The effects of hot-deboning on the physical meat quality and microbial quality and safety of ostrich *(Struthio camelus)* meat

by

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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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SUMMARY

The aim of this study was to investigate hot-deboning as an alternative excising method for the South African ostrich industry. The majority of research conducted on hot-deboning is on beef (pre-rigor meat), whilst research on the hot-deboning of ostrich meat (post-rigor meat) remains within a limited scope.

Fifteen ostriches were used for the study with the muscles hot-deboned (within 90 min post-mortem) from the left leg and cold-deboned (<4°C, 24 h post-mortem) from the right leg. Half of the sixteen hot-deboned muscles' weights were heavier ($p \leq 0.05$) than those cold-deboned. Five ostrich muscles: fan fillet (*M. iliofibularis*); rump steak (*M. iliotibialis lateralis*); big drum (*M. gastrocnemius, pars interna*); moon steak (*M. femorotibialis medius*) and triangle steak (*M. flexor cruris lateralis)* were used to establish meat quality at day three post-mortem. The varying ultimate pH (pH_u) values between muscles ($p = 0.01$) were still within the expected range for ostrich meat with the big drum having the highest pH_u (means ± standard deviation; 5.95 ± 0.16) linking with its low drip loss percentage (0.90% ± 0.30). The fan fillet had a more red (a* = 13.43 ± 1.21), saturated (Chroma = 16.58 ± 1.61) colour whereas the big drum was more blue (b* = 9.68 ± 1.52), with the lowest colour intensity (hue angle = 35.64 ± 3.79). Concerning Warner-Bratzler shear force values, the fan fillet was the most tender (35.34 N ± 8.26) in contrast with the moon steak (72.23 N ± 15.81) which can be linked to the high cooking loss percentage (37.05% ± 1.90) of the latter muscle.

A vacuum packaged ageing trial for 28 d post-mortem (0 - 4°C) was conducted on the above-mentioned five muscles. Hot-deboning did not have an effect on the pH_u ($p = 0.50$). Although a few significant differences in the colour coordinates (CIEL*a*b*, hue angle and Chroma) were found. Neither the cumulative moisture loss nor the cooking loss percentages showed significant differences between the hot- and cold-deboned muscles ($p > 0.05$). Concerning Warner-Bratzler shear force (WBSF) values, only the hot-deboned rump steak was tougher ($p \leq 0.05$) at day 28 post-mortem as compared to cold-deboned with a value that is still considered within the tender meat range (34.74 N vs. 26.55 N).

Microbiological analysis conducted on the aforementioned five muscles showed an absence of *Salmonella* spp. in all hot- and cold-deboned samples. Hot-deboning did not have an effect on the mean Aerobic Counts (AC) nor the *Enterobacteriaceae* counts over the 28 d ageing period ($p \leq 0.05$). Aerobic and *Enterobacteriaceae* counts for hot- and cold-deboned muscles were however higher in comparison with fresh meat standards used by the ostrich industry; whether this is standard or due to the specific execution of the experiment *per se* is unclear.

Regarding these findings, hot-deboning is deemed a suitable alternative to cold-deboning for the ostrich industry of South Africa.

OPSOMMING

Die doel van hierdie studie was om warmontbening as alternatiewe ontbeningsmetode vir die Suid-Afrikaanse volstruisindustrie te ondersoek. Die meerderhied van bestaande navorsing rakende warmontbening is gedoen op beesvleis (pre-rigor vleis), terwyl die omvang van navorsing oor die warmontbening van volstruisvleis (post-rigor vleis), steeds beperk is.

Fyftien volstruise is gebruik vir hierdie studie, met die spiere warm-ontbeen (binne 90 min post-mortem) van die linkerbeen en koud-ontbeen (<4°C, 24 h post-mortem) van die regterbeen. Die helfte van die sestien warm-ontbeende spiergewigte was swaarder ($p \leq 0.05$) as dié van die koud-ontbeende spiergewigte. Vyf volstruispiere: die *M. iliofibularis*; *M. iliotibialis lateralis*; *M. gastrocnemius, pars interna*; *M. femorotibialis medius* en *M. flexor cruris lateralis* is gebruik om die fisiese vleiskwaliteit op dag drie post-mortem vas te stel. Die varieërende uiteindelike pH (pH_u) waardes tussen spiere ($p = 0.01$) was steeds binne die verwagte reeks vir volstruisvleis met die *M. Gastrocnemius, pars interna* wat die hoogste pH_u gehad het (5.95 ± 0.16) en ooreenstem met sy lae dripverlies persentasie (0.90 ± 0.30). Die *M. iliofibularis* het ‘n meer rooi (a* = 13.43 ± 1.21), versadigde (16.58 ± 1.61) kleur gehad teenoor die *M. Gastrocnemius, pars interna* wat meer blou was (b* = 9.68 ± 1.52), met die laagste kleurintensiteit (35.64 ± 3.79). Rakende die Warner-Bratzler skeurkrag, was die *M. iliofibularis* die sagste (35.34 N ± 8.26) in kontras met die M*. femorotibialis medius* (72.23 N ± 15.81) wat toegeskryf kan word aan laasgenoemde se hoë kookverlies persentasie (37.05% ± 1.90).

‘n Verouderingsproef oor 28 d post-mortem (0 - 4°C) is uitgevoer op dieselfde vyf vakuumverpakte spiere. Warmontbening het nie ‘n effek op die pH_u ($p = 0.50$) gehad nie. Alhoewel ‘n paar beduidende verskille in die kleur-koördinate teenwoordig was, het warmontbening geen negatiewe effek gehad nie ($p > 0.05$). Nie die kumulatiewe vogverlies of die kookververlies persentasies het beduidende verskille tussen die warm- en koud-ontbeende spiere getoon nie ($p > 0.05$). Rakende Warner-Bratzler skeurkrag waardes was slegs die *M. iliotibialis lateralis* (34.74 N vs. 26.55) taaier ($p \leq 0.05$) op dag 28 post-mortem met ‘n waarde wat steeds beskou word binne die reeks vir sagte vleis.

Mikrobiologiese analise wat uitgevoer is op bogenoemde vyf spiere het ‘n afwesigheid van *Salmonella* spp. in alle warm- en koud-ontbeende monsters getoon. Warmontbening het nie ‘n effek op die gemiddelde Aërobiese Tellings of *Enterobacteriaceae* tellings oor die 28 d verouderingstydperk ($p \leq 0.05$) gehad nie. Aërobiese en *Enterobacteriaceae* tellings vir warm- en koud-ontbeende spiere was wel hoër in vergelyking met vars vleis standaarde wat gebruik word deur die volstruisindustrie.

Rakende hierdie bevindinge word warmontbening beskou as ‘n geskikte alternatief vir koue-ontbening vir die volstruisindustrie van Suid-Afrika.

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NOTES

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

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CHAPTER 1

Introduction

It is well-known that South Africa is considered the world leader in the global ostrich marketplace with 90% of produced commodities (meat, leather and feathers) exported. With this high amount of exports, South Africa comprises 75% of the global market with 45% skin, 45% meat and 10% feathers (Department of Agriculture, Forestry & Fisheries, 2017). In contrast to other livestock systems however, the enterprise is still relatively small and young with extensive ostrich farming commencing in the 1800's primarily for feathers (Cloete *et al*., 2012). The importance of ostrich meat has increased over time with foodservice traders, supermarkets, wholesalers and restaurants currently being the main ostrich markets. Ostrich products (meat, leather and feathers) are locally promoted and are exported through a free market system (prices controlled by an open market and consumers through supply and demand) (Department of Agriculture, Forestry & Fisheries, 2017).

Although ostrich meat has principally been exported in the past, at present a ban is placed on the export of ostrich meat due to the outbreak of highly pathogenic Avian Influenza in poultry at the end of 2017. Avian Influenza (AI) can be described as a highly contagious viral infection which can affect all bird species. Given that ostrich meat was mainly exported to European countries prior to the outbreak of AI, sanitary regulations as required by the European Union (EU) had to be met. This amongst other included that both deboning and packaging plants had to be EU approved for the export of ostrich meat to EU countries (Department of Agriculture, Forestry & Fisheries, 2017).

Currently the ostrich industry of South Africa uses cold-deboning as method of excising ostrich carcasses. Cold-deboning can be described as the excision of ostrich carcasses subsequent to refrigeration at 0 – 4°C for 24 h post-mortem (Hoffman *et al*., 2007). This method of excision is often referred to as conventional deboning, and it is important to note that this typically signifies deboning after the completion of *rigor mortis* (process characterised by muscles becoming stiff and inflexible) (Lawrie & Ledward, 2006a).

An alternative proposed deboning method for the South African ostrich industry is hot-deboning, which can be described as the practice where lean meat and fat are excised from carcasses before a great drop in body temperature occurs (Waylan & Kastner, 2004). Thus, with the occurrence of hot-deboning so soon after slaughter (within 2 h post-mortem), it is usually performed on pre-rigor meat in contrast to cold-deboning which is performed on post-rigor meat. Hot-deboning holds several potential advantages as hot-deboning not only occurs

prior to refrigeration, but completely eliminates the refrigeration of whole carcasses. However, the latter leads to the risk of cold-shortening (phenomenon when carcasses are refrigerated too soon after slaughter and selected muscles become rigid; dependent on temperatures below 10 – 15°C with pH > 6.0) (Lawrie & Ledward, 2006a). However, no risk of cold-shortening and/or pre-rigor contraction in ostrich muscles has been found due to the progression of *rigor mortis* within 45 min post-mortem. Consequently, the risk of rigor-shortening is ruled out with dressing (within 1 h post-mortem) and hot-deboning (within 2 h post-mortem) post-rigor. In addition, it has been found that ostrich muscles can reach a pH of ≤ 6.2 at temperatures higher than 10°C (Hoffman *et al*., 2007).

Hot-deboning has several benefits, of which the most prominent probably is that less refrigeration space is required for hot-deboned cuts in comparison with refrigeration of whole carcasses intended for cold-deboning. The lessened refrigeration space will have an economic advantage due to lower energy input utilised (Taylor *et al*., 1981; Powell *et al.,* 1982; Babiker & Lawrie, 1983; Spooncer, 1993; Stopforth & Sofos, 2005; Farouk *et al*., 2009; Pisula & Tyburcy, 2009). Pisula and Tyburcy (2009) found that 50 – 55% lower refrigeration space was required with a consequent saving of 40 – 50% refrigeration energy, whilst a 40 – 50% quicker turnover was achieved. Much smaller, purpose built refrigeration units will offer rapid chilling for deboned ostrich cuts and refrigeration of already deboned cuts lead to more efficient and uniform cooling of the meat (Taylor *et al*., 1981). Considering that the total chiller space needed for hot-deboned cuts is much lower, newly built plants will have much lower capital costs (Powell *et al*., 1982). However, this is only applicable when current plants are adapted or new plants are built (Spooncer, 1993).

The cost of purpose-built refrigeration plants or the remodelling of current plants can have high cost implications, which also extends to high costs of new equipment and retraining of staff (Pisula & Tyburcy, 2009). Well-trained staff that are skilled in hot-deboning can save up to 20% in labour required (Pisula & Tyburcy, 2009), as hot-deboning has proven to necessitate less effort leading to more carcasses excised in the same amount of time (Spooncer, 1993). According to the Meat Safety Act of 2000, the air flow temperature of a deboning room must be ≤ 12°C (Department of Agriculture, 2007). However, with hot-deboning the temperature of the meat is still high at the time of deboning (near the *in vivo* temperature of ~37°C) (Lawrie & Ledward, 2006b), therefore a higher ambient temperature in the deboning room can be implemented. The latter will create a more comfortable working environment whilst also contributing to a saving in energy costs as a lower air flow temperature does not need to be maintained (e.g. ≤ 12°C) (Department of Agriculture, 2007). Temperature is the most important factor influencing microbial growth (Lawrie & Ledward, 2006c), a higher ambient temperature during deboning might cause concern regarding microbial spoilage. The presence of environmental organisms within the deboning area is particularly of interest, as Hoffman *et al*. (2010) found *Pseudomonas* and *Shigella* to

be present within the slaughtering environment of an ostrich abattoir.

Most of the advantages of hot-deboning and the effect thereof on meat quality is applicable to species where the deboning is done pre-rigor with only the studies of Botha and co-workers on post-rigor ostrich meat being available in the scientific domain (Botha *et al*., 2006; 2007; Hoffman *et al*., 2006; 2007). Nonetheless, some of the advantageous of hot-deboned pre-rigor meat will also be applicable to post-rigor hot deboned ostrich meat and will be discussed further.

Hot-deboned meat has also shown improved functional properties and gives the opportunity to utilize each muscle according to its intrinsic properties post-mortem (Farouk *et al.*, 2009). Additionally, hot-deboned meat has a higher yield through the decrease of weight loss (i.e. moisture loss) which usually occurs during carcass refrigeration (Powell *et al*., 1982; Spooncer, 1993; Pisula & Tyburcy, 2009). Higher yields could also be ascribed to hot-deboned muscles being excised more cleanly from the bone (Spooncer, 1993). Pisula and Tyburcy (2009) further found the final weep loss, colour and tenderness of hot-deboned meat did not differ ($p > 0.05$) from cold-deboned meat. Although hot-deboned muscles have previously shown distorted shapes when compared to cold-deboned muscles (Pisula & Tyburcy, 2009), the risk of cold-shortening has definitely been one of the biggest concerning factors regarding the implementation of hot-deboning (Powell *et al*., 1982; Shaw & Powell, 1995; Spooncer, 1993; Farouk et al., 2009). Another concerning factor in the application of hot-deboning has been the risk of higher microbial contamination and proliferation of organisms in hot-deboned meat (Babiker & Lawrie, 1983; Stopforth & Sofos, 2005). When carcasses are contaminated with bacteria from the skin/hide during slaughter, the bacteria on carcass surfaces are chilled during the refrigeration period (24 h) associated with cold-deboning. This is a concern, as there is the risk that meat can encourage growth of pathogenic bacteria at refrigeration temperatures. However, it is important to note that once carcasses are chilled to an internal temperature of about 8°C, the pathogenic bacterial growth is very slow or even negligible. Nonetheless, with hot-deboning, however, the exposure and possible contamination of meat occurs at around 20 – 35°C, temperatures at which pathogenic bacteria can grow rapidly if the meat is not chilled quickly, e.g. within 2 h (Spooncer, 1993).

Research on the effects of hot-deboning has mainly been carried out on beef (Schmidt & Keman, 1974; Kastner & Russel, 1975; Taylor *et al*., 1981; Sheridan & Sherington, 1982; Babiker & Lawrie, 1983; Shaw & Powell, 1995; Farouk & Hall, 2000), whilst research on hot-deboned ostrich meat remains within a limited scope (Botha *et al.,* 2006; 2007; Hoffman *et al*., 2006; 2007). Botha *et al*. (2006) conducted a study on the physical meat quality characteristics of only one hot-deboned ostrich muscle, namely the big drum *(M. gastrocnemius, pars interna)* over an ageing period of 21 d post-mortem at 0 – 4°C. Furthermore, Botha *et al*. (2007) researched the effects of hot-deboning on the physical meat quality characteristics and microbial quality over 42 d refrigerated (0 – 4°C) storage of

two ostrich muscles, namely the big drum *(M. gastrocnemius, pars interna)* and fan fillet *(M. ioliofibularis).* A lack of research thus exists, particularly with regard to the muscle yields of hot-deboned post-rigor ostrich meat, as well as the physical meat quality of various other hot-deboned ostrich muscles (excluding the fan fillet and big drum muscles).

Therefore, the aim of this study is to investigate the muscle yields of all sixteen commercially deboned ostrich muscles in terms of hot-deboning (post-rigor) when compared to the cold-deboned muscles. An additional objective of this study is to evaluate the effect of hot-deboning (post- rigor) on both the physical meat quality characteristics (fresh ostrich meat), as well as the meat quality characteristics over an extended time period of 28 days (aged ostrich meat), of five representative ostrich muscles. Additionally, another objective is to establish the microbiological quality and safety of both the fresh and aged meat of the same five ostrich muscles. These muscles include the fan fillet *(M. iliofibularis)*, big drum *(M. gastrocnemius, pars interna)*, rump steak *(M. iliotibialis lateralis)*, moon steak *(M. femorotibialis medius)* and triangle steak *(M. flexor cruris lateralis)*. This knowledge will enable the ostrich industry of South Africa to gain better insight into the implementation of hot-deboning as alternative excising method, hopefully to gain economic strength with the current ban placed on exports and South Africa primarily being an exporter of ostrich meat.

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CHAPTER 2

Literature Review

2.1 Introduction

2.1.1 A historical overview of the ostrich (Struthio camelus)

The assembly of ostriches is substantially unlike any other prevailing in the class Aves. Ostriches are classified as part of the Ratitae family with a characteristic breastbone or sternum without a keel. Birds that are similar to ostriches, have individually uncharacteristic features which mainly include: a breastbone without a keel for the connection of the pectoral muscles; wings that are not necessary for flight or moving through water; feather spikes that are detached; and the advanced development of the diaphragm in comparison to other birds (De Mosenthal & Harting, 1964).

The key physical appearance traits of ostriches are the small head sitting on the well-developed, long neck stretching from a stout body with wings incapable of flying, all of which are assembled on the exceptionally sturdy, muscular legs and cushioned feet with two toes. Prehistorically, the ostrich has been referred to as the “camel-bird”, with characteristics such as bearing high temperatures without any water, whilst the physical appearance with an elongated neck and prevalent eyes can also be described as alike between the ostrich and the camel (De Mosenthal & Harting, 1964).

The earliest indication of the ostrich’s existence is said to date back to ancient times considering the recurrent mention thereof in both the Scripture and throughout history (De Mosenthal & Harting, 1964). Evidence shows that ostriches were found somewhat 20 million years ago (or more) around the Mediterranean Sea. Comprehensive illustrations of the ostrich bird were found in the prehistoric Egyptian tombs whilst rock drawings of the San (an indigenous group of people said to be ancestors of the earliest inhabitants of South Africa living in the Southern Kalahari region of the country), showed that they sought ostriches for food supply. Although the export of ostrich feathers from South Africa started as early as 1838, the farming of ostriches for feathers only started in 1863 (National Agricultural Marketing Council, 2003; Thondhlana & Shackleton, 2015).

When an ostrich is slaughtered, the entire bird is utilised. Historically, the skin was removed to protect the feathers, as these were of great value to the industry, including for wearing as a fashion accessory. However, ancient historians did not refer to the utilisation of ostrich feathers in terms of decorative purposes (De Mosenthal & Harting, 1964). Although the focus of the ostrich industry has primarily been ostrich feathers in the past, the focus of the ostrich industry has shifted from feathers to skin and therefore the production of meat. Nowadays the feathers

are removed preceding the removal of the skin (Hoffman, 2012). Furthermore, the consumption of ostrich meat is understood to have been a relatively common practice since ancient times. Although the texture of ostrich meat is described as being somewhat hard and chewy, the flavour has been regarded as acceptable and pleasant. However, with an adapted diet of lucerne, grain and clover, ostrich meat was consequently found to be tender and less dry (De Mosenthal & Harting, 1964).

2.1.2 The ostrich industry of South Africa

Ostrich farming in South Africa is not a novel concept and has been practiced for many years (De Mosenthal & Harting, 1964). In contrast to other livestock systems, however, the enterprise is still relatively small with extensive ostrich farming commencing in the 1800's primarily for feathers (Cloete *et al*., 2012). The origin of the ostrich industry of South Africa is said to be in the Beaufort West and Oudtshoorn regions, dating back to 1866 (De Mosenthal & Harting, 1964). The Swartland area, as well as the Southern Cape (25% of the flocks) and Little Karoo (65% of the flocks) are the regions where ostrich farming is predominantly practiced in South Africa (Brand *et al*., 2011). Leather became the prevailing commodity in 1975 following intensive ostrich production that started in the 1960's. In South Africa the ostrich industry accounts for approximately 70% of the global ostrich market (Duminy, 2016).

Consequently, South Africa has undoubtedly been considered the world leader in not only ostrich farming, but also in being the first to add value in the ostrich production chain, including the slaughtering, processing of meat and skin dyeing. Formerly, South Africa has principally been an exporter of ostrich meat and other related products, with approximately 90% of the ostrich prime cuts being exported to the European Union (EU), as well as feathers and leather (National Agricultural Marketing Council, 2003). Moreover, ostrich meat previously accounted for the largest amount of meat exported from South Africa, both in terms of quantity together with its commercial value. Conversely, the influence and contribution of ostrich meat to the local economy is not well-known (Brand *et al*., 2011).

Currently, a ban is placed on the export of ostrich meat (only sold to local markets within South Africa) due to the outbreak of Avian Influenza (AI) at the end of 2017. Thus, now more than ever, the ostrich industry of South Africa needs to look at ways in which production costs can be lowered in order to survive in the present difficult economic climate.

2.1.3 Muscles from the ostrich

The anatomical structure of ostrich muscles is generally compared to those of other birds and is related to the universal avian terminology that is commonly used. In describing the construction of ostrich muscles, as defined by George and Berger (1966), ostriches comprise of all the main muscle groups, except for the *M. iliacus* (a muscle known to evolve from the hip bone). Furthermore, the *M. gracilis* in the ostrich is also considered to be unique, and the femorotibial muscles has four muscles *(M. quadriceps femoris)*, similar to mammals, whereas

in all other birds, this muscle usually only comprises of three muscles (*M. femorotibialis*) (Mellett, 1985).

2.1.4 Physical and microbial meat quality

The term meat quality refers to a variety of factors, including yield, appearance and technological traits, as well as palatability, wholesomeness (microbiologically safe and nutritionally beneficial) and ethical quality (handling of animals to promote their well-being). Aspects which affect the microstructure of meat post-mortem, also have an effect on the water holding capacity (WHC) and colour of the meat. The latter is important as colour is a key trait that influences the physical appearance of meat, and is nearly the only standard which consumers use to evaluate the freshness of meat. An attractive meat colour leads to the consumer's assumption that the meat is fresh, where they typically expect meat to have a bright pink or red colour, as opposed to meat with a purple, grey or brown colour. Furthermore, WHC is significant for the technological value of meat. In fresh meat, drip or exudate due to poor WHC diminishes the appearance of the meat, resulting in overall weight loss of the meat, giving the perception of dryness when cooked (Warriss, 2000a).

Meat that is safe for consumption is one of the key components in producing wholesome meat. Meat should be free from microbiological pathogens, harmful chemicals and should not contain any parasites which may infect humans. When people consume meat, they want to do so without attaining food poisoning, without being subjected to high residue amounts in the meat (from preceding veterinary medication) and without consuming growth stimulating hormones and pesticides (Warriss, 2000a). When changes to meat quality is remarked as negative by consumers, it is often due to damage caused by microorganisms when microbes inside or on the surface of meat causes spoilage. These changes affect the appearance and/ or flavour and odour of meat. The spoilage microflora will affect the kind of organisms that appear, whilst its configuration will be determined by the inherent meat characteristics such as pH and water activity (a_w). The temperature at which, and the environment where, the meat is held, are external factors that will also influence the spoilage microorganisms (Gill, 2004).

2.2 Excising of ostrich carcasses

2.2.1 Overview of the current ostrich slaughter process in South Africa

Unlike game (free-ranging, wild, non-domesticated animals and birds that are legally hunted reared, slaughtered and commercially sold for food or for personal consumption), for the purpose of slaughter at export abattoirs, ostriches are commercially bred and not hunted. Ostriches are kept in lairages on the premises of slaughter after they have been conveyed live from the breeding/production farms. In the slaughter process, the feathers are collected to sell commercially, but the most important and primary product in the slaughter process (Fig. 2.1), is the skin; secondary to that is the meat. The local ostrich meat market only constitutes a small fraction of the market with the largest quantity being exported. Together with the

condemned material, the ostrich intestines originating from export birds, are considered prime products designated for value-added products such as pet food (Department of Agriculture and Rural Development, 2009).

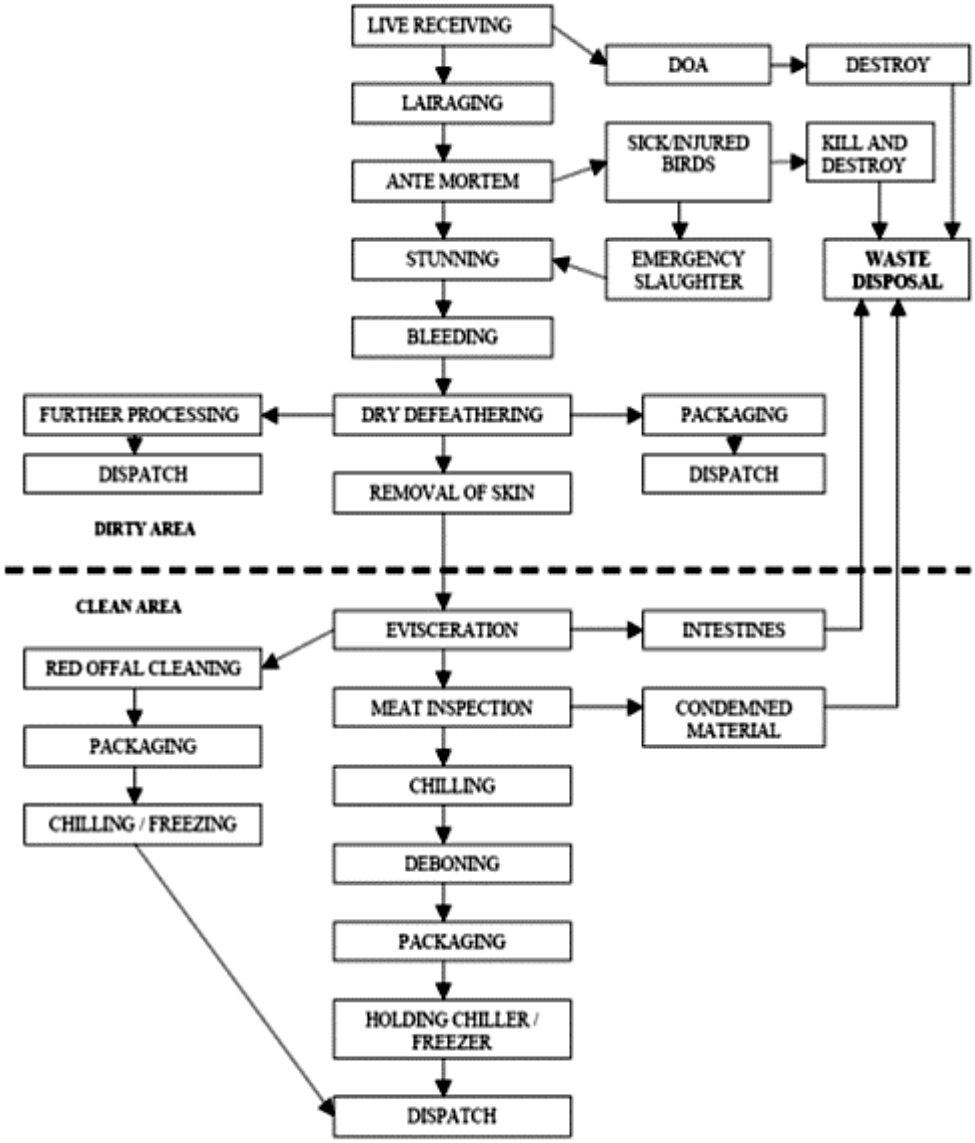

Figure 2.1: Ostrich slaughter process (Department of Agriculture and Rural Development, 2009).

2.2.2 Cold-deboning: Current excising method used in South Africa

As seen in Fig. 2.1, cold-deboning is performed after ostrich carcasses have been chilled (24 h). Deboning can be defined as follows: "*Deboning is the process whereby meat is removed from the skeletal bones and cut into retail cuts such as loin, rump or sirloin, whilst the bones are used for by-products such as bone-meal*" (Van Zyl, 1995).

Cold-deboning can be referred to as the conventional method of excising in the meat industry, where deboning is performed after *rigor mortis* have been completed on a chilled carcass. Consequently, extensive space for coolers are required, expending a large amount of energy (Waylan & Kastner, 2004). Nonetheless, with cold-deboning, processing properties

of meat are not negatively influenced due to *rigor* (warm) shortening (pre-rigor excision). Since the animal's body heat is dispersed at a slow rate, muscles are likewise not prone to the stiffening experienced with cold-shortening. The meat industry has continually been practicing cold-deboning, thus not necessitating consideration regarding the logistical deboning process or experiencing unknown difficulties with the meat grading/classification and its tenderness or technological properties (Ockerman & Basu, 2004).

The ostrich industry of South Africa currently uses cold-deboning as the method of excising whereby ostrich carcasses are refrigerated for 24 h post-mortem (0 - 4°C) before deboning is performed (Hoffman *et al*., 2007). According to the Meat Safety Act 2000, only ostrich carcasses that have been approved during meat inspection (Fig. 2.1), may be presented for deboning. Furthermore, after the deboning of carcasses and cutting of meat have been completed, packaging should take place as soon as possible for refrigerated or frozen storage within 1 h from the start of deboning. The ambient room temperature for cold-deboning should be 12°C or lower. In the instance of chilled meat, the core temperature of the meat should be lowered to 7°C within 12 h, whereas frozen meat should have a core temperature of -12°C before it can be dispatched (Department of Agriculture, 2007).

2.2.3 Hot-deboning: An alternative excising method

Hot-deboning is an alternative excising method, where carcasses are deboned before refrigeration. It has been described with varying terminology, including: hot-deboning; hot boning; hot processing; rapid processing; accelerated hot processing; processing prior to *rigor mortis*; and pre-rigor excision. Hot-deboning can be defined as the practice where fat and lean meat are detached from the carcass before a great drop in body temperature occurs (Waylan & Kastner, 2004). It is also typically performed on pre-rigor meat.

The Meat Safety Act of 2000 states that warm deboning (otherwise referred to as hot-deboning) of ostrich meat may be performed if in a singular operation the meat is conveyed straight from the dressing room to the deboning room, with the deboning room being in the same location as the dressing room. Moreover, it is required that deboning is performed straight after the meat has been conveyed, and the meat is only allowed if it is in accordance with permitted protocol of the provincial executive officer (Department of Agriculture, 2007).

Accordingly, hot-deboning is an alternative excising method which could provide several potential advantages for the ostrich industry of South Africa, since hot-deboned meat is usually excised 2 - 4 h post-mortem, whereas cold-deboned meat is excised 24 h post-mortem (Hoffman *et al.*, 2007). Hot-deboning was developed to decrease the use of energy and lessen the refrigeration space required, also greatly improving the turn-around time (thus more carcasses can be deboned and processed in less time) (Department of Agriculture, 2007). It has been proposed that hot-deboning in the instance of beef, can lower the amount of refrigeration energy with up to 50%, whilst refrigeration space can be reduced with up to 80%. Together with the latter economic benefit, a lower labour capacity and possibly enhanced

functional characteristics of hot-deboned meat also contribute to the advantages of hot-deboning (Waylan & Kastner, 2004).

Furthermore, the awareness and interest of hot-deboning is not only caused by the economic benefits due to the saving in refrigeration space and lowering of energy cost, but also because hot-deboned meat has shown improvements in functional meat properties. With hot-deboning, the early removal of muscles post-mortem enables each muscle to be utilized according to its optimal intrinsic properties (Farouk *et al*., 2005). Moreover, directly after carcasses are slaughtered, average carcass temperatures have been known to increase to 40°C due to metabolic activity in pre-rigor muscles. As carcasses are usually directly refrigerated prior to cold-deboning, this leaves room for proliferation of pathogenic and spoilage microorganisms in the period of cooling from 40°C to the chiller's temperature. With hot-deboning of carcasses, however, the removed muscles are chilled much quicker (Stopforth & Sofos, 2005).

In contrast with the above-mentioned potential advantages of hot-deboning, there are also challenges. Particularly with hot-deboning of pre-rigor meat the alteration of the meat cuts' form together with loss in tenderness and colour change are directly related to the meat quality. Also, the development of new excising techniques as well as hiring new staff and retraining current staff and possible increased inflexibility in production lines will have a direct economic impact. Stricter temperature regulations, firmer hygiene protocols and potential faster microbial growth might be plausible restraints of hot-deboning (Waylan & Kastner, 2004).

Taylor *et al*. (1981) studied hot-deboning of beef and found that the majority of weight loss through evaporation during chilling (as with conventional deboning) of beef carcasses, can be reduced by removing the muscles from the carcass immediately after slaughter, i.e. hot-deboning. Consequently, large chillers can be substituted for more compressed chillers as less refrigeration space is required. The direct chilling of meat cuts also lead to a more even cooling and less varying colour within the muscles. However, hot-deboning of beef resulted in a higher risk for microbial spoilage (possible contamination during deboning and packaging) with the proliferation of microbes deeper within muscles. Additionally, there is also the possibility of cold-shortening (refer to section 2.4.1.4) which is commonly associated with hot-deboning (Taylor *et al*., 1981).

To overcome the risk of cold-shortening associated with hot-deboning of beef (pre-rigor meat), Rosenvold *et al*. (2008) applied electrical stimulation (ES) in combination with wrapping of excised beef muscles. Wrapping (described as tight wrapping of meat in four layers of poly-ethylene cling film before it is placed in a waterproof poly-ethylene bag), was performed to imitate skeletal restraint commonly performed on beef carcasses to overcome cold-shortening. These wrapped beef muscles showed significantly lower shear force values indicating that wrapping and/or ES seemingly protects beef muscles against the undesirable outcome of high temperatures during hot-deboning (Rosenvold *et al*., 2008). However, it can

be noted that ostrich meat is especially unique in this regard with the early onset of *rigor mortis* (as early as 45 min post-mortem), eliminating the risk of cold-shortening (refer to section 2.4.1.4).

The packaging rate and exposure time of beef cuts was shown to directly affect the degree of loss in muscle weight. In the case where hot-deboning can be performed and packaging can be completed within 1 - 1.5 h, only 0.6% weight loss was experienced, which can further be reduced with faster processes (Taylor *et al*., 1981). Meat with less moisture loss by evaporation will have better quality. A relative extent of variability of the hot- vs. cold-deboned beef was found, especially in the amount of fat trimmed. The standard amount of fat trimming was unachievable with hot-deboned cuts. A difference of 2 - 6% was found in this regard, necessitating that the remaining fat on hot-deboned cuts be trimmed before retail. However, this is not regarded as a potential challenge with ostriches, since ostriches have very specific fat depots on the back, breast and in the abdomen (Sales *et al*., 1999; Horbalończuk *et al*., 2004). Nonetheless, hot-deboned beef cuts showed less variation in colour and had minimised drip loss which can be ascribed to the early-on deboning leading to faster cooling of muscles (Taylor *et al*., 1981).

Previous research on ostrich meat has shown that the temperature decline post-mortem is more rapid and consistent with hot-deboning than with muscles left on the carcass as with cold-deboning. Furthermore, hot-deboning has shown great potential as an alternate excising technique to be successfully implemented and carried out on ostrich carcasses. In most red meat species, hot-deboning is typically performed on pre-rigor muscles, however, Botha *et al*. (2006) indicated that ostrich muscles typically enter *rigor mortis* within 1 h post-mortem. Botha *et al*. (2006) further showed that this early entry into *rigor mortis* had a minor influence on ostrich meat quality. The biggest benefit in this regard, is the fact that hot-deboning is more economical in terms of processing time, therefore allowing for a quicker turn-around time as well as the refrigeration space saved since chilling of carcasses for 24 h (refer to Fig. 2.1) can be eliminated (Hoffman *et al*., 2007). Moreover, there is also the prospect to directly freeze hot-deboned ostrich muscles subsequent to excision. Since the onset of *rigor mortis* in ostriches is early (as early as 45 min post-mortem) and hot-deboning of ostrich meat is performed on post-rigor meat, hot-deboned muscles can be frozen immediately after excision without the risk of thaw-rigor. Thaw-rigor occurs when pre-rigor meat (e.g. beef) is frozen immediately after excision (hot-deboning), which is a more severe form of cold-shortening (refer to section 2.4.1.4). Although there is not a risk of cold-shortening or thaw-rigor with the rapid freezing of hot-deboned ostrich meat, chilling of post-rigor meat (e.g. ostrich) to the freezing point, may result in crystallisation of intracellular water within muscles which will eventually result in leakage upon thawing (Stopforth & Sofos, 2005).

2.3 Description of ostrich muscles

2.3.1 Meat from the ostrich

In the South African ostrich industry, raw meat cuts generally include the following: steaks; fillets; meat for biltong; minced dried sausage (known as *droëwors* in South Africa) meat, minced fresh sausage meat; and offcuts. When the percentage prime cuts, or the ostrich cuts with a higher retail worth is compared to similar pork, beef and lamb cuts, it becomes evident that 80 – 90% of the ostrich carcass has high value commercial cuts while meat cuts derived from the other animal species are in the region of 45%. The comparison can also be made for the two fillet cuts in the ostrich leg which encompasses 10%, whereas beef fillet only comprises 2% of the entire carcass (Mellett, 1985). Thus, in the words of Mellett, (1985): *"The ostrich is indeed the perfect meat animal."*

The largest portion of ostrich meat which is suitable to be sold as commercial cuts (75% of the meat), are situated in the hindquarters. The remaining meat on the carcass (25% of the meat), however needs refrigeration space which can become costly. Therefore, ostrich carcasses are already semi-hot-deboned even though the South African ostrich industry uses cold-deboning as method of excising. The former involves the removal of the femur from the acetabulum, while the relevant muscles are cut loose from the pelvic girdle; the connection of the internal obturator muscle to the ischium and pubis, is cut loose among the tendon inserting on the acetabulum. In addition to the removal of the legs within 2 h post-mortem, the internal obturator muscle is also removed. To attain an internal temperature of 7°C within 24 h, direct chilling at 0°C takes place after slaughter. Thereafter, cold-deboning is performed to excise the remaining muscles in the ostrich leg (Mellett, 1985).

2.3.2 Anatomical location of ostrich muscles

In the following section, the ostrich muscles will be described according to the main regions within the leg as described in detail by Mellett (1985). Firstly, the pre-acetabular region, including the tenderloin *(M. iliotibialis cranialis)* and tournedos (*M. ambiens).* Secondly, the acetabular region including the oyster fillet *(M. iliofemoralis externus)* and eye fillet (*M. iliofemoralis).* Thirdly, the post-acetabular region including the rump steak (*M. iliotibialis lateralis),* fan fillet (*M. iliofibularis),* triangle steak (*M. flexor cruris lateralis)*, small steak (*M. flexor cruris medialis)*, tender steak (*M. pubo-ischio-femoralis)* and long fillet (*M. obturatorius medialis).* Muscles of the thigh including the moon steak (*M. femorotibialis medius)* and minute steak (*M. femorotibialis externus).* Finally, muscles of the lower leg including: big drum (*M. gastrocnemius, pars interna)*, small drum (*M. gastrocnemius, pars intermedia),* flat drum (*M. gastrocnemius, pars externa)* and drum steak (*M. iliofibularis longus)* (Mellett, 1985; 1992; 1994). Figs 2.2 – 2.5 are illustrations of the anatomical locations of ostrich muscles and can be referred to for visual representation of each muscle that is described thereafter.

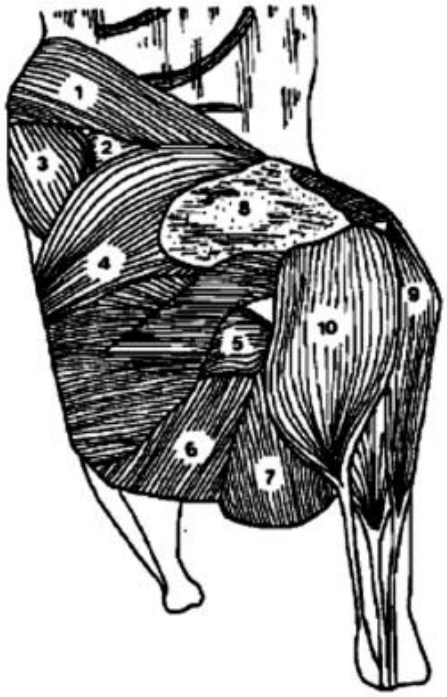

Fig. 2.2: Muscles of the superficial layer of the pelvic limb of the ostrich (lateral view of right leg).

1 Tenderloin (*M. iliotibialis cranialis*)
2 Tournedos (*M. ambiens*)
3 Oyster fillet (*M. iliofemoralis externus*)
4 Rump steak (*M. iliotibialis lateralis*)
5 Fan fillet (*M. iliofibularis*)
6 Triangle steak (*M. flexor cruris lateralis*)
7 Long fillet (*M. obturatorius medialis*)
8 Moon steak (*M. femorotibialis medius*)
9 Drum steak (*M. fibularis longus*)
10 Big drum (*M. gastrocnemius, pars interna*)
10 Small drum (*M. gastrocnemius, pars intermedia*)
10 Flat drum (*M. gastrocnemius, pars externa*)
(Mellett, 1994)

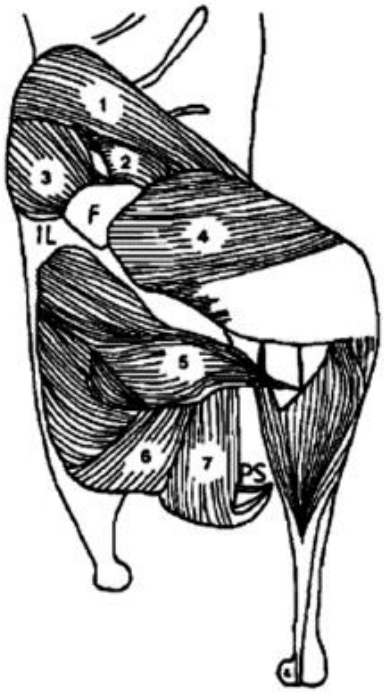

Fig. 2.3: Muscles of the second layer of the pelvic limb of the ostrich (lateral view of right leg).

