

THE EFFECTS OF HOT-DEBONING ON THE PHYSICAL  
QUALITY CHARACTERISTICS OF OSTRICH (*STRUTHIO  
CAMELUS*) *MUSCULARIS GASTROCNEMIUS, PARS  
INTERNA* AND *MUSCULARIS ILIOFIBULARIS*

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## DECLARATION

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

Signature: \_\_\_\_\_ Date: \_\_\_\_\_



## SUMMARY

The aim of this study was to investigate the effects of hot-deboning (1 h *post-mortem*) on the shelf-life and the physical meat quality characteristics, including tenderness, pH, purge (%), cooking loss (%), and raw meat colour of vacuum packed ostrich (*Struthio camelus var. domesticus*) meat cuts from the *M. gastrocnemius, pars interna* and the *M. iliofibularis* during *post-mortem* refrigerated aging for respectively 21 d at 4°C and 42 d at -3° to 0°C. The course of temperature (°C) decline, change in pH, as well as the effect of temperature (°C) on the course of *rigor mortis* were also investigated for the first 22 to 24 h *post-mortem* in the *M. gastrocnemius, pars interna* and the *M. iliofibularis*

Sensory evaluation indicated that hot-deboned *M. gastrocnemius, pars interna* was significantly tougher ( $P < 0.001$ ) and less juicy ( $P = 0.004$ ) than the cold-deboned (24 h *post-mortem*) muscles at 48 h *post-mortem*. Hot-deboned *M. gastrocnemius, pars interna* ( $2.05 \pm 0.18 \mu\text{m}$ ) also had shorter sarcomere lengths ( $P = 0.0001$ ) at 24 h *post-mortem* than the cold-deboned muscles ( $2.52 \pm 0.14 \mu\text{m}$ ). However, with *post-mortem* refrigerated aging beyond 5 d at 4°C, and for 14 d at -3° to 0°C, respectively, the difference in toughness between the hot-deboned and the cold-deboned *M. gastrocnemius, pars interna* was insignificant. In contrast to the *M. gastrocnemius, pars interna*, hot-deboning had no significant effect ( $P > 0.05$ ) on the tenderness of the *M. iliofibularis*.

Hot-deboning had no significant effect ( $P = 0.2030$ ) on the pH when hot and cold-deboned *M. gastrocnemius, pars interna* were aged at 4°C. In contrast, when aged at -3° to 0°C, muscle pH was significantly ( $P = 0.0062$ ) higher for the cold-deboned *M. gastrocnemius, pars interna* and *M. iliofibularis* ( $5.93 \pm 0.12$ ) than for hot-deboned *M. gastrocnemius, pars interna* and *M. iliofibularis* ( $5.91 \pm 0.11$ ).

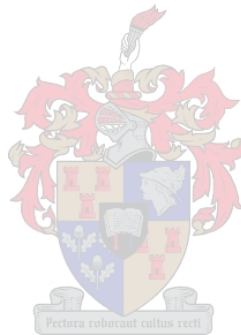
Hot-deboning had a significant negative effect ( $P < 0.0001$ ) on the water holding capacity of both the *M. gastrocnemius, pars interna* and the *M. iliofibularis*, causing the hot-deboned muscles to have more purge (%) during *post-mortem* aging than the cold-deboned muscles.

The effect of hot-deboning on the raw meat colour was mainly observed in the L\*-values, where the cold-deboned *M. gastrocnemius, pars interna* were significantly ( $P < 0.0042$ ) darker in colour ( $30.04 \pm 2.29$ ) than the hot-deboned muscles ( $30.71 \pm 1.88$ ) when aged at 4°C. In contrast, when muscles were aged at -3°-0°C, hot-deboning resulted in the *M. gastrocnemius, pars interna* ( $30.48 \pm 1.98$ ) to be significantly ( $P < 0.05$ ) darker in colour than the cold-deboned muscles ( $31.44 \pm 1.80$ ), while hot-deboning had no significant effect ( $P > 0.05$ ) on the L\*-values of the *M. iliofibularis*.

Hot-deboning had no significant effect on the shelf-life of meat cuts from both the *M. gastrocnemius, pars interna* and the *M. iliofibularis*, resulting in no increase in bacterial contamination prior to vacuum-packaging, nor in an increase in microbial counts during *post-mortem* storage for 42 d at -3° to 0°C.

Both the intact *M. gastrocnemius, pars interna* and the intact *M. iliofibularis*, when stored < 4°C, showed a rapid fall in muscle pH early *post-mortem*, reaching a mean minimum pH of  $6.07 \pm 0.41$  at approximately  $3.50 \pm 0.84$  h *post-mortem* and a mean minimum pH of  $5.81 \pm 0.07$  at approximately  $2.50 \pm 0.58$  h *post-mortem*, respectively. Furthermore, it was found that the muscle samples from the *M. gastrocnemius, pars interna*, maintained at 37°C, reached fully developed *rigor mortis* (maximum isometric tension) at the point of minimum muscle pH ( $5.76 \pm 0.13$ ).

With the rapid fall in pH (reaching a minimum pH at 2-4 h *post-mortem*), as well as the early onset (1 to 4 h) of *rigor mortis*, it was concluded that hot-deboning of ostrich muscles at 3 to 4 h *post-mortem* would be without detrimental effects on the eating quality in terms of meat tenderness.



## OPSOMMING

Die doel van hierdie studie was om die invloed van warmontbening (1 uur *post-mortem*) op die rakleef tyd, asook op die fisiese eienskappe, naamlik taaigheid, pH, "purge" (%), kookverlies (%) en kleur van vakuümverpakte volstruisvleis (*M. gastrocnemius, pars interna* en die *M. iliofibularis*), gedurende veroudering vir onderskeidelik 21 dae by 4°C en 42 dae by -3° tot 0°C, te evalueer. Daar was ook ondersoek ingestel na die daling in temperatuur (°C) en die verandering in pH gedurende die eerste 22 tot 24 uur *post-mortem*. Die invloed van temperatuur op die verloop van die ontwikkeling van *rigor mortis* was ook ondersoek.

Sensoriese evaluering het getoon dat warmontbeende *M. gastrocnemius, pars interna* betekenisvol taaier ( $P < 0.001$ ), asook minder sappig ( $P = 0.004$ ) was as die koudontbeende (24 uur *post-mortem*) spiere. Daar is ook gevind dat die warmontbeende *M. gastrocnemius, pars interna* betekenisvol ( $P = 0.0001$ ) korter "sarcomere" lengtes ( $2.05 \pm 0.18 \mu\text{m}$ ) getoon het as die koudontbeende spiere ( $2.52 \pm 0.14 \mu\text{m}$ ). Dog; veroudering by 4°C vir langer as 5 dae het veroorsaak dat daar geen noemenswaardige verskil in taaigheid tussen die warm- en koudontbeende *M. gastrocnemius, pars interna* was nie. In teenstelling met die *M. gastrocnemius, pars interna*, warmontbening het geen betekenisvolle effek ( $P > 0.05$ ) op die taaigheid van die *M. iliofibularis* getoon nie. Nie te min, alle spiere was sorgelyk in taaigheid na veroudering vir 14 dae by -3° tot 0°C.

Warmontbening het geen betekenisvolle effek ( $P = 0.2030$ ) op die pH van die *M. gastrocnemius, pars interna* getoon tydens veroudering by 4°C nie. In teenstelling hiermee, gedurende veroudering by -3° tot 0°C, was die pH van die koudontbeende *M. gastrocnemius, pars interna* en *M. iliofibularis* ( $5.93 \pm 0.12$ ) betekenisvol hoër ( $P = 0.0062$ ) as die pH van die warmontbeende *M. gastrocnemius, pars interna* en *M. iliofibularis* ( $5.91 \pm 0.11$ ).

Warmontbening het 'n noemenswaardige ( $P < 0.0001$ ), negatiewe invloed op die water-houdingsvermoë van beide die *M. gastrocnemius, pars interna* en die *M. iliofibularis* getoon, waar warmontbeende spiere meer "purge" (%) getoon het as die koudontbeende spiere.

Die effek van warmontbening op die kleur is hoofsaaklik waargeneem in die L\*-waardes. Koudontbeende *M. gastrocnemius, pars interna*, verouderd by 4°C, was noemenswaardig ( $P < 0.0042$ ) donkerder ( $30.04 \pm 2.29$ ) as die warmontbeende *M. gastrocnemius, pars interna* ( $30.71 \pm 1.88$ ). In teenstelling hiermee was warmontbeende *M. gastrocnemius, pars interna*, verouderd by -3° tot 0°C, noemenswaardig ( $P < 0.05$ ) donkerder ( $30.48 \pm 1.98$ ) as die koudontbeende *M. gastrocnemius, pars interna* ( $31.44 \pm$

1.80). Warmontbening het geen betekenisvolle ( $P > 0.05$ ) verskil veroorsaak in die  $L^*$ -waardes van die *M. Iliofibularis* nie.

Warmontbening het geen noemenswaardige effek op die rakleef tyd van beide die *M. gastrocnemius, pars interna* en *M. Iliofibularis*, verouderd by  $-3^{\circ}$  to  $0^{\circ}\text{C}$ , getoon nie. Daar was geen toename in mikrobiese groei of kontaminasie, asook geen toename in mikrobiologiese tellings gedurende die veroudering vir 42 dae by  $-3^{\circ}$  to  $0^{\circ}\text{C}$  nie.

Met opberging by  $< 4^{\circ}\text{C}$ , beide die *M. gastrocnemius, pars interna* en *M. iliofibularis* het 'n vinnig daling in pH getoon gedurende die eerste paar ure *post-mortem*. Die *M. gastrocnemius, pars interna* het 'n gemiddelde minimum pH van  $6.07 \pm 0.41$  bereik teen ongeveer  $3.50 \pm 0.84$  ure *post-mortem*. Die *M. iliofibularis* het 'n gemiddelde minimum pH van  $5.81 \pm 0.07$  bereik teen ongeveer  $2.50 \pm 0.58$  ure *post-mortem*. Daar was verder gevind dat by  $37^{\circ}\text{C}$  volledige *rigor mortis* in die *M. gastrocnemius, pars interna* bereik was (maksimum isometriese spanning) op die tydstip van die minimum pH ( $5.76 \pm 0.13$ ).

Met die vinnige tempo van pH daling (bereik 'n minimum pH binne 2 tot 4 ure *post-mortem*) en die voltooiing van volledige *rigor mortis* binne die eerste paar ure *post-mortem* (1 tot 4 ure), kan volstruispiere gevolglik binne die eerste 3 tot 4 ure *post-mortem* warm ontbeen word sonder enige nadelige effekte op die kwaliteit in terme van die taaiheid van die vleis.



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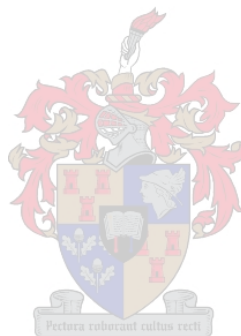




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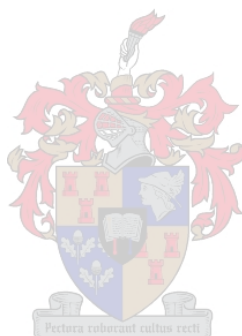
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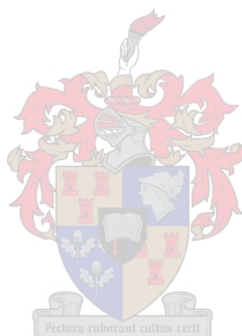
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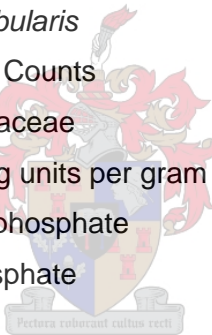
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## LIST OF ABBREVIATIONS

pH <sub>1</sub>	pH reading at 1 hour <i>post-mortem</i>
pH <sub>1 – 10 min</sub>	pH reading at 1 hour and 10 minutes <i>post-mortem</i>
pH <sub>24</sub>	pH reading at 24 hours <i>post-mortem</i>
pH <sub>48</sub>	pH reading at 48 hours <i>post-mortem</i>
T <sub>1</sub>	Temperature (°C) reading at 1 hour <i>post-mortem</i>
T <sub>1 – 10 min</sub>	Temperature (°C) reading at 1 hour and 10 minutes <i>post-mortem</i>
T <sub>24</sub>	Temperature (°C) reading at 24 hours <i>post-mortem</i>
T <sub>48</sub>	Temperature (°C) reading at 48 hours <i>post-mortem</i>
Hot <i>M. gastro</i>	Hot-deboned <i>M. gastrocnemius, pars interna</i>
Cold <i>M. gastro</i>	Cold-deboned <i>M. gastrocnemius, pars interna</i>
Hot <i>M. ilio</i>	Hot-deboned <i>M. iliofibularis</i>
Cold <i>M. ilio</i>	Cold-deboned <i>M. iliofibularis</i>
Intact <i>M. gastro</i>	Intact <i>M. gastrocnemius, pars interna</i>
Intact <i>M. ilio</i>	Intact <i>M. iliofibularis</i>
APC	Aerobic Plate Counts
EBC	Enterobacteriaceae
cfu.g <sup>-1</sup>	colony forming units per gram sample
ATP	Adenosine triphosphate
CP	Creatine phosphate
d	Days
h	Hours
min	Minutes

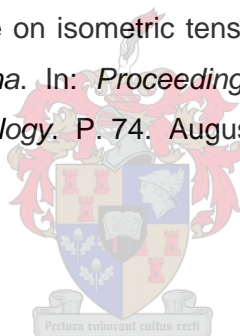


## NOTES

The language and style used in this thesis are in accordance with the requirements of the scientific journal, 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 the chapters has therefore been unavoidable.

Results from this study have been presented at the following Symposiums:

- Botha, S. St.C., Hoffman, L.C. & Britz, T.J. (2004). Muscle pH and temperature changes in ostrich *M. iliofibularis* and *M. gastrocnemius, pars interna* during the first 24 hours *post-mortem*. In: *Proceedings of the 2<sup>nd</sup> Joint Congress of the Grassland Society of Southern Africa and the South African Society of Animal Science*. P. 152. 28 June – 1 July 2004. Goudini, South Africa.
- Botha, S. St.C., Hoffman, L.C., Britz, T.J. , Nilsen, B.N. & Slinde, E. (2004). The effect of rigor-temperature on isometric tension, shortening and pH for ostrich *M. gastrocnemius, pars interna*. In: *Proceedings of the 50<sup>th</sup> International Congress of Meat Science and Technology*. P. 74. August 2004. Helsinki, Finland.



# Chapter 1

## INTRODUCTION

The consumer uses three sensory attributes, appearance, texture, and flavour to judge meat quality (Liu *et al.*, 1995). Visual appearance of meat products appears to be the most important as it strongly influences the consumers' evaluation and selection of meat and meat products (Fletcher, 2002). Fat content, fat colour and meat colour include the major contributing components to product appearance (Grunert, 1997; Fletcher, 2002). Colour is known to be the foremost selection criterion for the purchase of fresh meat and meat products. Consumers perceive fat as a negative criterion for health reasons (Dransfield, 2001), whereas the positive aspects of fat such as flavour and tenderness are not perceived as important (Grunert, 1997). Sales (1996) indicated that the intramuscular fat content of ostrich meat is below the range for mammalian as well as poultry muscle. With ostrich meat containing a lower percentage of fat than found in turkey and beef (Paleari *et al.*, 1998), it may be marketed as a healthier alternative to other red meats.

Meat colour is another meat quality attribute that influences appearance and it is commonly used by consumers as an indicator of freshness and therefore has an important impact on the acceptance of red meat (Issanchou, 1996). Visual appearance of red meat can be related to the probability of consumers making a purchase decision, with this probability decreasing as the appearance shifts from red to purple to brown (Carpenter *et al.*, 2001). In term of meat colour, raw ostrich meat has been described as slightly dark red to slightly cherry red (Paleari *et al.*, 1995). The colour range of raw beef is more towards a moderately cherry red colour. Thus, ostrich meat is darker in colour than beef.

Other examples of negative purchasing appearance traits include surface characteristics such as iridescence and exudates (Issanchou, 1996). Normally, fresh meat exudes fluid from cut surfaces *post-mortem*. This is known as "weep" or purge, and is more noticeable in pre-packaged meat cuts (Lawrie, 1998). The occurrence of purge is, amongst other factors, dependent on the water holding capacity (WHC) of the meat. Vacuum-packaged meat cuts with excessive liquid accumulation (purge) can negatively influence the visual appearance of the meat product, and is thus economically detrimental to meat suppliers.

It is suggested that the juiciness of meat depends on how loosely the water is bound in the meat. Water holding capacity affects meat appearance before cooking, the behaviour of the meat during cooking, and juiciness of the meat on mastication, thus making it an important meat quality attribute (Lawrie, 1998). It has been reported that cooking loss influences the juiciness of the meat but the relationship between cooking loss and the initial juiciness depends on the raw quality (pH) of the meat. Both the extent and the rate of *post-mortem* pH fall affect the water holding capacity in meat (Lawrie, 1998), and consequently, the juiciness. In the first place, the higher the ultimate pH, the greater the increase in water holding capacity, while the faster the rate of pH decline, the greater is the decrease in water holding capacity. Aaslyng *et al.* (2003) demonstrated that an increase in WHC or pH beyond a certain level did not decrease the cooking loss additionally and would therefore not result in an increase in juiciness.

It is well known that as the tenderness of meat increases, overall consumer acceptability increases (Cross & Stanfield, 1976). However, the importance of tenderness and juiciness depends on the products, as well as on the consumer. As toughness increases, the importance of flavour and juiciness in consumer satisfaction becomes more important (Miller *et al.*, 2001). It has been shown that tenderness of ostrich meat is similar to that of turkey and is more acceptable than beef (Paleari *et al.*, 1998). It is important to remember that meat tenderness is a function of production, age of the animal, processing and value adding as well as the meat preparation methods used by the consumer (Issanchou, 1996). The improvement of meat tenderness has great value, since consumers are willing to pay a higher price for tender meat (Miller *et al.*, 2001). However, acceptability of meat is largely determined by the large variation in tenderness (Dransfield *et al.*, 1982). Similarly to other meat animals, with the ageing of the ostrich, the tenderness of the meat tends to decrease (Mellett & Sales, 1997; Hoffman & Fisher, 2001). In South Africa, ostriches are slaughtered at an age of approximately 12 to 14 months in order to obtain high profit products, such as skin (for optimal leather quality), feathers and meat (Paleari *et al.*, 1998; Sales, 1999).

Overall meat quality is not only an inherent characteristic of the animal, but extrinsic factors such as *post-mortem* processing, handling and the environmental temperature (Lawrie, 1998) all play an important role in the final quality of the meat product. While the performance of hot-deboning is beneficial for the reduction in costs, time and refrigerator capacity and space (Pollok *et al.*, 1997), the possibility of cold-shortening could be detrimental for meat eating quality, particularly in terms of tenderness (Taylor *et al.*, 1980-81).

Hot-deboning was developed in response to commercial desires for reduction in both energy usage and refrigeration space requirements (Pollok *et al.*, 1997). The major commercial attraction of hot-deboning proved to be the considerable reduction in time, space and refrigeration capacity requirements. In contrast to these mentioned benefits of hot-deboning, eating quality would be reduced by the risk of cold-shortening (Taylor *et al.*, 1980-81). Fortunately, the increased risk of cold-shortening can be avoided by delayed chilling, but it is suggested by Taylor *et al.* (1980-81) that the maximum saving in time and cost are achieved when hot-deboning is preceded by electrical stimulation.

In general, temperature decline in hot-deboned muscles is faster and more uniform than in muscles left on the carcass (Van Laack & Smulders, 1992), which on the one hand is beneficial for controlling microbial spoilage (Lawrie, 1998). On the other hand, since the chilling and freezing is more rapid in hot-deboned meat cuts, the tendency for the occurrence of cold-shortening and super contraction of muscle fibres may be enhanced. When muscle temperature is reduced to below 10°C to 15°C while muscles are still in the early *pre-rigor* condition with a pH of approximately 6.0-6.4, there is a risk of cold-shortening. To avoid cold-shortening, it has been recommended to debone at muscle temperatures between 5°C and 15°C and then holding the vacuum-packed meat cuts at this temperature for at least 10 h *post-mortem* (Lawrie, 1998). Alternatively, electrical stimulation of the carcass immediately after slaughter could also be used to aid in preventing the occurrence of cold-shortening.

Sales & Mellett (1996) and Sales *et al.* (1996) found ostrich *M. iliofibularis* to have a rapid pH decline up to 2 h *post-mortem*, after which the pH started to increase. Morris *et al.* (1995) reported the most intense *post-mortem* pH decline for the ostrich *M. iliofibularis* and *M. gastrocnemius* to take place within 30 min after slaughter (not hot-deboned). Based on the results of Sales & Mellett (1996), the risk of cold-shortening would be reduced in the *M. iliofibularis* since it reached a pH = 6.20 at approximately 34 min *post-mortem*. Sales and co-workers (1996) reported that the noticeable high ultimate pH was reached rapidly at 2 h *post-mortem* in the *M. iliofibularis* ( $6.00 \pm 0.087$ ), and at 6 h *post-mortem* in the *M. gastrocnemius, pars interna* ( $6.12 \pm 0.056$ ). Therefore, it was concluded that there is a risk of cold-shortening in the *M. gastrocnemius, pars interna* if this muscle should be separated from the carcass after 30-45 min *post-mortem*. This would not be the case with the *M. iliofibularis*. With the fast rate of pH decline in ostrich muscles and the apparent absence of cold-shortening (Sales, 1994), the application of electrical stimulation to ostrich carcasses would appear to be an unnecessary aid to reduce the occurrence of cold-shortening and to improve the tenderness of ostrich meat.

Results reported in the literature on the effect of hot-deboning on the water holding capacity (WHC) of muscles, are contradictory. Miller *et al.* (1984) and Neel *et al.* (1987)



reported that hot-deboned primal pork muscles resulted in a higher WHC than in cold-deboned muscles. Similarly, results obtained by Taylor *et al.* (1980-81) confirmed that hot-deboning of beef muscles minimized drip loss due to more rapid cooling. However, electrical stimulation marginally increased drip loss compared to non-electrical stimulated meat when electrical stimulation was applied to the carcass before hot-deboning. It was suggested by Taylor *et al.* (1980-81) that the early fall in pH increased protein denaturation during chilling, and thus decreased the beneficial effect of hot-deboning on the degree of drip loss. On the other hand, Weakly *et al.* (1986) and Wiley *et al.* (1989) documented that drip loss increased after hot-deboning of pork muscles and subsequent chilling at 0°C to 2°C, while the results obtained by Van Laack & Smulders (1992) showed that hot-deboning hardly affected drip loss in pork. However, it is suggested that these contradicting results could be due to the different methods used in the individual studies to measure WHC, but also possibly because the chilling conditions applied could not result in appreciable differences in pH and temperature decline. It is therefore important to provide thorough information on the pH and temperature profiles during chilling.

Cross *et al.* (1979) and Cross & Tennent (1980) reported that, when electrically stimulated, hot-deboning resulted in less purge in vacuum packed beef cuts that had been stored for 7, 14 and 20 d compared to cold-deboned cuts. Griffin *et al.* (1992) also indicated that electrically stimulated, hot-deboned beef muscles (*M. longissimus thoracis et lumborum* and *M. semimembranosus*) showed lower visible purge in vacuum packages than non-stimulated, cold-deboned muscles. Griffin *et al.* (1992) suggested that if electrical stimulation was used in combination with hot-deboning, bovine muscles can be stored for 7 to 21 d in vacuum packages without any detrimental effects on subsequent retail display appearance.

In terms of the effect of hot-deboning on raw meat colour, Taylor *et al.* (1980-81) concluded that hot-deboning produced a more even colour across large bovine muscles because of the more rapid cooling made possible by early deboning. Cross *et al.* (1979) found that muscles removed at 1 h *post-mortem* were significantly darker than those removed at 48 h *post-mortem* after storage for 20 d. In contrast, Griffin *et al.* (1992) did not find any difference in colour after storage between electrically stimulated, hot-deboned and non-stimulated, cold-deboned beef muscles.

Over the last two decades, meat research has mainly concentrated on understanding and improving the technological processes of meat production to provide a better quality and variety of products to the consumer (Dransfield, 2001). The meat industry has now changed from a production-driven system to a consumer led industry. The meat industry can benefit from the knowledge on production obtained by meat

scientists and technologists, enabling the meat industry to respond to the goals set by consumers and society.

Currently in South Africa it is common practice to refrigerate ostrich carcasses for 24 h before deboning is performed (i.e. cold-deboning). However, with the benefits of hot-deboning in terms of the reduction in overall costs, time, space requirements and refrigerator capacity requirements, the ostrich industry is interested in the effects of hot-deboning on the shelf-life and eating quality of ostrich meat cuts. As discussed above, hot-deboning influences meat quality in several ways, and it would therefore be beneficial to investigate the effects of hot-deboning on the quality characteristics of ostrich meat for guidance towards future processing technologies.

The objectives of this study were to investigate the effects of hot-deboning on the physical quality characteristics of ostrich *M. gastrocnemius, pars interna* and *M. iliofibularis* to determine whether;

- hot-deboning would negatively affect the tenderness of the meat from the *M. gastrocnemius, pars interna* and the *M. iliofibularis* early *post-mortem* and during *post-mortem* aging,
- hot-deboning would cause cold-shortening in the *M. gastrocnemius, pars interna* muscles and thus lead to tougher meat,
- hot-deboning would decrease the shelf-life of vacuum packaged meat from the *M. gastrocnemius, pars interna* and the *M. iliofibularis* in terms of microbiological safety, raw meat colour and water holding capacity (purge) and whether
- hot-deboning would have negative effects on the overall holding and eating quality of vacuum-packaged ostrich meat cuts intended for export.

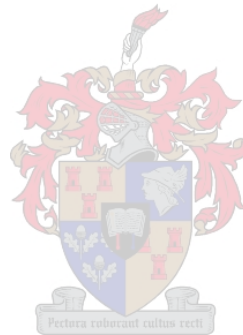
In addition to these objectives, it was also the objective of this study to investigate the development of *rigor mortis* at respectively 7°C and 37°C, as well as the pH profiles for ostrich *M. gastrocnemius, pars interna* and *M. iliofibularis* to obtain a better understanding of the *post-mortem* changes within ostrich muscles.

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## Chapter 2

### LITERATURE REVIEW

#### 1. BACKGROUND

South African ostrich abattoirs commonly refrigerate ostrich carcasses for 24 h *post-mortem* at  $< 4^{\circ}\text{C}$  before the muscles are excised (cold-deboning) and vacuum packed for retail and export purposes. However, with the benefits of hot-deboning, which include the reduction of time, space and costs, it is of great interest for the ostrich industry to know the effects of hot-deboning on the physical quality characteristics of meat. These include the pH (*post-mortem* glycolytic rate), tenderness, colour of raw meat, as well as the water holding capacity (WHC). To attain such information, it is therefore necessary to have an understanding of the biochemical and physical processes that occur in muscles *post-mortem*, and to know about the histological, biophysical and chemical characteristics of meat in general.

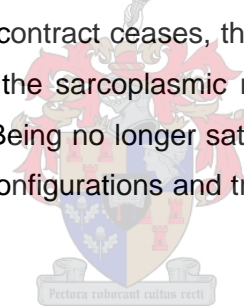


#### ***Muscle contraction***

##### ***Muscle contraction in vivo***

In normal resting muscles, myosin is prevented by troponin I from binding with actin by the magnesium complex of adenosine triphosphate ( $\text{MgATP}^{2-}$ ) (Lawrie, 1998) since  $\text{Mg}^{2+}$  strongly inhibits the rate of ATP (adenosine triphosphate) hydrolysis (Pearson & Young, 1989). The two most important ATP-splitting enzymes in normal resting muscles include myosin-ATPase and  $\text{Ca}^{2+}$ -ATPase (Pearson & Young, 1989). Myosin-ATPase is located in the myosin heads and possesses the highest potential ATPase activity of the muscle enzymes that can split ATP. The  $\text{Ca}^{2+}$ -ATPase is bound to the sarcoplasmic reticulum membrane and removes  $\text{Ca}^{2+}$  from the cytosol during the rest cycle of an ATP-dependent transport process. Since muscle membranes are not completely impermeable,  $\text{Ca}^{2+}$  slowly leaks into the cytosol and therefore  $\text{Ca}^{2+}$ -ATPase catalyses the breakdown of ATP to supply energy for “pumping” the  $\text{Ca}^{2+}$  back across the membranes in order to maintain resting physiological levels of  $\text{Ca}^{2+}$  and to assist in the prevention of muscle contraction.

Muscle contraction is initiated by a nerve impulse passing along the T-tubules, causing the sarcoplasmic reticulum membranes to lose some of the accumulated  $\text{Ca}^{2+}$  ions (Stromer *et al.*, 1974). This efflux of  $\text{Ca}^{2+}$  from sarcoplasmic reticulum membranes triggers muscle contraction since myosin-ATPase activity is stimulated by increased cytoplasmic  $\text{Ca}^{2+}$  levels (Pearson & Young, 1989). The sarcolemma temporarily loses its impermeability to potassium ( $\text{K}^+$ ) and sodium ions ( $\text{Na}^+$ ), and  $\text{Ca}^{2+}$  ions then dissociate from the calsequestrin where they are normally bound in the sarcotubular system (Lawrie, 1998). Consequently the  $\text{Ca}^{2+}$  concentration rises, saturating troponin C, the calcium-binding unit of the troponin complex. This causes a configuration change where the inhibitory protein, troponin I, no longer prevents actin from interacting with the  $\text{MgATP}^{2-}$  on the H-meromyosin heads of the myosin molecule. The contractile ATP-ase in the vicinity of the linkage is activated to split  $\text{MgATP}^{2-}$  to  $\text{MgADP}^-$ , providing the energy for the actin filament to be pulled inwards towards the centre of the sarcomere. The junction between actin and myosin is simultaneously broken. The process is repeated as long as there is an excess of  $\text{Ca}^{2+}$  ions to saturate the troponin C and myosin cross bridges link with the myosin-binding sites on actin at successively peripheral locations as the interdigitation continues. When the stimulus to contract ceases, the  $\text{Ca}^{2+}$  ions are actively pumped back into the sarcotubular system by the sarcoplasmic reticulum pump which depends upon ATP for the necessary energy. Being no longer saturated with  $\text{Ca}^{2+}$  ions, troponin C and troponin I return to their resting configurations and troponin I again prevents interaction of myosin and actin.



