarcomeres and therefore tough meat.

This means that meat colour may or may not be useful as a guide to beef tenderness (Swatland, 2004). Cannell *et al*. (1997) also reported a relationship between tenderness and colour parameters. A relationship between L* values and shear force was shown, but no correlation was found between b* values and shear force. The correlation between the L* values and shear force in the present investigation was low (r=-0.081, *P*=0.672) as was that between the b* values and shear force (r=-0.098, *P*=0.608) and was therefore not significant (*P*>0.05).

When muscles are cooked, the colour lightens and turns a brown-grey hue. Several factors affect the outcome of cooked beef colour, namely anatomical location and inherent biochemical composition, fat content, endpoint temperature, post-cooking temperature rise (Berry & Bigner-George, 2000; Berry & Bigner-George, 2001; Killinger *et al.*, 2000; Suman *et al.*, 2004), meat pH and muscle type (Young & West, 2001). Studies showed that a higher pH protects myoglobin from heat denaturation, allowing the maintenance of red of pink colour during and after cooking (Hunt *et al.*, 1999). A significant correlation between the pH and a*value of the cooked meat was found (r=0.532 and *P*=0.0025).

Chemical composition

The mean values for the proximate chemical composition and the mineral content of the *Biceps femoris* (BF), *Longissimus lumborum* (LL), *Rectus femoris* (RF), *Supraspinatus* (SS) and *Semitendinosus* (ST) are presented in Table 3.

Proximate chemical composition

Meat forms an important part of a balanced and varied diet (Morris, 1991). Numerous studies have highlighted the nutritional advantages of meat in terms of proteins, minerals, vitamins and certain fatty acids (Enser, 2000; Schönfeldt *et al*, 1996; Van Heerden & Schönfeldt, 2003; Webb, 2003). The disadvantage of meat with respect to its role in human disease is the excessive intake of meat which is rich in saturated fat and dietary cholesterol (Webb, 2003). Locally, the consumer has also taken notice of the fatness of red meat and its effect on human health (Naudé, 1994). Therefore, a comprehensive study was executed on the nutritional properties of 15 primal meat cuts from South African beef carcasses from the A age group containing 13% (lean) and 20% (medium) carcass fat, respectively (Schönfeldt *et al*., 1996). The muscles used in the present study were also from the A age group with a lean fat cover, however, the fat content of the muscles from the present study (Table 3) showed much lower fat percentages for beef muscles

than the data reported by other studies such as Schönfeldt *et al*. (1996; 1997b). Hoffman (2006) had fat percentages of more similar value to the muscles investigated in the present study.

Beef muscles do have some similarities with reference to chemical properties (Jiang, 1998). Lean muscle is constituted of approximately 75% water, 20% protein, 5% lipids, 1% carbohydrates and 1% minerals and vitamins, analysed as ash (Lawrie, 1985; Huff-Lonergan & Lonergan, 2005). Although beef muscles are based on a similar composition, some reports have indicated muscle differences in protein, fat and moisture (Strandine *et al*., 1949; Swift & Bauman, 1959).

A significant difference ($P \leq 0.05$) between the muscles with regard to moisture, lipid and ash content was found (Table 3). Protein content was the only component that did not differ significantly between the muscles (Table 3). However, the moisture, protein and ash contents are within the range of previously reported data (McKeith *et al*., 1985; Schönfeldt & Welgemoed, 1996; Huff-Lonergan & Lonergan, 2005).

From Table 3 the results showed that SS had the highest moisture content, a result supported by McKeith *et al*. (1985) and Jeremiah *et al*. (2003b). In McKeith *et al*. (1985) the SS was followed by the ST, BF, RF and LL with the lowest moisture content. The moisture results found in Table 3 indicated a similar ranking, however, BF was the muscle with the least moisture.

The fat content of the muscles used in this investigation is much lower than the reported results of Schönfeldt and Welgemoed (1996). Numerous reports on beef muscle differ in their percentage fat (Scollan, 2003as a result of a series of variables such as breed, feed and sex (Webb, 2003). South African beef carcasses differ to a great extent from overseas counterparts because of the significant contrast in carcass weight. The South African beef carcasses weigh considerably less (*ca.* 220 kg) and thus contain significantly less total fat (*ca.*18%) compared to American beef carcasses (Webb, 2003). Jeremiah *et al*. (1970) reported that intramuscular fat affects all palatability traits and accounts for 12-14% of the variation. Huff-Lonergan and Lonergan (2005) reports a lipid content of approximately 5% and Wood *et al.* (2003) also conclude that the lipid content of edible meat is approximately 2.5%. The average lipid content in edible meat from South African beef is approximately 2.5% (Webb, 2003). The fat percentage found in Table 3 showed BF had the highest fat percentage, followed by SS, RF, LL and ST. McKeith *et al*. (1985) found dissimilar results, with LL having the highest fat content, followed by RF, BF, ST and SS. Jeremiah *et al*. (2003b) also shows dissimilar results.

Numerous studies have documented an inverse relation between the moisture and fat content (McKeith *et al*., 1985; Savell *et al*., 1986; Park *et al*., 2000; Kim & Lee, 2003). The strong inverse and significant relationship between moisture and fat content (r=-0.475, *P*=0.0079) is illustrated in Table 3 by BF, which has the highest fat content and the lowest moisture content.

Furthermore, researchers are in disagreement regarding instrumental tenderness and the influence of marbling (the percentage fat in the muscle) on this characteristic. It is acknowledged that the palatability of meat is influenced by intramuscular fat (IMF) content, however, the specific relationship still seems unclear. Some studies have reported a positive relationship between tenderness and fat percentage, while others have found no evidence of any association (Fiems *et al*., 2000; Renard *et al*., 2001). Carpenter and King (1965) found a significant correlation between chops with variations in marbling scores and tenderness, while Parrish (1974) found no relationship between tenderness and percentage fat. Silva *et al*. (1999) did not find any relation between the intramuscular fat (1.9%) and the instrumental tenderness (r=0.06) and sensory tenderness (r=0.03). Furthermore, most studies suggested a slightly negative relation between shear force values and intramuscular fat content (Seideman, *et al*., 1987; Fiems *et al*., 2000; Park *et al*., 2000). In this investigation a strong and significant inverse relation was found for WBSF and fat content (r=-0.593, *P*=0.0005). The muscle with the highest fat content had the lowest shear force value and was therefore the most tender muscle among the selected muscles. *Biceps femoris*, for example, had the highest percentage of fat (3.04 ± 0.971%), and the lowest shear force value (46.26 ± 5.536 N), with ST having the lowest fat content (2.10 ± 0.435%) and the highest shear force value (86.23 ± 11.880 N). The *Longissimus lumborum* muscle showed a similar relationship with a lower fat content and higher shear force resistance.

Some researchers indicated a relation between cooking loss and fat content. Ozawo *et al*. (2000) report a significantly lower cooking loss with higher intramuscular fat content samples. Jeremiah *et al*. (2003b) also report a negative and significant association between muscle fat content and cooking loss. The fat percentage and cooking loss association in this investigation is inverse (r=-0.350, *P*=0.0582).

Mineral composition

Meat is a primary component and forms an important part of a balanced varied diet. Meat contributes a considerable proportion of the minerals required by the diet (Van Heerden & Schönfeldt, 2003) and the concentration of iron (Fe), zinc (Zn) and copper (Cu) in meat is

higher than the minerals provided by the rest of the diet as a whole (Williams, 1987). The results of this investigation indicated significant ($P \leq 0.05$) statistical differences in the mineral composition (Table 3) between muscles and accordingly some muscles can be considered as making a greater nutritional contribution to human dietary mineral requirements than others. Several other studies also indicated difference in mineral content between various muscles (Schönfeldt & Welgemoed, 1996; Hoffman, 2006).

The minerals phosphorus (P), potassium (K), calcium (Ca) and iron (Fe) in meat have received considerable attention (AMIF, 1960). Of these, P is quantitatively the most important, followed by K, Ca and Fe. It is well known that meat is an excellent food source of Fe and Zn, especially considering the higher bioavailability of the two minerals compared to that from plants (Lin *et al.*, 1989). Approximately 40% of the Fe in meat is haem iron and this form of iron is more available to humans that non-haem iron (Simonsen *et al.*, 1988).

Results from this investigation (Table 3) indicate significant differences ($P \leq 0.05$) between the beef muscle in phosphorus (P), potassium (K), sodium (Na), iron (Fe), zinc (Zn) and manganese (Mn). Phosphorus (P), K, Mg and Na, in that order, are the main contributors to the mineral content of the selected muscles. According to Schönfeldt and Welgemoed (1996), beef has a significant amount of P, K and Mg present, a result also depicted in Table 3. Beef is an excellent source of highly bioavailable Fe, Zn and Cu (Sanders; 1998). The high iron content of beef is highly beneficial, since the function of iron results from its ability to participate in oxidation and reduction reactions, hence forming an integral part of the human diet (Mahan & Escott-Stump, 2000).

Warriss (2000) also found the important minerals in beef to be P, K, Mg, Fe and Zn. Schönfeldt and Welgemoed (1996) found similar quantities in their results for the mineral composition of beef, except for K; Na and Cu were present in higher quantities. In the present investigation LL had the highest K (169.91 ± 5.086 mg/100 g meat), Ca (7.17 ± 0.164 mg/100 g meat), Cu (0.020 ± 0.0110 mg/100 g meat) and Mn (0.033± 0.0082 mg/100 g meat) content and significantly differed in these minerals from the other muscles, except for Ca and Cu. RF had the highest P content (196.30 ± 8.138 mg/100 g meat), which was significantly higher ($P \leq 0.05$) than that found in the other muscles, except for LL. SS had the highest quantities of the highly bioavailable minerals Fe (2.95 ± 0.350 mg/100 g meat) and Zn (6.03 ± 0.638 mg/100 g meat). SS also had the highest Na content and differed significantly in all these minerals from the other muscles. ST had only one mineral with the highest content, namely Mg (22.80 ± 0.845 mg/100 g meat). The general tendency was that LL had the higher mineral content and ST the lowest.

According to Lin *et al.* (1989), significant variation exists in the mineral content of retail cuts within a single carcass. The latter is the result of variation in muscle fibre type and physical activity between muscles (Kotula & Lusby, 1982). Substantial variability in the mineral content of meat is found in research studies because of the effects of age, feeding regimen, breed and geographical differences.

Fatty acid composition

Many studies established the increasing concern of the consumer regarding the nutritional quality and healthiness of the food they consume. One of the issues causing the most concern is the percentage fat consumed, the lipid composition of food, especially that of animal products (Hoffman *et al*., 2005) and the development of life-threatening diseases (Pike, 1999).

The quality of beef is determined by the fatty acid composition. Ways to manipulate the fatty acid composition of meat have become an increasingly popular research topic in recent years (Wood *et al*., 2003). The reason for this is the major role that meat play as part of our daily diet and the fat it contributes, especially the fatty acids (Wood *et al*., 2003). Saturated fatty acids (SFA) have been implicated in diseases associated with modern life, such as hypertension, coronary heart disease (Ulbricht & Southgate, 1991), various cancers and diabetes (Ashes *et al*., 2000). The South African Heart Foundation (2003) currently recommends that fat intake should not exceed 30% of the daily (total) energy intake, with 10% energy intake from saturated fatty acids. The unsaturated fatty acids (MUFA and PUFA), and in particular the omega-3 polyunsaturated fatty acid, are known to be advantageous for human health, and thus an increase in intake is recommended (Scollan, 2003; Raes *et al*., 2004). Therefore, the goal is to decrease the SFA content in food and/or the enrichment of food with certain fatty acids (n-3 fatty acid) (Hargis & Van Elswyk, 1993).

Fatty acid composition can be described by two nutritional indices, namely the PUFA: SFA (P:S) ratio and the *n*-6:*n*-3 ratio. For a healthy diet the United Kingdom recommends a minimal figure of 0.45 for the ratio of polyunsaturated (PUFA) to saturated acids (SFA) (P:S) (Department of Health, 1994). Values higher than 0.45 are considered optimum and healthier (Enser *et al.*, 1996). Typically, the P:S ratio for beef is around 0.1, which is not considered beneficial for human health (Enser *et al.*, 1998). With the other index, the ratio of the *n*-6:*n*-3 fatty acids, values higher than 4 are considered unhealthy.

Table 3. Means (±SD)[#] for proximate and mineral composition of selected beef muscles.

Chemical component	Muscle					LSD (*P*=0.05)
	***Biceps femoris* (BF)**	***Longissimus lumborum* (LL)**	***Rectus femoris* (RF)**	***Supraspinatus* (SS)**	***Semitendinosus* (ST)**	
Moisture (%)	73.17 ± 1.548[b]	73.84 ± 0.685[b]	74.18 ± 0.747[ab]	75.22 ± 0.931[a]	75.10 ± 0.936[a]	1.205
Protein (%)	20.21 ± 1.203[a]	19.78 ± 2.538[a]	20.28 ± 0.955[a]	20.28 ± 0.942[a]	20.71 ± 0.800[a]	NS
Lipid (%)	3.04 ± 0.971[a]	2.28 ± 0.335[bc]	2.54 ± 0.523[abc]	2.95 ± 0.617[ab]	2.10 ± 0.435[c]	0.700
Ash (%)	1.13 ± 0.081[b]	1.25 ± 0.098[a]	1.15 ± 0.020[ab]	1.10 ± 0.144[b]	1.14 ± 0.094[b]	0.102
Phosphorus (mg/100 g)	180.00 ± 9.928[b]	189.84 ± 7.922[a]	196.30 ± 8.138[a]	154.65 ± 9.573[d]	164.09 ± 5.198[c]	7.549
Potassium (mg/100 g)	166.77 ± 16.278[a]	169.91 ± 5.086[a]	163.63 ± 3.209[a]	145.50 ± 8.043[b]	161.49 ± 7.329[a]	10.36
Calcium (mg/100 g)	6.35 ± 1.301[a]	7.17 ± 0.164[a]	6.98 ± 0.199[a]	6.89 ± 0.622[a]	6.46 ± 0.984[a]	NS
Magnesium (mg/100 g)	21.78 ± 0.795[a]	21.88 ± 1.706[a]	22.49 ± 1.126[a]	21.85 ± 1.885[a]	22.80 ± 0.845[a]	NS
Sodium (mg/100 g)	12.05 ± 1.442[ab]	11.49 ± 0.569[bc]	11.15 ± 0.454[bc]	12.59 ± 0.974[a]	10.91 ± 0.316[c]	0.937
Iron (mg/100 g)	2.73 ± 0.608[a]	2.43 ± 0.740[ab]	1.91 ± 0.390[b]	2.95 ± 0.350[a]	2.11 ± 0.326[b]	0.592
Copper (mg/100 g)	0.018 ± 0.0075[a]	0.020 ± 0.0110[a]	0.022 ± 0.0040[a]	0.013 ± 0.0082[a]	0.017 ± 0.0052[a]	NS
Zinc (mg/100 g)	3.67 ± 0.409[cd]	4.05 ± 0.627[c]	4.63 ± 0.487[b]	6.03 ± 0.638[a]	3.54 ± 0.637[d]	0.469
Manganese (mg/100 g)	0.028 ± 0.0041[a]	0.033 ± 0.0082[a]	0.028 ± 0.0075[a]	0.015 ± 0.0055[b]	0.010 ± 0.0000[b]	0.007

[#] SD: Standard deviation.

[a, b, c, d] Means in the same row with different superscripts are significantly different (*P*≤0.05).

LSD: Least significant difference (*P*=0.05).

NS: Not Significant (*P*>0.05).

The *n*-6:*n*-3 ratio is usually expressed as the ratio of essential fatty acids C18:2n-6 (linoleic acid) : C18:3n-3 (linolenic acid). In beef the *n*-6:*n*-3 ratio is beneficially low and is normally less than three (Enser *et al.*, 1996; Scollan, 2003). In recent reviews (Connor, 2000; Williams, 2000) the recommended values for the mentioned ratios have been modified, with the recommended P:S ratio being higher than 0.7 (>0.7) and the *n*-6:*n*-3 ratio less than 5 (<5). The latter information is the driving force behind the development of different strategies to improve the fatty acid profile of meat and thereby supplying a healthier product (Scollan, 2003). Several studies have been published on how to manipulate the fatty acid composition of meat to achieve higher ratios of PUFA to SFA fatty acids and a more favourable balance between the n-6 and n-3 PUFA (Wood *et al.,* 2003; Raes, *et al*., 2004).

Fatty acids also influence specific meat-quality characteristics such as lipid and pigment oxidation, flavour and fat tissue firmness (Scollan, 2003; Wood *et al*., 2003). With an increasing PUFA level in the muscle, the oxidation reaction of the red myoglobin to brown metmyoglobin is accelerated (Renerre, 2000; Scollan, 2003; Wood *et al*., 2003). Tenderness and juiciness are not influenced by individual fatty acids, but by the total amount of fatty acids (Wood *et al*., 2003). The flavour effect of fatty acids is caused by the volatile, odourous products produced as a result of lipid oxidation during cooking and the reaction of these products with the Maillard reaction products to form other volatiles, which contribute to odour and flavour (Wood *et al*., 2003).

The intramuscular fat of beef typically consists of SFA (±47%), MUFA (±42%), and PUFA (±4%). The SFA consists of approximately 30% stearic acid (C18:0). The PUFA in beef contains significant amounts of *n*-3 PUFA, in particular α-linoleic (C18:3*n*-3) and eicosapentaenoic (C20:5*n*-3; EPA) docosahexaenoic (C22:6*n*-3; DHA) acids (Scollan, 2003). According to many reviews on fatty acid composition, the most important long-chain fatty acids are C18:3*n*-3, C20:5*n*-3, C22:5*n*-3, C22:6*n*-3, C18:2*n*-6, C29:4*n*-6 and C22:4*n*-6 and these will be discussed in more detail when presenting the results from this investigation.

Results of the effect of beef muscle on the fatty acid composition are shown quantitatively (mg/100 g) in Table 4 and qualitatively (percentage of total fatty acids) in Table 5. According to Enser *et al.* (1998), it is useful to present the fatty acid composition in mg per 100 g muscle, especially when calculating its nutritional value. Alternatively, the fatty acid composition can also be expressed as a percentage of the total identified fatty acids present. Although the percentage of fatty acids can be misleading, it still gives a fair indication of the distribution between the different fatty acids.

According to Table 5, the fatty acids with the highest concentration found within in all the muscles are oleic acid (C18:1*n*-9c) and palmitic acid (C16:0), followed by stearic acid (C18:0) and linoleic acid (C18:2*n*-6c). The SFA were approximately 50% in all the muscles, with C16:0 and C18:0 contributing the most, followed by a much lower C14:0. The MUFA were made up 40% of the total fatty acids, with C18:1*n*-9c followed by C16:1*n*-7, contributing substantially to the MUFA. With the PUFA (8-14%), C18:2*n*-6c followed by C20:3*n*-3 were the dominant fatty acids. The PUFA were the only FA that differed significantly in total among the muscles. An overview of fatty acid composition in beef is given by Raes *et al*. (2004) and the present investigation (Table 4 and 5) agrees with the reported data.

The results in Table 5 are also in accordance with beef loin data for the fatty acid content reported by Enser *et al.* (1996). Enser *et al*. (1998) also studied the effects of muscle (fibre type) on fatty acid profiles of beef. Results indicated that the differences in muscle fibre type between muscles is reflected in discrepancies in the fatty acid composition. A significantly greater proportion of phospholipids was found in the fat of red muscle compared to white fibre type muscles and therefore a higher percentage of polyunsaturated fatty acids (PUFA) (Raes *et al*., 2004)

According to Table 4, the total fatty acid content (mg/100 g meat) was the lowest for ST (11.33 ± 2.813) and the highest for BF (24.23 ± 8.523). The higher content of fatty acids of the BF muscle is caused by a significantly higher ($P \leq 0.05$) content of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), total unsaturated fatty acids (TUFA) and desirable fatty acids (DFA), compared to the other muscles. The significantly higher ($P \leq 0.05$) content of SFA (12.2 $\pm$ 4.954) can be attributed to the tendency towards a significantly higher ($P \leq 0.05$) content of palmitic acid (C16:0) and stearic acid (C18:0).

According to Table 5, the muscles differed significantly ($P \leq 0.05$) with regard to total saturated fatty acids (SFA). RF had the highest SFA (50%) content and ST the lowest (46%). The proportion of saturated fatty acids is a major risk factor for coronary heart disease (Santos-Silva *et al.*, 2002). However, stearic acid (C18:0), one of the dominant saturated fatty acid in the present study, is listed as a desirable fatty acid (DFA), with health-promoting advantages by lowering blood cholesterol.

The total percentage of monounsaturated fatty acids (MUFA) in the different muscles also differed significantly ($P \leq 0.05$). According to Table 5, oleic acid (C18:1*n*-9c) was the most abundant MUFA and BF contained the highest percentage of MUFA (37.7% $\pm$ 1.753).

Table 4. Means (±SD)[#] for fatty acid content (mg/100 g meat sample) of selected beef muscles.

Fatty acid	Muscle					LSD (*P*=0.05)
	***Biceps femoris* (BF)**	***Longissimus lumborum* (LL)**	***Rectus femoris* (RF)**	***Supraspinatus* (SS)**	***Semitendinosus* (ST)**	
Total FA	24.23 ± 8.523^{a}	17.34 ± 2.542^{ab}	20.48 ± 6.021^{a}	21.45 ± 6.818^{a}	11.33 ± 2.813^{b}	7.109
SFA						
C6:0	0.018 ± 0.0063^{b}	0.038 ± 0.0291^{a}	0.020 ± 0.0074^{b}	0.018 ± 0.0082^{b}	0.025 ± 0.0073^{ab}	0.016
C8:0	0.012 ± 0.0068^{ab}	0.004 ± 0.0056^{b}	0.009 ± 0.0047^{ab}	0.014 ± 0.0088^{a}	0.004 ± 0.0064^{b}	0.009
C10:0	0.014 ± 0.0158^{a}	0.008 ± 0.0071^{a}	0.012 ± 0.0117^{a}	0.007 ± 0.0061^{a}	0.004 ± 0.0062^{a}	NS
C12:0	0.022 ± 0.0113^{a}	0.016 ± 0.0092^{ab}	0.010 ± 0.0047^{b}	0.011 ± 0.0029^{b}	0.015 ± 0.0050^{ab}	0.009
C14:0	0.235 ± 0.1402^{ab}	0.344 ± 0.1633^{a}	0.320 ± 0.1962^{a}	0.288 ± 0.1800^{ab}	0.112 ± 0.0680^{b}	0.206
C16:0	6.54 ± 2.690^{a}	4.61 ± 1.031^{ab}	5.68 ± 1.929^{a}	5.19 ± 2.140^{a}	2.64 ± 0.873^{b}	2.249
C18:0	5.10 ± 2.075^{a}	3.33 ± 0.737^{ab}	4.31 ± 1.764^{a}	4.72 ± 1.725^{a}	2.34 ± 0.637^{b}	1.838
C20:0	0.052 ± 0.0125^{a}	0.032 ± 0.0073^{bc}	0.044 ± 0.0162^{ab}	0.044 ± 0.0098^{ab}	0.025 ± 0.0044^{c}	0.013
C22:0	0.023 ± 0.0035^{a}	0.012 ± 0.0094^{c}	0.017 ± 0.0015^{abc}	0.020 ± 0.0064^{ab}	0.013 ± 0.0075^{bc}	0.007
C24:0	0.077 ± 0.0280^{a}	0.042 ± 0.0073^{b}	0.070 ± 0.0215^{a}	0.083 ± 0.0391^{a}	0.079 ± 0.0177^{a}	0.027
MUFA						
C14:1	0.041 ± 0.0027^{a}	0.046 ± 0.0269^{a}	0.032 ± 0.0197^{a}	0.037 ± 0.0246^{a}	0.024 ± 0.0084^{a}	NS
C16:1*n*-7	0.616 ± 0.2670^{a}	0.428 ± 0.1097^{ab}	0.495 ± 0.1892^{a}	0.442 ± 0.1865^{a}	0.225 ± 0.0804^{b}	0.204
C18:1*n*-9t	0.151 ± 0.0975^{a}	0.053 ± 0.0101^{b}	0.068 ± 0.0190^{b}	0.082 ± 0.0388^{b}	0.038 ± 0.0113^{b}	0.057
C18:1*n*-9c	9.12 ± 3.178^{a}	6.45 ± 0.959^{bc}	7.16 ± 1.898^{ab}	8.03 ± 2.714^{ab}	4.12 ± 1.065^{c}	2.606
C20:1*n*-9	0.042 ± 0.0178^{a}	0.029 ± 0.0080^{bc}	0.034 ± 0.0104^{ab}	0.036 ± 0.0108^{ab}	0.019 ± 0.0059^{c}	0.010
C22:1*n*-9	0.012 ± 0.0070^{a}	0.008 ± 0.0063^{a}	0.010 ± 0.0053^{a}	0.013 ± 0.0069^{a}	0.012 ± 0.0031^{a}	NS
C24:1*n*-9	0.020 ± 0.0123^{a}	0.023 ± 0.0103^{a}	0.019 ± 0.0059^{a}	0.021 ± 0.0095^{a}	0.020 ± 0.0106^{a}	NS
PUFA						
C18:2*n*-6c	0.990 ± 0.477^{bc}	0.929 ± 0.169^{bc}	1.28 ± 0.502^{ab}	1.38 ± 0.238^{a}	0.869 ± 0.174^{c}	0.359
C18:2*n*-6t	0.054 ± 0.0252^{a}	0.035 ± 0.0062^{ab}	0.045 ± 0.0215^{a}	0.049 ± 0.0244^{a}	0.019 ± 0.0099^{b}	0.023

Table 4 (continue). Means (±SD)[#] for fatty acid content (mg/100 g meat sample) of selected beef muscles.

Fatty acid	Muscle					LSD (*P*=0.05)
	***Biceps femoris* (BF)**	***Longissimus lumborum* (LL)**	***Rectus femoris* (RF)**	***Supraspinatus* (SS)**	***Semitendinosus* (ST)**	
C18:3*n*-6	0.021 ± 0.0052^{a}	0.018 ± 0.0051^{a}	0.018 ± 0.0060^{a}	0.020 ± 0.0062^{a}	0.015 ± 0.0056^{a}	NS
C18:3*n*-3	0.062 ± 0.0271^{a}	0.037 ± 0.0155^{b}	0.077 ± 0.0284^{a}	0.073 ± 0.0343^{a}	0.045 ± 0.0205^{b}	0.020
C20:2*n*-6	0.016 ± 0.0029^{ab}	0.016 ± 0.0043^{b}	0.016 ± 0.0051^{ab}	0.021 ± 0.0079^{a}	0.016 ± 0.0029^{b}	0.005
C20:3*n*-6	0.086 ± 0.0210^{b}	0.073 ± 0.0111^{c}	0.082 ± 0.0164^{bc}	0.099 ± 0.0158^{a}	0.079 ± 0.0134^{bc}	0.010
C20:3*n*-3	0.287 ± 0.0604^{b}	0.207 ± 0.0342^{c}	0.262 ± 0.0576^{b}	0.341 ± 0.0554^{a}	0.271 ± 0.0267^{b}	0.050
C20:4*n*-6	0.022 ± 0.0119^{a}	0.020 ± 0.0044^{ab}	0.016 ± 0.0076^{ab}	0.014 ± 0.0039^{ab}	0.013 ± 0.0039^{b}	0.009
C20:5*n*-3	0.047 ± 0.0177^{a}	0.027 ± 0.0145^{b}	0.038 ± 0.0107^{ab}	0.031 ± 0.0155^{b}	0.033 ± 0.0083^{ab}	0.015
C22:2*n*-6	0.015 ± 0.0045^{b}	0.033 ± 0.0247^{a}	0.016 ± 0.0069^{b}	0.013 ± 0.0062^{b}	0.015 ± 0.0078^{b}	0.015
C22:5*n*-3	0.097 ± 0.0563^{a}	0.028 ± 0.0141^{b}	0.104 ± 0.0529^{a}	0.107 ± 0.0476^{a}	0.098 ± 0.0222^{a}	0.048
C22:6*n*-3	0.028 ± 0.0074^{a}	0.018 ± 0.0040^{b}	0.021 ± 0.0042^{ab}	0.018 ± 0.0093^{b}	0.018 ± 0.0049^{b}	0.008
SFA	12.2 ± 4.954^{a}	8.71 ± 1.646^{ab}	10.5 ± 3.826^{a}	10.4 ± 3.929^{a}	5.26 ± 1.566^{b}	4.211
MUFA	10.1 ± 3.700^{a}	7.04 ± 1.089^{bc}	7.82 ± 2.102^{ab}	8.66 ± 2.941^{ab}	4.46 ± 1.156^{c}	2.915
PUFA	1.73 ± 0.434^{bc}	1.44 ± 0.196^{c}	2.00 ± 0.590^{ab}	2.16 ± 0.233^{a}	1.49 ± 0.214^{c}	0.399
TUFA	11.8 ± 3.705^{a}	8.48 ± 1.168^{bc}	9.82 ± 2.458^{ab}	10.8 ± 2.917^{ab}	5.60 ± 1.259^{c}	3.002
DFA	16.9 ± 5.538^{a}	11.8 ± 1.618^{bc}	14.1 ± 3.863^{ab}	15.5 ± 4.502^{ab}	8.29 ± 1.862^{c}	4.632
P:S	0.17 ± 0.087^{b}	0.17 ± 0.041^{b}	0.20 ± 0.069^{b}	0.24 ± 0.118^{ab}	0.30 ± 0.075^{a}	0.093
n-6	1.19 ± 0.477^{bc}	1.09 ± 0.174^{c}	1.45 ± 0.504^{ab}	1.58 ± 0.226^{a}	1.01 ± 0.185^{c}	0.360
n-3	0.52 ± 0.118^{a}	0.32 ± 0.038^{b}	0.53 ± 0.120^{a}	0.55 ± 0.064^{a}	0.46 ± 0.056^{a}	0.099
n-6:*n*-3	2.44 ± 1.068^{bc}	3.45 ± 0.557^{a}	2.78 ± 0.690^{abc}	2.89 ± 0.551^{ab}	2.20 ± 0.379^{c}	0.671

[#] SD: Standard deviation.
[a, b, c] Means in the same row with different superscripts are significantly different (*P*≤0.05).
LSD: Least significant difference (*P*=0.05).
NS: Not Significant (*P*>0.05).
Abbreviations: SFA: Saturated Fatty Acids.
MUFA: Monounsaturated Fatty Acids.
PUFA: Polyunsaturated Fatty Acids.
TUFA: Total Unsaturated Fatty Acids.
DFA: Desirable Fatty Acids (C18:0 + TUFA).
P:S: Polyunsaturated:Saturated fatty acid ratio.
n-6 consists of C18:2, C18:3, C20:2, C20:4, C22:4.
n-3 consists of C18:3, C20:5, C22:5.

Table 5. Means (±SD)[#] of fatty acid composition (% of total fatty acids) of selected beef muscles.

Fatty acid	Muscle					LSD (*P*=0.05)
	***Biceps femoris* (BF)**	***Longissimus lumborum* (LL)**	***Rectus femoris* (RF)**	***Supraspinatus* (SS)**	***Semitendinosus* (ST)**	
SFA						
C6:0	0.082 ± 0.0299^{b}	0.834 ± 0.8505^{a}	0.110 ± 0.0612^{b}	0.106 ± 0.0819^{b}	0.2401 ± 0.1032^{b}	0.441
C8:0	0.056 ± 0.0366^{a}	0.020 ± 0.0280^{a}	0.044 ± 0.0250^{a}	0.077 ± 0.0686^{a}	0.037 ± 0.0665^{a}	NS
C10:0	0.074 ± 0.0937^{a}	0.046 ± 0.0423^{a}	0.057 ± 0.0639^{a}	0.038 ± 0.0357^{a}	0.033 ± 0.0515^{a}	NS
C12:0	0.091 ± 0.0415^{ab}	0.090 ± 0.0502^{ab}	0.049 ± 0.0299^{b}	0.061 ± 0.0398^{b}	0.136 ± 0.0598^{a}	0.060
C14:0	1.24 ± 0.626^{ab}	1.92 ± 0.706^{a}	1.46 ± 0.502^{ab}	1.25 ± 0.610^{ab}	0.93 ± 0.340^{b}	0.694
C16:0	26.5 ± 2.180^{a}	26.3 ± 2.112^{a}	27.5 ± 1.390^{a}	23.5 ± 2.870^{b}	22.9 ± 2.035^{b}	2.200
C18:0	20.8 ± 3.206^{a}	19.2 ± 2.391^{a}	20.7 ± 3.373^{a}	21.9 ± 2.464^{a}	20.6 ± 1.381^{a}	NS
C20:0	0.224 ± 0.0427^{a}	0.187 ± 0.0387^{a}	0.217 ± 0.0372^{a}	0.217 ± 0.0632^{a}	0.227 ± 0.0267^{a}	NS
C22:0	0.103 ± 0.0297^{a}	0.062 ± 0.0523^{a}	0.086 ± 0.0215^{a}	0.098 ± 0.0367^{a}	0.116 ± 0.0738^{a}	NS
C24:0	0.352 ± 0.1740^{b}	0.245 ± 0.0466^{b}	0.364 ± 0.1713^{b}	0.462 ± 0.3150^{b}	0.742 ± 0.2590^{a}	0.247
MUFA						
C14:1	0.162 ± 0.0723^{a}	0.250 ± 0.1207^{a}	0.149 ± 0.0527^{a}	0.166 ± 0.0839^{a}	0.223 ± 0.1057^{a}	NS
C16:1*n*-7	2.51 ± 0.368^{a}	2.45 ± 0.366^{a}	2.41 ± 0.480^{a}	2.01 ± 0.398^{b}	1.95 ± 0.320^{b}	0.390
C18:1*n*-9t	0.967 ± 0.6996^{a}	0.311 ± 0.0602^{b}	0.340 ± 0.0391^{b}	0.379 ± 0.1245^{b}	0.341 ± 0.0669^{b}	0.373
C18:1*n*-9c	37.7 ± 1.753^{a}	37.3 ± 2.932^{ab}	35.2 ± 2.460^{b}	37.1 ± 1.684^{ab}	36.3 ± 2.544^{ab}	2.109
C20:1*n*-9	0.175 ± 0.0418^{a}	0.165 ± 0.0410^{a}	0.162 ± 0.0243^{a}	0.172 ± 0.0444^{a}	0.172 ± 0.0338^{a}	NS
C22:1*n*-9	0.050 ± 0.0309^{b}	0.046 ± 0.0384^{b}	0.053 ± 0.0377^{b}	0.070 ± 0.0426^{ab}	0.112 ± 0.0343^{a}	0.048
C24:1*n*-9	0.090 ± 0.0560^{b}	0.141 ± 0.0714^{ab}	0.099 ± 0.0376^{b}	0.114 ± 0.0930^{ab}	0.189 ± 0.1154^{a}	0.088
PUFA						
C18:2*n*-6t	0.220 ± 0.0668^{a}	0.199 ± 0.0139^{a}	0.215 ± 0.0641^{a}	0.223 ± 0.0669^{a}	0.166 ± 0.0551^{a}	NS
C18:2*n*-6c	4.62 ± 2.799^{c}	5.45 ± 1.264^{bc}	6.38± 2.121^{abc}	7.16 ± 2.978^{ab}	7.95 ± 1.990^{a}	2.491

Table 5 (continue). Means (±SD)[#] of fatty acid composition (% of total fatty acids) of selected beef muscles.