1 Tenderloin (*M. iliotibialis cranialis*)
2 Tournedos (*M. ambiens*)
3 Oyster fillet (*M. iliofemoralis externus*)
4 Moon steak (*M. femorotibialis medius*)
5 Fan fillet (*M. iliofibularis*)
6 Triangle steak (*M. flexor cruris lateralis*)
7 Long fillet (*M. obturatorius medius*)
(Mellett, 1994)

Fig. 2.4: Muscles of the third and fourth layers of the pelvic limb of the ostrich (lateral view of right leg).

1 *M. iliotrochantericus cranialis*
2 *M. iliofemoralis internus*
3 *M. iliotrochantericus caudalis*
4 Tournedos (*M. ambiens*)
5 *M. femorotibialis accessorius*
6 Minute steak (*M. femorotibialis externus*)
7 *M. ischiofemoralis*
8 Eye fillet (*M. iliofemoralis*)
9 Tender steak (*M. pubo-ischio-femoralis*)
10 Small steak (*M. flexor cruris medialis*)
11 Triangle steak (*M. flexor cruris lateralis)*
12 Long fillet (*M. obturatorius medialis*)
(Mellett, 1994)

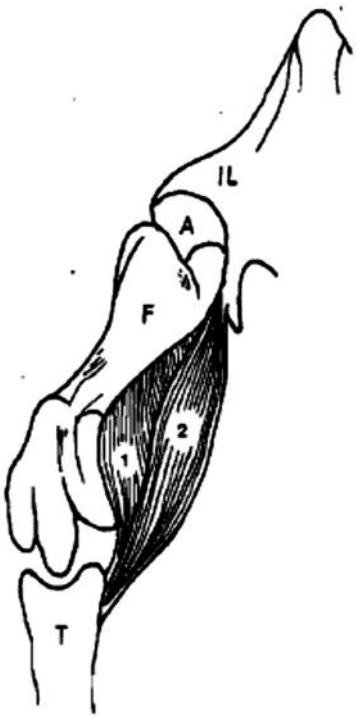

Fig. 2.5: Muscles of the upper leg of the ostrich (cranial view of right leg).

1 *M. femorotibialis internus*
2 *M. pectineus*
(Mellett, 1994)

2.3.2.1 Pre-acetabular region

- *M. iliotibialis cranialis* (tenderloin)

The *M. iliotibialis cranialis* shapes the craniolateral edge of the thigh and can be described as a strap-like, parallel-fibred muscle (Figs 2.2 and 2.3). The basis of this muscle is in the craniolateral rim of the pre-acetabular iliac crest. The attachment of the *M. iliotibialis cranialis* extends from the patella, consequently forming a part of the medial segment of the patellar ligament. The functionality of this muscle is twofold, firstly to make the extension of the knee joint possible and secondly to enable the hip to flex (Mellett, 1985; 1992; 1994; Smith *et al*., 2006). In commercial terms, this muscle is known as the tenderloin.

- *M. ambiens* (tournedos)

In the retail ostrich market, this muscle is well-known as the tournedos cut (Figs 2.2 and 2.3). The ostrich has a well-built ambiens, whilst it does not derive from the pubis and is not highly medial. This muscle, located on the adjacent exterior of the thigh caudal to the cranial *iliotibialis* muscle, is clearly noticeable. The form is cylindrical, narrowing to a thread-like tendon in the knee-joint area. From the pre-acetabular ilium, the ambiens stems from the lateral exterior, ventral to the basis of the medial trochantericus. The tendon stretches along the medial exterior of the patella with a crosswise direction. Both the involuntary grasping function and the tibiotarsus lengthening are the results of the ambiens' action (Mellett, 1985; 1992; 1994; Smith *et al*., 2006).

2.3.2.2 Acetabular muscles

- *M. iliofemoralis externus* (oyster fillet)

In the commercial ostrich trade in South Africa, the *M. iliofemoralis externus* is sold as the oyster fillet (Figs 2.2 and 2.3). This muscle can be defined as a slender, curved muscle enfolding a great part of the shallow pre-acetabular region of the ostrich hip. It is triangular in shape, spreading over the trochanter of the femur, whilst located above the dorsal part of the hip joint. This muscle is considered comparable to the *gluteus maximus* muscle found in mammals. The basis of the *M. iliofemoralis ext.* is in the pre-acetabular iliac crest's full dimension. The control of the rotation of the thigh is where this muscle's key function lies, although it does not assist greatly in the carrying of the thigh (Mellett, 1985; 1992; 1994; Smith *et al*., 2006).

- *M. iliofemoralis* (eye fillet)

An equal shape and size is found in the internal part of the *M. iliofemoralis* which is encompassed by the external part of this muscle (Fig. 2.4). The only distinction that is made for the internal part of the muscle, is that the internal muscle is curved on its lateral edge. In ostrich retail, the *M. iliofemoralis int.* is sold as the eye fillet, while being comparable to the

gluteus minimus muscle of mammals. Furthermore, the basis of this muscle is again to the pre-acetabular iliac crest's full dimension, whilst being ventral to the external part of the muscle. The internal throchanteric together with the external throchanteric controls the femur rotation in the acetabulum (Mellett, 1985; 1992; 1994; Smith *et al*., 2006).

- *M. iliotrochantericus caudalis*

The caudal *iliotrochantericus* is comparable to the cranial *iliotrochantericus* in terms of its form (Fig. 2.4). Additionally, the basis of this muscle is found ventral to and intermediate in the basis of both the internal and external trochanterici, on the lateral exterior of the pre-acetabular ilium. The medial rotation of the thigh is achieved through the function of this muscle (Mellett, 1985; 1992; 1994; Smith *et al*., 2006).

- *M. iliotrochantericus cranialis*

Mellett (1985) describes the *M. iliotrochantericus cranialis* as a muscle touching the craniolateral border of the trochanter of the femur, with a very small, spear-shaped muscle spreading in a caudo-ventral course (Fig. 2.4). The cranial tip of the basis of the *M. iliotrochantericus internus* and *externus* are ventral to the lateral exterior of the pre-acetabular ilium where the base of the *M. iliotrochantericus cranialis* lies. The medial rotation of the thigh is achieved through the function of this muscle (Mellett, 1985; 1992; 1994; Smith *et al*., 2006).

2.3.1.4 Post-acetabular region

- *M. iliotibialis lateralis* (rump steak)

The lateral *iliotibial* muscle stretches across practically both the whole post-acetabular exterior and femorotibial muscles of the thigh (Fig. 2.2). Three different parts of the muscle can clearly be identified, namely a cranial, medial and caudal segment (Mellett, 1985).

The cranial fragment can be described as smooth and widespread. At around half of the thigh the muscle fibres stop amongst an extremely delicate, widespread and translucent fascial sheath wrapping the remaining part of the thigh. Hidden beneath both heads of the *gastrocnemius* muscle, the remaining part of the thigh is hidden in the knee-joint area (Mellett, 1985). The region immediately caudal and ventral to the cranial fragment is wrapped by the medial fragment. The fleshy belly is hidden beneath the external segment of the *gastrocnemius* muscle with the medial fragment stretching beyond the cranial fragment (Mellett, 1985). Stretching from a ventral point of the cranial fragment, the caudal fragment is practically horizontally fibred, connecting the medial fragment to the joint of the knee (Mellett, 1985).

On the complete dimension of the postacetabular ilium, on and ventral to the iliac crest, the lateral *iliotibialis* is fleshy. The attachment is through the fascial sheath enfolding the femorotibialis, providing the development of the patellar tendon to the tibiotarsus. The function

of this muscle extends to supporting the abduction of the leg, as well as with the expansion of the thigh and shank, with lateral rotation of the tibiotarsus. Commercially this muscle translates to the rump steak cut (Mellett, 1985; 1992; 1994; Smith *et al*., 2006).

- *M. iliofibularis* (fan fillet)

Similar to the *M. biceps femoris* found in mammals, the *iliofibularis* is a very big, fleshy muscle with a triangular form, inhabiting the lateral region medial to the lateral *iliotibialis* (Figs 2.2 and 2.3). This muscle is generally also found to appear darker in colour compared to the rest of the muscles in die hind limb. The basis of this muscle is found in the fleshy fibres on the post-acetabular iliac crest beneath the lateral *iliotibialis*. Being a well-built flexor of the joint in the knee, as well as a key muscle for the movement of flightless birds such as the ostrich, this muscle is the well-known fan fillet which is sold in retail. Together with the *semitendinosus* and *semimembranosus*, this muscle is similar to the hamstring group found in mammals which are key in bending the knee joints (Mellett, 1985; 1992; 1994; Smith *et al*., 2006).

- *M. flexor cruris lateralis* (triangle steak)

Mellett (1985) describes this muscle as frequently called the *semitendinosus*, being similar to the *semitendinosus* in mammals (Figs 2.2 and 2.3). This muscle can be seen on the lateral exterior of the thigh, coming through the *iliotibialis lateralis*, with the narrowing form stretching across to the *iliofibularis*. The *semitendinosus* derives from the caudal margin of the ilium and ischium, while the attachment of this muscle is by a general tendon with the *semimembranosus* on the protuberance of the fibula. Furthermore, the *cruris lateralis* is also recognised as the triangle steak, and together with the *iliofibularis* and the *cruris medialis*, performs as flexor of the joint in the knee and expansion of the joint in the hip (Mellett, 1985; 1992; 1994; Smith *et al*., 2006).

- *M. flexor cruris medialis* (small steak)

This is the third part of the group that comprise of the hamstring muscle group in mammals, which is also known as the *semimembranosus* (Fig. 2.4). The *semimembranosus* is completely wrapped by the *semitendinosus* and is a very nearly cylindrical-shaped muscle. The muscle is joined to the *semitendinosus* by the round belly narrowing to an elongated tendon. The shaft of the ischium and the membrane amongst the ischium and ilium is where the *semimembranosus* find its origin. The small steak, as referred to in the trade, acts to assist in flexing the tibiotarsus and to withstand the expansion of the tibiotarus while other muscles are contracting (Mellett, 1985; 1992; 1994; Smith *et al*., 2006).

- *M. pubo-ischio-femoralis (*tender steak)

This muscle is commonly referred to as the *adductor femoris* muscle, comprising of two sections which differ in colour (Fig. 2.4). The outer part with a dark colour, the *pars*

superficialis, and the intermediate part with a light colour, the *pars internus*. They are however, extremely tightly connected, so that they are believed to principally be one muscle. The *adductor femoris* is on the lateral exterior of both the ischium and the pubis, while it is attached on the caudo-lateral exterior of the femur. With the *pubishiofemoralis* being an adductor of the femur, it also inhibits the onwards movement of the femur, thus being a postural muscle. This cut is well-known as the tender steak *(pars internus)* (Mellett, 1985; 1992; 1994; Smith *et al*., 2006).

- *M. isciofemoralis*

The *isciofemoralis* is characterised as an extremely short, dense, cylindrical-shaped muscle expanding in a cranio-lateral course from the ischium, in the direction of the trochanter of the femur (Fig. 2.4). The basis of this muscle is found in the area of a caudal to the *obturator foramen*, on the lateral exterior of the ischium. The attachment on the lateral exterior of the trochanter is transported by an extremely short, inflexible fastened tendon. The rotation of muscles away from the body is achieved by this muscle (Mellett, 1985; 1992; 1994; Smith *et al*., 2006).

- *M. obturatorius medialis* (long fillet)

This muscle enfolds the *incisuna puboishiadica*, being an extremely large muscle (Figs 2.2 and 2.4). The bulk of the muscle narrows to a tendon in the area of the *obturator foramen*, where it stretches across to touch the trochanter of the femur. The derivation of the internal obturator is on the lateral and medial exterior of the distal two thirds of the pubis, as well as on the whole medial exterior of the ischium. The horizontal tendon attaches on the caudal-lateral outside of the trochanter of the femur, caudo-ventral to the attachment of the *ischiofemoralis*. The caudo-lateral turning of the thigh is achieved through the function of the internal obturator. In the retail ostrich market, this muscle is sold as the long fillet (Mellett, 1985; 1992; 1994; Smith *et al*., 2006).

- *M. obturatorius lateralis*

Commercially the *M. obturator externus* is also sold as a long fillet. A small, fleshy bulk stretching to the caudo-lateral part of the trochanter, is found on the caudo-ventral edge of the acetabulum. In and on the caudo-lateral edge of the acetabulum, the basis of the external obturator is located. The attachment of the muscle is by a widespread tendon on the caudo-lateral exterior of the caudal part of the trochanter of the femur, whilst being caudal to and beneath that of the *ischiofemoralis* (Mellett, 1985; 1992; 1994; Smith *et al*., 2006).

2.3.1.5 Muscles of the thigh/ femoral muscles

- *M. femorotibialis medius* (moon steak)

Enfolding the lateral exterior of the upper leg of the ostrich, the external part of the *femorotibialis* muscle is the largest of the four fragments (Figs 2.2 and 2.3). The external *femorotibialis* derives from the aponeurosis on the lateral exterior of the femur. The tendon of the *iliotibialis lateralis* is connected by the level tendon of this muscle, to attach on the patellar ligament. More commonly the external *femorotibialis* is known as the moon steak (Mellett, 1985; 1992; 1994; Smith *et al*., 2006).

- *M. femorotibialis externus* (minute steak)

This muscle is closely related to the *rectus femoris*, but divides into two muscles in the ostrich (Fig. 2.4). The entire dimension of the femur (distal to the trochanter) is wrapped by the medial *femorotibialis*. The basis of this muscle is found on the entire extent of the lateral exterior of the femur and the level tendon is bound to the tendon of the *rectus femoris* (dorsally), as well as to that of the lateral *femorotibial* muscle (medially) and to the tendon of the *iliotibialis lateralis.* Amongst retailers, this ostrich muscle is recognised as the minute steak (Mellett, 1985; 1992; 1994; Smith *et al*., 2006).

- *M. femorotibialis internus*

The basis of the internal femorotibialis is on both the caudo-medial and medial length of the femur (Fig. 2.5). The tendon bounds the patellar ligament on the exterior (medial). This muscle attaches immediately on the tibiotarsus (Mellett, 1985; 1992; 1994; Smith *et al*., 2006).

2.3.1.6 Muscles of the lower leg

- *M. gastrocnemius* (big drum)

The largest muscle with the heaviest weight in the ostrich, is the *M. gastrocnemius* (Fig. 2.2). This muscle wraps practically the whole exterior (lateral, caudal and medial positions) of the lower leg. Deriving from the lateral exterior of the patellar ligament, is the *pars externa*. The other fragments in the crux are attached to the extremely compact tendon which is bent in a caudal course. The *pars media* derives from the medial exterior of the distal third of the femur. The tendon of the femur bounds the external section. The basis of the *pars interna* is found on the craniomedial position of the patellar ligament, whilst its tendon is also bound to that of the external as well as the medial fragments of the *gastrocnemius*. The function of this muscle is to expand the tarso-metatarsus. In ostrich retail, this muscle is better known as the big drum (*pars interna*), small drum *(pars intermedia)* and the flat drum *(pars externa)* (Mellett, 1985; 1992; 1994; Smith *et al*., 2006).

- *M. fibularis longus* (drum steak)

The cranial exterior of the lower leg is wrapped by the *peroneus longus*, which derives from the cranial position of the patellar ligament, as well as the patellar fascia and proximal edge of the tibiotarsus (Fig. 2.2). The attachment however, is found on the digits in the metarusus, derived from one of its flexors. This cut is commercially sold as the drum steak (Mellett, 1985; 1992; 1994; Smith *et al*., 2006).

2.4 Physical meat quality characteristics

2.4.1 pH

2.4.1.1 Post-mortem pH decline

Final or ultimate pH (pH_u) directly affects the colour, WHC, shelf-life, tenderness and flavour of meat. The pH_u will be reached at varying times post-mortem according to influencing factors such as variation amongst individual animals and amongst different muscles, whilst various measurements of pH post-mortem provide valuable insight into meat quality. The point at which the pH_u is reached post-mortem, is conditional to muscle type, species and pre-slaughter stress. The pH_u is consequently determined by the pH value measured during *rigor mortis*. Furthermore, post-mortem pH decline is a result of the conversion of glycogen to lactic acid (Honikel, 2004a).

The chemical process in which glycogen is converted to lactic acid, follows post-mortem glycolysis which occurs at death when all oxygen (O_2) is depleted from muscles. The latter process will last until all enzymatic working has stopped and a pH of 5.4 – 5.5, which signifies the iso-electric point, is reached. At this point, the pH_u is reached. With higher external temperatures (above ambient), the rate at which post-mortem glycolysis occurs increases accordingly. Moreover, with a decreasing temperature at 0 - 5°C, the degree to which post-mortem glycolysis takes place, also increases (Lawrie & Ledward, 2006a). During post-mortem glycolysis, myofibrillar proteins (actin and myosin) reach their iso-electric point, at which the protein molecules have no net electrical charge, and the muscles are inclined to lose the water normally bound to them. The initiation of this muscle denaturation precedes a reduced water binding ability, providing exudate which could ultimately lead to drip (Warriss, 2000b). Nonetheless, Adenosine Tri-Phosphate (ATP) keeps the cellular structure of the muscles intact, whilst also contributing to the tightening and working of muscles. In the physical transformation of meat post-mortem, the fibrillar structure is altered once the ATP is depleted and permanent attachments to fibres have been formed (Honikel, 2004b).

The rate and degree to which pH declines post-mortem, is greatly determined by a variety of factors such as muscle type, which can be classified as a native or inherent factor. Therefore, the post-mortem pH decline and pH_u of ostrich muscles, play a crucial role in determining the meat quality (Lawrie & Ledward, 2006a). Sales and Mellett (1996) showed that a particular muscle does not demonstrate the standard for all the individual muscles of the ostrich, and can be described as the most complicated of all the intrinsic factors (Lawrie,

2006a). Apart from muscle type, other intrinsic factors influencing post-mortem pH decline include species and inconsistency amongst animals, while extrinsic factors include temperature and the dispensation of drugs (Lawrie & Ledward, 2006a).

Sales and Mellett (1996) suggested that ostrich meat be assessed in accordance with red meat, since its colour appears to be darker than beef. The pH_u value of ostrich meat categorises it as an intermediate meat type, with a pH_u value varying between normal (pH < 5.8) and dark, firm and dry (DFD; pH > 6.2). Consequently, meat with a dark colour which negatively influences shelf-life, taste and its aptitude for nitrate absorption, is found (Sales & Mellett, 1996). The exhaustion of glycogen stores due to pre-slaughter stress is usually the cause of these damaging physical characteristics, but still gives the beneficial effect of water binding (Lawrie & Ledward, 2006a). The physical characteristics of meat is important within the broader red meat industry, as re-purchase of meat products by consumers is determined by the overall eating quality (Hutchison *et al*., 2010).

Hoffman *et al*. (2007) found that hot-deboned ostrich muscles reached the point of minimum pH_u slower in comparison with cold-deboned ostrich muscles. However, there was no meaningful dissimilarity in the minimum pH_u values. Over a period of 42 days of refrigeration, Botha *et al*. (2006) found the only interrelation of pH_u to be with the ageing time period. A difference between the two evaluated ostrich muscles (*M. gastrocnemius, pars interna* and *M. iliofibularis*) were found, for both hot- and cold-deboning as the time period increased. Both hot-deboned muscles had significantly lower mean pH_u values over the 42 d storage period, with pH_u values (for both muscles, hot- and cold-deboned) increasing towards day 42 post-mortem (Botha *et al*., 2006).

2.4.1.2 Development of rigor mortis

During the process of post-mortem glycolysis, the onset of *rigor mortis* takes place as the muscles become inflexible, leading to stiff chains of actomyosin being developed through the loss of ATP. The development of actomyosin is initially slow (the delay phase), followed by a fast phase, after which inflexibility of the muscles is unvarying. The point where the fast phase of *rigor mortis* is reached at a particular temperature, is dependant of the amount of ATP which is reduced gradually by the ATP-ase action of myosin (which is non-contractile) (Lawrie & Ledward, 2006a). In poultry meat (including ratites, i.e. ostriches), the development of *rigor mortis* is known to have a rapid onset (within 1 h post-mortem) due to the normal rapid pH decline post-mortem (Mozdziak, 2004).

Before the onset of *rigor mortis*, ATP acts to maintain the relaxed state of muscles through inhibiting the formation of actomyosin in resting muscles. During post-mortem glycolysis, with the breakdown of glycogen, the ATP concentration is sustained until glycolysis is limited by the absence of substrate or unfavourable circumstance for the enzymes. Tightening of muscles thus occur when the revival of ATP is eventually diminished, and ATP

is hydrolysed to ADP. The intensity of ATP can only be conserved for a short period through the re-synthesis of ADP and creatine phosphate (CP) (Lawrie & Ledward, 2006a). When ATP levels have dropped below the minimum required to keep muscles relaxed, *rigor mortis* will take place where actomyosin is formed through the bonding of thick and thin filaments of actin and myosin (Warriss, 2000b). The time to onset of *rigor mortis* can be correctly calculated with the knowledge of the early levels of ATP and CP, the early store of glycogen and the temperature. Together with these factors, the initial pH (45 min post-mortem) gives a good calculation to the time to onset of *rigor mortis* (Lawrie & Ledward, 2006a).

A lowering in water holding capacity (WHC) is characteristic of the onset of *rigor mortis*, and is not only a consequence of pH decline and the pH nearing the iso-electric point, but rather the effect of actomyosin development and the loss of ATP. Furthermore, there are at least two phases for water to be in, where some water is bound and the greater part is free. The cross-linking of actomyosin can be indicated through the volume of unvaried bound water in each phase, whilst the volume of free water moves around liberally between phases (Lawrie & Ledward, 2006a).

The key attributes of *rigor mortis* will be influenced by intrinsic factors including muscle type and species, as well as extrinsic factors including the temperature and ante-mortem stress, the latter influencing the levels of glycogen present in the muscles at death. These key attributes of *rigor mortis* include the early onset ATP and CP concentration levels; the early and enduring level of glycogen present; the initial, onset and pH_u (which is in fact a log transformed measurement of the H^+ concentration); and finally the ATP-ase action (Lawrie & Ledward, 2006a). The development of *rigor mortis* in ostriches is known to be within 45 min. Consequently, *rigor mortis* generally does not directly change or influence the deboning process of ostriches. Furthermore, when hot-deboning is performed for ostriches (at 2 - 4 h post-mortem), *rigor mortis* would already be complete (Hoffman *et al*., 2007).

2.4.1.3 Rigor (warm) shortening

During *rigor mortis*, the connection of actin-myosin bonds does not happen all at once. Ultimate association and attachment of the bonds occur once the remaining accessible chemical energy of the ATP molecule has been freed into the myosin opening. Thereafter, the bound actin and myosin remain in that state while the muscles lose flexibility and tighten to become rigid. Within this ongoing process, a small development of *rigor* shortening is caused through each arrangement of actomyosin. Apart from the case where muscles are exposed to greater temperatures, this shortening is minor (10 - 15%) (Honikel, 2004c).

Whilst only affecting a section of the muscles, shortening which take place during *rigor mortis,* is characterised as being permanent and therefore differentiates it from physiological shrinking. At temperatures between -1°C and 38°C, a degree of shortening happens in all muscles during post-mortem glycolysis. Minimum *rigor* shortening takes place at 15 - 20°C, with an increase seen in temperatures above 20°C. The degree of shortening at the start of

rigor mortis is interrelated to temperatures at 15°C. In the case where separated muscles (detached from the carcass), are subjected to temperatures below 14°C, loss in tenderness on cooking is caused by the muscles' predisposition to shorten. In the case where muscle shortening at the start of *rigor mortis* is inhibited, e.g. when the muscles are held inflexible on the carcass, the effect of temperature is not completely related to the level of toughness in cooking (Lawrie & Ledward, 2006a).

The pH decline during the development of *rigor mortis*, which causes the ATP levels to drop, thus triggers controlled warm-shortening which occur during normal *rigor mortis*. In standard commercial procedure, when muscles are retained on the carcass, some muscles will nevertheless only have partial freedom to tighten. Therefore, some fractions of the muscle may lengthen whereas others may shorten. However, a degree of toughening will still take place even when the carcass can largely be held inflexible until cold-deboning (Lawrie & Ledward, 2006b). Although muscles are not retained on the carcass with hot-deboning, but excised within 2 - 4 h post-mortem after the onset of *rigor mortis* 45 min post-mortem (Hoffman *et al*., 2007), the overall incidence of warm-shortening at a level which commonly occur in meat from all animals, is not ruled out specifically with hot-deboning of ostrich meat.

2.4.1.4 Cold-shortening

When carcasses are refrigerated too soon after slaughter, selected muscles are prone to become rigid. The latter refers to cold-shortening, which is dependent on temperatures below 10 - 15°C, at a pH above 6.0 (Lawrie & Ledward, 2006a), and becomes especially evident above pH 6.2; while ATP is still present in muscles (Honikel, 2004c). While *rigor*-shortening happens just before muscles stiffen during *rigor mortis*, cold-shortening is said to take place during the initial stage of *rigor mortis*. Muscles that experienced cold-shortening remain tough even at lengthy ageing time periods. This is ascribed to the fact that meat cannot be tenderised even with the continued enzymatic (calpains) working during ageing (Honikel, 2004c).

Babiker and Lawrie (1983) states that in the case of beef, muscles are subjected to microbial contamination during hot-deboning, emphasising quick chilling after deboning. With hot-deboning, quick cooling of the meat is easily attainable due to the much smaller surface volumes. Although rapid cooling of hot-deboned muscles is achieved, it does however lead to a higher risk of cold-shortening. Electrical stimulation (ES) is frequently applied when hot-deboning is performed, in order to prevent shortening of muscles upon the completion of *rigor mortis* (Sales & Mellett, 1996). The application of ES instantly after the post-mortem period rapidly lowers the pH below the level where it is prone to cold-shortening. Moreover, it is also evident that with the application of ES, it leads to improved tenderness (Babiker & Lawrie, 1983). It has however been found that ES has no effect on the meat tenderness of ostrich meat, specifically the *M. iliofibularis*, M. gastrocnemius, pars interna or *M. iliotibialis cranialis* muscles (Hoffman *et al*., 2009). This can in all likelihood be ascribed to the fact that ostriches develop *rigor mortis* very soon after slaughter (within 45 min) (Hoffman *et al*., 2007).

When excised, pre-rigor muscles are subjected to temperatures initiating cold- shortening, and as the level of pre-rigor shortening becomes greater, the level of toughness increases accordingly. The initial high temperature and protection of muscles located deep within carcasses, contribute to the rapid post-mortem glycolysis thereof, causing post-mortem glycolysis to be complete by the time refrigeration lowers the temperature under 15°C. Therefore, these muscles are not as prone to experience cold-shortening. Sales and Mellett (1996) found no viable variations in the cooling rate of ostrich muscles to have an effect on the post-mortem glycolysis (thus post-mortem pH decline). It was also found that there is no risk of cold-shortening in the ostrich muscles that were evaluated, due to the development of *rigor mortis* within 45 min post-mortem. Consequently, if ostriches are dressed (within 1 h post-mortem) and hot-deboned (2 – 4 h post-mortem) post-rigor, this could rule out cold-shortening. Additionally, in hot-deboned ostrich muscles, pH < 6.20 was reached at temperatures higher than 10°C, further emphasizing no great risk of cold-shortening in hot-deboned ostrich muscles (Hoffman *et al*., 2007).

2.4.2 Colour

The colour of fresh meat and meat products are often recognised as being the most important determining factor in the consumer's selection process (Jeremiah *et al*., 1972; Fletcher, 2002; Cornforth & Jayasingh, 2004). The freshness of meat is often judged upon colour by consumers, and it is well-known that consumers choose bright red meat above meat with a purple or brown colour (Carpenter *et al*., 2011).

The type and quality of the primary meat pigment, namely myoglobin, as well as the physical and chemical condition of the other constituents of meat influence the exterior appearance of meat (Cornforth & Jayasingh, 2004; Lawrie, 2006b). The intensity of muscular action affects the amount of myoglobin that is present where increased muscular activity will have an increased level of myoglobin. Muscle type, breed, species and age influence the level of myoglobin that is present in the muscles. The most prominent variety in colour of a meat surface, are the myoglobin molecules' chemical structure. The exposure of meat to air allows for O_2 to be bound to myoglobin, forming oxymyoglobin (MbO_2) within 30 min (blooming) (Cornforth & Jayasingh, 2004). Although 30 min is usually allowed for blooming time, in the case of ostrich meat it was found that the blooming time is 60 min (Leygonie, 2011). The most crucial chemical form in fresh meat is MbO_2 since it signifies the bright red colour for consumer preference. This bright red colour which only appears on the exterior meat surface, will principally be seen externally to where the ratio MbO_2: myoglobin is 1:1 (that constitutes approximately 84% of the total O_2 diffusion depth). In the case of cooked meat, the main pigment is the brown globin known as haemichromogen. A few other components influence the colour of brown, cooked meat: Maillard-type reactions amongst amino groups and reducing sugars; as well as carbohydrate caramelisation (surface browning) (Lawrie & Ledward, 2006b).

Meat colour and muscle pH_u have been found to be highly related, especially relating to DFD or pale, soft and exudative (PSE) meat as is commonly found in pork and less so in poultry and even less in most mammalian meat (Fletcher, 2002; Monin, 2004). Meat with a high pH_u, thus considerably above the iso-electric point, will have firmly packed fibres which exhibit a hurdle for O_2 diffusion. This, together with the remaining cytochrome enzyme action leads to a very thin layer of MbO_2 (bright red) being visible. Instead, an unlikeable, purplish- red colour of myoglobin itself dominates to such a degree that the meat will be apparently dark in colour. A darker red colour will prevail due to the distorted adsorption traits of the myoglobin caused by the higher pH_u. Meat with a lower pH_u will have less firmly packed fibres, resulting in more efficiently light scattering which will not appear dark in colour (Lawrie & Ledward, 2006b).

Furthermore, most prepacked fresh meat is placed in an oxygen permeable wrap, because the bright red colour of MbO_2 is an attractive quality in fresh meat. However, after a few days, the exterior of the meat oxidizes to metmyoglobin (MetMb) caused by the chiller's temperature. In vacuum packaging (common practice in the ostrich industry), the small amount of MetMb present is reduced by the lack of oxygen permeation, which in turn is replaced by the purplish red colour of myoglobin. Modified atmosphere packaging (MAP) where the air surrounding the meat is controlled with carbon dioxide (CO_2), is found to primarily be surrounded by traces of O_2 where MetMb will form. Thereafter, the MetMb changes back to myoglobin causing a purplish-red colour (Lawrie & Ledward, 2006b).

Botha (2005) found no significant difference amongst the raw meat colour (30 min blooming) of two hot- vs. cold-deboned ostrich muscles (*Muscularis gastrocnemius*, *pars interna* and *Muscularis iliofibularis*). The only meaningful difference was found between the individual ostriches for the same two muscles. Furthermore, the general pH_u of these two ostrich muscles showed a negative correlation with regards to muscle colour (Botha, 2005).

2.4.3 Water holding capacity (WHC)

Water holding capacity (WHC) of meat refers to the capability of meat to sustain its own (75% of muscle tissue) or added water when compression or heat is applied (Brewer, 2004). The latter is of great value as it directly influences the appearance and juiciness of meat. Furthermore, the WHC of meat also affects the cooking characteristics of meat. The *in vivo* WHC of meat is established through exudation of liquid in various forms: drip in uncooked meat that has been thawed; weep in uncooked meat that has not yet been frozen; and shrinkage in cooked meat. The gaps between the thin fibres of actin and the thick fibres of myosin is where most of the water in the muscles are present. Water is present under two conditions in muscles where each of the relative amounts are partially free or bound. There is only slight altering of bound water, whereas the amount of free water in the extracellular area increases (Lawrie & Ledward, 2006b).

In uncooked meat, weep or drip exudation is influenced by the amount of liquid freed from its connotation to muscle proteins. Post-mortem glycolysis will normally reach a pH_u of around

5.5 (iso-electric point) where a minimum WHC is found. Consequently, the degree to which the post-mortem pH declines will influence the WHC, where the higher the pH_u the smaller the reduction in the WHC will be. In the case where a remarkably low pH_u is reached, the result will be meat which is very moist post-mortem. Both extreme and moderate post-mortem glycolysis will thus have an effect on meat WHC. The tempo at which the post-mortem pH drops is also a crucial determining factor in the WHC of the meat. The faster the tempo at which the pH falls, the more damaging the denaturation effect will be and the more moisture will be lost (Lawrie & Ledward, 2006b).

Distinguishing aspects between muscles such as age, species and function are also known to affect the WHC through the effect on the tempo and degree of post-mortem pH decline (Lawrie & Ledward, 2006b). Additionally, the amount of fat also influences the WHC of uncooked meat, where muscles with a high amount of intramuscular fat is inclined to have an increased WHC. A possible explanation for this could be that the intramuscular fat releases the meat's microstructure whilst keeping more water bound (Lawrie & Ledward, 2006b). Nevertheless, ostrich meat is characteristically known for its low intramuscular fat content (Sales, 1996), thus indicating that the fat content in ostrich meat will not negatively affect its WHC.

The influencing factors for drip and weep in uncooked meat, are also relevant for cooked meat, with the changing factor being heat application. However, shrinkage of cooked meat is much greater than weight loss through drip and weep in uncooked meat, and is affected by factors such as cooking method, temperature and time of cooking. The high temperatures at which cooking takes place, will significantly decrease the WHC due to protein denaturation. The amount of shrinkage with cooking is interrelated to the loss of juiciness in cooked meat. The juiciness of meat is experienced with the first few chews where meat fluid is released giving the feeling of wetness; followed by continued juiciness caused by the stimulation of fat on salivation (Lawrie & Ledward, 2006b).

A considerably increased amount of weep was found in two hot-deboned ostrich muscles (*Muscularis gastrocnemius, pars interna* and *Muscularis iliofibularis*) in contrast with the same two muscles that were cold-deboned (during a 42 d period of refrigeration). This increase in weep of the hot-deboned muscles, was ascribed to the difference in deboning time. The considerably greater amount of weep in the vacuum-packed ostrich muscles, will negatively affect consumer perception of the meat quality at purchase. Nonetheless, hot-deboning of these two muscles did not seem to result in a higher cooking loss in comparison with the same two cold-deboned muscles (Botha *et al*., 2006).

2.4.2 Warner-Bratzler shear force

Of all the physical meat quality characteristics, meat texture and tenderness is currently valued as the most significant factor by consumers in determining eating quality (Tornberg, 1996; Lawrie & Ledward, 2006b). Although meat texture and tenderness is the most required eating

quality contributor (at the expense of colour and flavour), it is generally the most complicated to describe. Generally, texture can be described as a gathering of fibre bundles, with the size and number of fibres indicative of the size of the bundle. The roughness of the bundles is nonetheless not only established by the size of the bundles, but also through the amount of the perimysium (perimysial layer densely packed with coarse muscles) surrounding each bundle. Tenderness can be described in view of texture, through the initial ease of meat penetration by teeth, as well as the effort required to break meat into pieces and the residual amount subsequent to chewing (Lawrie & Ledward, 2006b).

The level of meat tenderness can be described through the correlation of three groups of protein: the sarcoplasm (sarcoplasmic reticulum, sarcoplasmic proteins); the myofibril (actin, myosin, tropomyosin); and connective tissue (the matrix mucopolysaccharides, elastin, reticulum, collagen) within muscles. The amount of tightening of the myofibrils, as well as the cooking temperature and muscle type, influence the significance of each of the three groups' relative contribution to tenderness. Although water-solubility of sarcoplasmic proteins might give the impression that they are not a factor in meat texture, on heating they coagulate, where after a part is attached to structural components within the muscle cell (Lawrie & Ledward, 2006b).

Post-mortem glycolysis is one of the common post-slaughter factors which influence meat texture and tenderness. The tempo at which pH declines post-mortem (followed by cooking), can be correlated to meat tenderness. Improved tenderness was found with a slow post-mortem pH decline where a higher pH is upheld together with a near *in vivo* temperature. This improved tenderness was however found in the instance where *rigor* shortening could not develop. The degree to which post-mortem glycolysis also affects meat tenderness, where loss of tenderness is seen when the pH_u rises from 5.5 to 6.0. However, at a pH_u above 6.0, tenderness further increases (Devine, 2004; Lawrie & Ledward, 2006).

Ageing is another post-slaughter factor which affect meat texture and tenderness. Ageing can be described as the development of meat tenderisation and happens through the endogenous muscle enzymes' presence (Devine, 2004). The loss in tenderness experienced during rigor-shortening, is slowly reversed through conditioning post-rigor. A shorter ageing time period is achieved at a higher temperature with a faster working of enzymes. This is seen in muscles comprising of certain proteolytic enzymes functioning more freely at 37°C as opposed to 5°C. This higher ageing temperature, does however play a part in creating a higher risk for microbial spoilage. In isolated areas of developing countries, the use of CO_2 versus mechanical chilling, have been implemented to rapidly chill hot-deboned meat. The advantage of this rapid chilling is seen in the stimulation of proteolytic enzymes that would improve tenderness, although there is a risk of cold-shortening with the use of very low temperatures (Lawrie & Ledward, 2006b).

Cooking of meat, can also vastly affect the texture and tenderness of meat. As with WHC, meat texture and tenderness is greatly influenced by cooking temperature, cooking time and the

cooking method. During the cooking process (application of heat), collagen (the main structural protein in meat) converts to gelatine. During this transition, myofibrillar proteins generally have increased toughness, whereas the connective tissue become increasingly tender. Cooking time is the most important factor influencing the collagen softening whilst the cooking temperature mostly affects the myofibrillar toughening. Cooking for an extended time period at fairly low temperatures are acceptable for meat high in connective tissue and contrariwise (Lawrie & Ledward, 2006b).

Botha *et al.* (2006) found that the hot-deboned *Muscularis gastrocnemius, pars interna* muscle from the ostrich initially showed toughness over a 42 day period of refrigeration in comparison with the same cold-deboned muscle. After a 14 day refrigeration period however, no meaningful difference in terms of the meat tenderness between the hot- vs. cold-deboned *Muscularis gastrocnemius, pars interna* and *Muscularis iliofibularis* muscles, was found (Botha *et al*., 2006).

2.5 Microbial quality and safety

2.5.1 General hygiene/safety practices in the meat industry

The possibility of meat products functioning as a source of food-borne pathogenic microorganisms has been an increasing subject of interest during recent years. Supervising authorities, public health agencies, the meat industry, researchers and consumers all have a particular interest in meat safety due to public awareness of food-borne disease epidemics caused by meat. The conditions of meat supply have therefore been advanced through the requirement of recognized prerequisites to enhance the hygienic status. Some of these requirements include the application of the following practices: sanitation standard operation procedures (SSOP); as well as a hazard analysis and critical control points (HACCP) programme. For the implementation of a HACCP programme, confirmation specifications and operating benchmarks must be set for the lowering of pathogens. Besides a HACCP programme, quality management (QM) systems are often used to guarantee constant product quality throughout the storage period (Kourtsoumanis & Sofos, 2004).

It might also be noteworthy to mention that circumstances under which meat is generally handled, processed and stored, are more important in considering the main microbial factor causing spoilage, rather than the concentration of microorganisms that are present early on (i.e. at slaughter). The circumstances of product treatment, control, and storage, are the key determinants of the small fragment of total initial microflora which become dominant through selection in the process of meat spoilage (Kourtsoumanis & Sofos, 2004).

Throughout deboning, the whole process is interrelated to good manufacturing practices (GMP) or good hygienic practices (GHP) during product processing, general hygienic procedures, sanitation operations as well as to temperature and time of deboning. Basic essentials include personnel education and training in a quick product throughput time, correct chilling of carcasses and the application of proper general and personal hygienic procedures. Good

management of these basics may retain contamination levels as near as possible to those found early on at slaughter (initially after deboning has been completed) (Kourtsoumanis & Sofos, 2004).

In an attempt to control the whole process from where the animal is received live to where meat products are dispatched (Fig. 2.1), the application of a quality control system, is key. An effective way to develop such a system, will be to rather focus on prevention than controlling and testing only the final product (Kourtsoumanis & Sofos, 2004). The relevance of proper hygiene and cleaning practices in the prevention of microbial spoilage, are emphasised by the Meat Safety Act of 2000. According to this act, a Hygiene Management System (HMS) for each room in an ostrich abattoir, including the deboning room, should be implemented. Within this HMS for the deboning room, a step-by-step cleaning method should be described, with an indication of how regular (specified time intervals) the cleaning should be executed. Together with this, information regarding all chemicals as well as the precise appliance of each chemical, should be mentioned (Department of Agriculture, 2007).

2.5.2 Causes of microbial contamination in meat

Infection of meat carcasses and cuts are very likely to occur during processing, and may sustain the growth of a variety of both pathogenic and spoilage microorganisms. Throughout the progression of slaughtering, dressing, chilling and excising, the exposed meat cuts may be infected by various sources. These sources commonly include: feed; hides; soil; water; air; intestines; lymph nodes: processing apparatus; and humans. Subsequently, contamination sources are not similar and may vary according to slaughtering and processing practices, which additionally influences the nature and extent of contamination. The quality and safety of the product will, however, not just be affected by the type of microorganisms present, but by the handling of the product as well. Furthermore, season, environmental derivation (geographic location) and individual animal traits will also influence contamination (Kourtsoumanis & Sofos, 2004).

Ultimately, contamination inflicted at some stage during the slaughtering process, can be minimised. Although microbial contamination can take place because of tissue wounds sustained through slaughter, through skin openings as well as through apparatus used during slaughter. Nonetheless, the latter can be limited by regulating procedures such as sufficient equipment sanitation and cleansing. However, the key microbial contamination sources throughout dressing remains the skin, feathers and other animals in a nearby vicinity. As seen in Fig. 2.1, slaughtering as well as defeathering and skin removal, is performed in the “dirty” area of the abattoir. With the first cut of the infected skin, microorganisms are passed to the muscle tissue which lies beneath the skin. Thereafter, the muscle tissue might be contaminated in the case where the skin folds over and comes into contact with it. The proximate environment to where the skin is removed, microorganisms conveyed from the knife with which skinning was performed, as well as worker’s hands which might be contaminated, also

contribute to contaminating muscle tissue. It can be remarked that as shown in Fig. 2.1, evisceration, which forms a part of carcass dressing, is nevertheless the first step performed in the “clean” abattoir area (Kourtsoumanis & Sofos, 2004).