### ***Muscle contraction post-mortem***

Shortening during the development of *rigor mortis* is reflected by a decrease in the sarcomere lengths of muscles in the *post-mortem* condition (Pearson & Young, 1989). Rigor shortening is explained by the release of  $\text{Ca}^{2+}$  ions from both mitochondria and the sarcoplasmic reticulum into the myofibrillar space at ATP concentrations sufficient for contraction (Honikel *et al.*, 1983). Whiting (1980) reported that the mitochondria are the first organelles to lose their *post-mortem* ability to sequester  $\text{Ca}^{2+}$  as the pH declines from 6.5 to 6.0, while the sarcoplasmic reticulum starts to lose its  $\text{Ca}^{2+}$  sequestering ability at pH values between 5.5 and 6.0. Hertzman *et al.* (1993) concluded that the  $\text{Ca}^{2+}$  release by mitochondria seems to be more important for rigor shortening than that by the sarcoplasmic reticulum.

In *post-mortem* muscles, the cells attempt to maintain ATP at physiological levels for as long as possible by minimising ATP-hydrolysis to essential processes (Pearson & Young, 1989). The development of *rigor mortis* does not occur until approximately half of

the ATP is depleted. With the decrease in ATP levels, there is not enough energy available for pumping  $\text{Ca}^{2+}$  back across the membranes and therefore  $\text{Ca}^{2+}$  slowly leaks into the cytosol. However, temperature and not ATP deficiency is the main cause of  $\text{Ca}^{2+}$  release from the mitochondria and sarcoplasmic reticulum, since the onset of shortening during the rigor process occurs at different and relatively high ATP levels at 15° and 37°C (Hertzman *et al.*, 1993). At low temperatures (below 10°C) the sarcoplasmic reticulum has a decreased ability to sequester and bind  $\text{Ca}^{2+}$  ions, while the mitochondria have a decreased  $\text{Ca}^{2+}$  binding capacity (Pearson & Young, 1989; Lawrie, 1998). At higher temperatures, Whiting (1980) reported that the  $\text{Ca}^{2+}$  uptake ability of the mitochondria decreased rapidly at temperatures above 20°C, while the  $\text{Ca}^{2+}$  uptake ability of the sarcoplasmic reticulum survived up to temperatures of > 37°C, but little activity remained at 49°C. The excess  $\text{Ca}^{2+}$  ions thus caused contraction of the muscle fibre bundles and the depletion of ATP leads to the formation of permanent cross-bridges between the actin and myosin filaments (actomyosin), which cannot be broken in the absence of ATP, leading thus to constant isometric tension (Pearson & Young, 1989).

## 2. MUSCLE TEMPERATURE

The total *pre-rigor* temperature history of muscles affects two important aspects of meat tenderisation: firstly, the degree of muscle shortening and secondly the modification of enzymes responsible for tenderisation (proteolysis) (Devine *et al.*, 1999). Over-effective chilling of hot carcasses can lead to toughness when the temperature of the muscles are reduced below approximately 10° to 15°C while they are still in the early *pre-rigor* condition with a pH of about 6.0-6.4 (Lawrie, 1998). At these conditions there is a tendency for shortening of the muscles and thus toughness on subsequent cooking. The greater the bulk of the carcass and the greater the amount of fat covering the carcass, the longer it will take to cool with a given air speed and temperature. The rate of *post-mortem* glycolysis increases with increasing external temperature above ambient; however, the rate also increases as the temperature at which it takes place decreases from about 5° to 0°C (Lawrie, 1998). With the application of hot-deboning, muscle temperature may drop to values of below 10° to 15°C while muscles are still in the early *pre-rigor* condition and since hot-deboned muscles are not attached to the carcass, muscle contraction and shortening is enhanced. However, the temperature decline in hot-deboned muscles is faster and more uniform than in muscles left on the carcass (Van Laack & Smulders, 1992), which is beneficial for controlling microbial spoilage (Lawrie, 1998) and therefore increasing the shelf-life.



The rate of *post-mortem* glycolysis will tend to be higher in muscles that are slow to cool, since higher temperatures are known to speed up the rate of chemical reactions (Pearson & Young, 1989; Lawrie, 1998). It is obvious that, in animal carcasses, various muscles will have different rates of *post-mortem* temperature decline according to the anatomical location of the muscles to the exterior and their degree of insulation. The rate of tenderisation early *post-mortem* would also be enhanced at higher muscle temperatures. However, pH is a detrimental factor influencing the activity of indigenous proteases, which are grouped by their optimum pH-values as follows: (i) the alkaline proteases; (ii) the neutral proteases that are activated by  $\text{Ca}^{2+}$ ; the calcium activated sarcoplasmic factors (CASF) or calpains; and (iii) the cathepsins or acid proteases (A, B, C, D, and L) (Pearson & Young, 1989). The role of the alkaline proteases are probably of minor importance since muscle pH soon falls below 7.0, but the CASF would remain active in muscles even after the pH have dropped below neutral. As the pH continues to decrease, the cathepsins (A, B, C, D, and L) may become active and cause additional degradation of the muscles.

### 3. **POST-MORTEM pH**

Anaerobic glycolysis occurs when oxygen is permanently removed from muscles *post-mortem*, leading to the conversion of glycogen to lactic acid and a subsequent fall in muscle pH (Lawrie, 1998). The conversion of glycogen to lactic acid will continue until a pH where the enzymes affecting the breakdown of glycogen, become inactivated. In typical muscles this pH is at a value of approximately 5.4-5.5, which is also the iso-electric point of the principal muscle proteins and consequently some loss in water holding capacity (WHC) is inevitable as the fall in muscle pH continues. The higher the ultimate pH, the less will be the decrease in WHC. Clearly the ultimate muscle pH, the extent and the rate of *post-mortem* pH decline, affects physical meat characteristics, such as colour, WHC and microbiological growth (Lawrie, 1998).

In addition, the rate of *post-mortem* glycolysis has an effect on the tenderness of aged meat, since it influences proteolytic enzyme activity (O'Halloran *et al.*, 1997). Morton *et al.* (1999) suggested that in beef, there is no correlation between the rate of tenderisation or ultimate meat tenderness and ultimate pH; however, there is an association between the rate of pH decline *post-mortem* and the rate of meat tenderisation. O'Halloran *et al.* (1997) demonstrated that fast glycolysing beef *M. longissimus thoracis et lumborum* were more tender than slow glycolysing muscles. It was concluded that low pH conditions in fast glycolysing muscles enhanced the release of

cathepsins B and L from the lysosomes, and that the activity of calpains were higher, while the calpastatin activity was lower.

The ultimate pH values (24 h *post-mortem*) of ostrich muscles suggest that ostrich meat may be classified as an intermediate meat type between normal (pH < 5.8) and extreme dark, firm and dry (DFD) (pH > 6.2) meat (Sales & Mellett, 1996). Sales & Mellett (1996) found a rapid *post-mortem* decline in pH for the *M. iliofibularis* muscle (the apparent ultimate pH was reached at 2 h *post-mortem*), thereafter this muscle showed an unusual increase in pH. It was suggested that, since this muscle reached a pH of 6.2 at less than 1 h *post-mortem*, there is no risk of cold shortening when separated from the carcass. On the other hand, the *M. gastrocnemius, pars interna* reached the ultimate pH values of 6.12 at approximately 6 h *post-mortem*. Based on the above mentioned data, Sales & Mellett (1996) thus concluded that in the *M. gastrocnemius, pars interna* muscles there is a risk of cold shortening when separated at 30 to 45 min *post-mortem*.

#### 4. DEVELOPMENT OF *rigor mortis*

The onset of *rigor mortis* is correlated with the disappearance of ATP (Tornberg, 1996; Lawrie, 1998) however, this does not occur across all muscles simultaneously (Hwang *et al.*, 2003). In the absence of ATP, actin and myosin combine to form rigid chains of actomyosin and the loss of extensibility, which is referred to as *rigor mortis*, is observed. In muscles which are free to shorten, the loss of tenderness during the onset of *rigor mortis* is directly related to the degree of interaction of actin and myosin filaments (shortening) at that time (Lawrie, 1998).

According to differences in ATP levels, the rigor process consists of two phases; a delay phase and a rapid phase (Tornberg, 1996; Lawrie, 1998). During the delay phase, the level of ATP is constant; the creatine phosphate (CP) levels decrease rapidly, while the formation of actomyosin proceeds slowly and there is a slow production of lactate (Tornberg, 1996). The time to the onset of the rapid phase is directly dependent on the level of ATP. During the period immediately *post-mortem*, ATP levels are slowly decreased by the surviving non-contractile ATP-ase activity of myosin. The level of ATP can be maintained for a short time by resynthesis from ADP (adenosine diphosphate) and CP. Also, anaerobic glycolysis can resynthesise ATP *post-mortem* when the stores of CP are depleted, but only inefficiently and the overall level of ATP falls. However, even if glycogen is abundant *post-mortem*, the resynthesis of ATP by anaerobic glycolysis cannot maintain it at a level sufficiently high to prevent the formation of actomyosin. Clearly, with low glycogen levels *post-mortem*, the decrease in ATP levels will proceed earlier.

Consequently, when the levels of CP are low enough, a rapid decline in the ATP (rapid phase) is initiated, accompanied by a shortening of the muscle and the development of a force under isometric conditions (i.e. developing tension while the muscle is prevented from contracting). Jungk *et al.* (1967) documented that 4 to 5  $\mu$ moles of ATP per gram of muscle was utilised by the time that tension development in rabbit *Psoas* and beef *Geniohyoideus* and *Semitendinosus* muscles, occurred.

In summary, during the development of *rigor mortis*, muscles become inextensible due to the sum of each muscle fibre going into full rigor, with irreversible cross bridge formation of the contractile components, actin and myosin (Hwang *et al.*, 2003). The muscle's sarcoplasmic reticulum and mitochondria loses their ability to bind calcium ions, utilise CP and ATP, produce lactic acid and develop tension (Schmidt *et al.*, 1970b). Due to the disappearance of ATP and the consequent formation of actomyosin, the onset of *rigor mortis* is accompanied by a lowering in water holding capacity (WHC) (Lawrie, 1998). However, the drop in pH, consequent approach of the muscle proteins to their iso-electric point, and denaturation of the sarcoplasmic proteins during the onset of *rigor mortis* also contribute to the loss in WHC.

During the onset of *rigor mortis*, not only longitudinal but also lateral contraction occurs (Tornberg, 1996). It was suggested that this decrease in cross-sectional area of the myofibrils (lateral contraction) during rigor is partly due to a fall in pH and partly due to the attachment of myosin heads to the actin. Both the longitudinal and lateral shrinkage of the myofibrils cause the fibres to shrink and the water that is left behind to accumulate, first along the perimysial network and later along the endomysial network, giving rise to extracellular compartments around both the fibres and the fibre bundles. These compartments of water give rise to a more viscous behaviour of raw meat compared to cooked meat. Since bridge formation between actin and myosin is the main cause of the lateral contraction during rigor, the degree of lateral contraction increases with shorter sarcomeres. On heating to temperatures above 60°C, when the contraction of the connective tissue begins, larger extracellular space would give more room for the connective tissue to contract without being restricted by the myofibrillar mass. Therefore, it was concluded by Tornberg (1996) that a more shortened muscle shows a higher cooking loss and a higher number of fibres per unit cross-area, leading to higher Warner-Bratzler peak shear force values.

The characteristics of *rigor mortis*: including the levels of ATP and CP initially and at onset; the initial pH value at onset and the ultimate pH value; the initial and residual stores of glycogen; the activities of ATP-ase and of the sarcoplasmic reticulum pump, will all vary according to intrinsic factors, such as species and type of muscle (Lawrie, 1998). Extrinsic factors, such as the degree of struggling before slaughter, the environmental

temperature, as well as the muscle temperature will also influence the above mentioned *rigor mortis* characteristics.

### ***Measurement of rigor mortis***

At present, the measurement of isometric tension and muscle shortening during the development of *rigor mortis* is performed with the use of the rigometer (Rigotech) (Fig. 1), where isometric tension is expressed as force per unit area and muscle shortening is expressed as percentage decrease of the initial muscle sample length (Devine *et al.*, 1999). Shortening of a muscle sample can be continuously followed during the rigor process as a function of time in a cell of constant temperature by maintaining the sample at constant length (i.e. isometrically) (Hertzman *et al.*, 1993). A typical analysis is performed by carefully cutting strips of muscles parallel to the fibre direction from muscles samples at approximately 30 min *post-mortem*. The length of the muscle strips is approximately 30-35 mm with a mass of approximately 1-2 g. The muscle strips are covered with a mixture of liquid paraffin and petroleum jelly to provide an anaerobic environment and to minimize dehydration. To minimize slippage of the muscle fibres, the ends of the muscle strips are glued with cyanoacrylate glue to the aluminium discs, which are applied to the apparatus. Muscles that are unrestrained (or under light load) will shorten as they develop *rigor mortis*, while muscles that are restrained by being maintained at a constant length will develop tension (Pearson & Young, 1989).



**Figure 1.** The rigometer (Rigotech®) is used to measure isometric tension ( $\text{mN}\cdot\text{mm}^{-2}$ ) and shortening (%) during the development of *rigor mortis* in the muscle strips at constant temperature ( $^{\circ}\text{C}$ ).

### ***Isometric tension and shortening***

The development of isometric tension during *rigor mortis* can be characterised by a delay period, which is shorter at  $37^{\circ}\text{C}$  than at  $15^{\circ}\text{C}$  (Hertzman *et al.*, 1993). Devine *et al.* (1999) measured isometric tension and muscle shortening with the use of the rigometer. For isometric tension, their results showed an initial lag phase followed by a steep increase for tension. This rate of tension development was different for each rigor temperature evaluated ( $15^{\circ}$ ,  $20^{\circ}$ ,  $25^{\circ}$ ,  $30^{\circ}$ , and  $35^{\circ}\text{C}$ ). At temperatures above  $15^{\circ}\text{C}$ , shortening started at pH of 6.3 in beef neck muscles, *M. sternomandibularis* and *M. mastoideus*, while rigor onset occurred at a pH of 6.25 when muscle were held at  $38^{\circ}\text{C}$  (Honikel *et al.*, 1983).

It was observed that the onset of shortening starts prior to the onset of isometric tension (Hertzman *et al.*, 1993) and before the onset of rigor (Honikel *et al.*, 1983). Generally, shortening of muscles should take place before the onset of rigor since contraction of muscle fibres needs a sufficient ATP concentration and an increase in the concentration of calcium ions around the myofibrils (Honikel *et al.*, 1983). Shortening occurs when myosin heads start to attach to actin (forming actomyosin). However, the muscle can still be extensible if there is enough ATP available (Hertzman *et al.*, 1993). With the onset of isometric tension, extensibility of the muscle is lost and the actomyosin

becomes irreversible. However, the rapid development of force during the onset of isometric tension does not start until all CP is depleted. Shortening, which could be both cold and *rigor mortis* shortening, is explained by the release of ionic calcium into the myofibrillar space at ATP concentrations sufficient for contraction (Honikel *et al.*, 1983). Both in the case of cold and *rigor mortis* shortening there is a high correlation between maximum shortening and the amount of ATP at shortening onset (Hertzman *et al.*, 1993).

Thus, in summary, the development of *rigor mortis* starts with the onset of shortening, which is followed by the start of the force development during the onset of the isometric tension (Hertzman *et al.*, 1993). Consequently the total depletion of CP and the onset of the rapid phase for rigor follow. Fully developed rigor, characterised by constant shortening, constant isometric tension and constant pH is reached approximately within the same time region.

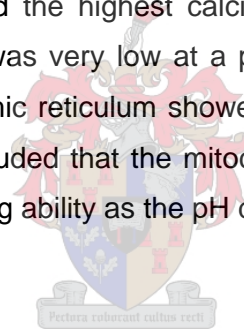
### ***Effect of post-mortem pH on rigor mortis***

The tenderness of cooked meat is inversely related to the rate of *post-mortem* pH fall with increasing tenderness when the pH fall is slow (Lawrie, 1998). The maintenance of a relatively high pH in combination with near *in vivo* temperatures for some time *post-mortem* may induce early conditioning changes by enzymes such as the calcium activated sarcoplasmic factors (CASF).

Regardless of rigor temperature, Fernandez & Tornberg (1994) demonstrated that maximum shortening during development of *rigor mortis* was not significantly affected by ultimate pH. However, regarding maximum isometric tension, these authors found a significant effect of ultimate pH only at high rigor temperatures (35°C). It was found that the maximum tension that developed in pig *Longissimus dorsi* muscle held at 35°C increased with increasing pH. This phenomenon was also demonstrated by Wood & Richards (1974) in chicken *Pectoralis major* muscle held at 23°C. Fernandez & Tornberg (1994) suggested that the positive correlation between ultimate pH and maximum isometric tension obtained at 35°C, but not at 12°C, could be explained by the denaturation of proteins at 35°C. A significant decrease in solubility of proteins was recorded at 35°C; while at 12°C there was no aggregation of proteins. This loss in solubility decreased continuously as pH increased until 6.0. The higher the ultimate pH, the further the enzymes are from their iso-electric points and consequently the less susceptible they are to denaturation. Thus, at 35°C, as the ultimate pH decreases, the contractile ability of the myofibrillar system would decrease because of denaturation.

As mentioned, the release of calcium ions into the actomyosin contractile system during the onset of rigor will initiate a shortening of the muscle and subsequent toughening of the meat (Lawrie, 1977). The decrease in pH at a constant temperature results in an accelerated release of  $\text{Ca}^{2+}$  ions (Kanda *et al.*, 1977). The *post-mortem* ability of the sarcoplasmic reticulum and the mitochondria to sequester calcium, which in their turn is influenced by *post-mortem* conditions (pH and temperature), will have a profound effect on the onset of *rigor mortis* (Lawrie, 1977). Whiting (1980) demonstrated the effects of pH on calcium uptake ability of isolated sarcoplasmic reticulum and mitochondria from the *Biceps femoris* of cattle. For the sarcoplasmic reticulum, activity increased as the pH declined to 6.5 and then rapidly decreased as the pH decreased below a pH-value of 6.0. Cassens & Cooper (1971) reviewed that sarcoplasmic reticulum isolated from white muscle seemed to have a higher total calcium uptake and initial rate of uptake compared to sarcoplasmic reticulum from red muscle. It was also found that the optimum pH for total calcium uptake was at a pH range of 6.5-7.0 for white and a range of 5.7-6.4 for red muscle sarcoplasmic reticulum.

The mitochondria showed the highest calcium uptake activity at pH 7.2, which rapidly declined at pH 6.5 and was very low at a pH-value of 5.5 (Whiting 1980). The mitochondria and the sarcoplasmic reticulum showed practically no activity near a pH of 5.0. From this it could be concluded that the mitochondria would be the first to lose its *post-mortem* calcium sequestering ability as the pH declines from 6.5 to 6.0.



### ***Influence of temperature on rigor mortis***

Development of *rigor mortis*, including shortening and isometric tension, is highly dependent on temperature as demonstrated by Hertzman *et al.* (1993), revealing that temperature, compared to the influence of the type of muscle and electrical stimulation (ES), is the dominating factor affecting the rate of rigor development. The degree of tension development and shortening during the onset of *rigor mortis* in muscles which are free to shorten, is also known to be a direct function of temperature up to 15°C (Lawrie, 1998). If isolated muscles are exposed to temperatures below 14°C at the time of *rigor mortis*, there is an increasing tendency to shorten, where this shortening is as great at 2°C as at 40°C. However, minimal shortening occurs at different temperature regimes for beef *M. longissimus thoracis et lumborum* and *M. semimembranosus* (Hertzman *et al.*, 1993; Olsson *et al.*, 1994). The maximum shortening and the consequential tenderness after 14 d of aging at 4°C obtained at different constant rigor temperatures revealed a minimal shortening range of 10° to 15°C for the *M. longissimus thoracis et lumborum*, and 7° to

13°C for the *M. semimembranosus* (Tornberg, 1996). The temperature-dependence of shortening and tenderness was greater for the *M. longissimus thoracis et lumborum* than for the *M. semimembranosus*, particularly in a region of 7° to 15°C rigor temperature when the *M. longissimus thoracis et lumborum* was more tender than the *M. semimembranosus*. It is thus important to develop rigor in this temperature range in order for the *M. longissimus thoracis et lumborum* to be tender. For the *M. semimembranosus*, it was shown that there is a high negative correlation between shortening (%) and ultimate tenderness both in the warm and the cold-shortening regions. In contrast, for the *M. longissimus thoracis et lumborum* this was only the case in the cold-shortening region from 1° to 10°C and not from 15° to 35°C. This suggests that besides shortening, enzymatic activity was greater in the *M. longissimus thoracis et lumborum* than in the *M. semimembranosus*, and that proteolysis seemed to govern the ultimate tenderness more in the case of the *M. longissimus thoracis et lumborum* in the temperature range of 7° to 15°C. As suggested by Dransfield (1993), there is an initiation of the calpains by the Ca<sup>2+</sup> being released during rigor, followed by a release of the inhibitor calpastatin, as pH decreases during the rigor process. Additionally, it was shown that  $\mu$  and m-calpain activity was substantially depleted at a rigor temperature of 35°C, whereas in muscle held at 15°C, little change in calpain activity in the *pre-rigor* period occurred (Tornberg, 1996). Proteolysis occurred predominantly in the *post-rigor* period and improved tenderness was reached in meat held at 15°C, as compared to 35°C. It was suggested that the lack of tenderisation at 35°C was caused primarily by the rapid depletion of calpains, rather than by calpastatin inhibition.

In the case of shortening, results obtained by Hertzman *et al.* (1993) indicated a delay period for samples at 15°C, while at 37°C shortening started immediately. In contrast, Fernandez & Tornberg (1994) found that the onset of shortening was not dependent on temperature. However, the quantity and rate of shortening is dependent on temperature, being much higher at 37°C than at 15°C (Hertzman *et al.*, 1993). As rigor temperature decreased, muscle shortening also decreased up to about 7°C with a greater percentage of maximum shortening at 1°C compared to 4°, 7° and 10°C (Olsson *et al.*, 1994). The time to reach the maximum shortening is significantly negatively correlated with rigor temperature (Devine *et al.*, 1999). However, results obtained by Olsson *et al.* (1994) showed shortening to be delayed and less intense at 7° and 10°C, while at 1° and 4°C shortening started almost immediately.

Hertzman *et al.* (1993) demonstrated that the difference in hours to obtain constant shortening or constant isometric tension during development of rigor in beef muscles is approximately 16 to 17 h between temperatures of 15° and 37°C. That is, at 37°C fully developed rigor was obtained 16 to 17 h earlier than at 15°C. It was similarly



demonstrated by Fernandez & Tornberg (1994) that the maximum values of shortening were reached at an earlier stage *post-mortem* at 35°C than at 12°C. Contrary to shortening, the onset of isometric tension was dependent upon temperature, with the onset being faster at 35°C than at 12°C. Consequently, the time to reach maximum muscle tension was highly negatively correlated with temperature (Devine *et al.*, 1999). Similar to shortening, the maximum value of isometric tension is also temperature dependent. It was found that the amount of muscle tension was minimal at 15°C and increased as the temperature increased (Devine *et al.*, 1999). Fernandez & Tornberg (1994) also found that maximum isometric tension was higher after rigor at 35°C compared to 12°C and the maximum values were also reached earlier at 35°C than at 12°C. Thus, the rate of onset of isometric tension and the rate of shortening development were higher at high temperatures. Hertzman *et al.* (1993) also found that the time to maximum tension was reduced as the temperature increased, however, in contrast, results obtained by Jungk & Marion (1970) did not indicate any effect of temperature on the time to reach maximum tension in turkey *Pectoralis major* muscle.

In a study conducted by Olsson *et al.* (1994) it was shown that temperature is a dominating factor with regard to the time course of *rigor mortis* compared to electrical stimulation (ES) and the type of muscle. The degree of shortening during rigor development is highly affected by temperature but not by ES (Hertzman *et al.*, 1993). It was found that maximum shortening and isometric tension were higher at 37°C, compared to 15°C, while ES did not reduce rigor shortening. Results from several authors (Locker & Hagyard, 1963; Honikel *et al.*, 1983; Olsson *et al.*, 1994) revealed that maximum shortening increases with decreasing temperature. Concerning the onset of shortening it was also found that there was a significant interaction between muscle and temperature (Olsson *et al.*, 1994).

The calcium sequestering stability and activity of both the sarcoplasmic reticulum and the mitochondria are temperature dependent (Whiting, 1980). It was demonstrated that the calcium uptake stability of the mitochondria rapidly decreased as temperatures increased above 20°C and was virtually non-existent after 30 min at 37°C. In contrast, temperature was not an important factor in sarcoplasmic reticular stability until temperatures were above 37°C. Regarding calcium uptake activity, both the sarcoplasmic reticulum and the mitochondria's rate of uptake increased with increasing temperatures; however, the sarcoplasmic reticulum's calcium uptake activity initially increased more rapidly than the mitochondria but did not increase at temperatures of 25° to 37°C. Results further suggested that a decline in muscle temperature into the cold-shortening temperature range (10° to 15°C) might have a more marked effect on the calcium accumulating ability of the mitochondria than that of the sarcoplasmic reticulum. It is thus

clear that mitochondria are more sensitive to temperature than the sarcoplasmic reticulum. Mitochondria are generally more labile than the sarcoplasmic reticulum and Whiting (1980) concluded that under normal aging and cold-shortening conditions, mitochondria could be the initial agents of calcium release.

## 5. RIGOR (WARM) SHORTENING

During *post-mortem* glycolysis, some shortening occurs in all muscles, which are free to shorten, at temperatures between  $-1^{\circ}$  and  $38^{\circ}\text{C}$ , with a minimum shortening at  $15^{\circ}$  to  $20^{\circ}\text{C}$  (Lawrie, 1998). Rigor shortening increases at temperatures above  $20^{\circ}\text{C}$ , while shortening at temperatures below  $10^{\circ}$  to  $15^{\circ}\text{C}$ , when the pH is still above 6.20, is referred to as cold-shortening. Rigor shortening occurs before the loss of extensibility, when ATP stores have been depleted and pH is at a minimum (Nuss & Wolfe, 1980-81). While, on the other hand, cold-shortening takes place at an earlier stage (Lawrie, 1998).

When shortening was prevented in beef muscles by tight wrapping and *rigor mortis* occurred at a range of temperatures of  $15^{\circ}$  to  $35^{\circ}\text{C}$ , the higher temperatures yielded tougher meat (according to shear force values) (Devine *et al.*, 1999). Even after aging at  $4^{\circ}\text{C}$  this difference in toughness did not decrease. With the measurement of calpain activity throughout the rigor process it was revealed that calpain activity remained constant at all temperatures until a pH of approximately 6.2, where after the activity decreased (Hwang *et al.*, 2003). Conditions of low pH and high temperature are known to denature the contractile proteins which are more stable at *rigor mortis* (Offer, 1991). Such conditions, in conjunction with greater autolysis of calpain at high temperatures could explain why proteolytic (aging) enzymes are reduced in effectiveness, leading to increased shear force (toughness) and reduced aging potential (Dransfield *et al.*, 1992). Extended duration at elevated *post-mortem* temperatures and low pH might be critical in terms of calpain inhibition and toughening of meat (Hwang *et al.*, 2003).

Honikel *et al.* (1983) explained that shortening is due to the release of  $\text{Ca}^{2+}$  ions into the myofibrillar space while ATP concentrations are high enough for contraction. Schmidt *et al.* (1970b) also demonstrated that the increase in free calcium in the sarcoplasm caused contraction during development of *rigor mortis* in pig *Longissimus dorsi* muscle. The sarcoplasmic reticulum has the ability to bind calcium ions ( $\text{Ca}^{2+}$ ) in an active process dependent on ATP utilisation (Cassens & Cooper, 1971). Stimulation of the muscle causes the sarcoplasmic reticulum to release small amounts of free  $\text{Ca}^{2+}$  that elicit contraction, while relaxation is caused by the binding of  $\text{Ca}^{2+}$  by the sarcoplasmic reticulum, which reduces the concentration of  $\text{Ca}^{2+}$  in the sarcoplasm to a critically low

level. According to Whiting (1980) a concentration of 3 mM ATP was required by both the sarcoplasmic reticulum and the mitochondria for maximum calcium uptake. It was demonstrated that the sarcoplasmic reticulum had 89% of its maximum calcium uptake activity at a concentration of 1 mM ATP, while the mitochondria had 76%. Hertzman *et al.* (1993) also found a high correlation coefficient between maximum shortening and ATP level at the onset of the rapid phase for shortening. This indicated that greater shortening was observed when higher energy levels are present *post-mortem*. Therefore, it was concluded that the greater shortening at 37°C compared to 15°C was due to the significant higher ATP level at the onset of the shortening rapid phase, which starts much sooner at 37°C than at 15°C. In addition, the stability of the calcium sequestering ability of especially mitochondria is decreased at high temperatures and at *post-mortem* pH-values of 6.5 to 6.0 (Whiting, 1980). Thus, the decreased stability of the calcium uptake ability of especially mitochondria, together with a faster pH decline at 37°C than at 15°C, might initiate rigor shortening at higher ATP levels, giving a larger maximum shortening at higher temperatures (Hertzman *et al.*, 1993).

## 6. COLD-SHORTENING

Cold-shortening is the response when muscles are exposed to low temperatures (normally below 10° to 15°C) early *post-mortem*, when ATP and pH (above 6.20) levels are still high (Nuss & Wolfe, 1980-81; Lawrie, 1998). On the other hand, rigor tension and shortening occurs much later and at temperatures between 0° and 37°C, reaching maximum values when ATP levels have been depleted and pH is at a minimum value (Nuss & Wolfe, 1980-81). Above a temperature of 12° to 15°C there is a contraction of muscle fibres at rigor, while below this temperature range a contraction occurs before rigor (Hwang *et al.*, 2003). Thus, above 15°C, rigor shortening is exclusively responsible for the shortening effects and this occurs when muscles become depleted of glycogen. With the decrease in temperature below 12°C a *pre-rigor* contraction or shortening takes place until rigor is completed. This shortening with falling temperature arises from increased cellular calcium ( $\text{Ca}^{2+}$ ) from the sarcoplasmic reticulum and mitochondria due to the failure of these organelles to sequester cytoplasmic calcium, which in turn activates actomyosin ATP-ase. When muscles go into rigor in a contracted condition, there is considerable shortening since the actin and the myosin filaments interpenetrate extensively, leading to tough meat when cooked (Lawrie, 1998).