Fatty acid	Muscle					LSD (*P*=0.05)
	***Biceps femoris* (BF)**	***Longissimus lumborum* (LL)**	***Rectus femoris* (RF)**	***Supraspinatus* (SS)**	***Semitendinosus* (ST)**	
C18:3*n* -6	0.101 ± 0.0546^{a}	0.109 ± 0.0366^{a}	0.097 ± 0.0487^{a}	0.105 ± 0.0567^{a}	0.137 ± 0.0651^{a}	NS
C18:3*n* -3	0.278 ± 0.1180^{bc}	0.212 ± 0.0580^{c}	0.389 ± 0.146^{a}	0.346 ± 0.122^{ab}	0.371 ± 0.152^{a}	0.076
C20:2*n*-6	0.073 ± 0.0243^{b}	0.092 ± 0.0310^{b}	0.080 ± 0.0166^{b}	0.116 ± 0.0724^{ab}	0.148 ± 0.0483^{a}	0.045
C20:3*n*-6	0.385 ± 0.1320^{b}	0.426 ± 0.0636^{b}	0.432 ± 0.1468^{b}	0.512 ± 0.1863^{b}	0.713 ± 0.1103^{a}	0.156
C20:3*n*-3	1.29 ± 0.429^{b}	1.21 ± 0.214^{b}	1.39 ± 0.527^{b}	1.81 ± 0.875^{b}	2.49± 0.560^{a}	0.670
C20:4*n*-6	0.096 ± 0.0424^{a}	0.120 ± 0.0363^{a}	0.091 ± 0.0660^{a}	0.074 ± 0.0421^{a}	0.116 ± 0.0350^{a}	NS
C20:5*n*-3	0.213 ± 0.107^{ab}	0.167 ± 0.0977^{b}	0.200 ± 0.0815^{ab}	0.171 ± 0.1039^{b}	0.304 ± 0.0800^{a}	0.106
C22:2*n*-6	0.066 ± 0.0184^{b}	0.208 ± 0.1802^{a}	0.076 ± 0.0193^{b}	0.097 ± 0.0674^{b}	0.145 ± 0.0990^{ab}	0.108
C22:5*n*-3	0.475 ± 0.3480^{b}	0.156 ± 0.0688^{b}	0.554 ± 0.3830^{ab}	0.4797 ± 0.4180^{b}	0.890 ± 0.2280^{a}	0.399
C22:6*n*-3	0.135 ± 0.0745^{ab}	0.109 ± 0.0369^{ab}	0.110 ± 0.0315^{ab}	0.088 ± 0.0453^{b}	0.164 ± 0.0407^{a}	0.058
SFA	49.5 ± 4.704^{ab}	50.0 ± 3.786^{ab}	50.5 ± 4.531^{a}	47.7 ± 3.951^{ab}	46.0 ± 2.725^{b}	4.329
MUFA	41.7 ± 2.470^{a}	40.7 ± 3.167^{ab}	38.5 ± 2.704^{b}	40.1 ± 1.985^{ab}	39.3 ± 2.763^{b}	2.288
PUFA	7.96 ± 3.494^{b}	8.46 ± 1.614^{b}	10.1 ± 2.851^{ab}	11.2 ± 4.483^{ab}	13.6 ± 2.863^{a}	3.663
TUFA	49.7 ± 4.646^{a}	49.1 ± 3.881^{a}	48.6 ± 4.560^{a}	51.2 ± 3.789^{a}	52.9 ± 2.710^{a}	NS
DFA	70.4 ± 2.356^{b}	68.3 ± 2.219^{b}	69.3 ± 1.671^{b}	73.1 ± 2.864^{a}	73.5 ± 1.867^{a}	2.442
n-6	5.50 ± 2.927^{c}	6.40 ± 1.345^{bc}	7.30 ± 2.118^{abc}	8.19 ± 3.263^{ab}	9.23 ± 2.126^{a}	2.669
n-3	2.39 ± 0.931^{b}	1.85 ± 0.192^{b}	2.76 ± 1.027^{b}	2.89 ± 1.320^{b}	4.22 ± 0.822^{a}	1.156

[#] SD: Standard deviation.
[a, b, c] Means in the same row with different superscripts are significantly different ($P \leq 0.05$).
LSD: Least significant difference (P=0.05).
NS: Not Significant (P>0.05).
Abbreviations: SFA: Saturated Fatty Acids.
MUFA: Monounsaturated Fatty Acids.
PUFA: Polyunsaturated Fatty Acids.
TUFA: Total Unsaturated Fatty Acids.
DFA: Desirable Fatty Acids (C18:0 + TUFA).
P:S: Polyunsaturated:Saturated fatty acid ratio.
n-6 consists of C18:2, C18:3, C20:2, C20:4, C22:4.
n-3 consists of C18:3, C20:5, C22:5.

The polyunsaturated fatty acid (PUFA) content also differed significantly ($P \leq 0.05$) between the muscles (Table 5), with BF and LL having a significantly lower percentage of approximately 8% and ST having the highest percentage of PUFA (13.6% $\pm$ 2.863). In the latter case linoleic acid (C18:2*n*-6) was the most abundant PUFA at 7.95% ± 1.990, followed by eicosatrienoic acid (C20:3*n*-3) at 2.49% ± 0.560% (Table 5).

As previously mentioned, the PUFA:SFA (also known as P:S) ratio and n-6:n-3 ratio are important in view of the nutritional guidelines. Table 4 contains the P:S, the *n*-6:*n*-3 and the desirable fatty acids (DFA) ratios. When judging the fatty acid content of food, nutritionists increasingly focus on the P:S, as well as the n-6:n-3 PUFA ratio. As previously mentioned, the Heart Foundation (2003) recommended values of 0.45 or above for the P:S ratio in dietary fats. The P:S ratio is presented as an important guideline representing the total impact of all SFA on the blood cholesterol content. P:S ratios are lower in ruminants than in non-ruminants because of biohydrogenation of dietary unsaturated fatty acids by ruminal micro-organisms (Banskalievaa *et al.*, 2000). C18:2*n*-6, the major plant fatty acid, is much lower in ruminant tissue and this low concentration leads to a P:S ratio below the recommended value of 0.45 (Warriss, 2000). Previous reports found P:S values from bovine meat between 0.11 and 0.15, which are lower than the recommended values (Geay *et al.*, 2001; Wood *et al.*, 2003) and therefore not favourable. Raes *et al*. (2003) found ratios ranging from 0.10 to 0.45 for the *Longissimus lumborum* muscle. P:S ratios of all the muscles in this investigation were within the range of the reported data, however, the present P:S muscle ratios were far below the recommended value of 0.45. The BF muscle had the lowest ratio of 0.17 ± 0.087 and the ST the highest ratio of 0.30 ± 0.075 (Table 4). According to Table 4 the n-6:n-3 ratio of the muscles are beneficially below than the ratio of 4.0 recommended by the British Department of Health (1994) and ranged from 2.20 ± 0.379 (ST) to 3.45 ± 0.557 (LL). The ratio data are supported by Raes *et al*. (2003), where n-6:n-3 ratios ranging from 2.46 to 11.5 for the *Longissimus lumborum* muscle were reported.

According to the health classification of Rhee (1992), the desirable fatty acids (DFA) is the sum of all the unsaturated fatty acids and C18:0. The DFA differed significantly ($P \leq 0.05$) between the muscles, with ST having the highest percentage (73.5 ± 1.867) of desirable for DFA (Table 5).

One of the most successful methods of manipulating muscle fat content, and consequently the quality of the meat product, is by changing the dietary intake of the animal with respect to the sources of long-chain PUFA (Wood & Enser, 1997). Grass is an important source of the beneficial fatty acid, α-linoleic acid (C18:3*n*-3), and consists of

approximately 60% of this fatty acid (Scollan, 2003). Therefore, providing a grass diet in comparison with concentrates increases the α-linoleic acid (C18:3) and other *n*-3 PUFA contents of the intramuscular fat of the beef muscle (Marmer *et al*., 1984; Enser *et al*., 1998). The *n*-3 PUFA, which occurs in greater proportions within the beef muscle after forage feeding, is C18:3*n*-3, C20:5*n*-3, C22:5*n*-3 and C22:6*n*-3. The fatty acids ratios are also influenced in grass-fed beef, i.e. 0.1 (P:S) and 1.5 (*n*-6:*n*-3) (Scollan, 2003). The latter P:S ratio is not desirable, but the *n*-6:*n*-3 ratio is beneficial (Wood & Enser, 1997). Animals fed a grain-based diet produced meat with higher concentrations of linoleic acid (C18:2) and the *n*-6 PUFA (Marmer *et al*., 1984; Enser *et al*., 1998). In addition, concentrate-fed beef has a high *n*-6:*n*-3 ratio, which is not beneficial to human health (Enser *et al*., 1998).

Descriptive sensory analysis

Sensory attributes play an undeniable role in consumer preference and acceptance (Risvik, 1994) and represent the eating quality of the product. Savell *et al.* (1978) considered appearance, tenderness and flavour to be the main sensory characteristics of meat quality perceived by consumers. Several authors agree (Jeremiah, 1982; Savell & Shackelford, 1992; Boleman *et al*., 1997) that flavour, juiciness and tenderness contribute the most to the consumer perception of beef palatability and the satisfaction derived from consuming beef. However, the prevalence of inconsistent tenderness of meat products is a major problem facing the modern beef industry (Morgan *et al*., 1991; Smith *et al*., 1992). Variation in texture (tenderness and juiciness) occurs due to age, muscle cut, pH and protein denaturation. To achieve the optimum texture quality, meat requires different heating temperatures and heating periods (Bouton *et al*., 1981; Stabursvik *et al*., 1984; Beilken, *et al*., 1986). Caporaso *et al*. (1978) reported that the intensity of juiciness, tenderness and flavour are significantly influenced by the heating temperature of the sample.

The conflicting results pertaining to the influence of fat level and fat composition on meat palatability are still under debate (Larick & Turner, 1990, Melton, 1990). There will always be some fat present in meat and it is in fact required to impart flavour and juiciness (Melton, 1990). Several studies concluded that the palatability of meat improves with the increase of marbling degree (Hoke & Hedrick, 1969; Tatum *et al*., 1980; Wood (1990)). Park *et al*. (2000) found that beef loins with high intramuscular fat had high scores in juiciness, tenderness and flavour. Savell *et al*. (1978) reported similar findings with a decrease in marbling level from slightly abundant to traces, resulting in the juiciness,

tenderness and flavour intensity ratings decreasing, while the shear force values increased. Wood (1990) indicated that the fat level has positive effects on the tenderness of meat. Then again, according to Jones *et al*. (1991) the degree of marbling had no effect on the overall tenderness of flavour intensity or desirability. However, in the case of steaks, a slight degree of marbling had higher juiciness scores than steaks with traces of marbling. Parrish *et al*. (1973) also found no significant variation in flavour due to marbling level.

It is evident that long-chain fatty acids do influence the sensory characteristics of beef and hence can be manipulated through feeding (Webb, 2004). Earlier researchers reported a negative correlation between SFA in the intramuscular fat with the flavour of beef (Westerling & Hendrick, 1979). The flavour of the *Longissimus* muscle was improved by oleic acid, whereas both stearic and palmitic acid resulted in a reduced beef flavour (Webb, 2004). No association of the fatty acids with juiciness and tenderness was reported in this study (Westerling & Hendrick, 1979).

The score means for sensory quality characteristics of the selected beef muscles are presented in Table 6. The scores allocated to the muscle characteristics coincide with scores reported in the literature (Schönfeldt *et al*., 1997a; Rhee *et al*., 2004; Jeremiah & Phillips, 2000). The sensory characteristics that differed significantly ($P \leq 0.05$) amongst the various muscles are beef aroma, juiciness (initial juiciness and sustained juiciness), tenderness (first bite and residue).

Beef aroma and flavour

According to Table 6, the beef aroma of BF (mean score of 6.88) was significantly higher than that of the other three samples ($P \leq 0.05$). Although there were no significant differences for flavour, the BF sample had the highest mean score of 6.55. Jeremiah *et al*. (2003c) report ST (5.00) and LL (5.19) to have the most bland beef flavour intensity, whereas BF and SS were higher in flavour intensity. Carmack *et al*. (1995) reported that, although the flavour of the samples did not differ significantly, BF had a tendency towards having a slightly higher flavour score. These results are supported by the present investigation.

The fact that there were no flavour differences in this investigation (Table 6) could be ascribed to the fact that all the muscles had a short ageing period. Ageing time is an important factor for the development of flavour precursors. Normally, beef muscles that were aged for 1 d do not have a specific aroma (Monsón *et al*., 2005).

The effect of forage versus grain finishing on beef flavour has been well researched, with meat from a grass-based diet having a distinctly stronger off-flavour than that from a grain-based diet (Varnam & Sutherland, 1995, Raes *et al*., 2004). Meat from animals fed on grass diets usually has an increased C18:3 fatty acid content and this normally leads to off-flavours, mainly due to the beef being more susceptible to oxidation (Reverte *et al*., 2003; Wood *et al*., 2003). In the present investigation none of the samples illustrated any off-flavours – this is expected from beef finished off in a feedlot.

Correlations between fatty acid composition and flavour scores are reported in numerous studies (Sañudo *et al*., 2000; Wood *et al*., 2003). In the latter studies the correlations were positive for 18:0, 18:1 (oleic) and 18:3 (Wood *et al*., 2003) and negative for 18:2 and 20:4 (Sañudo *et al*., 2000). In this investigation no significant association was found for aroma or flavour, possibly as a result of the fact that the aroma and flavour of the different muscles were very similar.

Initial and sustained juiciness

According to Schmidt (2002), juiciness is defined as a sensory attribute related to both the capacity of the muscle to release its constitutive water (initial juiciness) and the infiltrated fat content (sustained juiciness) (Table 1). Differences in the juiciness of meat can be attributed to the amount of water in the muscle and the intramuscular fat content of the meat sample (Andersen *et al*., 2004).

In the present investigation (Table 6) RF had the lowest mean score for initial juiciness (6.18 ± 0.897), as well as for sustained juiciness (5.48 $\pm$ 1.112). The other samples were all slightly juicier, with ST having a significantly higher ($P \leq 0.05$) mean score for initial juiciness (6.50 $\pm$ 0.972) and BF for sustained juiciness (5.86 $\pm$ 0.862). Previous studies found that RF and BF were juicier than ST (Jeremiah *et al*., 1971; Jeremiah *et al*. 2003c).

Silva *et al*. (1999) significantly correlated juiciness with tenderness. In the present results no significant correlation ($P > 0.05$) was found between juiciness and shear force, nor between initial and sustained juiciness. According to Jeremiah (1978), moisture content has a direct influence on juiciness. Again the correlation coefficients showed no significant association ($P > 0.05$) between moisture content and initial or sustained juiciness.

Table 6. Means (±SD)[#] for sensory characteristics of selected beef muscles.

Characteristic	Muscle				LSD (*P*=0.05)
	***Biceps femoris* (BF)**	***Longissimus lumborum* (LL)**	***Rectus femoris* (RF)**	***Semitendinosus* (ST)**	
Beef aroma[d]	6.88 ± 0.810[a]	6.52 ± 0.934[b]	6.39 ± 0.888[b]	6.63 ± 0.865[b]	0.233
Beef flavour[e]	6.55 ± 0.601[a]	6.36 ± 0.724[a]	6.39 ± 0.755[a]	6.54 ± 0.738[a]	NS
Initial juiciness[f]	6.32 ± 0.867[ab]	6.48 ± 0.953[a]	6.18 ± 0.897[b]	6.50 ± 0.972[a]	0.295
Sustained juiciness[g]	5.86 ± 0.862[a]	5.38 ± 0.964[b]	5.48 ± 1.112[b]	5.63 ± 0.964[ab]	0.257
First bite[h]	5.48 ± 0.874[c]	5.66 ± 0.959[c]	6.71 ± 0.825[a]	6.27 ± 0.904[b]	0.312
Residue[i]	6.07 ± 0.892[c]	6.07 ± 1.006[c]	7.00 ± 0.915[a]	6.66 ± 0.815[b]	0.293
Salty taste[j]	1.57 ± 0.806[a]	1.50 ± 0.831[a]	1.54 ± 0.873[a]	1.41 ± 0.781[a]	NS

[#] SD: Standard deviation.

[a, b, c] Means in the same row with different superscripts are significantly different (*P*≤0.05).

LSD: Least significant difference (*P*=0.05).

NS: Not Significant (*P*>0.05).

[d]Beef aroma: 1=Extremely untypical; 8=Extremely typical.

[e]Beef flavour: 1=Extremely untypical; 8=Extremely typical.

[f]Initial juiciness: 1=Extremely dry; 8=Extremely juicy.

[g]Sustained juiciness: 1=Extremely dry; 8=Extremely juicy.

[h]First bite: 1=Extremely tough; 8=Extremely tender.

[i]Residue: 1=Abundant; 8=None.

[j]Salty taste: 1=No salty taste; 8=Extremely salty.

Furthermore, several studies indicate a negative relationship between cooking loss and juiciness (Wood *et al*., 1995; Bejerholm & Aaslyng, 2003; Serra *et al*., 2004). Others indicated that cooking loss explained less than 10% of the juiciness variation and that the relationship between cooking loss and juiciness was dependent on the raw quality of the meat sample (Aaslyng *et al*., 2003). Bertram *et al*. (2005) therefore suggested that the total moisture content and cooking loss could not be used to explain the variation in juiciness. This was also the case in the present study.

First bite and residue

First bite, as well as residue, defines tenderness (Table 1). The muscles varied significantly ($P \leq 0.05$) in sensory tenderness scores (Table 6). For both first bite and residue RF showed the highest degree of tenderness and BF the lowest degree of tenderness. Tenderness ratings of beef muscles researched by McKeith *et al*. (1985), in a descending order of tenderness, are as follows: LL, RF, ST and then BF. Prost *et al*., 1975 and Rhee *et al*. (2004) found similar tenderness ratings as McKeith *et al*. (1985). Jeremiah *et al*. (2003c) report ST to be moderately tough overall, with BF and SS slightly deficient in overall tenderness, whereas RF and LL were more tender. BF was slightly deficient in overall tenderness.

The toughness of BF, in the present investigation, is possibly due to connective tissue content. Tenderness is improved when collagen with few intermolecular cross-links is converted to gelatin during cooking (60°C). However, when more cross-linkages that are intermolecular are present, moisture is squeezed out of the muscle, decreasing tenderness (Ledward, 1984).

The sensory tenderness of meat has been correlated with instrumental tenderness (shear force) (Jeremiah & Phillips, 2000; Peachey *et al*., 2002; Rhee *et al*., 2004; Wheeler *et al*., 2004). Chambaz *et al*. (2002) report a poor relationship between tenderness and shear force. However, other studies report a positive and significant relationship between instrumental and sensory tenderness (Bouton *et al*., 1975). Several reasons seem to be possible explanations for the variation in correlations, i.e. differences in the sensory panel, cooking methods, sample preparation and muscle used. Furthermore, the poor association that exists between the instrumental and sensory tenderness could possibly be explained by sampling variation and the development of stress and strain patterns within the mouth, during mastication of the meat sample. In the present investigation the association between first bite and shear force was not significant ($P > 0.05$), indicating a

poor relation, however, there was a significant correlation between residue and shear force (r=0.920, *P*<.0001).

Jeremiah *et al*. (1970) report that intramuscular fat affects tenderness indirectly. Most studies found a slightly positive association between intramuscular fat and sensory tenderness (Campion *et al*., 1975; Crouse & Smith, 1978; Dikeman *et al*., 1986; Seideman *et al*., 1987; Jones & Tatum, 1994; Wheeler *et al*., 1996; Fiems *et al*., 2000; Renard *et al*., 2001). In this investigation the correlation between sensory tenderness and fat content was not significant (*P*>0.05).

CONCLUSIONS

Variability in meat quality is a global problem, causing concern for both the meat industry as well as the consumer (Dransfield, 1992). The purpose of this study is to determine whether beef muscles differ significantly with regard to specific beef quality characteristics. This study confirms that different beef muscles from local origin differ significantly (*P*≤0.05) in their physical, chemical and sensory quality characteristics. Consumers will reject a meat product of poor quality. According to Koohmaraie (1996), it is important for the meat industry to address the problem of variability, in particular tenderness.

In order to optimise meat quality and thus minimise the variation in meat quality (Bertram *et al*., 2005), the mechanisms involved should be understood and identified (Koohmaraie, 1996; Koohmaraie *et al*., 2002; Anon., 2005b). Furthermore, it is important to gain a better understanding of the biochemical factors associated with meat ageing (Ouali, 1990), as well as the physical, histological and chemical characteristics of muscle; the process of meat production and of the way that processing can be manipulated and controlled to improve the palatability of single muscles and ensure consistent quality (Koohmaraie, 1996).

Several strategies can be used to approach this problem of inherent variation in meat. Firstly, all the critical control points causing variation should be regulated, and secondly, quality should be monitored carefully to identify unacceptable quality (Koohmaraie *et al*., 2003). In South Africa the only quality control point practised on a national basis is the so-called age classification. The age classification can account for part of the variation found in meat tenderness (Strydom, 2003), however, new processing techniques should be implemented to improve consistency and reduce variation. Furthermore, the traditional beef marketing system should be re-assessed and individual muscles and muscle groups should be merchandised based upon their inherent eating-

quality characteristics (Jeremiah *et al*., 2003c). In addition, post-mortem intervention or processing techniques, such as mechanical tenderising and/or infusion with a tenderising solution, could be implemented to reduce the variation in tenderness (Jeremiah *et al*., 2003c). Manipulating beef quality will only remain viable as long as it is practical, economical and does not detract from the intrinsic and extrinsic attributes of beef quality, or aspects such as "environmentally acceptability" or "ethnical beef production" (Webb, 2004).

This study justifies, and indicates a great scope for, further research on South African beef, especially with regard to the biological basis for variation in tenderness (Koohmaraie *et al*., 2003), muscle structure, sarcomere length, collagen content, muscle fibre characteristics (type and size), location or function, and myofibril fragmentation and how it affects beef muscle quality. This investigation also creates a platform to research a relatively new processing technique in South Africa that could be utilised to add value, diminish the variation that exists between beef muscles and thereby provide guaranteed quality, tenderness in particular. It is essential to capitalise on the discussed opportunities for the improvement of beef quality and its consistency for the survival of the beef industry (Jeremiah & Phillips, 2000; Jeremiah *et al*., 2003c).

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CHAPTER 4

PHYSICAL AND CHEMICAL PROPERTIES OF SELECTED BEEF MUSCLES INFUSED WITH A PHOSPHATE AND LACTATE BLEND

ABSTRACT

The consumer demands a beef product of consistent and acceptable tenderness. The infusion of beef muscles with a blend containing sodium and potassium salts, various phosphates and lactates has the potential to improve the current status of low meat consumption and inconsistent tenderness of the fresh beef products in South Africa. In the present investigation, various muscles from the left side of beef carcasses were infused, 3 d post-mortem, with a blend consisting of various sodium and potassium salts, di- and triphosphates and lactates, whilst the corresponding muscles from the right side were untreated and served as the control. The changes in beef quality over a 19-d period and the initial proximate and mineral composition of the muscles were also determined. The general findings suggest that an increase in tenderness concurrent with an acceptable beef colour resulted from the infusion with the mentioned blend. The chemical composition of the treated muscles was not negatively affected by the infusion and the mineral content of the treated muscles was increased, accordingly.

Keywords: Alkaline infusion, pH, water-binding capacity, instrumental tenderness, beef colour, proximate composition, mineral composition

INTRODUCTION

Attending to the consumer demand for fresh meat products of consistent quality is of great importance for achieving success in the meat industry and increasing beef consumption. A major quality weakness in the modern beef industry is the variability of beef quality, and in particular tenderness (Morgan *et al*., 1991b; Smith *et al*., 1992). Several studies on meat acceptability have indicated that consumers consider tenderness the most important attribute (Whipple *et al*., 1990) and surely the most desirable when meat is consumed, whether at home or in a restaurant (Huffman *et al*., 1996). Other important meat qualities that consumers also consider when buying meat are the meat freshness, juiciness and nutrients provided by the product (Boleman *et al*., 1995; Grunert, 1997). The consumer is

willing to pay for a more expensive product, expecting in return the assurance of a satisfactory tender product when purchasing meat (Boleman *et al*., 1997). This high standard of assured beef tenderness, stipulated by consumers, is one of the main concerns and objectives in the beef industry today, affecting the entire chain from production to retail (Smith *et al*., 1992).

Meat tenderness variation exists among species, animals within the same species and among different muscles held for different post-mortem periods (Polidori *et al*., 2000). A number of factors contribute to the variation in meat tenderness, such as biological and technological factors, i.e. ageing, marbling, connective tissue content, muscle contraction (Braghieri *et al*., 2005), muscle composition (Morris *et al*., 1997) and sarcomere length (O'Neill *et al*., 2004).

Connective tissue content is still believed to contribute extensively to tenderness variation. However, these stroma proteins undergo limited change during post-mortem ageing (storage). This has led to the conclusion that changes in the myofibrillar proteins are primarily responsible for post-mortem tenderisation and therefore controls the variations in the meat texture (Greaser, 2001). The exact mechanisms of post-mortem tenderisation are yet inconclusively understood, but many reports have confirmed that proteolysis of the myofibrillar proteins contributes mostly to the tenderisation of beef stored after slaughter (Koohmaraie, 1992; Taylor *et al*., 1995). Collagen-rich beef muscles are generally tough, even under the most favourable ageing conditions (Berge *et al*., 2001).

Beef colour is another important beef quality trait that has shown variation during retail display (Got *et al*., 1999). Assessing the colour of meat is important due to the fact that consumers consider colour their only point of reference when beef is judged before purchase. Even though colour is considered a poor guide to eating quality, consumers still base their purchase decisions on colour display (Young *et al*., 1999).

Therefore, to achieve guaranteed tenderness, consumer satisfaction and reduced economic loss in the beef industry (Robbins *et al*., 2003), the industry needs to find ways of improving meat palatability. Over the years several techniques and processes have been researched and applied in search of providing a solution for the problem of meat-quality variation and in particular that of tenderness. These corrective actions include the procedures of electrical stimulation (Dransfield *et al*., 1992), carcass suspension (Sørheim & Hildrum, 2002), natural ageing (Lawrie, 1998), blade tenderisation (Benito-Delgado *et al*., 1994), marinating (Scanga *et al*., 2000), injection (McGee *et al*., 2003) and explosion (Solomon *et al*., 1997).

Novel carcass suspension (stretching and restricting) technologies such as Tenderstretch^{TM} and Tendercut have been reported to increase the tenderness of muscles (Sørheim & Hildrum, 2002). Natural ageing has shown that storage of meat post-mortem at chilling temperature, whether it is carcasses and/or sub-primal cuts, results in the improvement of meat tenderness (Morgan *et al*., 1991a). Blade tenderisation is a mechanical technique for disrupting the muscle structure by inserting thin blades into the muscles and consequently the tenderness of the meat is improved. Even though this technique is convenient and effective, the high drip loss during storage and deceased shelf life make it a less acceptable application (Davis *et al*., 1977).

The application and success of meat-enhancing agents such as phosphates and salts have been well-documented (Kerth *et al*., 1995; Morris *et al*., 1997). Enhancing the flavour, tenderness and consumer acceptance of retail beef products and the ability to produce value-added and water-added beef products creates a growing market opportunity in the beef industry (Scanga *et al*., 2000). Several injection / infusion solutions have been developed over the years, which include mostly calcium and sodium salts.

Sodium lactate has been applied in the beef industry for its flavour-enhancing and shelf-life-extension properties (Duxbury, 1988; Maca *et al*., 1999). Consumers consider the salty taste observed when meat is subjected to sodium chloride treatment as favourable (Wheeler *et al*., 1993). This salt combination increases the ionic strength of the meat (Trout & Schmidt, 1984) thereby improving the water-binding capacity and decreasing the cooking losses of the meat (Pearson & Tauber, 1984). The cooking yield is also higher for lactate-treated muscles as lactate ions binds readily to water (Evans, 1992).

Sodium phosphate is also commonly used in meat processing and has been documented to increase protein solubility and the water-binding ability of meat (Hellendoorn, 1962; Trout & Schmidt, 1984). The injection of a brine containing sodium tripolyphosphate has been reported to reduce the Warner-Bratzler shear force resistance and increase the juiciness (Smith *et al*., 1984). Sodium chloride contributes mainly to the solubilisation of the myofibrillar proteins of meat.

Another solution consisting of calcium chloride ($CaCl_2$), infused into meat has been demonstrated to be successful in enhancing and accelerating post-mortem tenderisation (Koohmaraie *et al*., 1988, 1989, 1990; Koohmaraie & Shackelford, 1991; Morgan *et al*., 1991a; Wheeler *et al*., 1991). Utilising calcium accelerates the activation of calpain enzymes and causes the Z-disk proteins to degrade, resulting in the weakening of the myofibrillar structure (Koohmaraie, 1994). Despite the success in increased tenderness obtained with the use of $CaCl_2$, the detrimental colour (Wheeler *et al*., 1996), off-flavour

(Morgan *et al*., 1991) and increased purge (Kerth *et al*., 1995) have limited the use of this salt by commercial processors.

Phosphates are typically a component of many enhancement solutions applied in the modern beef industry, because of the phosphates' ability to increase the functionality of meat products, particularly via water binding (Hamm, 1970; Trout & Schmidt, 1983). Water retention in fresh muscles is based on a buffered (with phosphates) water solution with a pH that is more alkaline and further away from the isoelectric point of the meat. This action increases the water-holding capacity of the meat (Mandigo 2002).

Phosphates and sodium chloride (NaCl) both increase functionality via protein swelling (Paterson *et al*., 1988) and ionic strength and pH (Trout & Schmidt, 1984). This increased functionality leads to increased water retention (Trout & Schmidt, 1983) and improved tenderness and juiciness (Prestat *et al*., 2002). Therefore, the inclusion of salt and phosphate improve the yield and palatability characteristics and affects the colour and shelf-life. Contradictory colour results have been reported with the use of a phosphate and NaCl blend. Either an improved meat colour (Lee *et al*., 1998) or a meat colour that is diminished (Chen & Trout, 1991) is obtained with the infusion of such as blend.

The post-mortem storage (ageing) of beef at chill temperatures has been a practice for many years and remains an important procedure in the modern meat industry for producing tender meat (Koohmaraie *et al*., 1988). According to Chapter 3 and other research reports (Rhee *et al*., 2004) on muscle variation, it is clear that different muscles from one carcass react differently to post-mortem storage (Koohmaraie *et al*., 1988). A possible solution, which is a popular research topic in the beef industry of today, is the infusion of a blend containing salts, phosphates and lactates.

From the above, it is clear that the enhancement of beef via injection / infusion has been widely researched and used extensively in the United States of America (USA) and to a lesser extent in Europe (Robbins *et al*., 2002; Lawrence *et al*., 2004; Livingston *et al*., 2004). Solutions such as sodium lactate, sodium tripolyphosphate, sodium chloride and water infused into beef muscle, significantly improved tenderness and juiciness compared to non-treated control (Vote *et al*., 2000). These solutions could also be added to under-utilised meat cuts to improve their quality, in particular the meat tenderness and palatability.

The present study investigates a commercially available basting (Freddy Hirsch Tenderbite # 802539) consisting of sodium and potassium salts, various phosphates and lactates. This brine was used to infuse *Biceps femoris* (BF, silverside), *Rectus femoris* (RF), *Semitendinosus* (ST, eye of the silverside), *Supraspinatus* (SS, scotch fillet) and

Longissimus lumborum (LL, striploin) beef muscles. Previous research (Hoffman, 2006) has indicated that this specific blend increases the tenderness of meat significantly. However, the effect of the blend on beef qualities, with post-mortem ageing, has not yet been determined. Therefore, the first aim of this study is to determine the effect of a phosphate and lactate blend on the physical (pH, water-binding capacity, beef colour and shear force) and chemical properties (proximate and mineral composition) of selected beef muscles. A secondary aim is to determine whether the blend has any significant effect on the physical properties over a given time period.

MATERIALS AND METHODS

Animals and sampling

Beef carcasses representing South African beef breeds [Brahman × Simmentaler cross; n = 3, average mass = 300.73 kg and Charolais × Hereford cross; n = 3, average mass = 297.87 kg], finished off in a feedlot, were sourced from a commercial abattoir in Paarl, Western Cape, South Africa. At the abattoir, the animals were slaughtered, dressed and thereafter processed according to standard South African techniques and conditions. No electrical stimulation was applied to the carcasses. The animals were selected to represent culled steers from a typical commercial scenario, representative of the South African market.

The carcasses were classified as A2 according to the South African classification system (Government Notice No R. 1748, 26 June 1992). An A2 animal is a young animal of the A age group (no permanent incisors) with a fat code of 2, representing a lean fat cover (1-3 mm thick subcutaneous fat depth measured between the 10^{th} and 11^{th} rib, 50 mm from the midline of the cold unquartered carcass).

The whole, intact carcasses were chilled at *ca.* 2°C for 24 h in a cooling chamber before being weighed and quartered at the abattoir (Day 1). Twenty-four hours (Day 2) post-mortem the beef quarters were moved into a mobile cooling unit (set at 4°C) and transported to the Meat Science Laboratory at Stellenbosch University, where the carcasses were stored in the cooling facility at 4°C.

On the same day (Day 2; 24 h pm) both the left and right side *Biceps femoris* (BF, silverside), *Rectus femoris* (RF), *Semitendinosus* (ST, eye of the silverside), *Supraspinatus* (SS, scotch fillet) and *Longissimus lumborum* (LL, striploin), were removed from the carcasses. The muscles were trimmed of all visible subcutaneous fat and

superficial collagen, weighed, labelled, vacuum packed and stored in a cooler at *ca.* 4°C until further processing.

Sample preparation

On Day 3 (48 h pm) all the muscles were transported to the Freddy Hirsch Processing Plant, where they were removed from their packaging, demembraned, reweighed to determine the pre-infusion weight and the pH measured. Muscles from the right side of the carcass were left untreated and stored in a cooler at 2°C to be used as the control. The muscles from the left side were infused with a salt mixture containing sodium and potassium di- and triphosphates, lactate and chloride (Freddy Hirsch Tenderbite; PO Box 2554, Cape Town, 8000) at a pressure of 2.4 bar at 30 strokes per min on a Rühle Curing Centre IR56 (Rühle GmbH, D·79865, Grafenhausen, Germany) to give a calculated pumped gain of 15% with a retention of 12%. The basting mixture gave a calculated chemical composition of 75.75% water, 5.21% Na^+, 2.53% K^+, 3.45% P_2O_5 and 12.40% lactate. The treated meat samples were weighed immediately after infusion and after a resting (equilibration) period of 2 h to calculate the retained pumped gain. After 2 h the 10 muscles from both sides were divided into six portions to reflect six time intervals.