Succeeding evisceration and chilling of carcasses (24 h post-mortem), carcasses are excised (refer to Fig. 2.1). The only exception is in the instance of hot-deboning, where carcasses are deboned directly after dressing (thus immediately after evisceration). The key source of microbial contamination during deboning, is said to be incoming meat. The distribution degree of contamination is determined by contact with hands, apparatus and working surfaces which significantly affects the storage life and microbial quality of the subsequent meat cuts. Moreover, hands, apparatus and meat contact surfaces might transmit a great contamination amount and large numbers of bacteria which can rapidly accumulate on cutting surfaces (Kourtsoumanis & Sofos, 2004).

Additionally, Eisel *et al*. (1997) found that contamination due to handling, touching and moving of meat, becomes a higher risk during the deboning process. This wide-ranging handling of the meat makes it more prone to becoming contaminated with bacterial organisms. The degree to which this contamination takes place, is however relative to the rate and amount of meat throughput, the environmental setting, as well as the general hygiene associated with hands and equipment in the abattoir (such as instruments, knives, conveyor belts and working surfaces) (Eisel *et al*., 1997). Karama *et al*. (2003) found the slaughter procedures during skinning and evisceration in an ostrich abattoir to contribute to carcass contamination. Concerning Aerobic Plate Counts (APC), *Pseudomonas* spp. and *Staphylococcus aureus* counts, the original microbial load deposited on the carcass during skinning was maintained at regulated levels. A lower number of bacteria may however be deposited on carcasses if the skinning process can be performed with more care (Karama *et al*., 2003). Shange *et al*. (2019) found the initial bacterial load of game meat (black wildebeest; *Connochaetes gnou*) to not be caused by muscle pH (intrinsic meat quality), but rather through contamination during processing. Muscle pH did however have a greater effect on the spoilage of game with a high pH (DFD meat) in comparison with meat with a normal pH, as DFD meat spoiled sooner. From this study it was evident that the initial pH of game meat had an effect on the meat (aerobic refrigerated storage) (Shange *et al*., 2019).

Concerning meat yields, Hoffman *et al*. (2010) found cold trimming (after 24 h refrigeration of carcasses) of ostrich bruises to be more valuable. Cold trimming of bruises were also found to have a lower microbial load which is expected to contribute to a longer shelf life. Higher total aerobic viable counts were seen with the trimming of bruises on warm carcasses. Generally, with the management of trimming practices, losses caused by bruising of carcasses was halved while both increased hygiene control and higher meat yields followed (Hoffman *et al*., 2010a). Furthermore, the growth of predominant microbes on ostrich carcasses before and after overnight cooling in an abattoir and deboning plant, similarly showed cold trimming (mainly bruises) was beneficial over warm trimming regarding the

microbiological meat quality. The most hazardous point for carcass contamination was found to be pooled water in the abattoir, with Gram-negative pathogens being the most common contaminants. Air samples showed that *Pseudomonas* and *Shigella* were recurrently present in the abattoir emphasizing the management of total plant hygiene to produce ostrich meat with a satisfactory shelf life that is safe to consume (Hoffman *et al.,* 2010b).

Throughout the deboning process, the commercial cuts are detached from the hanging carcasses, after which it is usually consigned to stainless steel working areas/tables where extra fat is trimmed by hand. It is common practice to subsequently consign it to a conveyor belt which moves it to the next room where packaging and boxing is carried out. Finally, the boxed meat is commonly involved in two more final steps: firstly, it is transferred in a truck which is temperature controlled and not exceeding 10°C before it reaches the retail market; and finally the boxed meat is customarily frozen (-14 - 18°C). From there, the meat is dispatched and distributed at a later stage (Nel *et al*., 2004). In accordance with this cold-deboning process, hot-deboning is performed with the biggest apparent difference being the temperature at which excising takes place, as well as the temperature of the meat cuts after excision. The increased deboning temperature, can thus be described as a basis of concern for possible microbial contamination (Sheridan & Sherington, 1982; Reid *et al*., 2017).

Microbial contamination can mainly be overcome by vacuum packaging. However, the small amount of oxygen left over with vacuum packaging, can be utilised by microorganisms. Thereafter, CO_2 will be produced leading to a decrease in pH, creating flourishing conditions for bacterial growth, resulting in the production of lactic acid by facultative anaerobic bacteria (produces ATP either through aerobic respiration in the presence of O_2, or through fermentation in the absence of O_2). Vacuum packed meat will have a prolonged shelf-life due to the pH that is lower and the lack of oxygen that will prevent growth of bacteria (Aberle *et al*., 2001), which is particularly relevant to the ostrich industry since ostrich meat is mainly vacuum packed for export.

2.5.3 Meat spoilage: Factors influencing microorganism growth

Whether meat is spoiled through endogenous (infection of the animal whilst alive) or exogenous (post-mortem) contamination, it leads to spoilage recognised through unappealing physical changes in the meat. Amongst others, the key interdependent factors which affect the growth of microorganisms, are: an appropriate temperature, accessibility of moisture and a suitable pH range to enhance specific microorganism growth (Lawrie & Ledward, 2006c). Temperature is the most crucial factor which affects microbial growth. Hence, the tempo of microbial growth is correspondingly greater at a higher temperature. Although several meat microorganisms will grow in a temperature range of 1 – 65°C, organisms generally have a smaller temperature range in which they will grow optimally (Lawrie & Ledward, 2006c).

The moisture accessibility within meat is also key in determining microbial growth on meat. A reduced a_w, (water activity: in this context the relative water of a solution is the proportion

of its vapour pressure to that of pure water at the same temperature, which is inversely relative to the existing number of solute molecules), typically results in less bacterial- growth on meat surfaces. In prepacked meat with a small O_2 tension, microorganism growth might be restricted even at an increased a_w, due to the interrelation between a_w and O_2 accessibility (Lawrie & Ledward, 2006c).

The post-mortem pH_u of meat will have a meaningful effect on the microbial growth as the pH_u determines the level of endurance thereto. Bacterial growth is known to reach its peak at pH 7, whereas growth will be weaker at pH_u values of 4 or above 9. However, the optimum pH level of microbial growth is also affected by the synchronized action of all the factors influencing microbial growth (Lawrie & Ledward, 2006c).

With the performance of hot-deboning, Sheridan and Sheriton (1982) suggested that hot-deboned beef might have intrinsic characteristics which encourage microbial growth. These include an increased initial temperature, as well as cuts with a higher exterior a_w. However, Sheridan and Sheriton (1982) concluded that no loss of shelf-life was experienced in hot-deboned vacuum packed beef stored at normal refrigeration temperatures.

2.5.4 Microbial parameters considered by the South African ostrich industry

Actions during the progression of slaughtering and carcass dressing leads to the cut meat exterior being susceptible to contamination by Gram-negative as well as Gram-positive bacteria, yeasts and moulds. The animal's surroundings pre-slaughter might be one of the sources of contamination, as well as the animal's gastro-intestinal tract (GIT). The post-slaughter environment which influence the carcasses and meat cuts, might also be a source of contamination (Kourtsoumanis & Sofos, 2004).

In an effort to ensure proper and continued functioning of a microbial control system, the prevalence and intensity of occurrence of bacteria must, however, be known to perform as indicators. With emphasis on indicator organisms (defined as groups or clusters of bacteria which might reveal malfunctions or insufficiencies of processes created and applied for regulating pathogens), microbial observation is possible. Both total viable counts (TVC) of bacteria, and *Enterobacteriaceae* populations are implemented as indicator organisms for the microbial quality of ostrich meat. Pathogenic microorganisms which are indicative of the microbial safety of ostrich meat, include *Salmonella* spp. and *Listeria monocytogenes* (Kourtsoumanis & Sofos, 2004; Department of Agriculture, Forestry and Fisheries, 2010).

2.5.4.1 Enterobacteriaceae

Classes within the large *Enterobacteriaceae* family vary from those that are damaging to those that are risk-free, and is mainly found in the gut (GIT) flora of animals. This bacteria is characterised as Gram-negative, with a rod shape whilst being a facultative anaerobic bacteria (Magwedere *et al*., 2013). An ideal environment for the growth of this bacteria (dependant on the type of bacteria within this family), can cause food spoilage and lead to off odours (Ercolini

et al., 2008). Magwedere *et al*. (2013) noted that environmental hygiene levels can be reflected in *Enterobacteriaceae* counts, which can also be used as an indication of GMP.

The prevalence of *Enterobacteriaceae* in meat processing plants have mostly been found on surfaces where meat is handled. Stiles & Lai-King (1981) found that surfaces which do not have interaction with meat, were free of *Enterobacteriaceae*. Nonetheless, surfaces which had contact with meat during operations and processing, progressed to have a high *Enterobacteriaceae* load. This high bacterial load on surfaces consequently serves as source of *Enterobacteriaceae* contamination for other meat that comes into contact with the surfaces (before sanitation is performed). The exact point at which the contamination with *Enterobacteriaceae* becomes too high, is not easily defined (Stiles & Lai-King, 1981).

Aerobic plate counts (APC) of hot-deboned ostrich meat, also serve as a microbial quality parameter. Aerobic plate counts (APC) for two hot-deboned ostrich muscles *(Muscularis gastrocnemius, pars interna* and *Muscularis iliofibularis*), showed results within the accepted range (100 000 cfu/ cm^2 the maximum accepted value) determined by the South African standards for microbiological monitoring of meat. *Enterobacteriaceae* counts from the same two hot- deboned ostrich muscles, were also compliant with the South African standards for microbiological monitoring of meat (Botha *et al*., 2006). According to these standards for carcasses and meat cuts of ratites (ostriches), 35 samples have to be studied for *Enterobacteriaceae* analysis. The prescribed analysis method (ISO 21528-2) stipulated the maximum accepted *Enterobacteriaceae* range for export meat as 316 cfu/ cm^2 (Department of Agriculture, Forestry & Fisheries, 2010).

2.5.4.2 Salmonella spp.

Globally, the primary zoonotic agent is recognised as *Salmonella*, supporting the relative widespread knowledge of foodstuffs, feed and livestock contaminated with *Salmonella* in contrast with other zoonotic agents. *Salmonella* can be found in virtually all animals, including birds. This bacteria's aptness for adaption is consequently seen in the variety of environments in which *Salmonella* is found to act as zoonotic agent for humans. The importance of preventing *Salmonella* contamination is yet again confirmed through the fact that it is easily transmitted through foodstuffs and feed traded globally. Consequently, *Salmonella* has been identified as a subject of global trade, and as a result, involves the World Trade Organisation (WTO). The WTO has set universal regulations regarding analysing foodstuff and feed to lessen technical trade difficulties (Nielsen, 2004). According to the WTO, basic regulations in terms of food safety is included in the Sanitary and Phytosanitary Measures Agreement (SPS) of which South Africa is a member. The SPS states that is acceptable for countries to set their own regulation standards, but that it should be grounded on science. Requirements for inspection, regulation and approval measures, are also provided by SPS (World Trade Organisation, 2017). In the ostrich industry, this is especially of significance, since 90% of ostrich prime cuts produced in South Africa, are exported to the EU (National Agricultural

Marketing Council, 2003).

The number of contaminated carcasses at the end of the slaughter-line and the number of live animals carrying *Salmonella* spp. in their faeces are strongly correlated. Live animals transmitting *Salmonella* are three to four times more likely to result in a positive carcass than *Salmonella*-free animals. About 70% of carcasses contaminated with *Salmonella* is caused by the animals themselves being carriers. The remaining 30% of contaminated carcasses are caused by other animals being carriers (cross-contamination). The key risk factors for carcass contamination with *Salmonella*, as well as *Enterobacteriaceae*, is unsuitable procedures during evisceration and unsatisfactorily cleaned machines. Furthermore, insofar contamination with *Salmonella*, hygienic conditions of human carriers, walls, floors and ceilings within the slaughter-line is important to a lesser extent, and more-so carcass-specific with regards to the animals slaughtered on the implicated day (Berends *et al*., 1997).

In describing the characteristics of *Salmonella*, it forms part of the *Enterobacteriaceae* family alongside *Escherichia coli*, *Yersinia* and *Shigella* (Nielsen, 2004). Furthermore, it is a facultative anaerobic microorganism, whilst being non-spore forming and having a rod shape. The most common sources where *Salmonella* is populated, amongst others, are soil and water. Common foodborne foodstuffs that *Salmonella* has been related to however, include meat, poultry, eggs and dairy products (Ohtsuka *et al*., 2005).

As mentioned earlier, temperature is one of the most important factors which influence the growth of microorganisms (Lawrie & Ledward, 2006c). According to Ercolini *et al*. (2008), the temperature range at which *Salmonella* grows, varies from 5 - 47°C, where the ideal temperature for optimum growth is 35°C. However, the inhibition of *Salmonella* can be performed at exposure to 75°C for 10 min. *Salmonella* can grow at pH values of 4 to 9, with an optimal growth range between 6.5 and 7.5 (Ercolini *et al*., 2008).

As temperature is a major factor influencing the growth of *Salmonella*, growth could be amplified with the application of hot-deboning. Hot-deboning is performed on animals with a body temperature very near to the *in vivo* temperature of 37°C (Lawrie & Ledward, 2006b), which is very close to the optimum growth temperature of *Salmonella* at 35°C (Ercolini *et al*, 2008). Botha *et al.* (2006) found on hot deboned ostrich muscles that *Escherichia coli* counts were within the accepted range established by the South African Standards for the microbiological monitoring of meat for refrigerated export. Corresponding with these standards for carcasses and meat cuts of ratites (ostriches), the *E. coli* counts were within the prescribed maximum range of 10 cfu/cm^2 (of at least 35 individual samples analysed) (Department of Agriculture, Forestry and Fisheries, 2010). These results were found on two hot deboned ostrich muscles, namely the *Muscularis gastrocnemius, pars interna* and the *Muscularis iliofibularis* (Botha *et al*., 2006).

2.5.4.3 Listeria monocytogenes

This bacteria is characterised as being short, Gram-positive, rods. The combined term used to refer to the human illness caused by *Listeria monocytogenes*, is listeriosis of which the occurrence is mostly found after the intake of pre-cooked or ready-to-eat foods. The occurrence of listeriosis in meat animals however, is ascribed to the presence of listeriae in the animals' environment. Encephalitis, septicaemia and placentitis are the most frequently incurred forms of animal listeriosis. Influencing factors to animal listeriosis include: overpopulation; the introduction of new (contaminated) animals; abrupt alterations in diet; stress; and loss of teeth. Furthermore, the prevalence of healthy domestic and wild avian species carrying *Listeria monocytogenes* are known to be greater than in those of mammals. A possible explanation for this could be the pecking of contaminated materials such as soil and dirt (Brunic & Avery, 2004).

In South Africa, laboratory-confirmed listeriosis cases ranged between 60 – 80 cases per year on average prior to 2017 (Department of Health, 2018). However, an increased number of laboratory-confirmed listeriosis cases occurred in July 2017 with a listeriosis outbreak established in December 2017. Thereafter, ready-to-eat processed meat were found to be the source of the outbreak followed by a product recall which begun on 4 March 2018. Since the product recall, the number of new cases each week has decreased. Nonetheless, some of the involved products might not have been confiscated from consumer's homes or retail (Department of Health, 2018). Moreover, the incubation period of listeriosis can be up to 90 days, while cross-contamination can take place in the home and at retail (World Health Organization, 2018).

The widespread incidence of this pathogen, is especially due to its ever-presence in the environment in both poultry and red meat abattoirs. Considering that *Listeria monocytogenes* can stick to glass, rubber, polypropylene as well as stainless steel, the bacteria can inhabit walls, floor surfaces and equipment in processing plants. Due to this contamination of processing plants, meat products are often also contaminated. Apart from the regular contamination of meat surfaces, *Listeria* is sometimes found within the inner muscle tissue indicating possible pre-slaughter contamination. The growth of this pathogen is very dependent on temperature and pH. The growth constraint for *Listeria monocytogenes* is accordingly -0.4°C and pH 4.39 (Brunic & Avery, 2004). The O_2 accessibility, food type and inconsistency within the *Listeria* strain, affects the growth of this microorganism. Seemingly, *Listeria monocytogenes* has an increased ability to be unaffected by heat in comparison with other non-spore-forming foodborne pathogens (Brunic & Avery, 2004).

2.6 Conclusions

Ostrich farming in South Arica is not a novel concept and the South African ostrich industry is well-developed with an established export market (De Mosenthal & Harting, 1964). Globally, the South African ostrich industry accounts for approximately 70% of the ostrich marketplace

(Duminy, 2016). Consequently, South Africa has undoubtedly been considered the world leader in both ostrich farming and being the primary value-adding party to ostrich processing, including slaughtering, processing of meat and skin dyeing. With about 90% of ostrich prime cuts exported to the European Union (EU) in the past (National Agricultural Marketing Council, 2003), ostrich meat accounts for the largest amount of meat exported from South Africa, both in terms of quantity as well as its financial significance (Brand *et al*., 2011).

Cold-deboning (conventional excising technique) is currently used by the South African ostrich industry whereby ostrich carcasses are refrigerated for 24 h post-mortem (0 - 4°C) before deboning is performed (Hoffman *et al*., 2007). It is signified as cold-deboning because the carcasses are excised once the meat achieved the chiller's temperature. Consequently, extensive space for coolers are required, expending a great amount of energy (Waylan & Kastner, 2004).

Hot-deboning is a proposed alternative excising method for the South African ostrich industry where carcasses are deboned before refrigeration (2 - 4 h post-mortem). Accordingly, it holds several potential advantages for the ostrich industry of South Africa since the chilling of carcasses can completely be ruled out (Hoffman *et al.*, 2007). Hot-deboning was developed to decrease the use of energy and lessen the refrigeration space required, also greatly improving the turn-around time (thus more carcasses can be deboned and processed in less time) (Department of Agriculture, 2007). In most red meat species, hot-deboning is typically performed on pre-rigor muscles, however, Botha *et al*. (2006) indicated that ostrich muscles usually enter *rigor mortis* within 45 min post-mortem. Botha *et al*. (2006) further showed that this early entry into *rigor mortis* had a minor influence on ostrich meat quality. The biggest benefit in this regard, is the fact that hot-deboning is more economical in terms of processing time, therefore allowing for a quicker turn-around time as well as the refrigeration space saved since chilling of carcasses for 24 h (refer to Fig. 2.1) can be eliminated (Hoffman *et al*., 2007).

Anatomically, ostrich muscles are in accordance with other birds. The percentage prime cuts with a high retail value, however comprise of 80 – 90% of the ostrich meat (other animal species in the region of 45%) (Mellett, 1985). In terms of physical meat quality of hot-deboned ostrich cuts, the only known muscles in this regard are both the *Muscularis gastrocnemius, pars interna* and the *Muscularis iliofibularis*, which are the largest ostrich muscles (e.g. commercial cuts) (Botha *et al*., 2006). Especially with regards to ostrich meat, and the fact that almost all of the cuts are considered "prime cuts," the colour and weep loss of the vacuum packed cuts over an extended period are of great importance. Colour and weep loss are notably emphasised for ostrich meat, as ostrich meat is known to be darker in colour and weep loss is considerable in vacuum packed ostrich meat; causing both of these attributes to negatively affect consumer perception towards ostrich meat quality. Also, the microbial quality and safety of vacuum packed hot-deboned muscles over an extended period (as it is common practice in the ostrich industry to keep the refrigerated vacuum packed muscles for a number

of days until it reaches the export market), should be known (Botha *et al*., 2006).

Based upon previous research, it is expected that within the above-mentioned analysis, the advantageous (energy saving without a decrease in quality) effect of hot-deboning vs. cold-deboning of ostrich meat will become more apparent. Consequently, the research hypothesis states that the hot-deboning of ostrich meat will not have a negative influence on the meat quality or microbial quality and safety of the meat.

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CHAPTER 3

Muscle yields and physical meat quality characteristics of hot- vs. cold-deboned ostrich (*Struthio camelus*) meat

Abstract

Hot-deboning as an alternative excising method for the South African ostrich industry was investigated. Fifteen ostriches were used for the study with the muscles hot-deboned (within 90 min post-mortem) from the left leg and cold-deboned (<4°C, 24 h post-mortem) from the right leg. Half of the 16 hot-deboned muscles' weights were heavier ($p \leq 0.05$) than those cold-deboned. Five ostrich muscles: fan fillet (*M. iliofibularis*); rump steak (*M. iliotibialis lateralis*); big drum (*M. gastrocnemius, pars interna*); moon steak (*M. femorotibialis medius*) and triangle steak (*M. flexor cruris lateralis)* were used to establish physical meat quality characteristics at day 3 post-mortem. Hot-deboning did not have an effect on any of the physical meat quality parameters, but differences amongst muscles were found ($p \leq 0.05$). The varying pH_u values between muscles ($p = 0.01$) were still within the expected range for ostrich meat with the big drum having the highest pH_u (5.95 ± 0.16) linking with its low drip loss percentage (0.90% ± 0.30). The fan fillet had a more red (a* = 13.43 ± 1.21), saturated (Chroma = 16.58 ± 1.61) colour whereas the big drum was more blue (b* = 9.68 ± 1.52), with the least colour intensity (hue angle = 35.64 ± 3.79). Concerning Warner-Bratzler shear force values, the fan fillet was the most tender (35.34 N ± 8.26) in contrast with the moon steak (72.23 N ± 15.81) which can be linked to the latter's high cooking loss percentage (37.05% ± 1.90). Hot-deboning which provides several economic advantages for the South African ostrich industry can be considered.

Keywords: Hot-deboning, Cold-deboning, Ostrich, Muscle yields, Physical meat quality

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3.1 Introduction

South Africa has undeniably been considered the world leader in the ostrich marketplace in the past with approximately 90% of ostrich prime cuts, mainly consisting of whole muscles, principally being exported to the European Union (EU). The South African ostrich industry is known for being the first to add value to the ostrich production market, not only for ostrich farming, but also in the slaughtering, processing of meat and skin dyeing. These ostrich related products such as feathers and leather are also primarily exported from South Africa (National Agricultural Marketing Council, 2003; Department of Agriculture, Forestry & Fisheries, 2017). A ban is currently placed on the export of ostrich meat due to a "Not fit for the purpose laboratory competency in the National Chemical Control Program implementation and maintenance by the Department of Forestry and Fisheries (DAFF)" finding.

At present, cold-deboning is used as excising method in the ostrich industry of South Africa. The conditions at which cold-deboning is performed include the refrigeration of ostrich carcasses for 24 h post-mortem (0 – 4°C) prior to deboning (Hoffman *et al*., 2007). Cold-deboning can thus be referred to as the conventional excising method in the ostrich meat industry, and is known to be performed after *rigor mortis* is complete. Since excision is performed once the carcasses have achieved the chiller's temperature, it is signified as cold-deboning (Waylan & Kastner, 2004).

Hot-deboning, where carcasses are excised prior to refrigeration, is an alternative deboning method and is defined as the practice where lean meat and fat are detached from carcasses before a big drop in body temperature occurs (Waylan & Kastner, 2004). With excision performed 2 - 4 h post-mortem as opposed to 24 h post-mortem, hot-deboning is an alternative deboning method which provide several potential advantages for the South African ostrich industry, particularly as the ostrich typically starts going into *rigor mortis* within 45 min post-mortem (Hoffman *et al.,* 2007). The latter limits the risk of ostrich muscles developing cold-shortening which is commonly seen when pre-rigor meat is hot-deboned. The biggest proposed advantage of hot-deboning is the economic benefit of reduced energy costs due to less refrigeration space required (Farouk *et al*., 2009). It has been proposed that hot-deboning in the instance of beef, can lower the amount of refrigeration energy by up to 50%, whilst refrigeration space can be reduced with up to 80% (Waylan & Kastner, 2004). With the current ban placed on the export of ostrich meat, it is especially important to investigate ways in which production costs can be lowered for the ostrich industry to survive the difficult economic environment. Moreover, hot-deboning can be used as a technique to remove muscles very early on post-mortem to possibly enhance the functional properties of each muscle according to its intrinsic characteristics (Waylan & Kastner, 2004; Farouk *et al*., 2009).

The most important determining factor in the consumer's selection process when choosing meat, is often said to be the colour of fresh meat and meat products, where consumers judge the freshness of the meat upon colour (Jeremiah *et al*., 1972; Fletcher, 2002;

Cornforth & Jayasingh, 2004; Carpenter *et al*., 2011). It is important to note that although 30 min is usually allowed for blooming time (to reach the bright red colour), in the case of ostrich meat it was found that the optimum blooming time is 60 min (Leygonie, 2011). Ostrich meat is known to be darker in colour when it reaches the export market (after up to 42 d) due to its vacuum packed nature as well with its ultimate pH being in the intermediate range (Sales & Mellet, 1996). This negatively influences consumers purchasing behaviour which indicates that possible colour differences between hot- and cold-deboned ostrich meat at day three post- mortem, is of importance.

Meat texture and tenderness is the most important factors considered by consumers in determining eating quality (Tornberg, 1996; Lawrie & Ledward, 2006b). The fan fillet (*M. iliofibularis)* and big drum (*M. gastrocnemius, pars interna*) are known to have the highest shear force values between 24 h and three d post-mortem (Hoffman *et al.*, 2007), which signifies the period prior to post-mortem ageing which will be discussed in Chapter 4. Thus, the impact of hot-deboning on meat tenderness at day three post-mortem is also of particular interest for other economically important ostrich muscles.

This study was firstly carried out to determine whether there is a significant difference in the muscle yields between sixteen hot- vs. cold-deboned ostrich muscles. Secondly, to establish physical meat quality characteristics between five hot- and cold-deboned vacuum packed ostrich muscles at day three post-mortem. Five representative ostrich muscles (with a high commercial value) were used, including: fan fillet (*M. iliofibularis*); rump steak (*M. iliotibialis lateralis*); big drum (*M. gastrocnemius, pars interna*); moon steak (*M. femorotibialis medius*); and triangle steak (*M. flexor cruris lateralis*). Although Botha *et al*. (2006; 2007) studied the hot-deboning of ostrich meat, this research was only conducted on two commercial ostrich cuts/muscles namely the fan fillet (*M. iliofibularis*) and big drum (*M. gastrocnemius, pars interna*). This study focused on defining the physical meat quality characteristics (day three post-mortem) of the aforementioned five muscles to act as reference in describing the physical meat quality throughout a 28 d post-mortem ageing period (discussed in Chapter 4).

3.2 Materials and methods

3.2.1 Ostriches and muscle samples

A summary of the experimental procedure for the collection of ostrich muscle samples are presented in Fig. 3.1.

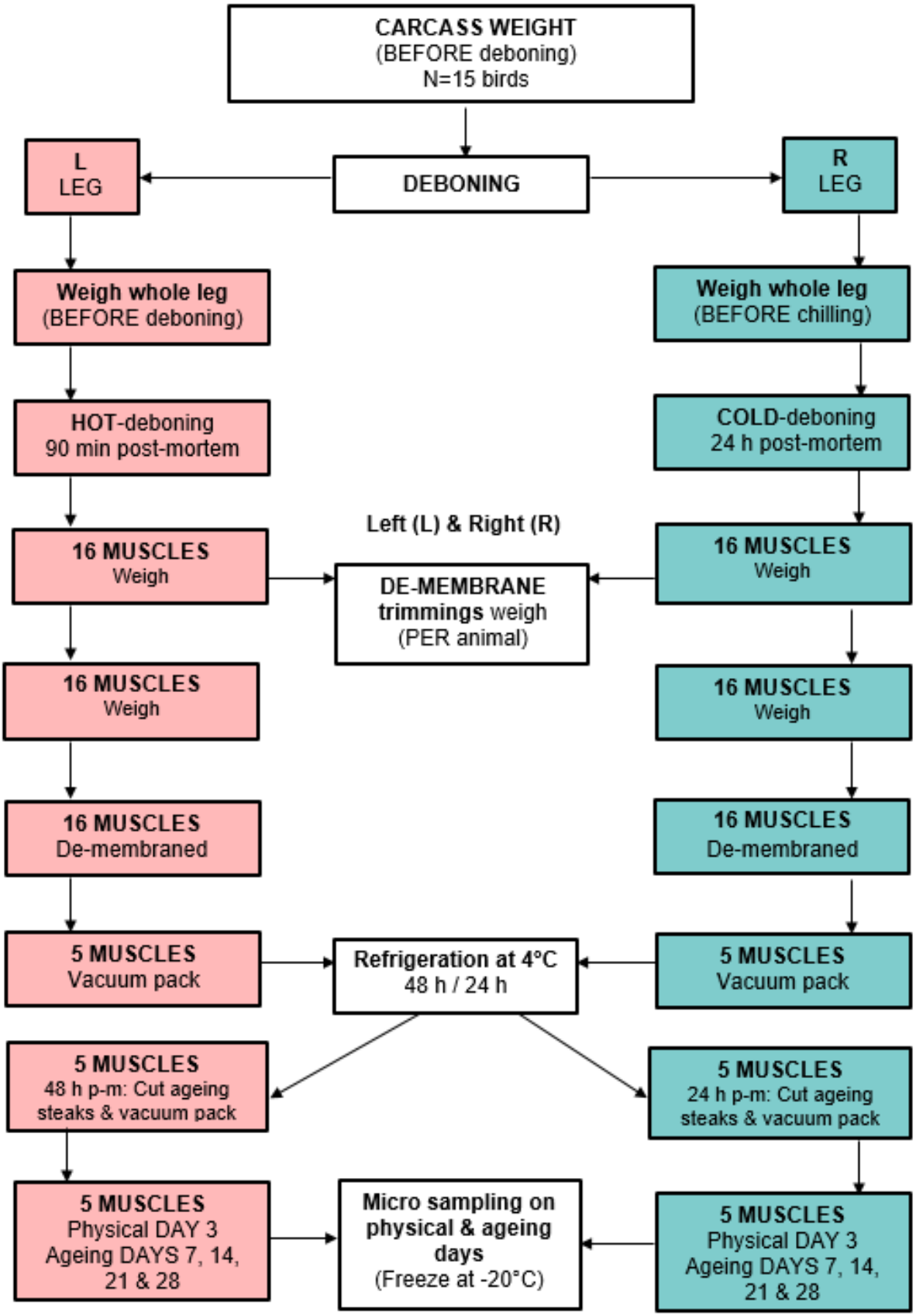

Figure 3.1 Summary of the experimental procedure for the collection of ostrich muscle samples.

Fifteen rested (approximately 24 h of lairage) ostriches (*Struthio camelus*), 10 months of age from the same farm (Oudtshoorn Research Farm of the Western Cape Department of Agriculture) were slaughtered as described by Hoffman (2012). The slaughter took place on the same day at the same EU approved abattoir in Oudtshoorn, South Africa, during August of 2017. The 15 ostriches were made up of three genotypes, namely six South African Black ostriches (SAB), three Zimbabwean Blue ostriches (ZB) and six Kenyan Red ostriches (KR). The dead weight of each ostrich was recorded prior to slaughter ("dirty" area of the abattoir), where-after the carcass weights (kg) were recorded subsequent to skinning and carcass dressing as soon as the carcasses entered the deboning area ("clean" area of the abattoir). After the death of the birds, the plucking of feathers, skinning as well as evisceration were completed between 60 and 90 min post-mortem before carcasses arrived at the deboning hall. Hot-deboning of the left leg from the carcasses were subsequently performed at approximately 90 min post-mortem, but definitely completed within 2 h post-mortem. Thereafter the removal of membranes and vacuum packaging of muscles followed, and were completed within 3 h post-mortem.

Each of the left legs were thus excised at approximately 90 min post-mortem (hot-deboning) where-after the leg weights (18.58 kg ± 2.04) were recorded, prior to the excision of the 16 individual muscles. After each of the 16 individual muscles (Table 3.1) were excised, each muscle was weighed individually before membranes (connective tissue surrounding the outer layer of a muscle defined as epimysium), were removed (Davies, 2004). The 16 individual de-membraned muscles were weighed again and the "clean muscle" yield calculated. The combined weight of goulash meat (weight of cubes/portions of meat trimmings suitable to be sold as goulash stew meat of all 16 individual muscles), meat trimmings and membrane trimmings (of all 16 individual muscles) were recorded per animal, respectively. Of the 16 individual muscles, the fan fillet (1.78 kg ± 0.18); rump steak (1.38 kg ± 0.27); big drum (1.07 kg ± 0.18); moon steak (0.88 kg ± 0.12) and triangle steak (0.46 kg ± 0.06) were used for further analyses and vacuum packed accordingly. The five vacuum packed muscles were refrigerated at < 4°C for 24 h post-mortem at the abattoir. The carcasses with the remaining right legs were refrigerated in the same refrigerator for 24 h post-mortem.

After 24 h post-mortem refrigeration at < 4°C, each of the 15 carcasses' right legs (17.35 kg ± 2.01) were excised (cold-deboning). Before excision of the 16 individual muscles, each of the right legs were weighed and the chiller weight loss calculated. After each of the 16 individual muscles were excised, each muscle was weighed individually before and after the epimysium was removed. Similarly, the combined weight of goulash meat; meat trimmings and membrane trimmings (of all 16 individual muscles) were recorded per animal respectively. Of the 16 individual muscles, the same five muscles: the fan fillet (1.70 kg ± 0.16); rump steak (1.29 kg ± 0.17); big drum (0.98 kg ± 0.28); moon steak (0.90 kg ± 0.13) and triangle steak (0.39 kg ± 0.06) were used for further analyses and vacuum packed accordingly. The five hot- and cold-deboned vacuum packed muscles (of all 15 ostriches) were transported from Oudtshoorn

to the laboratories at Stellenbosch University (395 km) in a refrigerated vehicle/ cool truck (< 4°C). The following day each of the hot- and cold-deboned five muscles were cut perpendicular to the longitudinal axis into 1.5 – 2.0 cm steaks for different ageing time periods according to the varying sizes of each muscle. Before the conduction of physical analysis on day 3 steaks, a representative 25 g sample of each muscle was cut, vacuum packaged and stored at -20°C for microbiological analysis. The remaining steaks at day 3 post-mortem were used to perform physical analyses after it was randomly assigned to each ageing time point.

Table 3.1 Description of 16 ostrich muscles' commercial names with scientific names

Commercial name	Scientific name
Tenderloin	*M. iliotibialis cranialis*
Oyster fillet	*M. iliofemoralis externus*
Small drum	*M. gastrocnemius, pars intermedia*
Tournedos	*M. ambiens*
Rump steak	*M. iliotbialis lateralis*
Fan fillet	*M. iliofibularis*
Triangle steak	*M. flexor cruris lateralis*
Eye fillet	*M. iliofemoralis*
Small steak	*M. flexor cruris medialis*
Tender steak	*M. pubo-ischio-femoralis*
Minute steak	*M. femorotibialis externus*
Long fillet	*M. obturatorius medialis*
Moon steak	*M. femorotibialis medius*
Flat drum	*M. gastrocnemius, pars externa*
Big drum	*M. gastocnemius, pars interna*
Drum steak	*M. fibularis longus*

3.2.2 Physical analysis

Muscle temperature (°C) and pH were recorded in the centre of each muscle steak with the use of a portable calibrated (standard buffers of pH 4.0 and 7.0) Crison pH25 pH meter (Alella, Barcelona, Spain) equipped with a pH and temperature probe. Readings were recorded at room temperature and the probe was rinsed with distilled water between every measurement.

The surface colour of the raw muscle steaks were determined using a Colour-guide 45°/0° colorimeter (BYK-Gardner, GmbH, Gerestried, Germany) and recorded according to the method described by Leygonie (2011). Prior to colour measurements, the 1.5 – 2.0 cm thick muscle steaks were allowed to bloom for 60 min at room temperature. Colour measurements were expressed by L* (lightness), a* (green-red) and b* (blue-yellow) coordinates of the CIELab colorimetric space (Boakye & Mittal, 1996; Konica Minolta, 2007)

and were recorded for each sample through five measurements at randomly selected positions. Hue angle (h_{ab}) for colour definition and Chroma (C*) for colour intensity were calculated using the CIE a * and b* values as follows:

Hue angle (h_{ab}) = $tan^{-1}\left(\frac{b*}{a*}\right)$

Chroma (C*) = $\sqrt{(a^*)^2 + (b^*)^2}$

Moisture loss was measured through drip loss and cooking loss percentages. The drip loss percentage in muscle samples were determined by cutting a sample from day 3 steaks and weighing it to establish the initial (reference) weight. Subsequently, samples were placed in inflated plastic bags (without the meat touching the sides of the bag) at ~2°C for 24 h. Thereafter, samples were blotted dry with an absorbent paper towel before the "after" weight was recorded. The drip loss was consequently expressed as a percentage of the initial sample weight:

Drip loss % = $\frac{\text{weight}_{\text{before}} - \text{weight}_{\text{after}}}{\text{weight}_{\text{before}}}$ X 100

The cooking loss percentage of each muscle sample was determined by using a preheated water bath (80°C) for 60 min (Honikel, 1998). After 60 min of cooking, cooked meat samples were removed from the water bath, where-after excess liquid which accumulated during the cooking process was drained after opening the seal from the vacuum bag. The samples were subsequently allowed to cool overnight and placed in a commercial refrigerator (~2°C). The mass of the cooled muscle samples were recorded the following day after each sample was blotted dry with absorbent paper towel. Accordingly, cooking loss was expressed as a percentage of the initial sample weight:

Cooking loss % = $\frac{\text{weight}_{\text{before}} - \text{weight}_{\text{after}}}{\text{weight}_{\text{before}}}$ X 100

The Warner-Bratzler shear force (WBSF) test was used to establish meat tenderness after the mass of the cooking loss samples was recorded (Honikel, 1998; Wheeler *et al*., 2005). An Instron Universal Testing Machine (Instron UTM, Model 2519-107) fitted with a Warner-Bratzler blade was used to measure the force in Newton (N) necessary to shear a block of meat perpendicular to its longitudinal axis. With the muscle fibres parallel to the longitudinal axis, a minimum of six blocks were cut from the cooking loss steaks to a size of 1 x 1 x 2 cm each. The 1 mm Warner-Bratzler blade had a cutting edge with a semi-circular shape (0.508 mm radius), whilst the opening of the blade was triangular. The Instron operated with a crosshead speed of 200 mm/min and had a load cell of 2 kN. The WBSF value of each sample was calculated as the mean of six Warner-Bratzler readings (North & Hoffman, 2015).

3.2.3 Statistical analyses

Statistica 64 version 13's (2015) VEPAC module was used to perform the statistical analyses (STATISTICA, 2011). Hot- vs. cold-deboning and with/without membranes were used as fixed effects in a mixed model repeated measures of analysis of variance (ANOVA) for the muscle yield results. For the physical meat quality characteristics, however, hot- vs. cold-deboning and muscle type were used as fixed effects in a mixed model repeated measures of ANOVA. The Fisher LSD (least significant differences) test was used for the multiple comparison test. It can be noted that animal was included as random effect while possible outliers were identified using normal probability plots. Significant influences were described as Means and Standard Deviation (SD). A significance level of 5% ($p \leq 0.05$) was used as guideline for detecting possible significant effects.

3.3 Results

3.3.1 Meat processing potential

The mean hot-deboned ostrich leg was 18.58 kg ± 2.04 in comparison with the cold-deboned 17.35 kg ± 2.01. Thus, the mean hot-deboned ostrich leg weight was 1.23 kg (6.62%) heavier than the mean cold-deboned leg weight ($p = 0.000$). An evaluation of the weights of the different muscles before and after the removal of membranes, showed that as expected, the muscles without membranes were lighter (Table 3.2). However, some muscles showed significant interactions when comparing the four different treatment groupings and these will be discussed in more detail.

Table 3.2 Hot- vs. cold-deboned ostrich muscle weights (g) with and without membranes as per muscle type and treatment (Mean ± SD)

	With Membranes		Without membranes	
Muscle Type	**Hot-deboned**	**Cold-deboned**	**Hot-deboned**	**Cold-deboned**
Tenderloin	658.73^{a} ± 0.074	601.93^{b} ± 0.073	566.47^{c} ± 0.660	503.87^{d} ± 0.069
Oyster fillet	646.07^{a} ± 0.074	581.27^{c} ± 0.080	592.07^{b} ± 0.074	535.80^{d} ± 0.074
Small drum	459.53^{a} ± 0.047	427.33^{b} ± 0.065	339.00^{c} ± 0.053	306.27^{d} ± 0.051
Tournedos	286.07^{a} ± 0.037	279.07^{a} ± 0.034	244.13^{b} ± 0.032	231.40^{c} ± 0.028
Rump steak	1597.40^{a} ± 0.218	1513.07^{a} ± 0.189	1448.73^{a} ± 0.171	1289.07^{b} ± 0.169
Fan fillet	1923.47^{a} ± 0.188	1840.60^{b} ± 0.171	1775.53^{c} ± 0.181	1699.93^{d} ± 0.159
Triangle steak	522.40^{a} ± 0.652	462.13^{b} ± 0.064	460.53^{c} ± 0.064	387.73^{d} ± 0.063
Eye fillet	532.86^{a} ± 0.091	527.00^{a} ± 0.083	469.13^{b} ± 0.079	464.47^{b} ± 0.088
Small steak	309.47^{a} ± 0.039	317.20^{a} ± 0.037	276.80^{b} ± 0.038	263.53^{b} ± 0.037
Tender steak	432.93^{a} ± 0.069	440.33^{a} ± 0.075	332.93^{b} ± 0.055	344.53^{b} ± 0.063
Minute steak	224.47^{a} ± 0.052	221.07^{a} ± 0.039	182.37^{b} ± 0.049	178.53^{b} ± 0.033
Moon steak	1014.67^{a} ± 0.143	1027.93^{a} ± 0.130	881.93^{b} ± 0.116	901.533^{b} ± 0.128
Long fillet	763.73^{a} ± 0.088	707.40^{b} ± 0.087	632.33^{bc} ± 0.08	574.67^{c} ± 0.087
Flat drum	951.400^{a} ± 0.141	929.33^{a} ± 0.152	797.87^{b} ± 0.137	728.200^{c} ± 0.123
Big drum	1302.47^{a} ± 0.120	1286.07^{a} ± 0.194	1074.67^{b} ± 0.183	1044.47^{b} ± 0.171
Drum steak	719.43^{a} ± 0.139	660.60^{ab} ± 0.110	586.27^{bc} ± 0.115	521.00^{c} ± 0.122
Total	12358.85^{a} ± 1.379	11822.33^{b} ± 1.283	10660.77^{c} ± 1.218	9975.00^{d} ± 1.156

$^{a-d}$ Means in the same row with different superscripts differed significantly between muscle weights with and without membranes ($p \leq 0.05$).