It was suggested by Cornforth *et al.* (1980) that the release of calcium ( $\text{Ca}^{2+}$ ) in the cold-shortening region is caused by the reduced calcium uptake ability of the calcium

accumulating systems as a result of the low temperature. At low temperatures, the  $\text{Ca}^{2+}$  pumps of the sarcotubular system are inhibited, causing an efflux of  $\text{Ca}^{2+}$  ions and continuous breakdown of ATP and enhanced activity of the contractile actomyosin ATP-ase (Lawrie, 1998). Kanda *et al.* (1977) showed that lowering of both the temperature and pH simultaneously increases  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum and this was in effect equivalent to cold-shortening. However, the effect of lowering either temperature or pH independently was greater (higher amount of  $\text{Ca}^{2+}$  released from the sarcoplasmic reticulum) than when the temperature and pH was lowered simultaneously.

According to Honikel *et al.* (1983) more ATP is split by contraction of the muscle due to cold-shortening *post-mortem* than at higher temperatures. Anaerobic glycolysis being the only source of ATP resynthesis after the depletion of creatine phosphate (CP), the velocity of glycolysis at low temperatures is not able to meet the demand for ATP resynthesis, resulting in a reduced ATP level in the muscle. Therefore, ATP starts to disappear at a higher pH and completion of rigor is obtained before the minimum pH-value is reached when rigor temperatures are below 5°C. In contrast, Nuss & Wolfe (1980-81) found that at temperatures of 5° and 0°C the drop in pH lags behind the fall in ATP and glycogen, presumably due to a greatly reduced rate of conversion of hexose-6-phosphate to triose phosphates. Maximum tension at low temperatures was attained several hours after minimum levels of ATP, glycogen and pH were reached, indicating that attainment of maximum rigor tension and time to maximum tension are not directly related to drop in ATP, glycogen or pH. It was indicated that the strong rigor tension which develops at temperatures below 5°C is unrelated to cold-shortening (Nuss & Wolfe, 1980-81).

With the rapid rates of cooling in hot-deboned vacuum-packaged meat cuts, cold-shortening would be readily induced, thus the application of electrical stimulation (ES) could help to avoid the occurrence of cold-shortening (Lawrie, 1998). Another way of avoiding muscles from cold-shortening is by cooling the muscles quickly to about 15°C and holding it at this temperature to allow the onset of *rigor mortis* after which the temperature can then be lowered as fast as is compatible with minimal surface dehydration and to minimize microbial growth (Lawrie, 1998).

## 7. ELECTRICAL STIMULATION

In the industry electrical stimulation (ES) is generally applied to overcome the occurrence of cold-induced toughening by accelerating *post-mortem* pH decline (Wu *et al.*, 1985; Stiffler *et al.*, 1986; Taylor & Tantikov, 1992). As reviewed by Hwang *et al.* (2003), the proposed areas by which electrical stimulation elicit changes in *post-mortem* muscles,

include: (i) prevention of cold-induced shortening by ensuring the development of *rigor mortis* under optimal conditions; (ii) physical disruption of the muscle fibre; and (iii) acceleration of proteolysis. Acceleration of proteolysis is seen as a secondary effect mediated through the time/pH/temperature interaction, consequently affecting tenderisation factors such as enzyme stability and activity. However, Olsson *et al.* (1994) suggested that ES influences tenderisation more than it prevents cold-shortening, since tenderness for beef *M. longissimus thoracis et lumborum* were improved by ES at 1° and 4°C but not for the *M. semimembranosus*.

Electrical stimulation accelerates the rate of *post-mortem* glycolysis and hence the onset of *rigor mortis* (Devine *et al.*, 2001) and thereby preventing cold-shortening by reducing the concentration of the ATP and other high-energy phosphates during rigor development (George *et al.*, 1980; Tornberg, 1996). Consequently, muscles enter rigor at higher temperatures and the meat commences to age rapidly at these higher temperatures, being ready to be sold to the consumer at an earlier stage. However, although Devine and co-workers (2001) found that stimulated ovine muscles reaches rigor earlier and at a slightly higher temperature than non-stimulated muscles, the non-stimulated muscles were tenderer than the stimulated muscles, but this difference was not significant. Nonetheless, these findings were consistent with earlier results by Devine *et al.* (1999), indicating that elevated rigor temperatures reduced tenderisation. Pommier *et al.* (1987) also found that meat from stimulated veal carcasses aged for 8 d was rated tougher compared to samples from non-stimulated carcasses. This increased toughness was explained by the rapid decline in muscle pH in the stimulated carcasses and the consequent autolysis of the proteolytic enzymes,  $\mu$ -calpain. Where stimulation caused a rapid pH decline (pH < 5.9 at 3 h post-stimulation), no significant reduction in shear force could be found between stimulated and non-stimulated carcasses (Soniya *et al.*, 1982). On the other hand, accelerated pH decline caused by stimulation will result in an increase in calcium levels, which in turn could increase *post-mortem* proteolysis (Hwang *et al.*, 2003). Under conditions of more moderate pH decline, Pommier (1992) found a decrease in toughness (as measured by shear force), which in part could be explained by an increase in proteolysis.

The question arises that if the temperature conditions for *rigor mortis* are constant, would there be any differences between stimulated and non-stimulated muscles (Hwang *et al.*, 2003)? Devine *et al.* (2001) found no significant difference in initial shear force, rate of change of shear force or final shear force attained between lamb muscles that were either electrically stimulated or non-stimulated and chilled at 10°C. Although there was significant variability between carcasses in the rate of aging, the rate of tenderisation was found to be similar for electrically stimulated or non-stimulated muscles when the factors

associated with different rigor and aging temperatures were reduced. However, as reviewed by Hwang *et al.* (2003), with a larger group of lambs under identical constant temperatures, the shear force values were lower in stimulated muscles.

Soares & Arêas (1995) demonstrated that electrical stimulated muscles from crossbred buffaloes reached their ultimate pH much sooner after slaughter than non-stimulated muscles. This effect indicated that the period to *rigor mortis* was considerably shortened by the electrical stimulus and thus electrical stimulation can significantly reduce the time necessary for *rigor mortis* to be achieved. Results from studies on muscle extensibility showed that the onset of *rigor mortis* occurred at pH-values of approximately 5.9 in electrical stimulated muscles (Honikel & Fischer, 1977). However, Olsson *et al.* (1994) could find no effect of ES on the amount of shortening or on the ultimate levels of shortening and isometric tension for beef *M. longissimus thoracis et lumborum* and *M. semimembranosus*. In the case of ostrich carcasses, results obtained by Morris *et al.* (1995) revealed that electrical stimulation of carcasses had no effect on *post-mortem* pH or muscle temperature declines.

In terms of meat eating quality, it has been suggested that electrical stimulation improves tenderness by preventing cold-shortening, but Bowles-Axe *et al.* (1983) also suggested that cooling regimes that avoid cold-shortening reduce the beneficial impact of electrical stimulation. Results from sensory analyses and shear force measurements showed that electrical stimulation produced significantly more tender buffalo meat compared to non-stimulated samples (Soares & Arêas, 1995). It was also revealed that electrical stimulated meat submitted to rapid cooling had better texture characteristics compared to non-stimulated meat submitted to conventional slow cooling.

Clearly, electrical stimulation of carcasses accelerates the onset of *rigor mortis*, preventing the incidence of cold-induced shortening when muscles are exposed to chilling conditions, which might cause this toughening effect (Hwang *et al.*, 2003). Corresponding to this increase in the rate of *post-mortem* glycolysis, early activation of proteolytic enzymes, such as the calpain system, which could hasten myofibrillar protein degradation, could generally contribute to meat tenderness. However, there is a balance between early activation of the calpain system and autolysis of these enzymes caused by a rapid pH decline at high temperatures.

## 8. MUSCLE FIBRE TYPE

Three types of muscle fibres have been identified by histochemical studies: (i) those that are red, slow-twitch and predominantly oxidative in metabolism (Type I); (ii) white fibres which are generally fast-twitch and predominantly glycolytic in metabolism (Type II); and (iii) two subdivisions of Type II: Type IIA with an appreciable capacity for oxidative metabolism and Type IIB which does not have a great oxidative metabolism activity (Lawrie, 1998). In terms of the general metabolism in muscle fibres, as early as the 1950's it was demonstrated that red fibres exhibit a greater lipase activity compared to white fibres (Cassens & Cooper, 1971). Enzymes involved in the complete oxidation of fat are more customary in red muscles (Lawrie, 1998). There is also a difference in glycogen metabolism between red and white muscle fibres where glycolysis is greater in white than in red fibres (Cassens & Cooper, 1971). The enzymes in the pathway from glycogen to lactic acid are more active in white muscles, while the pathway from glycogen to glucose is more active in red than white muscles (Lawrie, 1998). Results from Leseigneur-Meynier & Gandemer (1991) for total haem pigment content (as indicator of the redness of the muscle and closely related to its oxidative activity) and lactate dehydrogenase activity (LDH) (indicating glycolytic activity) showed that white glycolytic muscles (such as beef *M. longissimus thoracis et lumborum*) have a lower total haem pigment content, but higher LDH activity compared to red oxidative muscles (such as *Masseter* and *Diaphragm*). Results obtained from an investigation on lactate and pyruvate production indicated that white muscles depend more on glycogenolysis and glycolysis for energy production than do red fibres (Cassens & Cooper, 1971). All these findings are indicative of a greater glycolytic activity in white muscle fibres than in red fibres. Muscles with intermediate total haem pigment contents and LDH activities are regarded as an intermediate fibre type and muscles such as *Psoas major* and *Trapezius* are shown to be intermediate muscles (Leseigneur-Meynier & Gandemer, 1991).

There exists a definite difference in lipid content between red and white fibre types (Cassens & Cooper, 1971) and is specifically reflected in differences in fatty acid composition (Wood *et al.*, 2003). Histochemical investigations have established that red muscle fibres contain more lipids compared to white muscle fibres (Cassens & Cooper, 1971). White (glycolytic) muscles also contain less triglyceride, cholesterol and exhibit a lower content of polyunsaturated fatty acids (PUFA) than red muscles (Lawrie, 1998). However, results obtained by Leseigneur-Meynier & Gandemer (1991) showed that intramuscular lipid and triglyceride contents vary little with metabolic fibre type. In contrast to this, the total phospholipid and the PUFA content are strongly associated with the metabolic type. Regarding fatty acid profile, red muscle fibres have a higher proportion of

phospholipids than white fibres and therefore a higher percentage of polyunsaturated fatty acids (PUFA) (Wood *et al.*, 2003). Enser *et al.* (1998) demonstrated this by finding that the redder leg muscle *M. gluteobiceps* from steers, had a significant higher polyunsaturated to saturated fatty acid ratio (P:S) than the white glycolytic *M. longissimus*. These authors concluded that the higher P:S ratio in the red muscle is due to higher concentrations of mostly PUFA.

In a study conducted by Beecher *et al.* (1965) it was found that white fibres from the *M. semitendinosus* had a lower pH and glycogen content immediately after exsanguination, but equal contents of lactic acid 24 h after exsanguination compared to red fibres from the same muscle. This indicated that glycolysis is indeed greater in white muscles than in red (Cassens & Cooper, 1971), therefore, muscles which are predominantly composed of red fibres generally have a higher ultimate pH and lower acid buffering capacity than those which are predominantly composed of white fibres (Lawrie, 1998). The higher buffering capacity of the white fibres appeared to be due to their higher contents of inorganic phosphorus (~P) and of the dipeptide carnosine. The capability of white fibres to act on short burst of energy is aided by the relatively large store of energy-rich phosphorus and a low capacity for the aerobic resynthesis of ~P. On the other hand, red fibres with a capacity for sustained activity, have an ability to resynthesise ~P aerobically and a low ~P store.

Contradictory results have been found for the rate of onset and completion of *rigor mortis*, as well as for the occurrence of cold-shortening within white and red fibres. Both Briskey *et al.* (1962) and Beecher *et al.* (1965) found that the white fibres from the *M. semitendinosus* had a longer delay phase of *rigor mortis* than the red fibres from the same muscle. In contrast, investigating whole muscles, Schmidt *et al.* (1970a) demonstrated that white muscle (*M. longissimus dorsi*) from both stress-resistant and stress-susceptible pigs developed *rigor mortis* in a significantly shorter *post-mortem* period than red muscle (*Vastus lateralis*), although there were no significant difference in the initial levels of CP and ATP between white and red muscles. However, it was found that the white muscle (*M. longissimus dorsi*) had significantly higher initial levels of lactic acid than the red muscle. Also, the red muscle samples had a longer delay before the loss of extensibility (onset of *rigor mortis*) started to occur and proceeded to rigor completion more slowly compared to the white muscle samples. It was concluded by Schmidt *et al.* (1970a) that muscles with potentially fast-glycolysing (white fibres) ability would have a faster development of *rigor mortis*.

With respect to the influence of temperature, Nuss & Wolfe (1980-81) indicated that maximum rigor tension and the time to reach maximum tension did not differ between bovine muscles which contained large numbers of red fibres (*B. femoris* and *Vastus*



*lateralis*) and the muscle of low red fibre content (*M. semitendinosus*) over the temperature range of 15° to 37°C. However, significant differences between the predominantly red and white muscles were evident at 0°C and only the muscle consisting of mainly white fibres (*M. semitendinosus*) cold-shortened at 0°C, while none of the muscles cold-shortened at 5°C. The absence of cold-shortening at 5°C was explained by the delay in time before the muscle strips were exposed to low temperatures. The muscle strips were not exposed to low temperatures until 2 h *post-mortem*.

In contrast to the findings of Nuss & Wolfe (1980-81), results from fibre typing composition of beef *M. longissimus thoracis et lumborum* and *M. semimembranosus* by Olsson *et al.* (1994), demonstrated that *M. longissimus thoracis et lumborum* has significantly more oxidative (red) fibres than *M. semimembranosus* and therefore had a higher level of shortening. The *M. semimembranosus* contains significantly more white fibres and less intermediate fibres than *M. longissimus thoracis et lumborum*. It was also found that *M. longissimus thoracis et lumborum* had a significantly higher level of isometric tension. Kim *et al.* (2000) demonstrated that the rate of *post-mortem* glycolysis was faster in the *Psoas major*, having proportionally less white fibres (Type IIB), than in the *M. longissimus thoracis et lumborum* muscles, consequently having a significantly faster pH decline. Also, the depletion of ATP was faster in the *Psoas major* than in the *M. longissimus thoracis et lumborum*. Hertzman *et al.* (1993) also found a linear correlation between percentage oxidative fibres and maximum shortening in muscles. It has been demonstrated that muscles containing mainly white fibres (predominantly glycolytic) do not cold-shorten (Bendall *et al.*, 1976), however, Nuss & Wolfe (1980-81) found that bovine *M. semitendinosus*, containing predominantly white fibres, cold-shortened at 0°C.

In terms of the effects of electrical stimulation on muscle fibres, Devine *et al.* (1984) showed that red muscles did not exhibit an increase in rate of pH fall, but did show evidence of super-contraction when electrically stimulated. These authors also documented that red muscles are more susceptible to cold-shortening compared to white muscles. The rate of pH fall in white muscles was increased by stimulation and this muscle type had nearly no super-contraction.

Muscle type plays a major role in *post-mortem* tenderisation during the aging of beef (Klont *et al.*, 1998). The aging rate is faster in fast-twitch white muscles than in slow-twitch red muscles. The Z-lines in white muscles seems to be more labile than those in red muscles (Taylor *et al.*, 1995; Lawrie, 1998). It is also known that white muscles are less susceptible to cold-shortening than red muscles. This has been attributed to the greater ability of white muscles to control intramuscular concentrations of calcium ions, since they have a more effective sarcotubular system. In addition, the rate of tenderising during aging is minimal in muscles that have cold-shortened. O'Halloran *et al.* (1997)

showed that fast glycolysing muscles are more tender than slow glycolysing muscles. Thus, the exact tenderness and pH relationship varies between different muscles (Lawrie, 1998), since their rate and extent of *post-mortem* glycolysis differs from each other.

However, it is important to realise that variations observed between red and white muscle for one species do not necessarily hold true for all species (Nuss & Wolfe, 1980-1981), since Wood & Richards (1974) indicated that white, chicken *P. major* muscle cold-shortens, while Jungk & Marion (1970) found that turkey *P. major* muscle does not. Also, Busch *et al.* (1972) reported different tension responses of bovine compared to porcine and rabbit muscle at low temperatures.

In a study conducted by Marks *et al.* (1998), it was found that shear force values for ostrich meat aged for 1 h, 24 h and 7 d were all lower (more tender) than for beef aged for 7 d. It can thus be concluded that in the case for ostrich meat less aging time is needed to induce an acceptable level of tenderness. On the other hand, Pollok *et al.* (1997) suggested from their results that aging of ostrich meat is not necessary, since ostrich meat appears to be sufficiently tender and the improvement in tenderness with aging would be out-weighed by the potential increase in microbial growth and the subsequent decrease in shelf-life.



## 9. OTHER PHYSICAL MEAT QUALITY CHARACTERISTICS

### ***Raw meat colour***

The colour of meat is one of the major contributing components of appearance and it is known to be the foremost selection criterion for fresh meat and meat products (Risvik, 1994; Fletcher, 2002). Consumers use colour as an indicator for meat freshness and prefer red meat types with a bright red colour rather than meat with a purple or brown colour (Carpenter *et al.*, 2001). The bright, cherry-red colour for fresh red meat types is short lived, especially in red-meat cuts from which surface discolouration is inevitable and may be interpreted as an indication of unwholesomeness (Liu *et al.*, 1995).

The purple pigment observed in freshly cut meat is deoxymyoglobin (DeoxyMb), while the bright red colour of fresh meats is contributed to oxymyoglobin (MbO<sub>2</sub>) and represents the colour desired by the retailer and purchasers (Mackinney & Little, 1962; Liu *et al.*, 1995; Lawrie, 1998). With extended exposure to air, MbO<sub>2</sub> is oxidised to metmyoglobin (MetMb) in which a molecule of water substitutes for a molecule of oxygen resulting in a brown pigmentation. The myoglobin molecule consists of a haematin nucleus attached to a globulin protein (Lawrie, 1998). The haematin section contains a central iron atom in the reduced ferrous (Fe<sup>2+</sup>) form, surrounded by a ring of four pyrrole

nuclei. In DeoxyMb and MbO<sub>2</sub> the iron atom exists in the ferrous form, while in MetMb the iron has been oxidised to the ferric (Fe<sup>3+</sup>) form.

It was reported that meat colour and muscle pH are highly correlated, referring specifically to meat as being pale, soft and exudative (PSE-like) or dark, firm and dry (DFD-like) (Fletcher, 2002). These extreme conditions are strongly related to muscle pH and have been associated with poor functional properties. Darker meat is associated with higher muscle pH while lower pH values are associated with lighter meat and in the extreme cases seen as DFD and PSE respectively. The dark colour of meat with a high ultimate pH may be explained in two ways. In the first place, since the muscle proteins will be above their iso-electric point, much of the water in the muscle will still be associated with the proteins and the muscle fibres will be tightly packed together, inhibiting diffusion of the light (Lawrie, 1998). Together with this, the surviving activity of the cytochrome enzymes will be greater at higher pH values, causing the layer of red oxymyoglobin to decrease and the unpleasant, purplish-red colour of myoglobin to predominate. In the second place, the high ultimate pH alters the absorption characteristics of the myoglobin in such a way that the meat appears dark because its surface will not scatter light to the same extent as will the more "open" surface of meat with a lower pH value. On the other hand, in the case of PSE, the pale colour of the meat might be because of the relative absence of myoglobin, or the chemical change of the pigment due to very low pH values or an abnormally fast rate of pH fall *post-mortem*. In these cases the myoglobin is exposed to conditions that cause its oxidation to metmyoglobin, which has low colour intensity. In addition, the muscle structure is "open" and scatters light. However, muscles with a low pH in combination with high muscle temperature lead to an increased protein denaturation, causing the light scattering properties to increase and therefore the meat to appear lighter or paler in colour (Offer, 1991). Ledward *et al.* (1986) concluded that meat with an ultimate pH above 5.8 has better colour stability compared to similar meat with an ultimate pH of 5.6. Additionally, variations in poultry breast colour, primarily due to pH effects, significantly affect the shelf-life of the breast meat, as well as the water holding capacity (WBC) of the meat (Fletcher, 2002).

In terms of the different kinds of meat, beef and ostrich are described as being high in colour intensity and low in whiteness, while lamb and pork are described by lower colour intensity and increasing whiteness (Kubberod *et al.*, 2002). Paleari *et al.* (1998) documented that the colour of ostrich meat is darker in colour than beef and with the relatively high pH of ostrich meat, it has been classified as an intermediate meat type between normal (pH < 5.8) and extreme DFD (pH > 6.2) (Sales, 1996).

### ***Water holding capacity***

The water holding capacity of meat refers to its ability to keep water during the presence of external factors, for example refrigerated storage, cutting, mincing and heating (Sales, 1999). The water holding capacity affects the appearance of meat before cooking, its behaviour during cooking, the capacity of the meat to hold moisture when processed into a product, and the juiciness on mastication, making it an important attribute of meat quality (Lawrie, 1998; Sales, 1999). Most of the water in muscles is present in the myofibril spaces between the thick filaments of myosin and the thin filaments of actin and tropomyosin (Lawrie, 1998).

It is well known that both the extent and the rate of *post-mortem* pH fall affect the water holding capacity of meat (Lawrie, 1998) and consequently the juiciness of the cooked meat. In the first place, the higher the ultimate pH the greater the increase in water holding capacity, while the faster the rate of pH fall, the greater is the loss of water holding capacity. The point of minimum water holding capacity of the principal proteins in muscle is 5.4-5.5, which, under normal conditions, is the ultimate pH at 24 h *post-mortem* for beef, lamb and pork.

During *post-mortem* storage of meat, the pH tends to increase, causing a subsequent increase in the water holding capacity (Lawrie, 1998). This increase in the water holding capacity is possibly due to an increase in osmotic pressure, caused by the breakdown of protein molecules, and by changes in the ion-protein relationships, caused by the release of sodium ( $\text{Na}^+$ ) and calcium ( $\text{Ca}^{2+}$ ) ions and absorption of potassium ( $\text{K}^+$ ) ions. There is a net increase in charge through the absorption of  $\text{K}^+$  ions and the release of  $\text{Ca}^{2+}$  ions. Because of the absorption of  $\text{K}^+$  ions on to the muscle proteins, the net charge of the muscle proteins increases and thus the water holding capacity also increases. However, with further *post-mortem* storage, meat tends to lose more water due to denaturation of muscle proteins and their loss of water holding capacity. Chin & Keeton (1997) demonstrated that moisture loss or purge in vacuum packaged ostrich meat, stored at 2°C, increased as the *post-mortem* storage period increased from day 1 to day 14. These authors suggested that this reduction in water holding capacity was due to the loss of water binding (sarcolemmic) proteins with the purge. Purge in vacuum packaged meat could negatively influence the quality of the raw and cooked meat, as well as affect the juiciness of the meat when cooked (Pollok *et al.*, 1997).

## 10. CONCLUSIONS

With the low fat content of ostrich meat (Sales & Hayes, 1996; Sales, 1999), meat cuts from ostrich muscles may be marketed as a health product and as an alternative to other red meats such as beef and lamb. In addition, the export of ostrich meat to the European Union has increased during the past years with increasing demands from the South African ostrich industry. To meet these demands, the benefits of hot-deboning, which include the reduction in time, costs and space requirements, presents it self as an attractive means for improving future processing technologies in the ostrich industry. It is therefore of great value for the ostrich industry to gain information about the effects of hot-deboning on the quality of ostrich meat with regards to its physical and sensory qualities, as well as on the shelf-life of vacuum packed ostrich meat cuts.

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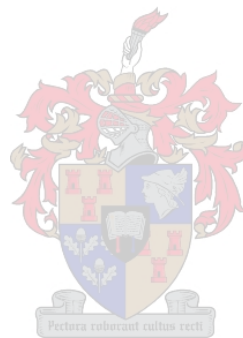
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## Chapter 3

### **Sensory properties of hot-deboned ostrich (*Struthio camelus var. domesticus*) *Muscularis gastrocnemius, pars interna***

#### **ABSTRACT**

Cold-deboning is currently practiced in South African ostrich abattoirs. However, the attractions of hot-deboning mainly include the reduction of costs and time, but there is always the risk of cold-shortening. The effects of hot-deboning of ostrich *M. gastrocnemius, pars interna* on meat sensory attributes including: ostrich aroma; initial juiciness; sustained juiciness; tenderness; residue and overall ostrich flavour, were investigated. Muscle pH at 48 h *post-mortem* ( $6.57 \pm 0.18$ ) was significantly negatively correlated ( $r = -0.7813$ ;  $P < 0.038$ ) to the mean Warner-Bratzler shear force values ( $71.28 \pm 18.62$  N.  $12.7 \text{ mm}^{-1}$  diameter) and positively correlated ( $r = 0.789$ ;  $P < 0.035$ ) to the mean scores for taste panel tenderness ( $66.39 \pm 15.45$ ) for hot-deboned muscles. After storage for 48 h *post-mortem*, the hot-deboned muscles were less juicy ( $P < 0.004$ ) and, according to both sensory tenderness scores and Warner-Bratzler shear force values, tougher ( $P < 0.0001$ ) than cold-deboned muscles. In contrast, cold-deboning resulted in less variation, and therefore would produce meat with more consistent eating quality in terms of texture than hot-deboned muscles.

**Keywords:** ostrich, hot-deboning, cold-deboning, muscle pH, sensory analysis, tenderness, juiciness, taste panel

#### **INTRODUCTION**

Tenderness is considered to be one of the most important attributes by consumers as an indicator of good eating quality (Risvik, 1994; Issanchou, 1996). It is generally accepted that juicy and tender meats are preferred to those that are less tender and less juicy; and that these attributes are generally the most important for the determination of preference in terms of texture (Risvik, 1994).

It is well known that there is a risk of toughening when muscles are hot-deboned. With hot-deboning, there is also the risk of cold-shortening in pre-rigor muscles if the temperature falls below 10°C while the pH is still high (pH > 6.0-6.2) and an adequate ATP (adenosine triphosphate) concentration is present (Pearson & Young, 1989). However, the major commercial attraction of hot-deboning is the considerable reduction in time, space and refrigeration capacity required (Taylor *et al.*, 1980-81). Hot-deboning also prevents weight loss due to evaporation during carcass chilling. It is thus essential that packaging be done without delay if weight losses from the cut surfaces of the hot meat exposed during deboning and packaging is to be minimised. In general, the temperature decline in hot-deboned muscles is faster and more uniform than in muscles left on the carcass (Van Laack & Smulders, 1992). This is beneficial for controlling microbial spoilage (Lawrie, 1998) and therefore increasing the shelf-life. Furthermore, Taylor *et al.* (1980-81) reported that hot-deboning minimised drip loss and produced a more even colour across the large muscles.

Ostrich muscles are apparently not susceptible to cold-shortening (Sales & Mellet, 1996). It was suggested by Sales and Mellet (1996) that the risk of cold-shortening would be reduced in the *M. iliofibularis* since it reached a pH of less than 6.20 at approximately 34 min after slaughter. The apparent ultimate pH was reached rapidly at 2 h *post-mortem* in the *M. iliofibularis* ( $6.00 \pm 0.087$ ), and at 6 h *post-mortem* in the *M. gastrocnemius, pars interna* ( $6.12 \pm 0.056$ ). It was thus concluded by Sales & Mellet (1996) that there is a risk of cold-shortening in the *M. gastrocnemius, pars interna* if this muscle is to be separated from the carcass at 30-45 min *post-mortem*, but not in the *M. iliofibularis*.

The aim of this study was to investigate the effect of hot-deboning (1 h *post-mortem*) on the eating attributes of ostrich *M. gastrocnemius, pars interna* muscles aged for only 48 h *post-mortem*, as perceived by a trained taste panel. The mechanical parameter Warner-Bratzler shear force values (N. 12.7 mm<sup>-1</sup> diameter) were also investigated to specifically gain a better indication of the effect of hot-deboning on tenderness.

## MATERIALS AND METHODS

### Ostriches and Sampling

Eight randomly selected, well rested (lairage of 12 h) ostriches (*Struthio camelus var. domesticus*) were slaughtered as described by Wotton & Sparrey (2002), on the same day during June 2004 at an EU approved abattoir in Malmesbury, South Africa. The left leg *M. gastrocnemius, pars interna* ( $0.96 \pm 0.18$  kg) from each carcass was removed at

approximately 1 h after slaughter (hot-deboned). The hot-deboned muscles were immediately vacuum packaged (AMSA, 1995) and stored for 24 h at  $< 4^{\circ}\text{C}$ . The rest of the carcass was also stored at  $< 4^{\circ}\text{C}$  in the same refrigerator at the abattoir before the right leg muscles ( $1.05 \pm 0.14$  kg) were excised according to commercial procedures at approximately 24 h *post-mortem* (cold-deboned muscles). The cold-deboned muscles were vacuum packaged and together with the hot-deboned muscles, transported in cooler boxes to Stellenbosch (60 km) and aged for an additional 24 h at an average temperature of  $4^{\circ}\text{C}$  ( $0^{\circ}\text{C}$  to  $7^{\circ}\text{C}$  variation). Sensory analysis of all the muscle samples was consequently conducted at 48 h *post-mortem* on the same day. Freezing of the muscles was avoided, since freezing and thawing causes a greater extent of tenderness than what the actual case of tenderness may be 48 h *post-mortem* (Watanabe & Devine, 1996). It is suggested that some ice crystal damage during freezing, storage and thawing may modify the process of meat aging.