The six time intervals reflect six successive periods of measurements, ranging from 4 to 19 d, i.e. Day 4, 7, 10, 13, 16 and 19. Meat cuts were cut cross-sectional to the muscle fibre for the determination of pH, purge loss, drip loss, cooking loss, colour and shear force of fresh beef muscle (4°C). It is known that the quality characteristics of the muscle vary across the muscle. Therefore, to reduce the inherent variability of the muscle, the samples were randomised. However, the same muscle segment of the left and right were compared experimentally with each other.

After the division, the muscles (sub-samples) were weighed, labelled, vacuum packed, stored in crates and transported back to the Meat Science Laboratory at Stellenbosch University and stored in the cooler at 4°C until collected for analysis on the pre-assigned day.

On the sampling date the samples allocated to the time interval were removed from the cooler for analyses. Upon analysing the physical characteristics of the muscle, the samples were dried by means of absorbent paper and reweighed for calculation of purge loss (exudate collected in the vacuum bag). Meat slices of approximately 1.5 cm thick were cut cross-sectional to the muscle fibre for the determination of instrumental colour (CIE Lab) of the raw (after a blooming period of 30 min) (Wulf & Wise, 1999) and cooked muscles, drip loss, cooking loss and instrumental tenderness of the cooked muscles.

The remaining samples were homogenised, vacuum packed and stored at −18°C until proximate chemical and mineral analyses could be conducted.

Throughout the trial an attempt was made to ensure that all activities were similar to a typical commercial scenario. Care was also taken throughout the investigation to ensure that the handling procedures were similar for the muscles from both sides of the same carcass.

Physical analyses

The physical characteristics determined from the deboned muscles were pH before and after infusion, the pumped gain and purge loss. The data collected from the sub-samples over the 19-d period were pH, purge loss, drip loss, cooking loss, raw and cooked colour and instrumental tenderness (Warner-Bratzler shear force resistance, WBSF).

pH measurement

The pH of the whole muscle was measured before and after infusion and the pH of the sub-samples was measured with each time interval. The pH measurements were conducted by using a penetrating glass electrode on a hand-held Crison pH/mV-507 meter with an automatic temperature compensator. The pH measurements were taken approximately in the middle of the muscle cut. The pH meter was re-calibrated after every fourth reading with pH 4.01 and pH 7.02 standard buffers and the electrode rinsed with distilled water between each measurement.

Water-holding properties

- Pumped gain and purge loss

The left-side muscles were weighed before and immediately after infusion to calculate the pumped gain, as well as 2 h after infusion (stored at 2°C) to calculate the retained pumped gain. The purge losses of the undivided infused muscles were calculated from the pumped gain measurements.

For the determination of purge loss of the sub-samples, all the sub-samples were weighed (approximately 100 g) prior to packaging and then re-weighed on the designated sampling date. The purge loss was calculated as weight loss expressed as a percentage of the initial weight of the sample (Honikel, 1998).

- Drip loss

For the determination of the drip loss (Honikel, 1998), 1.5 cm thick muscles samples, cut perpendicular to the longitudinal axis of the muscle, were weighed (approximately 100 g) and suspended with a wire (under atmospheric pressure) into an inflated polythene bag in such a way that the exudate did not come into contact with the sample, but collected in the bag. After 24 h at *ca.* 4°C, the samples were blotted with absorbent paper and re-weighed. The drip loss was calculated as weight loss expressed as a percentage of the initial weight of the sample (Honikel, 1998).

- Cooking loss

For cooking loss determination, samples of 1.5 cm thick were freshly cut, weighed (approximately 100 g) and placed in thin-walled plastic bags in a water-bath at 80°C. The samples were removed from the water-bath after 1 h and cooled under cold water. The meat was then removed from the bag, blotted dry and weighed. Cooking loss was calculated as the difference in sample weight before and after cooking, expressed as a percentage of the initial sample weight (Honikel, 1998).

Instrumental meat colour

Colour was evaluated according to the method described by Honikel (1998) to determine L*, a* and b* values using a Colour-guide 45°/0° colorimeter (Catalog No 6805; BYK-Gardner, USA) on the allocated sampling dates

The colour measurements for each sample were taken in triplicate at randomly selected positions. The readings were taken after a blooming period of 30 min (Wulf & Wise, 1999). The colour of the cooked meat was also measured after determination of the cooking loss. Both the fresh and cooked meat colour analyses were done on a white surface at an ambient temperature of 8°C.

Colour was expressed by the coordinates L*, a*, b* of the CIE Lab colorimetric space (CIE, 1978). The L*, a* and b* values are an indication of the lightness (black-white axis), redness (red-green spectrum) and yellowness (blue-yellow spectrum) of colour respectively. The hue-angle and the a* and b* chroma are psychometric correlates of perceived hue and chroma (Honikel, 1998) and were determined using the following equation (CIE, 1978):

Chroma: $C^* = \sqrt{(a^*)^2 + (b^*)^2}$ Hue angle: $h_{ab} = \tan^{-1}\left\{\frac{b^*}{a^*}\right\}$

Shear force resistance

After measurement of the cooking loss, the same samples were stored overnight in a refrigerator (4°C) and used for instrumental determination of tenderness. The shear force values of the cooked meat samples were obtained with a Warner-Bratzler shear (WBS) attachment (Voisey, 1976), fitted to an Instron Universal Testing Machine (Model 4444). Three randomly sampled cylindrical cores were cut from the centre of each cooked meat sample using a 1.27 cm diameter bore. Tenderness was measured as the maximum force (Newton) required to shear a 1.27cm diameter cylindrical core of cooked meat (perpendicular to the grain) at a crosshead speed of 200 mm/min. The mean was calculated for each muscle. A larger value indicated greater shear force and therefore tougher meat (Honikel, 1998).

Chemical analyses

Proximate chemical analyses

The total percentage of moisture, protein, fat and ash of the raw beef muscle samples were determined according to AOAC Methods (AOAC, 2002). The total lipid content was determined by extracting the fat with a 1:2 mixture of chloroform:methanol, according to the method of Lee *et al*. (1996). The moisture content was analysed by drying a 2.5 g sample at 100°C for a period of 24 h (Method 934.01, AOAC, 2002) and ashing was done by cremating the samples at 500°C for a period of 6 h. The protein content was determined by the Dumas combustion method (Method 968.06, AOAC, 2002) on the defatted samples using a FP528 Nitrogen Analyser.

Mineral analyses

The mineral composition of the meat was determined after ashing defatted meat samples. These samples (1-3 g) were air-dried and ground to pass through a 0.5 to 1.0 mm sieve. After this the samples were ashed overnight in a muffle furnace at 550°C. A 6 N hydrochloric acid (HCl) solution was prepared by diluting 500 ml of a 36% (m/m) HCl solution to 1 liter. After ashing, 5 ml of a 6 M HCl was added to dissolve the cooled sample. After this the samples were dried in a water bath. After cooling, a 5 ml 6 N nitric acid (HNO_3) solution was added to the samples. The 6 N HNO_3 solution was prepared by diluting 429 ml of a 65% (m/m) solution to 1 liter. After adding the latter solution, the samples were heated in a water bath and removed after boiling point was reached. The solution was subsequently filtered through filter paper into a 100 ml volumetric flask and diluted to volume with deionised water (Giron, 1973).

The concentrations of calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), sodium (Na), phosphorus (P), lead (Pb) and zinc (Zn) of the digestates were determined by using the Inductively Coupled Plasma Spectrometry (ICP) detection method (Method No AgriLASA 6.1.1) (Handbook of Feed & Plant Analysis, Volume 2).

Experimental design and statistical analyses

The experimental design for the deboned, whole muscles was a randomised complete block design with ten treatment combinations replicated in six blocks (animals/carcasses). The treatment design was a 2×5 factorial with the factors, two treatments (control and infused) and five muscles (BF, RF, ST, SS and LL). The pH and pumped data were measured before infusion and after 2 h equilibration (resting period) and differences were calculated. All these data were subjected to an analysis of variance using SAS statistical software Version 9.1 (SAS, 2003). The Shapiro-Wilk test was performed to test for non-normality (Shapiro & Wilk, 1965). Student's t-Least Significant Difference (LSD) was calculated at the 5% confidence level to compare treatment means of significant source effects (Ott, 1998).

A further statistical analysis was conducted on the muscles to test the effect of the infusion solution with a storage period of 19 d on the physical parameters (pH, purge loss, drip loss, cooking loss, shear force, and raw and cooked colour). The treatment design was a 2×5×6 factorial experiment replicated in six blocks (animals/carcasses). The factors were two treatments (control & infused), five muscles (BF, RF, ST, SS and LL) and six times (Days 4, 7, 10, 13, 16, 19). For all the above variables measured, analyses of variance were performed using SAS statistical software Version 9.1 (SAS, 2003). The Shapiro-Wilk test was performed to test for non-normality (Shapiro & Wilk, 1965). In some cases deviations from normality were the cause of one or two outliers, which were removed and reanalysed until the residuals were symmetrically distributed or normal. Where there was still significant evidence of non-normality, this could be ascribed to kurtosis rather than skewness. Interpretation of results was thus continued (Glass *et al.*, 1972). Student's t-Least Significant Difference (LSD) was calculated at the 5% confidence level to compare treatment means of significant source effects (Ott, 1998).

Another statistical analysis was conducted on the muscles to test for the effect of the infusion on the chemical parameters (proximate and mineral composition). The design was a 2×5 factorial experiment replicated in six blocks (animals/carcasses) with factors two treatments (control & infused) and five muscles (BF, RF, ST, SS and LL). Factorial analysis of variance were performed on the chemical constituents measured, using SAS

statistical software Version 9.1 (SAS, 2003). The Shapiro-Wilk test was performed to test for non-normality (Shapiro & Wilk, 1965). In some cases deviations from normality were the cause of one or two outliers, which were removed and reanalysed until the residuals were symmetrically distributed or normal. Where there was still significant evidence of non-normality, this could be ascribed to kurtosis rather than skewness. Interpretation of results was thus continued (Glass *et al.*, 1972). Student's t-Least Significant Difference (LSD) was calculated at the 5% confidence level to compare treatment means of significant source effects (Ott, 1998).

RESULTS AND DISCUSSION

Physical properties

The results from the deboned muscles infused with the phosphate and lactate blend on Day 3 (pre- and post-infusion pH, pumped gain) are given in Table 1. In Table 2 the mean values for the physical meat quality parameters of pH, water-binding properties and shear force resistance of the *Biceps femoris* (BF, silverside), *Rectus femoris* (RF), *Semitendinosus* (ST, eye of the silverside), *Supraspinatus* (SS, scotch fillet) and *Longissimus lumborum* (LL, striploin) sub-samples are displayed. In Table 3 the data for the quality measurements of the muscles over the 19 d were pooled and the muscles means are given. In the following section the various attributes will be discussed based on the treatment and time effect.

pH and pumped gain

Beef with a normal pH between 5.4 and 5.7 has a high storage quality when kept under chilled conditions (Gill & Newton, 1981) and also has a highly acceptable colour (Zhu & Brewer, 1998), a satisfactory beef flavour and is reliably tender (Simmons *et al*., 2000). The isoelectric point of meat proteins is about 5.3 (Hamm, 1960) and at this point the proteins have minimal water-holding capacity (WHC). The WHC increases steadily with pH on either side of the isoelectric point, partially indicating that the WHC of meat is improved with a pH of up to 5.7, thus on the alkaline side (Hamm, 1982). Several studies have shown that to improve the WHC of processed meat, the pH should be increased to a desired point (Young *et al*., 2005). This is achieved by adding an alkalinising agent to the meat product, such as alkaline polyphosphates (Shults *et al*., 1972; Puolanne *et al*., 2001). This agent aids the salt-induced solubilisation of myosin and increases water binding by increasing the pH (Young *et al*., 2005).

From Table 1 it is clear that the samples of both pre-infusion treatments were reasonably similar in initial pH values on the 3rd d post-mortem. Before infusion of the blend (Day 3; 72 h post-mortem), the pH of the control samples ranged from 5.45 ± 0.043 (LL) to 5.52 ± 0.055 (RF), and the pH of the samples earmarked for infusion ranged from 5.38 ± 0.035 (LL) to 5.53 ± 0.064 (SS). After infusion the pH of the infused muscles increased substantially to a pH range of 5.55 ± 0.189 for LL to 5.77 ± 0.232 for SS (Table 1). This latter increase in pH was expected and is supported by many studies, where the effect of an alkaline solution containing polyphosphates on muscle pH is researched (Baublits *et al*., 2005). The pH difference of the control and infused muscles (Table 1) illustrated significant differences before infusion ($P \leq 0.05$) for the LL muscle, whereas after infusion the pH difference between pre- and post-infusion muscles was not significant ($P > 0.05$) illustrating that infusion decreases pH differences between muscles.

Bendall (1967) reported that phosphates increased the volume of uncooked muscles and this statement is supported by the present investigation, with an increase in muscle volume after infusion. The percentage fluid retained (pumped gain) directly after the muscles were infused ranged from 18.05 ± 2.299 (BF) to 22.93 ± 3.312 (SS) at 0 h and then decreased to 13.73 ± 2.916 (LL) to 17.59 ± 3.928 (RF) after a 2 h stabilisation period (Table 1). Previous studies reported similar pumped gain values (Hoffman, 2006).

The results pertaining to the specific change of the pH in each muscle over time are given in Table 2. The pH of the infused samples differed significantly ($P \leq 0.05$) from that of the control over the 19 d, indicating that the phosphate blend increased the muscle pH of the infused samples substantially. The pH of the samples also changed significantly ($P \leq 0.05$) over the 19-d period. The general trend in both the control and infused muscles was that of an initial increase from Day 4 to 13, from whereon the pH started to decrease significantly ($P \leq 0.05$) from Day 13 to 16. Several authors reported that the alkalinity of the muscles and thus the pH is increased when muscles were treated with a blend containing phosphates (Boles & Shand, 2001; Baublits *et al*., 2005) and with the infusion of sodium lactate (Maca *et al.*, 1999).

Water-holding and water-binding capacity

The significant effect of the phosphate blend on the muscle pH illustrated in Table 2 should result in a significant effect on the water-binding abilities of the muscle (Honikel, 1987; Scanga *et al*., 2000; Baublits *et al*., 2005) and more specifically purge loss, drip loss and cooking loss (Briskey *et al*., 1960; Crouse *et al*., 1984). Several studies reported that steaks marinated in a solution of higher pH and strong buffering capacity have increased

water-binding ability compared to steaks left untreated or marinated in solutions with a pH close to, or below, the isoelectric point of meat (Trout & Schmidt, 1986; Boles & Shand, 2001; McGee *et al*., 2003; Baublits *et al*., 2005).

The drip formed in the retail packages influences the purchase decision of the consumer negatively and represents a portion of the product that cannot be used (Payne *et al*., 1998; Andersen *et al*., 2004). In the present investigation the fluid-loss measurements consisted of the determination of purge loss (collected in vacuum bags over time), drip and cooking loss observed within both the infused and control muscles. The control gives an indication of the normal fluid loss and of the water-holding capacity (WHC) of the meat under these circumstances, where fresh meat is stored in vacuum bags at a chill temperature. The WHC of muscles treated with a phosphate and lactate blend is known as the water-binding capacity (WBC) of the infused meat, which is the ability of the meat to bind added water (Boleman *et al*., 1995).

In the present investigation (Table 2) purge loss was slightly higher for the infused muscles than for the non-infused control treatment, but it was, however, not significant ($P>0.05$). Lawrence *et al*. (2003) also found similar results, i.e. a slightly higher but not significant purge loss in muscles treated with a salt solution. The addition of salt to a solution increases the ionic strength of the solution, thereby increasing the number of hydrophilic protein interactions, which causes an increase in the binding of free water (Lawrence *et al*., 2003).

The drip loss that occurs during storage of vacuum-packed meat is representative of the degree of moisture retention of the meat after infusion (Wheeler *et al*., 1993). In the present investigation the amount of drip loss was higher for the infused samples than for the control samples, with significant differences for BF, ST and SS ($P\leq0.05$) (Table 3). Several other studies reported this effect, with a consistent increase in WHC associated with an increase in salt content (Hamm, 1960; Sherman, 1962; Wheeler *et al*., 1993; Lennon *et al*., 2006).

Table 1. Interaction means (±SD)[#] for infusion data on Day 3 of beef muscles infused with a phosphate and lactate blend.

Muscle	Pre-infusion pH		pH difference (control[d] vs infused[e])	Post-infusion pH[f]	pH difference (pre[e] vs post[f] infusion)	Pumped gain (%) 0h[g]	Pumped gain (%) 2h[h]	Pumped gain difference (%)[g-h]
	Control[d]	Infused[e]		Infused		Infused	Infused	Infused
BF	$5.45_{b} \pm 0.022$	$5.42_{bc} \pm 0.038$	$-0.03_{ab} \pm 0.027$	$5.72_{a} \pm 0.266$	$0.31_{a} \pm 0.286$	$18.05_{b} \pm 2.299$	$14.81_{b} \pm 2.152$	$3.24_{b} \pm 1.824$
RF	$5.52_{a} \pm 0.055$	$5.47_{b} \pm 0.045$	$-0.06_{b} \pm 0.031$	$5.68_{a} \pm 0.144$	$0.21_{a} \pm 0.164$	$22.14_{a} \pm 3.601$	$17.59_{a} \pm 3.928$	$4.55_{b} \pm 1.005$
ST	$5.45_{b} \pm 0.034$	$5.40_{c} \pm 0.025$	$-0.05_{ab} \pm 0.050$	$5.68_{a} \pm 0.272$	$0.28_{a} \pm 0.264$	$19.43_{ab} \pm 4.881$	$15.72_{ab} \pm 4.797$	$3.71_{b} \pm 1.894$
SS	$5.51_{a} \pm 0.055$	$5.53_{a} \pm 0.064$	$0.02_{a} \pm 0.091$	$5.77_{a} \pm 0.232$	$0.24_{a} \pm 0.249$	$22.93_{a} \pm 3.312$	$16.12_{ab} \pm 2.407$	$6.81_{a} \pm 1.245$
LL	$5.45_{b} \pm 0.043$	$5.38_{c} \pm 0.035$	$-0.07_{b} \pm 0.031$	$5.55_{a} \pm 0.189$	$0.17_{a} \pm 0.208$	$20.53_{ab} \pm 4.126$	$13.73_{b} \pm 2.916$	$6.80_{a} \pm 1.578$
LSD (*P*=0.05)	0.047	0.054	0.066	0.265	0.279	3.577	2.695	1.891

[#] SD: Standard deviation.

BF: *Biceps femoris.*

RF: *Rectus femoris.*

ST: *Semitendinosus.*

SS: *Supraspinatus.*

LL: *Longissimus lumborum.*

$_{a, b, c}$ Means in the same column within a treatment and between muscles, with different subscripts are significantly different ($P \leq 0.05$).

[d,e] Pre-infusion pH: pH measured of both the control[d] and the infused[e] muscles before infusion.

pH difference[e-d] (control[d] vs injected[e]): the difference between the control and infused muscles before infusion.

[f] Post-infusion pH: pH measured of the infused muscles directly after infusion.

pH difference[f-e] (pre[e] vs post[f] infusion): the difference in pH between the infused muscles before and after infusion.

[g] Pumped gain (%) 0 h[g]: the amount of blend retained within the muscles directly after infusion.

[h] Pumped gain (%) 2 h[h]: the amount of blend retained within the muscles 2 h (resting period) after infusion.

Pumped gain difference[g-h]: the difference in pumped gain between the infused muscles before and after infusion.

LSD: Least significant difference (*P*=0.05).

Several authors reported a significant reduction in cooking loss when treating muscle with a salt solution similar to that of the present study (Bouton *et al*., 1982; Sheard *et al*., 1999). Despite the slightly higher cooking loss observed in the infused samples in the present investigation (Table 2), most of the infused muscles did not have significantly higher cooking loss values than the untreated muscles ($P>0.05$). The relatively similar cooking loss values of the control and infused muscles indicate that infusion did not have a negative effect on cooking loss in this investigation. However, there were differences within some of the muscles over storage time. For example, the BF and RF control and infused muscles differed significantly ($P\leq0.05$) from Day 4 to 13, after which both treatments stabilised and showed similar cooking losses ($P>0.05$). Generally, the cooking loss of the LL, ST and SS control and infused samples (Table 2) followed a similar pattern ($P>0.05$). Other authors also reported results of infused muscles indicating numerically higher cooking loss, but similar to the untreated muscles ($P>0.05$) (Baublits *et al*., 2006).

Table 3 illustrates the overall effect between treatments and between muscles for pH and water-binding capacity. The pH, purge, drip and cooking loss increased significantly ($P\leq0.05$) with infusion in most of the muscles.

Instrumental tenderness

Meat tenderness (Miller *et al*., 1995; Vestergaard *et al*., 2000) is the single most important factor in consumer acceptance (Taylor *et al*., 1995). Consumer complaints regarding tenderness and tenderness variation are of primary concern to the meat industry (Pearson, 1994; Sørheim & Hildrum, 2002).

The Warner-Bratzler shear force (WBSF) values of the various muscles measured over time are given in Table 2. A significant treatment effect ($P\leq0.05$) was achieved in the present study when a phosphate and salt solution was used to infuse the respective beef muscles, with significantly reduced WBSF values obtained for all the infused samples on the respective days. This result illustrates that infusion has a substantial and positive effect on meat tenderness. Vote *et al*. (2000) report significant treatment differences between control and infused samples. Stites *et al*. (1989) also found that when beef roasts were injected with a solution containing sodium tripolyphosphate and sodium chloride, the WBSF values were significantly lowered when compared to those of the control samples. Authors such as McGee *et al*. (2003) have shown that the injection of a sodium lactate-phosphate-chloride brine in beef inside round roasts resulted in decreased instrumental tenderness.

The time effect showed that all the muscles illustrated significant differences in tenderness ($P \leq 0.05$) over time (Table 2). Both the control and infused muscles showed a pattern of decreased shear force with time from Day 7 to 19. Therefore, over time a fair amount of conditioning (ageing and tenderisation) took place in both treatments. The initial shear force of some of the untreated and infused muscles was low on Day 4 and then it increased to Day 7. No clear explanation could be found to support this result. Reports on the effect of ageing on tenderness are contradictory, with some studies reporting no influence of ageing on WBSF, whereas others found a significant decrease in WBSF values throughout the ageing period, thus a significant improvement of the tenderness over time (French *et al*., 2001; Maria *et al*., 2003).

Table 3 illustrates the overall effect between treatments and between muscles for WBSF values. The shear force decreased substantially ($P \leq 0.05$) with infusion in all the muscles. The latter trend illustrates the positive effect of infusion on meat tenderness. Support muscles are reported to be more tender than locomotive muscles (Belew *et al*., 2003), however, with infusion this factor is not relevant, suggesting that the blend tenderises all the muscles to an acceptable level. In this investigation the infused muscles BF (38.90 N), RF (36.06 N) and LL (41.08 N) had significantly lower WBSF values than ST (47.63 N) and SS (47.26 N). The relatively high pH of the latter two samples could be ascribed to the initial high pH of the untreated samples.

Table 2. Interaction means (±SD)[#] for physical attributes of beef muscles infused with a phosphate and lactate blend and aged for 19 d.

Day	pH		Purge loss (%)		Drip loss (%)		Cooking loss (%)		WBSF (N)	
	Control	Infused	Control	Infused	Control	Infused	Control	Infused	Control	Infused
Biceps femoris (BF)										
4	$5.45_{ab}^{b} \pm 0.039$	$5.67_{bc}^{a} \pm 0.046$	$1.53_{b}^{a} \pm 0.487$	$2.93_{b}^{a} \pm 0.398$	$0.99_{ab}^{b} \pm 0.314$	$1.69_{a}^{a} \pm 0.334$	$34.22_{b}^{a} \pm 0.796$	$35.42_{c}^{a} \pm 1.854$	$46.26_{c}^{a} \pm 5.536$	$33.78_{b}^{b} \pm 5.110$
7	$5.45_{ab}^{b} \pm 0.044$	$5.65_{bc}^{a} \pm 0.011$	$2.80_{ab}^{a} \pm 0.886$	$4.36_{ab}^{a} \pm 1.463$	$1.10_{a}^{a} \pm 0.390$	$1.27_{b}^{a} \pm 0.407$	$41.55_{a}^{b} \pm 1.017$	$44.46_{a}^{a} \pm 1.818$	$60.86_{ab}^{a} \pm 6.246$	$47.19_{a}^{b} \pm 7.804$
10	$5.46_{ab}^{b} \pm 0.035$	$5.77_{a}^{a} \pm 0.100$	$3.53_{a}^{a} \pm 1.425$	$4.72_{a}^{a} \pm 1.825$	$0.98_{ab}^{a} \pm 0.120$	$1.12_{bc}^{a} \pm 0.303$	$41.12_{a}^{b} \pm 2.263$	$42.93_{ab}^{a} \pm 2.013$	$56.05_{abc}^{a} \pm 5.497$	$40.37_{ab}^{b} \pm 9.840$
13	$5.52_{a}^{b} \pm 0.029$	$5.72_{ab}^{a} \pm 0.064$	$2.92_{ab}^{b} \pm 1.844$	$4.56_{a}^{a} \pm 0.870$	$1.26_{a}^{b} \pm 0.191$	$1.61_{a}^{a} \pm 0.502$	$41.13_{a}^{b} \pm 0.940$	$44.35_{a}^{a} \pm 1.704$	$61.73_{a}^{a} \pm 13.64$	$45.95_{a}^{b} \pm 8.260$
16	$5.43_{b}^{b} \pm 0.046$	$5.63_{c}^{a} \pm 0.095$	$4.25_{a}^{a} \pm 1.591$	$5.37_{a}^{a} \pm 1.097$	$0.70_{b}^{a} \pm 0.121$	$0.81_{c}^{a} \pm 0.122$	$40.65_{a}^{a} \pm 0.796$	$42.00_{b}^{a} \pm 2.706$	$51.44_{bc}^{a} \pm 11.23$	$33.69_{b}^{b} \pm 7.859$
19	$5.34_{c}^{b} \pm 0.038$	$5.66_{bc}^{a} \pm 0.079$	$3.99_{a}^{a} \pm 1.184$	$4.03_{ab}^{a} \pm 1.054$	$1.13_{a}^{a} \pm 0.160$	$1.10_{bc}^{a} \pm 0.213$	$40.86_{a}^{a} \pm 1.483$	$41.45_{b}^{a} \pm 2.142$	$47.04_{c}^{a} \pm 12.05$	$32.42_{b}^{b} \pm 4.560$
Rectus femoris (RF)										
4	$5.42_{bc}^{b} \pm 0.034$	$5.65_{b}^{a} \pm 0.081$	$4.54_{a}^{a} \pm 2.089$	$5.87_{a}^{a} \pm 1.496$	$1.39_{ab}^{b} \pm 0.290$	$2.06_{a}^{a} \pm 0.339$	$37.32_{c}^{b} \pm 2.275$	$40.13_{b}^{a} \pm 2.162$	$58.68_{ab}^{a} \pm 11.82$	$41.53_{ab}^{b} \pm 5.809$
7	$5.46_{b}^{b} \pm 0.063$	$5.79_{a}^{a} \pm 0.116$	$3.92_{a}^{a} \pm 2.007$	$5.47_{a}^{a} \pm 1.650$	$1.24_{b}^{b} \pm 0.216$	$1.57_{b}^{a} \pm 0.275$	$40.36_{b}^{b} \pm 1.739$	$42.75_{a}^{a} \pm 2.617$	$61.00_{a}^{a} \pm 13.36$	$42.61_{a}^{b} \pm 8.977$
10	$5.50_{ab}^{b} \pm 0.058$	$5.78_{a}^{a} \pm 0.087$	$4.44_{a}^{a} \pm 1.195$	$5.43_{a}^{a} \pm 1.453$	$1.23_{b}^{a} \pm 0.117$	$1.21_{c}^{a} \pm 0.152$	$40.33_{b}^{b} \pm 1.003$	$42.06_{a}^{a} \pm 1.502$	$52.96_{abc}^{a} \pm 4.048$	$36.72_{abc}^{b} \pm 7.977$
13	$5.58_{a}^{b} \pm 0.082$	$5.79_{a}^{a} \pm 0.116$	$3.72_{a}^{b} \pm 1.308$	$6.64_{a}^{a} \pm 1.705$	$1.60_{a}^{a} \pm 0.190$	$1.41_{bc}^{a} \pm 0.220$	$40.03_{b}^{b} \pm 1.557$	$41.79_{a}^{a} \pm 1.697$	$50.08_{bc}^{a} \pm 8.457$	$32.72_{abc}^{b} \pm 2.489$
16	$5.47_{b}^{b} \pm 0.045$	$5.77_{a}^{a} \pm 0.099$	$4.55_{a}^{a} \pm 1.126$	$5.80_{a}^{a} \pm 1.466$	$1.16_{b}^{a} \pm 0.137$	$1.11_{c}^{a} \pm 0.315$	$42.44_{a}^{a} \pm 1.658$	$43.39_{a}^{a} \pm 2.615$	$51.52_{abc}^{a} \pm 8.748$	$27.44_{c}^{b} \pm 7.322$
19	$5.36_{c}^{b} \pm 0.058$	$5.63_{b}^{a} \pm 0.090$	$4.26_{a}^{b} \pm 0.871$	$6.50_{a}^{a} \pm 1.156$	$1.18_{b}^{a} \pm 0.290$	$1.27_{bc}^{a} \pm 0.187$	$40.84_{ab}^{a} \pm 2.471$	$42.05_{a}^{a} \pm 2.261$	$48.44_{c}^{a} \pm 9.766$	$35.32_{abc}^{b} \pm 8.998$
Semitendinosus (ST)										
4	$5.45_{b}^{b} \pm 0.030$	$5.73_{a}^{a} \pm 0.112$	$2.67_{b}^{b} \pm 1.100$	$6.61_{a}^{a} \pm 1.040$	$0.98_{ab}^{b} \pm 0.507$	$2.75_{a}^{a} \pm 0.392$	$39.02_{c}^{b} \pm 1.330$	$41.74_{bc}^{a} \pm 2.277$	$86.23_{ab}^{a} \pm 11.88$	$51.48_{a}^{b} \pm 9.642$
7	$5.43_{b}^{b} \pm 0.028$	$5.65_{ab}^{a} \pm 0.135$	$4.47_{a}^{b} \pm 1.27$	$7.77_{a}^{a} \pm 1.224$	$0.85_{b}^{b} \pm 0.327$	$1.42_{b}^{a} \pm 0.358$	$42.11_{a}^{a} \pm 0.914$	$43.64_{a}^{a} \pm 1.845$	$92.64_{a}^{a} \pm 14.43$	$48.56_{ab}^{b} \pm 8.630$
10	$5.48_{ab}^{b} \pm 0.053$	$5.65_{ab}^{a} \pm 0.084$	$3.72_{ab}^{b} \pm 1.486$	$8.08_{a}^{a} \pm 1.633$	$0.79_{b}^{a} \pm 0.297$	$0.84_{c}^{a} \pm 0.168$	$40.17_{bc}^{a} \pm 1.384$	$40.42_{c}^{a} \pm 2.894$	$79.10_{bc}^{a} \pm 19.42$	$48.99_{a}^{b} \pm 15.88$
13	$5.55_{a}^{b} \pm 0.041$	$5.72_{a}^{a} \pm 0.102$	$3.65_{ab}^{b} \pm 1.805$	$6.88_{a}^{a} \pm 1.312$	$1.27_{a}^{a} \pm 0.233$	$1.58_{b}^{a} \pm 0.481$	$41.70_{ab}^{a} \pm 0.845$	$42.66_{ab}^{a} \pm 1.542$	$82.35_{b}^{a} \pm 11.85$	$47.68_{ab}^{b} \pm 13.18$
16	$5.41_{bc}^{b} \pm 0.050$	$5.63_{b}^{a} \pm 0.126$	$5.16_{a}^{b} \pm 1.866$	$6.93_{a}^{a} \pm 1.648$	$0.73_{b}^{a} \pm 0.116$	$0.96_{c}^{a} \pm 0.298$	$40.69_{ab}^{a} \pm 1.100$	$41.45_{bc}^{a} \pm 2.340$	$80.09_{bc}^{a} \pm 19.82$	$50.46_{a}^{b} \pm 14.99$
19	$5.33_{c}^{b} \pm 0.053$	$5.58_{b}^{a} \pm 0.092$	$5.21_{a}^{b} \pm 2.634$	$7.97_{a}^{a} \pm 2.118$	$0.90_{b}^{a} \pm 0.076$	$1.04_{c}^{a} \pm 0.118$	$41.11_{ab}^{a} \pm 0.966$	$42.60_{ab}^{a} \pm 1.633$	$70.93_{c}^{a} \pm 7.007$	$38.77_{b}^{b} \pm 4.275$

Table 2 (continue). Interaction means (±SD)[#] for physical attributes of beef muscles infused with a phosphate and lactate blend and aged for 19 d.