Neither the tenderloin ($p = 0.256$), oyster fillet ($p = 0.202$) or small drum ($p = 0.922$) had significant interaction between treatments and muscle weights with or without membranes. However, there was significant differences regarding hot- vs. cold-deboned and with vs. without membranes of the aforementioned three muscles (Table 3.2). Although the hot- deboned tenderloin, oyster fillet and small drum had significantly higher mean muscle weights before the membranes were removed, both the hot- and cold-deboned tenderloin, oyster fillet and small drum mean muscle weights were, as expected, significantly lower after the membranes were removed. A significant interaction was seen between treatments and muscle weights with or without membranes in the tournedos ($p = 0.015$). Although the mean muscle weights for the tournedos with membranes did not differ significantly between hot- and cold- deboned, it did however differ between treatments after the membranes were removed (Table 3.2). The rump steak showed no interaction between treatments and muscle weights with or without membranes ($p = 0.094$). Furthermore, the rump steak was the only muscle which did not show significant differences between mean muscle weights for hot- and cold-deboning whatsoever,

however, the mean cold-deboned rump steak weight was significantly lower after the membranes were removed (Table 3.2). Although no significant interaction was seen between treatments and muscle weights with or without membranes in the fan fillet and triangle steak, significant differences occurred between hot- vs. cold-deboning and mean muscle weights with and without membranes (fan fillet: $p = 0.268$ and triangle steak: $p = 0.239$; Table 3.2). The fan fillet and triangle steak both had higher mean muscle weights when hot-deboned (with and without membranes), whilst the mean hot- and cold-deboned muscle weights with membranes were significantly higher compared to that without membranes (Table 3.2). No significant interaction was found between the mean muscle weights and treatments for the eye fillet ($p = 0.763$), tender steak ($p = 0.497$) and minute steak ($p = 0.933$) which all showed a non-significant difference between hot- and cold-deboning (Table 3.2). The small steak had an interaction ($p = 0.006$) between treatments and with or without membranes as seen in Table 3.2. As shown in Table 3.2, the aforementioned muscles did, however, have significant differences between the mean muscle weights with and without membranes. Nonetheless, the mean long fillet muscle weights with membranes showed a significant difference between hot- and cold-deboning (Table 3.2). Although there was no interaction between treatments and with or without membranes for the long fillet ($p = 0.935$), the mean muscles weights with and without membranes differed significantly. Moreover, the long fillet mean muscle weights were significantly higher for hot-deboning irrespective of with or without membranes (Table 3.2). No significant differences occurred between hot- and cold-deboning which also had no significant interaction with the mean muscle weights with or without membranes of the moon steak ($p = 0.500$), big drum ($p = 0.409$) or drum steak ($p = 0.195$). These muscles all still significantly differed in the mean muscle weights with and without membranes with the hot-deboned muscles constantly showing higher mean values (Table 3.2). A significant interaction between treatments and muscle weights with or without membranes was seen in the flat drum ($p = 0.027$). The flat drum was also the only muscle to show a significant difference between hot- and cold-deboning in the mean muscle weights with membranes, while the mean muscle weights furthermore showed significant differences between the measurements with and without membranes (Table 3.2). There was no interaction between treatments and with or without membranes ($p = 0.063$) when inspecting the total mean muscle weights of all four groups (Table 3.2). Although there was no interaction, both the mean hot- and cold-deboned groups differed (hot-deboned 536.52 g higher; $p = 0.000$), as well as the groups with and without membranes (hot-deboned 685.77 g higher; $p = 0.000$) (Table 3.2).

A summary of the hot- vs. cold-deboned goulash meat, meat trimmings and membrane trimmings (combined across replications ($n = 15$) and different muscles) are presented in Table 3.3.

Table 3.3 Weights of hot- vs. cold-deboned ostrich goulash meat, meat trimmings and membrane trimmings as per treatment (Mean ± SD)

	Treatment		
	Hot-deboned	**Cold-deboned**	**p-value**
Goulash meat	0.81 ± 0.26	0.96 ± 0.17	0.038
Meat trimmings	2.04 ± 0.50	2.22 ± 0.33	0.339
Membrane trimmings	1.64 ± 0.31	1.79 ± 0.24	0.160

$^{a-b}$ Least square means in the same row with different superscripts differed significantly between treatments ($p \leq 0.05$).

Only the mean goulash meat weights differed between hot- and cold-deboned ($p = 0.038$) with the cold-deboned goulash meat being significantly higher than that of the hot-deboned goulash meat (Table 3.3). Both the mean meat trimming ($p = 0.339$) and membrane trimming ($p = 0.160$) weights did not differ across treatments.

3.3.2 Physical analysis

Results of physical analyses (pH_u, drip loss %, cooking loss%, L*, a*, b*, hue angle, Chroma and WBSF) conducted on the fan fillet, rump steak, big drum, moon steak and triangle steak at day 3 post-mortem are presented in Table 3.4.

Table 3.4: Hot- vs. cold-deboned ostrich muscles pH_u, drip loss %, cooking loss %, L*, a*, b*, hue angle, Chroma and WBSF (N) values as per muscle type and treatment at day 3 post-mortem (Mean ± SD)

Treatment	Parameter measured	Fan fillet	Big drum	Rump steak	Moon steak	Triangle steak
Hot-deboned	pH_u	$5.94^{abc} \pm 0.11$	$6.01^{a} \pm 0.16$	$5.96^{bcd} \pm 0.16$	$5.92^{bcd} \pm 0.11$	$5.83^{d} \pm 0.05$
Cold-deboned		$5.93^{abc} \pm 0.09$	$5.96^{ab} \pm 0.15$	$5.91^{ab} \pm 0.29$	$5.93^{bcd} \pm 0.10$	$5.85^{cd} \pm 0.04$
Hot-deboned	Drip loss %	$1.33^{b} \pm 0.54$	$0.92^{cd} \pm 0.42$	$1.36^{b} \pm 0.83$	$1.34^{b} \pm 0.38$	$1.55^{ab} \pm 0.32$
Cold-deboned		$1.97^{a} \pm 0.96$	$0.88^{d} \pm 0.17$	$1.25^{bc} \pm 0.32$	$1.31^{bc} \pm 0.47$	$1.42^{a} \pm 0.30$
Hot-deboned	Cooking loss %	$35.01^{de} \pm 2.50$	$36.44^{b} \pm 1.74$	$35.91^{bcd} \pm 1.07$	$36.28^{bc} \pm 1.55$	$34.18^{e} \pm 1.35$
Cold-deboned		$35.13^{cde} \pm 1.92$	$36.09^{bcd} \pm 2.38$	$35.46^{bcde} \pm 1.19$	$37.82^{a} \pm 2.24$	$35.13^{cde} \pm 1.82$
Hot-deboned	L*	$30.44^{bc} \pm 2.21$	$29.32^{cde} \pm 1.84$	$28.75^{de} \pm 2.79$	$31.60^{a} \pm 1.64$	$27.19^{fg} \pm 1.74$
Cold-deboned		$30.07^{bcd} \pm 2.01$	$28.15^{ef} \pm 1.70$	$28.95^{de} \pm 1.55$	$31.02^{ab} \pm 2.12$	$26.97^{g} \pm 1.80$
Hot-deboned	a*	$13.58^{a} \pm 1.33$	$12.12^{de} \pm 1.46$	$12.89^{abcd} \pm 1.38$	$12.22^{cde} \pm 1.23$	$13.31^{a} \pm 1.27$
Cold-deboned		$13.28^{ab} \pm 1.08$	$11.90^{e} \pm 1.80$	$12.89^{abc} \pm 1.55$	$12.22^{bcde} \pm 1.44$	$13.40^{a} \pm 1.29$
Hot-deboned	b*	$9.90^{a} \pm 1.55$	$7.66^{c} \pm 1.89$	$8.69^{bc} \pm 2.10$	$8.62^{bc} \pm 2.25$	$9.04^{b} \pm 1.73$
Cold-deboned		$9.45^{ab} \pm 1.49$	$7.72^{c} \pm 1.79$	$9.11^{b} \pm 2.10$	$8.92^{b} \pm 1.87$	$8.88^{b} \pm 1.47$
Hot-deboned	Hue angle	$35.95^{a} \pm 3.68$	$32.07^{b} \pm 6.78$	$33.66^{b} \pm 6.57$	$35.01^{ab} \pm 6.93$	$33.57^{ab} \pm 6.94$
Cold-deboned		$35.30^{ab} \pm 3.90$	$33.04^{b} \pm 7.86$	$35.02^{ab} \pm 7.72$	$36.03^{ab} \pm 6.70$	$33.50^{b} \pm 5.17$
Hot-deboned	Chroma	$16.83^{a} \pm 1.74$	$14.43^{d} \pm 1.69$	$15.64^{bc} \pm 1.80$	$15.06^{cd} \pm 1.31$	$16.15^{abc} \pm 1.48$
Cold-deboned		$16.33^{ab} \pm 1.47$	$14.32^{d} \pm 1.64$	$15.93^{bc} \pm 1.69$	$15.24^{bcd} \pm 1.47$	$16.14^{abc} \pm 1.31$
Hot-deboned	WBSF	$35.51^{d} \pm 8.65$	$52.47^{bc} \pm 13.52$	$56.25^{b} \pm 16.31$	$71.52^{a} \pm 15.51$	$46.93^{c} \pm 13.47$
Cold-deboned		$35.16^{d} \pm 7.87$	$53.94^{bc} \pm 15.65$	$54.44^{bc} \pm 14.48$	$72.93^{a} \pm 16.11$	$49.58^{bc} \pm 14.12$

$^{a-f}$ Least square means in the same row for a specific parameter's measurement with different superscripts differed significantly between muscles ($p \leq 0.05$).

The mean day 3 pH_u values showed no interaction between muscle type and treatment ($p = 0.24$). The investigated muscles did however differ ($p = 0.01$) in comparison as shown in Fig. 3.2, whereas hot- vs. cold-deboning did not differ ($p = 0.85$; Table 3.4).

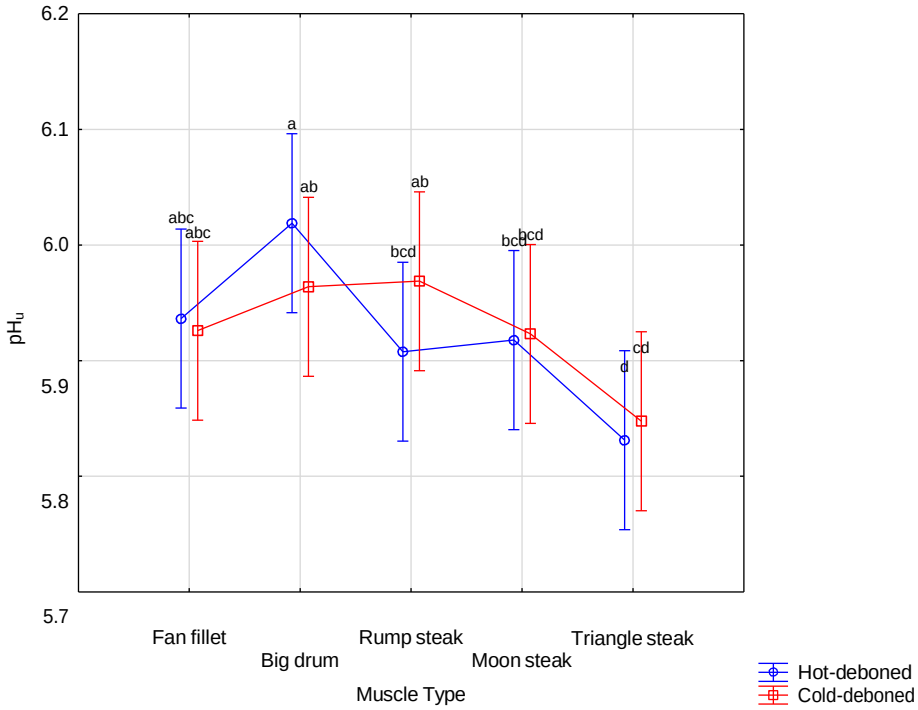

Figure 3.2 The impact of hot- vs. cold-deboning on the pH_u of the ostrich fan fillet; big drum; rump steak; moon steak and triangle steak at day 3 post-mortem ($p \leq 0.05$).

An interaction between muscle type and treatment ($p = 0.03$) was seen in the mean drip loss percentage at day 3 post-mortem. Although there was a significant interaction, hot- vs. cold-deboning did not differ ($p = 0.74$; Table 3.4), and the difference amongst muscles ($p = 0.00$) can be seen in Fig. 3.3. In fact, it was the fan fillet which had the highest drip loss percentage in the cold deboned samples compared to the hot deboned samples whilst the other muscles did not show statistical differences between hot or cold deboning when evaluated on an individual muscle basis even though the cold deboned muscles had a numerically lower percentage drip loss.

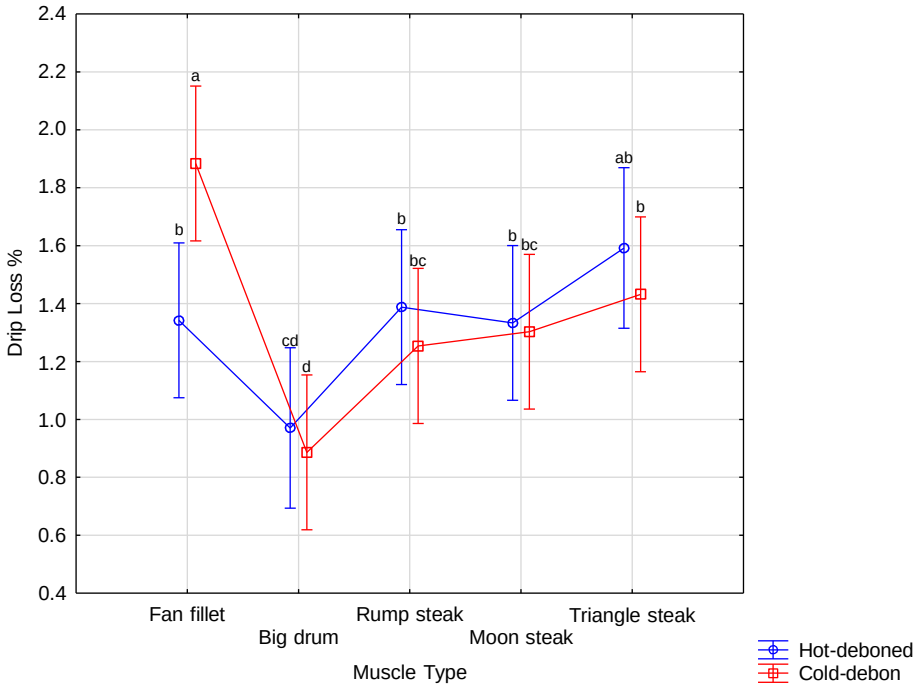

Figure 3.3 The impact of hot- vs. cold-deboning on the drip loss % of the ostrich fan fillet; big drum; rump steak; moon steak and triangle steak at day 3 post-mortem ($p \leq 0.05$).

The mean cooking loss percentage at day 3 post-mortem did not have an interaction between muscle type and hot- vs. cold-deboning ($p = 0.90$). As seen in Table 3.4, the muscles did however differ in comparison ($p = 0.00$) whilst the treatments showed no difference ($p = 0.27$).

No interaction was found between muscle type and treatment in the mean day 3 values for any of the colour parameters: L* ($p = 0.65$); a* ($p = 0.89$); b* ($p = 0.57$); hue angle ($p = 0.73$) and Chroma ($p = 0.66$) values. However, the muscles showed significant differences concerning the aforementioned colour parameters (Table 3.4). This was especially noticeable in the mean L* (Fig. 3.4), a* (Fig. 3.5) and b* (Fig. 3.6) values day 3 post-mortem. In contrast, as seen in Table 3.4, hot- vs. cold-deboning did not significantly differ in relation to any of the colour parameters.

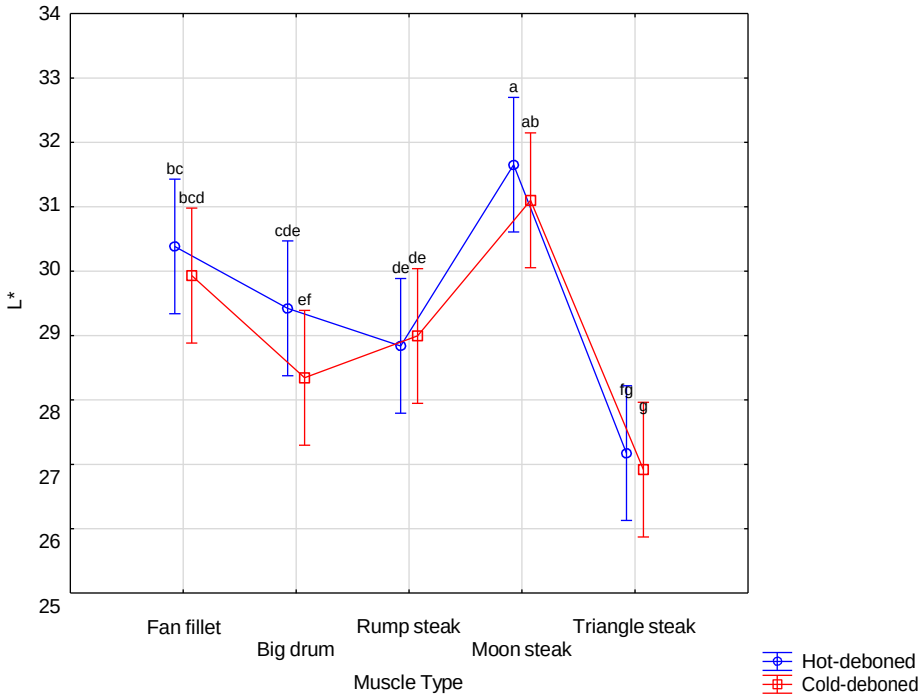

Figure 3.4 The impact of hot- vs. cold-deboning on the L* values of the ostrich fan fillet; big drum; rump steak; moon steak and triangle steak day 3 post-mortem ($p \leq 0.05$).

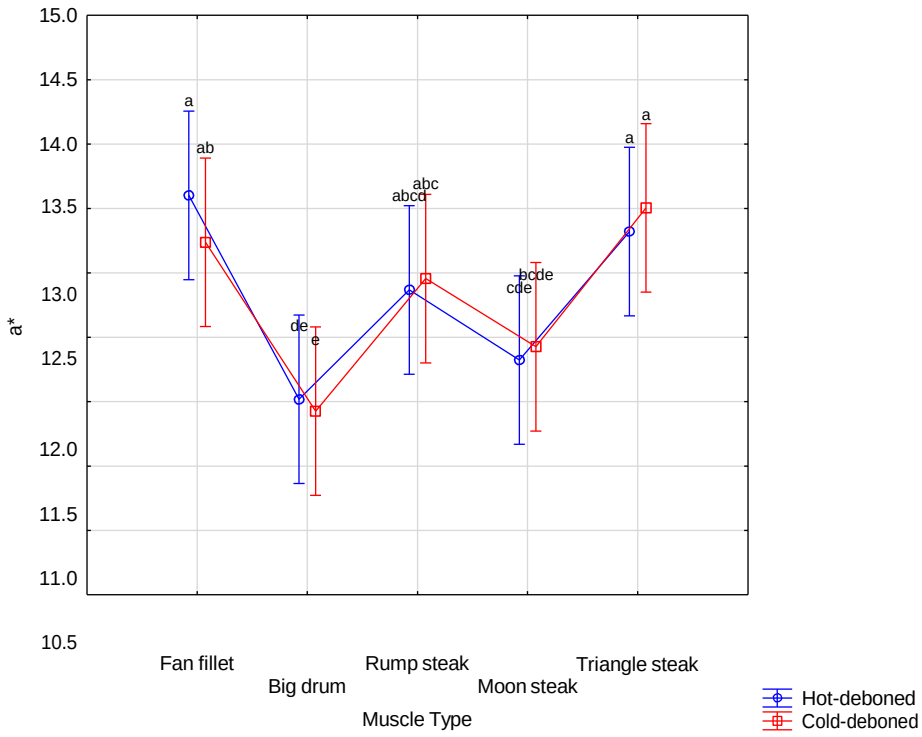

Figure 3.5 The impact of hot- vs. cold-deboning on the a* values of the ostrich fan fillet; big drum; rump steak; moon steak and triangle steak day 3 post-mortem ($p \leq 0.05$).

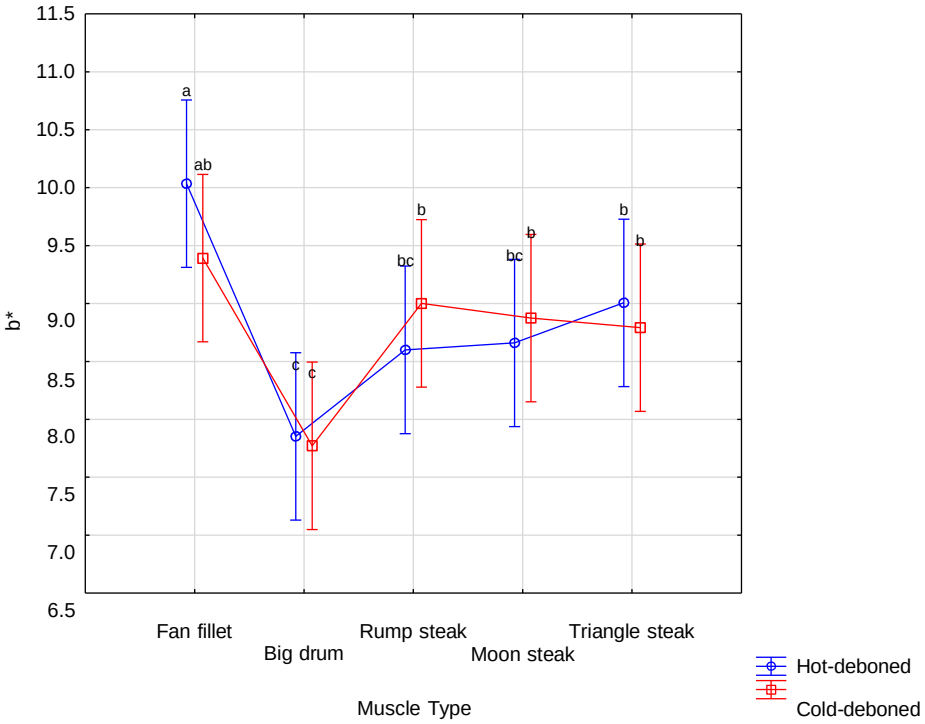

Figure 3.6 The impact of hot- vs. cold-deboning on the b* values of the ostrich fan fillet; big drum; rump steak; moon steak and triangle steak at day 3 post-mortem ($p \leq 0.05$).

The mean WBSF values day 3 post-mortem had no interaction between muscle type and treatment ($p = 0.68$). There was however a difference between muscles ($p = 0.00$) as illustrated in Fig. 3.7, whilst hot- vs. cold-deboning did not show any differences ($p = 0.72$; Table 3.4).

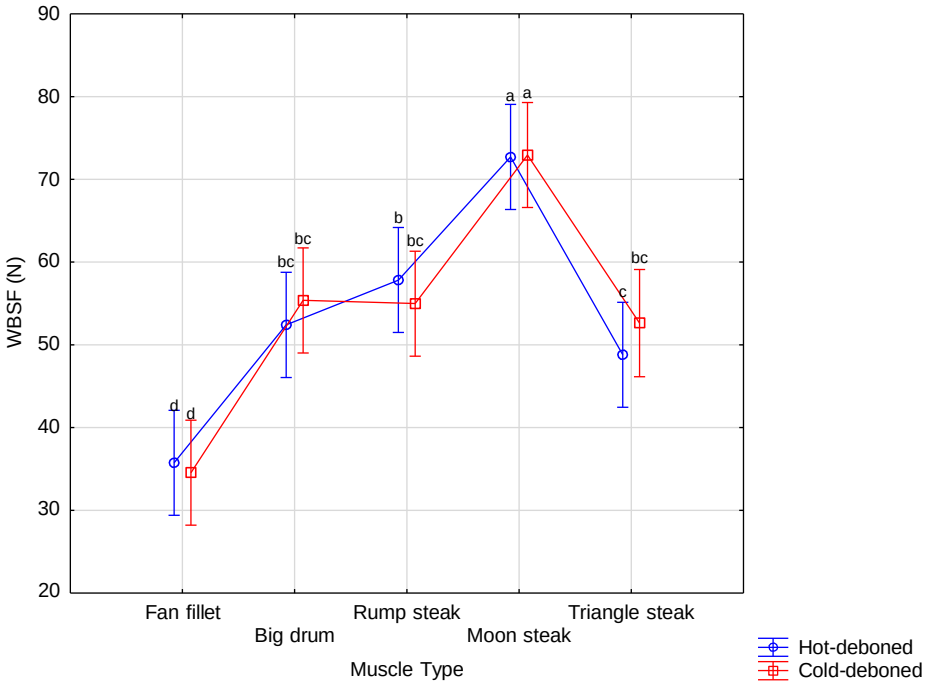

Figure 3.7 The impact of hot- vs. cold-deboning on the WBSF values of the ostrich fan fillet big drum; rump steak; moon steak and triangle steak at day 3 post-mortem ($p \leq 0.05$).

3.4 Discussion

Currently the ostrich industry in South Africa do not sell all sixteen commercially deboned ostrich muscles individually, but rather as individual grouped cuts (e.g. fillet or steak). Ostrich meat in South Africa is nevertheless sold fresh, frozen or processed (mainly as droëwors) to local markets. Frozen ostrich meat is sold as fillet, steak, goulash, mince, and patties. While fresh ostrich meat is grouped together from the sixteen commercial muscles, the fan fillet is the only muscle which is sold as such. However, the eye fillet is either sold individually or together with the tenderloin. The tenderloin is currently sold as a whole fillet at the highest price per kg comparing to all the other ostrich meat products. The oyster fillet, tournedos and long fillet are grouped together and sold as “small fillet” in the retail ostrich market. Moreover, the “small steak” currently sold in the South African ostrich market, is comprised of the small steak, small drum, tender steak, minute steak and triangle steak muscles. The “mixed portion ostrich steak” currently sold commercially include the rump steak, moon steak, flat drum, big drum and drum steak. It is interesting to note that the fan fillet, which is the only muscle sold as such, were found to be the most tender of all the investigated muscles. All of the other investigated muscles, including the big drum, rump steak, moon steak and triangle steak are sold as part of the “mixed portion ostrich steak.” The moon steak was found to be the most tough, whilst both the big drum and rump steak, although more tender than the moon steak, were also still within the range of tough meat (Destefanis

et al., 2007). It must however be taken into account that the meat is rarely sold as fresh meat at day three post-mortem, but rather as aged meat (discussed in Chapter 4).

Although ostriches are the largest of the Ratites which yield more meat per bird (Cooper & Horbańczuk, 2002), mainly the back, thighs and legs are utilised from carcasses (Balog & Almeida, 2007). Mellett (1985) stated that as much as 80 - 90% of ostrich carcasses are comprised of meat cuts whilst other species only reach 45%. Balog and Almeida (2007) however found the cold carcass yield of ostriches at approximately 51% with commercial muscle cuts comprising of 42% of the meat whilst Morris *et al*. (1995) found ostriches to have 62.5% removable lean meat. Girolami *et al*. (2003) found the three largest ostrich muscles (in weight) to be the big drum, rump steak and fan fillet (descending order); each comprising of over 6% of carcass weight. Similarly, in this study, the three largest muscles in descending order were the fan fillet, rump steak and big drum (Table 3.2), irrespective of treatment and with or without membranes. These hot-deboned muscles contributed 9.56% (fan fillet), 7.80% (rump steak) and 5.79% (big drum) to the hot-deboned leg weight, whilst the cold-deboned muscles similarly contributed 9.80% (fan fillet), 7.43% (rump steak) and 6.02% (big drum) to the cold-deboned leg weight.

Furthermore, as these three muscles are the heaviest in weight, and economically the most important for the industry, it can be emphasized that hot-deboning did not negatively affect the muscle yields of these three muscles. The hot- and cold-deboned fan fillet similarly lost 7.69% (147.44 g) and 7.64% (140.67 g) weight, respectively through membrane removal (Table 3.2). The hot-deboned rump steak however lost only 6.80% (148.67 g) whilst the cold-deboned rump steak lost 14.80% (224.00 g) weight through the removal of membranes (Table 3.2). For the big drum, the hot-deboned muscle likewise had a smaller weight loss (17.49%; 227.80 g) in comparison with the cold-deboned rump steak (18.79%; 241.60 g) (Table 3.2). It is worthwhile to mention that the big drum generally has a larger percentage of weight loss through the removal of membranes due to the anatomical location of the muscle. The big drum is situated in the superficial layer of the pelvic limb of the ostrich, thus creating a greater surface of membranes to remove, whereas the fan fillet and rump steak are located deeper within the pelvic limb (Smith *et al*., 2006).

Six muscles (tenderloin, oyster fillet, small drum, fan fillet, triangle steak and long fillet) of the sixteen commercial ostrich muscles showed a significant difference between hot- and cold-deboning for the mean muscle weights with membranes (Table 3.2). The mean hot-deboned muscle weights for these six muscles were higher ($p \leq 0.05$) than that of the cold-deboned muscles indicating that hot-deboning had higher muscle yields for 37.5% of the meat (0.41 kg higher). Furthermore, eight muscles (tenderloin, oyster fillet, small drum, tournedos, rump steak, fan fillet, triangle steak and flat drum) of the sixteen commercial ostrich muscles had significantly higher mean muscle weights without membranes for hot-deboning (Table 3.2) ($p \leq 0.05$) in comparison with cold-deboned mean muscle weights without membranes. This

amounts to 50% of the muscles/cuts showing higher yields for hot-deboning (0.46 kg per bird higher), which is important as the industry utilises muscles after membranes have been removed. Furthermore, hot-deboned muscles lost 13.74% (1698.08 g) in weight through the removal of membranes (expressed as a mean % of the original mean hot-deboned muscle weights), whilst cold-deboned muscles lost 15.63% (1847.33 g) in weight (expressed as a mean % of the original mean cold-deboned muscle weights), indicating that cold-deboned muscles lost significantly more weight through the removal of membranes ($p \leq 0.05$) (Table 3.2).

The higher mean muscle weights of the hot-deboned muscles can possibly be ascribed to the elimination of carcass chilling (24 h post-mortem) where a loss in muscle weight usually occurs; the fact that the ostrich has very little subcutaneous fat, but only localised fat depots (Sales *et al*., 1999) causes a higher than normal cold room weight loss (1.23 kg or 6.62 % over a 24 h chilling period). The excision of muscles from the ostrich leg during hot-deboning might also have been more “clean,” leaving less meat tissue on the bone. In cows, the meat yield was found to be 65.8% with hot-deboning, whereas the cold-deboned meat yield was 64.4% (Spooncer, 1993). Furthermore, hot-deboning generally requires less human energy with 49% less effort required to perform excision in the case of beef carcasses. Furthermore, the average time to hot- debone beef carcasses was found to be fourteen min in comparison with eighteen min to cold-debone. An increase in productivity was seen due to the lessened exertion (Spooncer, 1993).

Post-mortem glycolysis occurs at the death of the animal when all oxygen (O_2) is depleted from muscles. The amount of lactic acid produced following the aforementioned process influences the final or ultimate pH (pH_u) of meat (Lawrie & Ledward, 2006c). The post-mortem pH decline in meat is generally from pH 7.0 – 7.2 in skeletal muscles (Honikel, 2004) to a pH_u varying between normal (pH < 5.8) and dark, firm and dry (DFD; pH > 6.2) for ostrich meat which is classified as an intermediate meat type (Sales & Mellett, 1996; Balog & Almeida, 2007). A variety of factors, including muscle type influence the rate and degree to which pH declines post-mortem. Consequently, different muscles with different anatomical locations throughout the ostrich carcass will have varying rates of post-mortem pH decline with varying pH_u values (Sales & Mellett, 1996; Lawrie & Ledward, 2006a). The fan fillet is known to have a rapid pH decline and reaches the pH_u at 2 h post-mortem before an unusual increase in pH is normally seen. The rump steak has an intermediate pH decline and reaches the pH_u at three h post-mortem whilst the big drum and moon steak usually reaches the pH_u at a more normal rate towards six h post-mortem (Sales & Mellet, 1996).

The mean pH_u values of the fan fillet, big drum, rump steak, moon steak and triangle steak investigated in this study differed ($p = 0.01$; Table 3.4; Fig. 3.2), and can partly be ascribed to pre-slaughter glycogen levels differing between the different muscles bring about different pH_u values (Hoffman *et al*., 2007). Hoffman *et al*. (2008) found ten investigated ostrich muscles to have varying pH_u values. Furthermore, it can be noted that the

mean pH_u values of the investigated muscles ranged from 5.83 to 6.01 (Table 3.4) correlating with the typical pH range of ostrich meat (Sales & Mellet, 1996; Balog & Alemeida, 2007). However, hot-deboning did not significantly influence the mean pH_u at day three post-mortem (Table 3.4). This is similar to what Hoffman *et al.* (2007) found where hot- vs. cold-deboned fan fillet and big drum muscles showed no significant difference between the minimum pH_u values reached.

The water holding capacity (WHC) of meat affects both the drip in raw meat as well as the shrinkage in cooked meat. A key factor in determining the WHC of meat is the tempo at which the post-mortem pH declines, where a more rapid fall will have a more detrimental denaturation effect (Lawrie & Ledward, 2006b). Meat with intermediate to high pH_u values (such as ostrich) is, however, favourable for water binding resulting in a lower moisture percentage which will ultimately influence the cooking loss percentage (Hoffman *et al*., 2008). Both the mean drip loss and cooking loss percentages at day three post-mortem differed between the fan fillet, big drum, rump steak, moon steak and triangle steak ($p = 0.00$), whereas the treatments did not significantly differ (Table 3.4; Fig. 3.3). Hoffman *et al*. (2008) similarly found the drip and cooking loss percentage of six ostrich muscles to vary. In this study, it can be noted that the big drum had the lowest mean drip loss percentage (for both treatments) as a result of the high pH_u values of the hot- and cold-deboned big drum (Table 3.4). However, only the hot-deboned big drum showed a positive correlation between pH_u and drip loss percentage ($r = 0.49$; $p = 0.08$). Although the hot- deboned triangle steak was one of the smallest muscles in terms of weight (Table 3.2), it had the highest mean drip loss percentage. This could be as a result of the pH_u value of the hot-deboned triangle steak which was the lowest, and closest to the iso-electric point of pH_u 5.4 – 5.5 where minimum WHC is found (Lawrie & Ledward, 2006b). A positive correlation between pH_u and drip loss percentage was however only found for the cold-deboned triangle steak ($r = 0.58$; $p = 0.02$).

The most important determining factor in the consumer's purchasing selection process of meat and meat related products is often said to be the colour of the meat (Jeremiah *et al*., 1972; Fletcher, 2002; Cornforth & Jayasingh, 2004). It is well established that consumers choose meat with a bright red colour opposed to meat with a brown or purple hue (Carpenter *et al.,* 2011). The bright red colour is achieved through the exposure of meat to air which allows for O_2 to be bound to the primary meat pigment, namely myoglobin (Mb), forming oxymyoglobin (MbO_2) within 60 min in ostrich meat (blooming) (Lawrie & Ledward, 2006b; Leygonie, 2011). Muscle pH_u and meat colour are known to be interrelated particularly with regard to DFD or pale, soft and exudative (PSE) meat. Meat with a pH_u significantly exceeding the iso-electric point (pH 5.4 – 5.5) will have a purplish-red hue which appear as darker in colour and are not favoured by consumers (Lawrie & Ledward, 2006b).

Classified as an intermediate meat type with a pH_u varying between normal (pH < 5.8) and dark, firm and dry (DFD; pH > 6.2), ostrich meat is known to appear dark red making it comparable to other red meat despite its avian nature (Sales, 1996; Sales & Mellet, 1996; Balog & Almeida, 2007). The dark red colour of ostrich meat can also be ascribed to the high pigment content of ostrich meat (22 mg Fe/g) and is known to vary amongst muscles. The higher WHC of ostrich meat further affects colour through higher radiation absorption and lower reflection which results in darker meat (Balog & Almeida, 2007).

All of the investigated colour parameters (L*a*b*, hue angle and Chroma) at day 3 post-mortem showed significant differences between the fan fillet, big drum, rump steak, moon steak and triangle steak rather than between the deboning methods (Table 3.4). The moon steak had the highest mean L* value indicating increased lightness whereas the triangle steak had the lowest mean L* value suggesting a darker muscle colour (Table 3.4; Fig. 3.4). The fan fillet had the highest mean values for both a* and b* values suggesting a more red-yellow muscle colour, with the highest mean Chroma value indicative of a more saturated or vivid muscle colour (Table 3.4; Fig. 3.5/3.6). As the Chroma is calculated by an equation comprising of a* (red) and b* (yellow) values, results of a* and b* values will affect Chroma with an expected correlation as in the case of the fan fillet (Hoffman *et al*., 2008). The lowest mean a* and b* values were both found to be for the big drum suggesting a more green-blue muscle colour whilst overall having the least colour intensity with the lowest mean hue angle value (Table 3.4, Fig. 3.5/3.6). All of the significant colour differences were thus found between muscles, similar to what Hoffman *et al*. (2008) found for the colour parameters of ten investigated ostrich muscles which showed significant differences. Sales and Oliver-Lyons (1996) previously suggested the division of ostrich muscles according to colour groups for the optimal marketing of whole ostrich muscles.

The most significant factor in determining eating quality by consumers (at the expense of colour and flavour), is currently viewed as meat texture and tenderness (Tornberg, 1996; Lawrie & Ledward, 2006b). Meat tenderness is typically measured through Warner-Bratzler shear force (WBSF) and can be described as the initial ease of meat penetration by teeth followed by the exertion required to break meat into fragments and the remaining pieces successive to chewing (Lawrie & Ledward, 2006b). The tempo of post-mortem pH decline in meat can be related to the tenderness of the meat where increased tenderness is correlated to a slow pH decline post-mortem. The process of post-mortem glycolysis enables the onset of *rigor mortis* as muscle become inflexible, leading to stiff chains of actomyosin being developed through the loss of ATP. The ATP concentration is maintained until glycolysis is limited by the absence of substrate or unfavourable circumstance for the enzymes. A loss in meat tenderness is seen as the pH_u increases from 5.5 (iso-electric point) to 6 as a result of the degree of post-mortem glycolysis (Devine, 2004; Lawrie & Ledward, 2006a; Lawrie & Ledward, 2006b).

Tenderness is said to be the most valued characteristic of ostrich meat which has consistently showed lower WBSF values in comparison with beef. The tenderness of ostrich meat has previously been explained as a result of the muscle fibre arrangement being horizontal or angular, as well as low collagen level. Collagen is part of the protein complex forming part of the connective tissue which constructs meat texture (Balog & Almeida, 2007). It can also be noted that meat tenderness is highly correlated with cooking loss percentage. The connective tissue of meat primarily consisting of collagen, shrinks to one quarter of its original length when subjected to heat of ± 65°C. A consequent increase in meat toughness is seen as a result of the build-up in tension when heat is applied and fluid is lost from the muscle. Therefore, higher collagen concentrations in meat leads to increased levels of shrinkage when more fluid is exuded at high temperatures, resulting in meat with overall increased toughness (Hoffman *et al*., 2008). As seen in Table 3.4, the moon steak had one of the highest mean combined cooking loss percentages linking with the highest mean WBSF value. An analysis of the total collagen as well as type of collagen of this muscle, and the other ostrich muscles would be of interest to see whether this relationship discussed above is also applicable to ostrich muscles.

The mean WBSF values for the fan fillet, big drum, rump steak, moon steak and triangle steak at day three post-mortem differed ($p = 0.00$) whilst hot vs. cold-deboning did not show differences ($p = 0.72$). This is an important observation as it shows that hot-deboning (of post-rigor muscles) of ostrich meat does not lead to an increase in toughness as is experienced in other red meat species where the muscles are typically deboned pre-rigor (Pen *et al*., 2012; Sikes *et al*., 2014). This also means that no additional interventions such as wrapping of muscles imitating skeletal restraint to overcome the risk of cold-shortening (Rosenvold *et al*., 2008), needs to be conducted. Furthermore, in contrast with this study, it is interesting to note that Botha *et al*. (2007) found the hot-deboned big drum to be tougher than the cold-deboned big drum from 24 h to five days post-mortem.

From Table 3.4 it is clear that the moon steak had the highest mean WBSF values at day three with values above > 52.68 N which is considered to be tough meat. It can be noted that both the big drum and rump steak also had mean values classified as tough meat (Table 3.4). Furthermore, the triangle steak had mean values between 42.87 – 52.68 N placing it in the intermediate tenderness range (Table 3.4). The mean fan fillet values at day three (Table 3.4) had the lowest WBSF values < 42.87 N categorising it as tender meat (Destefanis *et al*., 2007). Balog and Almeida (2007) noted contrasting results regarding meat tenderness of different ostrich muscles with the fan fillet as most tender due to the lower collagen content of ostrich meat. Girolami *et al*. (2003) similarly found the fan fillet to be more tender compared to the big drum and rump steak, whilst Mellet and Sales (1997) found the rump steak and moon steak to be more tender than the fan fillet. Sales and Oliver-Lyons (1996) accordingly found the rump steak and moon steak to be more tender than the fan fillet.