Muscle pH and temperature ( $^{\circ}\text{C}$ ) of the left and right intact *M. gastrocnemius, pars interna* were measured with the use of a calibrated (standard buffers pH 4.0 and 7.0) portable Crison 506 pH-meter equipped with respectively a pH and temperature probe, at 1 h *post-mortem*. Muscle pH and temperature ( $^{\circ}\text{C}$ ) were recorded again immediately after the hot-deboned muscles were excised at approximately 10 min after the one-hour pH measurement was recorded, and similarly after the cold-deboned muscles were excised at approximately 24 h *post-mortem*. To avoid breakage of the seal of the vacuum package, measurements of pH and temperature were not recorded from hot-deboned muscles at 24 h *post-mortem*. Final muscle pH and temperature ( $^{\circ}\text{C}$ ) measurements were recorded at 48 h *post-mortem* before muscle samples were prepared for sensory analysis and Warner-Brazler shear force (N.  $12.7 \text{ mm}^{-1}$  diameter) measurements.

### Sample preparations

At 48 h *post-mortem*, meat portions of  $0.52 \pm 0.08$  kg were cut from the middle section of each muscle. The meat portions were placed in individual oven bags without added salt or spices and placed on open roasting pans. The four meat portions at a time were oven roasted (AMSA, 1995) in conventional electric ovens (Defy Model 835), preheated to  $160^{\circ}\text{C}$ , which were connected to a computerised electronic temperature control system (Viljoen *et al.*, 2001). The internal temperature of the meat portions was measured using a thermocouple probe inserted into the centre of the meat portions and roasting continued until a core temperature of  $68^{\circ}\text{C}$  was reached. At a core temperature of  $68^{\circ}\text{C}$ , the meat portions were removed from the oven and left to cool for 5 min. The cooked meat portions were cut into slices of approximately 1.5 to 2.0 cm thick, perpendicular to the fibre

direction. Cooked surfaces were removed from the slices (AMSA, 1995). Cubed samples of 1 cm<sup>3</sup> were then cut from these meat slices. Samples were individually wrapped in aluminium foil, placed in glass ramekins individually coded with three digit codes and preheated for 10 min at 100°C before being served to the panellists.

### **Sensory analysis**

The sensory panel consisted of 8 trained assessors previously selected for their flavour and texture sensitivity according to the guidelines of the American Meat Science Association (AMSA, 1995). The panel was further trained using the consensus method as described by Lawless & Heymann (1999). A 100 mm unstructured line scale, where the left side of the scale corresponded to the lowest intensity (zero) and the right hand side corresponded to the highest intensity (100), was used for attribute intensity evaluation. Separate samples of ostrich *M. gastrocnemius, pars interna* and *M. iliofibularis* (aged for approximately 7 d), as well as the hot and cold-deboned *M. gastrocnemius, pars interna* from one of the eight randomly selected ostriches, were used to train the panel for sensory attributes. The judges agreed on a consensus list of attributes for describing ostrich meat, which included intensity of ostrich aroma, impression of initial juiciness, sustained juiciness, impression of tenderness, the amount of residue, and overall ostrich flavour. Verbal definitions for the sensory attributes evaluated for the ostrich meat are given in Table 1. Hot and cold-deboned ostrich *M. gastrocnemius, pars interna* from a single carcass were used separately to familiarise the judges with differences in tenderness.

### **Sensory procedure**

Samples were served and evaluated during seven sessions, controlling for carcass by serving hot and cold-deboned samples from one carcass within the same session (AMSA, 1995). The panellists were seated individually at sensory booths, which were light and temperature controlled. Meat samples (individually wrapped in aluminium foil and preheated in an oven at 100°C), each coded with a three digit random code (AMSA, 1995), were presented in a complete randomised order according to carcass. The aroma of the samples was immediately assessed after unwrapping of the aluminium foil. Flavour and texture (tenderness) attributes were assessed on the entire sample. Still mineral water, unsalted biscuits and apple slices were available for assessors to cleanse their palates between samples when evaluating flavour.

**Table 1.** Verbal definition of sensory attributes for the sensory analysis of ostrich meat.

Attribute	Definition	Scale
Ostrich aroma intensity	Aroma associated with ostrich meat	0 = No ostrich meat aroma 100 = Strong ostrich meat aroma
Initial juiciness	The amount of fluid exuded on the cut surface when pressed between the thumb and forefinger	0 = Extremely dry 100 = Extremely juicy
Sustained juiciness	The degree of juiciness perceived after the first two to three chews between the molar teeth	0 = Extremely dry 100 = Extremely juicy
Tenderness	Impression of tenderness after the first two to three chews between the molar teeth	0 = Extremely tough 100 = Extremely tender
Residue	The amount of residue left in the mouth after the first twenty to thirty chews	0 = High amount of residue left 100 = No residue left
Overall ostrich flavour	Flavour associated with ostrich meat as a combination of taste and swallowing	0 = No ostrich flavour 100 = High ostrich flavour

### Physical tenderness

From each cooked meat portion, a slice of 1.5 to 2.0 cm thick was cooled for 24 h at 4°C before Warner-Bratzler shear force measurements were obtained as described by Wheeler *et al.* (2001) and Honikel (1998). Seven 12.7 mm wide cores were removed parallel to the muscle fibre from each muscle slice and placed in the Warner-Bratzler

device, with a load cell of 2.000 kN, which was attached to the Model 4444 Instron texture machine (Apollo Scientific cc, South Africa), so that the knife blade of the device cut across the fibres at right angles. The maximum (high peak) shear force value (N. 12.7 mm<sup>-1</sup> diameter) to shear a cylindrical core of cooked meat was recorded at a crosshead speed of 200 mm.min<sup>-1</sup>. Mean maximum shear force values were calculated from the shear force values recorded for seven cylindrical cores from each muscle sample and used in the statistical analyses.

### Statistical analyses

The results obtained by the eight judges were part of a complete randomised block design, performed with two treatments (hot and cold-deboning) replicated in seven blocks (ostrich carcasses). The sensory data were subjected to analysis of variance (ANOVA) using SAS version 8.2 statistical software (SAS, 1999), to evaluate different sources of variation in sensory attributes: ostrich aroma; initial juiciness; sustained juiciness; tenderness; residue and overall ostrich flavour. Ostrich, judge and deboning (hot or cold-deboned) were the main effects and a two way interaction between main effects was also included. Shapiro-Wilk tests were performed for testing non-normality (Shapiro & Wilk, 1965). Results from analysis of variance (ANOVA) and Shapiro-Wilk tests are summarised in Table 3. Correlation coefficients [r-values at the 5% significance level (*P*)] were calculated with the use of statistical software Statistica version 6 (StatSoft 2003). Correlation coefficients were calculated between the sensory attributes of ostrich aroma, initial juiciness, sustained juiciness, tenderness, residue and overall ostrich flavour from the raw data points for hot and cold-deboned muscles, respectively. Correlation coefficients between the data from muscle pH and temperature at 48 h *post-mortem* and the mean values for the sensory attributes of ostrich aroma, initial juiciness, sustained juiciness, tenderness, residue and overall ostrich flavour for the hot and cold-deboned muscles, respectively, as well as from the pooled data for the hot and cold-deboned muscles, were calculated.

## RESULTS AND DISCUSSION

### Muscle pH and temperature

The mean muscle pH of the cold-deboned muscles at 1 h *post-mortem* was 6.82 ± 0.10 (Table 2). This value did not differ (*P* > 0.05) from the pH (6.81 ± 0.15) determined for the hot-deboned muscles excised from the carcass at 1 h and 10 min *post-mortem* (T<sub>1 + 10 min</sub>).



It appears as though the excision of muscles after 1 h *post-mortem* did not cause the muscles to super contract, and there was also no occurrence of a sudden fall in muscle pH. The pH<sub>48</sub> of the hot and cold-deboned muscles did not differ significantly ( $P > 0.05$ ). However, the pH at 1 h ( $6.83 \pm 0.09$ ) differed ( $P < 0.05$ ) from the pH at 48 h ( $6.57 \pm 0.18$ ) *post-mortem* for hot-deboned muscles (Table 2), but not for the case of the cold-deboned muscles. This difference between the hot and the cold-deboned muscles could not be explained by muscle temperature, since no significant correlation ( $P > 0.05$ ) could be found between muscle pH and temperature. Also, there was no significant difference ( $P > 0.05$ ) between the temperature of the hot-deboned muscles right after excision ( $T_{1+10 \text{ min}}$ ) and the temperature of the cold-deboned muscles at 1 h *post-mortem* (Table 2). Muscle pH for the cold-deboned muscles did not differ significantly between the three different times *post-mortem* of 1 h ( $6.82 \pm 0.10$ ), 24 h ( $6.67 \pm 0.34$ ), and 48 h ( $6.63 \pm 0.24$ ). However, there was a decrease in pH from 1 to 48 h and therefore it is possible that, as was found with the hot-deboned muscles, pH might decrease further if the aging was extended beyond 48 h *post-mortem*.

At 1 h *post-mortem*, *M. gastrocnemius, pars interna* had reached a mean temperature of  $27.70^\circ \pm 3.02^\circ\text{C}$  (Table 2). During storage at  $< 4^\circ\text{C}$ , the temperature of the cold-deboned muscles, whilst still attached on the carcass, at 24 h *post-mortem* had decreased to below  $0^\circ\text{C}$  ( $-0.03^\circ \pm 0.29^\circ\text{C}$ ). Since the vacuum packaged hot-deboned muscles were kept under the same temperature conditions than the cold-deboned muscles, it was assumed that the temperature of the hot-deboned muscles was similar or even less than that of the cold-deboned muscles at 24 h *post-mortem*. The temperature of the hot-deboned muscles may have been lower than that of the cold-deboned muscles at 24 h *post-mortem*, since a larger surface area of the muscle was exposed to the ambient temperature compared to the cold-deboned muscles that were still attached to the carcass. Van Laack & Smulders (1992) showed in pork that the temperature decrease is more uniform and faster in hot-deboned muscles than in muscles left on the carcass.

Although the cold-deboned muscles were cooled at  $< 4^\circ\text{C}$  at the abattoir for approximately 24 h *post-mortem* before being excised, it is common practice in the meat industry for the cooler room to be at a temperature just below  $0^\circ\text{C}$  in order to ensure that the temperature of the cooler room is low enough when the refrigerator is filled with the day's warm carcasses. Therefore, the reason why the mean cold-deboned muscle temperature ( $-0.03^\circ \pm 0.29^\circ\text{C}$ ) was below  $0^\circ\text{C}$ , 24 h *post-mortem*. The higher temperatures of both the hot and the cold-deboned muscles at 48 h *post-mortem* can be explained by the fact that all muscles were transported to Stellenbosch at 24 h *post-mortem* and stored in a cooler room running at temperatures of  $0^\circ$  to  $7^\circ\text{C}$  for 24 h. However, it may be postulated that since hot and cold-deboned muscles from the same

ostrich were treated similarly at all times, the effect of the temperature differences would be constant within muscles.

**Table 2.** Mean ( $\pm$  Standard Deviation) muscle pH and temperature ( $^{\circ}\text{C}$ ) at 1 h *post-mortem*, right after hot-deboning (1 h and 10 min) and cold-deboning (24 h *post-mortem*), and at 48 h *post-mortem*.

Time <i>post-mortem</i> (h)	Mean $\pm$ Standard Deviation	
	Hot-deboned muscles	Cold-deboned muscles
Muscle pH		
pH <sub>1</sub>	6.83 $\pm$ 0.09 <sup>a</sup>	6.82 $\pm$ 0.10 <sup>a</sup>
pH <sub>1 + 10 min</sub>	6.81 $\pm$ 0.15 <sup>a</sup>	-
pH <sub>24</sub>	-	6.67 $\pm$ 0.34 <sup>ab</sup>
pH <sub>48</sub>	6.57 $\pm$ 0.18 <sup>b</sup>	6.63 $\pm$ 0.24 <sup>ab</sup>
Muscle temperature ( $^{\circ}\text{C}$ )		
T <sub>1</sub>	28.90 $\pm$ 3.16 <sup>c</sup>	29.36 $\pm$ 3.98 <sup>c</sup>
T <sub>1 + 10 min</sub>	30.83 $\pm$ 1.38 <sup>c</sup>	-
T <sub>24</sub>	-	-0.03 $\pm$ 0.29 <sup>d</sup>
T <sub>48</sub>	6.68 $\pm$ 0.39 <sup>e</sup>	7.70 $\pm$ 0.24 <sup>f</sup>

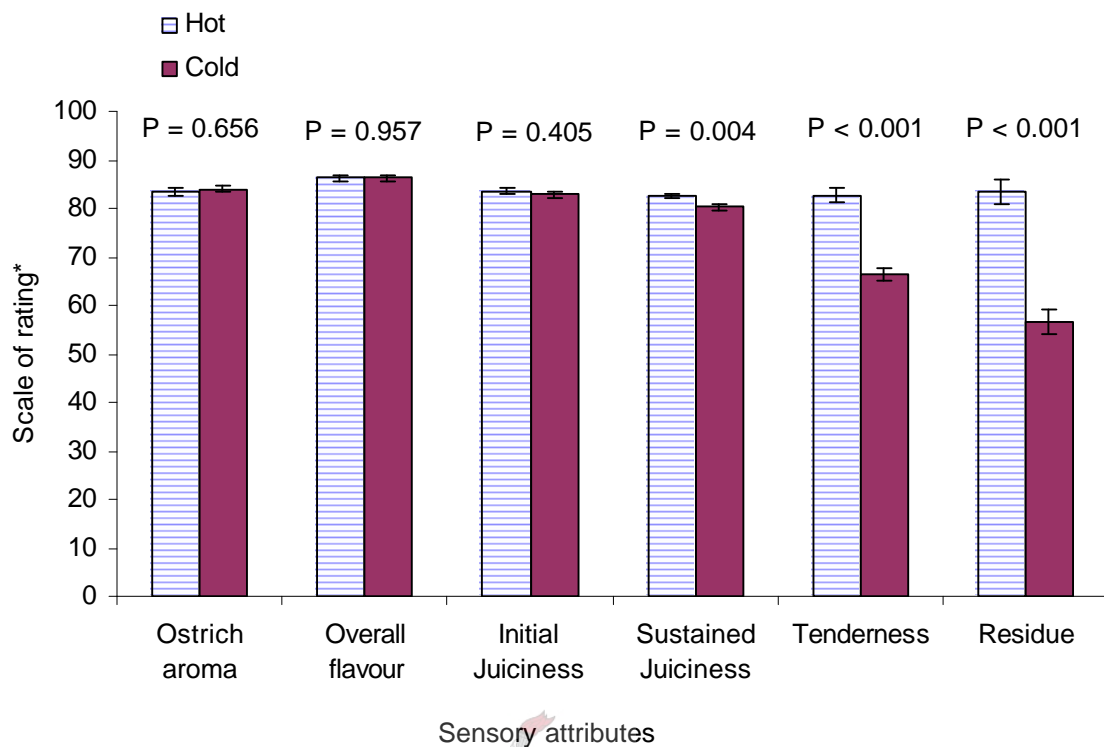
<sup>abc</sup> Different superscripts for pH differ at  $P < 0.05$ .

<sup>def</sup> Different superscripts for temperature differ at  $P < 0.05$ .

### Sensory attributes

The analysis of variance (ANOVA) of attributes: ostrich aroma; initial juiciness; sustained juiciness; tenderness; residue and overall ostrich flavour is presented in Table 3. It was found that the sensory panel was consistent in their judgements as there were no interactions ( $P > 0.05$ ) between judge and treatment (debone). There were also no significant two way interactions between ostrich and treatment (deboning) for any of the attributes. Significant interactions were observed, however for variables: ostrich aroma ( $P = 0.029$ ); initial juiciness ( $P = 0.001$ ) and sustained juiciness ( $P < 0.001$ ) between ostrich and judge. These interactions did not influence the interpretations for the main effect “deboning” and therefore hot-deboning directly influenced sensory attributes sustained juiciness, tenderness and residue.

The data obtained showed no differences in ostrich aroma and overall flavour ( $P > 0.05$ ) between hot and cold-deboned muscles (Fig. 1). This is similar to the results of Jeremiah *et al.* (1985) who reported no significant effect of hot-deboning on juiciness or beef flavour intensity for beef muscles. However, aroma was correlated ( $P < 0.05$ ) to overall flavour ( $r = 0.518$ ), where this correlation was higher for the cold-deboned muscles ( $r = 0.651$ ) than for the hot-deboned muscles ( $r = 0.374$ ).



**Figure 1.** Means ( $\pm$  Standard Errors) and Probability ( $P$ ) values of the F-ratio test for the main effect, deboning, of taste panel sensory scores for sensory attributes: ostrich aroma; overall ostrich flavour; initial juiciness; sustained juiciness; tenderness and residue for the hot and cold-deboned muscles, respectively. Hot and cold-deboned muscles differed for a particular attribute at the level of  $P < 0.05$ . \*Scale of rating: taste panel ratings were scored by ticking on an unstructured 100 mm line scale.

**Table 3.** Analyses of variance (ANOVA) of sensory attributes: ostrich aroma, initial juiciness, sustained juiciness, tenderness, residue, and overall ostrich flavour with ostrich, judge and deboning (debone) as main effects, the two way interaction between main effects, as well as the Shapiro-Wilk test for non-normality.

Source	df	Ostrich aroma		Initial juiciness		Sustained juiciness		Tenderness		Residue		Overall flavour	
		MS	P	MS	P	MS	P	MS	P	MS	P	MS	P
Ostrich	6	308.07	< 0.001	176.94	< 0.001	43.04	0.033	731.63	< 0.001	853.99	0.043	63.70	0.047
Judge	7	259.71	< 0.001	813.96	< 0.001	1322.58	< 0.001	528.24	0.001	2343.79	< 0.001	506.05	< 0.001
Ostrich *Judge	42	58.37	0.029	55.23	0.000	57.04	< 0.001	153.62	0.193	236.08	0.903	21.08	0.786
Debone	1	6.51	0.656	12.89	0.405	153.22	0.004	7491.57	< 0.001	20466.04	< 0.001	0.08	0.957
Debone *Judge	7	11.14	0.928	9.57	0.810	20.00	0.328	224.59	0.091	783.71	0.052	7.86	0.954
Debone *Ostrich	6	61.95	0.100	23.91	0.272	17.70	0.404	116.34	0.444	585.99	0.155	37.18	0.245
Error	42	32.25	-	18.21	-	16.76	-	117.34	-	353.48	-	26.96	-
Shapiro- Wilk			0.982		0.276		0.259		0.114		0.027		< 0.001

df – Degree of freedom

MS – Mean Square

P – Probability value of F-ratio test

\*Interaction between main effects

Taste panel scores for initial juiciness also showed no difference ( $P > 0.05$ ) between the hot and the cold-deboned muscles, while sustained juiciness was scored higher ( $P < 0.05$ ) for the cold-deboned ( $82.59 \pm 0.55$ ) than for the hot-deboned ( $80.25 \pm 0.55$ ) muscles. Initial juiciness was significantly ( $P < 0.05$ ) correlated to sustained juiciness for respectively the hot-deboned ( $r = 0.474$ ) and the cold-deboned ( $r = 0.512$ ) muscles. Cold-deboned muscle tenderness was positively correlated ( $P < 0.05$ ) to initial ( $r = 0.272$ ) and sustained juiciness ( $r = 0.533$ ). Tenderness for the hot-deboned muscles was not significantly ( $P > 0.05$ ) correlated to either initial ( $r = 0.149$ ) nor sustained juiciness ( $r = 0.201$ ).

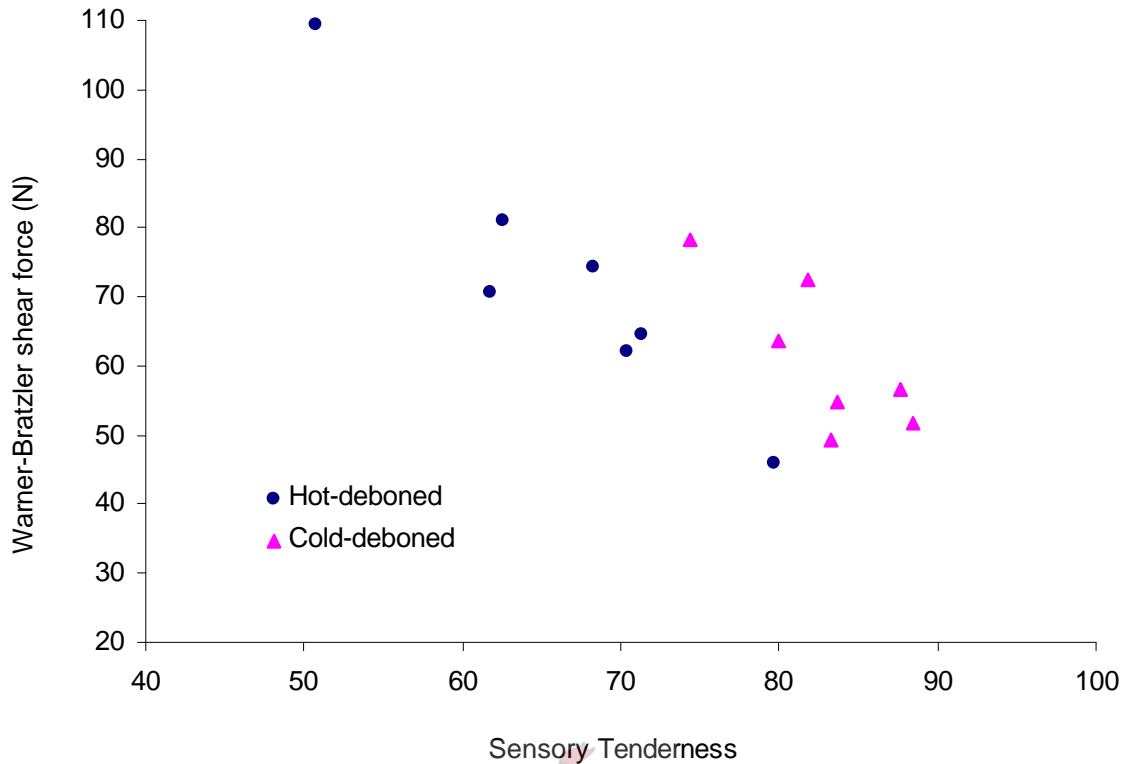
Results from both the sensory analysis and the Warner-Bratzler shear force measurements (N. 12.7 mm<sup>-1</sup> diameter) (Table 4) indicated that the hot-deboned muscles were tougher compared to the cold-deboned muscles at 48 h *post-mortem* ( $P < 0.05$ ). The data showed that hot-deboning also resulted in a larger variation in tenderness than the cold-deboned muscles. Warner-Bratzler shear force values were highly negatively correlated ( $P < 0.05$ ) with taste panel sensory tenderness scores for both the hot ( $r = -0.828$ ) and the cold-deboned ( $r = -0.792$ ) muscles (Fig. 2). This indicates that for this study shear force values were a good indicator of tenderness and a relative accurate method for the measurement of the sensory attribute tenderness.

**Table 4.** Mean values ( $\pm$  Standard Deviation) for tenderness as scored by the taste panel and mean Warner-Bratzler shear force values (N. 12.7 mm<sup>-1</sup> diameter) for respectively the hot and the cold-deboned *M. gastrocnemius, pars interna* at 48 h *post-mortem*.

Deboning	Means $\pm$ Standard Deviation	
	Tenderness Taste panel score*	Warner-Bratzler shear force (N. 12.7 mm <sup>-1</sup> diameter)
Hot-deboned muscles	66.39 $\pm$ 15.45 <sup>a</sup>	71.28 $\pm$ 18.62 <sup>a</sup>
Cold-deboned muscles	82.75 $\pm$ 12.52 <sup>b</sup>	58.12 $\pm$ 12.90 <sup>b</sup>

<sup>ab</sup> Different superscripts within a column differ at  $P < 0.05$ .

\*Higher values indicate more tender meat.



**Figure 2.** Correlations ( $P < 0.05$ ) between Warner-Bratzler shear force values (N. 12.7 mm<sup>-1</sup> diameter) and taste panel sensory scores for tenderness for the hot ( $r = -0.828$ ) and the cold-deboned ( $r = -0.792$ ) *M. gastrocnemius, pars interna*, respectively.

The amount of residue left in the mouth after the first twenty to thirty chews was higher ( $P < 0.05$ ) for the hot-deboned than for the cold-deboned muscles. The amount of mouth residue is in accordance with taste panel scores for tenderness, indicating that hot-deboned muscles were tougher compared to cold-deboned muscles. Both the hot-deboned ( $r = 0.719$ ) and the cold-deboned ( $r = 0.786$ ) muscles showed a high correlation ( $P < 0.05$ ) with residue and tenderness.

When the mean data from the hot and the cold-deboned muscles were pooled, the mean sustained juiciness was positively correlated ( $P < 0.05$ ) to the muscle pH at 48 h *post-mortem* ( $r = 0.575$ ) as well as to the mean taste panel tenderness scores ( $r = 0.598$ ). Muscle samples that were tender were therefore perceived as being juicier compared to less tender samples. However, Cameron *et al.* (1990) indicated that juiciness and tenderness are independent attributes.

When investigating the mean data for the hot-deboned and the cold-deboned muscles separately, muscle pH at 48 h *post-mortem* was negatively correlated ( $r = -0.781$ ) to mean Warner-Bratzler shear force values and positively correlated ( $r = 0.789$ ) to mean

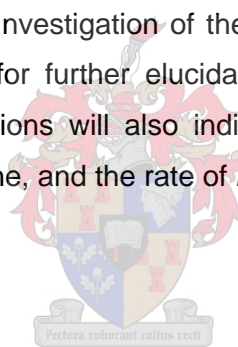
taste panel tenderness scores ( $P < 0.05$ ) only in the case of the hot-deboned muscles. The application of hot-deboning seemed to affect *post-mortem* glycolysis in such a manner that muscle pH had an affect on meat tenderness; i.e. the higher the pH the more tender the meat at was 48 h *post-mortem*. These results are in relationship with what has previously been reported for beef and lamb by several authors. It has been observed that a higher ultimate pH is related to more tender meat and that this improvement in tenderness at high pH values is the result of an increased proteolytic activity (Bouton *et al.*, 1982; Yu & Lee, 1986; Guignot *et al.*, 1994). However, different conclusions were reached for the relationship between ultimate pH and meat tenderness. While positive linear relationships were reported by Shackelford *et al.* (1994) for beef muscles, Bouton *et al.* (1982) for mutton, and by Guignot *et al.* (1994) for veal; others such as Purchas (1990), Jeremiah *et al.* (1991) and Purchas & Aungsupakorn (1993) found a curvilinear relationship with a minimum tenderness at an ultimate pH range of about 5.8-6.2. However, irrespective of this, the pH<sub>48</sub> values of the ostrich muscles in this investigation were in the range where both relationships (linear or curvilinear) would indicate more tender meat. Further investigation will be required to determine whether the relationship between muscle pH and tenderness is linear or curvilinear in ostrich muscles.

## CONCLUSIONS

The data from both the taste panel tenderness scores and Warner-Bratzler shear force measurements, indicated that hot-deboning of ostrich *M. gastrocnemius, pars interna* caused meat from this muscle to be tougher than that from cold-deboned muscles at 48 h *post-mortem*. Cold-deboned muscles were also juicier compared to the hot-deboned muscles, indicating that hot-deboning affects the water holding capacity of the muscles. This could in part be explained due to the slight difference in muscle pH at 48 h *post-mortem* between hot-deboned and cold-deboned muscles where cold-deboned muscles ( $6.63 \pm 0.24$ ) had a higher pH at 48 h *post-mortem* than the hot-deboned muscles ( $6.57 \pm 0.18$ ).

Cold-deboning also resulted in less variation in tenderness attributes when compared to hot-deboned muscles, and therefore would produce meat with a more consistent eating quality in terms of texture. Dransfield *et al.* (1982) concluded that acceptability to consumers was determined largely by the wide variation in tenderness. The question arises; however, whether the difference in tenderness between hot and cold-deboned muscles as found in the present study for ostrich *M. gastrocnemius, pars interna* would still prevail with further aging *post-mortem*? Earlier reports for beef (Smith *et al.*, 1978) showed that aging will increase tenderness, flavour and overall palatability of the

majority of muscle cuts when cooked by oven-broiling or roasting. On the other hand, it has been indicated that cold-shortening toughness could not fully be overcome by increased aging periods in the case of veal (Klont *et al.*, 2000). Further investigation is needed to explain the difference in tenderness between hot-deboned and cold-deboned ostrich muscles to conclude whether the difference is due to cold-shortening or reduced *post-mortem* proteolysis, and/or due to effects of *post-mortem* pH. It is well known that when *pre-rigor* muscle attains a temperature of below 10° to 15°C while the pH is above 6.0-6.4 and ATP levels are still high enough for muscle contraction to occur, there is a risk of cold-shortening and consequent toughening of the meat when cooked (Honikel *et al.*, 1983; Pearson & Young, 1989; Lawrie, 1998). Morton *et al.* (1999) suggested that in beef there is an association between the rate of pH decline *post-mortem* and the rate of meat tenderisation. O'Halloran *et al.* (1997) and Hwang & Thompson (2001) both reported that fast glycolysing muscles were more tender than slow glycolysing muscles. In the present study it was uncertain at which point in time of the *rigor mortis* process and course of pH decline the *M. gastrocnemius, pars interna* was when excision of these muscles was performed at 1 h *post-mortem*. Investigation of the course of *rigor mortis* and the *post-mortem* pH decline is required for further elucidation on the *post-mortem* changes in ostrich muscle. Such investigations will also indicate the time (hour *post-mortem*) of minimum pH, the rate of pH decline, and the rate of *rigor mortis* development.