Day	pH		Purge loss (%)		Drip loss (%)		Cooking loss (%)		WBSF (N)	
	Control	Infused	Control	Infused	Control	Infused	Control	Infused	Control	Infused
Supraspinatus (SS)										
4	$5.51_{bc}^{b} \pm 0.026$	$5.81_{bc}^{a} \pm 0.097$	$2.16_{b}^{b} \pm 0.572$	$5.09_{b}^{a} \pm 1.282$	$0.72_{b}^{b} \pm 0.116$	$1.26_{ab}^{a} \pm 0.402$	$40.45_{c}^{b} \pm 1.087$	$42.11_{c}^{a} \pm 1.785$	$69.71_{ab}^{a} \pm 8.950$	$54.21_{a}^{b} \pm 7.283$
7	$5.60_{a}^{b} \pm 0.051$	$5.88_{ab}^{a} \pm 0.065$	$3.17_{ab}^{b} \pm 0.663$	$5.28_{ab}^{a} \pm 1.134$	$0.79_{b}^{b} \pm 0.172$	$1.15_{ab}^{a} \pm 0.393$	$45.64_{a}^{a} \pm 1.739$	$46.39_{a}^{a} \pm 2.201$	$77.37_{a}^{a} \pm 10.87$	$47.23_{ab}^{b} \pm 3.842$
10	$5.64_{a}^{b} \pm 0.033$	$5.95_{a}^{a} \pm 0.119$	$3.45_{ab}^{b} \pm 0.800$	$6.11_{ab}^{a} \pm 1.572$	$0.85_{b}^{a} \pm 0.100$	$0.95_{b}^{a} \pm 0.292$	$45.04_{ab}^{a} \pm 0.559$	$45.11_{ab}^{a} \pm 1.423$	$70.80_{ab}^{a} \pm 9.375$	$48.11_{ab}^{b} \pm 3.219$
13	$5.59_{ab}^{b} \pm 0.079$	$5.84_{bc}^{a} \pm 0.118$	$3.15_{ab}^{b} \pm 1.151$	$6.20_{ab}^{a} \pm 1.500$	$1.52_{a}^{a} \pm 0.222$	$1.47_{a}^{a} \pm 0.534$	$43.72_{b}^{a} \pm 1.182$	$44.46_{b}^{a} \pm 1.194$	$63.57_{b}^{a} \pm 8.859$	$47.03_{ab}^{b} \pm 5.649$
16	$5.60_{a}^{b} \pm 0.047$	$5.86_{bc}^{a} \pm 0.080$	$3.64_{ab}^{b} \pm 1.660$	$6.25_{ab}^{a} \pm 1.395$	$0.86_{b}^{a} \pm 0.195$	$1.03_{b}^{a} \pm 0.214$	$44.55_{ab}^{a} \pm 1.459$	$43.83_{b}^{a} \pm 1.633$	$65.22_{b}^{a} \pm 3.509$	$45.95_{ab}^{b} \pm 8.342$
19	$5.48_{c}^{b} \pm 0.067$	$5.78_{c}^{a} \pm 0.076$	$4.15_{a}^{b} \pm 1.753$	$6.83_{a}^{a} \pm 1.955$	$0.96_{b}^{a} \pm 0.137$	$1.15_{ab}^{a} \pm 0.143$	$43.83_{b}^{a} \pm 2.138$	$44.14_{b}^{a} \pm 1.585$	$66.11_{b}^{a} \pm 6.237$	$41.04_{b}^{b} \pm 4.975$
Longissimus lumborum (LL)										
4	$5.40_{bc}^{b} \pm 0.026$	$5.55_{c}^{a} \pm 0.077$	$2.90_{b}^{b} \pm 0.781$	$4.96_{b}^{a} \pm 1.013$	$1.19_{b}^{a} \pm 0.171$	$1.22_{b}^{a} \pm 0.385$	$40.11_{abc}^{a} \pm 1.138$	$40.02_{bc}^{a} \pm 2.043$	$79.04_{a}^{a} \pm 16.67$	$54.73_{a}^{b} \pm 9.705$
7	$5.47_{ab}^{b} \pm 0.018$	$5.63_{bc}^{a} \pm 0.077$	$6.04_{a}^{a} \pm 1.128$	$6.39_{ab}^{a} \pm 1.434$	$1.91_{a}^{a} \pm 0.450$	$1.94_{a}^{a} \pm 0.349$	$40.90_{ab}^{a} \pm 1.436$	$41.71_{a}^{a} \pm 2.567$	$75.78_{a}^{a} \pm 20.17$	$49.21_{ab}^{b} \pm 19.90$
10	$5.45_{ab}^{b} \pm 0.057$	$5.70_{ab}^{a} \pm 0.071$	$5.76_{a}^{a} \pm 1.296$	$7.01_{a}^{a} \pm 0.893$	$1.01_{b}^{a} \pm 0.438$	$1.14_{b}^{a} \pm 0.454$	$39.83_{bc}^{a} \pm 1.249$	$39.74_{bc}^{a} \pm 1.769$	$63.96_{b}^{a} \pm 10.56$	$39.29_{bc}^{b} \pm 17.44$
13	$5.52_{a}^{b} \pm 0.046$	$5.73_{a}^{a} \pm 0.091$	$5.28_{a}^{a} \pm 1.256$	$6.15_{ab}^{a} \pm 0.819$	$1.28_{b}^{a} \pm 0.224$	$1.40_{b}^{a} \pm 0.249$	$38.54_{c}^{a} \pm 1.528$	$39.38_{c}^{a} \pm 2.604$	$54.93_{b}^{a} \pm 11.22$	$39.12_{c}^{b} \pm 17.98$
16	$5.42_{b}^{b} \pm 0.018$	$5.57_{c}^{a} \pm 0.088$	$5.62_{a}^{a} \pm 1.748$	$7.12_{a}^{a} \pm 1.845$	$1.06_{b}^{a} \pm 0.218$	$1.34_{b}^{a} \pm 0.373$	$41.14_{ab}^{a} \pm 1.261$	$41.14_{ab}^{a} \pm 1.862$	$58.69_{b}^{a} \pm 11.44$	$33.65_{c}^{b} \pm 12.44$
19	$5.33_{c}^{b} \pm 0.058$	$5.57_{c}^{a} \pm 0.073$	$5.37_{a}^{a} \pm 1.097$	$6.20_{ab}^{a} \pm 1.726$	$0.97_{b}^{a} \pm 0.164$	$1.16_{b}^{a} \pm 0.242$	$41.68_{a}^{a} \pm 1.155$	$41.36_{ab}^{a} \pm 0.958$	$58.56_{b}^{a} \pm 15.25$	$30.47_{c}^{b} \pm 8.662$
LSD *P*=0.05	1.968		1.568		0.324		1.654		10.01	

[#] SD: Standard deviation.

$_{a, b, c}$ Means in the same column within a treatment and between days, with different subscripts are significantly different ($P \leq 0.05$).

$^{a, b}$ Means in the same row within an attribute and between treatments, with different superscripts are significantly different ($P \leq 0.05$).

LSD: Least significant difference ($P=0.05$).

Table 3. Summary of interaction means (±SD)[#] for physical attributes of beef muscles infused with a phosphate and lactate blend and aged for 19 d.

Muscle	pH		Purge loss (%)		Drip loss (%)		Cooking loss (%)		WBSF (N)	
	Control	Infused	Control	Infused	Control	Infused	Control	Infused	Control	Infused
BF	$5.44_{b}^{b} \pm 0.065$	$5.68_{c}^{a} \pm 0.083$	$3.17_{c}^{b} \pm 1.523$	$4.33_{c}^{a} \pm 1.347$	$1.03_{b}^{b} \pm 0.288$	$1.24_{bc}^{a} \pm 0.428$	$39.92_{c}^{b} \pm 2.876$	$41.77_{b}^{a} \pm 3.634$	$53.90_{c}^{a} \pm 10.88$	$38.90_{bc}^{b} \pm 9.21$
RF	$5.46_{b}^{b} \pm 0.088$	$5.73_{b}^{a} \pm 0.113$	$4.24_{b}^{b} \pm 1.428$	$5.95_{b}^{a} \pm 1.465$	$1.30_{a}^{a} \pm 0.254$	$1.42_{a}^{a} \pm 0.390$	$40.22_{bc}^{b} \pm 2.302$	$42.03_{b}^{a} \pm 2.263$	$53.78_{c}^{a} \pm 10.19$	$36.06_{c}^{b} \pm 8.53$
ST	$5.44_{b}^{b} \pm 0.079$	$5.66_{c}^{a} \pm 0.115$	$4.16_{b}^{b} \pm 1.882$	$7.37_{a}^{a} \pm 1.541$	$0.92_{b}^{b} \pm 0.321$	$1.43_{a}^{a} \pm 0.728$	$40.80_{b}^{b} \pm 1.454$	$42.09_{b}^{a} \pm 2.238$	$82.20_{a}^{a} \pm 15.31$	$47.63_{a}^{b} \pm 11.79$
SS	$5.57_{a}^{b} \pm 0.075$	$5.86_{a}^{a} \pm 0.103$	$3.29_{c}^{b} \pm 1.279$	$5.96_{b}^{a} \pm 1.511$	$0.95_{b}^{b} \pm 0.307$	$1.17_{c}^{a} \pm 0.376$	$43.72_{a}^{a} \pm 2.241$	$44.34_{a}^{a} \pm 2.028$	$68.79_{b}^{a} \pm 9.00$	$47.26_{a}^{b} \pm 6.67$
LL	$5.43_{b}^{b} \pm 0.073$	$5.62_{d}^{a} \pm 0.102$	$5.16_{a}^{b} \pm 1.569$	$6.31_{b}^{a} \pm 1.446$	$1.22_{a}^{a} \pm 0.412$	$1.35_{ab}^{a} \pm 0.416$	$40.35_{bc}^{a} \pm 1.593$	$40.56_{c}^{a} \pm 2.093$	$65.70_{b}^{a} \pm 16.66$	$41.08_{b}^{b} \pm 16.30$
LSD *P*=0.05	0.033		0.640		0.132		0.668		4.083	

[#] SD: Standard deviation.

BF: *Biceps femoris.*

RF: *Rectus femoris.*

ST: *Semitendinosus.*

SS: *Supraspinatus.*

LL: *Longissimus lumborum.*

$_{a, b, c}$ Means in the same column within a treatment and between muscles, with different subscripts are significantly different ($P \leq 0.05$).

$^{a, b}$ Means in the same row within an attribute and between treatments, with different superscripts are significantly different ($P \leq 0.05$).

LSD: Least significant difference ($P=0.05$).

Instrumental meat colour

The appearance of the meat is the first important criterion that consumers take into account when judging meat quality (Barton-Gade *et al*., 1988; Mancini & Hunt, 2005). Consumers prefer beef to appear bright red (Carpenter *et al*., 2001), however, the latter is limited to only 3 to 5 d of retail display (Wheeler *et al*., 1993). Maintaining the bright red oxygenated colour of fresh retail beef is dependent on various factors, i.e. muscle pigment, pH, pre- and post-slaughter handling, and cut variability (Brody, 2000). Muscle type accounts for approximately half of the variances in colour stability and is an important controlling factor in the discolouration rate of beef muscle when exposed to oxygen (Hood, 1980; Varnam & Sutherland, 1995). Enhancement solutions also play a significant role in beef colour development (Baublits *et al*., 2005). Sodium lactate, for example, influences the colour parameters of beef, resulting in decreased L* and b* values and increased a* values. This effect becomes more apparent with increased levels of sodium lactate (Papadopoulos *et al*., 1991).

The parameters used in this investigation to evaluate the colour of the raw, as well as the cooked meat samples are the L*, a*, b* and chroma values, as well as hue angle.

The L* value gives an indication of lightness, with perfect white being a 100 and black a 0 (zero) (Papadopoulos *et al*., 1991). Overall there was no significant interaction between treatment and storage time ($P>0.05$). Although the L* values fluctuated during storage ($P<0.05$), there was no noticeable pattern (Table 4). Pooled over time and processing days (Table 5), L* values for the raw infused muscles ranged from 38.52 ± 2.361 (SS) to 42.97 ± 3.326 (RF). These results are supported by other studies, where a similar blend was used for infusion (Papadopoulos *et al*., 1991). From Table 4 it is clear that the L* values of the infused and untreated samples only differed significantly ($P\leq0.05$) in a limited number of cases. However, according to Table 5 (illustrating the overall effect), four of the infused samples had significantly ($P\leq0.05$) lower L* values than the control samples, indicating a significantly darker meat colour for the BF, RF, SS and LL infused samples. Lawrence *et al*. (2004) reported contradictory results to the results from the present investigation. Lawrence *et al*. (2004) found that beef samples treated with either a lactate or chloride solution, showed similar L* values to those of the control. Conversely, Baublits *et al*. (2005a) reported that the treated samples had higher L* values, thus lighter in colour than the control samples. However, the latter result was obtained with the inclusion of only phosphates (Baublits *et al*., 2005). With the addition of sodium chloride (NaCl) to the blend, as was the case in the present investigation, the overall colour becomes darker (Baublits *et al*., 2005; Hoffman, 2006).

The a* value measures chromaticity, with a positive value indicating redness and a negative value greenness. Therefore, greater a* values indicate a redder beef sample, whereas similar a* and b* values indicate a purple meat colour. In this investigation (Table 4) there was no interaction (P<0.05) between the sodium lactate level and storage time. However, there was a tendency for both the infused and control samples to increase in redness (higher a* values) with time, indicating a deterioration as the muscles became darker over time. With the addition of a salt solution, the redness of muscle samples have been observed to decrease and therefore a darker sample colour is obtained (Baublits *et al*., 2006). And the well-documented deterioration of fresh meat with storage even under vacuum packaging is the logical explanation of the decreasing meat colour. Pooled over time (Table 5), redness (a*) was significantly (P≤0.05) lower in all the treated samples with means for the raw muscles ranging from 13.67 ± 1.958 (RF) to 15.79 ± 1.772 (BF). Again, the results are supported by other studies, where a similar blend was used for infusion and the effect on colour parameters determined (Papadopoulos *et al*., 1991). Baublits *et al*. (2005b) also reported control muscles to have a redder colour (higher a* values) than the treated muscles. According to Baublits *et al*. (2005a), limited differences were observed between the control and muscles treated with phosphates and NaCl, however, there was a tendency for the phosphate and salt enhanced samples to have lower a* values, suggesting the deleterious effects of salt in meat colour (Baublits *et al*., 2006). In the present investigation much lower a* values were observed than reported by Baublits *et al*. (2005a).

The b* values also measure chromaticity with positive values indicating yellowness and negative values blueness, with a b* value of 0 (zero) indicating a grey appearance. With meat, greater b* values indicate a visual description of brown (Carpenter *et al*., 2001). In this investigation (Table 4) there was no consistent treatment effect, however, there was a tendency for the b* values to be lower in the treated samples. Furthermore, there was no significant interaction (P<0.05) between the phosphate and lactate blend and storage time, and therefore the b* value did not change significantly nor consistently during storage. The report by Papadopoulos *et al*. (1991), where a similar blend was used for infusion, showed comparable colour results. Pooled over time (Table 5) the means of the raw muscles treated with the blend ranged from 12.71 ± 1.612 (SS) to 14.90 ± 1.995 (ST). All the infused muscles had significantly lower b* values (P≤0.05), indicating a lower degree of brownness. Baublits *et al*. (2005b) also reported control muscles to have a more yellow colour (higher b* values) than the treated muscles.

Saturation index is defined by higher chroma values, indicating greater saturation or vividness of colour (Baublits *et al*., 2005). As illustrated in Table 4, as well the pooled data in Table 5 the treatment had a significant effect ($P \leq 0.05$) on all the muscles, with the infused muscles having significantly lower chroma values, i.e. degree of saturation. Lawrence *et al*. (2003) also report control muscles to have more intense red colour (higher chroma values) than the treated muscles. Baublits *et al*. (2005a) report similar degrees of vividness for both treated and untreated samples. The latter result suggests that phosphates can maintain or increase vividness, however, in combination with NaCl the vividness is hindered. In the present investigation, NaCl formed part of the blend and resulted in a poorer and less saturated raw colour. This again illustrates the negative effect of NaCl on beef colour (Baublits *et al*., 2005). The colour change over time (Table 4) was inconsistent and a non-significant change ($P > 0.05$) in all the muscles was observed within treatments and over time.

According to Baublits *et al*. (2005a), the inclusion of phosphate-based solutions increases or results in similar hue angles to those of the control samples. However, with the addition of NaCl the hue angle decreases, indicating a deterioration of redness when NaCl is included. In the present investigation the infusion had no significant ($P > 0.05$) effect on the hue angle (Table 4 and 5) and with time the pattern was inconsistent in both the control and infused samples. According to the pooled data (Table 5), four of the five muscles had similar hue angles ($P > 0.05$). Only the infused ST had a significantly higher hue angle and is supported by other research studies, which reported higher hue angles for infused muscles (Baublits *et al.,* 2005, Lawrence *et al*., 2003).

The results on the instrumental colour of the cooked samples are illustrated in Table 6. In general the blend did not affect the muscle lightness (L*) of the cooked muscles significantly (Table 6). Overall, however, the L* values of the infused samples were significantly higher ($P \leq 0.05$) and the infused samples were therefore slightly lighter in appearance (Table 7). No significant pattern ($P > 0.05$) over time with regard to lightness was visible within treatments (Table 6).

The a* value also showed no significant ($P > 0.05$) effect with regard to the treatment (Table 6). The change within treatment over time also indicated no pattern and suggests no significant ($P > 0.05$) change over time (Table 6). Overall (Table 7), the infused samples were generally significantly ($P \leq 0.05$) lower in cooked a* colour. The b* and chroma values followed similar patterns, i.e. significantly lower ($P \leq 0.05$) values in the infused muscles.

Table 4. Interaction means (±SD)[#] for colour attributes of the raw beef muscles infused with a phosphate and lactate blend and aged for 19 d.

Day	Raw L*		Raw a*		Raw b*		Raw chroma		Raw hue angle	
	Control	Infused	Control	Infused	Control	Infused	Control	Infused	Control	Infused
Biceps femoris (BF)										
4	$39.29_{a}^{a} \pm 1.758$	$39.90_{a}^{a} \pm 1.741$	$15.33_{e}^{a} \pm 1.470$	$14.19_{d}^{a} \pm 1.297$	$13.57_{bc}^{a} \pm 1.585$	$12.70_{c}^{a} \pm 1.535$	$20.50_{c}^{a} \pm 2.053$	$19.08_{c}^{a} \pm 1.823$	$41.46_{a}^{a} \pm 1.940$	$41.46_{a}^{a} \pm 2.577$
7	$39.78_{a}^{a} \pm 1.903$	$39.35_{a}^{a} \pm 1.387$	$16.06_{de}^{a} \pm 1.814$	$15.65_{bc}^{a} \pm 1.742$	$13.17_{c}^{a} \pm 2.674$	$14.46_{ab}^{a} \pm 1.643$	$20.87_{c}^{a} \pm 2.834$	$21.36_{ab}^{a} \pm 2.260$	$38.88_{a}^{b} \pm 4.039$	$42.61_{a}^{a} \pm 2.193$
10	$39.94_{a}^{a} \pm 2.637$	$38.51_{ab}^{a} \pm 2.285$	$16.95_{cd}^{a} \pm 2.006$	$14.97_{cd}^{b} \pm 0.778$	$15.05_{ab}^{a} \pm 2.173$	$13.03_{bc}^{b} \pm 1.817$	$22.69_{b}^{a} \pm 2.874$	$19.91_{bc}^{b} \pm 1.440$	$41.53_{a}^{a} \pm 1.605$	$40.88_{a}^{a} \pm 3.796$
13	$40.93_{a}^{a} \pm 2.601$	$39.02_{ab}^{a} \pm 1.950$	$18.34_{ab}^{a} \pm 1.377$	$16.26_{ab}^{b} \pm 1.432$	$16.35_{a}^{a} \pm 1.080$	$14.39_{ab}^{b} \pm 1.575$	$24.58_{a}^{a} \pm 1.637$	$21.75_{a}^{b} \pm 1.899$	$41.71_{a}^{a} \pm 1.445$	$41.61_{a}^{a} \pm 2.781$
16	$40.60_{a}^{a} \pm 1.796$	$40.52_{a}^{a} \pm 2.036$	$19.48_{a}^{a} \pm 1.206$	$17.44_{a}^{b} \pm 2.325$	$15.95_{a}^{a} \pm 1.047$	$14.66_{a}^{a} \pm 1.439$	$25.20_{a}^{a} \pm 1.356$	$22.80_{a}^{b} \pm 2.610$	$39.29_{a}^{a} \pm 1.939$	$40.11_{a}^{a} \pm 2.022$
19	$39.43_{a}^{a} \pm 1.157$	$36.94_{b}^{b} \pm 2.049$	$18.10_{bc}^{a} \pm 0.987$	$16.26_{ab}^{b} \pm 1.223$	$16.16_{a}^{a} \pm 1.236$	$14.35_{ab}^{b} \pm 1.777$	$24.30_{ab}^{a} \pm 1.444$	$21.76_{a}^{b} \pm 1.780$	$41.66_{a}^{a} \pm 1.565$	$41.34_{a}^{a} \pm 3.188$
Rectus femoris (RF)										
4	$49.74_{a}^{a} \pm 4.205$	$48.01_{a}^{a} \pm 1.868$	$13.19_{d}^{a} \pm 1.131$	$12.51_{d}^{a} \pm 0.766$	$15.43_{ab}^{a} \pm 1.392$	$14.75_{a}^{a} \pm 1.045$	$20.33_{c}^{a} \pm 1.502$	$19.38_{b}^{a} \pm 1.181$	$49.44_{a}^{a} \pm 2.767$	$49.65_{a}^{a} \pm 1.518$
7	$43.48_{b}^{a} \pm 2.746$	$41.07_{b}^{b} \pm 2.747$	$14.41_{cd}^{a} \pm 1.587$	$12.72_{cd}^{b} \pm 2.129$	$14.71_{b}^{a} \pm 1.538$	$12.06_{b}^{b} \pm 2.102$	$20.68_{c}^{a} \pm 1.800$	$17.65_{c}^{b} \pm 2.602$	$45.56_{b}^{a} \pm 3.479$	$43.32_{b}^{a} \pm 4.634$
10	$42.95_{b}^{a} \pm 1.749$	$42.04_{b}^{a} \pm 1.487$	$15.06_{bc}^{a} \pm 1.666$	$13.84_{abc}^{a} \pm 2.803$	$15.54_{ab}^{a} \pm 1.164$	$13.20_{b}^{b} \pm 1.594$	$21.67_{bc}^{a} \pm 1.797$	$19.19_{bc}^{b} \pm 2.958$	$45.99_{b}^{a} \pm 2.560$	$44.40_{b}^{a} \pm 4.230$
13	$44.66_{b}^{a} \pm 3.087$	$42.75_{b}^{a} \pm 3.189$	$16.32_{ab}^{a} \pm 1.371$	$14.25_{ab}^{b} \pm 1.916$	$15.59_{ab}^{a} \pm 1.137$	$13.43_{ab}^{b} \pm 1.896$	$22.61_{ab}^{a} \pm 1.187$	$19.64_{ab}^{b} \pm 2.513$	$43.73_{b}^{a} \pm 3.291$	$43.21_{b}^{a} \pm 2.737$
16	$45.21_{b}^{a} \pm 3.239$	$42.19_{b}^{b} \pm 2.175$	$16.01_{ab}^{a} \pm 1.229$	$13.64_{bcd}^{b} \pm 1.280$	$15.87_{ab}^{a} \pm 1.190$	$13.34_{ab}^{b} \pm 1.705$	$22.61_{ab}^{a} \pm 0.805$	$19.14_{bc}^{b} \pm 1.886$	$44.79_{b}^{a} \pm 3.822$	$44.33_{b}^{a} \pm 2.869$
19	$43.73_{b}^{a} \pm 2.959$	$41.52_{b}^{a} \pm 3.235$	$16.89_{a}^{a} \pm 1.296$	$15.05_{a}^{b} \pm 1.772$	$16.51_{a}^{a} \pm 1.790$	$14.78_{a}^{b} \pm 1.564$	$23.72_{a}^{a} \pm 1.276$	$21.16_{a}^{b} \pm 2.031$	$44.25_{b}^{a} \pm 4.327$	$44.44_{b}^{a} \pm 3.071$
Semitendinosus (ST)										
4	$44.06_{a}^{a} \pm 3.166$	$42.28_{a}^{a} \pm 2.814$	$15.06_{b}^{a} \pm 1.434$	$13.07_{b}^{b} \pm 1.655$	$15.25_{c}^{a} \pm 1.107$	$13.16_{c}^{b} \pm 1.321$	$21.46_{b}^{a} \pm 1.612$	$18.64_{c}^{b} \pm 1.488$	$45.46_{a}^{a} \pm 2.172$	$45.47_{bc}^{a} \pm 4.541$
7	$44.82_{a}^{a} \pm 4.364$	$44.07_{a}^{a} \pm 4.205$	$15.11_{b}^{a} \pm 1.569$	$12.80_{b}^{b} \pm 1.511$	$16.09_{abc}^{a} \pm 1.560$	$15.14_{ab}^{a} \pm 2.298$	$22.16_{ab}^{a} \pm 1.117$	$19.91_{bc}^{b} \pm 2.133$	$46.87_{a}^{a} \pm 4.935$	$49.60_{a}^{a} \pm 4.865$
10	$41.24_{b}^{a} \pm 1.759$	$42.15_{a}^{a} \pm 1.917$	$16.74_{a}^{a} \pm 1.530$	$13.75_{ab}^{b} \pm 2.335$	$16.80_{ab}^{a} \pm 1.252$	$15.47_{a}^{a} \pm 1.777$	$23.78_{a}^{a} \pm 0.905$	$20.78_{ab}^{b} \pm 2.311$	$45.17_{a}^{b} \pm 4.323$	$48.59_{a}^{a} \pm 4.984$
13	$43.34_{ab}^{a} \pm 2.814$	$42.80_{a}^{a} \pm 2.745$	$16.79_{a}^{a} \pm 1.319$	$14.83_{a}^{b} \pm 1.681$	$16.58_{abc}^{a} \pm 1.849$	$16.08_{a}^{a} \pm 2.064$	$23.65_{a}^{a} \pm 1.775$	$21.90_{a}^{b} \pm 2.509$	$44.68_{ab}^{a} \pm 3.501$	$47.36_{ab}^{a} \pm 2.227$
16	$45.35_{a}^{a} \pm 3.366$	$43.99_{a}^{a} \pm 3.024$	$16.01_{ab}^{a} \pm 1.675$	$14.42_{a}^{b} \pm 1.671$	$17.22_{a}^{a} \pm 0.464$	$15.93_{a}^{a} \pm 0.968$	$23.55_{a}^{a} \pm 1.268$	$21.55_{a}^{b} \pm 1.264$	$47.22_{a}^{a} \pm 2.901$	$48.05_{ab}^{a} \pm 3.987$
19	$43.56_{ab}^{a} \pm 3.893$	$43.62_{a}^{a} \pm 3.970$	$17.28_{a}^{a} \pm 1.874$	$14.02_{ab}^{b} \pm 1.095$	$15.37_{bc}^{a} \pm 1.077$	$13.63_{bc}^{b} \pm 1.873$	$23.19_{a}^{a} \pm 1.497$	$19.61_{bc}^{b} \pm 1.556$	$41.83_{b}^{a} \pm 3.833$	$44.09_{c}^{a} \pm 4.487$

Table 4 (continue). Interaction means (±SD)[#] for colour attributes of the raw beef muscles infused with a phosphate and lactate blend and aged for 19 d.

Day	Raw L*		Raw a*		Raw b*		Raw chroma		Raw hue angle	
	Control	Infused	Control	Infused	Control	Infused	Control	Infused	Control	Infused
Supraspinatus (SS)										
4	$39.99_{a}^{a} \pm 0.989$	$38.93_{a}^{a} \pm 1.928$	$14.76_{b}^{a} \pm 0.556$	$13.74_{b}^{a} \pm 1.365$	$12.88_{b}^{a} \pm 0.811$	$12.09_{ab}^{a} \pm 0.843$	$19.65_{b}^{a} \pm 0.793$	$18.42_{b}^{a} \pm 1.403$	$40.97_{a}^{a} \pm 1.895$	$41.40_{a}^{a} \pm 2.233$
7	$40.17_{a}^{a} \pm 3.023$	$37.09_{a}^{b} \pm 2.607$	$15.87_{b}^{a} \pm 1.062$	$13.93_{b}^{b} \pm 1.206$	$12.92_{b}^{a} \pm 1.076$	$11.53_{b}^{a} \pm 0.852$	$20.51_{b}^{a} \pm 0.875$	$18.14_{b}^{b} \pm 1.233$	$39.11_{a}^{a} \pm 3.446$	$39.66_{a}^{a} \pm 2.610$
10	$40.67_{a}^{a} \pm 1.377$	$38.61_{a}^{a} \pm 1.803$	$18.26_{a}^{a} \pm 0.993$	$15.87_{a}^{b} \pm 0.938$	$15.83_{a}^{a} \pm 1.048$	$12.58_{ab}^{b} \pm 1.439$	$24.18_{a}^{a} \pm 1.352$	$20.27_{a}^{b} \pm 1.508$	$40.90_{a}^{a} \pm 1.184$	$38.32_{a}^{a} \pm 2.216$
13	$40.73_{a}^{a} \pm 2.051$	$39.14_{a}^{a} \pm 3.873$	$18.77_{a}^{a} \pm 1.753$	$15.74_{a}^{b} \pm 1.631$	$15.88_{a}^{a} \pm 1.400$	$13.60_{a}^{b} \pm 2.657$	$24.59_{a}^{a} \pm 2.204$	$20.84_{a}^{b} \pm 2.888$	$40.24_{a}^{a} \pm 0.979$	$40.49_{a}^{a} \pm 3.130$
16	$41.13_{a}^{a} \pm 2.218$	$38.58_{a}^{b} \pm 2.092$	$17.83_{a}^{a} \pm 0.677$	$15.68_{a}^{b} \pm 0.973$	$14.95_{a}^{a} \pm 1.067$	$13.53_{a}^{a} \pm 1.256$	$23.34_{a}^{a} \pm 0.928$	$20.74_{a}^{b} \pm 1.403$	$39.80_{a}^{a} \pm 2.166$	$40.70_{a}^{a} \pm 2.016$
19	$42.28_{a}^{a} \pm 1.687$	$38.77_{a}^{b} \pm 1.631$	$17.84_{a}^{a} \pm 0.561$	$14.79_{ab}^{b} \pm 0.848$	$15.68_{a}^{a} \pm 0.730$	$12.94_{ab}^{b} \pm 1.448$	$23.79_{a}^{a} \pm 0.594$	$19.69_{ab}^{b} \pm 1.485$	$41.32_{a}^{a} \pm 4.686$	$41.06_{a}^{a} \pm 2.147$
Longissimus lumborum (LL)										
4	$38.72_{c}^{a} \pm 1.417$	$38.16_{b}^{a} \pm 1.723$	$13.82_{c}^{a} \pm 1.048$	$13.58_{cd}^{b} \pm 1.004$	$12.32_{c}^{a} \pm 0.900$	$12.08_{b}^{a} \pm 0.775$	$18.57_{c}^{a} \pm 1.103$	$18.23_{c}^{a} \pm 0.770$	$41.62_{ab}^{a} \pm 2.409$	$41.67_{ab}^{a} \pm 3.150$
7	$40.56_{abc}^{a} \pm 1.725$	$39.42_{ab}^{a} \pm 1.141$	$16.54_{b}^{a} \pm 0.906$	$14.36_{bcd}^{b} \pm 1.205$	$14.15_{b}^{a} \pm 1.415$	$12.73_{ab}^{a} \pm 1.633$	$21.87_{b}^{a} \pm 1.342$	$19.23_{bc}^{b} \pm 1.904$	$40.56_{ab}^{a} \pm 2.101$	$41.37_{ab}^{a} \pm 2.159$
10	$39.39_{bc}^{a} \pm 2.107$	$38.59_{ab}^{a} \pm 1.899$	$16.04_{b}^{a} \pm 0.948$	$13.44_{d}^{b} \pm 1.494$	$14.80_{ab}^{a} \pm 0.334$	$13.09_{ab}^{b} \pm 0.894$	$21.86_{b}^{a} \pm 0.756$	$18.89_{bc}^{b} \pm 1.022$	$42.75_{a}^{a} \pm 1.711$	$44.32_{a}^{a} \pm 4.357$
13	$41.24_{ab}^{a} \pm 2.026$	$39.67_{ab}^{a} \pm 2.186$	$17.25_{ab}^{a} \pm 0.887$	$14.83_{bc}^{b} \pm 1.423$	$15.87_{a}^{a} \pm 0.658$	$13.93_{a}^{b} \pm 0.341$	$23.47_{ab}^{a} \pm 0.891$	$20.39_{ab}^{b} \pm 1.224$	$42.58_{a}^{a} \pm 1.553$	$43.29_{a}^{a} \pm 2.311$
16	$40.17_{abc}^{a} \pm 1.630$	$39.62_{ab}^{a} \pm 1.178$	$16.31_{b}^{a} \pm 1.370$	$15.09_{b}^{a} \pm 0.914$	$14.55_{ab}^{a} \pm 0.932$	$13.34_{ab}^{a} \pm 0.767$	$21.90_{b}^{a} \pm 1.265$	$20.20_{ab}^{b} \pm 0.559$	$41.74_{ab}^{a} \pm 2.742$	$41.49_{ab}^{a} \pm 2.988$
19	$41.91_{a}^{a} \pm 1.248$	$40.74_{a}^{a} \pm 1.064$	$18.38_{a}^{a} \pm 0.456$	$16.79_{a}^{b} \pm 0.903$	$15.02_{ab}^{a} \pm 0.835$	$13.81_{a}^{a} \pm 1.278$	$23.76_{a}^{a} \pm 0.471$	$21.76_{a}^{b} \pm 1.333$	$39.25_{b}^{a} \pm 1.896$	$39.37_{b}^{a} \pm 2.205$
LSD *P*=0.05	2.336		1.277		1.528		1.631		3.108	

[#] SD: Standard deviation.

$_{a, b, c, d, e}$ Means in the same column within a treatment and between days, with different subscripts are significantly different ($P \leq 0.05$).

$^{a, b}$ Means in the same row within an attribute and between treatments, with different superscripts are significantly different ($P \leq 0.05$).

LSD: Least significant difference ($P=0.05$).

Table 5. Summary of interaction means (±SD)[#] for colour attributes of the raw beef muscles infused with a phosphate and lactate blend and aged for 19 d.

Muscle	Raw L*		Raw a*		Raw b*		Raw chroma		Raw hue angle	
	Control	Infused	Control	Infused	Control	Infused	Control	Infused	Control	Infused
BF	$40.0_{c}^{a} \pm 1.982$	$39.04_{b}^{b} \pm 2.124$	$17.38_{a}^{a} \pm 2.005$	$15.79_{a}^{b} \pm 1.772$	$15.04_{bc}^{a} \pm 2.053$	$13.93_{b}^{b} \pm 1.703$	$23.02_{a}^{a} \pm 2.699$	$21.11_{a}^{b} \pm 2.245$	$40.75_{b}^{a} \pm 2.424$	$41.33_{cd}^{a} \pm 2.724$
RF	$45.0_{a}^{a} \pm 3.677$	$42.97_{a}^{b} \pm 3.326$	$15.31_{c}^{a} \pm 1.809$	$13.67_{c}^{b} \pm 1.958$	$15.61_{ab}^{a} \pm 1.400$	$13.59_{bc}^{b} \pm 1.830$	$21.94_{b}^{a} \pm 1.789$	$19.36_{c}^{b} \pm 2.346$	$45.63_{a}^{a} \pm 3.693$	$44.89_{b}^{a} \pm 3.805$
ST	$43.8_{b}^{a} \pm 3.367$	$43.15_{a}^{a} \pm 3.18$	$16.16_{b}^{a} \pm 1.694$	$13.81_{c}^{b} \pm 1.728$	$16.22_{a}^{a} \pm 1.404$	$14.90_{a}^{b} \pm 1.995$	$22.96_{a}^{a} \pm 1.558$	$20.40_{b}^{b} \pm 2.127$	$45.20_{a}^{b} \pm 3.881$	$47.19_{a}^{a} \pm 4.398$
SS	$40.83_{c}^{a} \pm 2.001$	$38.52_{b}^{b} \pm 2.361$	$17.22_{a}^{a} \pm 1.724$	$14.96_{b}^{b} \pm 1.412$	$14.69_{c}^{a} \pm 1.637$	$12.71_{d}^{b} \pm 1.612$	$22.68_{a}^{a} \pm 2.236$	$19.68_{c}^{b} \pm 1.942$	$40.39_{b}^{a} \pm 2.054$	$40.27_{d}^{a} \pm 2.471$
LL	$40.33_{c}^{a} \pm 1.928$	$39.36_{b}^{b} \pm 1.694$	$16.39_{b}^{a} \pm 1.666$	$14.68_{b}^{b} \pm 1.575$	$14.45_{c}^{a} \pm 1.386$	$13.16_{cd}^{b} \pm 1.151$	$21.91_{b}^{a} \pm 1.950$	$19.78_{bc}^{b} \pm 1.624$	$41.41_{b}^{a} \pm 2.313$	$41.92_{c}^{a} \pm 3.169$
LSD *P*=0.05	0.953		0.521		0.624		0.666		1.269	

[#] SD: Standard deviation.

BF: *Biceps femoris.*

RF: *Rectus femoris.*

ST: *Semitendinosus.*

SS: *Supraspinatus.*

LL: *Longissimus lumborum.*

$_{a, b, c, d}$ Means in the same column within a treatment and between muscles, with different subscripts are significantly different ($P \leq 0.05$).