3.5 Conclusions

Hot-deboned muscles without membranes had significantly higher mean muscle yields than the same muscles when processed cold accounting for 50% of the sixteen commercial cuts, which is especially relevant as the ostrich industry utilises meat after membranes have been removed. Hot-deboning did not significantly influence the physical meat quality parameters (at day three post-mortem) for any of the five investigated muscles including the fan fillet *(M. iliofibularis)*, rump steak *(M. iliotibialis lateralis)*, big drum *(M. gastrocnemius, pars interna)*, moon steak *(M. femorotibialis medius)* or triangle steak *(M. flexor cruris lateralis).*

Although significant differences in the physical meat quality were seen amongst muscles, results of the hot-deboned muscles matched that of the cold-deboned muscles. Thus, with regards to muscle yields and physical meat parameters as investigated on five ostrich muscles at day three post-mortem, hot-deboning is a suitable alternative to cold-deboning. The dressing of ostrich carcasses and excision of commercial ostrich cuts ready to be vacuum packed or further processed within two h post-mortem (opposed to 24 h post-mortem as with cold-deboning), has beneficial cost implications.

A significant amount of cost can be reduced with the elimination of a typical 24 h chilling of carcasses in terms of the lessening of refrigeration space, which lowers the cost of energy and can contribute to a quicker throughput rate at the abattoir. The higher muscle yield percentage of hot-deboned meat can have further beneficial cost implications and is particularly relevant at this stage as the South African ostrich industry finds itself in a difficult economic climate due to the ban on exports. Although hot-deboning is considered to be a fitting alternative deboning method for the South African ostrich industry considering muscle yields and physical meat characteristics, the physical meat characteristics over an extended time period post-mortem needs to be further investigated. This is of great relevance for the industry as vacuum packed ostrich muscles do not necessarily reach local markets at day three post-mortem. Additionally, the microbial shelf-life stability of hot deboned muscles needs to be quantified as it is postulated that the exposure of hot muscles to the environment could lead to a higher microbial contamination and faster growth rate of the microbes. This is particularly relevant when the muscles have had their membranes removed.

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CHAPTER 4

Physical meat quality characteristics of five hot- vs. cold-deboned ostrich (*Struthio camelus*) muscles during post-mortem ageing

Abstract

Hot-deboning as an alternative excising method for the South African ostrich industry was investigated. Fifteen ostriches were used for the study with the muscles hot-deboned (within 90 min post-mortem) from the left leg and cold-deboned (24 h post-mortem) from the right leg. Five ostrich muscles: fan fillet (*M. iliofibularis*); rump steak (*M. iliotibialis lateralis*); big drum (*M. gastrocnemius, pars interna*); moon steak (*M. femorotibialis medius*); and triangle steak (*M. flexor cruris lateralis)* were used to conduct a vacuum packaged ageing trial for 28 d post-mortem (0 - 4°C). Hot-deboning did not have an effect on the pH_u ($p = 0.50$). Although a few significant differences in the colour coordinates (CIEL*a*b*, hue angle and Chroma) were found between treatments across different muscles, hot-deboning had no major negative effect ($p > 0.05$). Neither the cumulative weep loss nor the cooking loss percentages showed significant differences between the hot- and cold-deboned muscles ($p > 0.05$). Concerning Warner-Bratzler shear force (WBSF) values, only the hot-deboned rump steak (34.74 vs. 26.55 N) was tougher ($p \leq 0.05$) at 28 d post-mortem compared to the cold-deboned steak with a value which is nonetheless considered within the tender meat range. Significant differences were greater amongst the ageing time intervals than between hot- vs. cold-deboning. Regarding these findings, hot-deboning is deemed a suitable alternative for the ostrich industry of South Africa.

Keywords: Hot-deboning, Cold-deboning, Ostrich, Physical quality, Age

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4.1 Introduction

The South African ostrich industry is currently limited to only supplying ostrich meat to local markets since a ban has been placed on the export of ostrich meat. Thus, now more than ever, for the South African ostrich industry to survive the difficult economic climate and regain status as leader in the global ostrich marketplace (National Agricultural Marketing Council, 2003; Department of Agriculture, Forestry and Fisheries, 2017) once the ban is lifted, the industry needs to investigate ways in which economic strength can be obtained. Hot-deboning, which is defined as the practice where lean meat and fat is removed from a carcass before a big drop in body temperature occurs (performed within 2 h post-mortem) is a way in which the industry can do so (Waylan & Kastner, 2004; Hoffman *et al*., 2007). Through hot-deboning, the chilling of carcasses for 24 h post-mortem, as is currently done with cold-deboning, can be eliminated which greatly reduces energy costs. However, for hot-deboning to be applied, not only the physical meat quality characteristics should be known, but also the longer term meat quality characteristics. This is especially crucial should the ban on exports be lifted, as it is known that ostrich meat can take up to 42 d to reach the export market (Botha *et al*., 2007).

Ageing can be referred to as the progression of meat tenderisation which happens through the presence of endogenous muscle enzymes. It is one of the post-slaughter factors greatly influencing meat texture and tenderness as these endogenous muscle enzymes are known to break down structural muscle proteins during the process of ageing post-mortem. It is postulated that amongst possible enzymes, Calpains are most probably responsible for the tenderisation in red meat and poultry (Warriss, 2000; Devine, 2004; Thomas *et al*., 2004).

There are various factors affecting ageing, including the animal's history leading to slaughter, i.e. pre-slaughter stress (Hoffman *et al*., 2012), as well as temperature during *rigor*, shortening of muscles and the duration and temperature of ageing (Devine, 2004; Lawrie & Ledward, 2006b; Botha *et al*., 2006; 2007). Different muscles from the same animal are distinguished to have varying ageing rates which is considerably influenced by the latter factors (Warriss, 2000; Devine, 2004; Botha *et al*., 2007). Moreover, a period of time usually passes before meat is consumed, and the loss in tenderness experienced during rigor-shortening is slowly reversed through ageing post-rigor (Warriss, 2000; Lawrie & Ledward, 2006b).

Concerning meat quality, the most important demonstration of post-mortem muscle protein denaturation is the loss in water holding capacity (WHC) (Lawrie & Ledward, 2006b). In the case of ostrich meat, possible meat quality differences between hot- and cold-deboned meat over an extended time period is especially relevant as vacuum packed ostrich meat takes up to 42 d to reach the export market. The amount of purge that accumulates during the ageing time period in hot-deboned meat is particularly notable since a significantly higher purge percentage has previously been found in hot-deboned ostrich meat (Botha *et al*., 2007).

Although ostrich meat is not currently exported, vacuum packed ostrich muscles are still kept for a considerable number of days before being sold locally.

This study was carried out to determine the physical meat quality characteristics of hot- and cold-deboned vacuum packed ostrich muscles throughout a 28 d ageing period. Five representative ostrich muscles (of which the physical meat quality was established at day three post-mortem; Chapter 3), were used in the ageing trial: fan fillet (*M. iliofibularis*); rump steak (*M. iliotibialis lateralis*); big drum (*M. gastrocnemius, pars interna*); moon steak (*M. femorotibialis medius*); and triangle steak (*M. flexor cruris lateralis*). Although Botha *et al*., (2006) conducted a similar ageing trial between hot- vs. cold-deboned ostrich muscles, a 21 d ageing time period was carried out on only one high value commercial ostrich cut, namely the big drum (*M. gastrocnemius, pars interna*). For this study, an ageing time period of 28 d was chosen as 28 d is accepted as the benchmark for microbiological analysis where decline is usually observed (discussed in Chapter 5) (Sheridan & Sherington, 1982).

4.2 Materials and methods

4.2.1 Ostriches and muscle samples

Following the materials and methods section described in Chapter 3, physical analysis was performed on the ageing muscle steaks.

Each of the five hot- and cold-deboned muscles (Table 4.1) from 15 ostriches were cut perpendicular to the longitudinal axis into 1.5 – 2.0 cm steaks for different ageing time periods according to the varying sizes of each muscle (Table 4.2). The fan fillet (*M. iliofibularis*) was cut into five steaks for five ageing time points (3, 7, 14, 21 and 28 d post-mortem). The rump steak (*M. iliotibialis lateralis*) was cut into four steaks for four ageing time points (3, 14, 21 and 28 d post-mortem). Both the big drum (*M. gastrocnemius, pars interna*) and moon steak (*M. femorotibialis medius*) were cut into three steaks for three ageing time points (3, 14 and 28 d post- mortem). The triangle steak (*M. flexor cruris lateralis*) were cut into two steaks for two ageing time points (3 and 28 d post-mortem).

After each muscle was cut, each steak was individually weighed, randomly assigned to the above-mentioned ageing time points and vacuum packed (with the exception of day three steaks), and stored in a commercial refrigerator (0 - 4°C).

Table 4.1 Description of ostrich muscles used for ageing trial as per commercial and scientific names

Commercial name	Scientific name
Fan fillet	*M. iliofibularis*
Rump steak	*M. iliotbialis lateralis*
Big drum	*M. gastocnemius, pars interna*
Moon steak	*M. femorotibialis medius*
Triangle steak	*M. flexor cruris lateralis*

Table 4.2 Overview of the experimental layout as per main effects (hot- vs. cold-deboning, muscle type and ageing time points)

Deboning Treatment	Muscle	Weight (g)*	Ageing time points (days post-mortem)				
Hot-deboned	Fan fillet	1775.53 ± 0.181	1	7	14	21	28
	Rump steak	1448.73 ± 0.171	1		14	21	28
	Big drum	1074.67 ± 0.183	1		14		28
	Moon steak	881.93 ± 0.116	1		14		28
	Triangle steak	460.53 ± 0.064	1				28
Cold-deboned	Fan fillet	1699.93 ± 0.159	1	7	14	21	28
	Rump steak	1289.07 ± 0.169	1		14	21	28
	Big drum	1044.47 ± 0.171	1		14		28
	Moon steak	901.533 ± 0.128	1		14		28
	Triangle steak	387.73 ± 0.063	1				28

*Mean muscle weights without membranes ± Standard Deviation

4.2.2 Physical analysis

Muscle pH and temperature; raw meat colour (CIEL*a*b* colour coordinates, hue angle and Chroma); cumulative weep loss percentage; cooking loss percentage and Warner-Bratzler shear force measurements were recorded at each of the above-mentioned ageing intervals (Table 4.2) as described in the materials and methods section in Chapter 3.

The cumulative weep loss percentage in the steak samples were determined by weighing steak samples prior to vacuum packaging to establish the initial weight as well as after samples were removed from the vacuum packaging at each ageing time point. Steak samples were blotted dry with absorbent paper towel before the “after” weight was recorded. The weep loss was consequently expressed as a percentage of the initial sample weight:

$$\text{Weep loss \%} = \frac{\text{weight}_{\text{before}} - \text{weight}_{\text{after}}}{\text{weight}_{\text{before}}} \text{ X } 100$$

4.2.3 Statistical analyses

Statistica 64 version 13's (2015) VEPAC module was used to perform statistical analyses (STATISTICA, 2011). Hot- vs. cold-deboning and day were used as fixed effects in a mixed model repeated measures of ANOVA. The Fisher LSD (least significant differences) test was used for the multiple comparison test. It can be noted that animal was included as random effect while possible outliers were identified using normal probability plots. Significant influences were described as Means and Standard Deviation (SD). A significance level of 5% ($p \leq 0.05$) was used as guideline for detecting possible significant effects.

4.3 Results

4.3.1 pH

Results of the pH_u conducted on the fan fillet, rump steak, big drum, moon steak and triangle steak are presented in Table 4.3.

Table 4.3 Hot- vs. cold-deboned ostrich muscle pH_u as per muscle type and treatment over the 28 d ageing period (Mean ± SD)

Muscle Type*		**Hot-deboned**	**Cold-deboned**			
Fan fillet		6.19^{a} ± 0.26	6.15^{a} ± 0.18			
Rump steak		6.02^{b} ± 0.38	5.99^{bc} ± 0.15			
Big drum		5.94^{bc} ± 0.45	6.01^{bc} ± 0.15			
Moon steak		5.99^{bc} ± 0.11	5.99^{bc} ± 0.11			
Triangle steak		5.90^{c} ± 0.08	5.91^{bc} ± 0.08			
		Ageing days post-mortem#				
		3	**7**	**14**	**21**	**28**
Fan fillet	Hot-deboned	5.94^{c} ± 0.11	6.30^{a} ± 0.31	6.26^{ab} ± 0.27	6.23^{ab} ± 0.20	6.21^{b} ± 0.22
	Cold-deboned	5.93^{c} ± 0.09	6.21^{ab} ± 0.16	6.23^{ab} ± 0.17	6.20^{ab} ± 0.14	6.18^{ab} ± 0.14
Rump steak	Hot-deboned	5.91^{b} ± 0.16		6.00^{ab} ± 0.03	5.99^{ab} ± 0.03	5.98^{ab} ± 0.02
	Cold-deboned	5.97^{ab} ± 0.29		6.02^{a} ± 0.06	5.99^{ab} ± 0.05	5.99^{ab} ± 0.03
Big drum	Hot-deboned	6.01^{ab} ± 0.16		6.03^{ab} ± 0.08		5.98^{ab} ± 0.06
	Cold-deboned	5.96^{b} ± 0.15		6.06^{a} ± 0.18		6.02^{ab} ± 0.11
Moon steak	Hot-deboned	5.92^{c} ± 0.11		6.06^{a} ± 0.12		6.00^{b} ± 0.07
	Cold-deboned	5.93^{c} ± 0.10		6.03^{ab} ± 0.10		6.02^{b} ± 0.08
Triangle steak	Hot-deboned	5.83^{b} ± 0.05				5.93^{a} ± 0.04
	Cold-deboned	5.85^{b} ± 0.04				5.98^{a} ± 0.05

*$^{a-c}$ Means with different superscripts differed significantly ($p \leq 0.05$).

#$^{a-c}$ Means in the same double row per muscle with different superscripts differed significantly between ageing time points ($p \leq 0.05$).

There was no interaction between treatments and muscle type (p = 0.50) for the pooled mean pH_u values (per hot-deboned and per cold-deboned) over the 28 d ageing time period (Table 4.3). The mean hot vs. cold-deboned pH_u values of the five aged muscles over the 28 d ageing time period are represented in Fig. 4.1. Although no statistically significant differences were seen between hot- vs. cold-deboning, the pooled mean pH_u values showed significant differences between muscles, irrespective of treatment (Fig. 4.1).

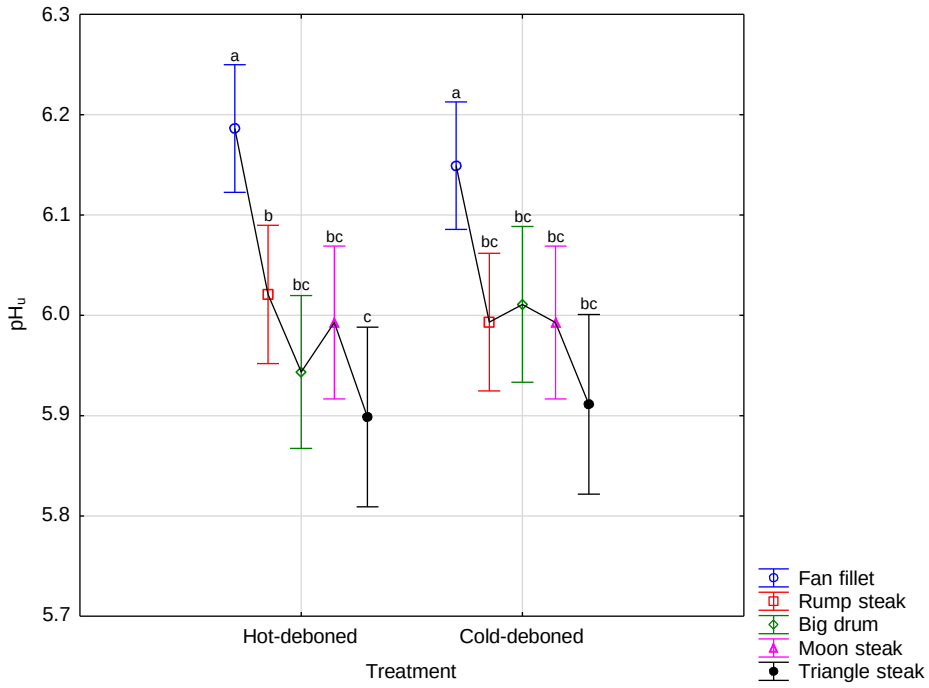

Figure 4.1 The impact of hot- vs. cold-deboning on the pH_u of the ostrich fan fillet; rump steak; big drum; moon steak and triangle steak over the 28 d ageing period. Significant difference is indicated by different letters ($p \leq 0.05$).

No interaction was found between hot- vs. cold-deboning and the ageing time points (p = 0.80) in the mean pH_u values of the fan fillet (Table 4.3) over the course of the 28 d ageing period. The mean hot- vs. cold-deboned pH_u values of the fan fillet over the 28 d ageing period are represented in Fig. 4.2. No difference was seen between the hot- and cold-deboned fan fillets, but a significant increase in pH_u was seen for both treatments between days 3 and 7 post-mortem (Fig. 4.2). After day 7, the pH_u of the cold-deboned fan fillet did not change, whereas the pH_u of the hot-deboned fan fillet significantly declined between days 7 and 28 post-mortem as seen in Fig. 4.2.

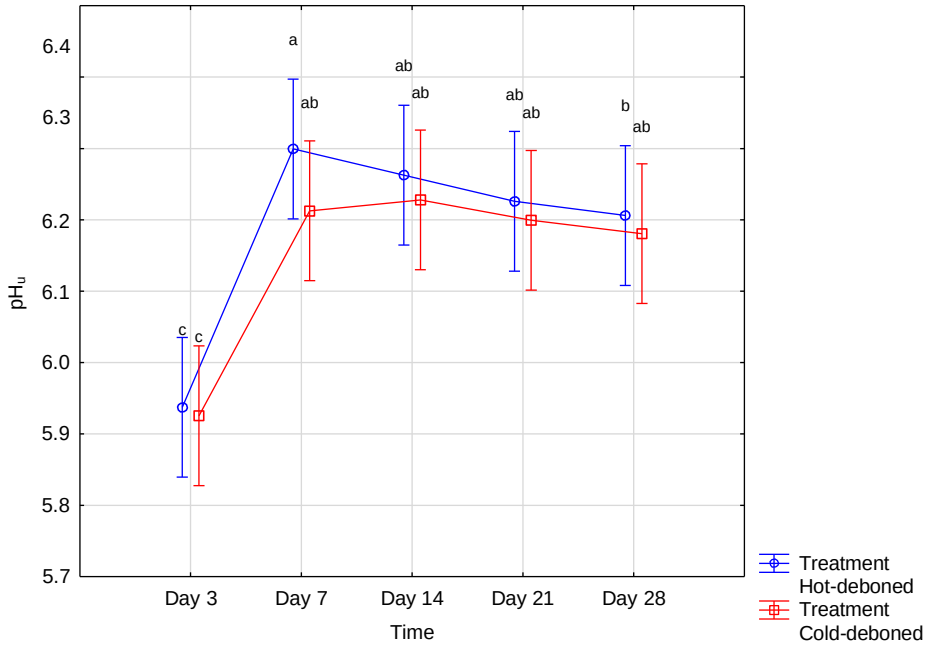

Figure 4.2 The impact of hot- vs. cold-deboning on the pH_u of the ostrich fan fillet over the ageing period 28 d post-mortem. Significant difference is indicated by different letters ($p \leq 0.05$).

There was no interaction between the ageing time points or treatments ($p = 0.75$), in the mean pH_u values of the hot- vs. cold-deboned rump steak over the 28 d ageing period (Table 4.3). As shown in Fig. 4.3 however, the pH_u of the cold-deboned rump steak was significantly higher at day 7 post-mortem when compared to the pH_u of the hot-deboned rump steak on day 3 post-mortem.

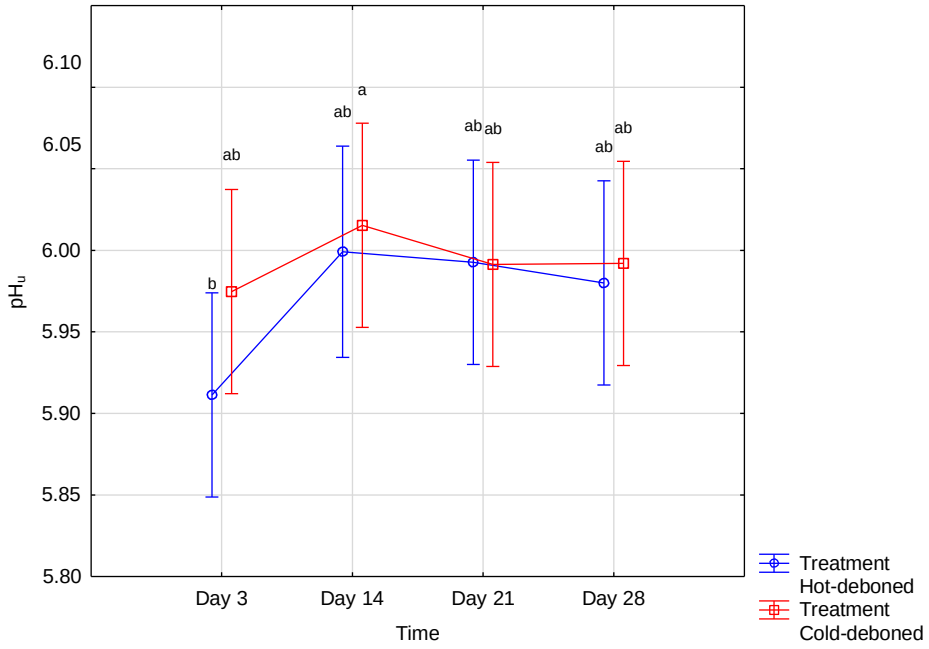

Figure 4.3 The impact of hot- vs. cold-deboning on the pH_u of the ostrich rump steak over the ageing period 28 d post-mortem. Significant difference is indicated by different letters ($p \leq 0.05$).

The mean pH_u values of the big drum showed no interaction between the ageing time points or between hot- vs. cold-deboned ($p = 0.18$) over the 28 d ageing period (Table 4.3). Hot- vs. cold-deboning showed no difference in pH_u whilst the only significant difference between ageing time points was seen between days 3 and 14 for the cold-deboned big drum (Table 4.3).

The moon steak had no interaction between ageing time points or treatments ($p = 0.10$), with no significant differences between the mean pH_u values (hot- and cold-deboned) over the ageing period of 28 d (Table 4.3). Although there was no interaction, differences were seen between all the ageing time points (days 3, 14 and 28 post-mortem) as indicated in Table 4.3. A significant increase in the mean pH_u values of both treatments were seen between days 3 and 14, followed by a significant decrease between days 14 and 28 only in the hot-deboned moon steak (Table 4.3).

No interaction between hot- vs. cold-deboning and ageing time points were seen in the mean pH_u values of the triangle steak over the 28 d ageing period ($p = 0.95$; Table 4.3). The mean pH_u values did not differ across treatments, but did however significantly increase from day 3 to day 28 post-mortem (Table 4.3).

4.3.2 Surface colour

- *CIE L**

The mean raw meat colour coordinates (CIE L*) of the hot- vs. cold-deboned ostrich muscles are represented in Table 4.4.

An interaction between treatments and ageing time points was found in the mean CIE L* values of the fan fillet (p = 0.00; Table 4.4) over the 28 d ageing period. As seen in Fig. 4.4, a significant difference between hot- and cold-deboning was found on day 7 post-mortem, where the hot-deboned fan fillet had a significantly higher mean CIE L* value (also significantly higher than the mean CIE L* values on day 3). Differences between ageing time points (irrespective of treatment) were seen between days 3, 7, 14 and 28 post-mortem (Fig. 4.4). The mean CIE L* values increased until day 14 with no significant change between days 14 and 21, followed by a significant decrease towards day 28 post-mortem (Fig. 4.4).

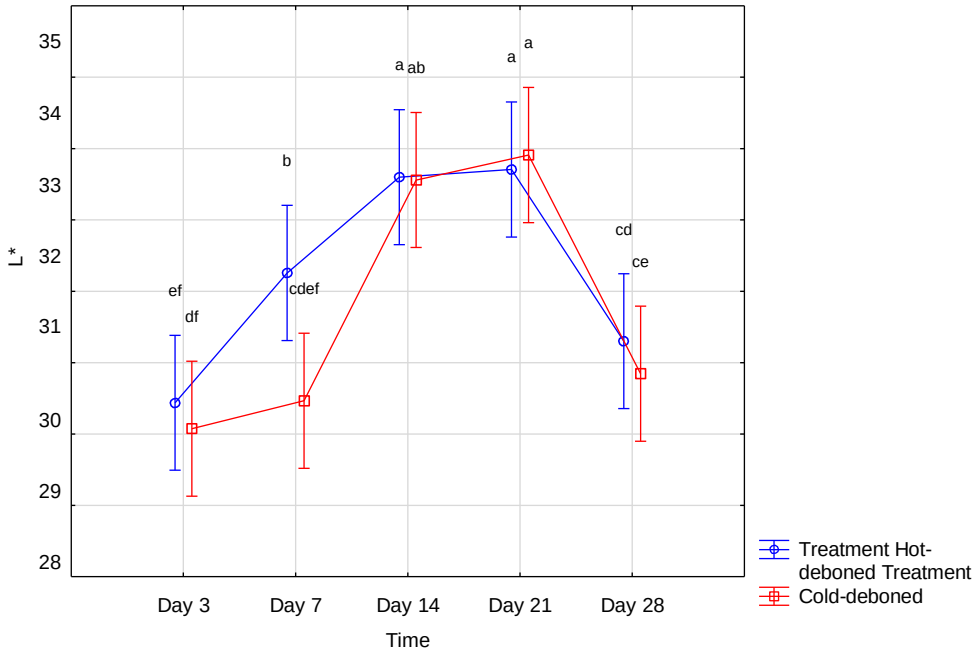

Figure 4.4 The mean CIE L* values of hot- vs. cold-deboned ostrich fan fillet over the ageing period 28 d post-mortem. Significant difference is indicated by different letters ($p \leq 0.05$).

Table 4.4 Hot- vs. cold-deboned ostrich muscle CIE L* as per muscle type and treatment over the 28 d ageing period (Mean ± SD)

Measurement	Muscle type	Treatment	Ageing days post-mortem				
			3	**7**	**14**	**21**	**28**
L*	Fan fillet	Hot-deboned	$30.44^{ef} \pm 2.21$	$32.24^{b} \pm 2.76$	$33.60^{a} \pm 2.59$	$33.71^{a} \pm 2.35$	$31.30^{cd} \pm 2.49$
		Cold-deboned	$30.07^{df} \pm 2.01$	$30.48^{cdef} \pm 1.89$	$33.56^{ab} \pm 2.35$	$33.91^{a} \pm 2.07$	$30.85^{ce} \pm 2.17$
	Rump steak	Hot-deboned	$28.75^{e} \pm 2.79$		$32.41^{b} \pm 1.93$	$33.27^{a} \pm 2.64$	$31.31^{cd} \pm 2.28$
		Cold-deboned	$28.95^{e} \pm 1.55$		$32.20^{abc} \pm 2.30$	$31.74^{bc} \pm 2.07$	$30.90^{d} \pm 1.90$
	Big drum	Hot-deboned	$29.32^{c} \pm 1.84$		$32.26^{a} \pm 2.07$		$29.64^{b} \pm 2.22$
		Cold-deboned	$28.15^{d} \pm 1.79$		$32.30^{a} \pm 1.56$		$29.73^{bc} \pm 2.02$
	Moon steak	Hot-deboned	$31.60^{cd} \pm 1.64$		$33.97^{a} \pm 2.12$		$32.43^{b} \pm 1.92$
		Cold-deboned	$31.02^{d} \pm 2.12$		$33.36^{a} \pm 1.99$		$31.48^{bc} \pm 1.48$
	Triangle steak	Hot-deboned	$27.19^{c} \pm 1.74$				$30.64^{a} \pm 1.80$
		Cold-deboned	$26.97^{c} \pm 1.80$				$29.07^{b} \pm 1.77$

$^{a-f}$ Means in the same double row per muscle with different superscripts differed significantly between ageing time points ($p \leq 0.05$).

An interaction was seen between the mean CIE L* values of the hot- and cold-deboned rump steak and ageing time points (p = 0.00; Table 4.4) over the 28 d ageing period. As shown in Fig. 4.5, the mean CIE L* values of the rump steak had significant differences at every ageing time point (3, 14, 21, 28 d post-mortem). Hot- and cold-deboning did however only differ on day 21, where the mean hot-deboned CIE L* value was significantly higher (also significantly higher than the mean CIE L* values of both days 14 and 28; Fig. 4.5). Furthermore, the mean CIE L* values of the hot-deboned rump steak significantly increased until day 21 where after it significantly decreased at day 28 post-mortem (Fig. 4.5). In turn, the mean CIE L* values of the cold-deboned rump steak only significantly increased between days 3 and 14 where after it significantly decreased between days 14 and 28 post-mortem (Fig. 4.5).

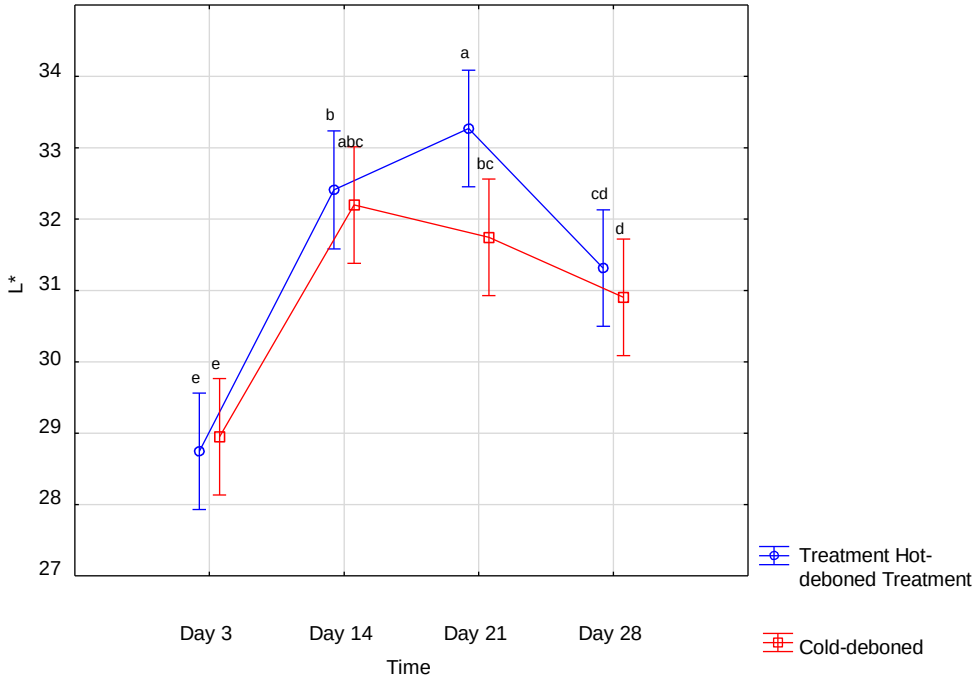

Figure 4.5 The mean CIE L* values of hot- vs. cold-deboned ostrich rump over the ageing period 28 d post-mortem. Significant difference is indicated by different letters (p ≤ 0.05).

The mean CIE L* values of the big drum showed an interaction concerning treatments and the respective ageing time points (p = 0.00) over the 28 d ageing period (Table 4.4). Furthermore, the varying ageing time points (days 3, 14 and 28 post-mortem) showed significant differences with the only difference between hot- and cold-deboning found on day 3 post-mortem as shown in Table 4.4. The mean CIE L* values of both the hot- and cold-deboned big drum

significantly increased between days 3 and 14, where after it significantly decreased between days 14 and 28 post-mortem (Table 4.4).

The mean hot vs. cold-deboned CIE L* values of the moon steak over the 28 d ageing time period had no interaction between hot- vs. cold-deboning and ageing time points (p = 0.13; Table 4.5). The individual ageing time points (days 3, 14 and 28 post-mortem) did however show differences with a significant increase at 14 d post-mortem for both treatments (Table 4.5).

An interaction occurred between treatments and ageing time points (p = 0.00) in the mean CIE L* values of the triangle steak (Table 4.5). The mean L* values of the triangle steak increased significantly between days 3 and 28 post-mortem, with hot- and cold-deboning only significantly differing on day 28 (Table 4.5).

- *CIE a**

The mean raw meat colour coordinates (CIE a*) of the hot- vs. cold-deboned ostrich muscles are represented in Table 4.5.

There was an interaction between treatments and ageing time points in the mean CIE a* values of the hot- vs. cold-deboned fan fillet (p = 0.00; Table 4.5) over the course of the 28 d ageing period. As seen in Fig. 4.6, there was differences between ageing time points (days 3, 7, 14, 21 and 28), whereas hot- and cold-deboning only differed at day 28 post-mortem. The mean a* value of the hot-deboned fan fillet was significantly lower at day 28 in comparison with both the cold-deboned muscle at day 28 as well as with day 21 post-mortem (Fig. 4.6). Moreover, a significant increase in the mean CIE a* values until day 14 were seen where after it significantly decreased at 21 d and again at 28 d post-mortem (Fig. 4.6).

Table 4.5 Hot- vs. cold-deboned ostrich muscle CIE a* as per muscle type and treatment over the 28 d ageing period (Mean ± SD)

Measurement	Muscle type	Treatment	Ageing days post-mortem				
			3	**7**	**14**	**21**	**28**
a*	Fan fillet	Hot-deboned	$13.58^{e} \pm 1.33$	$14.38^{cd} \pm 1.71$	$15.22^{ab} \pm 1.88$	$14.62^{cd} \pm 1.44$	$11.86^{f} \pm 1.95$
		Cold-deboned	$13.28^{e} \pm 1.08$	$14.46^{d} \pm 1.73$	$15.70^{a} \pm 1.33$	$14.93^{cd} \pm 1.69$	$13.31^{f} \pm 1.48$
	Rump steak	Hot-deboned	$12.89^{d} \pm 1.38$		$14.67^{a} \pm 1.63$	$13.46^{bc} \pm 2.02$	$11.12^{e} \pm 1.49$
		Cold-deboned	$12.89^{cd} \pm 1.55$		$15.12^{a} \pm 1.91$	$13.68^{b} \pm 1.92$	$11.53^{e} \pm 1.57$
	Big drum	Hot-deboned	$12.12^{b} \pm 1.46$		$13.85^{a} \pm 1.45$		$11.72^{b} \pm 1.27$
		Cold-deboned	$11.90^{b} \pm 1.80$		$13.63^{a} \pm 1.64$		$12.26^{b} \pm 1.39$
	Moon steak	Hot-deboned	$12.22^{bc} \pm 1.23$		$14.50^{a} \pm 1.58$		$10.94^{d} \pm 1.43$
		Cold-deboned	$12.22^{b} \pm 1.44$		$14.39^{a} \pm 1.67$		$11.60^{c} \pm 1.26$
	Triangle steak	Hot-deboned	$13.31^{a} \pm 1.27$				$11.23^{b} \pm 1.49$
		Cold-deboned	$13.40^{a} \pm 1.29$				$11.34^{b} \pm 1.64$

$^{a-f}$ Means in the same double row per muscle with different superscripts differed significantly between ageing time points ($p \leq 0.05$).

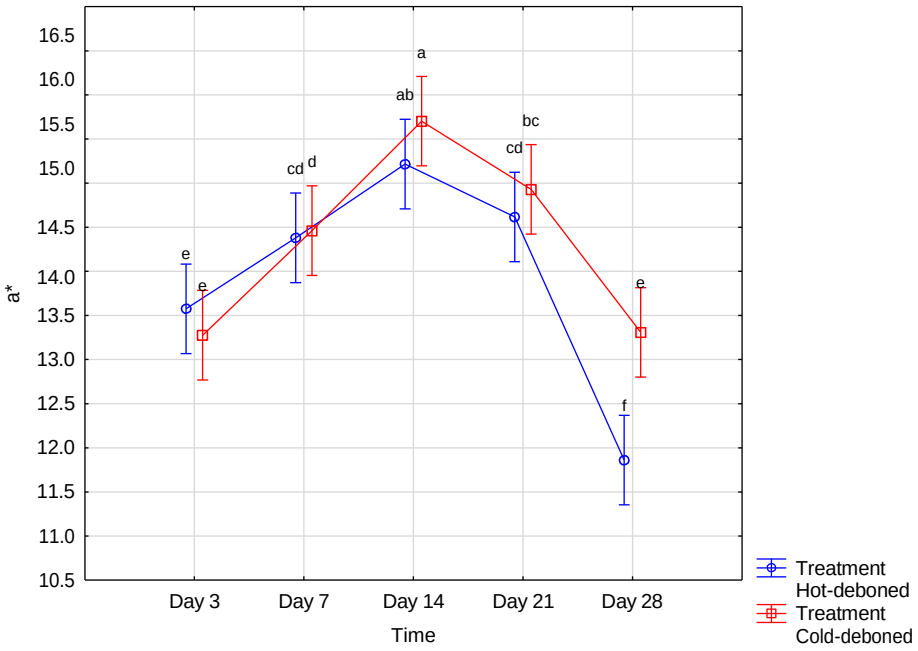

Figure 4.6 The mean CIE a* values of hot- vs. cold-deboned ostrich fan fillet over the ageing period 28 d post-mortem. Significant difference is indicated by different letters ($p \leq 0.05$).

The mean CIE a* values of the rump steak over the 28 d ageing period showed no interaction between treatments and ageing time points ($p = 0.53$; Table 4.5). Although there was no interaction, significant differences were seen between the ageing time points at days 3, 14, 21 and 28 post-mortem (Fig. 4.7). A significant increase was seen in the mean CIE a* values at day 14 where after it significantly decreased at both 21 and 28 d post-mortem (Fig. 4.7).

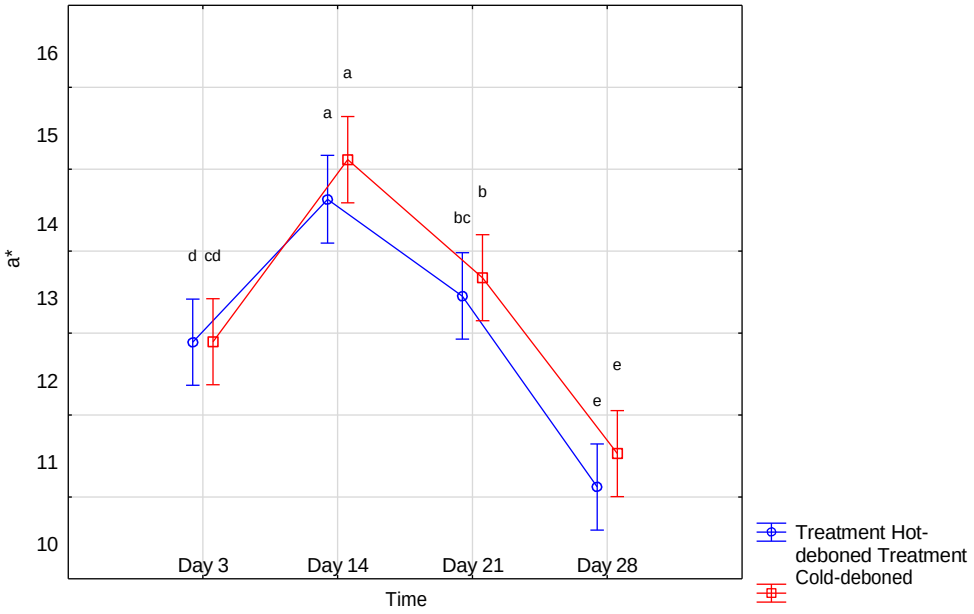

Figure 4.7 The mean CIE a* values of hot- vs. cold-deboned ostrich rump steak over the ageing period 28 d post-mortem. Significant difference is indicated by different letters ($p \leq 0.05$).

There was an interaction between treatments and ageing time points ($p = 0.02$) in the mean CIE a* values of the hot- and cold-deboned big drum over the 28 d ageing period (Table 4.5). Furthermore, hot- and cold-deboning did not differ, but both treatments showed a significant increase in the mean CIE a* values at day 14 followed by a significant decrease at both 21 and 28 days post-mortem (Table 4.5).

The mean CIE a* values of the moon steak had an interaction between treatments and ageing time points ($p = 0.02$) over the 28 d ageing period (Table 4.5). A significant difference between hot- and cold-deboned was seen on day 28 post-mortem, with all ageing time points (days 3, 21 and 28 post-mortem) differing (Table 4.5). The mean CIE a* values of the moon steak significantly decreased from day 14 to day 28 after the initial increase between days 3 and 14 (Table 4.5).

No interaction was found between treatments and ageing time points for the mean CIE a* values of the triangle steak ($p = 0.97$; Table 4.5). Although there was no significant difference between hot- and cold-deboned, the mean CIE a* values decreased significantly from day 3 to day 28 post-mortem (Table 4.5).

- *CIE b**

The mean raw meat colour coordinates (CIE b*) of the hot- vs. cold-deboned ostrich muscles are represented in Table 4.6.

The mean CIE b* values of the fan fillet over the 28 d ageing period indicated an interaction between treatments and ageing time points as seen in Table 4.6 (p = 0.02). No differences were found between hot- and cold-deboning (when comparing hot- vs cold-deboning at the same ageing time point), but significant differences between the ageing time points were seen (Fig. 4.8). The mean CIE b* values of the hot-deboned fan fillet showed a significant decrease between days 14 and 21 after which it significantly increased at day 28 post-mortem (Fig. 4.8). On the other hand, the mean CIE b* values of the cold-deboned fan filled significantly decreased from day 7 to day 21 increasing significantly at day 28 post-mortem (Fig. 4.8).