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## Chapter 4

### **Physical meat quality characteristics of hot-deboned ostrich (*Struthio camelus var. domesticus*) *Muscularis gastrocnemius, pars interna* during *post-mortem* aging**

#### **ABSTRACT**

With hot-deboning of muscles, there is a risk of shortening and subsequent toughening of the meat. However, with refrigerated aging this phenomenon may be negated. Vacuum-packaged hot and cold-deboned ostrich *M. gastrocnemius, pars interna* were stored for 21 d at 4°C to investigate the effects of hot-deboning on the physical quality characteristics of ostrich meat during *post-mortem* refrigerated storage. At 24 h *post-mortem*, hot-deboned muscles ( $2.05 \pm 0.18 \mu\text{m}$ ) had shorter sarcomeres ( $P < 0.0001$ ) compared to cold-deboned muscles ( $2.52 \pm 0.14 \mu\text{m}$ ). Muscle pH did not differ ( $P = 0.2030$ ) between hot and cold-deboned muscles during *post-mortem* storage. Hot-deboned muscles were tougher ( $P < 0.05$ ) than cold-deboned muscles from 24 h up to 5 d *post-mortem*. Hot-deboning caused significantly ( $P < 0.0001$ ) more purge (%) to accumulate in the vacuum packages of the hot-deboned muscles ( $1.83 \pm 1.31\%$ ) than in the vacuum packages of the cold-deboned muscles ( $0.67 \pm 0.75\%$ ) during the 21-day aging period. Although hot-deboning caused muscles to be tougher than the cold-deboned muscles, with *post-mortem* aging at 4°C beyond 5 d this toughness was found to be insignificant and it was concluded that hot-deboning of the *M. gastrocnemius, pars interna* may be performed in the ostrich meat industry without effects on potential consumption, since consumers seldom consume meat aged for less than 7 d.

**Keywords:** hot-deboning, pH, tenderness, sarcomere length, water holding capacity (WHC), purge, cooking loss, aging, colour

## INTRODUCTION

Cold-shortening is the response when muscles are exposed to low temperatures below 10° to 15°C early *post-mortem*, while the pH is above 6.20, and ATP levels are still high enough to support shortening of the muscle fibres (Nuss & Wolfe, 1980-81; Pearson & Young, 1989; Lawrie, 1998). With the rapid cooling rate of hot-deboned vacuum-packaged meat cuts, cold-shortening and consequent toughening of the meat would be induced (Lawrie, 1998). Sales & Mellet (1996) suggested that ostrich muscles are not susceptible to cold-shortening. However, these authors concluded that there is a risk of cold-shortening in the *M. gastrocnemius, pars interna* if this muscle is to be separated from the carcass at 30 to 45 min *post-mortem*.

Aging or conditioning of unprocessed meat is one technique to enhance tenderness. However, Sales *et al.* (1996) suggested that it would not be beneficial to age ostrich *M. gastrocnemius, pars interna* for more than 3.5 d, since it was found by these authors that shear force values did not decrease significantly with an additional 7 d of aging after the initial 3.5 d. Results obtained by Marks *et al.* (1998) indicated that ostrich meat may not require an aging time beyond 1 h prior to deboning to ensure sufficient tenderness. However, results from the previous study (Chapter 3 of this thesis) indicated that deboning of the *M. gastrocnemius, pars interna* at approximately 1 h *post-mortem* caused muscles to be tougher than the cold-deboned muscles after aging for only 48 h.

Purge in vacuum-packaged meat could negatively influence the quality of the meat and also the juiciness of the meat when cooked (Pollok *et al.*, 1997), however, the water holding capacity of meat changes during *post-mortem* storage. The extent of *post-mortem* pH decline will affect the water holding capacity of muscles and the higher the ultimate pH, the lower the decrease in water holding capacity (Lawrie, 1998). The point of minimum water holding capacity of the principal proteins in muscles is at a pH of 5.4 to 5.5. After reaching a minimum pH, the muscle pH then tends to increase with subsequent aging. This is due to an increase in osmotic pressure resulting from the breakdown of protein molecules, as well as a net increase in charge through absorption of K<sup>+</sup> ions and release of Ca<sup>2+</sup> ions. Therefore, the water holding capacity of muscle proteins will also increase with increasing aging (Tirupal *et al.*, 1998; Lawrie, 1998). On the other hand, with continued aging, one of the most important manifestations of the *post-mortem* denaturation of muscle proteins is the loss of water holding capacity (Lawrie, 1998).

Since hot-deboned *M. gastrocnemius, pars interna* were found to be tougher than the cold-deboned muscles at 2 d (48 h) *post-mortem* (Chapter 3 of this thesis), the aims of this study were to determine whether hot-deboning would cause *M. gastrocnemius, pars interna* to have shorter sarcomeres than cold-deboned muscles, resulting in hot-deboned muscles being tougher. Furthermore, to determine whether the toughness of hot-deboned

*M. gastrocnemius, pars interna* at 2 d *post-mortem* would prevail after aging for 21 d *post-mortem*, hot and cold-deboned muscles were vacuum packed and aged for 21 d at 4°C. The effects of hot-deboning on the physical meat qualities: pH, shear force, purge (%) and cooking loss (%) were therefore investigated during the *post-mortem* storage period of 21 d at 4°C. Preference for meat is not only affected by texture, but also strongly affected by changes in colour and appearance (Risvik, 1994), thus, the effect of hot-deboning on raw ostrich meat colour was also investigated during *post-mortem* storage for 21 d at 4°C. Since Warner-Bratzler shear force values (N.12.7 mm<sup>-1</sup> diameter) were highly correlated to taste panel sensory scores for tenderness of ostrich *M. gastrocnemius, pars interna* in the previous research study (Chapter 3 of this thesis), shear force values were accepted as an indicator for meat tenderness in the present research project.

## MATERIALS AND METHODS

### Ostriches and muscle samples

Eight rested (approximately 12 h of lairage) ostriches (*Struthio camelus var. domesticus*), between 10 to 14 months of age, were slaughtered as described by Wotton & Sparrey (2002), on different days at the same EU approved ostrich abattoir at Malmesbury, South Africa, during February 2004. Each left *M. gastrocnemius, pars interna* (1.1 ± 0.2 kg) were excised at approximately 1 h *post-mortem* (hot-deboned). Two slices of approximately 1 cm thickness were removed from the centre of the hot-deboned muscles for determination of sarcomere length. The rest of the muscle was vacuum packed in vacuum bag with the following specifications: standard thickness: 80 micron ± 10% variation, weight: 80 g.m<sup>-2</sup> ± 10% variation, oxygen transmission: 38 cm<sup>3</sup>.m<sup>-2</sup>.24h<sup>-1</sup> ± 20% variation, water vapour transmission: 2.8 g.m<sup>-2</sup>.24h<sup>-1</sup> ± 20% variation, and carbon dioxide transmission: 195 cm<sup>3</sup>.m<sup>-2</sup>.24h<sup>-1</sup> ± 20% variation. The vacuum packed muscles were then refrigerated at < 4°C until 24 h *post-mortem* in the same refrigerator (at the ostrich abattoir) where the ostrich carcasses were kept before cold-deboning of the right leg muscles (the same carcasses from which the hot-deboned muscles were removed) were performed 24 h *post-mortem*.

After 24 h *post-mortem*, refrigerated at < 4°C, the right leg muscles (1.2 ± 0.2 kg) from the same carcasses were cold-deboned, samples for sarcomere length determination were removed (as described above), and the remaining muscles were vacuum-packed. All muscle samples were then transported to the laboratories at the University of Stellenbosch (60 km) in an insulated container.

The muscles were then cut into eight 1.5 to 2.0 cm thick slices. The slices were individually weighed, vacuum-packed, and randomly assigned to aging periods of 1 (approximately 24 h *post-mortem*), 2, 3, 5, 7, 14 and 21 days *post-mortem* in a commercial refrigerator at a mean temperature of 4°C (0° to 7°C variation).

### **Sarcomere Length**

The samples for sarcomere length determinations were taken from muscle slices (1 cm thick), cut from the centre of the muscles at 1 h *post-mortem* from hot-deboned *M. gastrocnemius, pars interna* and at 24 h *post-mortem* from both the hot and the cold-deboned muscles. Meat cubes of approximately 1 cm<sup>3</sup> were cut from the centre of the 1 cm thick muscle slices and immediately fixed in 2.5% (m/v) glutaric dialdehyde solution A [2.5% glutaric dialdehyde, 0.1 M potassium chloride (KCl), 0.039 M boric acid (H<sub>3</sub>BO<sub>3</sub>), and 5 mM ethylenediaminetetra-acetic acid disodium salt dehydrate (Tritriplex III)] and stored at 4°C for 24 h. After 24 h, the meat cubes were transferred to a higher concentration of 2.5% glutaric dialdehyde solution B [2.5% glutaric dialdehyde, 0.25 M potassium chloride (KCl), 0.29 M boric acid (H<sub>3</sub>BO<sub>3</sub>), and 5 mM ethylenediaminetetra-acetic acid disodium salt dehydrate (Tritriplex III)] and stored in this solution at 4°C until homogenised. The samples were homogenised in fresh 2.5% glutaric dialdehyde solution B with a Polytron homogeniser (Lasec, South Africa) at a speed of 27 000 min<sup>-1</sup> for 20 s. The homogenised samples were stored at 4°C until microscopically analysed.

Muscle fibres from individual muscle samples were transferred to glass slides, making sure they remained moist with 2.5% glutaric dialdehyde solution B before being covered with a cover glass slip. Images of ten individual muscle fibres from each glass slide were taken with a digital camera (Nikon, DXM 1200, USA) connected to a light microscope (Nikon, Eclipse E600), using the 40x objective and accompanying software program (Nikon, ACT-1, USA). Sarcomere lengths were measured with the use of an image analysis software program (Simple PCI, Version 4.0, Compix Inc. USA), by counting three sets of 10 consecutive sarcomeres from each fibre image. The mean sarcomere length for each muscle fibre image was then calculated.

### **Physical characteristics**

Muscle temperature, pH, percentage purge, raw meat colour (CIE lightness L\*, a\* and b\* colour coordinates), percentage cooking loss (after cooking for an hour at 80°C in a water bath) and Warner-Bratzler shear force (N. 12.7 mm<sup>-1</sup> diameter) measurements were

recorded at the respective aging intervals of 1 (24 h *post-mortem*), 2, 3, 5, 7, 14 and 21 d *post-mortem*.

Muscle temperature ( $^{\circ}\text{C}$ ) and pH were recorded at room temperature ( $18^{\circ}$ - $19^{\circ}\text{C}$ ) with the use of a calibrated (standard buffers of pH 4.0 and 7.0) portable Crison 506 pH-meter equipped with a pH and temperature probe. Although muscles were stored at an average temperature of  $4^{\circ}\text{C}$ , all muscle samples were at an average temperature of  $10.10^{\circ} \pm 3.23^{\circ}\text{C}$  (between  $7^{\circ}$  and  $14^{\circ}\text{C}$ ) when the pH measurements were recorded throughout the 21-day aging period. The muscle temperature was above  $4^{\circ}\text{C}$  since muscle samples were removed from the refrigerator and kept at room temperature ( $18^{\circ}$ - $19^{\circ}\text{C}$ ) until all measurements were recorded. In addition, the temperature of the refrigerator fluctuated between  $0^{\circ}$  and  $7^{\circ}\text{C}$  throughout the 21-day storage period, causing some muscle samples to be higher than  $4^{\circ}\text{C}$  when they were removed from the refrigerator.

To determine the purge (%), muscle samples were weighed ( $88.09 \pm 19.09$  g) after they were removed from the vacuum package and blotted dry with tissue paper. Purge was then expressed as a percentage of the initial weight of the muscle sample.

The colour of the raw muscle slices were recorded according to the method described by Honikel (1998) with the use of a Colour-guide 45 $^{\circ}$ /0 $^{\circ}$  colorimeter (Cat no: 6805; BYK-Gardner, USA). Muscle slices (1.5 to 2.0 cm thick) were allowed to “bloom” for 30 min at room temperature ( $18^{\circ}$  to  $19^{\circ}\text{C}$ ) prior to colour measurements. Colour measurements were recorded in triplicate for each sample at randomly selected positions and expressed by the coordinates  $L^*$ ,  $a^*$  and  $b^*$  of the CIELab colorimetric space (MINOLTA, 1998). In the colour space,  $L^*$  indicates lightness and  $a^*$  and  $b^*$  are the chromaticity coordinates, where  $a^*$  is the red-green range, and  $b^*$  the yellow-blue range of the colour spectrum. The Hue angle ( $h_{ab}$ ) ( $^{\circ}$ ) and Chroma ( $C^*$ ) were also calculated as follows, using the  $L^*$ ,  $a^*$  and  $b^*$  values:

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

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

The Hue angle ( $h_{ab}$ ) is defined as starting at the positive side of the  $a^*$  axis of the chromaticity diagram and is expressed in degrees ( $^{\circ}$ ), meaning that  $0^{\circ}$  would indicate red and  $90^{\circ}$  would indicate yellow. Chroma ( $C^*$ ) is a measure of the difference from a grey of the same lightness (Mackinney & Little, 1962). Chroma ( $C^*$ ) has a value of 0 at the centre of the chromaticity diagram (central grey) and extending outwards according to the

distance from the centre, indicating that the colour increases in brightness (MINOLTA, 1998).

Cooking loss (%) of muscle slices was determined by placing the weighed raw meat sample; sealed in a plastic bag, in a water bath (preheated to 80°C) for 1 h (Honikel, 1998). After an hour, the cooked meat samples were removed, allowed to cool down under running water and the mass was recorded after excess water was blotted with tissue paper. Cooking loss was expressed as a percentage of the initial mass ( $89.46 \pm 19.77$  g) of the muscle sample (Honikel, 1998).

The same muscle samples that were used to determine the cooking loss were used for assessment of tenderness. The muscle samples were refrigerated (4°C) and stored over-night before tenderness was determined the following day by using a Warner-Bratzler device as described by Wheeler *et al.* (2001) and Honikel (1998). A load cell of 2.000 kN was attached to the model 4444 Instron texture machine (Apollo Scientific cc, South Africa). Seven 12.7 mm wide cores were removed parallel to the muscle fibre from the cooked muscle slice of 1.5 to 2.0 cm in thickness and placed in the Warner-Bratzler device, so that the knife blade of the device cut across the fibres at a right angle. The maximum shear force value (N.  $12.7 \text{ mm}^{-1}$  diameter) to shear a cylindrical core of cooked meat was recorded at a crosshead speed of  $200 \text{ mm} \cdot \text{min}^{-1}$ . Mean maximum shear force values were calculated from the recorded shear force values for seven cylindrical cores from each muscle sample and used in the statistical analyses.



### Statistical analyses

A  $2 \times 7$  factorial experiment was performed in a randomised complete block design with eight blocks (ostrich carcasses). The factors were the two deboning treatments (hot and cold) and *post-mortem* aging time [day 1 (approximately 24 h *post-mortem*), 2, 3, 5, 7, 14 and 21]. The data were subjected to factorial analysis of variance (ANOVA) using SAS version 8.2 statistical software (SAS, 1999). Shapiro-Wilk tests were performed for testing non-normality (Shapiro & Wilk, 1965). Results from the factorial analysis of variance (ANOVA) and Shapiro-Wilk tests for dependable variables: sarcomere length, muscle pH, Warner-Bratzler shear force (N.  $12.7 \text{ mm}^{-1}$  diameter), percentage purge, percentage cooking loss, colour coordinates  $L^*$ ,  $a^*$ ,  $b^*$ , Hue angle ( $h_{ab}$ ) ( $^\circ$ ) and Chroma ( $C^*$ ); with ostrich, deboning and aging time (d) as main effects, as well as two way interaction between aging time and deboning, are summarised in Tables 1, 2, 5, 7, 10, 12, 14, 15, 16, and 17.

When deviations from normality were detected, outliers were removed until data were symmetrical or normal distributed (Glass *et al.*, 1972). Student's t-Least Significant



Difference was calculated at the 5% confidence level to compare means for significant effects (Ott, 1998).

Exponential models were fitted to the Warner-Bratzler shear force values using SAS version 8.2 statistical software (SAS, 1999). The function of this model was:

$$y = ax^b$$

where:  $y$  = Warner-Bratzler shear force value (N. 12.7 mm<sup>-1</sup> diameter) at time  $x$ ;  $x$  = *post-mortem* aging time in days;  $a$  = intercept, and  $b$  = slope. The regression coefficients, intercept ( $a$ ) and slope ( $b$ ) of the fitted model, were then analysed by analysis of variance (ANOVA) using SAS version 8.2 (SAS, 1999) and the results are summarised in Table 4.

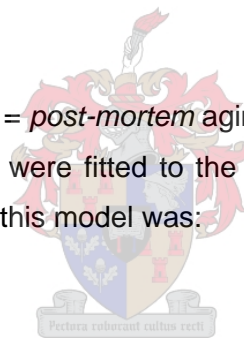
Trend lines were fitted to the pH values using Microsoft Excel (2004). Third order polynomial trend lines gave the best fit as determined by the R<sup>2</sup>-value (R<sup>2</sup> = 0.9799 ± 0.0093). The function represented by the trend lines was:

$$y = ax^3 + bx^2 + cx + d$$

Where:  $y$  = pH values at time  $x$ ;  $x$  = *post-mortem* aging time in days, and  $d$  = intercept.

Linear regression models were fitted to the purge (%) values using SAS version 8.2 (SAS, 1999). The function of this model was:

$$y = ax \pm b$$



where:  $y$  = purge (%) values at time  $x$ ;  $x$  = *post-mortem* aging time in days;  $a$  = intercept, and  $b$  = slope. The regression coefficients, intercept ( $a$ ) and slope ( $b$ ) of the fitted model, were then analysed by analysis of variance (ANOVA) using SAS version 8.2 (SAS, 1999).

Pearson correlation coefficients [ $r$ -values at the 5% significance level ( $P$ )] were calculated with the use of statistical software Statistica version 6 (StatSoft 2003) as well as using the linear regression procedure (Prod CORR) of SAS version 8.2 (SAS, 1999). In the cases where there were no significant differences in variables between hot and cold-deboned muscles, correlation coefficients were calculated for the pooled values. Correlation coefficients [ $r$ -values at the 5% significance level ( $P$ )] were calculated from the raw data points for hot and cold-deboned muscles respectively between the different variables (pH, Warner-Bratzler shear force, purge, cooking loss and raw meat colour) as well as with storage time. Correlation coefficients between muscle pH at 24 h *post-mortem* and the mean values for hot and cold-deboned muscles respectively, as well as from the hot and cold-deboned pooled mean values, were also calculated.

## RESULTS AND DISCUSSION

The analysis of variance (ANOVA) of the dependable variable sarcomere length ( $\mu\text{m}$ ) is presented in Table 1. The sarcomere length data was normally distributed ( $P = 0.9884$ ) and no transformation of data was required for further analysis. There was no significant difference in sarcomere length between individual ostrich carcasses ( $P = 0.2030$ ). Deboning, as well as time *post-mortem* (1 or 24 h), had a significant effect on sarcomere length ( $P < 0.05$ ). Deboning at 1 h *post-mortem* caused hot-deboned muscles to have shorter sarcomeres ( $2.05 \pm 0.18 \mu\text{m}$ ) at 24 h *post-mortem* than the cold-deboned muscles ( $2.52 \pm 0.14 \mu\text{m}$ ) removed and measured at 24 h *post-mortem* (Fig. 1). Similarly, at 1 h *post-mortem* the sarcomere lengths for the hot-deboned muscles ( $2.18 \pm 0.06 \mu\text{m}$ ) were significantly shorter ( $P < 0.05$ ) than the sarcomere length for the cold-deboned muscles at 24 h *post-mortem* ( $2.52 \pm 0.14 \mu\text{m}$ ).

Results reported in the literature for cold-deboned and cold-shortened beef *M. longissimus thoracis et lumborum*, indicated that cold-shortened muscles had shorter sarcomeres than cold-deboned muscles (Bouton *et al.*, 1973; Smulders *et al.*, 1990; Silva *et al.*, 1999). The shorter sarcomeres ( $P > 0.05$ ) at 24 h for the hot-deboned muscles ( $2.05 \pm 0.18 \mu\text{m}$ ) in this study compared to sarcomere lengths measured at 1 h *post-mortem* ( $2.18 \pm 0.06 \mu\text{m}$ ) can most probably be explained by the shortening of muscle fibres during rigor development while the muscles were not attached to the carcass and therefore were free to shorten. In addition, when muscles that are free to shorten are exposed to temperatures below  $10^{\circ}$  to  $15^{\circ}\text{C}$  during the development of *rigor mortis*, there is the occurrence of cold-shortening and super contraction under the influence of the cold temperatures. The hot and the cold-deboned muscles were refrigerated at  $< 4^{\circ}\text{C}$  during the first 24 h *post-mortem*, while during the blooming and measuring period all the muscle samples were at an ambient temperature of  $18^{\circ}$  to  $19^{\circ}\text{C}$ , therefore resulting in the hot ( $11.29^{\circ} \pm 3.99^{\circ}\text{C}$ ) and the cold-deboned muscles ( $11.36^{\circ} \pm 5.15^{\circ}\text{C}$ ) to be at a relative high temperature after 24 h *post-mortem*. The insignificant difference ( $P > 0.05$ ) in muscle temperature after 24 h *post-mortem* between the hot and the cold-deboned muscles indicate that all the muscle samples were treated similarly at all times and that the increase in temperature during the blooming and measuring period did not influence the main effects. It can therefore be hypothesised that both the hot and the cold-deboned muscles had some degree of cold-shortening during refrigeration at  $< 4^{\circ}\text{C}$ , but since the cold-deboned muscles were attached to the carcass, super contraction of the muscle fibres was prevented.

During the first hour *post-mortem*, muscle fibres started to shorten slightly due to the onset of *rigor mortis*. At 1 h *post-mortem*, muscles were excised from the carcass (also causing a slight shortening of the muscle fibres) and the muscle fibres were thus fixed while they were in the early phase of the onset of *rigor mortis* with little shortening. This could explain the low degree of shortening observed at 1 h *post-mortem*.

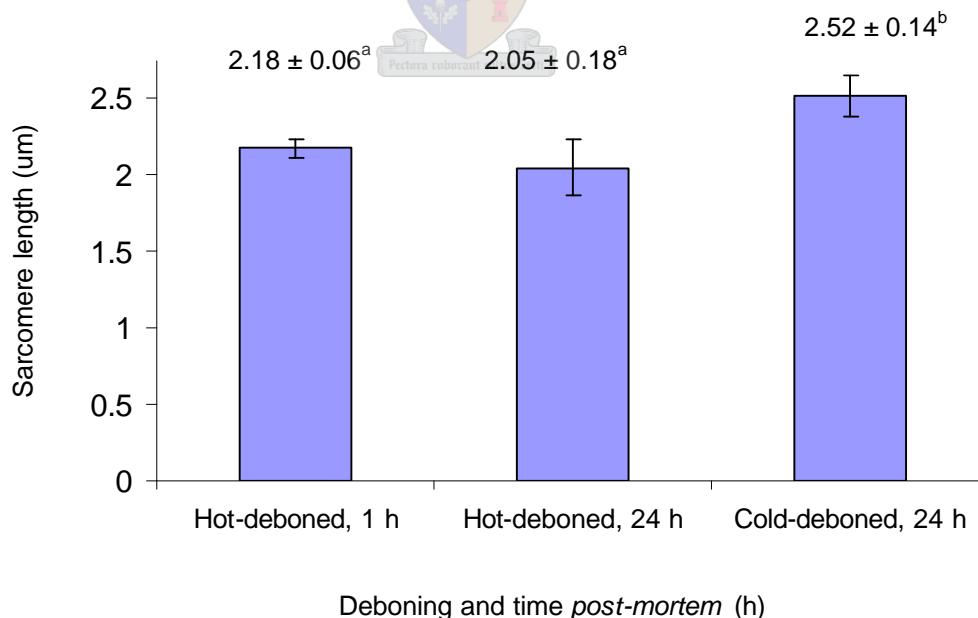
**Table 1.** Analysis of variance (ANOVA) of the dependable variable sarcomere length ( $\mu\text{m}$ ) with ostrich carcass, deboning (debone) and time (h) *post-mortem* as main effects, as well as the Shapiro-Wilk test for non-normality.

Sarcomere length ( $\mu\text{m}$ )			
Source	df	MS	P
Ostrich carcass	7	0.0243	0.2030
Debone	1	0.7019	< 0.0001
Time (h) <i>post-mortem</i>	1	0.0935	0.0267
Error	10	0.0139	
Shapiro-Wilk			0.9884

df – Degree of freedom

MS – Mean Square

P – Probability value of F-ratio test



**Figure 1.** Mean ( $\pm$  Standard Deviation) sarcomere length ( $\mu\text{m}$ ) for the hot-deboned and the cold-deboned *M. gastrocnemius, pars interna* at respectively 1 h and 24 h *post-mortem*. <sup>ab</sup> Different superscripts indicate differences at  $P < 0.05$ .

The mean sarcomere length for the cold-deboned muscles at 24 h *post-mortem* ( $2.52 \pm 0.14 \mu\text{m}$ ) was longer than that previously reported by Sales (1996) for sarcomere length in ostrich *M. gastrocnemius, pars interna* at approximately 40 h *post-mortem* ( $2.34 \pm 0.14 \mu\text{m}$ ). It can be hypothesised that the difference in sarcomere length found in this study compared to what has been reported by Sales (1996) is due to difference in locations within the *M. gastrocnemius, pars interna* from where samples were collected, as well as due to the different method used by Sales (1996) to measure the sarcomere length of the muscle samples.

The analysis of variance (ANOVA) of the dependable variable Warner-Bratzler shear force (N.  $12.7 \text{ mm}^{-1}$  diameter) is presented in Table 2. Although all ostriches were slaughtered at the same age (10 to 14 months) and were handled similarly before and during the slaughter practices (Wotton & Sparrey, 2002), significant differences in shear force values (N.  $12.7 \text{ mm}^{-1}$  diameter) were observed between individual ostrich carcasses ( $P < 0.0001$ ). As the ostriches were not from the same flock or producer, various extrinsic factors (for example nutrition, intensive or extensive farming practices and the duration of transportation) may have been the cause of this variation in tenderness. Similarly, some birds might have been more susceptible to pre-slaughter stress than others, resulting therefore in different levels of glycogen and ATP concentrations within the muscles between individual carcasses. All these factors are known to influence the quality characteristics of meat (Lawrie, 1998).

There was a significant interaction ( $P = 0.0175$ ) between deboning and aging for the variable tenderness (shear force values; N.  $12.7 \text{ mm}^{-1}$  diameter). Hot and cold-deboned muscles had different initial tenderness values at 24 h *post-mortem* and there was also a slight difference in the rate of increase in tenderness during the 21-day storage period at  $4^{\circ}\text{C}$  between the hot and the cold-deboned muscles (Table 3).

Irrespective of the interaction between the main effects “deboning” and “aging”, hot-deboning had a significant effect on tenderness ( $P < 0.0001$ ), while *post-mortem* aging also influenced ( $P < 0.0001$ ) shear force values during the 21-day aging period (Table 2).

**Table 2.** Analysis of variance (ANOVA) of the dependable variable mean Warner-Bratzler shear force (N. 12.7 mm<sup>-1</sup> diameter) with ostrich carcass, deboning (debone) and aging time (d) as main effects, as well as the two way interaction between aging time (d) and deboning, and the Shapiro-Wilk test for non-normality.

<b>Mean Warner-Bratzler shear force</b>			
<b>(N. 12.7 mm<sup>-1</sup> deameter)</b>			
<b>Source</b>	<b>df</b>	<b>MS</b>	<b>P</b>
Ostrich carcass	7	934.281	< 0.0001
Debone	1	2654.045	< 0.0001
Aging	6	570.000	< 0.0001
Debone*Aging	6	190.057	0.0175
Error	75	68.690	
Shapiro-Wilk			0.7047

df – Degree of freedom  
MS – Mean Square  
P – Probability value of F-ratio test  
\*Interaction between main effects

A large variation in the tenderness of meat samples was observed between the individual ostriches ( $P < 0.05$ ), but on average both the hot and the cold-deboned muscles showed a gradual increase in tenderness ( $P < 0.05$ ) as the time of aging increased from day 1 to day 21 (Table 3). The highest toughness was found to be at 24 h and 3 d *post-mortem* for both the hot and the cold-deboned muscles. However, it was noticed that as the *post-mortem* aging time increased, the variation in tenderness between the individual ostrich carcasses decreased. This was particularly noteworthy on days 14 and 21. It was suggested by Sales *et al.* (1996) that aging after 3.5 d *post-mortem* would not increase the tenderness of the *M. gastrocnemius, pars interna* further. However, in this study it was found that cold-deboned *M. gastrocnemius, pars interna* increased further in tenderness from day 3 to day 5 of aging, after which the tenderness did not increase significantly any further up to day 21. However, in the hot-deboned muscles, there was still a significant ( $P < 0.05$ ) increase in tenderness from day 5 to day 21.

Cold-deboned muscles were significantly more tender ( $P < 0.05$ ) than hot-deboned muscles during the first 3 d *post-mortem*, however, the cold-deboned muscles showed similar shear force values on day 14 and on day 21 to the hot-deboned muscles (Table 3). When sarcomere length and shear force values, both sampled and measured 24 h *post-mortem*, for respectively the hot and the cold-deboned muscles were investigated, there was no significant correlation between shear force values and sarcomere length in the

case of cold-deboned muscles ( $r = -0.659$ ;  $P = 0.108$ ). In contrast, sarcomere length for the hot-deboned muscles was significantly negatively correlated to shear force values ( $r = -0.888$ ;  $P = 0.0180$ ), indicating that hot-deboning resulted in some degree of shortening during the first 24 h *post-mortem*, which would explain the higher shear force values obtained for hot-deboned muscles at 24 h *post-mortem* ( $83.63 \pm 14.18$  N.12.7 mm<sup>-1</sup> diameter), compared to that of the cold-deboned muscles ( $72.12 \pm 11.64$  N. 12.7 mm<sup>-1</sup> diameter) (Table 3). When the mean Warner-Bratzler shear force values at 24 h *post-mortem* from hot and cold-deboned ostrich muscles for the present study were pooled, there was also a significant negative correlation between tenderness and sarcomere length at 24 h *post-mortem* ( $r = -0.690$ ;  $P = 0.0090$ ), indicating that tenderness was associated with long sarcomeres. Shortened sarcomeres are generally associated with tough meat (Currie & Wolfe, 1980) and it was previously concluded by Smulders *et al.* (1990) that muscle shortening is a major determinant of tenderness when muscle tissue has not entered *rigor mortis* at the time of deboning.