$^{a, b}$ Means in the same row within an attribute and between treatments, with different superscripts are significantly different ($P \leq 0.05$).

LSD: Least significant difference ($P=0.05$).

With the hue angle calculations (Table 7) the infused muscles had slightly higher values than the control samples, however, only the infused ST and LL samples were significantly higher ($P \leq 0.05$). Thus, overall the infused cooked samples appeared redder. Other research also reported higher hue angles for infused muscles (Baublits *et al*., 2005, Lawrence *et al*., 2003).

The mechanism by which sodium lactate (NaLac) affects cooked colour muscle is unknown. Maca *et al*. (1999) concluded that NaLac had a protective effect on the meat colour and acted as a stabiliser. This was observed in the treated muscles of this investigation, i.e. having a slightly redder colour than the control sample. The conversion of myoglobin to metmyoglobin in the cooked product could also have been influenced by the pH, further explaining the above treatment effect of a more stable red cooked beef colour. These observations are, however, pure speculation; the exact mechanism by which NaLac affects meat colour is still not known and future research is suggested to determine the effect of NaLac on meat colour (Maca *et al*., 1999).

In conclusion to this section on instrumental meat colour, it can be concluded that colour values fluctuated during the storage of raw and cooked beef over the 19 d and no clear pattern could be found.

Chemical composition

The proximate chemical composition values were determined using the muscles samples taken from Day 4, i.e. the *Biceps femoris* (BF, silverside), *Rectus femoris* (RF), *Semitendinosus* (ST, eye of the silverside), *Supraspinatus* (SS, scotch fillet) and *Longissimus lumborum* (LL, striploin). The results are presented in Table 8. The mineral content of the above-mentioned muscles are shown in Table 9.

Proximate chemical composition

The selected beef muscles were compared with regard to percentage moisture, protein, lipid and ash content (Table 8). The proximate chemical composition of the control sample of this investigation is similar to that reported for beef (Sayed *et al.,* 1999; Hoffman, 2006). The results of the infused muscles presented in Table 8 are in agreement with what is expected when a solution consisting of water and several minerals such as phosphates, potassium and sodium is infused into beef muscle, i.e. an increase in moisture and ash content and a decrease in protein and lipid content (Hoffman, 2006).

Table 6. Interaction means (±SD)[#] for colour attributes of the cooked beef muscles infused with a phosphate and lactate blend and aged for 19 d.

Day	Cooked L*		Cooked a*		Cooked b*		Cooked chroma		Cooked hue angle	
	Control	Infused	Control	Infused	Control	Infused	Control	Infused	Control	Infused
Biceps femoris (BF)										
4	$38.88_{ab}^{a} \pm 2.800$	$42.00_{a}^{a} \pm 2.129$	$5.74_{bc}^{a} \pm 0.896$	$5.72_{a}^{a} \pm 0.501$	$13.11_{c}^{a} \pm 0.785$	$12.55_{c}^{a} \pm 0.417$	$14.34_{d}^{a} \pm 0.629$	$13.82_{c}^{a} \pm 0.439$	$66.30_{bc}^{a} \pm 3.998$	$65.44_{b}^{a} \pm 1.911$
7	$39.67_{ab}^{a} \pm 3.208$	$40.92_{a}^{a} \pm 2.515$	$5.91_{bc}^{a} \pm 1.186$	$6.18_{a}^{a} \pm 0.703$	$15.35_{a}^{a} \pm 0.805$	$14.69_{ab}^{a} \pm 0.610$	$16.50_{bc}^{a} \pm 0.889$	$15.96_{a}^{a} \pm 0.627$	$68.96_{ab}^{a} \pm 3.896$	$67.14_{ab}^{a} \pm 2.458$
10	$41.36_{a}^{a} \pm 1.374$	$42.51_{a}^{a} \pm 3.431$	$5.48_{c}^{a} \pm 0.711$	$5.25_{a}^{a} \pm 0.538$	$15.87_{a}^{a} \pm 0.338$	$14.64_{ab}^{b} \pm 0.457$	$16.82_{ab}^{a} \pm 0.201$	$15.58_{ab}^{b} \pm 0.470$	$70.95_{a}^{a} \pm 2.608$	$70.28_{a}^{a} \pm 1.874$
13	$41.75_{a}^{a} \pm 4.417$	$41.80_{a}^{a} \pm 4.710$	$5.92_{bc}^{a} \pm 1.217$	$5.83_{a}^{a} \pm 0.809$	$15.99_{a}^{a} \pm 0.853$	$14.98_{a}^{b} \pm 0.891$	$17.10_{ab}^{a} \pm 0.794$	$16.10_{a}^{b} \pm 0.837$	$69.67_{a}^{a} \pm 4.221$	$68.70_{ab}^{a} \pm 3.049$
16	$36.90_{b}^{b} \pm 3.529$	$40.56_{a}^{a} \pm 3.779$	$6.86_{a}^{a} \pm 0.865$	$5.61_{a}^{b} \pm 0.631$	$14.36_{b}^{a} \pm 1.052$	$14.12_{b}^{a} \pm 0.863$	$15.94_{c}^{a} \pm 0.889$	$15.22_{b}^{a} \pm 0.719$	$64.40_{c}^{b} \pm 3.625$	$68.27_{ab}^{a} \pm 2.972$
19	$41.48_{a}^{a} \pm 1.580$	$41.80_{a}^{a} \pm 1.824$	$6.51_{ab}^{a} \pm 0.847$	$5.76_{a}^{a} \pm 0.685$	$15.97_{a}^{a} \pm 0.793$	$15.23_{a}^{a} \pm 0.728$	$17.27_{a}^{a} \pm 0.993$	$16.29_{a}^{b} \pm 0.801$	$67.85_{ab}^{a} \pm 1.975$	$69.27_{a}^{a} \pm 2.117$
Rectus femoris (RF)										
4	$50.52_{a}^{a} \pm 2.709$	$50.78_{a}^{a} \pm 2.992$	$4.15_{b}^{a} \pm 0.531$	$3.99_{a}^{a} \pm 1.562$	$16.47_{b}^{a} \pm 0.505$	$16.65_{a}^{a} \pm 0.734$	$17.01_{b}^{a} \pm 0.536$	$17.19_{a}^{a} \pm 0.878$	$75.77_{ab}^{a} \pm 1.727$	$76.58_{a}^{a} \pm 4.853$
7	$46.21_{b}^{a} \pm 4.447$	$47.03_{b}^{a} \pm 3.365$	$4.87_{ab}^{a} \pm 0.984$	$4.09_{a}^{a} \pm 0.576$	$17.06_{ab}^{a} \pm 0.446$	$15.72_{b}^{b} \pm 0.769$	$17.85_{a}^{a} \pm 0.391$	$16.32_{b}^{b} \pm 0.631$	$73.95_{abc}^{a} \pm 3.324$	$75.05_{ab}^{a} \pm 2.473$
10	$45.82_{b}^{a} \pm 3.681$	$45.46_{b}^{a} \pm 3.368$	$5.12_{a}^{a} \pm 0.801$	$4.31_{a}^{a} \pm 0.920$	$16.69_{b}^{a} \pm 0.398$	$15.75_{b}^{b} \pm 0.663$	$17.56_{ab}^{a} \pm 0.250$	$16.39_{b}^{b} \pm 0.533$	$72.72_{bc}^{a} \pm 3.008$	$74.53_{ab}^{a} \pm 3.536$
13	$45.21_{b}^{a} \pm 3.854$	$44.84_{b}^{a} \pm 3.427$	$5.22_{a}^{a} \pm 0.548$	$4.74_{a}^{a} \pm 0.818$	$16.90_{ab}^{a} \pm 0.868$	$16.22_{ab}^{a} \pm 0.959$	$17.80_{a}^{a} \pm 0.652$	$16.99_{ab}^{b} \pm 0.722$	$72.33_{c}^{a} \pm 2.875$	$73.38_{ab}^{a} \pm 3.579$
16	$45.62_{b}^{a} \pm 2.936$	$47.53_{b}^{a} \pm 3.174$	$5.18_{a}^{a} \pm 1.054$	$4.00_{a}^{b} \pm 0.888$	$16.67_{b}^{a} \pm 0.741$	$16.24_{ab}^{a} \pm 0.685$	$17.50_{ab}^{a} \pm 0.490$	$16.79_{ab}^{a} \pm 0.527$	$72.64_{bc}^{b} \pm 3.898$	$76.07_{ab}^{a} \pm 3.436$
19	$49.69_{a}^{a} \pm 4.273$	$46.95_{b}^{a} \pm 3.265$	$3.98_{b}^{a} \pm 1.232$	$4.83_{a}^{a} \pm 0.551$	$17.53_{a}^{a} \pm 0.611$	$15.81_{b}^{b} \pm 0.395$	$18.04_{a}^{a} \pm 0.606$	$16.59_{ab}^{b} \pm 0.436$	$77.21_{a}^{a} \pm 3.890$	$72.91_{b}^{b} \pm 1.789$
Semitendinosus (ST)										
4	$42.42_{a}^{a} \pm 3.653$	$45.34_{a}^{a} \pm 4.337$	$7.06_{a}^{a} \pm 0.764$	$6.54_{a}^{a} \pm 1.242$	$15.67_{c}^{b} \pm 0.773$	$16.96_{a}^{a} \pm 0.585$	$17.23_{ab}^{b} \pm 0.504$	$18.23_{a}^{a} \pm 0.475$	$65.72_{c}^{a} \pm 3.246$	$68.90_{b}^{a} \pm 4.081$
7	$42.11_{a}^{a} \pm 4.049$	$44.70_{a}^{a} \pm 3.410$	$6.16_{ab}^{a} \pm 1.032$	$5.35_{b}^{a} \pm 1.235$	$15.76_{bc}^{a} \pm 0.945$	$15.86_{bc}^{a} \pm 0.998$	$16.97_{b}^{a} \pm 0.573$	$16.81_{bc}^{a} \pm 0.829$	$68.56_{bc}^{a} \pm 4.411$	$71.25_{ab}^{a} \pm 4.586$
10	$42.34_{a}^{b} \pm 4.46$	$46.39_{a}^{a} \pm 4.948$	$5.12_{c}^{a} \pm 0.887$	$4.70_{b}^{a} \pm 1.158$	$16.13_{abc}^{a} \pm 0.931$	$16.21_{abc}^{a} \pm 0.595$	$16.98_{b}^{a} \pm 0.707$	$16.93_{bc}^{a} \pm 0.565$	$72.24_{a}^{a} \pm 3.658$	$73.87_{a}^{a} \pm 4.004$
13	$43.35_{a}^{a} \pm 4.59$	$46.19_{a}^{a} \pm 2.608$	$5.76_{bc}^{a} \pm 1.264$	$5.15_{b}^{a} \pm 0.634$	$16.68_{a}^{a} \pm 0.930$	$16.46_{ab}^{a} \pm 0.618$	$17.71_{a}^{a} \pm 0.629$	$17.28_{b}^{a} \pm 0.718$	$70.91_{ab}^{a} \pm 4.464$	$72.74_{a}^{a} \pm 1.735$
16	$43.13_{a}^{a} \pm 5.54$	$45.79_{a}^{a} \pm 4.481$	$5.44_{bc}^{a} \pm 1.357$	$4.75_{b}^{a} \pm 0.775$	$15.91_{abc}^{a} \pm 0.897$	$15.54_{c}^{a} \pm 0.632$	$16.89_{b}^{a} \pm 0.521$	$16.29_{c}^{a} \pm 0.493$	$71.01_{ab}^{a} \pm 5.332$	$72.97_{a}^{a} \pm 3.092$
19	$45.25_{a}^{a} \pm 4.698$	$47.10_{a}^{a} \pm 2.316$	$5.26_{bc}^{a} \pm 1.019$	$5.11_{b}^{a} \pm 0.334$	$16.52_{ab}^{a} \pm 0.808$	$15.62_{c}^{b} \pm 0.658$	$17.38_{ab}^{a} \pm 0.641$	$16.46_{c}^{b} \pm 0.562$	$72.24_{a}^{a} \pm 3.756$	$71.87_{ab}^{a} \pm 1.656$

Table 6 (continue). Interaction means (±SD)[#] for colour attributes of the cooked beef muscles infused with a phosphate and lactate blend and aged for 19 d.

Day	Cooked L*		Cooked a*		Cooked b*		Cooked chroma		Cooked hue angle	
	Control	Infused	Control	Infused	Control	Infused	Control	Infused	Control	Infused
Supraspinatus (SS)										
4	$39.87_{a}^{a} \pm 2.658$	$39.90_{a}^{a} \pm 3.997$	$6.90_{a}^{a} \pm 0.754$	$6.27_{a}^{a} \pm 0.690$	$15.30_{b}^{a} \pm 0.525$	$14.76_{abc}^{a} \pm 0.998$	$16.82_{b}^{a} \pm 0.639$	$16.08_{a}^{b} \pm 0.882$	$65.69_{b}^{a} \pm 2.121$	$66.95_{b}^{a} \pm 3.037$
7	$38.50_{a}^{a} \pm 3.002$	$38.47_{a}^{a} \pm 2.632$	$7.04_{a}^{a} \pm 0.965$	$6.23_{a}^{a} \pm 0.403$	$15.92_{ab}^{a} \pm 0.682$	$14.58_{c}^{b} \pm 0.940$	$17.45_{ab}^{a} \pm 0.532$	$15.90_{a}^{b} \pm 0.956$	$66.13_{b}^{a} \pm 3.478$	$66.74_{b}^{a} \pm 1.316$
10	$39.32_{a}^{a} \pm 2.388$	$38.81_{a}^{a} \pm 2.827$	$7.09_{a}^{a} \pm 0.725$	$6.38_{a}^{a} \pm 0.610$	$15.96_{ab}^{a} \pm 0.898$	$14.65_{bc}^{b} \pm 0.769$	$17.55_{a}^{a} \pm 0.642$	$16.02_{a}^{b} \pm 0.532$	$65.83_{b}^{a} \pm 3.275$	$66.36_{b}^{a} \pm 2.908$
13	$38.86_{a}^{a} \pm 3.452$	$39.42_{a}^{a} \pm 2.790$	$5.93_{b}^{a} \pm 0.931$	$5.11_{b}^{a} \pm 0.678$	$16.22_{a}^{a} \pm 0.806$	$15.48_{a}^{a} \pm 0.968$	$17.32_{ab}^{a} \pm 0.495$	$16.34_{a}^{b} \pm 0.716$	$69.80_{a}^{a} \pm 3.802$	$71.57_{a}^{a} \pm 3.278$
16	$37.36_{a}^{a} \pm 2.029$	$39.00_{a}^{a} \pm 2.367$	$6.67_{ab}^{a} \pm 0.673$	$5.98_{ab}^{a} \pm 0.652$	$16.30_{a}^{a} \pm 0.516$	$15.42_{ab}^{b} \pm 0.629$	$17.65_{a}^{a} \pm 0.578$	$16.59_{a}^{b} \pm 0.449$	$67.74_{ab}^{a} \pm 2.099$	$68.75_{ab}^{a} \pm 2.761$
19	$39.04_{a}^{a} \pm 3.757$	$40.29_{a}^{a} \pm 2.353$	$6.69_{ab}^{a} \pm 0.701$	$6.31_{a}^{a} \pm 0.665$	$16.11_{a}^{a} \pm 1.044$	$15.30_{abc}^{b} \pm 0.796$	$17.49_{ab}^{a} \pm 0.911$	$16.59_{a}^{b} \pm 0.695$	$67.38_{ab}^{a} \pm 2.814$	$67.55_{b}^{a} \pm 2.670$
Longissimus lumborum (LL)										
4	$49.39_{a}^{a} \pm 3.395$	$48.19_{a}^{a} \pm 4.910$	$5.33_{a}^{a} \pm 1.296$	$4.37_{a}^{b} \pm 1.124$	$17.56_{a}^{a} \pm 1.235$	$15.29_{a}^{b} \pm 0.792$	$18.42_{a}^{a} \pm 1.382$	$15.94_{a}^{b} \pm 0.779$	$73.31_{ab}^{a} \pm 3.482$	$74.07_{c}^{a} \pm 4.082$
7	$46.97_{ab}^{a} \pm 4.089$	$49.31_{a}^{a} \pm 2.596$	$5.41_{a}^{a} \pm 0.797$	$4.22_{ab}^{b} \pm 0.692$	$15.81_{b}^{a} \pm 0.504$	$15.59_{a}^{a} \pm 0.363$	$16.74_{c}^{a} \pm 0.510$	$16.17_{a}^{a} \pm 0.406$	$71.08_{b}^{b} \pm 2.776$	$74.90_{bc}^{a} \pm 2.367$
10	$48.27_{ab}^{a} \pm 3.973$	$51.06_{a}^{a} \pm 4.337$	$4.34_{b}^{a} \pm 1.204$	$3.41_{bc}^{b} \pm 1.584$	$16.99_{a}^{a} \pm 0.542$	$15.54_{a}^{b} \pm 0.359$	$17.59_{b}^{a} \pm 0.330$	$15.99_{a}^{b} \pm 0.325$	$75.62_{a}^{a} \pm 4.176$	$77.62_{ab}^{a} \pm 5.739$
13	$49.62_{a}^{a} \pm 4.821$	$50.70_{a}^{a} \pm 3.901$	$4.28_{b}^{a} \pm 0.795$	$3.74_{abc}^{a} \pm 0.997$	$17.04_{a}^{a} \pm 0.068$	$15.79_{a}^{b} \pm 0.691$	$17.59_{b}^{a} \pm 0.174$	$16.26_{a}^{b} \pm 0.785$	$75.92_{a}^{a} \pm 2.527$	$76.70_{abc}^{a} \pm 3.340$
16	$45.42_{b}^{b} \pm 4.012$	$49.57_{a}^{a} \pm 2.193$	$4.21_{b}^{a} \pm 0.645$	$3.15_{c}^{b} \pm 0.524$	$16.77_{a}^{a} \pm 0.724$	$15.80_{a}^{b} \pm 0.426$	$17.31_{bc}^{a} \pm 0.543$	$16.13_{a}^{b} \pm 0.485$	$75.83_{a}^{a} \pm 2.708$	$78.69_{a}^{a} \pm 1.728$
19	$46.74_{ab}^{b} \pm 3.275$	$50.07_{a}^{a} \pm 2.738$	$4.30_{b}^{a} \pm 0.895$	$3.17_{c}^{b} \pm 0.618$	$16.82_{a}^{a} \pm 0.651$	$15.54_{a}^{b} \pm 0.398$	$17.40_{bc}^{a} \pm 0.636$	$15.90_{a}^{b} \pm 0.430$	$75.61_{a}^{a} \pm 3.035$	$78.39_{a}^{a} \pm 2.236$
LSD *P*=0.05	3.178		0.930		0.795		0.722		3.350	

[#] SD: Standard deviation.

$_{a, b, c, d}$ Means in the same column within a treatment and between days, with different subscripts are significantly different ($P \leq 0.05$).

$^{a, b}$ Means in the same row within an attribute and between treatments, with different superscripts are significantly different ($P \leq 0.05$).

LSD: Least significant difference ($P=0.05$).

Table 7. Summary of interaction means (±SD)[#] for colour attributes of the cooked beef muscles infused with a phosphate and lactate blend and aged for 19 d.

Muscle	Cooked L*		Cooked a*		Cooked b*		Cooked chroma		Cooked hue angle	
	Control	Infused	Control	Infused	Control	Infused	Control	Infused	Control	Infused
BF	$40.00_{c}^{b} \pm 3.298$	$41.60_{c}^{a} \pm 3.060$	$6.07_{b}^{a} \pm 1.018$	$5.72_{a}^{a} \pm 0.666$	$15.11_{c}^{a} \pm 1.306$	$14.37_{d}^{b} \pm 1.097$	$16.33_{c}^{a} \pm 1.233$	$15.49_{c}^{b} \pm 1.043$	$68.02_{c}^{a} \pm 3.905$	$68.18_{d}^{a} \pm 2.757$
RF	$47.18_{a}^{a} \pm 4.040$	$47.10_{b}^{a} \pm 3.585$	$4.75_{c}^{a} \pm 0.974$	$4.33_{c}^{b} \pm 0.943$	$16.89_{a}^{a} \pm 0.671$	$16.07_{a}^{b} \pm 0.750$	$17.62_{a}^{a} \pm 0.575$	$16.71_{a}^{b} \pm 0.670$	$74.10_{a}^{a} \pm 3.490$	$74.75_{b}^{a} \pm 3.434$
ST	$43.10_{b}^{b} \pm 4.334$	$45.92_{b}^{a} \pm 3.614$	$5.80_{b}^{a} \pm 1.197$	$5.27_{b}^{b} \pm 1.084$	$16.11_{b}^{a} \pm 0.888$	$16.11_{a}^{a} \pm 0.819$	$17.19_{b}^{a} \pm 0.627$	$17.00_{a}^{a} \pm 0.864$	$70.12_{b}^{b} \pm 4.543$	$71.93_{c}^{a} \pm 3.530$
SS	$38.83_{c}^{a} \pm 2.836$	$39.31_{d}^{a} \pm 2.743$	$6.72_{a}^{a} \pm 0.837$	$6.05_{a}^{b} \pm 0.728$	$15.97_{b}^{a} \pm 0.790$	$15.03_{c}^{b} \pm 0.883$	$17.83_{ab}^{a} \pm 0.659$	$16.25_{b}^{b} \pm 0.728$	$67.09_{c}^{a} \pm 3.138$	$67.99_{d}^{a} \pm 3.108$
LL	$47.73_{a}^{b} \pm 3.970$	$49.82_{a}^{a} \pm 3.456$	$4.64_{c}^{a} \pm 1.037$	$3.67_{d}^{b} \pm 1.039$	$16.83_{a}^{a} \pm 0.845$	$15.59_{b}^{b} \pm 0.524$	$17.51_{a}^{a} \pm 0.826$	$16.07_{b}^{b} \pm 0.539$	$74.56_{a}^{b} \pm 3.452$	$76.73_{a}^{a} \pm 3.698$
LSD *P*=0.05	1.2975		0.3797		0.3246		0.2947		1.3677	

[#] SD: Standard deviation.

BF: *Biceps femoris.*

RF: *Rectus femoris.*

ST: *Semitendinosus.*

SS: *Supraspinatus.*

LL: *Longissimus lumborum.*

$_{a, b, c, d}$ Means in the same column within a treatment and between muscles, with different subscripts are significantly different ($P \leq 0.05$).

$^{a, b}$ Means in the same row within an attribute and between treatments, with different superscripts are significantly different ($P \leq 0.05$).

LSD: Least significant difference ($P=0.05$).

The percentage moisture was influenced significantly ($P \leq 0.05$) by the infusion of a phosphate and lactate blend – three of the five muscles had increased moisture content. The protein content of the infused BF and RF muscles were significantly lower ($P \leq 0.05$) than that of the control samples. The control and infused muscles were very similar in fat content, except for the BF muscles, where the expected lower fat content of enhanced meat was obtained (Hoffman, 2006) with the addition of a water-based solution. Because of the infusion blend containing several minerals such as potassium and sodium; significant differences ($P \leq 0.05$) in the ash content between the treated and control muscles were expected, as shown in Table 8.

It became clear from Chapter 3 that the various beef muscles within a carcass differ in chemical composition. Although the muscles differed in proximate composition in this investigation (Table 8), the differences between muscles within treatments showed no definite pattern. However, it was observed that the BF muscle had the lowest moisture content and highest fat content compared to the other beef muscles. Other studies have also reported this inverse relationship (Delgado *et al*., 2005).

Mineral composition

Meat contributes a considerable proportion of the minerals required in the human diet (Van Heerden & Schönfeldt, 2003) and the concentration of iron (Fe), zinc (Zn) and copper (Cu) in meat is higher than the minerals provided by the rest of the diet as a whole (Williams, 1987). The results of this investigation indicated significant ($P \leq 0.05$) differences in the mineral composition (Table 9) between muscles. Several other studies also indicated differences in mineral content between various muscles (Schönfeldt & Welgemoed, 1996; Hoffman, 2006).

The ash content of the infused samples (Table 9) indicated an increase in mineral composition as a result of the blend. Therefore, significant differences in mineral content between treatments are expected. The infused muscle had higher values for K, Na, and Cu, which is expected, because both K and Na are present in the blend infused into the beef muscles. The effect of the infusion on the various muscles is in accord with previously reported data (Hoffman, 2006).

Table 8. Interaction means (±SD)[#] for proximate chemical composition of beef muscles infused with a phosphate and lactate blend.

Muscle	Moisture (%)		Protein (%)		Lipid (%)		Ash (%)	
	Control	Infused	Control	Infused	Control	Infused	Control	Infused
BF	$73.17_{b}^{a} \pm 1.548$	$73.61_{c}^{a} \pm 1.489$	$20.21_{a}^{a} \pm 1.203$	$18.13_{bc}^{b} \pm 0.814$	$3.04_{a}^{b} \pm 0.971$	$3.71_{a}^{a} \pm 0.726$	$1.13_{ab}^{b} \pm 0.081$	$1.73_{b}^{a} \pm 0.121$
RF	$74.18_{ab}^{b} \pm 0.747$	$75.84_{ab}^{a} \pm 1.325$	$20.28_{a}^{a} \pm 0.955$	$17.19_{c}^{b} \pm 2.14$	$2.54_{ab}^{a} \pm 0.523$	$2.54_{b}^{a} \pm 0.783$	$1.15_{ab}^{b} \pm 0.020$	$1.91_{a}^{a} \pm 0.087$
ST	$75.10_{a}^{b} \pm 0.936$	$76.99_{a}^{a} \pm 1.250$	$20.71_{a}^{a} \pm 0.800$	$19.25_{ab}^{a} \pm 1.214$	$2.10_{b}^{a} \pm 0.435$	$1.70_{c}^{a} \pm 0.261$	$1.14_{ab}^{b} \pm 0.094$	$1.89_{a}^{a} \pm 0.094$
SS	$75.22_{a}^{b} \pm 0.931$	$76.84_{a}^{a} \pm 1.191$	$20.28_{a}^{a} \pm 0.942$	$18.82_{abc}^{a} \pm 1.043$	$2.95_{a}^{a} \pm 0.617$	$2.47_{b}^{a} \pm 0.378$	$1.06_{b}^{b} \pm 0.144$	$1.74_{b}^{a} \pm 0.122$
LL	$73.84_{b}^{a} \pm 0.685$	$74.62_{bc}^{a} \pm 1.16$	$19.78_{a}^{a} \pm 2.538$	$20.16_{a}^{a} \pm 1.190$	$2.28_{b}^{a} \pm 0.335$	$2.51_{b}^{a} \pm 0.780$	$1.25_{a}^{b} \pm 0.098$	$1.72_{b}^{a} \pm 0.106$
LSD *P*=0.05	1.260		1.681		0.582		0.116	

[#] SD: Standard deviation.

BF: *Biceps femoris.*

RF: *Rectus femoris.*

ST: *Semitendinosus.*

SS: *Supraspinatus.*

LL: *Longissimus lumborum.*

$_{a, b, c}$ Means in the same column within a treatment and between muscles, with different subscripts are significantly different ($P \leq 0.05$).

$^{a, b}$ Means in the same row within a component and between treatments, with different superscripts are significantly different ($P \leq 0.05$).

LSD: Least significant difference ($P=0.05$).

Table 9. Interaction means (±SD)[#] for mineral composition (mg/100 g) of beef muscles infused with a phosphate and lactate blend.

Mineral component (mg/100 g)	Muscle										LSD (*P*=0.05)
	Biceps femoris (*BF*)		*Rectus femoris* (*RF*)		*Semitendinosus* (*ST*)		*Supraspinatus* (*SS*)		*Longissimus lumborum* (*LL*)		
	Control	Infused	Control	Infused	Control	Infused	Control	Infused	Control	Infused	
Phosphorus	180.0_{ab}^{a} ± 9.928	157.9_{b}^{a} ± 29.78	196.3_{a}^{a} ± 8.138	178.5_{ab}^{a} ± 25.04	164.1_{bc}^{a} ± 5.198	184.0_{a}^{a} ± 35.53	154.6_{c}^{a} ± 9.572	172.8_{ab}^{a} ± 14.57	189.8_{a}^{a} ± 7.922	158.8_{b}^{b} ± 25.42	23.58
Potassium	166.8_{ab}^{b} ± 16.28	191.7_{b}^{a} ± 28.93	163.6_{ab}^{b} ± 3.209	199.7_{ab}^{a} ± 26.53	161.5_{ab}^{b} ± 7.329	215.4_{a}^{a} ± 35.92	145.5_{b}^{b} ± 8.043	196.6_{ab}^{a} ± 20.06	169.9_{a}^{a} ± 5.086	187.5_{b}^{a} ± 12.40	22.56
Calcium	6.35_{a}^{a} ± 1.301	4.94_{b}^{b} ± 1.167	6.98_{a}^{a} ± 0.199	4.73_{b}^{b} ± 0.889	6.46_{a}^{a} ± 0.984	5.64_{b}^{a} ± 0.878	6.89_{a}^{a} ± 0.622	7.89_{a}^{a} ± 1.813	7.17_{a}^{a} ± 0.164	4.94_{b}^{b} ± 0.809	1.126
Magnesium	21.78_{a}^{a} ± 0.795	16.29_{ab}^{b} ± 1.130	22.49_{a}^{a} ± 1.126	15.35_{b}^{b} ± 2.093	22.80_{a}^{a} ± 0.845	16.34_{ab}^{b} ± 1.491	21.85_{a}^{a} ± 1.885	17.56_{a}^{b} ± 0.922	21.88_{a}^{a} ± 1.706	16.41_{ab}^{b} ± 1.943	1.895
Sodium	12.05_{a}^{b} ± 1.442	24.43_{bc}^{a} ± 5.879	11.15_{a}^{b} ± 0.454	25.74_{b}^{a} ± 4.298	10.91_{a}^{b} ± 0.316	26.27_{b}^{a} ± 3.537	12.59_{a}^{b} ± 0.974	30.99_{a}^{a} ± 5.358	11.49_{a}^{b} ± 0.569	21.30_{c}^{a} ± 3.108	3.968
Iron	2.73_{a}^{a} ± 0.608	1.99_{b}^{b} ± 0.561	1.91_{b}^{a} ± 0.390	1.73_{b}^{a} ± 0.346	2.11_{b}^{a} ± 0.326	1.58_{b}^{b} ± 0.232	2.95_{a}^{a} ± 0.350	2.68_{a}^{a} ± 0.368	2.15_{b}^{a} ± 0.323	1.63_{b}^{b} ± 0.201	0.454
Copper	0.018_{ab}^{a} ± 0.008	0.025_{ab}^{a} ± 0.005	0.022_{a}^{a} ± 0.004	0.023_{b}^{a} ± 0.005	0.017_{ab}^{b} ± 0.005	0.032_{a}^{a} ± 0.004	0.013_{b}^{b} ± 0.008	0.027_{ab}^{a} ± 0.008	0.016_{ab}^{a} ± 0.005	0.022_{b}^{a} ± 0.008	0.008
Zinc	3.48_{a}^{a} ± 0.610	2.28_{d}^{b} ± 0.628	4.49_{b}^{a} ± 0.554	3.37_{b}^{b} ± 0.187	3.54_{cd}^{a} ± 0.637	2.45_{cd}^{b} ± 0.772	6.03_{a}^{a} ± 0.638	4.74_{a}^{b} ± 0.433	4.05_{bc}^{a} ± 0.627	2.91_{bc}^{b} ± 0.768^{f}	0.540
Manganese	0.028_{a}^{a} ± 0.004	0.027_{a}^{a} ± 0.005	0.028_{a}^{a} ± 0.008	0.020_{b}^{b} ± 0.000	0.010_{b}^{b} ± 0.000	0.020_{b}^{a} ± 0.009	0.015_{b}^{a} ± 0.005	0.015_{b}^{a} ± 0.005	0.033_{a}^{a} ± 0.008	0.030_{a}^{a} ± 0.000	0.006

[#] SD: Standard deviation.

$_{a, b, c, d}$ Means in the same row within a treatment and between muscles, with different subscripts are significantly different ($P \leq 0.05$).

$^{a, b}$ Means in the same row within a component and between treatments, with different superscripts are significantly different ($P \leq 0.05$).

LSD: Least significant difference ($P=0.05$).

CONCLUSIONS

One of the main objectives of the beef industry is to produce a product of consistent quality, which complies with the consumer needs and satisfies the demand for a high-quality beef product (Kerth *et al*., 1995). In the present study the effect of a blend containing sodium and potassium salts, di- and triphosphates and lactates on the pH, water-binding capacity, instrumental meat colour and instrumental tenderness during post-mortem ageing were investigated. The initial proximate and mineral composition of the treated muscles was also determined. The general findings suggest than an increase in tenderness concurrent with an acceptable beef colour resulted from the infusion with the mentioned blend.

Infusion of a salt mixture containing sodium and potassium di- and triphosphates, lactate and chloride is one of the methods that can be used by South African meat processors to improve traditionally less tender beef cuts. Thereby tenderness in all beef cuts is guaranteed and consumer demands for a higher-quality and more convenient product are met.

Several corrective actions mentioned in this study have been investigated by researchers to overcome toughness problems, reduce tenderness variability and increase consumer satisfaction with regard to beef quality (Scanga *et al*., 2000; Baublits *et al*., 2005; Hoffman, 2006). The similarities of the brine solutions applied within these studies and the success achieved by other reported studies, such as Hoffman (2006), give an indication of the success that the blend used in the present investigation could accomplish in the South African beef industry.

In conclusion, the infusion of beef muscles with a commercial basting mixture containing sodium and potassium salts, di- and triphosphates and lactates was an effective means of lowering shear force values, without affecting the colour and water-binding abilities negatively. Therefore, the blend containing sodium and potassium salts, di- and triphosphates and lactates could be implemented in the current industry practice as a feasible and effective means of improving the tenderness of beef with no detrimental effects on other physical and chemical properties.

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CHAPTER 5

SENSORY AND PREFERENCE TESTING OF SELECTED BEEF MUSCLES INFUSED WITH A PHOSPHATE AND LACTATE BLEND

ABSTRACT

Consumers expect beef products to be juicy and tender. In the present investigation four beef muscles: *Biceps femoris* (BF, silverside), *Longissimus lumborum* (LL, striploin), *Rectus femoris* (RF) and *Semitendinosus* (ST, eye of the silverside), were infused 3 d post-mortem with a blend consisting of sodium and potassium salts, di- and triphosphates and lactates. After a storage period of 24 h at 4°C, the muscles were stored at −18°C until the descriptive sensory analysis could be performed. The BF and LL were also used in a consumer preference test to determine the overall degree of liking. The infused samples were significantly more juicy and tender than the control samples ($P \leq 0.05$). Furthermore, the infused samples, especially the LL sample, illustrated a significantly ($P \leq 0.05$) higher degree of liking than the non-infused samples. The enhancement of beef muscle with a phosphate, salt and lactate blend showed a degree of liking by the total group of target consumers. The infusion of beef muscles with a commercial blend containing sodium and potassium salts, di- and triphosphates and lactates could be applied successfully in the meat industry to enhance the sensory attributes of beef.