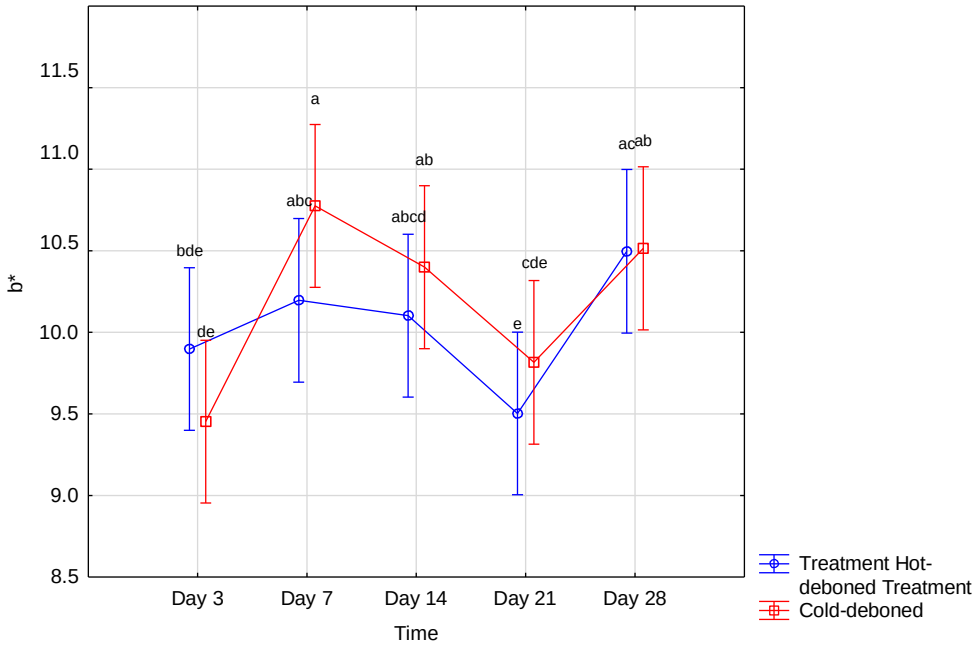

Figure 4.8 The mean CIE b* values of hot- vs. cold-deboned ostrich fan fillet over the ageing period 28 d post-mortem. Significant difference is indicated by different letters (p ≤ 0.05).

Table 4.6 Hot- vs. cold-deboned ostrich muscle CIE b* as per muscle type and treatment over the 28 d ageing period (Mean ± SD)

Measurement	Muscle type	Treatment	Ageing days post-mortem				
			3	**7**	**14**	**21**	**28**
b*	Fan fillet	Hot-deboned	$9.90^{bde} \pm 1.55$	$10.19^{abc} \pm 1.68$	$10.10^{abcd} \pm 1.56$	$9.50^{e} \pm 1.60$	$10.52^{ac} \pm 1.65$
		Cold-deboned	$9.45^{de} \pm 1.49$	$10.78^{a} \pm 1.74$	$10.39^{ab} \pm 1.38$	$9.82^{cde} \pm 1.50$	$10.53^{ab} \pm 1.55$
	Rump steak	Hot-deboned	$8.63^{bcd} \pm 2.06$		$8.51^{cd} \pm 1.69$	$8.40^{cd} \pm 1.66$	$8.34^{cd} \pm 1.63$
		Cold-deboned	$9.05^{abc} \pm 2.20$		$9.48^{a} \pm 1.70$	$8.08^{d} \pm 1.78$	$9.29^{ab} \pm 1.72$
	Big drum	Hot-deboned	$7.71^{b} \pm 1.85$		$7.94^{b} \pm 1.68$		$8.87^{a} \pm 1.41$
		Cold-deboned	$7.72^{b} \pm 1.80$		$8.09^{b} \pm 1.81$		$9.34^{a} \pm 1.68$
	Moon steak	Hot-deboned	$8.62^{abcd} \pm 1.87$		$8.30^{bd} \pm 1.77$		$8.76^{ac} \pm 1.70$
		Cold-deboned	$8.99^{ab} \pm 1.70$		$9.10^{ab} \pm 1.88$		$8.50^{cd} \pm 1.63$
	Triangle steak	Hot-deboned	$9.04^{a} \pm 1.73$				$8.80^{a} \pm 1.50$
		Cold-deboned	$8.88^{a} \pm 1.47$				$9.15^{a} \pm 1.48$

$^{a-e}$ Means in the same double row per muscle with different superscripts differed significantly between ageing time points ($p \leq 0.05$).

The mean CIE b* values of the rump steak over the 28 d ageing period showed an interaction between treatments and ageing time points (p = 0.00; Table 4.6). As shown in Fig. 4.9, differences between hot- and cold-deboned was seen at 14 and 28 d post-mortem where the cold-deboned rump steak had significantly higher mean b* values. The mean CIE b* values of the hot-deboned rump steak did not significantly change between days 3 and 14, whereas the mean CIE b* values of the cold-deboned rump steak significantly increased after day 3 (Fig. 4.9). Furthermore, the mean CIE b* values of the hot-deboned rump steak increased between day 21 and day 28 post-mortem, whilst that of the cold-deboned rump steak did not significantly change (Fig. 4.9).

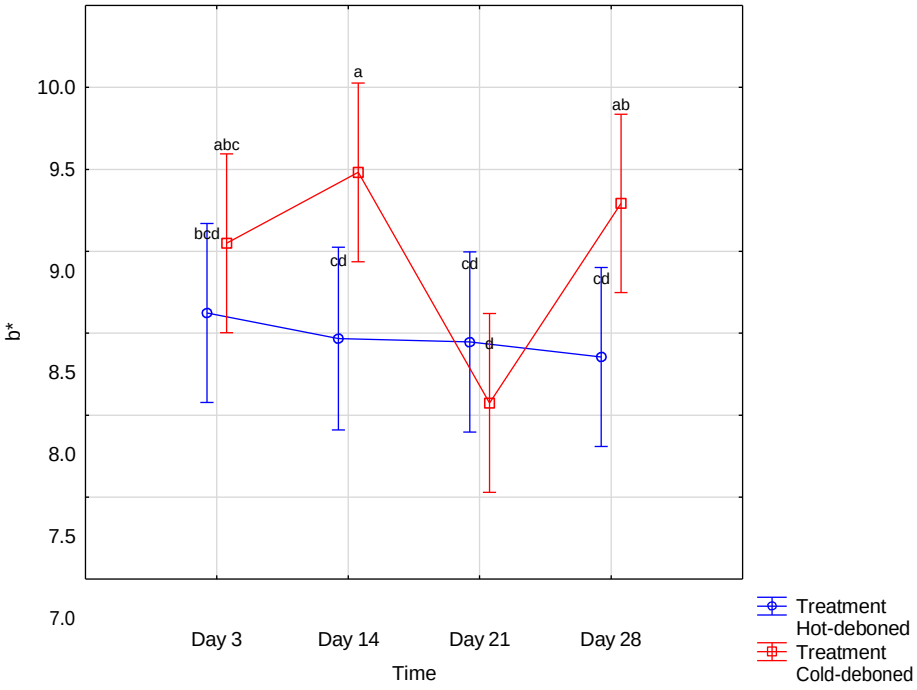

Figure 4.9 The mean CIE b* values of hot- vs. cold-deboned ostrich rump steak over the ageing period 28 d post-mortem. Significant difference is indicated by different letters (p ≤ 0.05).

The mean CIE b* values of the big drum had no interaction between treatments and ageing time points ($p = 0.44$) over the 28 d ageing period (Table 4.6). Although the difference between hot- and cold-deboning was non-significant, a significant increase in the mean CIE b* values was seen between days 14 and 28 (Table 4.6).

An interaction was found between treatments and ageing time points in the mean CIE b* values of the moon steak over the course of the 28 d ageing period ($p = 0.01$; Table 4.6). The hot- vs. cold-deboned moon steak significantly differed on days 14 and 28 where the cold-deboned moon steak had significantly higher mean CIE b* values (Table 4.6). As seen in Table 4.6, the mean CIE b* values of the hot-deboned moon steak showed a decrease between days 3 and 14, with an increase between days 14 and 28. In contrast, the mean CIE b* values of the cold-deboned moon steak slightly increased between days 3 and 14 and decreased between days 14 and 28 (Table 4.6).

No interaction was found between treatments and ageing time points for the mean CIE b* values of the triangle steak ($p = 0.12$) over the 28 d ageing period (Table 4.6). Accordingly, there was no difference between hot- and cold-deboning nor between the ageing time points (Table 4.6).

- *Hue angle*

The mean raw meat hue angle values of the hot- vs. cold-deboned ostrich muscles are represented in Table 4.7.

Table 4.7 Hot- vs. cold-deboned ostrich muscle hue angle values as per muscle type and treatment over the 28 d ageing period (Mean ± SD)

Measurement	Muscle type	Treatment	Ageing days post-mortem				
			3	**7**	**14**	**21**	**28**
Hue angle	Fan fillet	Hot-deboned	$35.95^{cd} \pm 3.68$	$34.33^{def} \pm 7.53$	$33.59^{ef} \pm 4.25$	$32.95^{f} \pm 4.74$	$40.51^{a} \pm 8.95$
		Cold-deboned	$35.30^{cde} \pm 3.90$	$36.65^{bc} \pm 4.85$	$33.49^{f} \pm 3.87$	$32.83^{f} \pm 5.84$	$38.37^{b} \pm 4.72$
	Rump steak	Hot-deboned	$33.54^{cd} \pm 6.53$		$30.11^{e} \pm 5.01$	$31.63^{de} \pm 11.05$	$36.86^{ab} \pm 7.40$
		Cold-deboned	$34.81^{bc} \pm 7.55$		$32.14^{de} \pm 4.99$	$30.50^{e} \pm 6.19$	$38.85^{a} \pm 6.84$
	Big drum	Hot-deboned	$32.30^{bc} \pm 6.55$		$29.40^{d} \pm 7.29$		$37.10^{a} \pm 5.92$
		Cold-deboned	$33.04^{b} \pm 7.86$		$30.75^{cd} \pm 7.12$		$37.27^{a} \pm 6.69$
	Moon steak	Hot-deboned	$35.01^{bc} \pm 6.93$		$29.26^{d} \pm 6.46$		$40.48^{a} \pm 9.93$
		Cold-deboned	$36.30^{b} \pm 6.30$		$32.89^{c} \pm 8.72$		$36.15^{b} \pm 6.15$
	Triangle steak	Hot-deboned	$33.57^{b} \pm 6.94$				$38.08^{a} \pm 6.30$
		Cold-deboned	$33.50^{b} \pm 5.17$				$39.00^{a} \pm 6.76$

$^{a-f}$ Means in the same double row per muscle with different superscripts differed significantly between ageing time points ($p \leq 0.05$).

An interaction between treatments and ageing time points was seen in the mean fan fillet hue angle values ($p = 0.01$) over the 28 d ageing period (Table 4.7). A shown in Fig. 4.10, the mean hue angle values differed significantly between hot- and cold-deboned at days 7 and 28 post-mortem. The cold-deboned mean hue angle of the fan fillet was significantly higher than hot-deboned at day 7 post-mortem (also significantly higher than cold-deboned at days 3 and 14), whereas the hot-deboned fan fillet had a significantly higher mean hue angle value at day 28 post-mortem (also significantly higher than day 21; Fig. 4.10). Thus, the mean hue angle values of the cold-deboned fan fillet significantly increased between days 3 and 7, significantly decreased between days 7 and 14, and significantly increased again between 21 and 28 d post-mortem (Fig. 4.10). A similar increase between days 21 and 28 was also seen in the mean hue angle of the hot-deboned fan fillet (Fig. 4.10).

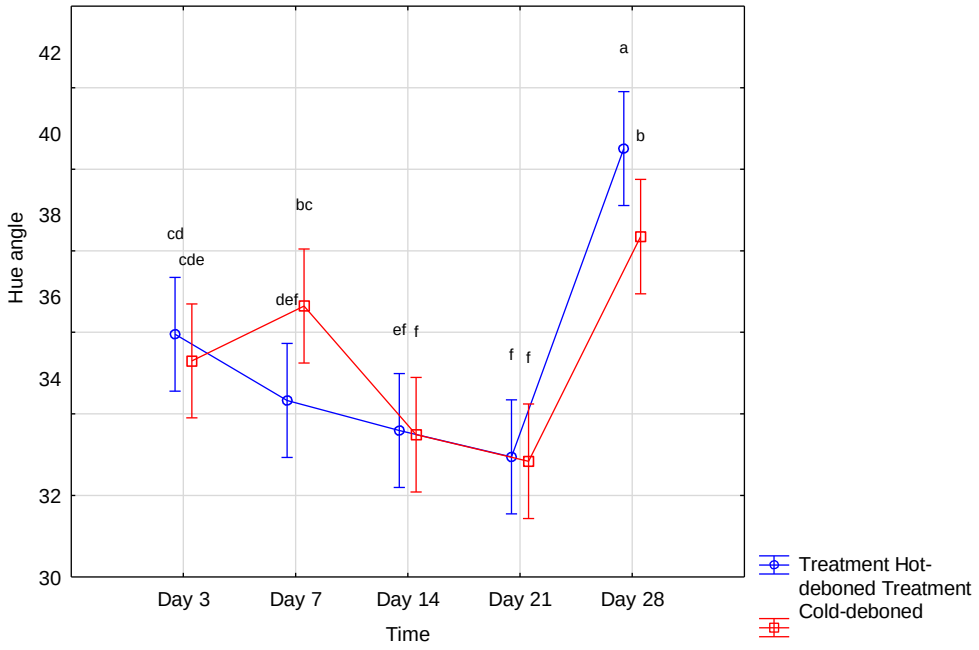

Figure 4.10 The mean hue angle values of hot- vs. cold-deboned ostrich fan fillet over the ageing period 28 d post-mortem. Significant difference is indicated by different letters ($p \leq 0.05$).

No interaction between treatments and ageing time points was seen in the mean rump steak hue angle values (p = 0.19) over the 28 d ageing period (Table 4.7). Significant differences were however seen in the mean hue angle values between the ageing time points of both the hot- and cold-deboned rump steak (Fig. 4.11). A significant decrease in the mean hue angle values was seen between days 3 and 14, where after a significant increase was seen between days 21 and 28 (Fig. 4.11).

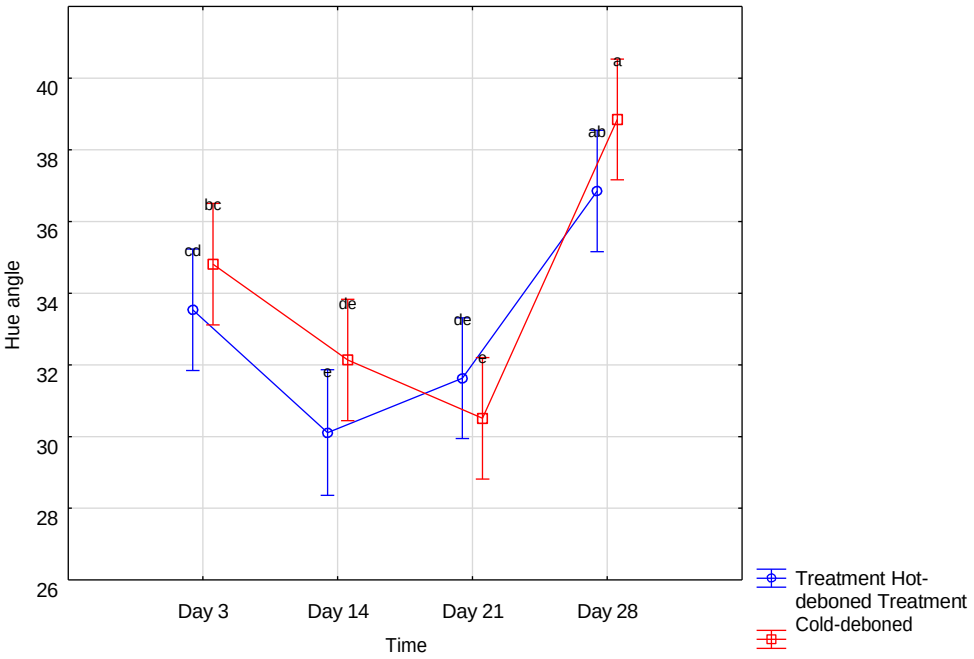

Figure 4.11 The mean hue angle values of hot- vs. cold-deboned ostrich rump steak over the ageing period 28 d post-mortem. Significant difference is indicated by different letters (p ≤ 0.05).

Over the course of the 28 d ageing period, no interaction occurred between treatments and ageing time points (p = 0.76) for the mean hue angle values of the big drum (Table 4.7). All of the ageing time points differed, with a significant decrease between days 3 and 14 and a significant increase between days 14 and 28 (Table 4.7).

The mean hue angle values of the moon steak had an interaction between treatments and ageing time points (p = 0.00) over the course of the 28 d ageing period (Table 4.7). The mean hue angle values of the moon steak differed significantly across all of the ageing time points (days 3, 14 and 28 post-mortem), while the hot-deboned moon steak had a significantly lower mean hue angle value on day 14 post-mortem (Table 4.7).

As seen in Table 4.7, the mean hue angle values of the triangle steak had no interaction between treatments and ageing time points (p = 0.48). Furthermore, there was also no

difference between hot- and cold-deboning, but a significant increase between days 3 and 28 post-mortem did occur (Table 4.7).

- *Chroma*

The mean raw meat Chroma values of the hot- vs. cold-deboned ostrich muscles are represented in Table 4.8.

The mean Chroma values of the fan fillet over the 28 d ageing period had an interaction between treatments and ageing time points (p = 0.00; Table 4.8). All of the ageing time points (days 3, 7, 14, 21 and 28 post-mortem) had significant differences with a significantly lower mean Chroma value seen for the hot-deboned fan fillet at day 28 post-mortem (significantly lower than day 21 as well as cold-deboned at day 28 post-mortem; Fig. 4.12). Both the hot- and cold-deboned mean Chroma values of the fan fillet significantly increased from day 3 to day 21 where after it significantly decreased towards day 28 post-mortem (Fig. 4.12).

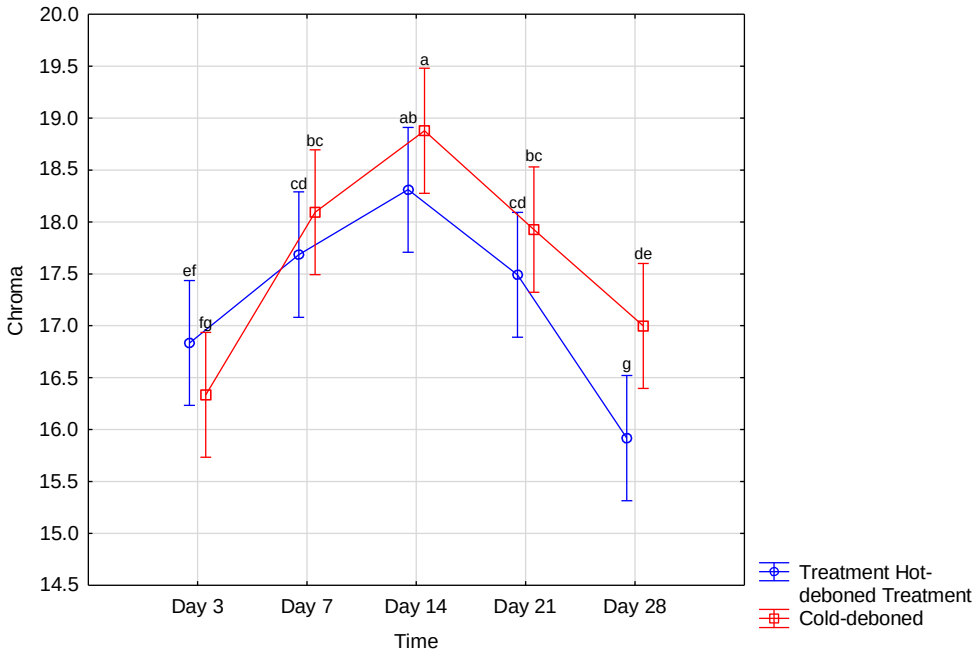

Figure 4.12 The mean Chroma values of hot- vs. cold-deboned ostrich fan fillet over the ageing period 28 d post-mortem. Significant difference is indicated by different letters (p ≤ 0.05).

Table 4.8 Hot- vs. cold-deboned ostrich muscle Chroma values as per muscle type and treatment over the 28 d ageing period (Mean ± SD)

Measurement	Muscle type	Treatment	Ageing days post-mortem				
			3	**7**	**14**	**21**	**28**
Chroma	Fan fillet	Hot-deboned	$16.83^{ef} \pm 1.74$	$17.68^{cd} \pm 1.87$	$18.31^{ab} \pm 2.06$	$17.49^{cd} \pm 1.61$	$15.96^{g} \pm 1.93$
		Cold-deboned	$16.33^{fg} \pm 1.47$	$18.09^{bc} \pm 1.95$	$18.87^{a} \pm 1.45$	$17.94^{bc} \pm 1.81$	$17.00^{de} \pm 1.62$
	Rump steak	Hot-deboned	$15.60^{bc} \pm 1.77$		$17.07^{a} \pm 1.72$	$15.92^{b} \pm 1.95$	$14.02^{d} \pm 1.34$
		Cold-deboned	$15.91^{b} \pm 1.70$		$17.74^{a} \pm 1.80$	$16.02^{b} \pm 1.93$	$14.91^{c} \pm 1.51$
	Big drum	Hot-deboned	$14.43^{b} \pm 1.70$		$16.07^{a} \pm 1.32$		$14.77^{b} \pm 1.14$
		Cold-deboned	$14.35^{b} \pm 1.63$		$15.97^{a} \pm 1.40$		$15.52^{a} \pm 1.24$
	Moon steak	Hot-deboned	$15.06^{bc} \pm 1.31$		$16.76^{a} \pm 1.70$		$14.09^{d} \pm 1.57$
		Cold-deboned	$15.26^{b} \pm 1.46$		$17.08^{a} \pm 1.81$		$14.46^{cd} \pm 1.36$
	Triangle steak	Hot-deboned	$16.15^{a} \pm 1.48$				$14.35^{b} \pm 1.41$
		Cold-deboned	$16.14^{a} \pm 1.31$				$14.67^{b} \pm 1.38$

$^{a-g}$ Means in the same double row per muscle with different superscripts differed significantly between ageing time points ($p \leq 0.05$).

An interaction was found between treatments and ageing time points in the mean Chroma values of the rump steak over the 28 d ageing period (p = 0.04; Table 4.8). Moreover, no significant difference was seen between hot- and cold-deboning when compared at each ageing time point, whereas all the ageing points (days 3, 14, 21 and 28 post-mortem) did however differ (Fig. 4.13). For both treatments a significant increase in the mean Chroma values was seen from days 3 to 14 where after the mean values significantly decreased at both 21 and 28 d post-mortem (Fig. 4.13).

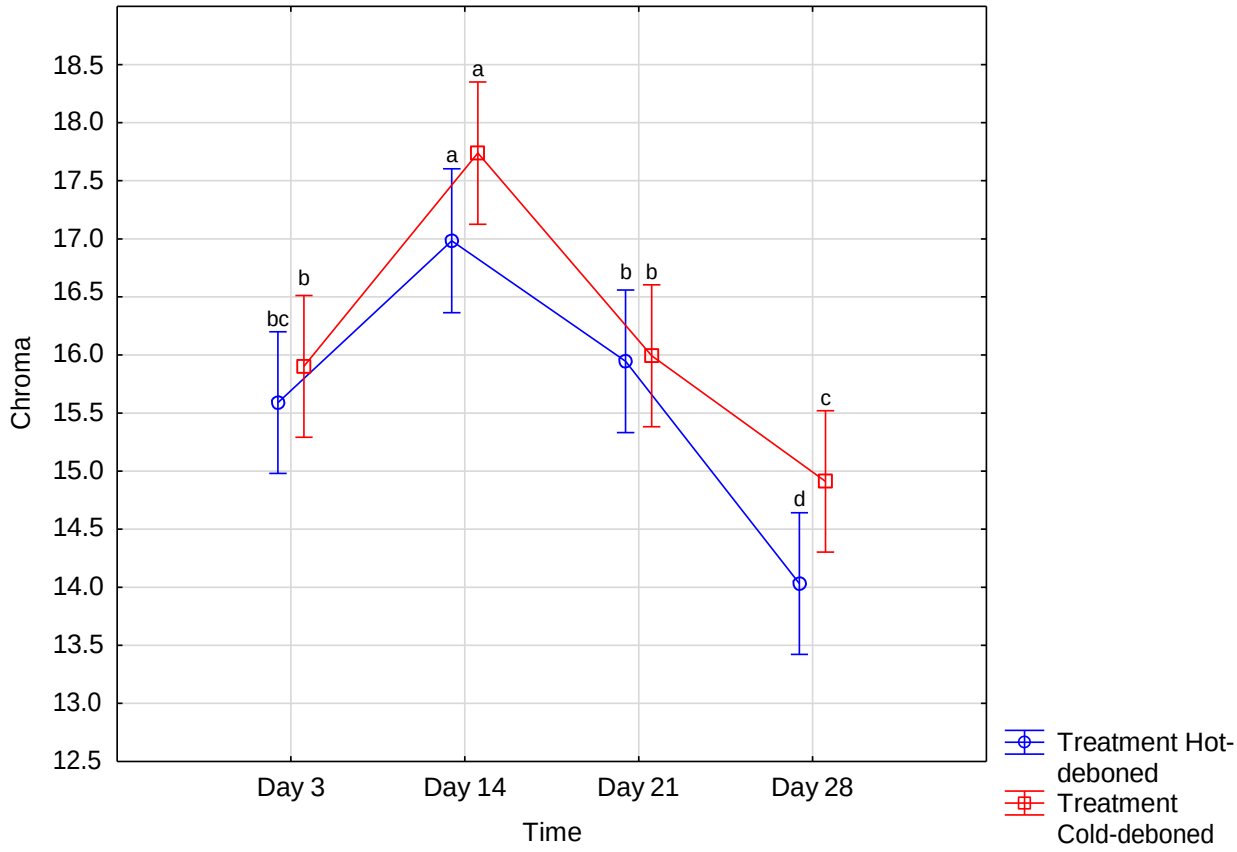

Figure 4.13 The mean Chroma values of hot- vs. cold-deboned ostrich rump steak over the ageing period 28 d post-mortem. Significant difference is indicated by different letters (p ≤ 0.05).

The mean Chroma values of the big drum showed an interaction between treatments and ageing time points (p = 0.00) over the 28 d ageing period (Table 4.8) The mean Chroma values for both the hot- and cold-deboned big drum significantly increased between days 3 and 14, whereas the mean values for only the cold-deboned big drum significantly decreased between days 14 and 28 post-mortem (Table 4.8).

The mean Chroma values of the moon steak had no significant interaction between treatments and ageing time points (p = 0.69) over the 28 d ageing period (Table 4.8). A difference was seen between all of the ageing time points at days 3, 14 and 28 with no significant difference between hot- and cold-deboning (Table 4.8). Both the treatments' mean Chroma values had a significant increase at day 14 followed by a significant decrease at day 28 (Table 4.8).

No interaction was found between treatments and ageing time points (p = 0.26) for the mean triangle steak Chroma values (Fig. 3.32) over the 28 d ageing period (Table 4.8). Furthermore, the hot- and cold-deboned triangle steak did not differ, but did however significantly decrease between days 3 and 28 post-mortem (Table 4.8).

4.3.3 Moisture loss

- *Cumulative weep loss %*

The cumulative weep loss percentage of the hot- and cold-deboned ostrich muscles over the ageing period 28 d post-mortem is given in Table 4.9.

No significant interaction between treatments and ageing time points was seen in the mean fan fillet weep loss percentage over the 28 d ageing period (p = 0.62; Table 4.9). There was however a significant difference between hot- and cold-deboning on 21 d post-mortem which also signified the ageing point with the highest mean weep loss percentage for both treatments (Fig. 4.14). Moreover, the mean weep loss percentage of the hot-deboned fan fillet had a significant increase between days 7 and 21, whilst the cold-deboned fan filled showed significant differences for all of the ageing time points apart from between days 7 and 14 post-mortem (Fig. 4.14). For both the hot- and cold-deboned mean weep loss percentage, a gradual increase in the mean weep loss percentage was observed between days 3, 7, 14 and 21 followed by a decrease, (significant for cold- deboned), between 21 and 28 d post-mortem (Fig. 4.14).

Table 4.9 Hot- vs. cold-deboned ostrich muscle weep loss % as per muscle type and treatment over the 28 d ageing period (Mean ± SD)

Measurement	Muscle type	Treatment	Ageing days post-mortem				
			3	**7**	**14**	**21**	**28**
Weep loss %	Fan fillet	Hot-deboned	$1.33^{e} \pm 0.54$	$1.99^{cde} \pm 0.97$	$2.12^{cd} \pm 1.13$	$2.96^{b} \pm 0.97$	$2.58^{bcd} \pm 1.08$
		Cold-deboned	$1.97^{de} \pm 0.96$	$2.80^{bc} \pm 1.58$	$2.45^{bcd} \pm 1.16$	$3.87^{a} \pm 1.35$	$2.76^{bc} \pm 1.42$
	Rump steak	Hot-deboned	$1.36^{f} \pm 0.83$		$2.89^{de} \pm 0.62$	$3.47^{bc} \pm 0.71$	$3.96^{a} \pm 0.87$
		Cold-deboned	$1.20^{f} \pm 0.36$		$2.79^{e} \pm 0.61$	$3.27^{cd} \pm 0.56$	$3.87^{ab} \pm 0.65$
	Big drum	Hot-deboned	$0.92^{c} \pm 0.42$		$1.81^{b} \pm 0.55$		$2.80^{a} \pm 0.74$
		Cold-deboned	$0.88^{c} \pm 0.17$		$1.74^{b} \pm 1.22$		$2.53^{a} \pm 1.24$
	Moon steak	Hot-deboned	$1.34^{c} \pm 0.38$		$2.38^{b} \pm 0.49$		$3.27^{a} \pm 0.68$
		Cold-deboned	$1.31^{c} \pm 0.47$		$2.49^{b} \pm 0.66$		$3.20^{a} \pm 0.76$
	Triangle steak	Hot-deboned	$1.55^{b} \pm 0.32$				$3.72^{a} \pm 0.89$
		Cold-deboned	$1.42^{b} \pm 0.30$				$3.85^{a} \pm 0.71$

$^{a-g}$ Means in the same double row per muscle with different superscripts differed significantly between ageing time points ($p \leq 0.05$).

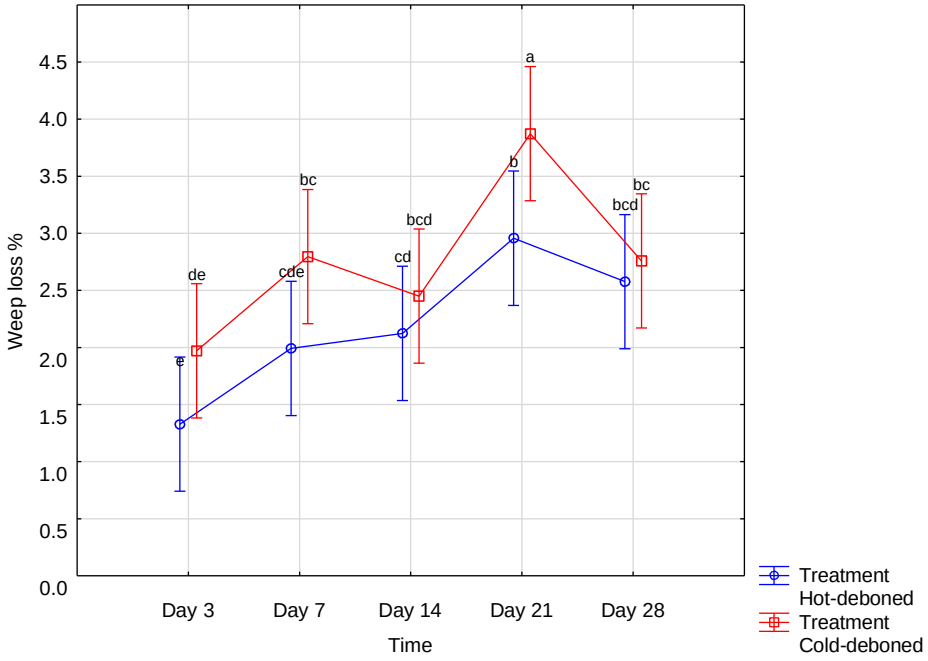

Figure 4.14 The mean weep loss % of hot- vs. cold-deboned ostrich fan fillet over the ageing period 28 d post-mortem. Significant difference is indicated by different letters ($p \leq 0.05$).

As seen in Table 4.9, the mean weep loss % for the rump steak had a no interaction between treatments and ageing time points ($p = 0.99$) throughout the 28 d ageing period. Although there was no significant difference between hot- and cold-deboning when compared at each ageing time point, a significant increase was seen between the mean weep loss % at all of the ageing time points (days 3, 14, 21 and 28 post-mortem; Fig. 4.15). The highest mean weep loss % was seen at day 28 post-mortem for both treatments (Fig. 4.15).

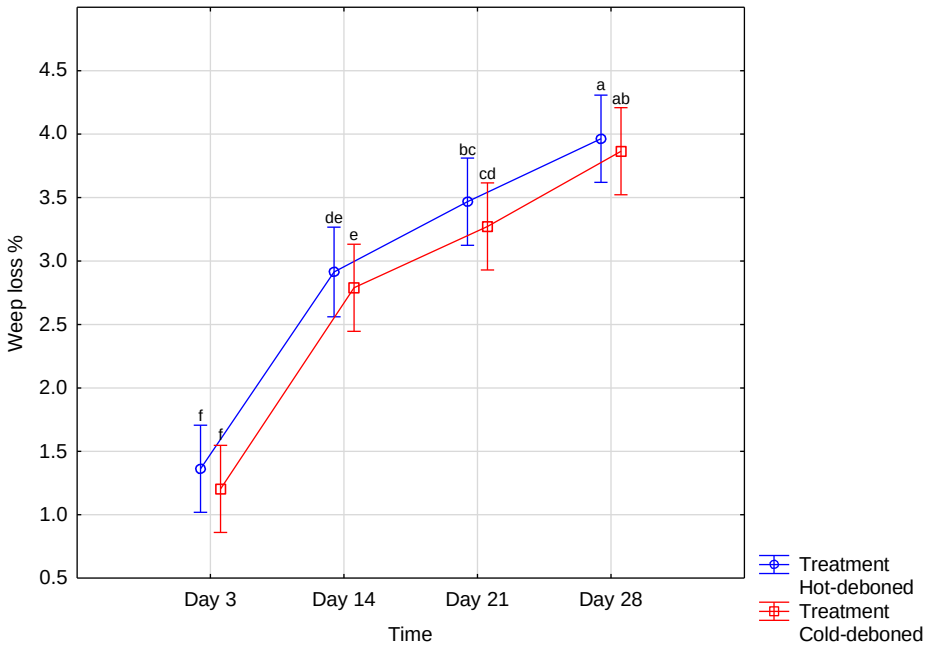

Figure 4.15 The mean weep loss % of hot- vs. cold-deboned ostrich rump steak over the ageing period 28 d post-mortem. Significant difference is indicated by different letters ($p \leq 0.05$).

The mean weep loss % for the big drum had no interaction between treatments and ageing time points ($p = 0.81$) throughout the 28 d ageing period. There was however a significant increase in all the ageing time points (days 3, 14 and 28 post-mortem) for both treatments, with the highest mean weep loss % reached at 28 d post-mortem (Table 4.9).

No interaction occurred between treatments and ageing time points for the mean weep loss % of the moon steak ($p = 0.75$) throughout the 28 d ageing period (Table 4.9). Although the increase between all the ageing time points (days 3, 14 and 28 post-mortem) were significant, the hot- and cold-deboned moon steak did not differ significantly (Table 4.9). The highest mean weep loss % for both the hot- and cold-deboned moon steak occurred at day 28 post-mortem (Table 4.9).

The mean weep loss % of the triangle steak had no interaction between treatments and ageing time points ($p = 0.27$) over the 28 d ageing period (Table 4.9). Furthermore, as seen in Table 4.9, hot- and cold-deboning did not have an effect on the mean weep loss % whereas the ageing time points did increase significantly between days 3 and 28 post-mortem

- *Cooking loss %*

The cooking loss % of the hot- and cold-deboned ostrich muscles over the ageing period 28 d post-mortem is given in Table 4.10.

There was no interaction between treatments and ageing time points for the mean cooking loss % of the fan fillet ($p = 0.54$; Table 4.10). Although the treatments did not differ, the mean cooking loss % of both the hot- and cold-deboned deboned fan fillet was significantly lower at day 7 post-mortem (significantly lower than days 3 and 14; Fig. 4.16). Thus, both the hot- and cold-deboned mean cooking loss % decreased from day 3 to day 7 after which a gradual increase was seen to reach the highest mean cooking loss % at 28 d post-mortem (Fig. 4.16).

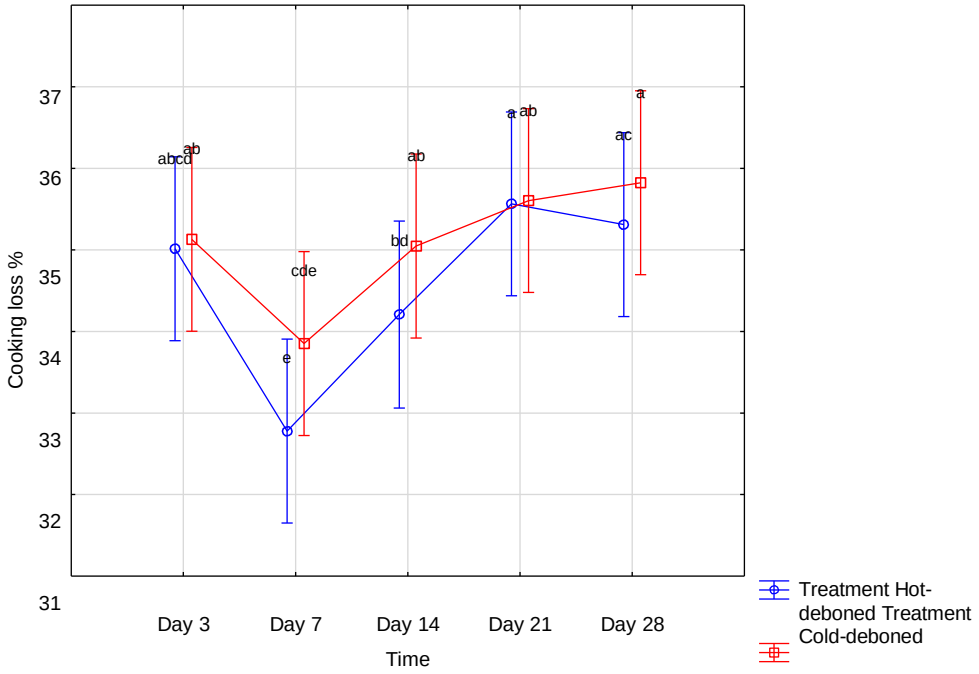

Figure 4.16 The mean cooking loss % of hot- vs. cold-deboned ostrich fan fillet over the ageing period 28 d post-mortem. Significant difference is indicated by different letters ($p \leq 0.05$).

Table 4.10 Hot- vs. cold-deboned ostrich muscle cooking loss % as per muscle type and treatment over the 28 d ageing period (Mean ± SD)

Measurement	Muscle type	Treatment	Ageing days post-mortem				
			3	**7**	**14**	**21**	**28**
Cooking loss %	Fan fillet	Hot-deboned	35.01^{abcd} ± 2.50	32.78^{e} ± 2.25	34.34^{bd} ± 1.97	35.56^{a} ± 2.26	35.31^{ac} ± 2.66
		Cold-deboned	35.13^{ab} ± 1.92	33.85^{cde} ± 2.18	35.05^{ab} ± 2.04	35.61^{ab} ± 2.11	35.82^{a} ± 2.00
	Rump steak	Hot-deboned	35.91^{cd} ± 1.07		36.58^{ab} ± 0.97	36.87^{ab} ± 1.12	35.75^{d} ± 1.27
		Cold-deboned	35.60^{d} ± 1.22		36.76^{ac} ± 1.48	37.04^{a} ± 1.41	36.01^{bd} ± 0.91
	Big drum	Hot-deboned	36.44^{a} ± 1.74		36.52^{a} ± 1.88		37.03^{a} ± 1.32
		Cold-deboned	36.09^{a} ± 2.38		36.65^{a} ± 1.30		36.19^{a} ± 2.06
	Moon steak	Hot-deboned	36.28^{d} ± 1.55		37.48^{abc} ± 1.61		36.83^{bcd} ± 1.23
		Cold-deboned	37.82^{ab} ± 2.24		38.57^{a} ± 1.60		36.89^{cd} ± 1.53
	Triangle steak	Hot-deboned	34.18^{a} ± 1.35				35.23^{b} ± 1.33
		Cold-deboned	35.13^{a} ± 1.82				34.80^{b} ± 1.88

$^{a-g}$ Means in the same double row per muscle with different superscripts differed significantly between ageing time points ($p \leq 0.05$).

As seen in Table 4.10, no interaction was found between treatments and ageing time points (p = 0.63) for the cooking loss of the rump steak. As indicated further in Fig. 4.17, no significant difference occurred between the hot- and cold-deboned mean rump steaks' cooking loss %. However, the ageing time points (days 3, 14 and 28) did differ with a significant increase in the mean cooking loss % between days 3 and 14 followed by a significant decrease at day 28 post-mortem (Fig. 4.17).