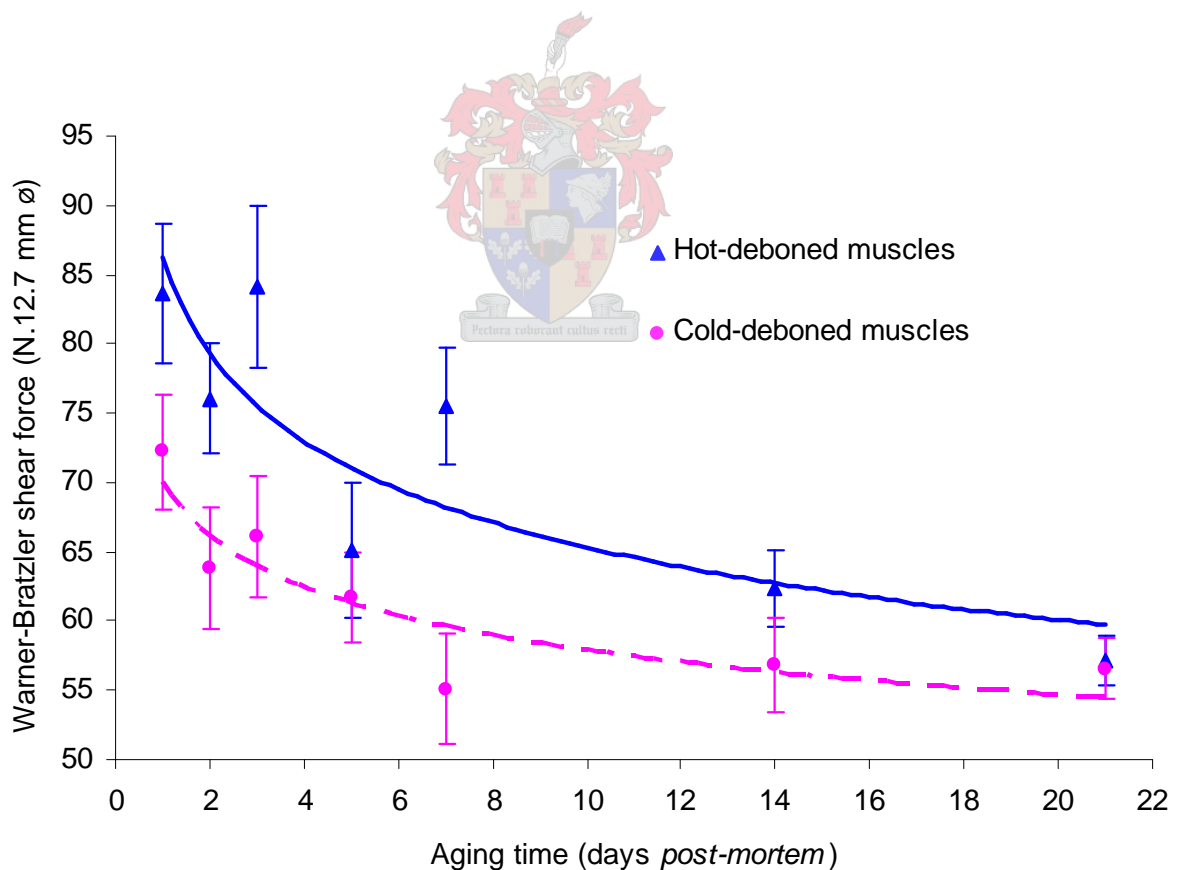
**Table 3.** Mean Warner-Bratzler shear force values (N. 12.7 mm<sup>-1</sup> diameter) for the hot-deboned and the cold-deboned *M. gastrocnemius, pars interna* muscle on the individual aging days (1, 2, 3, 5, 7, 14 and 21) *post-mortem*.

Warner-Bratzler shear force (N. 12.7 mm <sup>-1</sup> diameter)		
Aging Time (d)	Hot-deboned	Cold-deboned
1	83.63 ± 14.18 <sup>a</sup>	72.21 ± 11.64 <sup>bc</sup>
2	76.07 ± 11.22 <sup>ab</sup>	63.78 ± 12.41 <sup>cdef</sup>
3	84.15 ± 16.54 <sup>a</sup>	66.14 ± 12.35 <sup>cd</sup>
5	65.08 ± 13.74 <sup>cde</sup>	61.72 ± 9.13 <sup>def</sup>
7	75.53 ± 12.08 <sup>ab</sup>	55.12 ± 11.34 <sup>f</sup>
14	62.36 ± 7.74 <sup>def</sup>	56.77 ± 9.71 <sup>ef</sup>
21	57.20 ± 5.10 <sup>def</sup>	56.51 ± 6.19 <sup>ef</sup>

<sup>abc</sup> Different superscripts differ at  $P < 0.05$ .

Both the hot and the cold-deboned muscles showed an exponential ( $P < 0.05$ ) decline in shear force values (Fig. 2) over the 21-day *post-mortem* aging period. The analysis of variance (ANOVA) of the regression coefficients: intercept (a) and slope (b) of the fitted exponential model ( $y = ax^b$ ) for Warner-Bratzler shear force values (N. 12.7 mm<sup>-1</sup>

diameter) are presented in Table 4. Hot-deboned muscles (intercept of  $91.66 \pm 27.54$  N.  $12.7 \text{ mm}^{-1}$  diameter) were initially tougher ( $P = 0.041$ ) than cold-deboned muscles (intercept of  $69.35 \pm 11.96$  N.  $12.7 \text{ mm}^{-1}$  diameter). Although there was no significant difference ( $P = 0.169$ ) in the rate of decline in shear force values between the hot ( $-0.075 \pm 0.042$ ) and cold-deboned ( $-0.127 \pm 0.077$ ) muscles, the rate for the hot-deboned muscles was faster, which resulted in the hot-deboned muscles having a similar tenderness value to that of the cold-deboned muscles after *post-mortem* aging for 5 d (Table 3). It must be kept in mind that the temperature of the refrigerator was at times above  $4^{\circ}\text{C}$  ( $0^{\circ}\text{--}7^{\circ}\text{C}$  variation) and that the higher temperature could have had an effect on the tenderness of the meat. High temperatures enhance the *post-mortem* proteolytic enzyme activity in meat (Lawrie, 1998), and therefore it can be hypothesised that if the muscle temperature in the present study did not increase above  $4^{\circ}\text{C}$ , it would have taken longer than 5 d for the hot-deboned and the cold-deboned muscles to reach similar levels of tenderness.



**Figure 2.** Exponential decrease in shear force ( $\pm$  Standard Error) with *post-mortem* aging time from day 1 to day 21 for respectively the ( $\blacktriangle$ ) hot-deboned ( $y = 91.660x^{-0.1273}$   $R^2 = 0.7462$ ) and the ( $\bullet$ ) cold-deboned ( $y = 69.348x^{-0.0746}$ ;  $R^2 = 0.8092$ ) muscles.

**Table 4.** Analysis of variance (ANOVA) of the regression coefficients: intercept (a) and slope (b) of the exponential model ( $y = ax^b$ ) for Warner-Bratzler shear force value (N. 12.7 mm<sup>-1</sup> diameter) with model, ostrich carcass and deboning (debone) as main effects, as well as the Shapiro-Wilk test for non-normality.

Source	df	Intercept (a)		Slope (b)	
		MS	P	MS	P
Ostrich carcass	7	583.393	0.221	0.003	0.717
Debone	7	1991.308	0.041	0.011	0.169
Error	1	317.803		0.005	
Shapiro-Wilk			0.069		0.790

df – Degree of freedom

MS – Mean Square

P – Probability value of F-ratio test

The analysis of variance (ANOVA) of dependable variable muscle pH is presented in Table 5. Data from the pH results were normally distributed ( $P = 0.3535$ ) and therefore further analysis could be made without any transformation of the data. As expected, muscle pH differed between individual ostrich carcasses ( $P < 0.0001$ ), which probably can be explained by intrinsic variation found naturally between ostriches, as well as different levels of *ante mortem* stress and consequent differences in the levels of *post-mortem* muscle glycogen (Lawrie, 1998) between individual ostriches. No significant interaction was observed between the main effects “deboning” and “aging” ( $P = 0.9028$ ). Similarly, the muscle pH between the hot and cold-deboned muscles did not differ significantly ( $P = 0.2030$ ), while *post-mortem* aging had a significant effect on muscle pH ( $P < 0.0001$ ) (Table 6).



**Table 5.** Analysis of variance (ANOVA) of the dependable variable muscle pH with ostrich carcass, deboning (debone) and aging time (d) as main effects, as well as the two way interaction between aging time (d) and deboning, and the Shapiro-Wilk test for non-normality.

Muscle pH			
Source	df	MS	P
Ostrich carcass	7	0.056	< 0.0001
Debone	1	0.013	0.2030
Aging	6	0.056	< 0.0001
Debone*Aging	6	0.003	0.9028
Error	86	0.008	
Shapiro-Wilk			0.3535

df – Degree of freedom

MS – Mean Square

P – Probability value of F-ratio test

\*Interaction between main effects

**Table 6.** Mean pH ( $\pm$  Standard Deviation) for the pooled hot-deboned and cold-deboned *M. gastrocnemius, pars interna* muscle at the respective *post-mortem* aging days (1 d to 21 d).

Aging Time (d)	Muscle pH
1	5.83 $\pm$ 0.09 <sup>ab</sup>
2	5.84 $\pm$ 0.10 <sup>ab</sup>
3	5.84 $\pm$ 0.09 <sup>ab</sup>
5	5.87 $\pm$ 0.01 <sup>a</sup>
7	5.87 $\pm$ 0.08 <sup>a</sup>
14	5.80 $\pm$ 0.05 <sup>b</sup>
21	5.70 $\pm$ 0.18 <sup>c</sup>

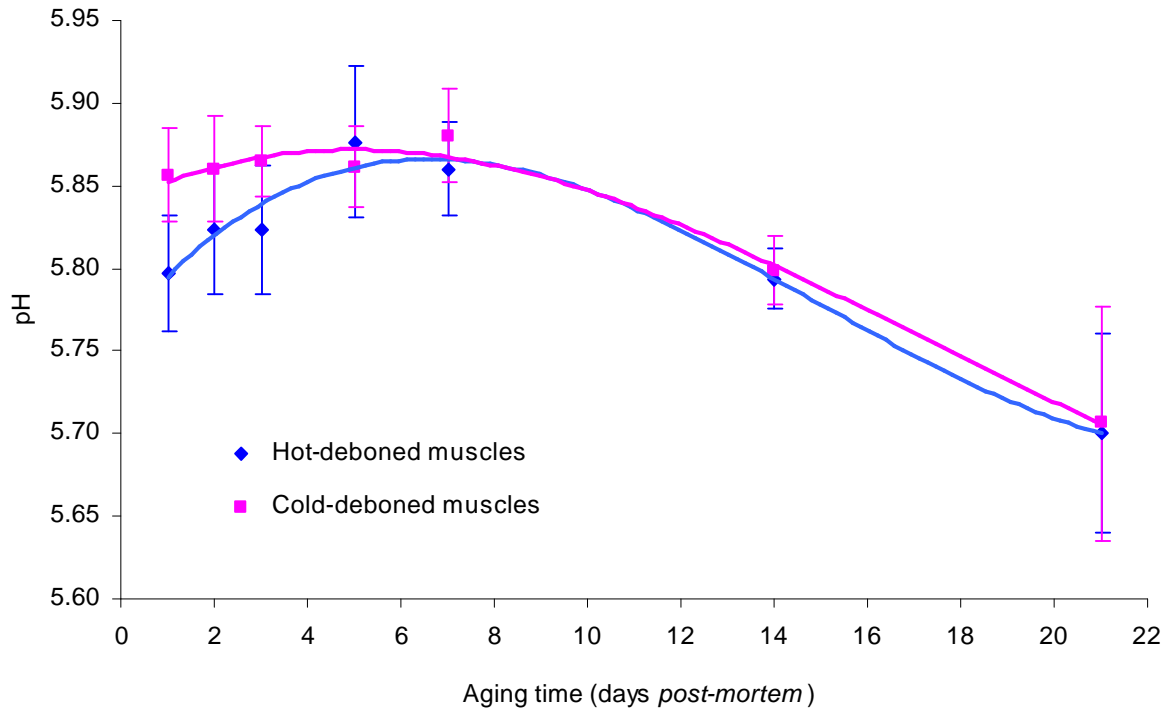
<sup>abc</sup> Different superscripts within a column differ at  $P < 0.05$ .

Muscle pH at 24 h *post-mortem* (pH<sub>24</sub>) for the hot (5.80  $\pm$  0.10) and cold-deboned (5.86  $\pm$  0.08) muscles when pooled resulted in pH<sub>24</sub>-values of 5.83  $\pm$  0.09 (Table 6), that were analogous to the pH range of pH<sub>24</sub> values (5.9 to 6.3) previously reported for ostrich muscles (Morris *et al.*, 1995; Sales, 1996). The pH<sub>24</sub> for ostrich muscles is well above the 5.5 value as reported to be the ultimate or minimum pH (at 24 or 48 h *post-mortem*) for

beef, veal and pig muscles (Pearson & Young, 1989; Lawrie, 1998). The mean ultimate pH-value of 5.5 for emu muscles (Berge *et al.*, 1997) is also lower to that found for ostrich muscles. However, the pH of ostrich meat tends to fall in the same high pH range generally found in the leg muscles of the conventional poultry species (6.0 to 6.4) (Touraille *et al.*, 1981; Paleari *et al.*, 1998; Barbut, 1993).

The trend lines for the mean muscle pH over the aging time of 21 d *post-mortem* for respectively the hot and the cold-deboned muscles are illustrated in Fig. 3. At the time of *post-mortem* pH measurements, all muscle samples were at an average temperature of  $10.10^{\circ} \pm 3.23^{\circ}\text{C}$  (between  $7^{\circ}$  and  $14^{\circ}\text{C}$ ). This high temperature for the muscle samples at the time of measurement could be explained by the fact that the muscle samples were removed from the refrigerator and kept at room temperature ( $18^{\circ}$  to  $19^{\circ}\text{C}$ ) until all measurements including purge (%), pH, temperature and raw meat colour (after an 30 min blooming period), were obtained. However, since all muscle samples were stored at the same temperature(s) and were treated similarly throughout the storage and measuring periods, it is argued that variation in temperature did not influence the main effects “deboning” and “aging”. The decline in muscle temperature during the first 24 h *post-mortem* has an important effect on muscle  $\text{pH}_{24}$  (Lawrie, 1998). The rate of *post-mortem* glycolysis will tend to be higher in muscles which do not have a fast rate of decline in temperature, since higher temperatures are known to speed up the rate of chemical reactions (Pearson & Young, 1989; Lawrie, 1998). It has been reported that the early *post-mortem* temperature decline in hot-deboned beef muscles is faster than in muscles left on the carcass (Van Laack & Smulders, 1992; Van Laack *et al.*, 1994).

Investigation of the effect of hot-deboning on the early *post-mortem* temperature decline and pH changes in ostrich muscles could elucidate on differences in initial tenderness between hot and cold-deboned *M. gastrocnemius, pars interna* (Chapter 6 of this thesis). From the data in Fig. 3 it can be seen that the *post-mortem* pH increased during the first 7 d to a value of  $5.87 \pm 0.08$ , there after decreasing sharply to a minimum value of  $5.70 \pm 0.18$  at day 21.



**Figure 3.** Third order polynomial trend lines for muscle pH ( $\pm$  Standard Error) over aging time of 21 d *post-mortem* for respectively the ( $\blacklozenge$ ) hot-deboned ( $y = 7 \times 10^{-5}x^3 - 0.0033x^2 + 0.0343x + 5.7635$ ;  $R^2 = 0.9733$ ) and the ( $\blacksquare$ ) cold-deboned ( $y = 3 \times 10^{-5}x^3 - 0.0016x^2 + 0.0135x + 5.8401$ ;  $R^2 = 0.9864$ ) muscles.

For percentage purge, a significant interaction ( $P = 0.0016$ ) between deboning and aging was observed (Table 7), indicating that the hot and the cold-deboned *M. gastrocnemius, pars interna* differed significantly at the individual aging days (Table 8) and also differed in the rate of increase in purge (%) as the time of *post-mortem* aging increased (Fig. 4). Both the hot and the cold-deboned *M. gastrocnemius, pars interna* showed an increase in purge ( $P < 0.05$ ) as the time of aging increased (Table 8). Chin & Keeton (1997) similarly demonstrated that drip loss in vacuum packaged ostrich meat increased with *post-mortem* storage of 14 d at 2°C. However, in the present study, the muscle samples were stored at temperatures between 0° and 7°C. This higher temperature at which the muscle samples were stored could have caused muscles to have a higher percentage purge than if the muscles were stored at lower temperatures. Tirupal *et al.* (1998) demonstrated the effect of *post-mortem* storage temperature on the water holding capacity of meat. These authors found that the maximum loss of water holding capacity was obtained at a slower rate in samples stored at a low temperature (4°C) compared to meat samples stored at higher temperatures (9° and 15°C).

In the case of the hot-deboned muscles in this study, the greatest increase in purge occurred after 14 d of storage, while cold-deboned muscles had the greatest increase at 21 d *post-mortem*. The highest percentage of purge was measure at day 21 of the aging period for the both hot ( $3.57 \pm 0.81\%$ ) and the cold-deboned ( $1.35 \pm 0.94\%$ ) muscles. However, further investigation is required to determine whether purge (%) would increase to values higher than what have been found in this study if the time of refrigerated aging *post-mortem* is to be increased beyond 21 d. Vacuum packed ostrich meat cuts predestined for export are commonly refrigerated for 42 d before reaching the consumer. Vacuum-packaged meat cuts with high amounts of purge negatively influence the visual appearance of the meat product, which is economically detrimental to meat suppliers (Lawrie, 1998). High levels of liquid accumulation in vacuum-packaged meat cuts also increase the risk of microbial spoilage.

Irrespective of the interaction between the main effects “deboning” and “aging”, the hot-deboned muscles had significantly ( $P < 0.0001$ ) more purge (%) than the cold-deboned muscles (Table 8). The higher percentage of purge for the hot-deboned muscles can partly be explained by the shorter sarcomeres measured at 24 h *post-mortem* for the hot-deboned muscles compared to the cold-deboned muscles. Tornberg (1996) also demonstrated that beef muscles with shorter sarcomeres showed lower levels of water holding capacity compared to muscles which had not shortened.

**Table 7.** Analysis of variance (ANOVA) of the dependable variable percentage purge with ostrich carcass, deboning (debone) and aging time (d) as main effects, as well as the two way interaction between aging time (d) and deboning, and the Shapiro-Wilk test for non-normality.

Source	Purge (%)		
	df	MS	P
Ostrich carcass	7	2.636	0.0001
Debone	1	36.500	< 0.0001
Aging	6	6.784	< 0.0001
Debone*Aging	6	2.148	0.0016
Error	86	0.546	
Shapiro-Wilk			0.9713

df – Degree of freedom

MS – Mean Square

P – Probability value of F-ratio test

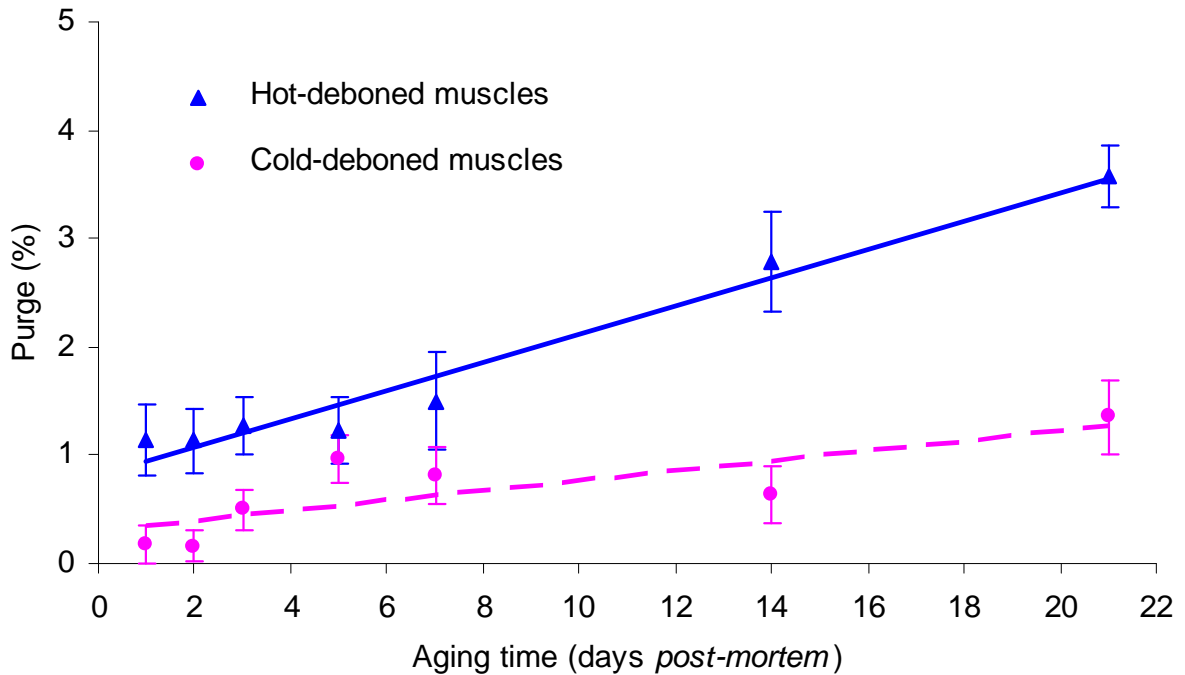
\*Interaction between main effects

**Table 8.** Mean percentage purge ( $\pm$  Standard Deviation) for respectively the hot and the cold-deboned *M. gastrocnemius, pars interna* muscles at the individual *post-mortem* aging days (1 d to 21 d).

Purge (%)		
Aging Time (d)	Hot-deboned muscles	Cold-deboned muscles
1	1.15 $\pm$ 0.93 <sup>cde</sup>	0.18 $\pm$ 0.51 <sup>f</sup>
2	1.13 $\pm$ 0.84 <sup>cde</sup>	0.16 $\pm$ 0.43 <sup>f</sup>
3	1.27 $\pm$ 0.75 <sup>cd</sup>	0.50 $\pm$ 0.54 <sup>fe</sup>
5	1.22 $\pm$ 0.88 <sup>cde</sup>	0.96 $\pm$ 0.63 <sup>cde</sup>
7	1.50 $\pm$ 1.25 <sup>c</sup>	0.81 $\pm$ 0.73 <sup>cdef</sup>
14	2.78 $\pm$ 1.29 <sup>b</sup>	0.64 $\pm$ 0.73 <sup>def</sup>
21	3.57 $\pm$ 0.81 <sup>a</sup>	1.35 $\pm$ 0.94 <sup>cd</sup>

<sup>abc</sup> Different superscripts indicate differences at  $P < 0.05$ .

The linear trend lines fitted to the increase in purge (%) over storage time of 21 d *post-mortem* are presented in Fig. 4. The analysis of variance (ANOVA) of the regression coefficients: intercept (a) and slope (b) of the fitted linear model ( $y = ax + b$ ) for percentage purge are presented in Table 9. The intercept for respectively hot ( $0.771 \pm 0.634\%$ ) and cold-deboned ( $0.333 \pm 0.435\%$ ) muscles did not differ significantly ( $P = 0.1770$ ), while the slope for hot-deboned ( $0.133 \pm 0.028$ ) muscles was higher ( $P < 0.0020$ ) than that for the cold-deboned ( $0.044 \pm 0.056$ ) muscles. Hot-deboned muscles therefore had a faster rate of increase in purge during the *post-mortem* storage period compared to the cold-deboned muscles.



**Figure 4.** Linear increase in percentage purge ( $\pm$  Standard Error) over *post-mortem* aging time of 21 d for respectively the ( $\blacktriangle$ ) hot-deboned ( $y = 0.1303x + 0.8167$ ;  $R^2 = 0.9687$ ) and the ( $\bullet$ ) cold-deboned ( $y = 0.0462x + 0.3088$ ;  $R^2 = 0.6309$ ) muscles.

**Table 9.** Analysis of variance (ANOVA) of the regression coefficients: intercept (a) and slope (b) of the linear model ( $y = ax + b$ ) for percentage purge with model, ostrich carcass and deboning (debone) as main effects, as well as the Shapiro-Wilk test for non-normality.

Source	df	Intercept (a)		Slope (b)	
		MS	P	MS	P
Ostrich carcass	7	0.351	0.3150	0.002	0.2610
Debone	7	0.768	0.1170	0.032	0.0020
Error	1	0.240		0.001	
Shapiro-Wilk			0.3300		0.9950

df – Degree of freedom

MS – Mean Square

P – Probability value of F-ratio test

The analysis of variance (ANOVA) of the dependable variable percentage cooking loss is presented in Table 10. The data for percentage cooking loss was normally distributed ( $P = 0.3805$ ) and transformation of the data was not required. Individual ostrich carcasses had significant ( $P < 0.0001$ ) different percentages of cooking loss, which

can partly be explained by the different pH-values observed for the individual ostrich carcasses ( $P < 0.0001$ , Table 1), since there was a significant correlation ( $r = -0.278$ ;  $P = 0.0039$ ) between pH and cooking loss (%) throughout the 21-day aging period. This indicated that as the pH decreased with increasing aging time, the cooking loss increased. Aaslyng *et al.* (2003) similarly demonstrated that pork with a low pH (below 5.4) had a higher cooking loss (%) than pork with a high pH (above 5.8) and normal pH (between 5.4 and 5.8). These authors concluded that water holding capacity and pH influence cooking loss; however the relationship seemed to be non-linear.

There was no significant interaction between deboning and aging ( $P = 0.8625$ ) as pertaining to percentage cooking loss. Hot-deboning at approximately 1 h *post-mortem* caused no significant ( $P = 0.1234$ ) difference in cooking loss (%) between the hot ( $36.66 \pm 4.29\%$ ) and the cold-deboned ( $35.65 \pm 3.96\%$ ) muscles. On the other hand, aging influenced cooking loss significantly ( $P = 0.0043$ ), causing an increase in cooking loss (%) as aging time increased (Table 11).

**Table 10.** Analysis of variance (ANOVA) of the dependable variable percentage cooking loss with ostrich carcass, deboning (debone) and aging time (d) as main effects, as well as the two way interaction between aging time (d) and deboning, and the Shapiro-Wilk test for non-normality.

Cooking loss (%)			
Source	df	MS	P
Ostrich carcass	7	94.824	< 0.0001
Debone	1	25.022	0.1234
Aging	6	35.602	0.0043
Debone*Aging	6	4.361	0.8625
Error	86	10.335	
Shapiro-Wilk			0.3805

df – Degree of freedom

MS – Mean Square

P – Probability value of F-ratio test

\*Interaction between main effects

**Table 11.** Mean percentage cooking loss ( $\pm$  Standard Deviation) for the pooled hot and cold-deboned *M. gastrocnemius, pars interna* muscle at the individual *post-mortem* aging days (1 d to 21 d).

Aging Time (d)	Cooking loss (%)
1	37.37 $\pm$ 4.68 <sup>ab</sup>
2	35.42 $\pm$ 3.45 <sup>bc</sup>
3	34.77 $\pm$ 4.34 <sup>c</sup>
5	35.62 $\pm$ 4.22 <sup>bc</sup>
7	34.53 $\pm$ 4.76 <sup>c</sup>
14	36.78 $\pm$ 2.46 <sup>abc</sup>
21	38.44 $\pm$ 3.84 <sup>a</sup>

<sup>abc</sup> Different superscripts differ at  $P < 0.05$ .

The analysis of variance (ANOVA) of the dependable colour variable  $L^*$  is presented in Table 12. The non-normality ( $P = 0.0323$ ) for the data of the  $L^*$ -values was due to Kurtosis (-0.806) and therefore the data were analysed further without transformation of the data (Glass *et al.*, 1972). Individual ostrich carcasses differed significantly in  $L^*$ -values ( $P < 0.0001$ ). No significant interaction was observed between deboning and aging ( $P = 0.2774$ ), while both main effects “deboning” and “aging” influenced the lightness ( $L^*$ -values) of the raw meat colour significantly ( $P < 0.05$ ). In general, cold-deboned muscles had significantly ( $P < 0.0042$ ) lower mean  $L^*$ -values ( $30.04 \pm 2.29$ ) over the 21-day aging period (Table 13) compared to the hot-deboned muscles ( $30.71 \pm 1.88$ ). As the aging time increased from day 1 to day 21, the hot-deboned and the cold-deboned muscles became slightly lighter (higher  $L^*$ -values;  $P = 0.0155$ ) in appearance (Table 13) which can be explained by the occurrence of oxidation and denaturation of myoglobin (Lawrie, 1998). Similar results were found by Otremba *et al.* (1999) for the combined mean  $L^*$ -values for intact and ground ostrich meat. These authors found that an initial mean  $L^*$ -value of 29.68 increased to 32.87 during a 28-day storage period at 0°C in vacuum bags.



**Table 12.** Analysis of variance (ANOVA) of the dependable colour variable L\* with ostrich carcass, deboning (debone) and aging time (d) as main effects, as well as the two way interaction between aging time (d) and deboning and the Shapiro-Wilk test for non-normality.

Colour L*			
Source	df	MS	P
Ostrich carcass	7	40.739	< 0.0001
Debone	1	11.755	0.0042
Aging	6	3.813	0.0155
Debone*Aging	6	1.729	0.2774
Error	79	1.354	
Shapiro-Wilk			0.0323

df – Degree of freedom

MS – Mean Square

P – Probability value of F-ratio test

\*Interaction between main effects

**Table 13.** Mean ( $\pm$  Standard Deviation) L\* values (lightness) at the respective *post-mortem* aging days (1 d to 21 d) for the hot-deboned and the cold-deboned *M. gastrocnemius, pars interna* muscles.