Keywords: Meat quality; enhanced beef; descriptive sensory analysis; preference testing

INTRODUCTION

Beef consumption is declining in South Africa (RMRDT, 2004) and studies overseas reveal a similar problem (Morgan *et al*., 1991). Tenderness is the most important meat palatability characteristic for consumers (Jeremiah, 1982; Huffman *et al*., 1996; Dransfield, 2003) and the prevalence of inconsistent tenderness is a major problem facing the beef industry today (Morgan *et al*., 1991; Smith *et al*., 1992). The meat industry needs to address this problem by striving to meet consumer demands and at the same time it has to keep up with changing consumer trends. Consumer acceptability and perception of quality are the key determinants of the success of a meat product.

From Chapter 3 it is clear that variations in beef muscle do exist, especially with regard to the sensory attributes of beef such as tenderness and juiciness. In order to reduce this variation and to diminish its negative impact, several interventions and treatments have been suggested and applied in the meat industry. One such treatment is the infusion of the meat with a salt mixture. The use of calcium enhancement has been researched extensively and, despite increased tenderness, the negative effects pertaining to the detrimental colour (Wheeler *et al*., 1996) and flavour effects (Wheeler *et al*., 1993; 1997) still need to be resolved satisfactorily. Another intervention is the marinating of meat in acidic solutions to soften and flavour meat (Berge *et al*., 2001; Offer & Knight, 1988). The injection / infusion of fresh, whole muscles with a solution of water and ingredients such as salt, phosphates, antioxidants and flavourings to tenderise, add juiciness and enhance shelf-life qualities (Lawrence *et al*., 2003, Lawrence *et al*., 2004; Anon., 2005) is also a popular technique.

Chapter 4 described how various beef muscles were infused with a phosphate and lactate blend to achieve a meat product of enhanced physical and chemical characteristics, and also a reduction in the variation of specific characteristics such as instrumental tenderness. It is assumed that the latter technique will also result in a positive effect on the sensory properties of the product. Results from previous studies indicate that descriptive sensory analyses have shown increased juiciness and tenderness scores with the enhancement of beef with phosphate and salt containing solutions (Vote *et al*., 2000; Hoffman, 2006).

Therefore, the first aim of this study is to determine the effect of a commercially available basting (Freddy Hirsch Tenderbite) consisting of sodium and potassium salts, various phosphates and lactates on the sensory attributes of beef muscles as determined by descriptive sensory analysis. The second aim is to determine the acceptability of the basting by consumer sensory analysis.

MATERIALS AND METHODS

Animals and sampling

Beef carcasses representing South African beef breeds [Brahman × Simmentaler cross; n = 3, average mass = 300.73 kg and Charolais × Hereford cross; n = 3, average mass = 297.87 kg] finished off in a feedlot, were sourced from a commercial abattoir in Paarl, Western Cape, South Africa. At the abattoir, the animals were slaughtered, dressed and thereafter processed according to standard South African techniques and conditions. No

electrical stimulation was applied to the carcasses. The animals were selected to represent slaughter steers from a typical commercial scenario, representative of the South African market.

The carcasses were classified as A2 according to the South African classification system (Government Notice No R. 1748, 26 June 1992). An A2 animal is a young animal of the A age group (no permanent incisors) with a fat code of 2, representing a lean fat cover (1-3 mm thick subcutaneous fat depth measured between the 10^{th} and 11^{th} rib, 50 mm from the midline of the cold unquartered carcass). The whole, intact carcasses were chilled at *ca.* 2°C for 24 h in a cooling chamber before being weighed and quartered at the abattoir (Day 1). Twenty-four hours (Day 2) post-mortem (pm) the beef quarters were and transported in a mobile cooling unit (set at 4°C) to the Meat Science Laboratory at Stellenbosch University, where the carcasses were stored in the cooling facility at 4°C. On the same day (Day 2; 24 h pm) both the left and right side *Biceps femoris* (BF, silverside), *Longissimus lumborum* (LL, striploin), *Rectus femoris* (RF), *Semitendinosus* (ST, eye of the silverside) were removed from the carcasses. The muscles were trimmed of all visible subcutaneous fat and superficial collagen, weighed, labelled, vacuum packed and stored in a cooler at *ca.* 4°C until further processing.

Sample preparation

On Day 3 (48 h pm) all the muscles were transported to the Freddy Hirsch Processing Plant where the left-side muscles were removed from their packaging, demembraned and infused. Muscles from the right side of the carcass were left untreated and stored in a cooler at 2 °C to be used as the control. The muscles were infused with a cooled (2°C) salt mixture containing sodium and potassium di- and triphosphates, lactate and chloride (Freddy Hirsch Tenderbite; PO Box 2554, Cape Town, 8000) at a pressure of 2.4 bar at 30 strokes per min on a Rühle Curing Centre IR56 (Rühle GmbH, D·79865, Grafenhausen, Germany) to give a calculated pumped gain of 15% with a retention of 12%. The basting mixture gave a calculated chemical composition of 75.75% water, 5.21% Na^+, 2.53% K^+, 3.45% P_2O_5 and 12.40% lactate. After a resting (equilibration) period of 2 h the infused meat samples were vacuum packed and all the muscles were transported back to the Meat Science Laboratory at Stellenbosch University and stored in the cooler (4°C).

Twenty-four hours after infusion the samples were removed from the cooler and packaged for specific analyses. The samples were divided into two portions, firstly for physical and chemical analyses (Chapter 4) and secondly for sensory analysis (descriptive

sensory analysis and preference testing). The samples intended for sensory analysis were labelled, vacuum packed and frozen at −18°C (±10 months).

Throughout the trial an attempt was made to ensure that all activities were similar to a typical commercial scenario. Care was also taken throughout the investigation to ensure that the handling procedures were similar for the muscles from both sides of the same carcass.

Descriptive sensory analysis

Two treatments of beef, i.e. untreated beef muscle and beef muscle infused with a phosphate and lactate blend, were tested for selected beef attributes using an eight-point ordinal scale (AMSA, 1995).

Sample preparation

The vacuum-packed meat samples taken from both sides of the carcass were defrosted at a temperature of 2 to 4°C for a period of 48 h prior to cooking on their pre-assigned sensory analysis dates. Per carcass, eight samples of meat (2 treatments × 4 muscles) were used for each sensory analysis session. The left and right *Biceps femoris* (BF, silverside), *Longissimus lumborum* (LL, striploin), *Rectus femoris* (RF) and *Semitendinosus* (ST, eye of the silverside) were analysed.

The meat samples were cut to a uniform size and placed in a coded oven bag on aluminium foil covered metal racks. A temperature probe was inserted into the centre of the meat sample. Temperature changes were monitored with thermocouples connected to hand-held digital recorders. Samples were oven-roasted at 160°C in two conventional electric Defy 835 ovens connected to a computerised electronic temperature control system (Viljoen *et al.*, 2001) to an internal temperature of 68°C. After cooking, the meat was allowed to rest for 5 min, in which time an endpoint temperature of 72°C was reached.

Cubed samples (1.5 × 1.5 cm) were taken from the middle of each sample and wrapped individually in aluminium foil. The samples were placed in preheated glass ramekins, which were marked with random three-digit codes and positioned in a preheated oven of 100°C. The samples were removed from the oven and evaluated within 10 min by the sensory panel (AMSA, 1995).

Sensory analysis

An analytical sensory analysis panel consisting of eight members was used to analyse the sensory attributes of the selected muscles. The panellists were selected and trained

according to the guidelines for the sensory analysis of meat of the American Meat Science Associations (AMSA, 1995) using the generic descriptive analysis technique (Lawless & Heymann, 1998). The panellists were trained in two sessions on the attributes to be evaluated. The meat was analysed in five sessions over a period of 3 d, with eight samples (muscles) from one carcass per session.

The meat was analysed using the standard questionnaire of the American Meat Science Association (AMSA, 1995). This is an eight-point ordinal scale from low in intensity (1) to extremely high in intensity (8). The score sheet was compiled and refined by the panel during the training session. One attribute was added to the standard questionnaire, namely salty taste. The descriptive sensory analysis performed on the meat included beef aroma, beef flavour, initial impression of juiciness, sustained juiciness, first bite, residue and salty taste (Table 1).

Table 1. Definitions of the sensory attributes used for descriptive sensory analysis of beef.

Attribute and Scale	Definition
Beef aroma 1=Extremely untypical; 8=Extremely typical	Characteristic aroma associated with the meat of the animal species
Beef flavour 1=Extremely untypical; 8=Extremely typical	Characteristic flavour associated with the meat of the animal species
Initial juiciness 1=Extremely dry; 8=Extremely juicy	Amount of fluid exuded on the cut surface when pressed between the thumb and forefinger
Sustained juiciness 1=Extremely dry; 8=Extremely juicy	Amount of water perceived during mastication
First bite 1=Extremely tough; 8=Extremely tender	Force needed to compress the meat sample between molar teeth on the first bite
Residue 1=Abundant; 8=None	Amount of connective tissue remaining after most of the sample has been masticated
Salty taste 1=No salty taste; 8=Extremely salty	Taste on the tongue associated with sodium ions

Panellists were seated in individual booths in a light-controlled and temperature controlled room. Each judge received eight samples from each carcass per session (a set of four samples was served). The serving order of the samples was completely randomised for each session and each panellist. The panel used crackers, apple slices and distilled water to cleanse the palate in-between samples (AMSA, 1995).

Consumer preference testing

Two treatments of beef, i.e. untreated beef muscle (right side) and beef muscle infused with a phosphate and lactate blend (left side), were tested for degree of liking using a nine-point hedonic scale (Lawless & Heymann, 1998).

Sample preparation

Four samples of meat (2 treatments × 2 muscles) were used for the consumer sensory analysis. Both the left and right *Biceps femoris* (BF, silverside) and *Longissimus lumborum* (LL, striploin), were analysed. After the vacuum-packed meat samples were defrosted for 48 h at 3°C, a stock mixture was prepared for each muscle according to its weight and treatment (Table 2). The stock mixture consisted of the beef muscle, water, carrots, onions, celery, pepper, bay leaves and salt (Table 2). Due to the salty taste of the infused sample, no additional salt was added to this sample during the cooking process.

Table 2: Stock mixture for the two beef treatments of *Biceps femoris* and *Longissimus lumborum.*

Ingredient	Weight (g)			
	BFC	**BFI**	**LLC**	**LLI**
Beef muscles	2382.00	2659.00	1555.00	1353.30
Water	587.86	582.78	383.75	246.60
Carrots	215.54	213.68	140.71	108.76
Onions	156.76	155.40	102.33	79.09
Celery	117.56	116.56	76.75	59.32
Salt	11.75	-*	7.68	-*
Pepper	4.70	4.67	3.06	2.38
Bay leaves	0.72	0.69	0.47	0.35

* No additional salt was added during the cooking process to the infused muscles samples.

BFC: *Biceps femoris Control.*

BFI: *Biceps femoris Infused*.

LLC: *Longissimus lumborum Control.*

LLI: *Longissimus lumborum Infused.*

Each meat sample was placed in a coded oven bag into an oven pan. A temperature probe was inserted into the centre of each meat sample. The temperature changes were monitored with thermocouples connected to hand-held digital recorders. The samples were cooked in a Hobart Combi Steamer at 160°C to an internal temperature

of 68°C. After cooking, the meat was allowed to rest for 5 min, in which time an endpoint temperature of 72°C was reached.

Cubed samples (2.0 x 2.0 cm) were taken from the middle of each sample and wrapped individually in aluminium foil. The samples were placed in preheated glass ramekins, which were marked with random three-digit codes and positioned in a preheated oven of 100°C for less than 10 min. Upon removal from the oven, the samples were evaluated within 10 min by the consumer panel (AMSA, 1995).

Preference testing

The degree of liking of the four samples (2 treatments × 2 muscles) of beef was measured using the nine-point hedonic scale (AMSA, 1995). Internationally, the nine-point hedonic scale has been studied widely and has been found to be extremely useful in the hedonic assessment of foods and beverages. In this type of sensory analysis the subject is asked to indicate which term best describes his/her attitude towards the samples tested using a scale with nine categories ranging from Dislike extremely (1) to Like extremely (9). This test uses unbranded products and gives an indication of preference as well as acceptance, but not an indication of purchase intent. Furthermore, consumers should not give reasons for their preference (Lawless & Heymann, 1998).

The questionnaire was completed by 100 consumers (37% male; 63% female) from the age group of 18 up to an age group of 31+. The consumers were non-vegetarian and had to consume beef on a regular basis. The consumers were asked to complete a questionnaire comparing the four samples (sets of two, infused vs control) in front of them. The samples were presented in a completely randomised order. The consumers used tap water (21°C) to cleanse the palate in-between samples (AMSA, 1995).

Experimental design and statistical analyses

The experimental design for the descriptive panel was a randomised complete block design with eight treatment combinations replicated in six blocks (animals/carcasses). The treatment design was a 2 × 4 factorial with the factors, two treatments (control and infused) and four muscles (BF, LL, RF and ST). The sensory characteristics were measured on an eight-point ordinal scale.

The experimental design for the preference testing was a randomised complete block design with 4 treatment combinations replicated in 6 blocks (animals/carcasses) The treatment design was a 2 × 2 factorial with factors two treatments (control & infused) and two muscles (BF and LL). A random selection of 100 consumers scored the

treatments according to degree of liking, twenty consumers for each of the first 4 blocks and ten for the last two blocks. The degree of liking was measured by using a nine-point hedonic scale and the liking score data were subjected to analysis of variance (Lawless & Heymann, 1998).

For both trials, analysis of variance was performed on the data using SAS statistical software Version 9.1 (SAS, 2003). The Shapiro-Wilk test was performed to test for non-normality (Shapiro & Wilk, 1965). In some cases deviations from normality were the cause of one or two outliers, which were removed and the data reanalysed until the residuals were symmetrically distributed or normal. Where there was still significant evidence of non-normality, this could be ascribed to kurtosis rather than skewness. Interpretation of results was thus continued (Glass *et al.*, 1972). Student's t-Least Significant Difference (LSD) were calculated at the 5% confidence level to compare treatment means of significant source effects (Ott, 1998).

RESULTS AND DISCUSSION

Descriptive sensory analysis

The score means for the sensory quality characteristics of the selected beef muscles for the control and infused samples are presented in Table 3.

Beef aroma and flavour

According to Table 3, the treated and untreated samples did not differ significantly with regard to aroma and flavour ($P>0.05$). Therefore, in the present investigation the infusion had no negative effect on the beef aroma or on the beef flavour of the product. The score means of the untreated samples indicated that the samples (control and enhanced) had a typical beef aroma and flavour. Some researchers have found results of poorer overall beef flavour with the infusion of a phosphate and salt blend (Morris *et al.,* 1997; Hoffman, 2006), whereas other studies report a more intense flavour with enhancing a meat product with a salt solution (Baublits *et al*., 2006a). Sheard *et al*. (1999) report a similar or poorer flavour intensity with the application of a phosphate blend. For aroma a significant ($P\leq0.05$) difference was observed between muscles regardless of the treatment (Table 3). The BF control sample was rated highest for aroma (6.875 ± 0.810), significantly higher than that of the other untreated muscles. Similar results were obtained for the aroma of the injected samples, with infused BF having the more typical beef aroma ($P\leq0.05$) than the rest of the treated muscles.

The flavour of the control samples as well as that of the injected samples did not differ significantly ($P>0.05$). Therefore, the four muscles in both treatment groups had a similar typical beef flavour (Table 3). This result could be due to the short ageing period (3 d) of the muscles before freezing (−18°C). Ageing time is an important factor for the development of strong flavour precursors (Monsón *et al*., 2005). However, other reports also indicated no significant differences in beef flavour intensity between muscle types, indicating a similar flavour between muscles across all treatments (Baublits *et al*., 2006c).

Initial and sustained juiciness

Initial juiciness is an indication of the amount of fluid exuded on the cut surface when pressing the sample between the thumb and forefinger, whereas sustained juiciness is an indication of the juiciness during mastication (Table 1). According to Table 3, all the injected samples illustrated a significantly ($P\leq0.05$) higher degree of initial and sustained juiciness than the untreated samples. Several studies support the results of the present investigation, where improved juiciness was observed when blends of similar composition were infused into beef muscles (Baublits *et al*., 2005, Baublits *et al*., 2006b; Lennon *et al*., 2005) Hoffman (2006) reported similar results, with the exception that the mean score for the infused sample was slightly lower. This is most likely because older animals were used in the mentioned study (Hoffman, 2006) and muscles from older animals have less moisture, although more fat, which could result in lower scale ratings for juiciness.

In this investigation (Table 3), the RF sample was considered the least juicy. This tendency was significant ($P\leq0.05$) for the control as well as for the injected muscles.

Although infusion increased the overall juiciness of both parameters, the ranking remained similar. Therefore the RF cannot be made more juicy than the LL, if both muscles were treated the same. The order for the highest score changed for initial juiciness (LL) and sustained juiciness (RF). Although the RF sample showed a higher fat content than LL in Chapter 4, the difference was not significant ($P>0.05$) and therefore fat content is probably not an explanation for the change in juiciness. From the pH results obtained in Chapter 4 it is shown that the RF had a significantly higher ($P\leq0.05$) pH (5.73) than the LL (5.62). This is a more plausible explanation for the change in juiciness, with a higher pH resulting in a higher water-holding capacity (Honikel, 1987).

Table 3. Mean scores (±SD)[#] for sensory characteristics of beef muscles infused with a phosphate and lactate blend.

Characteristic	Muscle								LSD (*P*=0.05)
	Biceps femoris (BF)		*Longissimus lumborum* (LL)		*Rectus femoris* (RF)		*Semitendinosus* (ST)		
	Control	Injected	Control	Injected	Control	Injected	Control	Injected	
Beef aroma[d]	$6.875_{a}^{a} \pm 0.810$	$6.839_{a}^{a} \pm 0.848$	$6.518_{bc}^{a} \pm 0.934$	$6.536_{b}^{a} \pm 0.894$	$6.393_{c}^{a} \pm 0.888$	$6.518_{b}^{a} \pm 0.809$	$6.625_{b}^{a} \pm 0.865$	$6.446_{b}^{a} \pm 0.851$	0.2315
Beef flavour[e]	$6.554_{a}^{a} \pm 0.601$	$6.679_{a}^{a} \pm 0.716$	$6.357_{a}^{a} \pm 0.724$	$6.518_{a}^{a} \pm 0.763$	$6.393_{a}^{a} \pm 0.755$	$6.554_{a}^{a} \pm 0.658$	$6.536_{a}^{a} \pm 0.738$	$6.518_{a}^{a} \pm 0.809$	0.2067
Initial juiciness[f]	$6.321_{b}^{a} \pm 0.897$	$6.464_{a}^{a} \pm 0.953$	$6.537_{a}^{a} \pm 0.905$	$6.482_{a}^{a} \pm 0.809$	$6.179_{b}^{a} \pm 0.897$	$6.071_{b}^{a} \pm 0.871$	$6.491_{a}^{a} \pm 0.979$	$6.357_{a}^{a} \pm 0.883$	0.2617
Sustained juiciness[g]	$5.857_{a}^{b} \pm 0.862$	$6.161_{a}^{a} \pm 1.005$	$5.375_{b}^{b} \pm 0.964$	$5.804_{c}^{a} \pm 0.999$	$5.482_{b}^{b} \pm 1.112$	$5.857_{bc}^{a} \pm 0.962$	$5.625_{ab}^{b} \pm 0.964$	$6.071_{ab}^{a} \pm 0.783$	0.2544
First bite[h]	$5.482_{c}^{b} \pm 0.874$	$5.893_{c}^{a} \pm 1.155$	$5.661_{c}^{b} \pm 0.959$	$6.464_{b}^{a} \pm 0.830$	$6.714_{a}^{b} \pm 0.825^{b}$	$7.054_{a}^{a} \pm 0.818$	$6.268_{b}^{b} \pm 0.904$	$6.625_{b}^{a} \pm 0.926$	0.2917
Residue[i]	$6.071_{c}^{b} \pm 0.892$	$6.429_{c}^{a} \pm 0.912$	$6.167_{c}^{b} \pm 0.885$	$6.907_{b}^{a} \pm 0.807$	$7.000_{a}^{b} \pm 0.915$	$7.339_{a}^{a} \pm 0.721$	$6.661_{b}^{b} \pm 0.815$	$7.018_{b}^{a} \pm 0.674$	0.2661
Salty taste[j]	$1.571_{a}^{b} \pm 0.806$	$2.630_{a}^{a} \pm 0.938$	$1.500_{a}^{b} \pm 0.831$	$2.661_{a}^{a} \pm 0.978$	$1.536_{a}^{b} \pm 0.873$	$2.500_{a}^{a} \pm 0.894$	$1.411_{a}^{b} \pm 0.781$	$2.759_{a}^{a} \pm 0.845$	0.2854

[#] SD: Standard deviation.

$_{a, b, c}$ Means in the same row within a treatment and between muscles, with different subscripts are significantly different ($P \leq 0.05$).

$^{a, b}$ Means in the same row within a sensory attribute and between treatments, with different superscripts are significantly different ($P \leq 0.05$).

LSD: Least significant difference (P=0.05).

[d]Beef aroma: 1=Extremely untypical; 8=Extremely typical.

[e]Beef flavour: 1=Extremely untypical; 8=Extremely typical.

[f]Initial juiciness: 1=Extremely dry; 8=Extremely juicy.

[g]Sustained juiciness: 1=Extremely dry; 8=Extremely juicy.

[h]First bite: 1=Extremely tough; 8=Extremely tender.

[i]Residue: 1=Abundant; 8=None.

[j]Salty taste: 1=No salty taste; 8=Extremely salty.

First bite and residue

First bite as well as residue gives an indication of the tenderness of a sample (Table 1). In both these parameters (Table 3) the tenderness of all the injected samples were enhanced significantly ($P \leq 0.05$). This result has also been illustrated by a number of researchers (Papadopoulos *et al.,* 1991; McGee *et al*., 2003; Baublits *et al.,* 2005; Hoffman; 2006).

The mean scores for tenderness give an indication of which part of the scale the descriptive panel used. For the injected samples the mean scores for the tenderness were mostly on the upper part of the scale between 6 (moderately tender) and 7 (very tender) (Table 3). Hoffman (2006) reported much lower scores, possibly because much older animals were used and thus a slightly tougher result could be expected.

Within treatments, the four muscle samples also differed significantly ($P \leq 0.05$) in tenderness (Table 3). For both first bite and residue the RF samples were rated significantly higher in tenderness than the other three samples: 7.054 ± 0.818 and 7.339 ± 0.721 respectively. Similar results were reported by McKeith *et al*. (1985), Jeremiah *et al*. (2003) and Rhee *et al*. (2004).

From the scores allocated, the order of muscles rank is similar in both the treatments and attributes. This is a similar scenario as previously discussed with the overall juiciness. In other words, the muscles with the highest rank cannot be made more tender, if all the muscles were treated the same. This muscle ranking of sensory tenderness is supported by the instrumental tenderness reported in Chapter 4. The RF muscle is rated the most tender (highest residue and first bite score), and also gave the lowest shear force value within both treatments.

Salty taste

The infused samples were rated significantly higher ($P \leq 0.05$) in salty taste than the untreated samples (Table 3). Within treatments, i.e. between muscles, no significant ($P > 0.05$) differences were detected. These results indicate that all the injected samples had a similar but slight salty taste, whereas the untreated samples were all deficient of a typical salty taste. In previous research and developmental studies a commercial phosphate and lactate blend resulted in a strong salty taste (Hoffman, 2006). After improvements were made, the blend resulted in a desirable and slight salty taste (unpublished data), similar to that of the present investigation.

Consumer preference

Table 4 presents the analysis of variance (ANOVA) for liking scores and Table 5 shows the means of the scores. According to Table 5, the total group of consumers found the infused LL sample significantly more acceptable ($P \leq 0.05$) than both untreated muscles. This higher degree of liking for the treated LL sample is probably because this sample illustrated a high degree of sensory tenderness (Table 3). According to Jeremiah (1982), tenderness of beef is the main palatability attribute to influence consumer acceptance. Although the infused BF samples were not regarded as the most tender sample (Table 3), their acceptability was not significantly different ($P > 0.05$) from that of the infused LL sample (Table 4). This is not in agreement with the data obtained from the trained panel, where the BF and LL samples were scored significantly ($P \leq 0.05$) different in first bite and residue. Again, the BF was scored as less tender than the LL by the trained panel.

Table 4. Analysis of variance table for degree of liking score.

Source	DF	Mean Square	Pr > F
Carcass	5	32.4407	0.0019
Muscle	1	4.1823	0.3684
Treatment	1	55.9690	0.0040
Muscle x Treatment	1	8.3621	0.2095
Experimental error	15	4.8632	0.0462
Sample error	373	2.8377	-
Total corrected	376	-	-
Non-normality	-	Pr < W	0.0075

DF: Degrees of freedom.

Table 5. Scores means (±SD)[#] for degree of liking of beef muscles infused with a phosphate and lactate blend.

Muscle x Treatment	Replications	Degree of liking score[c]
BF Control	98	5.429 ± 1.833^{b}
BF Infused	100	5.900 ± 1.636^{ab}
LL Control	100	5.360 ± 2.008^{b}
LL Infused	99	6.394 ± 1.760^{a}
LSD (P=0.05)	-	0.667

[#] SD: Standard deviation.

[a, b] Means with different superscripts are significantly different ($P \leq 0.05$).

LSD: Least significant difference (P=0.05).

BF Control: *Biceps femoris control*; BF Infused: *Biceps femoris infused.*

LL Control: *Longissimus lumborum* control; LL Infused: *Longissimus lumborum infused.*

[c]Degree of liking: 1= Dislike extremely; 9=Like extremely.

CONCLUSIONS

The aim of this study was to determine the effect of a phosphate and lactate blend on the sensory attributes of various beef muscles, as well as whether the consumer would find the infused beef muscles acceptable.

The infused samples were significantly more juicy and tender than the control samples ($P \leq 0.05$). Furthermore, the infused samples, especially the LL sample, illustrated a significantly ($P \leq 0.05$) higher degree of liking amongst the consumers than the non-infused samples. Therefore, it can be concluded that infusion of beef muscles with a commercial blend containing sodium and potassium salts, di- and triphosphates and lactates can be applied successfully in the meat industry to enhance the sensory attributes of beef. Furthermore, this does not influence the palatability of the meat negatively; in fact, it is preferred by the meat-eating consumer.

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CHAPTER 6

PHYSICAL, MICROBIOLOGICAL AND CHEMICAL QUALITY OF BEEF *Longissimus thoracis et lumborum* MUSCLE INFUSED WITH A PHOSPHATE AND LACTATE BLEND

ABSTRACT

The modern consumer is increasingly concerned about consuming healthy and safe products, which include meat. Consumers expect meat products to have the required nutritional value, be wholesome, fresh, and have acceptable juiciness, taste and tenderness. In the present investigation the *Longissimus thoracis et lumborum* muscle from the left side of beef carcasses were infused 4 d post-mortem with a blend of sodium and potassium salts, di- and triphosphates and lactates, whilst the corresponding muscles from the right side were untreated and served as the control. After a storage period of 24 h at 4°C, the changes in pH and temperature, as well as the loss in purge and colour change of the muscles, were measured. Chemical attributes such as protein, fat and ash were also determined. The purge loss, colour change and the microbial growth on the muscle samples over a 10-d storage period (4°C, in oxygen permeable PVC) was determined. The meat colour analysis indicated greater loss of colour during this period in the infused muscle (lower a*, b* and chroma values) than the control sample. Physical analyses indicated a significant difference ($P \leq 0.05$) regarding the purge loss, with the treated sample losing 2.78% more purge in the 10 d. The results show that the phosphate and lactate blend infused into beef samples extended the microbiological shelf-life of beef loins by 1 d. The proximate composition of the meat samples taken from the first sampling day showed that the treated loin had a higher moisture and ash content, and lower lipid and protein content. All of these attributes have an impact on the buying decision made by the consumer. The phosphate-lactate blend can successfully be used to extend the shelf-life of beef and improve the safety at refrigerated temperatures with little effect on the physical (purge loss) and chemical attributes. However, the excessive decrease in colour stability observed in the infused steaks could negatively affect the purchasing decision of the consumer.

Keywords: Purge loss, colour, microbiological growth, shelf-life, proximate composition

INTRODUCTION

The demand for meat is at risk of being adversely affected as a result of consumer concerns about several aspects of meat consumption and production (Harrington, 1994). These consumer concerns are driving the market by increasing the demand for healthy and safe products, including meat products. Consumers view safety in this respect as a meat product with a relatively long shelf-life, produced with a minimal degree of processing under hygienic and animal-friendly conditions (Snijders & Van Knapen, 2002). Furthermore, consumers expect the meat products on the market to have the required nutritional value, be wholesome, fresh, lean, and have adequate juiciness, flavour and tenderness (Dransfield, 2003).

Meat is an excellent source of nutrients that are essential for normal human growth and development (Lushbough & Schweigert, 1960). As mentioned above, the palatability attributes of the meat product also form the basis for consumer acceptance and appeal (Weir, 1960). Despite the excellent dietary value and palatability of meat, the risks associated with meat consumption are possibly the most commonly acknowledged reason for declining meat consumption (Richardson *et al*., 1994).

Given the abundance of nutrients found in meat, it forms the ideal environment required by bacteria, yeasts and moulds to promote their growth (Lawrie, 1985). It is for this reason that meat is the most perishable of all the important foods (Gill, 1996).

In an attempt to ensure safe meat and as a means to extend the shelf-life of the meat product, the temperature and time of storage should be managed. According to Waites (1988), the main factor in controlling the development of micro-organisms during storage is not the composition of the flora initially present, but the conditions of storage. The shelf-life of red meat at refrigerated temperatures (0°-4°C) is, however, very limited. The shelf-life is approximately 10 to 14 d for beef (Warriss, 2000). Spoilage of chilled beef at refrigeration temperatures is due to the proliferation of various bacteria, yeasts and moulds on the meat surface (Jensen, 1954; Borch *et al*., 1996). The numbers and types of micro-organisms initially present and their subsequent growth have a significant impact on the shelf-life of the meat. The dressing process is responsible for causing more than 99% of the initial contamination (Labadie, 1999). Merely 10% of the initial microbes present have the ability to grow at refrigerated temperatures and an even smaller percentage of microbes are able to produce spoilage. Environmental factors such as temperature, gaseous atmosphere (oxygen availability), pH, moisture availability of the substrate and

salt content is microbe-specific and will consequently affect their growth rate and activity (Evans & Niven, 1960; Borch *et al*., 1996).

Borch *et al*. (1996) defined spoilage as a specified maximum microbial level or an off-flavour/off-odour or appearance that is undesirable. Fortunately, pertaining to a specified maximum microbial level, there are legally set microbiological standards to monitor the safety of most food products, including meat. A microbiological scale was developed by Fung (1986) to indicate the spoilage potential of meat. A bacterial count of 0 log to 2 log colony-forming units (CFU)/g on the meat is considered low. When a count reaches 3 log to 4 log CFU/g, the spoilage is considered intermediate. A count of 5 log to 6 log CFU/g is considered a high degree of meat spoilage. A count of 7 log CFU/g is considered the "Index of Spoilage", because when the number reaches higher than 8 log CFU/g, the meat will have developed an odour, and at 9 log CFU/g, slime will appear on the meat surface. The ICMSF (International Commission on Microbiological Specifications for Foods) (1986) also recommends a general viable count of less than 1×10^7 CFU.g^{-1}.

There are numerous techniques that can be applied to reduce or eliminate growth and preserve meat for longer periods, such as various packaging alternatives (Forrest *et al*., 1975) and the incorporation of sodium and potassium lactates (De Vegt, 1997). Aerobically stored meat is rapidly spoiled by bacteria which cause discoloration and off-odours (Labadie, 1999). The dominant organisms on meat after a few days of storage, at temperatures between 0 and 7°C, are *Pseudomonas* spp (Molin & Ternström, 1982). The rapid growth rate of *Pseudomonas* spp results in meat shelf-life being only a matter of days (Dainty, 1983; Borch *et al*., 1996).

In the present investigation a commercially available basting (Freddy Hirsch Tenderbite) consisting of sodium and potassium salts, various phosphates and lactates, was used to infuse the *Longissimus thoracis et lumborum* (loin) beef muscle. From previous research (Hoffman, 2006) and Chapters 4 and 5 in this thesis it is known that this blend increases the tenderness of meat. The effect of the blend on the shelf-life of beef has, however, not yet been determined. Therefore, the first aim of this study is to determine the effect of the phosphate and lactate blend on the shelf-life of the meat product and the synchronised result on the physical qualities of the meat. A secondary aim was to determine whether the blend has any negative implications pertaining to the chemical composition of the meat.

MATERIALS AND METHODS

Animals and sampling

The carcasses and meat samples were selected so as to represent steaks from a typical commercial scenario, representative of the South African market. Three beef carcasses were sourced from a commercial meat processing plant in the Western Cape, South Africa. The animals were slaughtered, dressed and processed according to standard South African techniques. Electrical stimulation was applied to the carcasses.

Four days post-mortem (pm) (Day 1 of trial) both the left and right *Longissimus thoracis et lumborum* muscles (LTL, loin) were removed from the hind quarter of the refrigerated (4°C) carcasses. The loin was dissected from the carcass between the 4th and 5th last rib to the last lumbar vertebrae. The muscles were trimmed of all visible fat and superficial collagen, according to industrial procedures at the commercial plant. The samples were tagged and weighed and the pH measured at the cranial end, in the eye of the muscle using a penetrating glass electrode on a portable Crison pH/mV-507 meter.

Sample preparation

The muscles from the right side of the carcass were left untreated and stored in a cooler at 4°C (Day 1) to be used as the control sample. The muscles from the left side of the carcass were infused (Day 1) with a salt mixture (pH 8.33; temperature −1.7°C) containing sodium and potassium di- and triphosphates, lactate and chloride (Freddy Hirsch Tenderbite; PO Box 2554, Cape Town, 8000) at a pressure of 1.9 bar at 33 cycles per min on a Rühle Curing Centre IR56 (Rühle GmbH, D·79865, Grafenhausen, Germany) to give a calculated pumped gain of 15% with a retention of 12%. The basting mixture gave a calculated chemical composition of 75.75% water, 5.21% Na^+, 2.53% K^+, 3.45% P_2O_5 and 12.40% lactate. The treated meat samples were weighed immediately after infusion and stored overnight in the same cooler (4°C) in which the control samples were stored (Day 1).

Twenty-four hours after infusion (Day 2) the left-side muscles were re-weighed to calculate the retained pumped gain. On Day 2 the six muscles from both sides were divided into five portions to reflect five time intervals. Meat slices, approximately 20 mm thick, were cut cross-sectional to the muscle fibre for the determination of pH, drip loss, colour and microbial growth of fresh beef muscle (4°C).

The quality characteristics of the muscle vary across the muscle. Therefore, to reduce the inherent variability of the muscle, the samples were randomised. However, the same muscle segment of the left and right were compared experimentally with each other.

The five time intervals reflect five successive shelf-life periods, ranging from 2 to 10 d, i.e. Day 2, 4, 6, 8 and 10. Day 1 accounts for the deboning, trimming and infusion of the muscle. The steaks were weighed, labelled and packed separately on black styrofoam trays (Foamotray 69) and over wrapped with heat-shrinkable oxygen-permeable polyvinyl chloride (PVC) cling film (Savell, 2003) (O_2 transmission, 12 000 $cm^3.m^{-2}.24h^{-1}$; CO_2 transmission 76 000 $cm^3.m^{-2}.24h^{-1}$) in a similar manner to packaging used by the retail industry. The meat samples were stored in crates and transported to the Meat Science Laboratory at Stellenbosch University and stored in a cooler (set at 4°C) until analysed on the pre-assigned day.