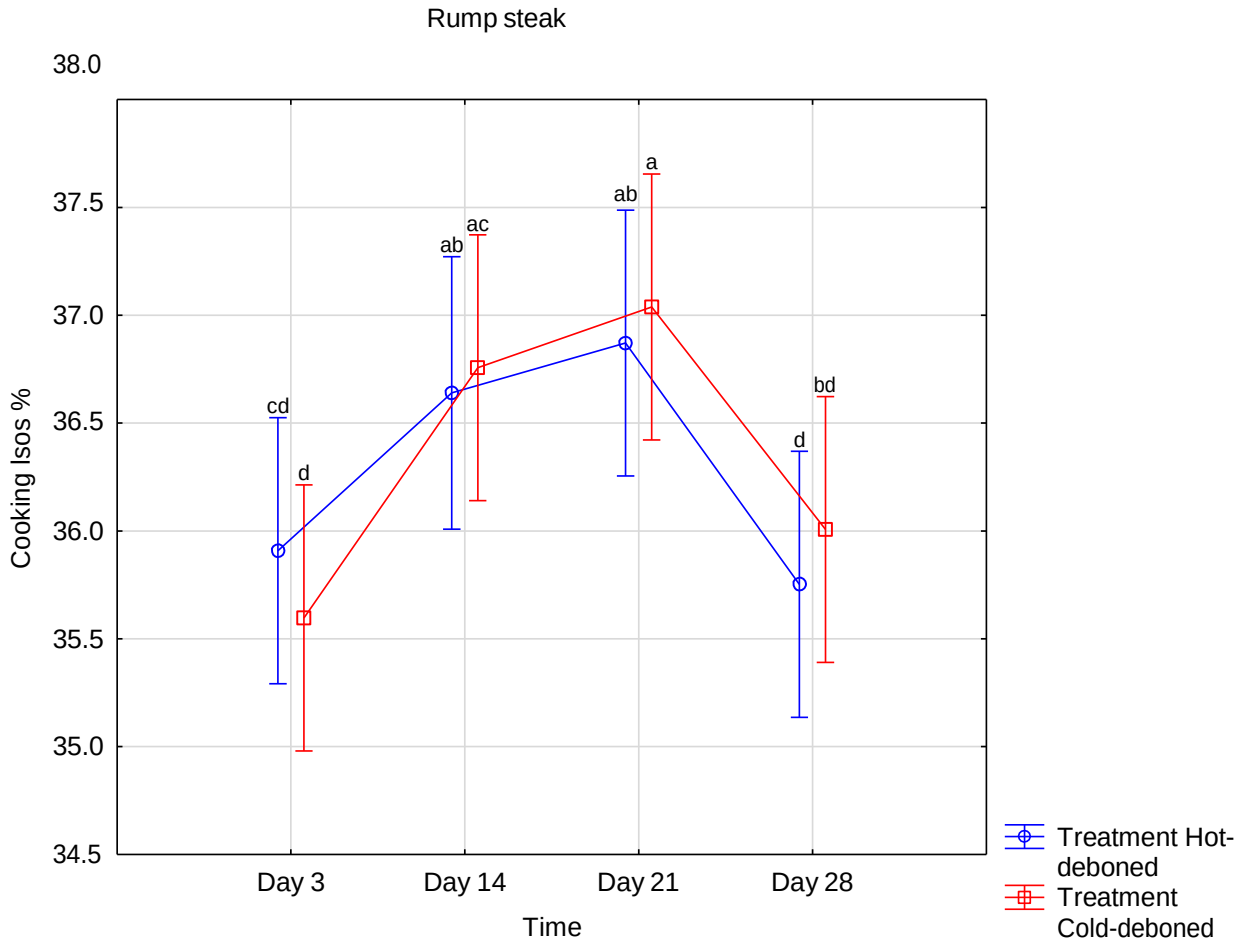

Figure 4.17 The mean cooking loss % of hot- vs. cold-deboned ostrich rump steak over the ageing period 28 d post-mortem. Significant difference is indicated by different letters (p ≤ 0.05).

The mean cooking loss % of the big drum showed no interaction between treatments and ageing time points (p = 0.52). Furthermore, there was no significant difference between hot- and cold-deboning or between the ageing time points at days 3, 14 or 28 post-mortem (Table 4.10). The highest mean cooking loss % was seen at day 28 post-mortem for both the hot- and cold-deboned big drum (Table 4.10).

No interaction occurred between treatments and ageing time points in the mean cooking loss % of the moon steak ($p = 0.07$; Table 4.10). The mean cooking loss % of the cold-deboned moon steak was significantly higher than the hot-deboned at day 3 post-mortem and further showed a significant increase towards day 14 before significantly decreasing towards day 28 (Table 4.10). The mean cooking loss % for the hot-deboned moon steak however only showed a significant increase between days 3 and 14 post-mortem (Table 4.10). Thus, the highest mean cooking loss % for both the hot- and cold-deboned moon steak was found to be 14 d post-mortem (Table 4.10).

No interaction was found between treatments and ageing time points of the mean cooking loss % of the triangle steak ($p = 0.09$) over the 28 d ageing period (Table 4.10). Although non-significant, the mean cooking loss % of the hot-deboned triangle steak increased from days 3 to 28, whereas the cold-deboned triangle steak non-significantly decreased from 3 to 28 d post-mortem (Table 4.10).

4.3.4 Warner-Bratzler shear force (WBSF)

The Warner-Bratzler shear force values (N) of the 5 aged ostrich muscles are presented in Table 4.11. The mean WBSF values are given in terms of the hot- vs. cold-deboned muscles at each ageing time point.

No interaction was found between the mean fan fillet WBSF values and ageing time points over the 28 d ageing period ($p = 0.31$; Table 4.11). The lowest mean WBSF value for the hot-deboned fan fillet was reached 21 d post-mortem (also the lowest WBSF value overall), and did not increase towards 28 d post-mortem. The mean WBSF values for both the hot- and cold-deboned fan fillet exponentially decreased ($y = -0.08 + (0.62)(1 - 0.87)^{x}$) over the 28 d (x) ageing period ($R^2 = 0.36$; Fig. 4.18).

Table 4.11 Hot- vs. cold-deboned ostrich muscle Warner-Bratzler shear force (WBSF, N) per muscle, treatment and ageing period (Mean ± SD)

Measurement	Muscle type	Treatment	Ageing days post-mortem				
			3	**7**	**14**	**21**	**28**
WBSF	Fan fillet	Hot-deboned	35.78^{a} ± 8.47	28.02^{bdf} ± 9.76	27.20^{bcdefg} ± 8.81	26.04^{ceg} ± 8.39	26.31^{bcdefg} ± 10.31
		Cold-deboned	35.16^{a} ± 7.87	29.89^{bc} ± 7.27	28.90^{bcde} ± 9.56	27.27^{defg} ± 8.05	26.55^{fg} ± 8.45
	Rump steak	Hot-deboned	56.23^{a} ± 15.75		44.13^{b} ± 15.42	37.57^{cd} ± 10.83	34.74^{d} ± 12.14
		Cold-deboned	54.44^{a} ± 14.48		43.91^{bc} ± 14.53	34.55^{d} ± 13.50	26.55^{e} ± 8.45
	Big drum	Hot-deboned	51.92^{a} ± 13.03		42.63^{b} ± 10.66		35.00^{cd} ± 9.34
		Cold-deboned	53.94^{a} ± 15.65		38.19^{bc} ± 12.61		31.04^{d} ± 11.25
	Moon steak	Hot-deboned	71.70^{a} ± 15.50		56.11^{b} ± 15.91		48.23^{c} ± 13.01
		Cold-deboned	73.16^{a} ± 16.05		59.60^{b} ± 16.05		43.54^{c} ± 12.68
	Triangle steak	Hot-deboned	46.93^{a} ± 13.47				39.33^{b} ± 13.57
		Cold-deboned	49.58^{a} ± 14.12				33.86^{b} ± 11.48

$^{a-g}$ Means in the same double row per muscle with different superscripts differed significantly between ageing time points ($p \leq 0.05$).

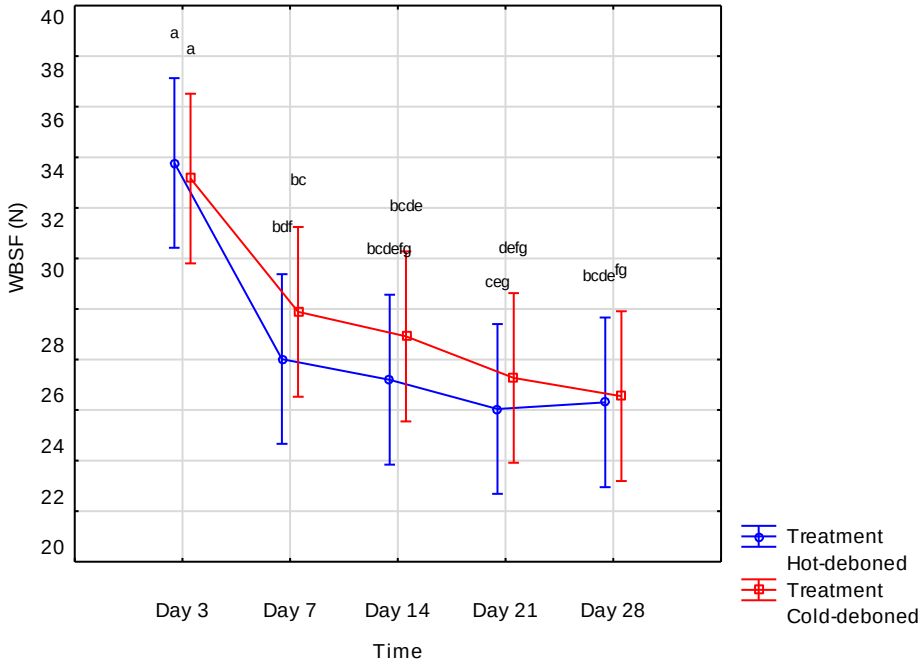

Figure 4.18 The ostrich fan fillet WBSF values over the course of the ageing period 28 d post-mortem. Different letters indicate significant difference ($p \leq 0.05$).

The mean rump steak WBSF values (N) showed an interaction between hot- and cold-deboning and ageing time points ($p = 0.00$) over the course of the 28 d ageing period (Table 4.11). The rump steak (Fig. 4.19) did not differ between hot- and cold-deboning days 3, 14 or 21 post-mortem. There was however a difference at day 28, where the hot-deboned rump steak had a significantly higher mean WBSF value (Fig. 4.19). Moreover, all of the ageing time points (days 3, 7, 14 and 21 post-mortem) differed significantly. Both the hot- and cold-deboned rump steak had decreasing WBSF values ($y = -27.52 + (27.81)(1 - 0.71)^x$) over the 28 d (x) ageing period as seen in Fig. 4.19 ($R^2 = 0.58$).

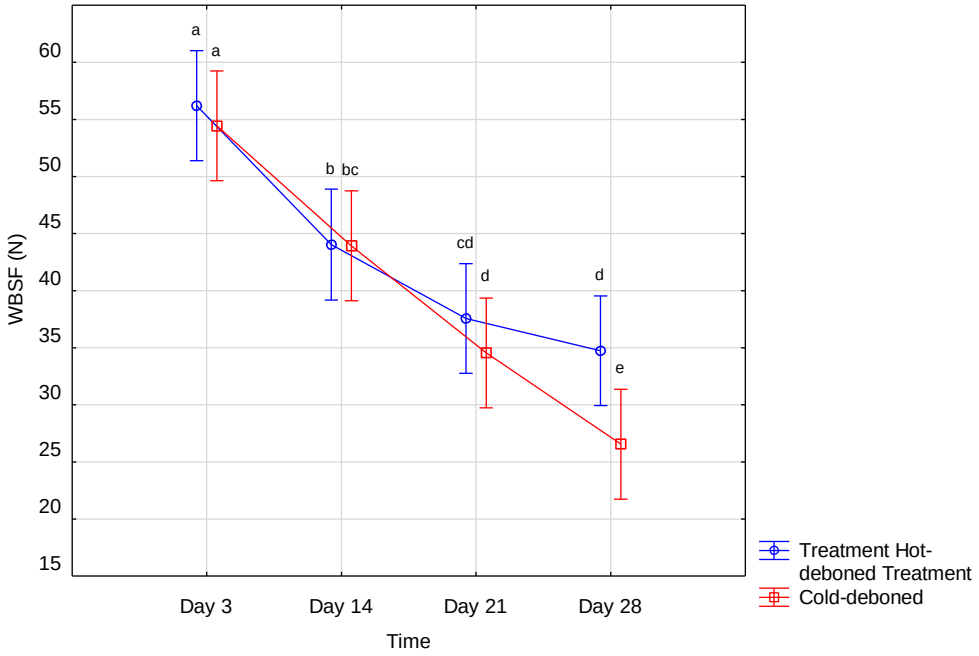

Figure 4.19 The ostrich rump WBSF values over the course of the ageing period 28 d post-mortem. Different letters indicate significant difference ($p \leq 0.50$).

The mean WBSF values for the big drum showed an interaction between hot- vs. cold-deboning and ageing time points ($p = 0.00$) over the 28 d ageing period (Table 4.11). Although significant differences were found between the ageing time points between days 3, 14 and 28 post-mortem, there were no differences in the mean WBSF values between hot- and cold-deboned at the given ageing time points. The big drum showed a descending trend in the WBSF values over the 28 d ageing period with the lowest WBSF values reached at day 28 post-mortem (Table 4.11).

An interaction was found between treatments and ageing time points ($p = 0.00$) for the mean WBSF values of the moon steak (Table 4.11). The moon steak showed descending WBSF values for both treatments over the course of the 28 d ageing period (Table 4.11). Although no difference was found between hot- and cold-deboning, significant decrease was seen between the ageing days 3, 14 and 28 with the lowest mean WBSF values reached at 28 d post-mortem (Table 4.11).

An interaction was seen between treatments and ageing time points in the mean WBSF values of the triangle steak ($p = 0.00$) over the 28 d ageing period (Table 4.11). The hot- and cold-deboned triangle steak showed significant difference in the mean WBSF values on both 3 and 28 d post-mortem (Table 4.11). On day 3, the cold-deboned triangle steak had a higher mean WBSF value, whereas the hot-deboned triangle steak had a higher mean WBSF value

on day 28 (Table 4.11). Although the ageing time points differed significantly, the difference between hot- and cold-deboning was non-significant (Table 4.11).

4.4 Discussion

It is well-known that the glycogen content of muscles at slaughter usually influences the pH decline and the ultimate pH that is reached (Zarasvand *et al*., 2012). As seen in Table 4.3 the mean pH_u values at the individual ageing days ranged from a pH_u value of 5.83 upwards to reach a maximum value of pH_u 6.06. Although a value of pH_u 6.06 is above the typical intermediate range of ostrich meat varying between normal (pH < 5.8) and dark, firm and dry (DFD; pH > 6.2), the mean pH_u values over the 28 d ageing period (Table 4.3), shows the pH_u values are still within the aforementioned range (Sales & Mellett, 1996).

Given that no significant differences were found between the mean pH_u values of hot- and cold-deboned fan fillet, rump steak, big drum, moon steak and triangle steak muscles over the 28 d ageing period ($p = 0.50$), it can be concluded that hot-deboning does not have an effect (significantly different to that of cold-deboning), on the post-mortem pH decline or pH_u in the relevant ostrich muscles (Table 4.3; Fig. 4.1).

Although no interaction between the five muscles and treatments (hot- vs. cold-deboned) over the ageing period of 28 d was found in the mean pH_u values ($p = 0.50$), differences between the five individual muscles (irrespective of the deboning method), were found ($p = 0.00$). This could be ascribed to the fact that muscle type is the most complicated intrinsic factor influencing pH_u due to different anatomical muscle locations and functions within a carcass (Lawrie & Ledward, 2006a).

The fan fillet showed no significant differences between the mean hot- and cold-deboned pH_u values over the ageing period 28 d post-mortem ($p = 0.80$). There was however a significant increase in the fan fillet pH_u for both treatments between days 3 and 7 (Fig. 4.2). This is similar to that found by Sales and Mellett (1996) concerning an unusual increase in the pH of the fan fillet over time after the initial pH decline. Moreover, there was no significant difference between hot- and cold-deboning in the pH_u values of the big drum (Table 4.3) ($p = 0.18$). Botha *et al*. (2007) similarly found no significant difference in the pH_u values between hot- and cold-deboned big drum muscles over an ageing period of 21 d post-mortem. However, the cold-deboned big drum did indicate a significant increase in the mean pH_u values between days 3 and 14 post-mortem where after it decreased non-significantly at day 28 post-mortem (Table 4.3). Meat colour, water holding capacity (WHC) and tenderness is known to be directly affected by the pH_u of meat (Honikel, 2004). As soon as the pH_u is reached, denaturation of one of the sarcoplasmic proteins namely myoglobin, which is the main muscle pigment, speeds up oxidation of its iron to the ferric form resulting in the muscle pigment turning brown (metmyoglobin). Although not a broad process, it is still considered important, as it occurs near exposed surfaces. Such factors such as dryness can introduce denaturation and

discolouration which is also linked to persisting activity of oxygen consuming enzymes at 0°C (Lawrie & Ledward, 2006b).

For all of the investigated colour parameters (L*a*b*, hue angle and Chroma) significant differences were observed between the ageing time points, with only a few significant differences between hot- vs. cold-deboning (Tables 4.4 – 4.8). Overall, hot-deboned muscles matched cold-deboned muscles in terms of differences and trends between ageing time points (Tables 4.4 – 4.8).

The mean CIE L* values of the fan fillet, rump steak, big drum and moon steak of both treatments showed a gradual increase between days 3 and 21, followed by a decrease between days 21 and 28 post-mortem (with values still significantly higher at day 28 compared to day 3; Table 4.4). This trend might be ascribed to microbial degradation which will be further discussed in Chapter 5. The triangle steak similarly had a higher mean CIE L* value at day 28 showing an increase in lightness throughout the 28 d ageing period (Table 4.4). Similar to what was found on day 3 post-mortem (Chapter 3), the moon steak continually showed the highest mean CIE L* values indicative of a lighter muscle colour whereas the triangle steak had the darkest muscle colour in terms of mean CIE L* values (Table 4.4). Although significant differences in the mean CIE L* values were seen between ageing time points, hot-deboned muscles were significantly lighter at day 7 for the fan fillet, day 21 for the rump steak and at day 28 for the triangle steak (Table 4.4).

The mean CIE a* values of the fan fillet, rump steak, big drum and moon steak of both treatments showed a gradual increase between days 3 and 14 after which a decrease was seen towards day 28 (Table 4.5). The mean CIE a* values of these muscles at day 28 post-mortem was generally lower than the starting values at day 3, which was also true in the case of the triangle steak (Table 4.5). Over the course of the 28 d ageing period, the fan fillet had the highest mean CIE a* values (Table 4.5) suggesting a more red muscle colour which correlates with the results at day 3 post-mortem (Chapter 3). Furthermore, the moon steak had the lowest mean CIE a* values over the 28 d ageing period (Table 4.5) indicating a more green muscle colour. Although the moon steak had the lowest mean CIE a* values (Table 4.5), it did not differ significantly from the mean CIE a* values of the big drum which had the lowest values at day 3 post-mortem (Chapter 3).

The mean CIE b* values of the different muscles showed varying trends over the 28 d ageing period (Table 4.6). The fan fillet did however show the highest mean CIE b* values over the 28 d period (Table 4.6) suggesting a more yellow colour (yellow-red as it had the highest mean CIE a* values) which corresponds with day 3's post-mortem findings (Chapter 3). Difference between treatments was only seen in the rump steak which had significantly higher mean CIE b* values at days 14 and 28 post-mortem for cold-deboning (Table 4.6). Moreover, the moon steak showed the lowest mean CIE b* values indicative of a more blue colour (blue-green as the moon steak had the lowest mean CIE a* values). Although the big drum had the lowest mean CIE b* values at day 3 post-mortem (Chapter 3), the moon steak and big

drum did not significantly differ throughout the 28 d ageing period (Table 4.6).

The mean hue angle values of the rump steak, big drum and moon steak decreased between days 3 and 21 where after it increased at day 28 post-mortem (Table 4.7). The hot-deboned fan fillet however showed an initial increase between days 3 and 7, followed by a similar trend as the aforementioned muscles (Table 4.7). The only significant differences in mean hue angle values between hot- and cold-deboning were seen in the fan fillet at days 7 and 28 post-mortem. Moreover, linking with the highest mean CIE a* values, the fan fillet generally had the highest mean hue angle and Chroma values over the 28 d ageing period (Table 4.6 – 4.8), signifying that the fan fillet was the muscle with the most red, most vivid colour, as well as the highest colour intensity. This is similar to the results found at day 3 post-mortem (Chapter 3). Furthermore, the big drum had the lowest mean hue angle values (least colour intensity) over the course of the 28 d (Table 4.7), similar to the mean hue angle values of the big drum at day 3 post-mortem (Chapter 3).

The mean Chroma values over the duration of the 28 d ageing period showed that the fan fillet, rump steak, big drum and moon steak increased from day 3 towards day 14, where after it decreased towards day 28 post-mortem (Table 4.8). The mean Chroma values of the hot-deboned fan fillet, rump steak and big drum were significantly lower at day 28 post-mortem (Table 4.8). Correlating with the lowest mean CIE a* and b* values, the lowest mean Chroma value was seen in the moon steak (Table 4.8). This signifies that the moon steak had the lowest saturation in muscle colour over the ageing period together with the increased lightness also seen in the moon steak (Table 4.4).

The high pigment content (22 mg Fe/g meat) and the state thereof in ostrich meat is known to be the reason for the distinguishing red colour variation amongst muscles (Balog & Almeida, 2007). The fan fillet (*M. iliofibularis*) and triangle steak (*M. flexor cruris lateralis*) are known to have a higher pigment content which presents a darker or more intense colour in these muscles. Furthermore, ostrich meat colour (CIEL* values in particular), may be influenced by slaughter procedures or feeding practises. Additionally, the higher bound water content (due to the high muscle pH) in ostrich meat causes a decreased reflection and an increased absorption of radiance contributing to the darker colour of ostrich meat. This emphasizes the effect of WHC on meat colour (Balog & Almeida, 2007).

The WHC of meat is of great importance since it has a direct influence on the juiciness and appearance of meat (Brewer, 2004) and can be defined as the ability of meat to sustain its own or added water when compression or heat is applied. The amount of liquid freed from its binding to muscle proteins in uncooked meat is referred to as drip or weep exudation and is expressed as a cumulative weep loss percentage over the course of the ageing 28 d post-mortem. The point of minimum WHC is found at the iso-electric point (pH 5.4 – 5.5) of the meat (muscle proteins) which generally signifies the pH_u. The changing factor in the moisture loss of cooked meat is heat application. Factors such as cooking time, temperature and method influence shrinkage during cooking (expressed as cooking loss percentage) which is generally

much greater than the weight loss through drip/weep exudation in uncooked meat (Lawrie & Ledward, 2006c).

Hot- and cold-deboned muscles showed a gradual increase in the mean weep loss percentage of the rump steak, big drum, moon steak and triangle steak over the progression of the ageing period, with the highest weep loss percentage at day 28 post-mortem (Table 4.9). The mean weep loss percentage of the fan fillet (both treatments), however only showed a gradual increase until day 21 where after a decrease occurred towards day 28 (Table 4.9). Thus, both the hot- and cold- deboned fan fillet had the highest mean weep loss percentage at day 21 post-mortem (Table 4.9). Furthermore, significant differences were seen between ageing time points, whilst the only significant difference between treatments was seen in the fan fillet at day 21 (Table 4.9). The cold-deboned fan fillet had a significantly higher mean weep loss percentage at day 21 post-mortem as seen in Table 4.9. Similar to the mean drip loss percentage of the big drum at day 3 post-mortem (Chapter 3), the mean weep loss percentage of the big drum over the 28 d ageing period was the lowest (Table 4.9).

This finding is in contrast with Botha *et al.* (2007) who found that the hot-deboned big drum showed a significantly higher cumulative weep loss percentage over a 21 d ageing period than the cold-deboned big drum. It can be noted that membranes were not removed from the big drum in the study of Botha *et al*., (2007), whereas membranes were removed from the muscles used in this study. Membranes are defined as connective tissue surrounding the outer layer of a muscle (epimysium), and consequently it can be postulated that the WHC was influenced by the presence of connective tissue and time of deboning post-mortem (Davies, 2004).

The mean cooking loss percentage of the different muscles showed varying trends over the 28 d ageing period (Table 4.10). Although there were significant differences between ageing time points, the only difference between treatments was seen in the moon steak at day 3, where the cold-deboned muscle had a significantly higher mean cooking loss percentage (Table 4.10). Furthermore, the moon steak also had the highest mean cooking loss percentage throughout the 28 d ageing period, correlating with the results found at day 3 post-mortem (Chapter 3). Comparable to the study that Botha *et al*. (2007) conducted, no significant difference was found in the cooking loss percentage between the hot- and cold-deboned big drum.

During the progression of ageing, the structure of protein in meat denatures. This denaturation can be described as an intramolecular or physical reorganisation excluding hydrolysis of chemical bonds linking amino acids of polypeptide chains. The denatured proteins are especially prone to attack by proteolytic enzymes. Although there has been much debate about which proteins go through proteolysis, collagen and elastin of connective tissue seems to undergo the least extensive proteolysis. A complex correlation between pH_u and proteolysis exist where the extent of proteolysis is believed to be comparatively higher at a high pH_u as has been found in ostrcih meat (Lawrie & Ledward, 2006b).

All of the investigated muscles showed a gradual decrease in mean WBSF values over the progression of the 28 d ageing period with the highest mean WBSF at day 3, and the lowest mean WBSF at day 28 post-mortem (Table 4.11). The ageing time points did differ significantly, whilst the only difference between treatments were seen in the significantly higher hot-deboned rump steak at day 28 post-mortem (Table 4.11).

Although the hot-deboned rump steak had a significantly higher mean WBSF value at day 28 post-mortem with 34.74 N, the value still falls within the range of tender meat (< 42.87 N), as reported by Destefanis *et al*. (2008). Additionally, both the mean hot- and cold-deboned WBSF values decreased significantly between days 3, 14, 21 and 28 post-mortem. Significantly decreasing mean WBSF values were seen for both treatments in the big drum and moon steak at days 3, 14 and 28 post-mortem. Botha *et al*. (2007) found the hot-deboned big drum to be tougher up to 5 d post-mortem where-after no significant difference in comparison to cold-deboned muscles could be found (up to 21 d post-mortem). In this study, there was no significant difference found between hot- and cold-deboning in the mean WBSF of either the big drum or moon steak between days 3, 14 and 28 post-mortem (Table 4.11). It can however be noted that the moon steak had the highest mean WBSF values over the course of the 28 d, which correlates with the high cooking loss % of the moon steak (Table 4.10). This is similar to the mean WBSF values of the moon steak at day 3 post-mortem, which was also found to be the toughest muscle (Chapter 3). Nonetheless, at day 28 post-mortem, both the hot- (48.23 N) and cold-deboned (43.54 N) moon steak had tenderness values classified as intermediate ranging from 42.87 – 52.68 N (Destefanis *et al*., 2008).

Ostrich meat is generally known and appreciated for its tenderness which makes it especially noteworthy to mention that all five investigated muscles decreased in toughness over the ageing period reaching the most tender mean WBSF reading at 28 d post-mortem (regardless of treatment) (Table 4.11). The connective tissue within ostrich muscles forms part of the collagen to protein ratio (low collagen level of 0.44 on average) and is responsible for the meat texture. Moreover, the muscle fibre arrangement within ostrich muscles are known to be crosswise which may contribute to the meat tenderness (Balog & Almeida, 2007).

4.5 Conclusions

Hot-deboned muscles did not significantly differ from cold-deboned muscles in terms of the physical meat quality parameters investigated over a 28 d ageing period post-mortem. The five investigated muscles, including the fan fillet *(M. iliofibularis);* rump steak *(M. iliotibialis lateralis);* big drum *(M. gastrocnemius, pars interna);* moon steak *(M. femorotibialis medius)* and triangle steak *(M. flexor cruris lateralis),* did however generally show differences between muscle type and between ageing time points.

While the mean pH_u values of all of the muscles over the 28 d period were within in the expected range for ostrich meat (hot- and cold-deboned), differences occurred between the various muscles. Concerning meat colour, the various muscles also showed significant

differences in comparison and between ageing time points, whilst only differing in selected instances in terms of treatment. It can thus be concluded that hot-deboning had no major negative effect on the colour of ostrich meat over a 28 d ageing period. The results of hot-deboned muscles matched that of cold-deboned muscles when assessing all of the investigated colour parameters. It can be noted that the fan fillet (*M. iliofibularis*) had the most red-yellow (a*; b*), most vivid (hue angle) muscle colour, with the highest colour intensity (Chroma). Furthermore, the moon steak (*M. femorotibialis medius*) had the most green-blue (a*; b*) muscle colour with the least colour saturation (Chroma), as well as having the lightest (L*) muscle colour over the progression of the 28 d ageing period. Concerning WHC, all of the investigated muscles showed a gradual increase in the mean weep loss percentage with the highest loss reached at day 28 post-mortem. Only the fan fillet (*M. iliofibularis)* had the highest mean loss percentage at day 21 with a significantly higher mean weep loss percentage seen in the cold-deboned muscle. Moreover, all of the investigated muscles showed a gradual decrease in mean WBSF values over the progression of the 28 d ageing period with the highest mean WBSF at day 3, and the lowest mean WBSF at day 28 post-mortem. The ageing time points did differ significantly, whilst the only difference between treatments were seen in the significantly higher hot-deboned rump steak (*M. iliotibialis*) at day 28 post-mortem (with values within the range of tender meat). The moon steak (*M. femorotibialis medius*) *was* the toughest throughout the 28 d ageing period correlating with its high cooking loss percentage.

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CHAPTER 5

Microbiological quality and safety of five hot- vs. cold-deboned ostrich (*Struthio camelus*) muscles during post-mortem ageing

Abstract

Hot-deboning as an alternative excising method for the South African ostrich industry was investigated. Five ostriches were used for the study with the muscles hot-deboned (within 90 min post-mortem) from the left leg and cold-deboned (24 h post-mortem) from the right leg. Five ostrich muscles: fan fillet (*M. iliofibularis*); rump steak (*M. iliotibialis lateralis*); big drum (*M. gastrocnemius, pars interna*); moon steak (*M. femorotibialis medius*); and triangle steak (*M. flexor cruris lateralis)* used in a vacuum packaged ageing trial for 28 d post-mortem (0 - 4°C), were used to evaluate the microbial load when compared to that found on d 3. An absence of *Salmonella* spp. was found in all hot- and cold-deboned samples. Hot-deboning did not have an effect on the mean Aerobic Counts (AC) nor the *Enterobacteriaceae* counts over the 28 d ageing period, and matched that of cold-deboned muscles ($p \leq 0.05$). Aerobic and *Enterobacteriaceae* counts for hot- and cold-deboned muscles (initial and over the ageing period), were however higher in comparison with fresh meat standards used by the industry; whether this is standard or due to the specific execution of the experiment *per se* is unclear. The microbial quality therefore necessitates further investigation for the South African ostrich industry.

Keywords: Hot-deboning, Cold-deboning, Ostrich, Microbial quality, Ageing

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5.1 Introduction

One of the key elements in producing wholesome meat is that it should be safe for consumption. Meat should not include any parasites which may infect humans and should be free from harmful chemicals and microbiological pathogens (Warriss, 2000). The negative changes in meat observed by consumers are often due to microorganisms when microbes inside or on the surface of meat cause spoilage. The temperature and environment at which meat is held are external factors that will affect the presence and growth of spoilage microorganisms (Gill, 2004).

During recent years, the possibility of meat products acting as a source of food-borne pathogenic microorganisms has been a rising topic of interest. Deliberating the main microbial factor causing spoilage, the conditions under which meat is generally handled, processed and stored, are more important than the concentration of microorganisms that are present early on (i.e. at slaughter). Numerous sources may infect exposed meat cuts throughout the process of slaughtering, dressing, chilling and excising. Generally, these sources include: feed, hides, soil, water, air, intestines, lymph nodes, processing apparatus and humans. The key microbial contamination sources throughout dressing however remains the skin, feathers and other animals in a nearby vicinity. Incoming meat is said to be the main source of microbial contamination during deboning (Kourtsoumanis and Sofos, 2004; Hoffman *et al*., 2010b; Shange *et al*., 2018; 2019).

During skinning and evisceration in an ostrich abattoir, slaughter procedures were found to contribute to carcass contamination. Concerning Aerobic Plate Counts (APC), *Pseudomonas* spp. and *Staphylococcus aureus* counts, regulated levels of the original microbial load deposited on the carcass during skinning, was maintained. If the skinning process can be performed with more care, a lower number of bacteria may however be deposited on carcasses (Karama *et al*., 2003; Hoffman *et al*., 2010b; Shange *et al*., 2019).

In the past, South Africa has principally been an exporter of ostrich meat (National Agricultural Marketing Council, 2003; Department of Agriculture, Forestry & Fisheries, 2017). However, with the current ban that is placed on the export of ostrich meat, emphasis is placed on investigating ways in which the South African ostrich industry can lower production cost to gain economic strength. Presently, cold-deboning is used as excising method where carcasses are chilled for 24 h post-mortem (0 – 4°C) preceding deboning (Hoffman *et al*., 2007). The conventional deboning of ostriches is thus performed after the completion of *rigor mortis* once carcasses have achieved the chiller's temperature.

An alternative deboning method where carcasses are excised prior to refrigeration, namely hot-deboning, can be described as the practice where lean meat and fat are detached from carcasses before a big drop in body temperature occurs (Waylan & Kastner, 2004). With hot-deboning, excision is performed 2 – 4 h post-mortem opposed to 24 h post-mortem, and

holds several possible advantages for the South African ostrich industry. Since ostriches normally enter *rigor mortis* within 45 min post-mortem (Hoffman *et al*., 2007), the risk of ostrich muscles developing cold-shortening which is frequently seen when pre-rigor meat is hot-deboned, can be limited. A major proposed benefit of hot-deboning is the economic advantage of reduced energy costs due to less refrigeration space required. Furthermore, it has been suggested that the functional properties of each muscle can be enhanced according to its intrinsic characteristics as muscles are removed very early on post-mortem (Waylan & Kastner, 2004; Farouk *et al.*, 2009).

The most critical factor influencing microbial growth is however temperature. Consequently, a higher temperature is known to facilitate greater microbial growth. Organisms commonly have a small temperature range in which they will grow optimally, but the general temperature ranges between 1 and 60°C (Lawrie & Ledward, 2006). With the performance of hot-deboning, Sheridan and Sheriton (1982) suggested that hot-deboned beef might have intrinsic characteristics which encourage microbial growth. These include an increased initial temperature, as well as cuts with a higher exterior a_w. However, Sheridan and Sheriton (1982) concluded that no loss of shelf-life was experienced in hot-deboned vacuum packed beef stored at normal refrigeration temperatures.

Although hot-deboning can cause concern in terms of the microbial quality and safety of the meat due to the higher deboning temperature, it might also be advantageous. Due to metabolic activity in pre-rigor muscles, average carcass temperatures have been known to increase to 40°C following slaughter. While carcasses are usually chilled from 40°C to the chiller's temperature prior to deboning, this leaves scope for the proliferation of spoilage and pathogenic microorganisms. With hot-deboning of carcasses however, carcasses are deboned and packaged (normally anaerobically) while still hot, and with ostriches developing *rigor mortis* within 45 min post-mortem, this can contribute to resisting microbial spoilage of meat (Stopforth & Sofos, 2005).

The prevalence and intensity occurrence of bacteria must be known to function as indicators in an effort to ensure proper and continued functioning of a microbial control system. With emphasis on indicator organisms (defined as groups or clusters of bacteria which might reveal malfunctions or insufficiencies of processes created and applied for regulating pathogens), microbial observation is possible. Both Aerobic Counts (AC) of bacteria, and *Enterobacteriaceae* populations are implemented as indicator organisms for the microbial quality of ostrich meat. Pathogenic microorganisms which are indicative of the microbial safety of ostrich meat, include *Salmonella* spp. (Kourtsoumanis and Sofos, 2004; Department of Agriculture, Forestry and Fisheries, 2017). This is especially important for hot-deboning as the membranes (connective tissue surrounding the outer layer of a muscle defined as epimysium), of muscles are removed (Davies, 2004). This implicates that muscles are more susceptible to microbial degradation before vacuum packaging due to organisms present in the environment of the abattoir, specifically the deboning room (Hoffman *et al*., 2010b).

This study was carried out to determine the microbiological quality and safety (AC, *Enterobacteriaceae* and *Salmonella* spp.) of five hot- and cold-deboned vacuum packed ostrich muscles, stored at 0 – 4°C, throughout a 28 d ageing time period (days 3, 7, 14, 21 and 28). Microbiological analysis was conducted on five representative ostrich muscles used in the ageing trial (Chapter 4), including: fan fillet (*M. iliofibularis*); rump steak (*M. iliotibialis lateralis*); big drum (*M. gastrocnemius, pars interna*); moon steak (*M. femorotibialis medius*); and triangle steak (*M. flexor cruris lateralis*). Although Botha *et al*., (2007) conducted a post-mortem storage trial (including microbiological analysis) between hot- vs. cold-deboned ostrich muscles, a 42 d ageing time period was carried out on only two high value commercial ostrich cuts, namely the big drum (*M. gastrocnemius, pars interna*) and fan fillet (*M. iliofibularis*). The microbiological analysis on the aforementioned two muscles were also only carried out on two ageing time intervals namely days 1 and 42 post-mortem. It can be mentioned that vacuum packed ostrich muscles which were exported prior to the ban on ostrich meat, could take up to 42 d to reach the export market. Nonetheless, this ageing trial was conducted over a period of 28 d. This ageing time period with seven day intervals progressing to 28 days (days 3, 7, 14, 21 and 28 post-mortem) was chosen, as 28 d is accepted as the benchmark for microbiological analysis where decline is usually observed. Trends over the progression of the time period can also be seen more clearly because analysis is performed on all of the ageing intervals (Sheridan & Sherington, 1982).

5.2 Materials and methods

5.2.1 Ostriches and muscle samples

Following the materials and methods section described in Chapters 3 and 4, microbiological analysis was performed on both physical and ageing samples.

Table 5.1 Description of ostrich muscles used for ageing trial as per commercial and scientific names

Fan fillet	*M. iliofibularis*
Rump steak	*M. iliotbialis lateralis*
Big drum	*M. gastocnemius, pars interna*
Moon steak	*M. femorotibialis medius*
Triangle steak	*M. flexor cruris lateralis*

A sample for microbiological analysis (25 g) was cut aseptically from each steak at day 3 (physical analysis), after which it was vacuum packaged and frozen at -20°C (Chapter 3, section 3.2). A 25 g sample was also aseptically cut at each of the ageing time points during the progression of the 28 d ageing period (Table 5.2). Microbiological samples were then vacuum packed and stored at -20°C. The cutting of all samples for microbiological analyses was done so before any physical meat quality tests were performed, and was done with a sterile knife, tweezer and cutting board to cut a representative 25 g sample of each steak (around the outer layer).

Table 5.2 Overview of the microbiological sampling days as per main effects (hot- vs. cold-deboning, muscle type and ageing time points)

Number of animals	**Deboning Treatment**	**Muscle**	**Ageing period (days post mortem)**				
# 1 – 15	Hot-deboned	Fan fillet	3	7	14	21	28
		Rump steak	3		14	21	28
		Big drum	3		14		28
		Moon steak	3		14		28
		Triangle steak	3				28
	Cold-deboned	Fan fillet	3	7	14	21	28
		Rump steak	3		14	21	28
		Big drum	3		14		28
		Moon steak	3		14		28
		Triangle steak	3				28

5.2.2 Microbiological analysis

Samples for each muscle taken at every time point (Table 5.2: days 3, 7, 14, 21 and 28, or part thereof) were frozen (-20°C) until microbial analyses could commence. Microbial analysis was performed on the five muscles removed from five ostriches (randomly selected).

- Sample preparation: Tempo system

  As seen in Fig. 5.1, prior to analysis, samples were thawed overnight at 4°C. Samples were used for assessing Aerobic Counts (AC) as well as *Enterobacteriaceae* using the Tempo system (BioMérieux, South Africa). A 25 g sample was placed in 225 ml Oxoid buffered peptone water (BPW) after which it was placed in a stomacher (260 r/p/m for 1 min).

- Sample preparation: *Salmonella* spp.

  The samples prepared for analysis with the Tempo system was consequently used for the detection of *Salmonella* spp. using the ISO 6579 method (Fig. 5.1).

- Detection of microorganisms: Tempo system

  The Tempo system makes use of fully automated tests for the enumeration of different microorganisms as quality indicators. For both the *Enterobacteriaceae* and AC tests, fractional amounts of each of the prepared samples were introduced to the Tempo vials containing reconstituted media (Fig. 5.1). Thereafter, specific volumes of autoclaved distilled water was added to the Tempo vials comprising of reconstituted media. By means of the Tempo filler, the substance of the vials was transferred to Tempo cards where each of the cards comprises of 48 wells, 15 of each of the three volumes (225, 22.5, 2.5 µL). One millilitre of each sample was used for both the *Enterobacteriaceae* and AC tests formulating a 1/40 Tempo dilution. Furthermore, all cards were incubated for 24 h, the *Enterobacteriaceae* cards at 35°C and the AC cards at 30°C. After the incubation period, the results were analysed by the Tempo Reader system which utilises detection software to determine which wells tested positive. Conditional to the investigated microorganism, positive wells could either increase or decrease in fluorescence. Moreover, based on the Most Probable Number (MPN) tables, the software make use of both the dilution of the sample and the positive wells' volumes to mathematically determine the cfu/g of the sample (Versetti *et al*., 2007; Owen *et al*., 2010).

- Detection of microorganisms: *Salmonella* spp.

  As seen in Fig. 5.1, Oxoid BPW was used as pre-enrichment for the 25 g meat samples which was incubated at a temperature of 37 ± 1°C. After an incubation period of 18 ± 2 h, 0.1 ml of the pre-enriched BPW sample was added to 10 ml Oxoid Rappaport-Vassiliadis (RVS) culture for the selective enrichment phase (24 ± 3 h at 41.5 ± 1°C). Next, the incubated RVS culture was streaked onto Oxoid Xylose Lysine deoxycholate (XLD) agar, to subsequently incubate for 24 ± 3 h at 37 ± 1°C. Finally, after the streaked XLD plates were incubated for 24 ± 3 h at 37 ± 1°C, the plates were checked for the occurrence of colonies. *Salmonella* colonies are typically black in the middle with a lightly transparent region of reddish colour (Lee *et al*., 2015).