Aging Time (d)	Hot-deboned muscles	Cold-deboned muscles
1	29.43 $\pm$ 2.71 <sup>e</sup>	29.59 $\pm$ 1.57 <sup>cde</sup>
2	30.12 $\pm$ 2.11 <sup>bcde</sup>	30.41 $\pm$ 1.62 <sup>abcde</sup>
3	29.91 $\pm$ 2.29 <sup>bcde</sup>	30.56 $\pm$ 2.01 <sup>abcde</sup>
5	29.49 $\pm$ 2.07 <sup>de</sup>	30.70 $\pm$ 1.34 <sup>abcd</sup>
7	29.34 $\pm$ 2.31 <sup>e</sup>	31.47 $\pm$ 1.06 <sup>a</sup>
14	30.81 $\pm$ 2.42 <sup>abc</sup>	30.81 $\pm$ 2.47 <sup>abc</sup>
21	31.06 $\pm$ 2.48 <sup>ab</sup>	31.44 $\pm$ 2.74 <sup>a</sup>

<sup>abcd</sup> Different superscripts differ at  $P < 0.05$ .

The analysis of variance (ANOVA) of the dependable colour variable a\* is presented in Table 14. The data for the a\*-values was normally distributed ( $P = 0.3616$ ) and transformation of the data was not required for further analysis. No significant interaction was observed between the main effects “deboning” and “aging” ( $P = 0.2005$ ).

Cross *et al.* (1979) indicated that beef muscles removed at 1 h *post-mortem* were significantly darker compared to muscles removed at 48 h *post-mortem* after storage for 20 d.

In contrast to the findings for beef muscles (Cross *et al.*, 1979), there was no significant difference ( $P = 0.6918$ ) for the  $a^*$ -values between the hot ( $13.89 \pm 1.98$ ) and the cold-deboned ( $13.7 \pm 1.91$ ) ostrich *M. gastrocnemius, pars interna* (Table 18) in the present study. Aging *post-mortem* also had no significant effect ( $P = 0.8545$ ) on the chromaticity coordinate  $a^*$  of the Lab colour space (CIELab).

**Table 14.** Analysis of variance (ANOVA) of the dependable colour variable  $a^*$  with ostrich carcass, deboning (debone) and aging time (d) as main effects, as well as the two way interaction between aging time (d) and deboning and the Shapiro-Wilk test for non-normality.

Colour $a^*$			
Source	df	MS	<i>P</i>
Ostrich carcass	7	25.990	< 0.0001
Debone	1	0.352	0.6918
Aging	6	0.967	0.8545
Debone*Aging	6	3.259	0.2005
Error	87	2.228	
Shapiro-Wilk			0.3616

df – Degree of freedom

MS – Mean Square

*P* – Probability value of F-ratio test

\*Interaction between main effects

The analysis of variance (ANOVA) of the dependable colour variable  $b^*$  is presented in Table 15. The data for the  $b^*$ -values were normally distributed ( $P = 0.2364$ ) and transformation of the data was not required for further analysis. The  $b^*$ -values for individual ostrich carcasses differed significantly ( $P < 0.0001$ ) between muscle samples. Similar to the colour variable  $a^*$ , there was no significant interaction between deboning and aging for the  $b^*$ -values ( $P = 0.1664$ ). The time of deboning (at approximately 1 h *post-mortem* or at 24 h *post-mortem*) had no significant effect ( $P = 0.2364$ ) on the  $b^*$ -values for the hot-deboned ( $8.62 \pm 2.13$ ) and the cold-deboned muscles ( $8.22 \pm 1.79$ ) (Table 18). Aging *post-mortem* also had no significant effect ( $P > 0.05$ ) on the  $b^*$ -values.

**Table 15.** Analysis of variance (ANOVA) of the dependable colour variable  $b^*$  with ostrich carcass, deboning (debone) and aging time (d) as main effects, as well as the two way interaction between aging time (d) and deboning and the Shapiro-Wilk test for non-normality.

Colour $b^*$			
Source	df	MS	P
Ostrich carcass	7	15.693	< 0.0001
Debone	1	4.321	0.2364
Aging	6	1.274	0.8638
Debone*Aging	6	4.753	0.1664
Error	87	3.033	
Shapiro-Wilk			0.2364

df – Degree of freedom

MS – Mean Square

P – Probability value of F-ratio test

\*Interaction between main effects

The analysis of variance (ANOVA) of the dependable colour variable Hue angle ( $^{\circ}$ ) is presented in Table 16. The Hue angle data was normally distributed ( $P = 0.4447$ ) and transformation of the data was not required for further analysis. In contrast to the colour variables:  $L^*$ ,  $a^*$  and  $b^*$ , the Hue angle values did not differ significantly between individual ostrich carcasses ( $P = 0.4046$ ). Similar to the chromaticity coordinates,  $a^*$  and  $b^*$ , the Hue angle values (Table 18) also did not differ between the hot-deboned and the cold-deboned muscles ( $P = 0.4593$ ). Aging *post-mortem* also had no significant effect ( $P = 0.7699$ ) on the Hue angle values of the muscle samples.

**Table 16.** Analysis of variance (ANOVA) of the dependable variable Hue angle ( $h_{ab}$ ) ( $^{\circ}$ ) with ostrich carcass, deboning (debone) and aging time (d) as main effects, the two way interaction between aging time (d) and deboning, as well as the Shapiro-Wilk test for non-normality.

Hue angle ( $h_{ab}$ ) ( $^{\circ}$ )			
Source	df	MS	<i>P</i>
Ostrich carcass	7	30.225	0.4046
Debone	1	15.947	0.4593
Aging	6	15.830	0.7699
Debone*Aging	6	35.064	0.3064
Error	87	28.861	
Shapiro-Wilk			0.4447

df – Degree of freedom

MS – Mean Square

*P* – Probability value of F-ratio test

\*Interaction between main effects

The analysis of variance (ANOVA) of the dependable colour variable Chroma ( $C^*$ ) is presented in Table 17. The Chroma data was normally distributed ( $P = 0.3776$ ) and transformation of the data was not required for further analysis. Chroma values (Table 18) differed significantly between individual ostrich carcasses ( $P < 0.0001$ ), whilst neither deboning nor *post-mortem* aging had any significant effect ( $P > 0.05$ ) on the Chroma values and therefore, the hot and cold-deboned muscles did not differ at the respective days of aging (Table 18).

**Table 17.** Analysis of variance (ANOVA) of the dependable variable Chroma (C\*) with ostrich carcass, deboning (debone) and aging time (d) as main effects, the two way interaction between aging time (d) and deboning, as well as the Shapiro-Wilk test for non-normality.

Source	Chroma (C*)		
	df	MS	P
Ostrich carcass	7	39.225	< 0.0001
Debone	1	1.216	0.5283
Aging	6	1.078	0.9049
Debone*Aging	6	4.250	0.2234
Error	87	3.034	
Shapiro-Wilk			0.3776

df – Degree of freedom  
 MS – Mean Square  
 P – Probability value of F-ratio test  
 \*Interaction between main effects

**Table 18.** Mean ( $\pm$  Standard Deviation) a\*, b\*, Hue angle ( $h_{ab}$ ) ( $^{\circ}$ ) and Chroma (C\*) values for the hot and the cold-deboned muscles pooled over the 21 d *post-mortem* storage period.

Deboning	Mean $\pm$ Standard Deviation			
	a*	b*	Hue angle ( $h_{ab}$ ) ( $^{\circ}$ )	Chroma (C*)
Hot-deboned	13.89 $\pm$ 1.98	8.62 $\pm$ 2.13	31.66 $\pm$ 5.03	16.37 $\pm$ 2.53
Cold-deboned	13.78 $\pm$ 1.91	8.22 $\pm$ 1.79	30.89 $\pm$ 5.64	16.15 $\pm$ 2.09
Probability value (P) of F-ratio test <sup>1</sup>	0.6918	0.2364	0.4593	0.5283

<sup>1</sup>Probability value (P) of F-ratio test as calculated for the analysis of variance (ANOVA).

When the a\* and b\*-values were correlated with the L\*-values, it was found that, although a\* and b\* did not change as aging time (d) increased, both a\*-values ( $r = 0.203$ ;  $P = 0.0427$ ) and b\*-values ( $r = 0.300$ ;  $P = 0.0024$ ) increased as lightness (L\*) increased ( $P < 0.05$ ) over the 21-day storage period.

Overall muscle pH (pooled over the storage period of 21 d *post-mortem*) was poorly, but significantly ( $P < 0.05$ ) negatively correlated to  $L^*$ -values ( $r = -0.383$ ;  $P < 0.0001$ ),  $a^*$ -values ( $r = -0.247$ ;  $P = 0.0105$ ),  $b^*$ -values ( $r = -0.235$ ;  $P = 0.0149$ ), and Chroma ( $C^*$ ) values ( $r = -0.285$ ;  $P = 0.0029$ ). As muscle pH decreased, muscles became lighter (higher  $L^*$  values) and had a redder (higher  $a^*$ -values) as well as a more yellow (higher  $b^*$  values) colour. Raw meat with a low pH is generally lighter (or paler) with higher measured  $L^*$ -values than meat with a high pH (Offer, 1991; Swatland, 2004). The relationship between lightness and pH of raw meat is caused by light scattering, where there is a minimum penetration and a maximum reflectance of light from raw meat with a low pH.

## CONCLUSIONS

The shorter sarcomeres for the hot-deboned muscles ( $2.12 \pm 0.14 \mu\text{m}$ ) at 24 h *post-mortem* compared to that found for the cold-deboned muscles ( $2.52 \pm 0.14 \mu\text{m}$ ), could explain the greater toughness found for hot-deboned muscles during the first 5 d of *post-mortem* storage. Hot-deboning also caused the muscles to have a higher percentage purge (lower water holding capacity) during the *post-mortem* storage period. It was concluded by Tornberg (1996) that for beef, a more shortened muscle shows a higher cooking loss (lower water holding capacity) and higher numbers of fibres per unit cross-area, leading to higher Warner-Bratzler peak shear force values. However, the shorter sarcomeres and tougher meat found for hot-deboned ostrich *M. gastrocnemius, pars interna* does not seem to pose a great problem in terms of eating quality, since the difference in tenderness between hot and cold-deboned muscles disappeared with further aging beyond 5 d *post-mortem*. In the ostrich meat industry and with the export of chilled vacuum packaged ostrich meat, consumers seldom consume ostrich meat aged for less than 7 d.

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## Chapter 5

### The effect of hot-deboning on the physical meat quality characteristics of ostrich (*Struthio camelus var. domesticus*) *Muscularis gastrocnemius, pars interna* and *Muscularis iliofibularis* during *post-mortem* storage

#### ABSTRACT

Hot-deboning does not influence the tenderness of different anatomically located muscles to the same extent. The effects of hot-deboning on the physical meat quality characteristics, as well as on the shelf-life of ostrich *M. gastrocnemius, pars interna* and the *M. iliofibularis*, were investigated during an aging period of 42 d. The hot-deboned *M. gastrocnemius, pars interna* were initially tougher ( $P < 0.05$ ) than the cold-deboned muscles, while hot-deboning had no significant effect on ( $P > 0.05$ ) the shear force values of the *M. iliofibularis*. At 14 d *post-mortem* there was no significant ( $P > 0.05$ ) difference in tenderness between the hot-deboned *M. gastrocnemius, pars interna* ( $68.01 \pm 12.89$  N.  $12.7 \text{ mm}^{-1}$  diameter) and the hot-deboned *M. iliofibularis* ( $62.29 \pm 11.29$  N.  $12.7 \text{ mm}^{-1}$  diameter), nor between the cold-deboned *M. gastrocnemius, pars interna* ( $59.54 \pm 7.37$  N.  $12.7 \text{ mm}^{-1}$  diameter) and the cold-deboned *M. iliofibularis* ( $58.07 \pm 9.78$  N.  $12.7 \text{ mm}^{-1}$  diameter). Muscle pH was significantly ( $P = 0.0062$ ) higher for cold-deboned muscles ( $5.93 \pm 0.12$ ) than for hot-deboned muscles ( $5.91 \pm 0.11$ ) throughout the 42-day aging period. Hot-deboning had a significant effect ( $P < 0.0001$ ) on the water holding capacity of the muscles, causing hot-deboned muscles to have more purge ( $3.36 \pm 2.33\%$ ) during aging of 42 d compared to the cold-deboned muscles ( $2.14 \pm 2.06\%$ ). The cold-deboned muscles had significantly ( $P < 0.05$ ) higher L\*-values ( $31.67 \pm 1.76$ ) and higher a\*-values ( $13.52 \pm 1.42$ ) than the hot-deboned muscles during the 42-day aging period. All muscle samples were within the South African Standards for the microbiological monitoring of meat for refrigerated ( $< 10^4 \text{ cfu.g}^{-1}$ ) export as determined by APC. The counts for *E. coli* from all muscles were also below the South African Standards for the microbiological monitoring of refrigerated meat ( $< 10^1 \text{ cfu.g}^{-1}$ ) and it was concluded that hot-deboning had no negative effect on the shelf-life of ostrich muscles. Results from this study indicated that there was a greater risk of toughening in the *M. gastrocnemius, pars interna* when hot-deboned, than in the *M. iliofibularis*, but with refrigerated aging the initial difference in

toughness between the hot and the cold-deboned *M. gastrocnemius, pars interna* was negated.

**Keywords:** hot-deboning, tenderness, pH, microbial contamination, purge, cooking loss, colour, shelf-life

## INTRODUCTION

Within the ostrich carcass, various muscles will have different rates of *post-mortem* temperature decline and subsequent pH profiles according to the anatomical location of the muscles to the exterior, as well as due to their degree of insulation (Lawrie, 1998). In addition, when considering hot-deboning, the degree of cold-shortening will also differ in the various muscles due to different fibre type compositions. White, fast-twitch fibres (Type II) are less susceptible to cold-shortening than red, slow-twitch (Type I) fibres as these fibres have a less well developed sarcotubular system, and thus a reduced ability to sequester and bind  $\text{Ca}^{2+}$  at low temperatures (Pearson & Young, 1989; Lawrie, 1998).

The largest muscle in the ostrich carcass is the *M. gastrocnemius* (*M. gastrocnemius, pars interna* and *M. gastrocnemius, pars externa* attached) followed by the *M. iliofibularis* (Marks *et al.*, 1998). However, the ostrich *M. iliofibularis* has the largest individual mass (Sales, 1996) and is located deeper within the ostrich leg than the *M. gastrocnemius*. *Post-mortem* glycolysis and the rate of pH fall is faster in intact muscles with a larger mass and which are located deeper within the carcass. This is ascribed to the higher initial *in vivo* temperatures when compared to muscles located at the periphery of the carcass (Lawrie, 1998). The rate of tenderisation during early *post-mortem* would also be enhanced in muscles which are at higher temperatures since the extent of proteolysis is temperature dependent, and is greater at for example 37°C than at 5°C. The higher temperatures at deeper locations within the carcass could lead to a faster rate of microbial spoilage and may also lead to a greater water holding capacity loss of the proteins. O'Halloran *et al.* (1997a) and Hwang & Thompson (2001) both reported that fast glycolysing muscles were more tender than slow glycolysing muscles. O'Halloran *et al.* (1997a) also suggested that the increased tenderness of fast glycolysing muscles might be caused by early *post-mortem* proteolysis. The rate of *post-mortem* pH fall also influences the water holding capacity of muscles (Lawrie, 1998). The water holding capacity of muscles is at a minimum in the pH range of 5.4 to 5.5 (i.e. the isoelectric point). On subsequent conditioning of the meat, the pH then tends to increase.

Meat with a high pH *post-mortem* favours the rapid growth of spoilage bacteria during aging and thus leading to a shortening of the meat's shelf-life. Ostrich meat has been classified as an intermediate meat type between normal (pH < 5.8) and extreme DFD (dark, firm and dry) meat (pH > 6.2) (Mellett & Sales, 1996). Ostrich meat has a higher pH compared to beef, mutton or pork (Mellett & Sales, 1996; Sales, 1996). It was suggested by Pollok *et al.* (1997) that vacuum-packed ostrich steaks should not be stored for more than 14 d, as the steaks would become microbiologically unacceptable after 21 d of refrigeration at 2°C. In addition to the contribution of a high pH to the dark colour of ostrich meat, the intense red colour of ostrich meat can also be ascribed to the high concentration of haem pigment (22-30 µg Fe.g<sup>-1</sup>) (Heinze *et al.*, 1986; Sales, 1996) when compared to that of beef muscle (16-20 µg Fe.g<sup>-1</sup>) (Berge *et al.*, 1993). This is impartial as meat colour is used as a cue by the consumer for freshness (Issanchou, 1996).

Hot-deboning caused *M. gastrocnemius, pars interna* to have shorter sarcomeres and to be tougher than the cold-deboned muscles for 5 d *post-mortem* (Chapter 4 of this thesis). The aims of this study were firstly to investigate whether hot-deboning would also cause toughening of the *M. iliofibularis*. Secondly to investigate the effects of hot-deboning on the physical quality characteristics of *M. gastrocnemius, pars interna* and *M. iliofibularis* in terms of pH changes, purge (%), cooking loss (%) and raw meat colour during an extended refrigerated (-3° to 0°C) storage of 42 d *post-mortem*, a typical scenario within the ostrich meat industry. Thirdly, due to the high pH reported for ostrich muscles (Mellett & Sales, 1996; Sales, 1996), microbiological evaluations were done to determine whether hot-deboning would have detrimental effects on the shelf-life and consumer safety of ostrich meat. The main aims of this study were therefore to investigate the effects of hot-deboning on the physical quality characteristics, as well as on the shelf-life of two economically important ostrich muscles, the *M. gastrocnemius, pars interna* (commercially referred to as the Big Drum) and the *M. iliofibularis* (commercially referred to as the Fan Fillet).

## MATERIALS AND METHODS

### Ostriches and muscle samples

Twelve randomly selected, well rested (lairage of 12 h) ostriches (*Struthio camelus var. domesticus*) of 10 to 14 months were slaughtered (nett carcass weight; 42.72 ± 3.95 kg), as described by Wotton & Sparrey (2002), over 5 days during December 2003 at an EU Abattoir in Malmesbury, South Africa. The left leg *M. gastrocnemius, pars interna* (*M. gastro*) and left leg *M. iliofibularis* (*M. ilio*) were excised at approximately 2 h *post-mortem*

to obtain hot-deboned muscles, weighing respectively  $1.14 \pm 0.12$  kg and  $1.62 \pm 0.24$  kg. Muscles were cut into ten 1.5 to 2.0 cm thick slices, weighed and individually vacuum-packed in vacuum bags (standard thickness: 80 micron  $\pm 10\%$  variation, weight:  $80 \text{ g} \cdot \text{m}^{-2} \pm 10\%$  variation, oxygen transmission:  $38 \text{ cm}^3 \cdot \text{m}^{-2} \cdot 24\text{h}^{-1} \pm 20\%$  variation, water vapour transmission:  $2.8 \text{ g} \cdot \text{m}^{-2} \cdot 24\text{h}^{-1} \pm 20\%$  variation and carbon dioxide transmission of  $195 \text{ cm}^3 \cdot \text{m}^{-2} \cdot 24\text{h}^{-1} \pm 20\%$  variation). The vacuum packed meat samples were then randomly assigned to aging periods of 1 (approximately 24 h *post-mortem*), 2, 3, 5, 7, 14, 21, 28, 35 and 42 d *post-mortem* and aged at  $-3^\circ$  to  $0^\circ\text{C}$ .

The right leg *M. gastrocnemius, pars interna* and *M. iliofibularis* were left intact on the same carcasses from which the hot-deboned muscles were excised. The intact muscles were refrigerated for 24 h at  $< 4^\circ\text{C}$  before being excised to obtain cold-deboned muscles. Their mass was  $1.08 \pm 0.15$  kg and  $1.70 \pm 0.14$  kg for the *M. gastrocnemius, pars interna* and *M. iliofibularis*, respectively. The cold-deboned muscles were also cut into ten 1.5 to 2.0 cm thick slices, individually vacuum-packed, weighed, randomly assigned to aging periods of 1 (approximately 24 h *post-mortem*), 2, 3, 5, 7, 14, 21, 28, 35 and 42 d *post-mortem* and aged at  $-3^\circ$  to  $0^\circ\text{C}$ , adjacent to the hot-deboned muscles in the refrigerator of the abattoir. The HACCP records at the abattoir showed that the refrigerator temperature fluctuated between  $-3^\circ$  and  $0^\circ\text{C}$ , but never increased above  $0^\circ\text{C}$ .

### Physical characteristics

Muscle temperature ( $^\circ\text{C}$ ), pH, purge (%), raw meat colour (CIE lightness  $L^*$ ,  $a^*$  and  $b^*$  colour coordinates), cooking loss (%) (after cooking for an hour at  $80^\circ\text{C}$  in a water bath) and Warner-Bratzler shear force (N.  $12.7 \text{ mm}^{-1}$  diameter) measurements were recorded at the respective aging intervals of 1 (approximately 24 h *post-mortem*), 2, 3, 5, 7, 14, 21, 28, 35 and 42 d *post-mortem*, after the muscle samples were transported in a cooler box from the abattoir to the facilities of the University of Stellenbosch.

Muscle temperature ( $^\circ\text{C}$ ) and pH were measured at room temperature ( $18^\circ$  to  $19^\circ\text{C}$ ) using a calibrated (standard buffers of pH 4.0 and 7.0) portable Crison 506 pH-meter, equipped with pH and temperature probes. At their time of pH measurement, all the muscle samples were at an average temperature of  $15.09^\circ \pm 3.56^\circ\text{C}$  (between  $10^\circ$  and  $18^\circ\text{C}$ ) as the muscle samples had been transported for approximately 1 h from the abattoir to the facilities of the University of Stellenbosch (60 km) in a cooler box. All the muscle samples were also maintained at room temperature ( $18^\circ$  to  $19^\circ\text{C}$ ) until all physical measurements were obtained, therefore, resulting in an increase in sample temperatures. It was argued that, since all the muscle samples were treated similarly at all times, this increase in muscle sample temperature did not influence the main effects.

To determine the purge (%), muscle samples were weighed ( $82.08 \pm 17.32$  g and  $116.04 \pm 25.23$  g for respectively the *M. gastrocnemius, pars interna* and the *M. iliofibularis*) after they had been removed from the vacuum package and blotted dry with tissue paper. Purge was then expressed as a percentage of the initial mass of the muscle sample.

The colour of the raw muscle slices (1.5-2.0 cm thick) was recorded according to the method described by Honikel (1998) with the use of a Colour-guide 45°/0° colorimeter (Cat no: 6805; BYK-Gardner, USA). Muscle slices were allowed to “bloom” for 30 min at room temperature (18° to 19°C) prior to colour measurements. Colour measurements were recorded in triplicate for each sample at randomly selected positions and expressed by the coordinates  $L^*$ ,  $a^*$  and  $b^*$  of the CIELab colorimetric space (MINOLTA, 1998). In the colour space,  $L^*$  indicated lightness and  $a^*$  and  $b^*$  are the chromaticity coordinates, where  $a^*$  is the red-green range, and  $b^*$  the yellow-blue range of the colour sphere. The Hue angle ( $h_{ab}$ ), (°) and Chroma ( $C^*$ ) were also calculated as follows, using the  $L^*$ ,  $a^*$  and  $b^*$  values:

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

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

The Hue angle ( $h_{ab}$ ) is defined as starting at the positive side of the  $a^*$  axis of the chromaticity diagram and is expressed in degrees (°), meaning that 0° would indicate red and 90° would indicate yellow. Chroma ( $C^*$ ) is a measure of the difference from a grey of the same lightness (Mackinney & Little, 1962). Chroma ( $C^*$ ) has a value of 0 at the centre of the chromaticity diagram (central grey) and extending outwards according to the distance from the centre, indicating the colour increases in brightness (MINOLTA, 1998).

Cooking loss (%) of the muscles slices were determined by placing the weighed raw meat samples; individually sealed in plastic bags, in a water bath (preheated to 80°C) for 1 h (Honikel, 1998). After an hour, the cooked meat was removed, allowed to cool down under running water and the mass was recorded after excess water was blotted with tissue paper. Cooking loss was expressed as a percentage of the initial mass ( $80.22 \pm 16.90$  g and  $111.60 \pm 24.56$  g for respectively the *M. gastrocnemius, pars interna* and the *M. iliofibularis*) of the muscle sample (Honikel, 1998).

Since Warner-Bratzler shear force values (N. 12.7 mm<sup>-1</sup> diameter) had been significantly correlated to taste panel sensory tenderness scores (Chapter 3 of this thesis), shear force values were accepted as an indicator of meat tenderness in this study. The same muscle samples that were used to determine the cooking loss were stored over-

night at 4°C before tenderness was determined the following day. Assessment of tenderness was done as described by Wheeler *et al.* (2001) and Honikel (1998), by using a Warner-Bratzler device, with a load cell of 2.000 kN, attached to the Model 4444 Instron texture machine (Apollo Scientific cc, South Africa). Seven 12.7 mm wide cores were removed parallel to the muscle fibre from the cooked muscle slice of 1.5 to 2.0 cm in thickness and placed in the Warner-Bratzler device, so that the knife blade of the device cut across the fibres at right angle. The maximum shear force value (N. 12.7 mm<sup>-1</sup> diameter) to shear a cylindrical core of cooked meat was recorded at a crosshead speed of 200 mm.min<sup>-1</sup>. Mean maximum shear force values were calculated from the shear force values recorded for seven cylindrical cores from each meat sample and used in the statistical analyses.

### Microbiological tests

Meat samples were cut from both the hot-deboned (right after excision) and the cold-deboned *M. gastrocnemius, pars interna* and *M. iliofibularis* at respectively 24 h *post-mortem* and at day 42 of the aging period to determine the Aerobic Plate Counts (APC), *Escherichia coli* (*E. coli*), EBC (Enterobacteriaceae) and *Pseudomonas*. Enterobacteriaceae and *Escherichia coli* were determined as these organisms are commonly used to assess the level of faecal contamination of foods.

The microbiological analyses were done by Quantum Analytical Service (Pty) Ltd., (12 Voortrekker Road, Malmesbury 7300, South Africa). For sample preparation, the meat samples were placed in individual filter bags, where each sample was made up to volume with 85% NaCl solution to produce a solution of 10<sup>1</sup>. The filter bags were then placed in a bag-mixer blender so as to crush and mix the meat samples to obtain meat suspensions from which further dilutions for microbiological testing were conducted. Dilutions of 10<sup>-1</sup> were prepared for testing *Pseudomonas* (0.1 ml/petri-dish) on pseudomonas selective agar, incubated for 48 h at 35°C. Dilutions of 10<sup>-2</sup> were prepared to test for Aerobic Plate Count (APC), using the petrifilm method #990.12 as listed in the AOAC Official Methods of Analysis (2002). The petrifilms were incubated for 48 h at 35°C before APC counts were obtained. For testing *E. coli* and EBC (Enterobacteriaceae), dilutions of 10<sup>-1</sup> were prepared and the respective petri-dish methods #996.02 and #998.08, as listed in the AOAC Official Methods of Analysis (2002), were used to obtain *E. coli* and EBC (Enterobacteriaceae) counts after incubation for 24 h at 35°C.

### Statistical analyses

A 2 x 10 factorial experiment was performed in a randomised complete block design with eight blocks (ostrich carcasses). The factors were the two deboning treatments (hot and cold) and *post-mortem* aging time [1 (approximately 24 h *post-mortem*), 2, 3, 5, 7, 14, 21, 28, 35 and 42 d]. The data were subjected to factorial analysis of variance (ANOVA) using SAS version 8.2 statistical software (SAS, 1999). Shapiro-Wilk tests were performed for testing non-normality (Shapiro & Wilk, 1965). The percentage variation for the dependable variables were calculated from the sum of squares (SS) and total sum of squares as obtained from the factorial analysis of variance (ANOVA), using the following

$$\text{equation: \% Variation} = \frac{SS}{\sum SS} \times 100\%$$

Results from the factorial analysis of variance (ANOVA), the Shapiro-Wilk tests, and the percentage variation for dependable variables: muscle pH, Warner-Bratzler shear force, percentage purge, percentage cooking loss, colour coordinates L\*, a\*, b\*, Hue angle ( $h_{ab}$ ) and Chroma (C\*); with ostrich, deboning and aging time (days) as main effects, as well as the three way interaction between muscle, deboning and aging, and the respective two way interactions between muscle and deboning, muscle and aging, and deboning and aging, are summarised in Tables 1, 2, 3, 4, 5, 7, 8, 10, 11 and 12.

When deviations from normality were detected, outliers were removed until data were symmetrical or normal distributed (Glass *et al.*, 1972). The removal of outliers from the data caused the degree of freedom (df) for error in the analysis of variance (ANOVA) to differ for the respective dependable variables: muscle pH, Warner-Bratzler shear force, percentage purge, percentage cooking loss, colour coordinates L\*, a\*, b\*, Hue angle ( $h_{ab}$ ) and Chroma (C\*). Student's t-Least Significant Difference was calculated at the 5% confidence level to compare means for significant effects (Ott, 1998).

Various trend lines were fitted to the pH values using Microsoft Excel (2004). The third order polynomial trend lines gave the best fit according to the calculated R<sup>2</sup>-values. The function represented by the trend lines is depicted by the following equation:

$$y = ax^3 + bx^2 + cx + d$$

Where: y = pH values at time x; x = *post-mortem* aging time in days, and d = intercept.

Linear regression models were fitted to the purge (%) values using SAS version 8.2 (SAS, 1999). The function of this model was:

$$y = ax \pm b$$



where:  $y$  = values at time  $x$ ;  $a$  = intercept;  $x$  = *post-mortem* aging time in days;  $b$  = slope. The regression coefficients, intercept ( $a$ ) and slope ( $b$ ) of the fitted model, were then analysed by analysis of variance (ANOVA) using SAS version 8.2 (SAS, 1999) as summarised in Table 4.

Pearson correlation coefficients [ $r$ -values at the 5% significance level ( $P$ )] were calculated with the use of statistical software Statistica version 6 (StatSoft 2003) as well as using the linear regression procedure (Proc CORR) of SAS version 8.2 (SAS, 1999). In the cases where there were no significant differences in variables between hot and cold-deboned muscles, correlation coefficients were calculated for the pooled values. Correlation coefficients were calculated from the raw data points for hot and cold-deboned muscles respectively between the different variables (pH, Warner-Bratzler shear force, purge, cooking loss and raw meat colour) as well as with storage time.