Throughout the trial, an attempt was made to ensure that all activities were similar to those of a typical commercial scenario. Care was also taken throughout the investigation to ensure that the handling procedures were similar for the muscles from both sides of the same carcass.

On the sampling date the samples allocated to that time interval were removed from the cooler for analyses. Upon completion of the physical and microbial analyses, the samples were homogenised, vacuum packed and stored at −18°C until proximate chemical analysis could be conducted. Care was taken to limit the sample handling and prevent contamination.

Physical analyses

The physical characteristics determined from the whole loin were pH before and after infusion, the pumped gain and purge loss. The data collected from the raw sub-samples/steaks over the 10-d period were pH, purge loss and instrumental colour.

pH and temperature

The pH of the muscle cut was measured before and after infusion and the pH of the sub-samples was measured with each time interval. All of the measurements were conducted by using a penetrating glass electrode on a hand-held Crison pH/mV-507 meter with an automatic temperature compensator. The pH measurements were taken approximately in the middle of the muscle cut. The pH meter was re-calibrated after every fourth reading with pH 4.01 and pH 7.02 standard buffers and the electrode rinsed with distilled water between each measurement.

Pumped gain and purge loss

The left-side muscles were weighed before and immediately after infusion to calculate the pumped gain, as well as 24 h after infusion (stored at 2°C) to calculate the retained pumped gain. For the determination of purge loss of the sub-samples, all the sub-samples were weighed (approximately 100 g) on Day 2 prior to packaging and then re-weighed on the designated sampling date. The purge loss was calculated as weight loss expressed as a percentage of the initial weight of the sample (Honikel, 1998).

Instrumental meat colour

Colour was evaluated according to the method described by Honikel (1998). The colour of the fresh control and infused *Longissimus thoracis et lumborum* (LTL) muscle samples were recorded on Day 2 (24 h after infusion), 4, 6, 8 and 10 (2 d = 48 h ± 2 h) using a Colour-guide 45°/0° colorimeter (Catalogue No 6805; BYK-Gardner, USA). The colour measurements for each sample were taken directly though the packaging material (cling wrap), in triplicate at randomly selected positions. The samples were continuously being exposed to oxygen due to the oxygen-permeable packaging material. Therefore, blooming was found to be unnecessary. Any subsequent microbiological contamination was also prevented in this manner.

Colour was expressed by the co-ordinates L*, a*, b* of the CIE Lab colorimetric space (CIE, 1978). The hue-angle and the a* and b* chroma are psychometric correlates of perceived hue and chroma that were determined using the following equations (CIE, 1978):

Chroma: $C^* = \sqrt{(a^*)^2 + (b^*)^2}$ Hue angle: $h_{ab} = \tan^{-1}\left\{\frac{b^*}{a^*}\right\}$

Microbiological analyses

After the physical parameters were executed, the muscle was microbiologically analysed by determining the total cell counts of the bacteria and yeast population on the beef loin samples (stored at 4°C) on Day 2, 4, 6, 8 and 10. With this analyses the shelf-life of the infused meat could be determined.

Growth of micro-organisms isolated from beef

Ten grams of meat from each steak sample was cut from the centre of the eye muscle. The samples were homogenised with 90 ml of sterile dilution liquid (0.1% (w/v) peptone, 0.85% (w/v) NaCl) and serially diluted. The dilutions were plated out on selected media

agar plates and incubated at the optimal growth temperature of the specific test organism for a specified period. The different selective growth media used to isolate the micro-organisms from beef loin samples are depicted in Table 1. The number of viable cells (CFU/g) (colony forming units/g) was determined after the various incubation times expired.

Table 1. The different selective growth media used to isolate micro-organisms from beef loin samples .

Selective Agar/Medium	Micro-organisms	Incubation conditions	Colony characteristics
MRS Agar, pH 5.6	Lactic acid bacteria	30°C, 72 h to 5 d, aerobic	Catalase-negative cells, rods and cocci
Plate Count Agar	Aerobic colony count	26°C, 48 h	All colonies
CASO Agar	*Pseudomonas*	26°C, 72 h	Oxidase-positive cells, Gram-negative rods
Yeast Extract Glucose Agar	Yeast and moulds	26°C, 72 h to 5 d	Yeast and moulds
KRANEP Agar	Micrococci and staphylococci	37°C, 48 h	Catalase-positive cells, cocci

Proximate chemical analyses

The LTL muscle samples from both sides of the carcass sampled on Day 2 were cut into smaller portions, minced three times through a 2 mm sieve to ensure homogeneity, vacuum packed and stored at −18°C until analysed chemically. The total percentages moisture, protein, fat and ash of the raw beef muscle samples were determined according to AOAC Methods (AOAC, 2002). The total lipid content was determined by extracting the fat with a 1:2 mixture of chloroform: methanol according to the method of Lee *et al*. (1996). The moisture content was analysed by drying a 2.5 g sample at 100°C for a period of 24 h (Method 934.01, AOAC, 2002) and ashing was done by cremating the moisture samples at 500°C for a period of 6 h. The protein content was determined by the Dumas combustion method (Method 968.06, AOAC, 2002) on the defatted samples using a FP528 Nitrogen Analyser.

Experimental design and statistical analyses

The experimental design for the physical parameters data from the muscle cuts (pH, purge loss) was a randomised complete block design with four treatment combinations replicated in three blocks (animals/carcasses). The treatment design was a 2×2 factorial with the factors, two treatments (control and infused) and two times (Day 1 and 2).

The experimental design for the physical parameters data from the sub-samples (pH, purge loss, colour) was a randomised complete block design with ten treatment combinations replicated in three blocks (animals/carcasses). The treatment design was a 2×5 factorial with the factors, two treatments (control and infused) and six times (Day 2, 4, 6, 8, 10). A second-order polynome function was also fitted over time on the means for each treatment and the coefficients were compared. For the colour characteristics the difference was analysed in the change in colour from Day 2 to Day 10 (Value on allocated day - Initial value on Day 2) as well as per day, with the same treatment design for the sub-samples as mentioned above. The data collected from the microbiological analysis were also transformed to log = LOG10(x+1) data.

The experimental design for the chemical parameters data from the sub-samples was a randomised complete block design with four treatment combinations replicated in three blocks (animals/carcasses). The treatment design was a 2×1 factorial with the factors, two treatments (control and infused) and one time (Day 2).

Analysis of variance was performed on the data using SAS statistical software Version 9.1 (SAS, 2003). The Shapiro-Wilk test was performed to test for non-normality (Shapiro & Wilk, 1965). In some cases deviations from normality were the cause of one or two outliers, which were removed and reanalysed until the residuals were symmetrically distributed or normal. Where there was still significant evidence of non-normality, this could be ascribed to kurtosis rather than skewness. Interpretation of results was thus continued (Glass *et al.*, 1972). Student's t-Least Significant Difference (LSD) was calculated at the 5% confidence level to compare treatment means of significant source effects (Ott, 1998).

RESULTS AND DISCUSSION

Physical properties

pH and temperature

A fundamental change that occurs in the conversion of muscle to meat is the process of muscle acidification. The variation in the rate and extent of acidification influences

important meat quality characteristics such as colour and water-holding capacity of the meat. Directly after deboning and trimming (Day 1; 4 d pm) the pH of the control and left-side loin was 5.30 ± 0.106 and 5.32 ± 0.067, respectively, and did not differ significantly (*P*=0.8003) between the two carcass sides. After infusion (blend = pH 8.33), the pH of the left-side muscle was initially 5.58 ± 0.287 (Day 1), which decreased to 5.46 ± 0.038 on Day 2 (24 h). The pH of the control sample was 5.40 ± 0.104 on Day 2 (5 d pm) and did not differ significantly from the infused muscle (*P*>0.05). However, as expected, the pH of the infused muscle was higher than the control. Previous research studies found higher pH values in muscle infused with alkaline phosphates, particularly polyphosphates (Offer & Knight, 1988; Lawrence *et al*., 2004).

The temperature (°C) of the control and infused muscles before infusion did not differ significantly, which indicates similar handling procedures for all the muscles from all the carcasses. The post-infusion temperature of the treated muscle (4.50 ± 0.200°C) was lower than the control (6.00 ± 0.100°C) because of the low temperature of the injection fluid (−1.7°C). The significance in temperature variation between Day 1 and Day 2 is a result of the two different coolers used for storage of the whole carcasses and the meat samples (muscle cuts).

In Table 2a and in Figure 1 the pH over time of the control (C) and infused (I) steak samples is shown. The pH range for the control samples over the 10 d was typical of the ultimate pH (pHu) of aged beef muscle, which is 5.4 to 5.5 (Lawrie, 1998). As expected, the treated samples had a significantly higher pH than the control immediately after injecting up to and including Day 4 (Figure 1). Earlier reports found that one of the main effects of phosphates on raw meats is elevation of the pH from the isoelectric point of approximately 5.5 towards the alkaline side. By increasing the alkalinity of the meat, the water-holding and water-binding capacities are increased (Shults *et al*., 1972; Lawrence *et al*., 2004). Although the infused samples had a higher pH than the control from Day 6 to Day 8, this was not significant (Table 2a). Interestingly, the decline in the pH of the infused muscles resulted in their attaining a lower pH than the control on Day 10 (5.39 ± 0.165 and 5.46 ± 0.148 respectively).

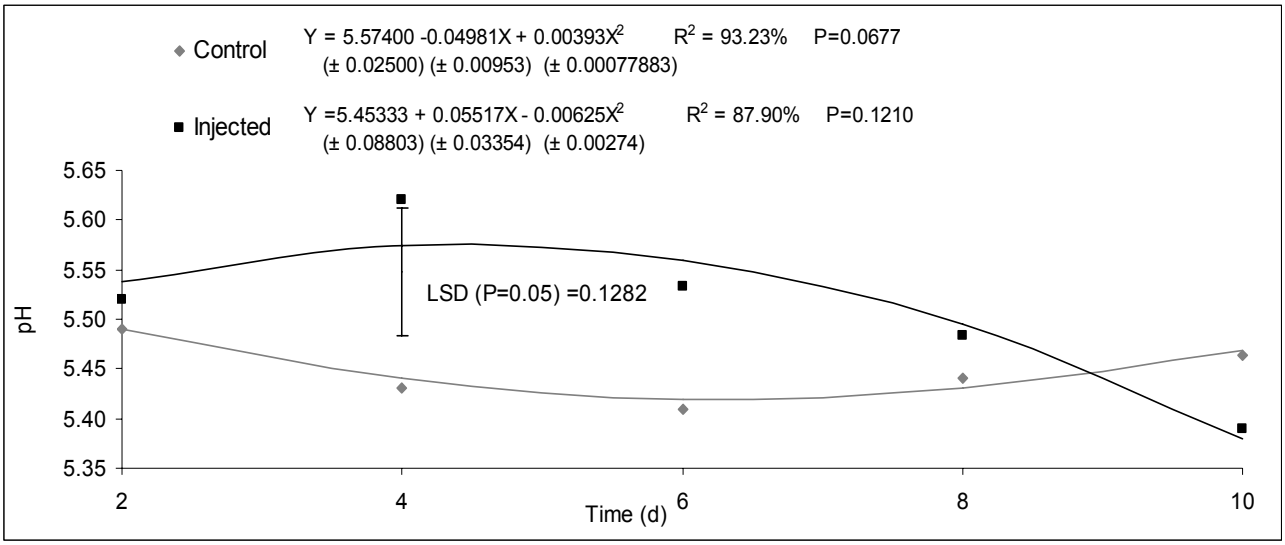

Figure 1. pH change of beef *Longissimus thoracis et lumborum* (LTL) muscle infused with a phosphate and lactate blend over a period of 10 d.

Pumped gain and purge loss

In this investigation the *Longissimus thoracis et lumborum* (LTL) muscle was infused 4 d post-mortem. The exudate that formed in the crates after the meat was stored at 4°C for 24 h was noted as purge and the retained pumped gain determined. The infused loins (LTL) initially gained 11.89 ± 3.181% by weight during the treatment, though after a resting period of 24 h the infused muscle had a retained pumped gain of 6.72 ± 0.899% (4.58 ± 1.893% purge loss). This result stresses the importance of allowing a "dripping period" in a commercial scenario, so that excess unbound water can drain off prior to cutting and packing the infused muscle. If this is not done, excess purge could accumulate in the packaging resulting in consumer aversion (Payne *et al*., 1998; Rogers, 2001). These results coincide with previous reported data, where a similar result of fluid uptake by the loin muscle was found (Lawrence *et al*., 2004). The control muscle had a purge loss of 0.23 ± 0.016% after a storage period of 24 h.

The role of various salts, particularly phosphates, for enhancing the water-holding capacity of muscle is well known. In the present investigation the infused solution contained phosphates and Foster (2004) found that by adding alkaline phosphates, in particular polyphosphates, the water-holding capacity of muscle is improved.

The question arises whether the water (pumped gain) will be retained throughout the storage period of the samples or whether it would slowly ooze out as purge. The percentage water in a meat product changes depending on how the meat is treated and on time passed (Offer & Knight, 1988). The loss of water can be due to evaporation, drip and cooking, with drip being defined as the dilute solution of sarcoplasmic proteins. The

quality of the meat product is reduced with the formation of drip, leading to lower yield and unsightly appearance when collected in the tray that presents the meat (Payne *et al*., 1998; Warriss, 2000).

Overall the infused samples had a significantly ($P \leq 0.05$) higher purge loss, because of the water added via the solution injected (Table 2a). Despite these higher values, both the control and the infused muscle had purge losses well in the range of beef muscle with a normal pH. According to Offer and Knight (1988), the amount of drip lost from steaks is in the range of 2-6% of the weight of lean meat after 4 d of chill storage.

The change in percentage purge loss over time is depicted in Figure 2. The amount of purge from the control samples increased with time and decreased from Day 8 onwards, however, this change in purge over the 10 d was not significant. The infused muscle also had an increase in purge loss from Day 2 to Day 8 and from then on the purge loss decreased. Several studies indicated that purge increases with storage time, with the largest losses occurring during the first 7 d of storage (Boles & Swan, 2002).

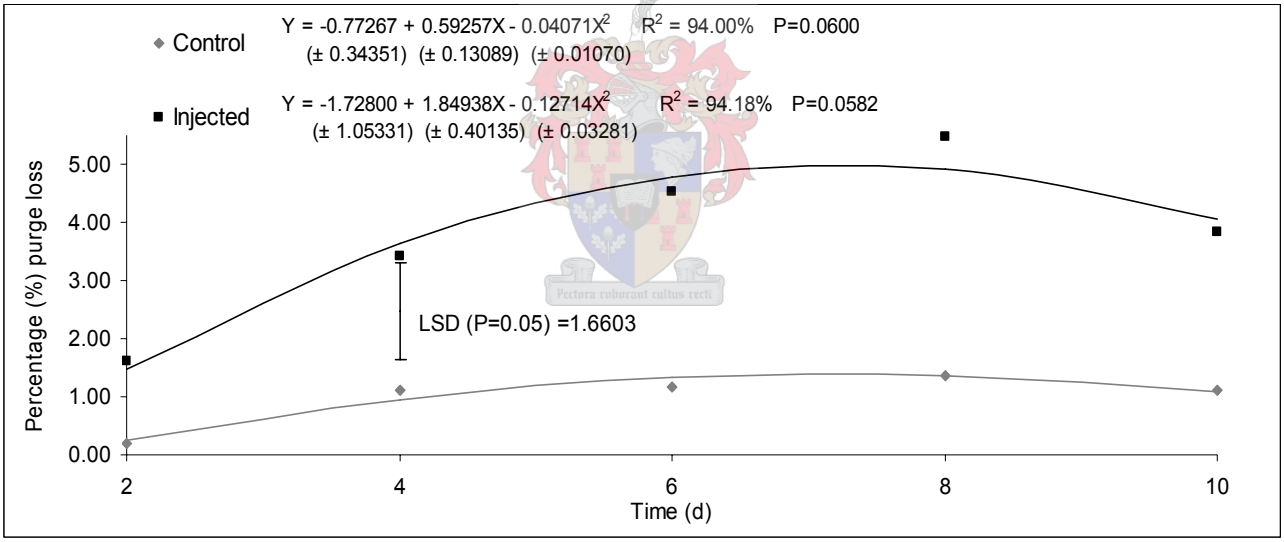

Figure 2. Percentage purge loss of beef *Longissimus thoracis et lumborum* muscle infused with a phosphate and lactate blend over a period of 10 d.

Table 2a. Interaction means (±SD)[#] for physical attributes of beef *Longissimus thoracis et lumborum* muscle infused with a phosphate and lactate blend over a period of 10 d.

Treatment	Day	pH	Purge loss	Initial-L*	Day-L*	Difference-L*	Initial-a*	Day-a*	Difference-a*
Control	2	5.49 ± 0.086^{abc}	0.19 ± 0.226^{c}	36.53 ± 2.257^{a}	36.53 ± 2.257^{c}	0.00 ± 0.000^{c}	15.99 ± 2.167^{ab}	15.99 ± 2.167^{a}	0.00 ± 0.000^{ab}
Control	4	5.43 ± 0.055^{bc}	1.12 ± 0.930^{c}	36.53 ± 2.733^{a}	39.14 ± 1.179^{ab}	2.61 ± 1.554^{ab}	15.35 ± 1.567^{abc}	15.31 ± 0.333^{ab}	-0.03 ± 1.372^{ab}
Control	6	5.41 ± 0.118^{bc}	1.18 ± 0.733^{c}	37.14 ± 0.882^{a}	39.75 ± 0.973^{a}	2.61 ± 0.420^{ab}	16.29 ± 3.632^{a}	15.74 ± 1.275^{a}	-0.55 ± 2.413^{ab}
Control	8	5.44 ± 0.056^{bc}	1.37 ± 0.563^{c}	36.42 ± 1.280^{a}	39.05 ± 0.899^{ab}	2.63 ± 1.269^{ab}	15.66 ± 1.564^{abc}	13.82 ± 1.938^{b}	-1.84 ± 1.318^{b}
Control	10	5.46 ± 0.148^{bc}	1.10 ± 0.115^{c}	36.18 ± 2.500^{a}	37.40 ± 1.470^{abc}	1.21 ± 1.132^{bc}	15.22 ± 3.427^{abcd}	10.88 ± 1.955^{c}	-4.34 ± 2.031^{c}
Infused	2	5.52 ± 0.225^{ab}	1.61 ± 1.409^{c}	36.99 ± 1.872^{a}	36.99 ± 1.872^{bc}	0.00 ± 0.000^{c}	14.26 ± 3.355^{abcd}	14.26 ± 3.355^{ab}	0.00 ± 0.000^{ab}
Infused	4	5.62 ± 0.090^{a}	3.42 ± 2.031^{b}	36.46 ± 2.146^{a}	38.80 ± 1.491^{abc}	2.34± 1.294^{ab}	14.09 ± 2.410^{bcd}	14.62 ± 2.091^{ab}	0.53 ± 0.452^{a}
Infused	6	5.53 ± 0.110^{ab}	4.54 ± 1.542^{ab}	34.58 ± 0.858^{a}	38.73 ± 0.193^{abc}	4.15 ± 0.667^{a}	13.74 ± 3.205^{cd}	13.84 ± 0.983^{b}	0.10 ± 2.666^{ab}
Infused	8	5.48 ± 0.049^{bc}	5.48 ± 1.193^{a}	35.57 ± 1.308^{a}	37.81 ± 2.614^{abc}	2.24 ± 1.317^{b}	14.20 ± 2.665^{abcd}	8.33 ± 1.045^{d}	-5.87 ± 1.620^{cd}
Infused	10	5.39 ± 0.165^{c}	3.82 ± 1.483^{ab}	35.89 ± 0.563^{a}	36.97 ± 1.345^{bc}	1.08 ± 1.211^{bc}	13.22 ± 2.480^{d}	5.31 ± 1.358^{e}	-7.91 ± 1.539^{d}
LSD (*P*=0.05)		0.1282	1.6603	2.5743	2.3785	1.8338	2.1051	1.8142	2.3004

[#] SD: Standard deviation.

[a, b, c, d, e] Means in the same column with different superscripts are significantly different (*P*≤0.05).

LSD: Least significant difference (*P*=0.05).

Initial-L*: L* measured on Day 2.

Day-L*: L* measured on allocated day.

Difference-L*: Change in L* from Day 2 to allocated day.

Table 2b (continue). Interaction means (±SD)[#] for physical attributes of beef *Longissimus thoracis et lumborum* muscle infused with a phosphate and lactate blend over a period of 10 d

Treatment	Day	Initial-b*	Day-b*	Difference-b*	Initial-Chr	Day-Chr	Difference-Chr	Initial-Hue_{ab}	Day-Hue_{ab}	Difference-Hue_{ab}
Control	2	15.23 ± 2.627^{ab}	15.23 ± 2.627^{a}	0.00 ± 0.000^{a}	22.09± 3.354^{abc}	22.09 ± 3.354^{a}	0.00 ± 0.000^{a}	43.48 ± 1.544^{b}	43.48 ± 1.544^{d}	0.00 ± 0.000^{cd}
Control	4	15.20 ± 0.475^{ab}	14.25 ± 0.856^{ab}	-0.96 ± 0.969^{ab}	21.62 ± 1.215^{abc}	20.92 ± 0.736^{ab}	-0.70 ± 0.506^{ab}	44.81 ± 2.919^{ab}	42.90 ± 1.516^{d}	-1.91 ± 4.414^{cd}
Control	6	16.51 ± 2.055^{a}	14.24 ± 1.308^{ab}	-2.27 ± 0.894^{bcd}	23.22 ± 3.989^{a}	21.23± 1.821^{ab}	-1.99 ± 2.287^{ab}	45.76 ± 3.192^{ab}	42.12 ± 0.367^{d}	-3.64 ± 3.419^{d}
Control	8	15.94 ± 1.775^{ab}	13.86 ± 1.798^{ab}	-2.08 ± 0.403^{bc}	22.34 ± 2.357^{ab}	19.58 ± 2.601^{b}	-2.77 ± 1.213^{b}	45.48 ± 0.555^{ab}	45.11 ± 1.418^{cd}	-0.37 ± 1.789^{cd}
Control	10	15.93 ± 1.305^{ab}	12.57 ± 0.668^{bc}	-3.36 ± 1.289^{cde}	22.10 ± 2.984^{abc}	16.64 ± 1.795^{c}	-5.45 ± 2.036^{c}	46.73 ± 5.593^{ab}	49.37 ± 3.461^{bc}	2.65 ± 2.631^{bc}
Infused	2	14.45 ± 2.564^{b}	14.45 ± 2.564^{ab}	0.00 ± 0.000^{a}	20.31 ± 4.173^{bc}	20.31 ± 4.173^{ab}	0.00 ± 0.000^{a}	45.62 ± 1.786^{ab}	45.62 ± 1.786^{cd}	0.00 ± 0.000^{cd}
Infused	4	14.78 ± 2.051^{ab}	14.46 ± 0.346^{ab}	-0.32± 1.962^{ab}	20.43 ± 3.142^{bc}	20.60 ± 1.578^{ab}	0.17 ± 1.636^{a}	46.47 ± 0.996^{ab}	44.88 ± 3.968^{cd}	-1.59 ± 3.063^{cd}
Infused	6	14.70 ± 2.380^{ab}	12.67 ± 2.535^{bc}	-2.03 ± 2.500^{bc}	20.13 ± 3.925^{bc}	18.86 ± 1.459^{bc}	-1.27 ± 2.971^{ab}	47.19 ± 1.968^{a}	42.18 ± 7.145^{d}	-5.00 ± 7.174^{d}
Infused	8	15.10 ± 2.013^{ab}	11.02 ± 1.441^{cd}	-4.08 ± 1.112^{de}	20.74 ± 3.253^{abc}	13.82 ± 1.770^{d}	-6.92 ± 1.640^{cd}	46.93 ± 2.016^{ab}	52.91 ± 0.884^{b}	5.98 ± 2.582^{b}
Infused	10	14.59 ± 1.611^{ab}	9.57 ± 1.071^{d}	-5.02 ± 1.576^{e}	19.70 ± 2.820^{c}	10.96 ± 1.591^{e}	-8.74 ± 2.176^{d}	48.05 ± 2.586^{a}	61.29 ± 3.392^{a}	13.24 ± 0.808^{a}
LSD (*P*=0.05)		1.9744	2.3973	1.9823	2.5725	2.5103	2.3039	3.5205	5.2951	5.8669

[#] SD: Standard deviation.

[a, b, c, d, e] Means in the same column with different superscripts are significantly different (*P*≤0.05).

Control: Sample left untreated.

Infused: Sample infused with a phosphate and lactate blend.

LSD: Least significant difference (*P*=0.05).

Initial-b*: L* measured on Day 2.

Day-b*: L* measured on allocated day.

Difference-b*: Change in b* from Day 2 to allocated day.

Chr: Chroma.

Instrumental meat colour

The appearance of the meat is the first important criterion that the consumer takes into account when judging meat quality (Barton-Gade *et al*., 1988; Mancini & Hunt, 2005). One of the major problems experienced with using high gas-permeable plastic film to over-wrap meat in trays is the unacceptable discolouration that develops (Savell, 2003). The plastic film allows easy penetration of oxygen from the air to the meat in the pack, where oxygen reacts with the myoglobin to give bright red oxymyoglobin. However, the stability of this colour is short lived, often only 1 to 2 d, before oxidation to metmyoglobin (grey-brown) becomes a problem. Consumers prefer beef to appear bright red because of the association with freshness (Carpenter *et al*., 2001). Both the infused and control samples showed rapid colour deterioration due to formation of metmyoglobin (Table 2a, b). The changes in colour over time for the colour components are shown in Figure 3-7.

Initial colour values do not differ significantly between the control and infused samples, which is favourable (Table 2a, b). With time the infused and control samples both increase in the L* value (lightness), indicating that the product becomes lighter. However, from Day 6 onwards both the treatments became more grey as a result of decreased L* values. No significant difference in lightness was found between the two treatments on Day 10 (Figure 3).

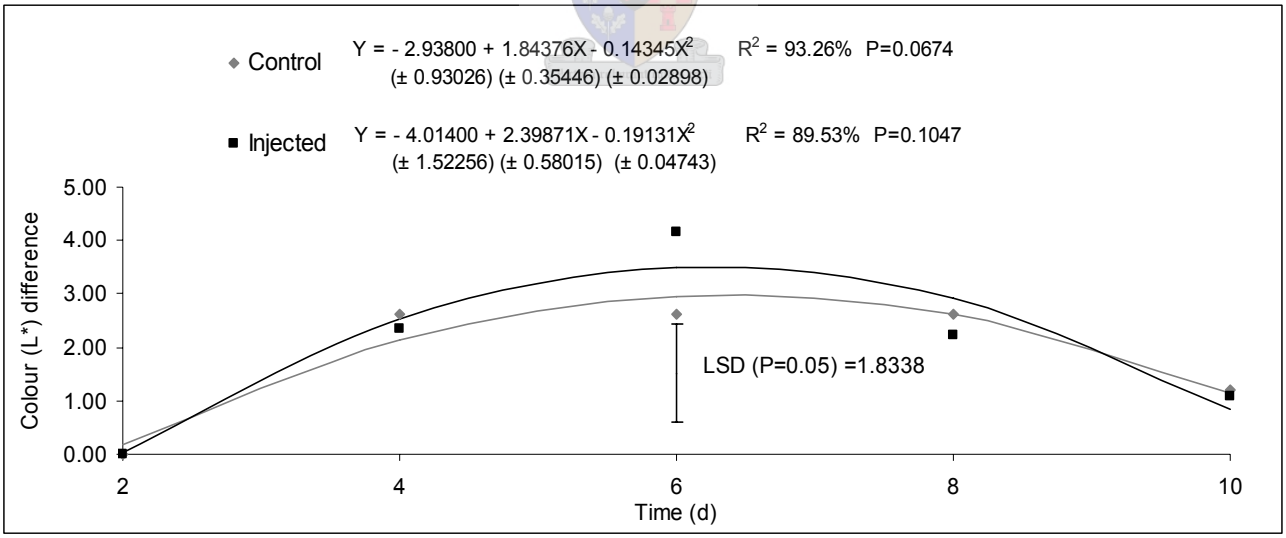

Figure 3. Colour change in lightness (L*) of beef *Longissimus thoracis et lumborum* muscle infused with a phosphate and lactate blend over a period of 10 d.

The higher the a* value, the more red (as opposed to green) the sample is and the higher the b* value, the more yellow (as opposed to blue) the sample. The control samples (Table 2a) maintained a stable a* (red) value for 6 d before decreasing significantly. Although the infused samples also showed a stable red colour for 6 d, the colour deterioration was more drastic (Figure 4).

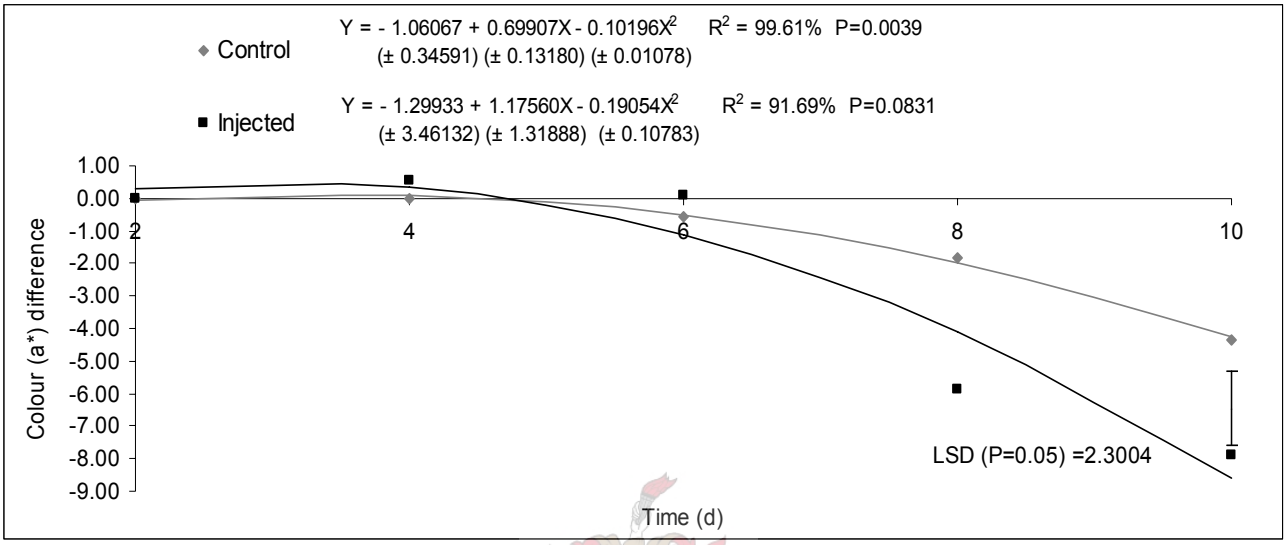

Figure 4. Colour change in a* (red vs. green) of beef *Longissimus thoracis et lumborum* muscle infused with a phosphate and lactate blend over a period of 10 d.

The b* value (Table 2b) of both the infused and control samples decreased with time, signifying a product less yellow and more dark towards a blue undertone. The control and infused samples did not differ significantly in yellowness ($P<0.05$) over the 10 d, although the infused samples showed a more rapid colour deterioration than the control and therefore appeared darker on Day 10 (Figure 5).

The chroma values (Table 2b) of both the infused and control samples decreased over the 10-d period, once more indicating the products were becoming less saturated in colour and therefore appearing duller. The control and infused samples differed significantly in chroma/saturation ($P\leq0.05$) over the 10-d period. As expected, the infused samples showed a more rapid loss in colour than the control (Figure 6).

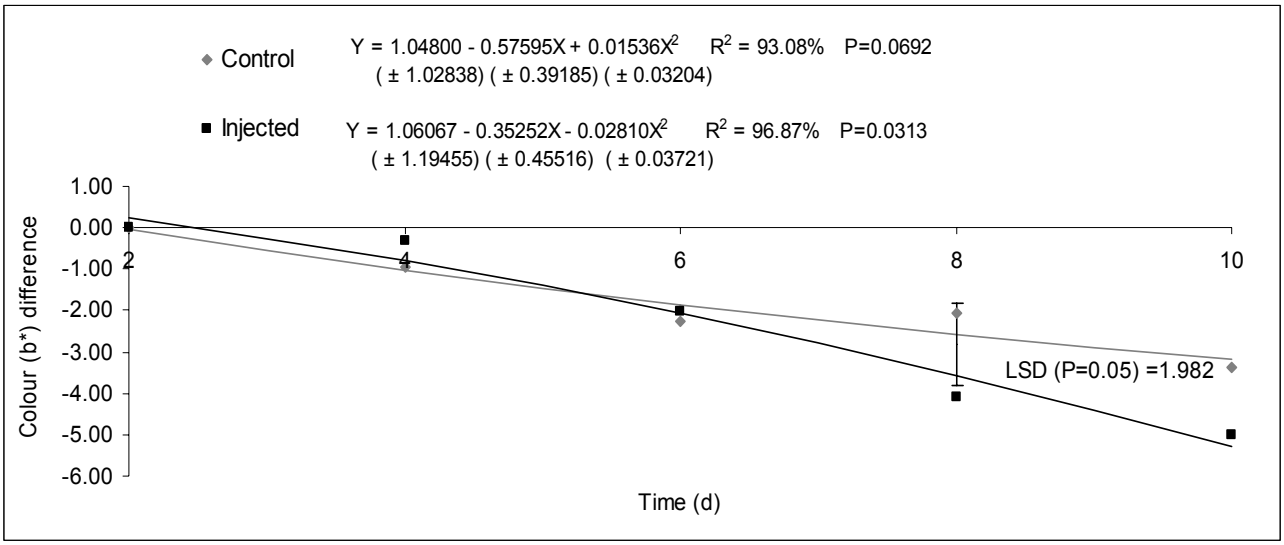

Figure 5. Colour change in b* (yellow vs. blue) of beef *Longissimus thoracis et lumborum* muscle infused with a phosphate and lactate blend over a period of 10 d.