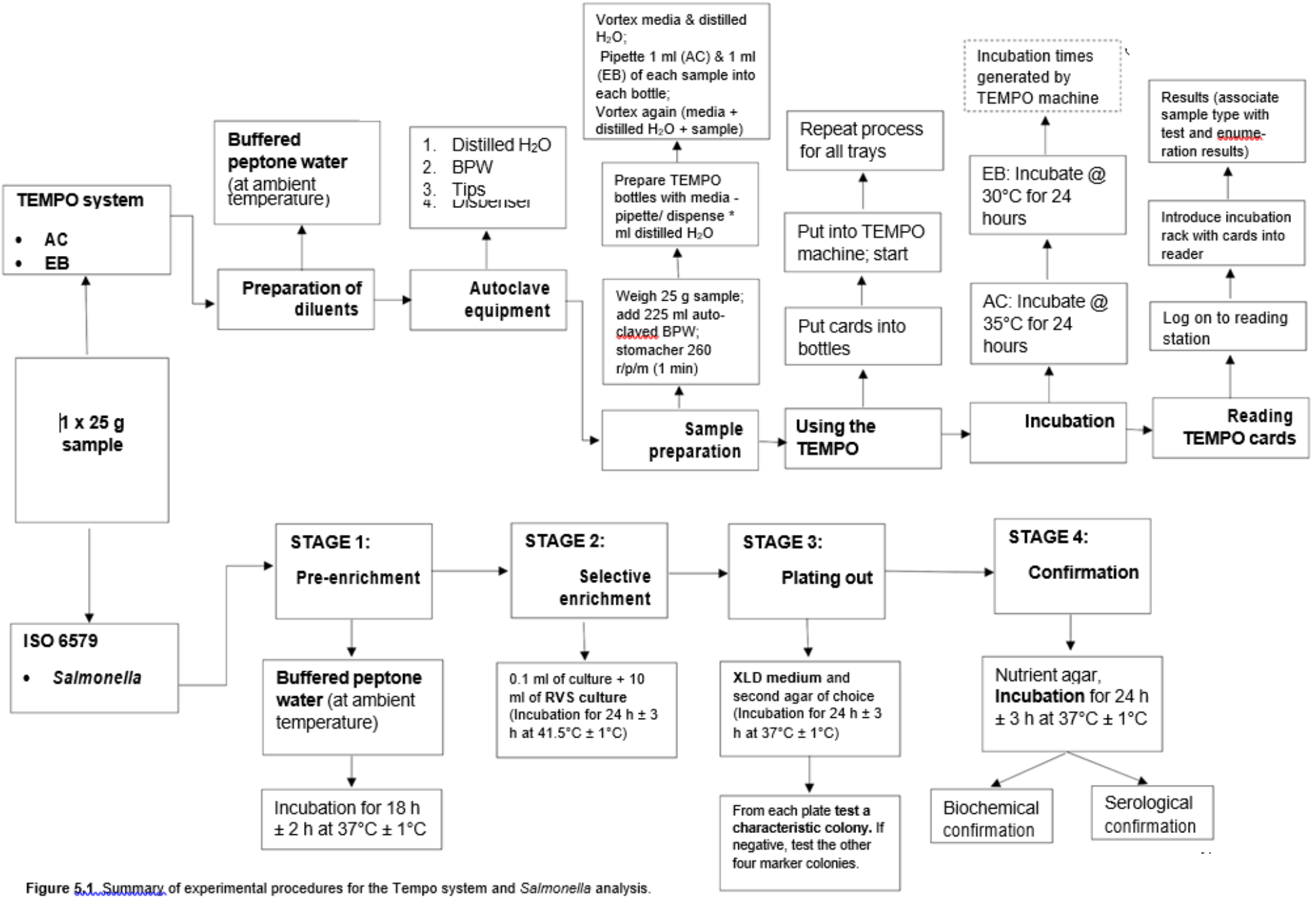

Figure 5.1 Summary of experimental procedures for the Tempo system and *Salmonella* analysis.

5.2.3 Statistical analyses

Statistica 64 version 13 (2015) VEPAC module was used to perform statistical analyses (STATISTICA, 2011). Hot- vs. cold-deboning and day were used as fixed effects in a mixed model repeated measures of analysis of variance (ANOVA). The Fisher LSD (least significant differences) test was used for the multiple comparison test. It can be noted that animal was included as random effect while possible outliers were identified using normal probability plots. Significant influences were described as Means and Standard Deviation (SD). A significance level of 5% ($p \leq 0.05$) was used as guideline for detecting possible significant effects.

5.3 Results

5.3.1 Aerobic Counts (AC)

Mean Aerobic Counts (AC) of the five investigated muscles (Table 5.1) over the 28 d ageing period (Table 5.2) are represented in Table 5.3.

Table 5.3 Aerobic Counts (cfu/g) of hot- vs. cold-deboned muscles as per treatment and time over the 28 d ageing period (Mean ± SD)

Muscle type	Treatment	Ageing days post-mortem				
		3	7	14	21	28
Fan fillet	Hot-deboned	16872^{b} ± 21756.58	33960^{ab} ± 21684.28	49000^{a} ± 0.00	49000^{a} ± 0.00	49000^{a} ± 0.00
	Cold-deboned	32500^{ab} ± 22710.13	39620^{a} ± 20974.32	49000^{a} ± 0.00	49000^{a} ± 0.00	49000^{a} ± 0.00
Rump steak	Hot-deboned	49000^{a} ± 0.00		49000^{a} ± 0.00	49000^{a} ± 0.00	46600^{a} ± 5366.56
	Cold-deboned	49000^{a} ± 0.00		49000^{a} ± 0.00	49000^{a} ± 0.00	49000^{a} ± 0.00
Big drum	Hot-deboned	32025^{a} ± 20194.12		49000^{a} ± 0.00		49000^{a} ± 0.00
	Cold-deboned	31870^{a} ± 23815.11		41400^{a} ± 16994.12		49000^{a} ± 0.00
Moon steak	Hot-deboned	49000^{a} ± 0.00		39204^{a} ± 21904.07		49000^{a} ± 0.00
	Cold-deboned	32400^{a} ± 18702.94		40400^{a} ± 19230.18		41800^{a} ± 10733.13
Triangle steak	Hot-deboned	41020^{a} ± 17843.82				49000^{a} ± 0.00
	Cold-deboned	49000^{a} ± 0.00				49000^{a} ± 0.00

$^{a-b}$ Means in the same double row per muscle with different superscripts differed significantly between ageing time points ($p \leq 0.05$).

The mean AC values of the fan fillet showed no interaction between treatments and time over the 28 d ageing period (p = 0.62). As seen in Table 5.3, there was no difference (p = 0.39) between hot- vs. cold-deboning, but there was a difference between days 3 and 7 (p = 0.00). Fig. 5.2 shows the increase in mean AC between days 3 and 7 post-mortem which occurred for both treatments.

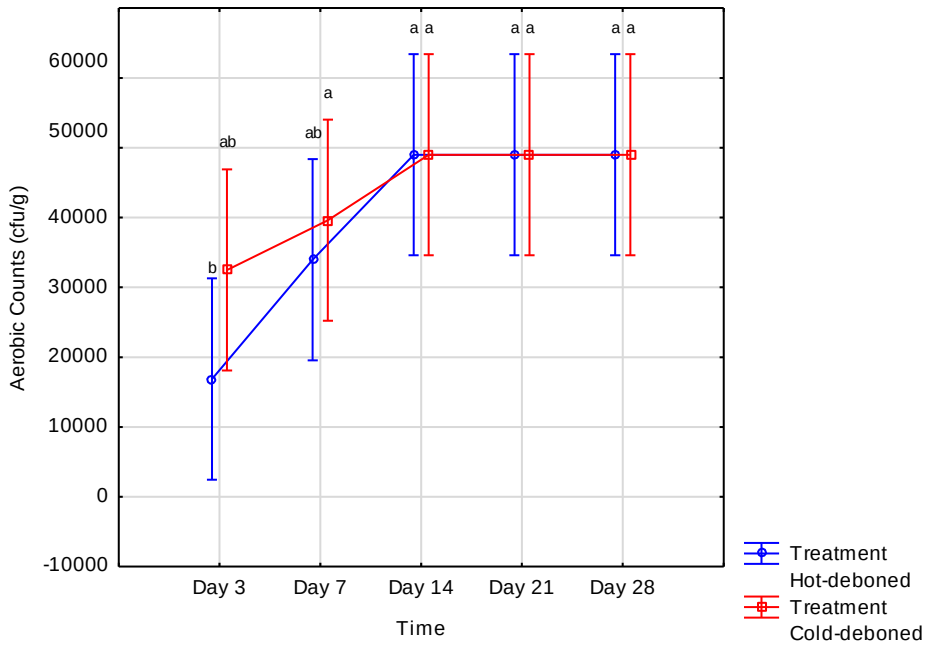

Figure 5.2 Mean Aerobic Counts of hot- vs. cold-deboned ostrich fan fillet over the ageing period 28 d post-mortem. Significant difference is indicated by different letters (p ≤ 0.05).

No interaction was seen between hot- vs. cold-deboning and time in the mean AC values of the rump steak throughout the 28 d ageing period (p = 0.43; Table 5.3). As seen in Fig. 5.3, the values of the cold-deboned rump steak stayed consistent throughout the 28 d ageing period, whilst the hot-deboned rump steak, although non-significant, showed a decrease between days 21 and 28 post-mortem. Furthermore, there was no difference between treatments (p = 0.39) or between ageing time points (p = 0.43) in the mean AC values of the rump steak over the 28 d ageing period (Table 5.3).

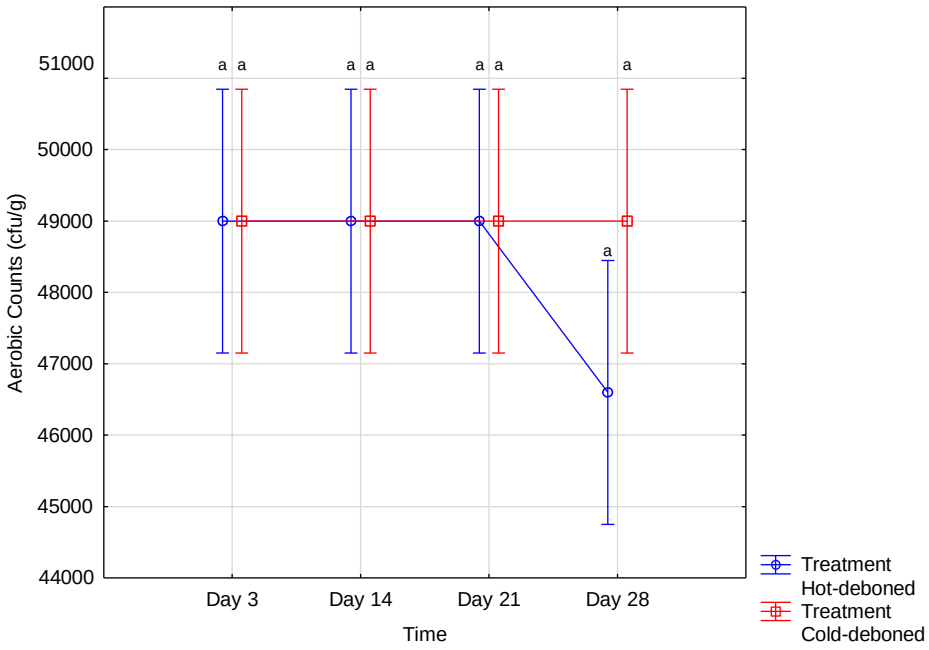

Figure 5.3 Mean Aerobic Counts of hot- vs. cold-deboned ostrich rump steak over the ageing period 28 d post-mortem. Significant difference is indicated by different letters ($p \leq 0.05$).

Neither the mean AC values of the big drum ($p = 0.84$), moon steak ($p = 0.32$) or triangle steak ($p = 0.37$) showed interactions between hot- vs. cold-deboning and ageing time. The only of these muscles which did not show a gradual increase in the mean AC values over the 28 d ageing period, was the hot-deboned moon steak which showed a non-significant decrease between days 3 and 14 before increasing towards day 28 (Table 5.3).

5.3.2 Enterobacteriaceae

Mean *Enterobacteriaceae* values of the five investigated muscles (Table 5.1) over the 28 d ageing period (Table 5.2) are represented in Table 5.4.

Table 5.4 *Enterobacteriaceae* counts (cfu/g) of hot- vs. cold-deboned muscles as per treatment and time over the 28 d ageing period (Mean ± SD)

Muscle type	Treatment	Ageing days post-mortem				
		3	7	14	21	28
Fan fillet	Hot-deboned	21.0^{c} ± 0.00	27.5^{a} ± 28.15	4677.5^{c} ± 7007.01	35598^{ab} ± 21023.15	43400^{bc} ± 12521.98
	Cold-deboned	33604.2^{a} ± 22348.32	29404^{a} ± 26832.93	30414.2^{a} ± 25509.25	42200^{a} ± 15205.26	49000^{a} ± 0.00
Rump steak	Hot-deboned	986.2^{d} ± 1532.95		590^{ab} ± 744.63	6302.5^{cd} ± 6318.07	28580^{abc} ± 21037.63
	Cold-deboned	32880^{bcd} ± 22840.14		24014.2^{ab} ± 23625.18	29430^{ab} ± 26797.37	38800^{a} ± 14703.74
Big drum	Hot-deboned	15.25^{b} ± 6.08		50.6^{b} ± 42.51		20940^{b} ± 18671.31
	Cold-deboned	10^{b} ± 0.00		27^{a} ± 6.93		32666.67^{a} ± 18876.79
Moon steak	Hot-deboned	45.6^{b} ± 50.96		4239.6^{ab} ± 9369.42		34160^{b} ± 20638.02
	Cold-deboned	9876^{ab} ± 21871.28		12286.75^{a} ± 24475.51		27213.2^{ab} ± 21384.65
Triangle steak	Hot-deboned	73.8^{a} ± 104.91				11564^{a} ± 21003.65
	Cold-deboned	50.8^{a} ± 67.98				3504.6^{a} ± 3568.06

$^{a-d}$ Means in the same double row per muscle with different superscripts differed significantly between ageing time points ($p \leq 0.05$).

The mean *Enterobacteriaceae* values of the fan fillet showed no interaction between treatments and ageing time points (p = 0.15) throughout the 28 d ageing period. As seen in Table 5.4, treatments did however differ (p = 0.01), as well as the ageing time points (p = 0.00). Moreover, as indicated by Fig. 5.4, both the hot- and cold-deboned fan fillet showed an increase in mean *Enterobacteriaceae* values towards day 28 post-mortem.

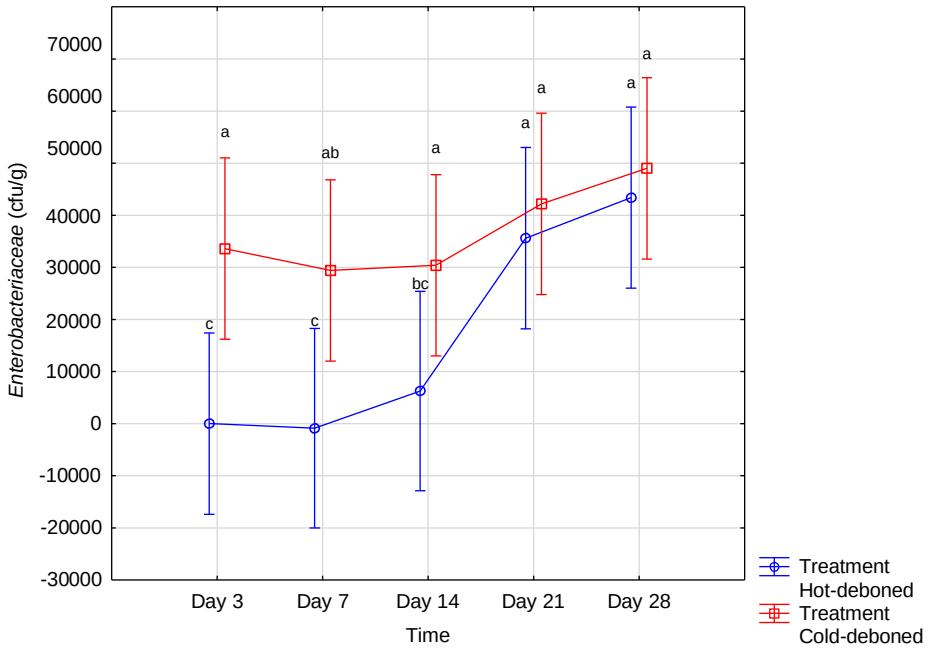

Figure 5.4 Mean *Enterobacteriaceae* counts of hot- vs. cold-deboned ostrich fan fillet over the ageing period 28 d post-mortem. Significant difference is indicated by different letters (p ≤ 0.05).

There was no interaction between hot- vs. cold-deboning and ageing time points (p = 0.36) in the mean *Enterobacteriaceae* values over the 28 d ageing period for the rump steak. Both treatments (p = 0.02) and time points (p = 0.02) did however differ (Table 5.4). The only decrease, although non-significant, was seen between days 3 and 14 in the mean cold-deboned values (Figure 5.5).

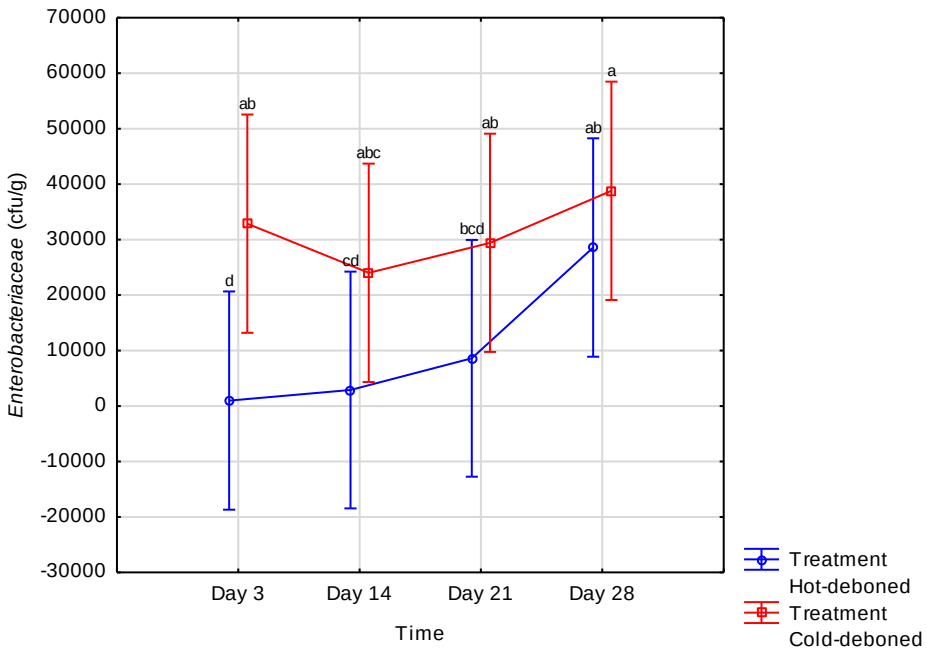

Figure 5.5 Mean *Enterobacteriaceae* counts of hot- vs. cold-deboned ostrich rump steak over the ageing period 28 d post-mortem. Significant difference is indicated by different letters ($p \leq 0.05$).

The mean *Enterobacteriaceae* values of the big drum ($p = 0.67$), moon steak ($p = 0.56$) and triangle steak ($p = 0.37$) showed no interactions between hot- vs. cold-deboning and ageing time points (Table 5.4). Both the big drum ($p = 0.55$) and moon steak ($p = 0.62$) had no differences between treatments, whilst significant differences between ageing time points occurred (Table 5.4). The triangle steak however showed no difference between hot- vs. cold-deboning ($p = 0.37$) or between ageing time points ($p = 0.24$; Table 5.4). Additionally, all three these muscles increased in mean *Enterobacteriaceae* values over the course of the 28 d ageing trial (Table 5.4).

5.3.3 Salmonella spp.

Salmonella was absent in a 25 g sample in all of the investigated muscles at all of the ageing time points throughout the 28 d ageing period, for both hot- and cold-deboned muscles.

5.4 Discussion

Meat undergoes wide-ranging handling and is subjected to surfaces that are predisposed to contamination throughout the deboning process. The degree of contamination is, however, dependent on throughput of meat, the local environment, as well as cleanliness and temperature of utensils, especially cutting tables, conveyor belts and knives (Nel *et al*., 2004a; Shange *et al*., 2019). Additionally, vacuum packaging is adept in extending the shelf life of meat by deterring microbial contamination (Voloski *et al*., 2016).

As frozen microbiological samples were used in this study (described in section 5.2), it can be highlighted that although freezing can be defined as a method of food preservation that slows down the deterioration in foods through microbiological activity, microbial growth is only paused through freezing (Berry *et al*., 2008). Storage at low temperatures slows microbial reactions or reduces the rate at which changes take place, but does not completely stop them. Therefore, residual microbial or endogenous enzyme activity present in a product may continue and can ultimately spoil a product. (Berry *et al*., 2008). It is also a well-known fact that food products can perform as cryoprotectans for bacteria enabling its survival at freezing temperatures for long time periods (Adam & Moss, 2008).

It is important to note that microbiological analysis was only conducted on frozen samples in this study due to the experimental layout leading to practical limitations which prevented microbiological analysis on fresh meat as well. It is therefore not assumed that the frozen samples are representative of the microbiological standard of the fresh meat or that the true ageing effect in terms of microbiology was recorded. Although the initial characteristics of meat is well preserved through freezing, the freezing process is known to convert most of the water in meat (50 – 75% by weight water dependant of species), into ice (Heinz & Hautzinger, 2007). This crystallisation will impact the meat, and might lead to a microbiological result not true of the ageing effect.

By now, it is well established that hot-deboning has several advantages, including reduced energy inputs, decreased chiller space, lower drip loss and is estimated to cause a 4% higher carcass yield (Kotula, 1981; Sheridan & Sherrington; 1982; Reid *et al*., 2017). In this study, it was found that hot-deboned ostrich muscles (without membranes) had a 6.43% higher yield than the cold-deboned muscles (Chapter 3, section 3.1.1). Meat processing conditions can also be optimised through the more efficient heat transfer from muscles with an increased surface to volume relation. Meat trimmings can likewise be ground much sooner for the manufacture of products such as patties and sausages (Kotula, 1981).

The disadvantages of hot-deboning however include darker meat, of which the shape (particularly when removed pre-rigor) as well as the shelf life can be influenced due to the higher temperature at which excision is performed (Reid *et al*., 2017). Since the surface area of the meat is increased dramatically early on in the marketing chain, it leads to the possibility of high microbial contamination (at higher temperatures) which will shorten the shelf life. The

potential growth of bacteria is a public health concern because of the elevated excision temperature at which hot-deboning is performed (Kotula, 1981). It must be considered that after muscles are hot-deboned, muscles are exposed to a higher temperature for a longer period of time in comparison with cold-deboned muscles (with cold-deboning carcasses are immediately chilled at 0 – 4°C after slaughter). The risk of microbial contamination and increased growth rates is thus much greater with the higher temperature persisting from the time of deboning (within 2 h post-mortem), performed at the temperature of the animal which is still near the *in vivo* temperature (37°C), throughout the process of membrane-removal, until the muscles are vacuum packed. Hot-deboned meat thus necessitates rapid cooling to inhibit/ retard microbial growth. Most abattoirs will be eager to change procedures in order to benefit from reduced production costs though (Smith, 1979).

Aerobic Counts (AC) are widely used to establish the general degree of microbial contamination (Nel *et al*., 2004a). No significant difference between hot- vs. cold-deboning was seen in any of the investigated muscles over the 28 d ageing period (Table 5.3). Botha *et al*. (2006) similarly found no significant differences between hot- and cold-deboned fan fillet and big drum muscles in terms of Aerobic Plate Counts (APC comparable with AC used in this study). Sheridan and Sherington (1982) conversely found AC of hot-deboned beef similar to or higher than cold-deboned beef with the hot-deboned muscles showing fluctuating trends. Reid *et al*. (2017) found Total Viable Counts (TVC comparable with AC used in this study) of hot- vs. cold-deboned beef to show no initial (day 0) difference. However, in contrast with this study, a gradual increase in counts were seen with hot-deboned muscles significantly higher throughout a six week ageing period. The greatest counts were reached at the six week mark for both the hot- and cold-deboned muscles showing that for both deboning methods, there is risk of contamination. The hot-deboned muscles are handled more, leading to a higher danger of cross-contamination and higher counts throughout the ageing period. The cold-deboned muscles in turn have greater aerobic exposure time leading to growth prior to vacuum packaging contributing to the high load at six weeks post-mortem (Reid *et al.,* 2017).

The fan fillet and big drum muscles both had lower mean AC loads at day 3 in comparison with the other muscles (Table 5.3; Fig. 5.2). The lower initial AC loads might be due to where these muscles are situated anatomically, which is deeper within the ostrich pelvic limb (Smith *et al*., 2006). Although the mean AC load of the fan fillet and big drum muscles increased towards day 14 (Table 5.3), the initial exposure of these muscles to environmental organisms are thus more limited (Hoffman *et al*., 2010a; 2010b). Similar to the fan fillet and big drum, during the first two weeks of storage, bacterial numbers increased notably in cold-deboned beef (Hulankova *et al.*, 2018). Furthermore, it is interesting to note that the rump steak had the highest mean initial AC load which prevailed throughout the 28 d ageing period (Table 5.3; Fig. 5.3). The high initial AC load of the rump steak can also be ascribed to its anatomical muscle location within the superficial layer of the ostrich pelvic limb (Smith *et al*., 2006), suggesting a larger surface area of the muscle comes into contact with

environmental organisms, as found by Hoffman *et al*. (2010a; 2010b). This is also comparable with findings of Karama *et al*. (2003) where the primary deposited bacterial load during skinning of ostriches, was maintained.

Although hot-deboned muscles' AC generally matched that of cold-deboned muscles throughout the 28 d ageing period, the actual AC load of both treatments were relatively high throughout the ageing period. Currently in the ostrich industry (export approved), a product specification of < 1 x 10^4 cfu/g is used for Total Bacterial Counts (TBC comparable with AC's used in this study) of fresh meat. Initial AC loads at day 3 post-mortem were higher than the aforementioned range for all of the investigated muscles (Table 5.3). Over the progression of the 28 d period, the AC's further showed to also be higher than the range used by the industry (Table 5.3). It can be postulated that these high AC were due to the removal of the membranes which left the muscles much more susceptible to spoilage through environmental organisms (Hoffman *et al*., 2010a; 2010b). The huge variation in SD values (Table 5.3) suggests that the data analysed was not homogenous and could be an indication of difference in procedures. As muscles might have been contaminated at day 3 (initially), it would be expected that AC loads throughout the 28 d ageing period would progressively increase, as the initial contamination of muscles would enable microbial spoilage even if muscles were subsequently vacuum packed. However, Botha *et al*. (2006) in contrast found both hot- and cold-deboned fan fillet and big drum muscles to have APC's (APC comparable with AC in this study), < 2000 cfu/g at 24 h post-mortem, and < 1000 at 42 d post-mortem (vacuum-packaged storage at 0 – 4°C). These results were below the South African Standards for microbiological monitoring of meat for refrigerated export (< 1000 cfu/g) (Botha *et al*., 2006).

Enterobacteriaceae counts offer a general view of the presence of organisms on the product (Nel *et al*., 2004a). In general, mean *Enterobacteriaceae* counts of hot-deboned muscles did not significantly differ from cold-deboned muscles over the 28 d ageing period (Table 5.4). Although the treatments did not significantly differ, relatively high numbers of *Enterobacteriaceae* was present in the investigated muscles (Table 5.4). The ostrich industry currently uses a product standard of < 1 x 10^3 cfu/g for *Enterobacteriaceae* in fresh meat suitable for export purposes. As seen in Table 5.4, all of the hot-deboned muscles at day 3 post-mortem were within this range, whilst only the cold-deboned big drum and triangle steak at day 3 were within the range used by the industry. From day 7 onwards, none of the mean *Enterobacteriaceae* counts of the investigated muscles however presented within the range used by the industry (Table 5.4) (Hoffman *et al*., 2010b).

Similarly, *Enterobacteriaceae* counts were found in relatively high numbers in all ostrich samples by Alonso-Calleja *et al*. (2004) showing the ability of *Enterobacteriaceae* to grow at refrigeration temperatures in vacuum-packed products. However, no relationship was seen between *Enterobacteriaceae* values and AC, implicating that fluctuations in *Enterobacteriaceae* do not lead to resembling changes in AC populations (Alonso-Calleja *et al*., 2004). Holman *et al*. (2017) however showed cold-deboned beef had *Enterobacteriaceae*

counts < 10 cfu/g over the progression of a 5 week ageing period. Thus, there was an absence of sufficient microbial growth to cause spoilage over the ageing period (Holman *et al*., 2017) which is in contrast with findings in this study.

Similar to the increase of mean *Enterobacteriaceae* counts of the fan fillet (Fig. 5.4) and rump steak (Fig. 5.5) between days 3 and 14, cold-deboned goat meat also showed an increase in both AC and *Enterobacteriaceae* counts over the progression of a 14 d ageing period (Sabow *et al*., 2016). Both the hot-deboned fan fillet and rump steak showed significantly lower mean *Enterobacteriaceae* counts in comparison with the cold-deboned muscles throughout the ageing period (Table 5.4; Fig. 5.4). Generally, all of the muscles showed varying trends over the 28 d ageing period with no one clear point where *Enterobacteriaceae* counts reach the highest value for all muscles (Table 5.4). This might be ascribed to the fact that the source of *Enterobacteriaceae* on meats has been located to meat-handling work surfaces. Surfaces that do not typically have interaction with meat did not present as *Enterobacteriaceae* pools. Moreover, it was found that any accumulation of *Enterobacteriaceae* during the day seems to be regulated by sanitation practices. However, work surfaces that builds up exceptionally high contaminating loads of *Enterobacteriaceae* during the day, can perform as a persistent source of contamination. This can make the process of identifying the stage at which surfaces become unsanitary, problematic (Stiles & Lai-King, 1981). The high SD values (Table 5.4) is an indication that the data was not uniform and that variation could have occurred in the procedure that was followed or between the animals that were analysed.

A definite correlation has been observed between pH and most microbial counts, including AC and *Enterobacteriaceae* (Balog & Almeida, 2007). This could signify that higher pH values benefits microbial growth (Shange *et al*., 2018; 2019). Otremba (1999) found a high pH in meat together with microbial loads from day 7 onwards in refrigerated vacuum packed ostrich meat. Alonso-Calleja *et al*., 2004 similarly found pH to be deemed an important consideration in microbiological quality of meat. The proliferation of microorganisms has been found at pH_u of 6, and over the progression of time, increased bacterial loads may cause a shorter shelf life of meat (Balog & Almeida, 2007). In this study, however, a definite correlation could not be made between pH and microbial counts over the 28 d ageing period, as microbiological analysis was only conducted on 5 birds and the number of measurements and their high variation were insufficient to reach a clear conclusion. Furthermore, it can be mentioned that

Addressing microbiological quality and safety of meat; in some instances cold-deboning has been proven the safer option. Hoffman *et al*. (2010a) found cold trimming (after 24 h refrigeration of carcasses) of ostrich bruises to have a lower microbial load compared to warm trimming (post-evisceration) which is expected to contribute to a longer shelf life. With the trimming of bruises on warm carcasses, higher total aerobic viable counts were found (Hoffman *et al*., 2010a). Furthermore, cold trimming (mainly bruises) was similarly found

beneficial considering the growth of predominant microbes on ostrich carcasses before and after overnight cooling in an abattoir and deboning plant. Moreover, Gram-negative pathogens were discovered as the most common contaminants in pooled water in the abattoir, which was correspondingly considered the most hazardous point for carcass contamination. In producing ostrich meat which is safe to consume with an acceptable shelf life, total plant hygiene was emphasized through air samples showing *Pseudomonas* and *Shigella* to be recurrently present in the abattoir (Hoffman *et al.,* 2010b).

Salmonella is recognised as a zoonotic agent which arises globally, and is mainly found in the intestinal tract of humans and animals. Foodborne disease can be the consequence of consumption of certain strains of *Salmonella*. Eggs, poultry, meat and meat products are frequently recognised as vehicles of salmonellosis to humans. The required levels to cause salmonellosis range from 10^7 – 10^9 cells/kg. At levels of 10^5/g however, the likelihood of food poisoning is strong. It is also important to note that *Salmonella* can continue surviving on foods for an extended period of time (Department of Health, 2000).

In this study, an absence of *Salmonella* was found for all of the investigated muscles at all of the ageing time points for both deboning methods (Table 5.5). This is a positive outcome as inefficient slaughtering practices may be a source of *Salmonella* considering intestinal substance is known to comprise of *Salmonella* which may ultimately contaminate the meat. Workers transmitting *Salmonella* may also infect meat as *Salmonella* is one of the organisms which commonly originates from infected workers. Cross-contamination can occur once the production line is contaminated with *Salmonella* and the organism manifests itself on the hands of workers, and on equipment and machinery within the abattoir. The deboning room in particular is a greatly labour intensive environment where highly perishable foodstuff is handled (Nel *et al*., 2004).

5.5 Conclusions

Hot-deboned muscles did not significantly differ from cold-deboned muscles in terms of microbial load as investigated over the 28 d ageing period post-mortem on the five investigated muscles. An absence of *Salmonella* was found in all samples over the 28 d ageing period for both hot- and cold-deboning, indicating that slaughtering practices were hygienic and that the ostriches were *Salmonella* free. Relatively high mean Aerobic and *Enterobacteriaceae* counts were however found for all of the hot- and cold-deboned muscles, initially (day 3 post-mortem), as well as throughout the 28 d post-mortem. All of the mean Aerobic and *Enterobacteriaceae* counts were higher than the fresh meat standards used by the industry. A contributing factor to the high Aerobic and *Enterobacteriaceae* counts found in this study, might have been the fact that muscle membranes were removed prior to packaging which could make the muscles more susceptible to microbial contamination through environmental organisms. Further investigation into the environmental hygiene in the abattoir, principally the deboning room, could

help elucidate this. Considering all of these factors, hot-deboning is seen as a suitable alternative excising method for the ostrich industry of South Africa, as hot-deboning did not influence the microbial quality and safety of the meat negatively.

5.6 References

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CHAPTER 6

General Conclusions

The ostrich industry of South Africa currently uses cold-deboning as method of excising whereby ostrich carcasses are refrigerated for 24 h post-mortem (0 - 4°C) before deboning is performed (Hoffman *et al*., 2007). Hot-deboning, where post-rigor meat is removed from carcasses before refrigeration (within 2 – 4 h post-mortem), is however an alternative excising method for the South African ostrich industry (Waylan & Kastner, 2004). Hot-deboning holds several potential advantages for the ostrich industry of South Africa as found by Botha *et al*., (2006; 2007), of which the most prominent is the saving of refrigeration space of up to 50 – 55% in traditional livestock situations (Wayler & Kastner, 2004; Pisula & Tyburcy, 2009). This saving in refrigeration space amounts to greatly reduced running energy costs with a 40 – 50% saving that can be made, whilst a 40 – 50% quicker turnover rate can be achieved (Pisula & Tyburcy, 2009). In the ostrich industry, standard chillers refrigerate ±150 ostrich carcasses. A saving of 50% in refrigeration space thus amounts to double the number of carcasses that can be refrigerated in the same chiller. However, as whole carcasses are not chilled with hot-deboning, the saving in refrigeration needs to be evaluated in terms of vacuum packaged meat. With hot-deboning, excised, vacuum packaged commercial cuts are refrigerated instead of whole carcasses, and refrigeration requirements are therefore aimed at chilling already processed, vacuum packaged meat. In all probability, the energy saved in an ostrich facility will therefore be even more than the ~50% discussed for other livestock species particularly if it is taken into account that ostrich muscles, after deboning, are frequently blast frozen rather than chilled.

Currently in the ostrich industry, the sixteen deboned commercial cuts (fresh meat) are processed and sold as “small fillet,” “small steak,” and “mixed steak portions,” with only the fan fillet, tenderloin and eye fillet sold as such. Thus, with hot-deboning, the excised cuts would immediately be processed as aforementioned, and chilled as ready-to-be-sold products. It is important to highlight the current ban that is placed on the export of ostrich meat due to the outbreak of Avian Influenza (AI) at the end of 2017. Thus, now more than ever it is crucial for the ostrich industry of South Africa (world leader in the ostrich market with 90% of prime cuts exported), to investigate ways in which production costs can be lowered to gain economic strength (National Agricultural Marketing Council, 2003; Department of Agriculture, Forestry & Fisheries, 2017).

For existing or already built abattoirs, the lower costs of construction through building smaller cooling chambers, will not be applicable (Spooncer, 1993). The saving of energy might rather be through the usage of less cooling chambers, e.g. one instead of two chillers. Alternatively, all chillers could still be utilized, which will in turn give a higher throughput rate

and be of great value during peak slaughtering periods. Further research needs to be conducted in terms of drawing a cost-analysis of how much energy cooling chambers utilize for ostriches specifically, and what the saving in refrigeration space (through hot-deboning), would translate to economically. The Meat Safety Act of 2000 does make provision for the hot-deboning of ostrich meat, and as stated by the Department of Agriculture (2007), the room where meat is cut and packed must be upheld at or below 12°C in terms of air temperature, mainly to ensure microbial safety as the meat typically has a temperature < 6°C. With hot-deboning however, one of the advantages could include raising the air temperature of the deboning room as the meat temperatures will still be high (> 25°C), although this might require regulation changes. This gives the possibility of performing hot-deboning at a higher ambient temperature which is more comfortable for workers. Further research needs to be conducted on ambient hot- deboning temperatures that will still be microbiologically safe.

Besides the advantages of hot-deboning discussed above, hot-deboned meat is known to have a higher yield through reduced weight loss usually experienced during carcass refrigeration (Powell *et al*., 1982; Spooncer, 1993; Pisula & Tyburcy, 2009), as well as through muscles excised more cleanly from the bone (Spooncer, 1993). The muscle yields of hot-deboned ostrich muscles are not known at present. This study consequently found 50% of the meat of hot-deboned muscles without membranes (sixteen commercial cuts) had significantly higher mean muscle yields than the same muscles when processed cold (Chapter 3). This is especially relevant as the ostrich industry utilises meat after membranes have been removed, together with the fact that all sixteen deboned cuts are sold commercially and are of economic importance (whether exported to the European Union or sold locally).

The ostrich is uniquely suited for hot-deboning as it enters *rigor mortis* within 45 min post-mortem implying that hot-deboning is performed on post-rigor meat (Hoffman *et al*., 2007). As hot-deboning is performed on post-rigor meat, the risk of cold-shortening (phenomenon where muscles are prone to become rigid when meat is refrigerated too soon after slaughter at temperature below 10 – 15°C at a pH > 6.0) and pre-rigor shortening, can be eliminated (Lawrie & Ledward, 2006; Hoffman *et al.,* 2007). As hot-deboning of ostrich muscles do not hold the risk of cold- and/or pre-rigor shortening, the idea of freezing the muscles directly after excision, might be explored. Also, in this study, hot-deboning did not significantly influence the physical meat quality parameters at day 3 post-mortem (Chapter 3) or over the 28 d ageing period (Chapter 4) for any of the five investigated muscles including the fan fillet *(M. iliofibularis);* rump steak *(M. iliotibialis lateralis);* big drum *(M. gastrocnemius, pars interna);* moon steak *(M. femorotibialis medius)* or triangle steak *(M. flexor cruris lateralis).* Both the fresh meat (day 3 post-mortem) and the aged meat (28 d period) did however show significant differences amongst muscles, with results of the hot-deboned muscles continually matching that of the cold-deboned muscles.

Hot-deboning of ostrich meat is advantageous in many aspects, but cause for concern

regarding microbial spoilage exists due to the higher temperature of meat at which hot-deboning is performed (Stopforth & Sofos, 2005). Botha *et al*. (2007) found microbiological results of only two hot-deboned ostrich muscles namely the fan fillet (*M. iliofibularis*) and big drum (*M. gastrocnemius, pars interna*) muscles with membranes, at two ageing time points (1 and 42 days post-mortem), to be favourable. In this study, hot-deboning did not have an effect on the mean Aerobic Counts (AC) nor the *Enterobacteriaceae* counts of five investigated muscles without membranes over the 28 d ageing period, and matched that of cold-deboned muscles (Chapter 5). Both AC and *Enterobacteriaceae* counts for hot- and cold-deboned muscles (initial and over the ageing period), were however higher in comparison with fresh meat standards used by the industry; whether this is standard or due to the specific execution of the experiment *per se* is unclear. The microbial quality therefore necessitates further investigation for the South African ostrich industry. An absence of *Salmonella* spp. was however found in all hot- and cold-deboned samples indicating that slaughtering practices were microbiologically safe.

Considering the muscle yields of sixteen commercially deboned ostrich cuts (Chapter 3); in addition to the physical meat quality characteristics of five ostrich muscles at day 3 post-mortem (Chapter 3), as well as over a 28 d ageing period (Chapter 4); including the microbiological quality and safety of these five ostrich muscles throughout a 28 d ageing period; hot-deboning is a suitable alternative excising method for the South African ostrich industry. However, for the practical implementation of hot-deboning within the ostrich industry of South Africa, it is recommended that a cost-analysis of refrigeration units used in an ostrich abattoir be conducted to economically translate the saving of refrigeration space through hot-deboning. Additionally, findings of the microbial load in this study suggests further research be conducted pertaining to the organisms present in the environment of the abattoir. Further research into the direct freezing of hot-deboned ostrich muscles (no risk of cold-shortening), might also prove valuable as ostrich meat is exported vacuum packaged in a frozen state. The current ban on the export of ostrich meat will in all likelihood be lifted in the future, and as South Africa is known to principally be an exporter of ostrich meat, this will prove helpful in further streamlining and optimising the packaging process of ostrich muscles and the costs involved.

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