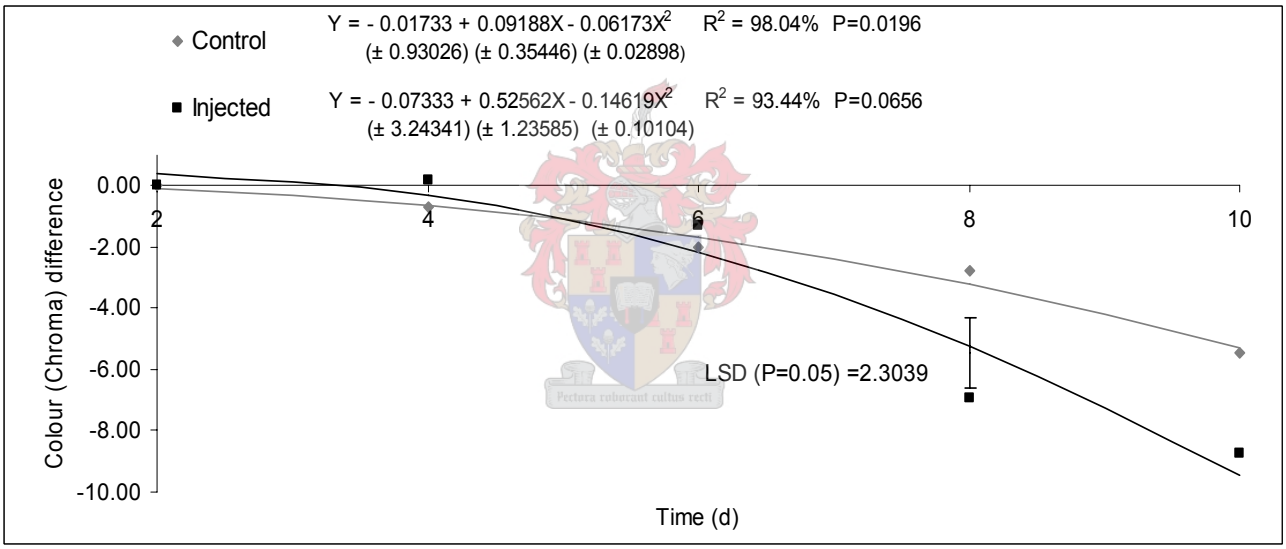

Figure 6. Colour change in chroma (saturation) of beef *Longissimus thoracis et lumborum* muscle infused with a phosphate and lactate blend over a period of 10 d.

Initially the hue_{ab} value for both the control and infused samples decreased slightly (Table 2b). However, from Day 6 both the treatments started to regain colour intensity. This significant increase in hue_{ab} values clearly demonstrated the unacceptable development of a brown meat colour. The control and infused samples differed significantly in hue_{ab} ($P \leq 0.05$) over the 10-d period. The infused samples showed a more rapid increase in colour intensity during this period (Figure 7), thereby displaying a browner meat colour than the control on Day 10.

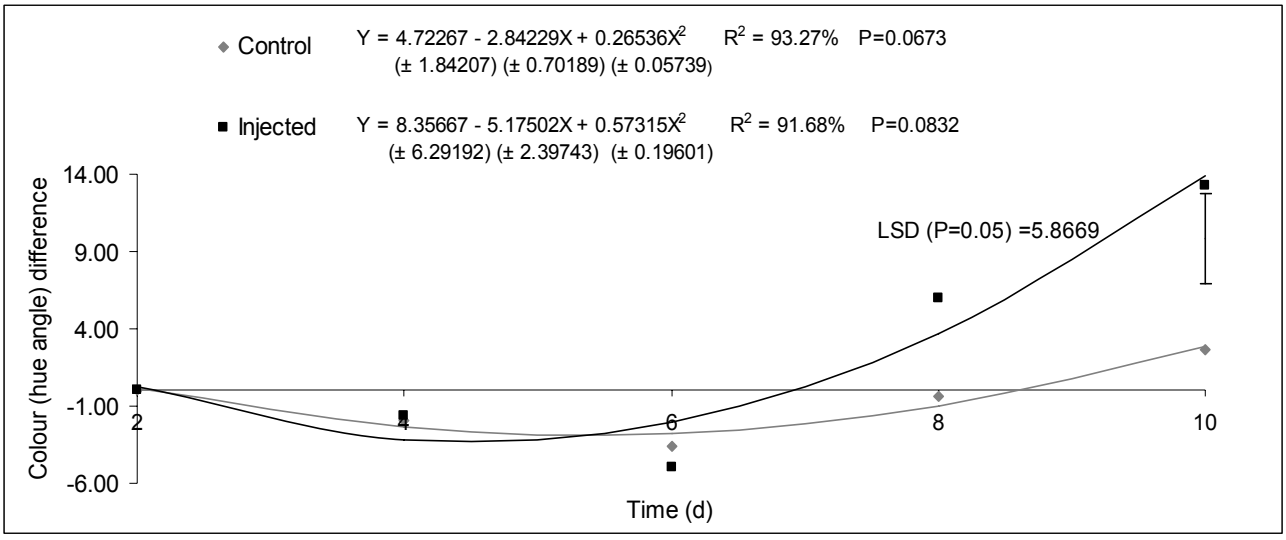

Figure 7. Colour change in hue_{ab} (intensity) of beef *Longissimus thoracis et lumborum* muscle infused with a phosphate and lactate blend over a period of 10 d.

Throughout the investigation the control samples maintained a red/light brown colour and the treated group a dark red/brown colour. What is interesting is that the results clearly indicate that both the control and infused muscle showed extensive discolouration by Day 6, giving the meat an appearance that would most probably not appeal to the consumer. The colour of fresh beef during retail display is an important attribute used by the consumers to judge the quality of the meat product and ultimately determine their purchase decision. Consumers desire a bright, cherry-red muscle tissue as an indication of freshness. Unfortunately, a natural process in beef is the regression of the desired cherry-red colour and the appearance of brown hues. This colour change normally occurs before microbial spoilage has manifested (Morgan, 2003).

Microbiological growth

The surface of carefully handled carcasses may contain up to 10^3 to 10^4 organisms cm^{-2} (the total viable count). When poor handling and hygiene occur, this amount could rise to more than 10^6 organisms cm^{-2} and, furthermore, off-odours often start developing at approximately 10^7 to 10^8 organisms cm^{-2} (Warriss, 2000; Fung *et al*., 2001). The legally set microbiological standard for meat is a generally viable count of less than 7 log_{10} CFU/g (Fung, 1986; ICMSF, 1986; Sallam & Samejima, 2004).

The number of bacteria and yeasts isolated from the beef *Longissimus thoracis et lumborum* muscle samples are listed in Table 3. Although the infused muscles, initially had significantly higher cell counts pertaining to all the media used, the initial number of micro-organisms present on the meat for both treatments was well below the previously

mentioned spoilage limit of 7 $\log_{10}$ CFU/g. The infused muscles had higher cell counts, possibly as a result of increased handling and/or contamination by the needles of the injection machine used (Wheeler *et al*., 1993) and the lack of a meat safety system such as HACCP (Hazard Analysis and Critical Control Point system) at the commercial processing plant.

Although the lactic acid bacteria (LAB) on the MRS-agar increased significantly in cell count (Figure 8), this increase was insufficient to cause spoilage in either the control or infused samples. Sakala *et al*. (2002) found that LAB is the most predominant spoilage bacteria in vacuum-packed beef, hence the lack of growth in an aerobic environment. Also the lactic acid bacteria in general are slower-growing bacteria and have a slow spoilage potential (Gill, 1996).

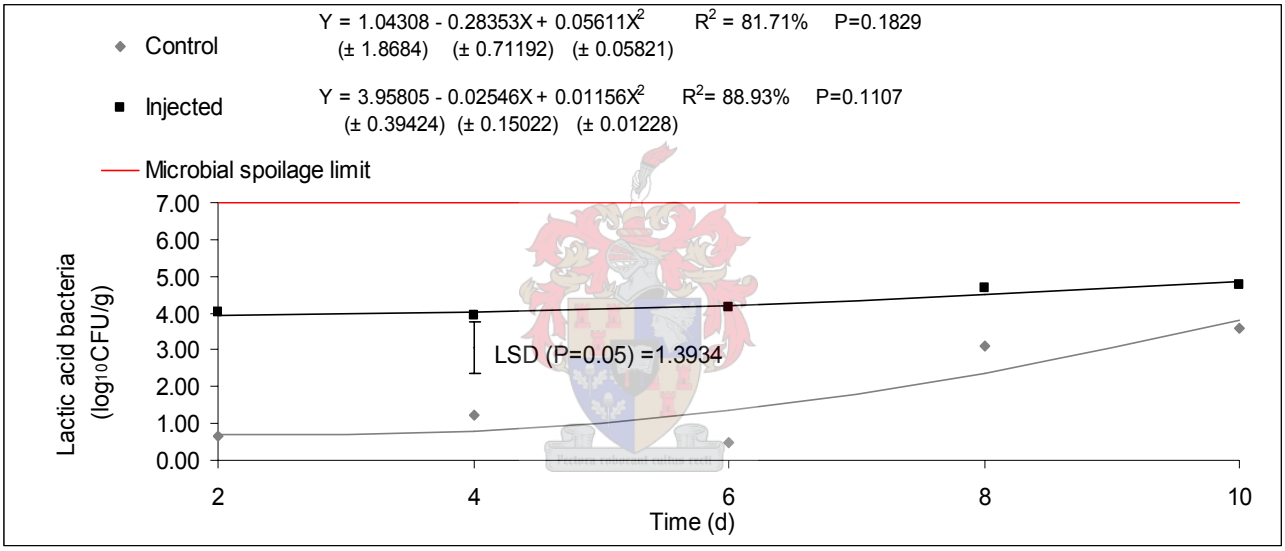

Figure 8. Growth of lactic acid bacteria (MRS-agar) on beef *Longissimus thoracis et lumborum* muscle infused with a phosphate and lactate blend over a period of 10 d.

The growth on the PCA-agar from the control muscles increased immediately from Day 2 onwards and already reached the spoilage limit (7 $\log_{10}$ CFU/g) by Day 8 (Figure 9). The infused sample also increased in growth, but at a significantly slower rate and reached the spoilage limit by Day 10. The total aerobic cell count (PCA-agar) differed significantly between the two treatments, indicating that the blend significantly impaired microbial growth. This result is supported by several shelf-life studies on fresh meat products, where NaL treatment significantly delayed the microbial growth and extended the shelf-life of the meat (Maca *et al*., 1999; Mandigo, 2002; Foster, 2004; Sallam & Samejima, 2004).

Table 3. Interaction means (±SD)[#] for colony forming units per gram (log_{10} CFU.g^{-1}) of beef *Longissimus thoracis et lumborum* muscle infused with a phosphate and lactate blend on various selective agar over a period of 10 d.

Treatment	Day	MRS	PCA	CASO	YE	KRANEP
Control	2	0.64 ± 1.117^{c}	4.01 ± 0.151^{f}	4.16 ± 0.163^{e}	4.22 ± 0.191^{f}	n/d
Control	4	1.20 ± 2.087^{c}	5.22 ± 0.577^{e}	5.48 ± 0.720^{d}	5.11 ± 0.522^{e}	n/d
Control	6	0.50 ± 0.868^{c}	5.15 ± 0.047^{e}	5.39 ± 0.038^{d}	5.30 ± 0.089^{e}	n/d
Control	8	3.12 ± 0.936^{b}	7.21 ± 0.374^{b}	7.41 ± 0.348^{ab}	7.45 ± 0.630^{bc}	n/d
Control	10	3.59 ± 0.125^{ab}	7.76 ± 0.384^{a}	7.92 ± 0.322^{a}	8.22 ± 0.348^{a}	n/d
Infused	2	4.02 ± 0.563^{ab}	4.94 ± 0.105^{e}	5.39 ± 0.302^{d}	5.25 ± 0.192^{e}	n/d
Infused	4	3.92 ± 0.058^{ab}	5.36 ± 0.353^{e}	6.28 ± 0.303^{c}	6.15 ± 0.339^{d}	n/d
Infused	6	4.17 ± 0.023^{ab}	5.97 ± 0.150^{d}	7.21 ± 0.109^{b}	7.12 ± 0.080^{c}	n/d
Infused	8	4.69 ± 0.158^{a}	6.60 ± 0.375^{c}	7.45 ± 0.173^{ab}	7.27 ± 0.123^{bc}	n/d
Infused	10	4.77 ± 0.120^{a}	7.51 ± 0.119^{ab}	7.82 ± 0.231^{a}	7.75 ± 0.173^{ab}	n/d
LSD (*P*=0.05)		1.3934	0.5158	0.5662	0.5727	n/c

[#] SD: Standard deviation.

[a, b, c, d, e, f] Means in the same column with different superscripts are significantly different ($P \leq 0.05$).

n/d: Not detected.

n/c: Not calculated.

LSD: Least significant difference ($P=0.05$).

MRS: De Man-Rogosa-Sharpe medium (Biolab, Merck Laboratories, Milnerton, South Africa).

PCA: Plate Count Agar (Biolab).

CASO: Casein-peptone Soymeal-peptone Agar (Biolab).

YE: Yeast Extract Agar (Biolab).

KRANEP: Potassium thiocyanate Actidion Sodium azide Egg-yolk Pyruvate Agar Basis (Fluka, Merck Laboratories, Milnerton, South Africa.

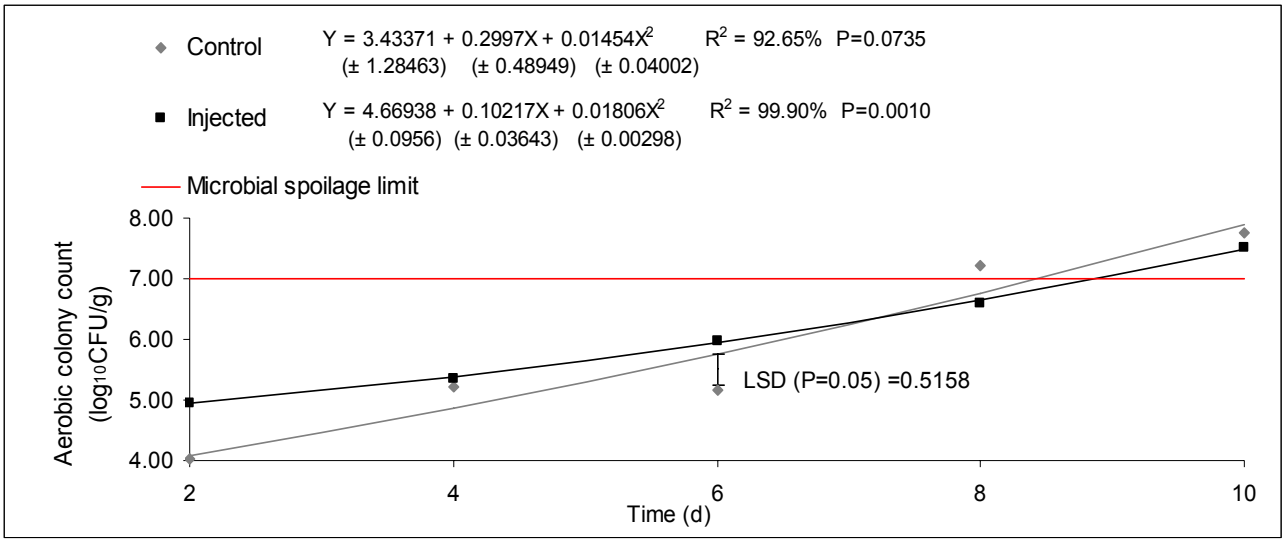

Figure 9. Growth of aerobes (PCA-agar) on beef *Longissimus thoracis et lumborum* muscle infused with a phosphate and lactate blend over a period of 10 d.

The *Pseudomonas* on the control CASO-agar followed the same growth trend as the micro-organisms on the control PCA-agar and reaches the limit (7 $\log_{10}$ CFU/g) by Day 8 (Figure 10). The infused sample increased in growth at a significantly faster rate than the control and already reached the spoilage limit (7 $\log_{10}$ CFU/g) by Day 6. Under aerobic conditions the dominant spoilage organisms are the strictly aerobic pseudomonads (Labadie, 1999). From Tables 3 and 4 it is evident that the *Pseudomonas* contributes the most to the spoilage of the beef *Longissimus thoracis et lumborum* muscle samples in this experiment.

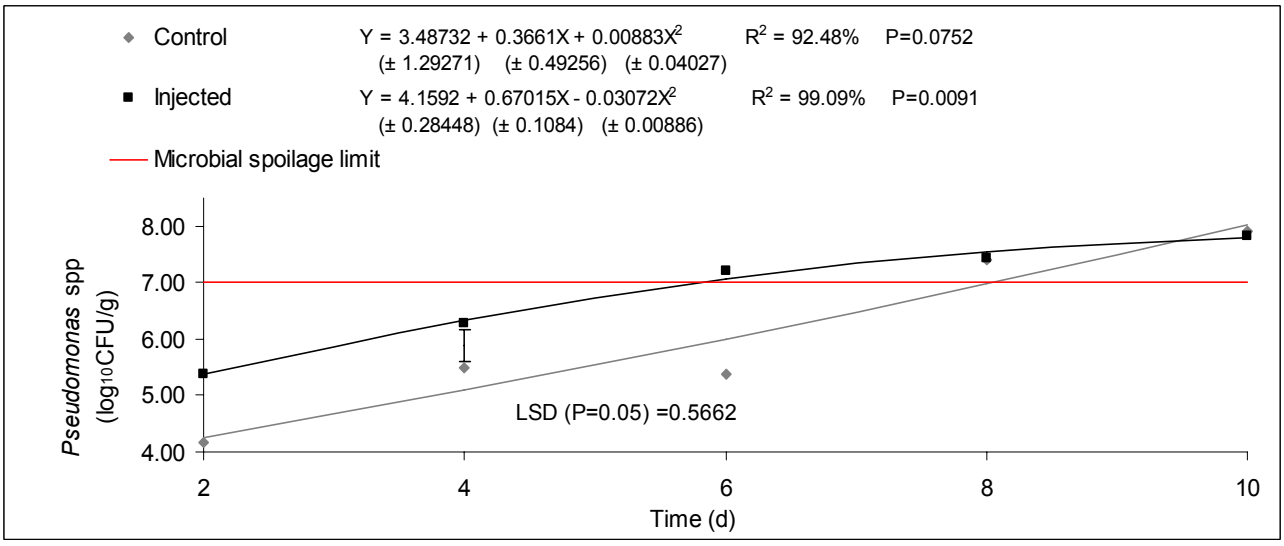

Figure 10. Growth of *Pseudomonas* spp (CASO-agar) on beef *Longissimus thoracis et lumborum* muscle infused with a phosphate and lactate blend over a period of 10 d.

The yeast and moulds (YE-agar) followed a similar trend (Figure 11) as the bacteria on the CASO-agar.

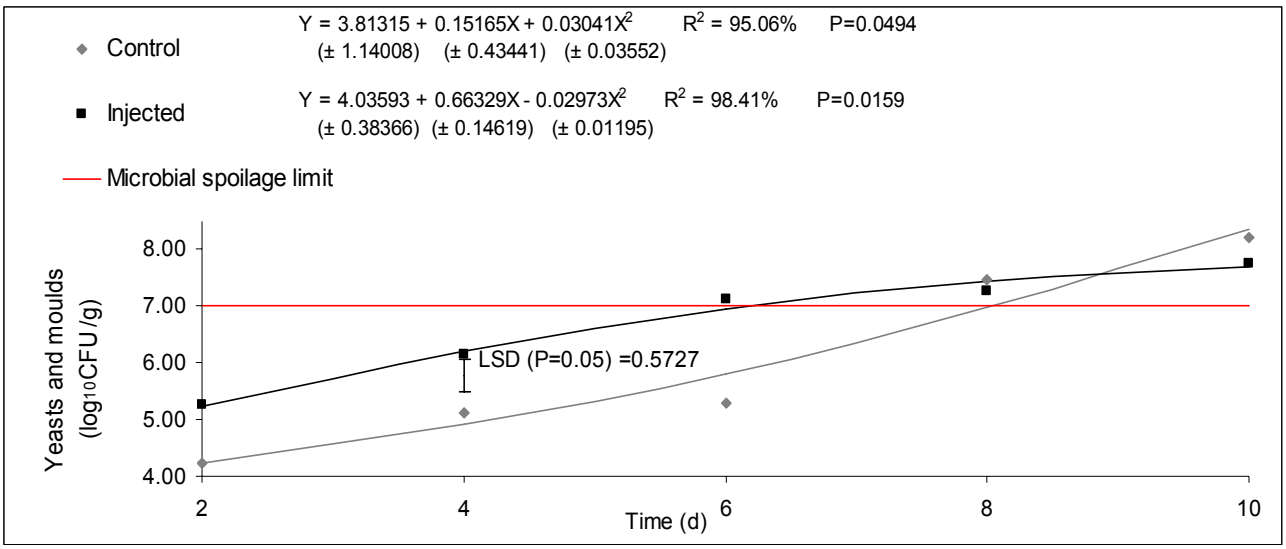

Figure 11. Growth of yeast and moulds (YE-agar) on beef *Longissimus thoracis et lumborum* muscle infused with a phosphate and lactate blend over a period of 10 d.

Micrococci and *staphylococci* (KRANEP-agar) could not be detected and appears to be absent on both the infused and the control beef samples.

It was concluded from the growth on the different isolation media (Table 4) that the *Pseudomonas,* yeast and moulds were the dominant micro-organisms contributing to the viable aerobic counts and as such the spoilage of the meat. *Pseudomonas* grows under aerobic conditions (0° to 7°C), explaining its rapid growth and contribution to spoilage of meat products (Borch *et al*., 1996; Gill, 1996; Labadie, 1999). The maximum acceptable microbial level for red meat (7 $\log_{10}$ CFU/g) (Fung, 1986; ICMSF, 1986) of the untreated samples was reached by 8 d and the treated sample reached this level by 10 d. Therefore, in general the infusion of a lactate blend is effective against microbial growth in the beef. This result was also obtained in various research studies, where lactate was applied to extend the shelf-life of beef (De Vegt, 1997; Eckert *et al*., 1997; Maca *et al*., 1999; Mandigo, 2002; Foster, 2004). However, the growth of specific micro-organisms was not successfully reduced with the infusion of the lactate blend. As mentioned earlier, the infused muscles probably had higher cell counts due to increased handling and temperature fluctuation and contamination by the needles of the injection machine used (Wheeler *et al*., 1993; Gill, 1996). The higher moisture content, as a result of the infusion of the blend containing 75.75% water, also contributes to a more favourable environment for microbial growth (Labadie, 1999).

Table 4. Means (±SD)[#] for main effects of a phosphate and lactate blend on the colony forming units (log_{10} CFU.g^{-1}) of beef *Longissimus thoracis et lumborum* muscle on various selective agar over a period of 10 d.

Attribute	Control	Infused	*P*-value	LSD (*P*=0.05)
MRS	1.81 ± 1.677 [b]	4.31 ± 0.427 [a]	<0.0001	0.6232
PCA	5.87 ± 1.478 [a]	6.08 ± 0.966 [a]	0.0779	0.2307
CASO	6.07 ± 1.476 [b]	6.83 ± 0.934 [a]	<0.0001	0.2532
YE	6.06 ± 1.604 [b]	6.71 ± 0.944 [a]	<0.0001	0.2561
KRANEP	n/d	n/d	n/c	n/c

[#] SD: Standard deviation.

[a, b] Means in the same row with different superscripts are significantly different (*P*≤0.05).

n/d: Not detected.

n/c: Not calculated.

LSD: Least significant difference (*P*=0.05).

MRS: De Man-Rogosa-Sharpe medium (Biolab, Merck Laboratories, Milnerton, South Africa).

PCA: Plate Count Agar (Biolab).

CASO: Casein-peptone Soymeal-peptone Agar (Biolab).

YE: Yeast Extract Agar (Biolab).

KRANEP: Potassium thiocyanate Actidion Sodium azide Egg-yolk Pyruvate Agar Basis (Fluka, Merck Laboratories, Milnerton, South Africa).

Proximate chemical composition

The proximate composition was determined on samples originating from Day 2 to establish that the muscle samples used are representative of normal beef. The proximate composition of the loin from the two treatments is summarised in Table 5. The proximate composition of the control is similar to that reported for beef (Sayed *et al*., 1999; Hoffman, 2006).

The moisture content of the treated LTL (loin) were 74.37 ± 2.605%, which concurs with previous research of a *Longissimus lumborum* muscle sample infused with a similar salt solution, which contained 75.90% moisture (Hoffman, 2006). Surprisingly, the moisture content of the treated and control samples did not differ significantly (*P*>0.05), despite the fact that water had been infused and a weight gain of 6.72 ± 0.899% was recorded. The result is not statistical significant possibly because of too few carcasses being used as replications (L.C. Hoffman, personal communication, 2004).

The protein content of the *Longissimus thoracis et lumborum* muscle (LTL) used in this investigation showed (Table 5) a highly significant difference (*P*≤0.05) between the

treated and control samples. The treated sample contained a protein content of 20.67 ± 1.556%, while the control sample showed 22.75 ± 1.468% proteins. Similar values were obtained in a study with *Longissimus lumborum* muscles from old cows, which contained 23.83% protein in the untreated sample and a 20.67% protein for the salt treated sample (Hoffman, 2006).

Table 5. Means (±SD)[#] for chemical composition on Day 2 of beef *Longissimus thoracis et lumborum* muscle infused with a phosphate and lactate blend.

Chemical constitute	Control	Infused	*P*-value	LSD (*P*=0.05)
Moisture (g/100 g)	73.04 ± 2.097[a]	74.37 ± 2.605[a]	0.0855	1.7852
Protein (g/100 g)	22.75 ± 1.468[a]	20.67 ± 1.556[b]	0.0064	0.7200
Lipid (g/100 g)	3.04 ± 0.902[a]	2.99 ± 0.972[a]	0.5657	0.3155
Ash (g/100 g)	1.01 ± 0.491[a]	1.48 ± 0.105[a]	0.1807	1.0008

[#] SD: Standard deviation.

[a, b] Means in the same row with different superscripts are significantly different (*P*≤0.05).

LSD: Least significant difference (*P*=0.05).

In this investigation the results indicated a fat percentage of 3.04 ± 0.902% for the control and 2.99 ± 0.972% for the infused sample. Although no significant difference (*P*>0.05) exists between the fat and moisture content, the trend of a lower fat content in the infused muscle is seen (Table 5).

In the loin (LTL) the ash content obtained for the control samples was 1.01 ± 0.491% and 1.48 ± 0.105% for the infused muscle. Although the difference is not significant (Table 5), the infused sample displayed a slightly higher ash content than the control because of the added salt/minerals to the left-side muscle. Previous research showed that *Longissimus lumborum* muscle samples infused with salts significantly increased in ash content by 1.16% (Hoffman, 2006).

CONCLUSIONS

The aim of this study was to determine the effect of the phosphate and lactate blend on microbial growth and whether the blend had any negative implications as pertaining to the physical and chemical qualities.

The main effect of treatment with a phosphate and lactate blend versus no treatment was highly significant (*P*≤0.05) for most of the attributes tested. The loin infused

with a phosphate and lactate blend was perceived to be more brown and darker than the control sample, especially towards the end of the shelf-life period (10 d). The microbiological spoilage limit of the total aerobic cell counts for the control sample was reached by Day 8, whereas the infused sample reached the limit by Day 10. The significant difference means that the phosphate and lactate blend extended the shelf-life of beef loin samples by more than 1 d. A feasible reason for this short extension could be the source of the meat as the main contributor of meat contamination with spoilage and pathogenic bacteria. This observation is supported by the fact that most of the microbes on the meat are acquired during the dressing process (Merck, undated). A need exists for the application of safety systems like HACCP (Kennedy *et al*., 2000; Panisello *et al*., 2000) to lower the chance of contamination of the meat before packaging. The appearance of the beef loin due to the colour deterioration would most probably not appeal to the consumer. Other packaging methods, with more controlled atmospheres such as vacuum packaging and modified atmosphere packaging (MAP), could be applied and investigated to determine if the shelf-life and the colour-life could be extended.

Therefore, in conclusion, it can be stated that infusion of *Longissimus thoracis et lumborum* (LTL) with a commercial blend containing sodium and potassium salts, di- and triphosphates and lactates was to some degree effective against the proliferation of lactic acid bacteria, aerobic micro-organisms, *Pseudomonas* spp and yeast and moulds, however not to the extend to increase the shelf-life significantly. The blend however has the potential to improve the safety of the meat at refrigerated temperatures with little effect on the physical qualities, pertaining to the purge loss and on the chemical attributes. However, the rapid colour deterioration observed in the steaks that were stored for 10 d under a gas-permeable film will undeniably impact negatively on the consumer's purchasing decision.

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CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

Variability in meat quality is a global problem, causing concern in terms of decreased meat consumption and a declining market share for the meat industry and uncertain product quality for the consumer. One of the main attributes indicating high variability in the beef industry is tenderness. The focus on this characteristic is the result of tenderness being the key attribute that determines whether the consumer will repurchase the product and also the attribute the consumer rates most highly when purchasing and consuming beef. However, tenderness is a meat-quality characteristic that is highly inconsistent, resulting in the beef industry struggling to guarantee homogenous tenderness of beef products.

Several strategies can be used to approach this problem of inherent variation in meat, such as age classification, which is already applied in the South African beef industry. The age classification can account for part of the variation found in meat tenderness (Strydom, 2003), however, new processing techniques should be implemented to improve consistency and reduce variation. Post-mortem intervention or processing techniques, such as mechanical tenderising and/or infusion with a tenderising solution, should be implemented to reduce tenderness variability (Jeremiah *et al*., 2003) and increase consumer satisfaction with regard to beef quality (Scanga *et al*., 2000; Baublits *et al*., 2005; Hoffman, 2006). For several years international studies have been reporting on the implementation of a brine solution infused into a beef cut to increase tenderisation. However, in the South African meat industry this is a fairly new concept, which is in need of extensive research and tapping of a novel market in South Africa. Therefore, one of the leading companies in South Africa has developed such as solution containing salt, phosphates and lactate for application in enhancing meat tenderness. The similarities of the brine solutions applied in overseas studies and the success achieved, along with other reported studies by South African researchers (Hoffman, 2006), gives an indication of the success that the blend used in the present investigation could accomplish in the South African beef industry. The effect of this basting mixture, currently employed in the South African meat market, to improve the tenderness of beef muscle is increasingly questioned in terms of its effect on the physical, nutritional and sensory quality of the beef. However, before the effect of the brine on the meat quality of beef is researched, the inherent meat-

quality variation that exists within a carcass between beef muscles pertaining to the physical, chemical and sensory qualities should be investigated.

Research on meat-quality variability of various beef muscles is lacking in the South African beef market. Therefore, the variation found among beef muscles within a carcass and the effect on the physical, chemical and sensory quality characteristics of South Africa beef muscles was the first objective of this study. The first study (Chapter 3) of the research chapters confirmed that different beef muscles from local origin differ significantly ($P \leq 0.05$) in their characteristics.

One of the main objectives of the beef industry is to produce a product of consistent quality, which complies with consumer needs and satisfies the demand for a high-quality beef product (Kerth *et al*., 1995), in particular tenderness (Koohmaraie, 1996). This is where an enhancing solution becomes relevant in order to decrease this problem of variation between muscle properties. The effect of this brine on tenderness and other meat qualities when injected after 7 d of storage has been researched (Hoffman, 2006). However, the immediate effect after infusion, 3 d post-mortem and up to 19 d post-mortem, has not been studied.

Therefore, the second aspect of study undertaken was to determine the effect of a brine consisting of sodium chloride, di- and tripolyphosphate, and lactate on the physical properties, nutrient content and sensory characteristics and consumer acceptability of selected South African beef muscles.

The general findings suggest that variation between muscles characteristics can be reduced by applying a solution of salts, phosphates and lactate to the muscles. Over the 19 d an increase in tenderness concurrent with a darker but acceptable beef colour resulted from the infusion with the mentioned blend. No significant differences ($P > 0.05$) in water-binding ability (purge loss, drip loss and cooking loss) of the infused samples, packed in vacuum bags, was found compared to the control muscles over time. With respect to the sensory characteristics the infused samples were significantly more juicy and tender than the control samples ($P \leq 0.05$). Furthermore, the infused samples, especially the *Longissimus lumborum* (LL) sample, illustrated a significantly ($P \leq 0.05$) higher degree of liking amongst the consumer panellists than the non-infused samples.

With the infused meat product thus found acceptable by the target consumer, the importance of whether the infused meat samples could be a viable retail product was investigated.

Therefore, a third aspect of the study was to determine the effect of the phosphate and lactate blend on the shelf-life of the beef product. The microbiological spoilage limit of

the total aerobic cell counts for the control sample was reached by Day 8, whereas the infused sample reached the limit by Day 10. The significant difference means that the phosphate and lactate blend is able to extend the shelf-life of beef loin samples by more than 1 d. The steaks used in the shelf-life study were packaged in trays covered with oxygen-permeable plastic. As a result the raw colour deteriorated more rapidly than when the meat cuts were packed in vacuum bags. The steaks in the overwrap tray packaging also demonstrated higher percentages of fluid loss. Therefore, despite being microbiologically safe (less than 7 $\log_{10}$ CFU/g) (Fung, 1986; ICMSF, 1986), the appearance of brown colour and drip formed in the tray packaging would probably not appeal to the consumer.

However, in this field of study, there are concerns with regard to the microbiological environment created. The increased pH and modified atmosphere packaging used in the industry may be a highly beneficial environment for the growth of organisms such as *Clostridium* (T.J. Britz, personal communication, 2005). These concerns about the blend composition and microbiological growth of pathogens warrant further research.

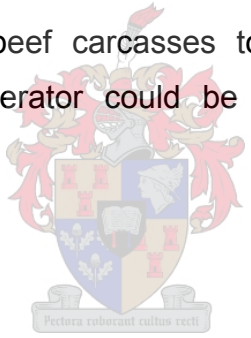
A further possible reason for the short extension of 1 d with the use if the infusion technique could be the source of the meat. The main contributor of meat contamination with spoilage and pathogenic bacteria could probably be found during the dressing process (Merck, undated). A need exist for the application of safety systems like HACCP (Kennedy *et al*., 2000; Panisello *et al*., 2000) to reduce the chances of contamination of the meat before packaging.

Another concern that was mentioned in this study was the possible consumer concern about fresh meat being infused / injected with a solution of some kind. The consumer is opposed to the idea of buying a seemingly fresh, unprocessed beef cut, when in fact it has been processed or tampered with and no indication of this was given to the consumer. The consumer's other main concern is paying for an inexpensive solution, namely 'water', which is the main component of the blend added to the meat product to increase tenderness and juiciness. Other concerns raised had to do with the formation of visible drip in the poly-vinyl chloride packaging and the purge formation in the vacuum packaging.

The only plausible solution for all these concerns raised by the consumer is to educate the consumer about the infusion / injection technology and provide information on the subject and the positive enhancement of meat quality and, most importantly, the tenderness of beef. Education on the components present in the solution is recommended to emphasise that all the ingredients in the solution are naturally present in the live animal

and therefore no ill effects on human health are expected on the intake of this solution. Other positive qualities that could be presented with respect to the infusion of meat is the probability of a decreased salt intake, due to the infused solution containing salt components, which is sufficient for flavour development during preparation and consumption. The amount of sodium chloride (NaCl) present in the blend is below the recommended value of 120 mg/100 g sample of the South African Heart Foundation (2003). It therefore supports a healthier lifestyle by contributing to decreased intake of sodium, as recommended to the modern consumer (Heart Foundation, 2003). Therefore, consumer education with demonstration at the retail outlets and tasting sessions, together with the distribution of pamphlets, should improve consumer knowledge of meat-enhancing technologies.

Despite all the issued raised, the immediate effect of the blend on the tenderness of the beef muscles, as early as 3 d after slaughter and 1 d after infusion, is a highly positive outcome as opposed to natural ageing, where storage space and time are necessary. Therefore, it can be speculated that with this infusion the requirement of natural ageing of long storage periods (14 d) for beef carcasses to obtain tenderness and thus the occupation of space in the refrigerator could be eliminated, which is economically beneficial.



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