## RESULTS AND DISCUSSION

The analysis of variance (ANOVA) of the dependable variable Warner-Bratzler shear force values (N. 12.7 mm<sup>-1</sup> diameter) is presented in Table 1. The Shapiro-Wilk test indicated that the Warner-Bratzler shear force data was not normally distributed ( $P < 0.001$ ). However, this non-normality was due to Kurtosis (-0.116), which, according to Glass *et al.* (1972), does not have a significant effect on further analyses of the data.

The probability values for the F-ratio test ( $P$ -values) indicated that there was a significant three way interaction ( $P = 0.0067$ ) between muscle, deboning and aging, as well as a significant two way interaction between muscle and deboning ( $P = 0.0116$ ). The percentage of variance (Table 1) explained by these interactions was less (< 3%) than the percentage of variance can be explained by the two way interaction between muscle and aging (7.56%). The percentage of variance explained by the main effects "ostrich carcass" (8.78%), "muscle" (11.47%) and "aging" (19.50%) was also higher than the percentage of variance explained by the three way interaction (2.76%) and the two way interaction between muscle and deboning (0.76%). Therefore, the two way interaction between muscle and aging, and the main effects "ostrich carcass", "muscle", "deboning" and "aging" will be discussed further.

**Table 1.** Analysis of variance (ANOVA) of the dependable variable mean Warner-Bratzler shear force (N. 12.7 mm<sup>-1</sup> diameter) with ostrich carcass, muscle, deboning (debone) and aging time (d) as main effects, as well as the two and three way interactions between main effects, and the Shapiro-Wilk test for non-normality.

Warner-Bratzler shear force (N. 12.7 mm <sup>-1</sup> diameter)				
Source	df	MS	P	% Var
Ostrich carcass	10	826.063	< 0.0001	8.78
Muscle	1	10790.459	< 0.0001	11.47
Debone	1	2426.409	< 0.0001	2.58
Aging	9	2038.470	< 0.0001	19.50
Muscle*Debone	1	717.943	0.0116	0.76
Muscle*Aging	9	790.028	< 0.0001	7.56
Debone*Aging	9	206.143	0.0587	1.97
M*D*A	9	288.651	0.0067	2.76
Error	376	111.615		44.61
Shapiro-Wilk			0.0018	

df – Degree of freedom

MS – Mean Square

P – Probability value of F-ratio test

% Var – Percentage variation explained by that particular effect

\*Interaction between main effects

M\*D\*A – Interaction between main effects Muscle, Debone and Aging

The significant two way interaction between muscle and aging ( $P < 0.0001$ ) indicated that as the time of aging increased from day 1 to day 42, the *M. gastrocnemius, pars interna* and the *M. iliofibularis* differed in the increase in tenderness as illustrated in Fig. 1. Throughout the 42-day aging period, both the hot and the cold-deboned *M. gastrocnemius, pars interna* were tougher than the hot and the cold-deboned *M. iliofibularis*. Initial toughness (approximately 24 h *post-mortem*) was significantly ( $P < 0.05$ ) higher for the *M. gastrocnemius, pars interna* ( $76.97 \pm 13.82$  N. 12.7 mm<sup>-1</sup> diameter) than for the *M. iliofibularis* ( $68.56 \pm 11.10$  N. 12.7 mm<sup>-1</sup> diameter). This greater toughness for the *M. gastrocnemius, pars interna* persisted up to 14 d *post-mortem*. In contrast to the findings of Sales (1994), similar results were found by Girolami *et al.* (2003) and Marks *et al.* (1998) where both Warner-Bratzler shear force values and taste panel scores indicated that the *M. gastrocnemius, pars interna* was tougher than the *M. iliofibularis*. Berge *et al.* (1997) also found that the *M. iliofibularis* was more tender than the *M. gastrocnemius lateralis* and *M. gastrocnemius medialis* in the Emu. The difference in tenderness between *M. iliofibularis* and *M. gastrocnemius, pars interna* can partly be

explained by the higher collagen content of *M. gastrocnemius, pars interna* ( $6.11 \pm 1.52 \text{ g.kg}^{-1}$ ) compared to *M. iliofibularis* ( $2.99 \pm 0.84 \text{ g.kg}^{-1}$ ) (Sales, 1996). Similarly, emu *M. gastrocnemius lateralis* ( $6.6 \text{ g.kg}^{-1}$ ) contain a higher total intramuscular collagen content compared to the *M. iliofibularis* ( $4.0 \text{ g.kg}^{-1}$ ) (Berge *et al.*, 1997). In addition, it has been indicated that marbling (the total fatty acid content of meat), affects eating quality, especially in terms of tenderness and juiciness (Wood *et al.*, 2003). Although the *M. iliofibularis* has a higher composition of intramuscular fat ( $0.92 \pm 0.227 \text{ g.100g}^{-1}$ ) than the *M. gastrocnemius, pars interna* ( $0.43 \pm 0.126 \text{ g.100g}^{-1}$ ) (Sales & Hayes, 1996), the small difference in intramuscular fat between these two muscles would probably not explain the difference in tenderness between the *M. gastrocnemius, pars interna* and the *M. iliofibularis*.

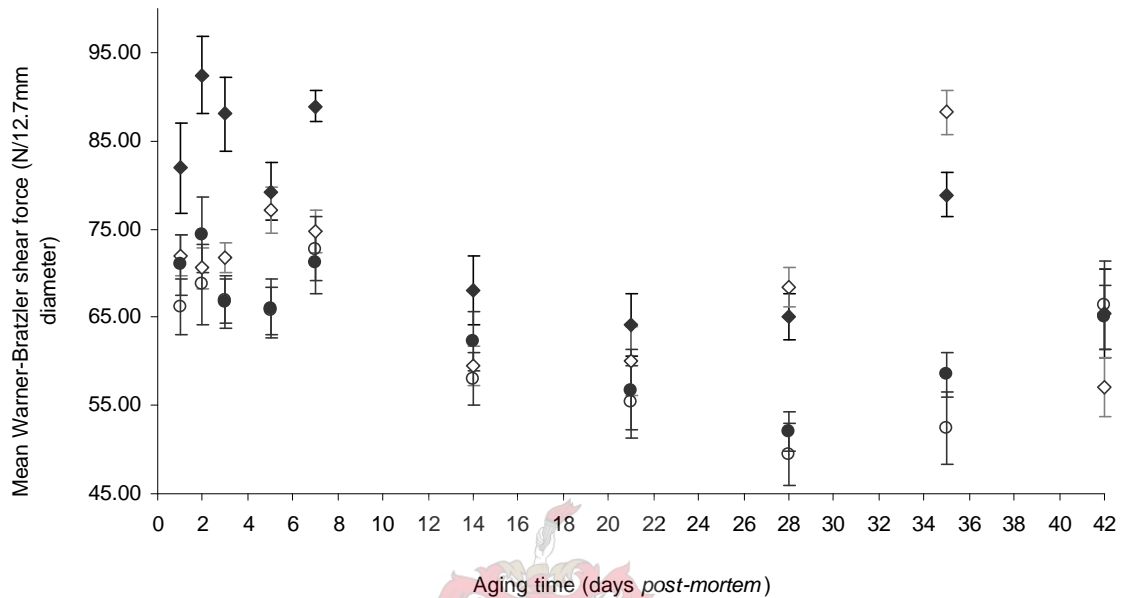
On day 14 of the 42-day aging period (Fig. 1), there was no significant ( $P > 0.05$ ) difference in tenderness between the hot-deboned *M. gastrocnemius, pars interna* ( $68.01 \pm 12.89 \text{ N. } 12.7 \text{ mm}^{-1}$  diameter) and the hot-deboned *M. iliofibularis* ( $62.29 \pm 11.29 \text{ N. } 12.7 \text{ mm}^{-1}$  diameter), nor between the cold-deboned *M. gastrocnemius, pars interna* ( $59.54 \pm 7.37 \text{ N. } 12.7 \text{ mm}^{-1}$  diameter) and the cold-deboned *M. iliofibularis* ( $58.07 \pm 9.78 \text{ N. } 12.7 \text{ mm}^{-1}$  diameter). Sales *et al.* (1996) found that the tenderness of *M. iliofibularis* increased significantly from 3.5 d to 10.5 d of aging *post-mortem*, while that of the *M. gastrocnemius, pars interna* did not increase any further in tenderness with an additional 7 d after the initial 3.5 d of aging. In this study, it was found that both the hot and the cold-deboned *M. gastrocnemius, pars interna* and *M. iliofibularis* increased in tenderness from day 3 to day 14, where after there was no further increase ( $P > 0.05$ ) in tenderness up to day 42. On day 42, the hot-deboned *M. gastrocnemius, pars interna* ( $65.49 \pm 16.54 \text{ N. } 12.7 \text{ mm}^{-1}$  diameter), the cold-deboned *M. gastrocnemius, pars interna* ( $57.10 \pm 11.00 \text{ N. } 12.7 \text{ mm}^{-1}$  diameter), the hot-deboned *M. iliofibularis* ( $65.02 \pm 12.10 \text{ N. } 12.7 \text{ mm}^{-1}$  diameter), and the cold-deboned *M. iliofibularis* ( $66.37 \pm 16.88 \text{ N. } 12.7 \text{ mm}^{-1}$  diameter) were all similar in tenderness ( $P > 0.05$ ), indicating that aging up to 42 d *post-mortem* will offset the initial higher toughness of the *M. gastrocnemius, pars interna*.

As expected, the Warner-Bratzler shear force values (N.  $12.7 \text{ mm}^{-1}$  diameter) differed significantly between individual ostrich carcasses ( $P < 0.0001$ ). Although hot-deboning had a significant effect ( $P < 0.0001$ ) on the Warner-Bratzler shear force values, the percentage of variance explained by deboning was less (2.58%) than the percentage of variance explained by the main effect "muscle" (11.47%), indicating therefore that the hot-deboned ( $76.95 \pm 15.86 \text{ N. } 12.7 \text{ mm}^{-1}$  diameter) and the cold-deboned ( $69.80 \pm 12.33 \text{ N. } 12.7 \text{ mm}^{-1}$  diameter) *M. gastrocnemius, pars interna* were tougher than respectively the hot-deboned ( $64.38 \pm 12.88 \text{ N. } 12.7 \text{ mm}^{-1}$  diameter) and the cold-deboned ( $62.11 \pm 13.93 \text{ N. } 12.7 \text{ mm}^{-1}$  diameter) *M. iliofibularis* throughout the 42-day aging period. None the less,

it was observed that, within muscles, hot-deboning caused larger differences in tenderness between the hot and the cold-deboned *M. gastrocnemius, pars interna* than for the *M. iliofibularis*. After 24 h *post-mortem* (day 1), the hot-deboned *M. gastrocnemius, pars interna* ( $81.91 \pm 17.04$  N.  $12.7 \text{ mm}^{-1}$  diameter) were significantly tougher ( $P < 0.05$ ) than the cold-deboned *M. gastrocnemius, pars interna* ( $72.03 \pm 7.55$  N.  $12.7 \text{ mm}^{-1}$  diameter), while there was no significant difference in the initial (day 1) tenderness between the hot-deboned ( $70.96 \pm 11.51$  N.  $12.7 \text{ mm}^{-1}$  diameter) and the cold-deboned *M. iliofibularis* ( $66.17 \pm 10.65$  N.  $12.7 \text{ mm}^{-1}$  diameter). The greater toughness of the hot-deboned muscles compared to the cold-deboned muscles could in part be explained by some degree of shortening and/or super contraction when muscles were excised at 1 h *post-mortem* (Chapter 4 of this thesis) and the temperature of the muscles fell below  $10^{\circ}\text{C}$  while muscle pH and ATP concentrations were still sufficient for muscle contraction. In addition, the degree of shortening also depends on fibre type composition, since white, fast-twitch fibres (Type II) are less susceptible to cold-shortening than red, slow-twitch (Type I) fibres (Pearson & Young, 1989; Lawrie, 1998). It can therefore be hypothesised that the *M. gastrocnemius, pars interna* contain more red (Type I) fibres than *M. iliofibularis* because of the higher initial shear force values obtained for the hot-deboned *M. gastrocnemius, pars interna* (Table 5), indicating the possibility of some degree of cold-shortening and super contraction (Chapter 4 of this thesis). The insignificant difference for the initial shear force values (as well as throughout the 42-day aging period) between the hot and the cold-deboned *M. iliofibularis*, indicated that the risk of cold-shortening was not as great in this muscle as compared to the *M. gastrocnemius, pars interna*. Sales & Mellett (1996) also suggested that there is a risk of cold-shortening in the *M. gastrocnemius, pars interna* if this muscle is to be separated from the carcass and cooled at 30-45 min *post-mortem* but not in the *M. iliofibularis*. The results found by Sales & Mellett (1996) showed that the *M. iliofibularis* reached a pH = 6.20 at approximately 30 min *post-mortem*.

The main effect “aging” caused the largest percentage of variation (19.50%) within Warner-Bratzler shear force values and had a highly significant ( $P < 0.0001$ ) effect on tenderness. Aging time (days) was significantly ( $P < 0.05$ ) correlated with the Warner-Bratzler shear force values ( $r = -0.250$ ), indicating that tenderness increased as the aging time increased from day 1 to day 42 for all the muscle samples. Although there were fluctuations within the tenderness data throughout the 42-day aging period, which may be due to differences in connective tissue content between muscles and/or between Warner-Bratzler shear force samples, all the muscles were similar in tenderness on day 42 (Fig. 1).

It was also found that the Warner-Bratzler shear force values were poorly, but significantly correlated with pH ( $r = -0.187$ ;  $P < 0.001$ ), indicating that muscles with a higher pH were more tender.



**Figure 1.** Warner-Bratzler shear force values ( $N \cdot 12.7 \text{ mm}^{-1}$  diameter) with standard error bars for the (◇) hot-deboned and the (○) cold-deboned *M. gastrocnemius, pars interna*, and the (●) hot-deboned and the (◆) cold-deboned *M. iliofibularis* respectively at the individual aging days (1 d to 42 d).

The analysis of variance (ANOVA) of the dependable variable muscle pH is presented in Table 2. The non-normality ( $P < 0.0001$ ) for the pH data was due to Kurtosis (1.602) and therefore further analyses of the data were performed without transformation of the data (Glass *et al.*, 1972). The only significant interaction ( $P < 0.0001$ ) was observed between muscle and aging, indicating that as the aging time increased from day 1 to day 42, the change in pH for the hot and the cold-deboned *M. gastrocnemius, pars interna* differed ( $P < 0.05$ ) significantly from the change in pH for the hot and the cold-deboned *M. iliofibularis* (Figure 2). However, this interaction between muscle and aging caused only 3.32% of variation in pH compared to the 26.53% of variation caused by “ostrich carcass” and the 35.25% of variation caused by the main effect “aging”.

The significant effect of deboning ( $P = 0.0062$ ), where the hot-deboned muscles had significantly ( $P < 0.05$ ) lower pH values ( $5.91 \pm 0.11$ ) throughout the 42-day storage period compared to the cold-deboned muscles ( $5.93 \pm 0.12$ ), caused only 0.61% of the variation in the pH data, and therefore the main effects “ostrich carcass” and “aging” are discussed further.

**Table 2.** Analysis of variance (ANOVA) of the dependable variable muscle pH with ostrich carcass, muscle, deboning (debone) and aging time (d) as main effects, as well as the two and the three way interactions between main effects, and the Shapiro-Wilk test for non-normality.

Source	Muscle pH			
	df	MS	P	% Var
Ostrich carcass	10	0.142	< 0.0001	26.53
Muscle	1	0.165	< 0.0001	3.09
Debone	1	0.033	0.0062	0.61
Aging	9	0.209	< 0.0001	35.25
Muscle*Debone	1	0.0002	0.8224	0.004
Muscle*Aging	9	0.020	< 0.0001	3.32
Debone*Aging	9	0.006	0.1992	1.00
M*D*A	9	0.003	0.7872	0.45
Error	367	0.004		29.74
Shapiro-Wilk			< 0.0001	

df – Degree of freedom

MS – Mean Square

P – Probability value of F-ratio test

% Var – Percentage variation explained by that particular effect

\*Interaction between main effects

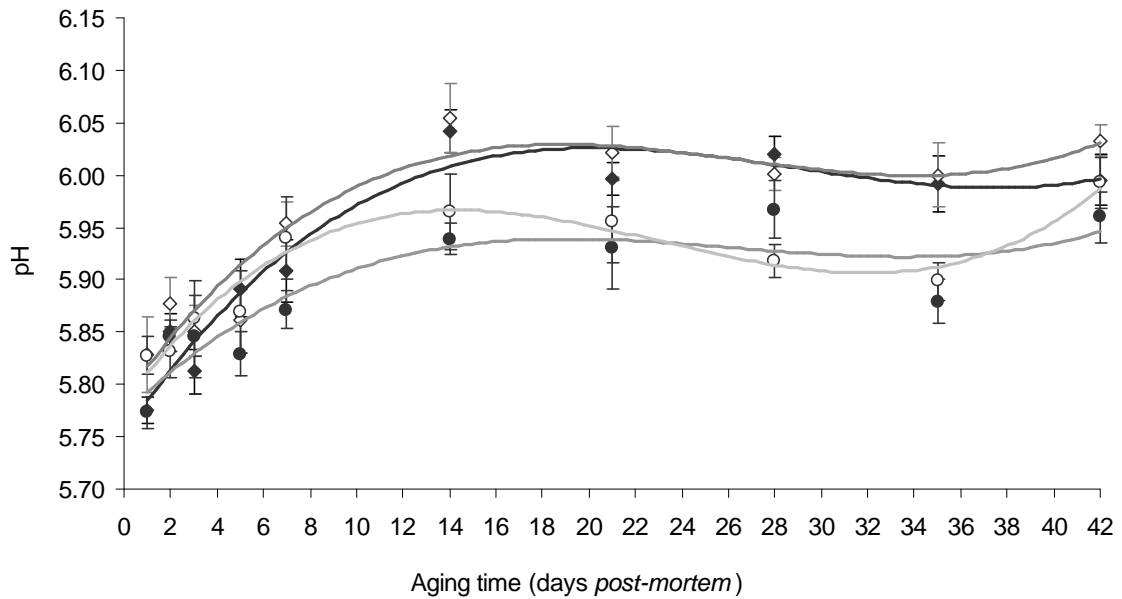
M\*D\*A – Interaction between main effects Muscle, Debone and Aging

As expected, there were significant differences ( $P < 0.0001$ ) for pH between individual ostrich carcasses, indicating that the ostriches had different levels of *post-mortem* glycogen in their muscles. The difference in pH between the individual ostrich carcasses can probably also be explained by intrinsic variation found naturally between ostriches, as well as different levels of *ante mortem* stress and consequent differences in the levels of *post-mortem* muscle glycogen between the individual ostriches.

Aging had a significant effect ( $P < 0.001$ ) on pH, with the pH increasing as the time of aging ( $-3^{\circ}$ - $0^{\circ}$ C) increased from day 1 to day 42 (Fig. 2). Muscle pH (hot and cold-

deboned *M. iliofibularis* and *M. gastrocnemius, pars interna* pooled) was positively correlated ( $P < 0.05$ ) with aging time ( $r = 0.294$ ) and the pH generally increased from  $5.80 \pm 0.08$  at day 1 (24 h *post-mortem*) to a value of  $6.00 \pm 0.08$  at day 42. The pH at 24 h *post-mortem* ( $pH_{24}$ ) for *M. gastrocnemius, pars interna* and *M. iliofibularis*, was higher ( $P < 0.05$ ) in the case of the cold-deboned than the hot-deboned muscles, indicating that the rate of *post-mortem* pH change during the first 24 h *post-mortem* was faster in the case of the cold-deboned muscles. In contrast to the difference in pH between the hot and the cold-deboned muscles, the  $pH_{24}$  did not differ significantly ( $P > 0.05$ ) between the hot-deboned *M. gastrocnemius, pars interna* ( $5.78 \pm 0.04$ ) and the hot-deboned *M. iliofibularis* ( $5.77 \pm 0.05$ ), nor between the cold-deboned *M. gastrocnemius, pars interna* ( $5.83 \pm 0.12$ ) and the cold-deboned *M. iliofibularis* ( $5.83 \pm 0.06$ ).

The trend for the change in pH over aging time was best described ( $R^2 = 0.8965 \pm 0.0563$ ) by third order polynomial trend lines (Fig. 2). From the data in Fig. 2 it can be seen that, irrespective of the time of deboning, the *M. gastrocnemius, pars interna* obtained higher pH values ( $P > 0.05$ ) during the aging period than the *M. iliofibularis*. It can also be seen that the pH increased sharply during the first 14 d, after which the pH decreased slowly, and then started to increase again on day 42. This profile of *post-mortem* pH is similar to the pH profile found for the *M. gastrocnemius, pars interna* aged for 21 d as reported in Chapter 4 of this thesis, where the pH increased during the first 7 d, after which it decreased to a value of  $5.70 \pm 0.18$  at day 21. In the present study the initial increase in pH occurred over 14 d and not during the first 7 d as reported for *M. gastrocnemius, pars interna* in Chapter 4 of this thesis. The difference in the pH profiles between the two research studies (Chapters 4 and 5 of this thesis) can be explained by the difference in aging temperature. The *M. gastrocnemius, pars interna* in Chapter 4 of this thesis were aged at a temperature of  $4^{\circ}\text{C}$ , while all muscles from the present study was aged at a temperature of between  $-3^{\circ}$  to  $0^{\circ}\text{C}$ . Aging temperature significantly affects the pH profile of meat during *post-mortem* aging (Tirupal *et al.*, 1998) and it can therefore be hypothesised that the lower aging temperature in the present study caused *post-mortem* glycolysis to be slowed; the rate of increase in pH and subsequent decrease in pH to be slower, causing the initial increase in pH to continue over 14 d instead of 7 d.



**Figure 2.** Third order polynomial trend lines (with standard error bars) for the change in pH over aging time (d) for respectively the (◇) hot-deboned ( $y = 10^{-5}x^3 - 0.0013x^2 + 0.0333x + 5.7525$ ;  $R^2 = 0.9412$ ) and the (○) cold-deboned ( $y = 2 \times 10^{-5}x^3 - 0.0013x^2 + 0.0316x + 5.7866$ ;  $R^2 = 0.9110$ ) *M. gastrocnemius, pars interna* and the (●) hot-deboned ( $y = 10^{-5}x^3 - 0.0009x^2 + 0.0216x + 5.7719$ ;  $R^2 = 0.8153$ ) and the (●) cold-deboned ( $y = 2 \times 10^{-5}x^3 - 0.0015x^2 + 0.0301x + 5.7832$ ;  $R^2 = 0.9453$ ) *M. iliofibularis*.

The analysis of variance (ANOVA) of the dependable variable percentage purge is presented in Table 3. The non-normality ( $P < 0.0001$ ) for the data for percentage purge was due to Kurtosis and therefore further analyses of the data were performed without transformation of the data (Glass *et al.*, 1972). Although there were significant two way interactions between deboning and aging ( $P = 0.0006$ ), and between muscle and aging ( $P = 0.0023$ ), the percentages of variation of respectively 4.13% and 1.29% caused by these interactions, were low compared to the percentages of variation caused by the main effects “ostrich carcass” (5.44%), “muscle” (10.11%), “deboning” (7.20%), and “aging” (16.43%); and therefore these are discussed further.

Significant differences ( $P < 0.0001$ ) for purge (%) were found between individual ostrich carcasses. The main effect “muscle” also had a significant effect ( $P < 0.0001$ ) on percentage purge, where the *M. gastrocnemius, pars interna* had less purge (%) than the *M. iliofibularis* (Fig. 3). As illustrated in Fig. 3, the cold-deboned *M. gastrocnemius, pars interna* had the lowest percentage purge, while the hot-deboned *M. iliofibularis* had the highest percentage purge throughout the 42-day aging period.



Hot-deboning resulted in ( $P = 0.0001$ ) both the *M. gastrocnemius, pars interna* and the *M. iliofibularis* to lose more moisture (higher values for purge) during the 42-day aging period compared to the cold-deboned muscles.

**Table 3.** Analysis of variance (ANOVA) of the dependable variable purge (%) with ostrich carcass, muscle, deboning (debone) and aging time (d) as main effects, as well as the two and three way interactions between main effects, and the Shapiro-Wilk test for non-normality.

Purge (%)				
Source	df	MS	P	% Var
Ostrich carcass	10	12.278	< 0.0001	5.44
Muscle	1	228.259	< 0.0001	10.11
Debone	1	162.434	< 0.0001	7.20
Aging	9	41.204	< 0.0001	16.43
Muscle*Debone	1	29.141	0.0023	1.29
Muscle*Aging	9	4.040	0.2315	1.61
Debone*Aging	9	10.361	0.0006	4.13
M*D*A	9	2.213	0.6947	0.88
Error	386	3.092		52.90
Shapiro-Wilk			< 0.0001	

df – Degree of freedom

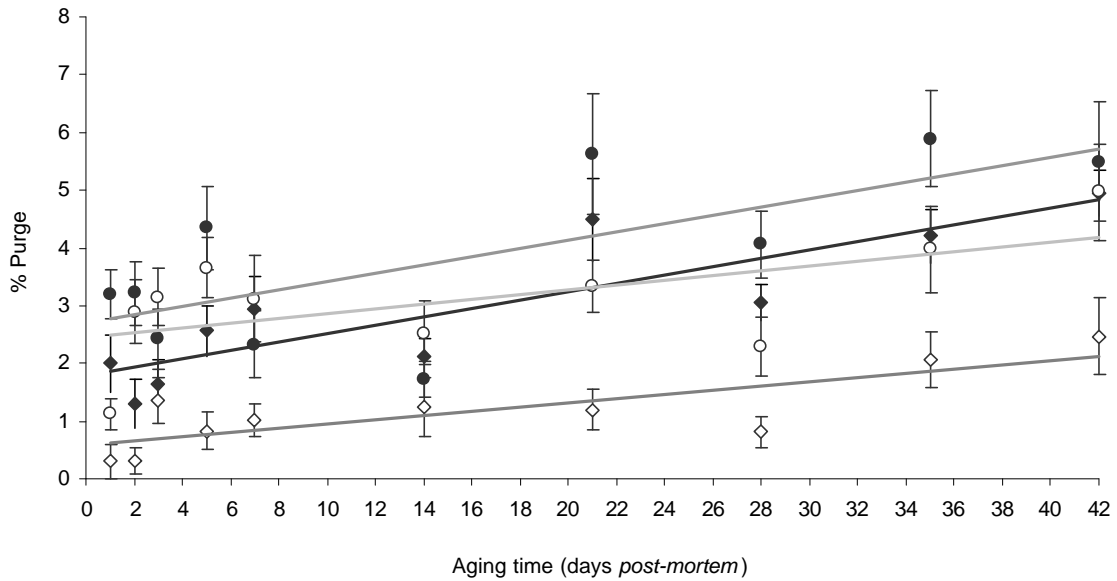
MS – Mean Square

P – Probability value of F-ratio test

% Var – Percentage variation explained by that particular effect

\*Interaction between main effects

M\*D\*A – Interaction between main effects Muscle, Debone and Aging



**Figure 3.** Linear trend lines (with standard error bars) fitted from day 1 to day 42 for the increase in percentage purge with *post-mortem* aging time (d) for respectively the (○) hot-deboned ( $y = 0.0723x + 1.7818$ ;  $R^2 = 0.7445$ ) and the (◇) cold-deboned ( $y = 0.0369x + 0.5795$ ;  $R^2 = 0.6360$ ) *M. gastrocnemius, pars interna* and the (●) hot ( $y = 0.0713x + 2.707$ ;  $R^2 = 0.5081$ ) and the (◆) cold-deboned ( $y = 0.0417x + 2.4447$ ;  $R^2 = 0.3647$ ) *M. iliofibularis*.

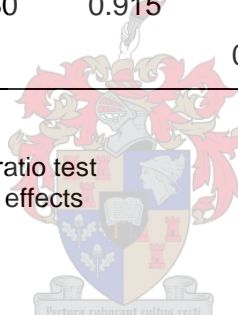
The analysis of variance (ANOVA) of the regression coefficients: intercept (a) and slope (b) of the linear model ( $y = ax + b$ ) for purge (%) are presented in Table 4. There was no significant interaction between muscle and deboning for the intercept values ( $P = 0.0669$ ), nor for the slope values ( $P = 0.7915$ ), therefore the main effects: “muscle” and “deboning” are discussed. From the data in Fig. 3 it can be seen that the hot-deboned ( $2.00 \pm 1.64\%$ ) and the cold-deboned ( $0.30 \pm 1.00\%$ ) *M. gastrocnemius, pars interna* had a significant lower ( $P < 0.05$ ) initial (day 1) purge (%) than the hot-deboned ( $3.20 \pm 1.38\%$ ) and the cold-deboned *M. iliofibularis* ( $1.13 \pm 0.89\%$ ). However, no significant difference ( $P > 0.05$ ) in the rate of increase in purge was observed between the hot-deboned *M. gastrocnemius, pars interna* ( $0.0631 \pm 0.0172$ ) and the hot-deboned *M. iliofibularis* ( $0.0704 \pm 0.0667$ ). Similarly, there was also no significant difference ( $P > 0.05$ ) in the rate of increase in purge between the cold-deboned *M. gastrocnemius, pars interna* ( $0.0369 \pm 0.0304$ ) and the cold-deboned *M. iliofibularis* ( $0.0371 \pm 0.0499$ ). Irrespective of the main effect: “muscle”, the time of deboning had a significant effect ( $P = 0.0221$ ) on the initial purge (intercept), as well as on the rate of increase in purge as the time of aging increased from day 1 to day 42.

Hot-deboned muscles had a higher initial purge ( $2.27 \pm 1.16\%$ ) than the cold-deboned ( $1.57 \pm 1.49\%$ ) muscles, and the rate of increase in purge was faster in the case of the hot-deboned ( $0.0667 \pm 0.0477$ ) than the cold-deboned ( $0.0370 \pm 0.040$ ) muscles.

**Table 4.** Analysis of variance (ANOVA) of the regression coefficients: intercept (a) and slope (b) of the linear model ( $y = ax + b$ ) for purge (%) with model, ostrich carcass and deboning (debone) as main effects, the two way interactions between main effects, as well as the Shapiro-Wilk test for non-normality.

Source	df	Intercept (a)		Slope (b)	
		MS	P	MS	P
Ostrich carcass	10	2.159	0.0338	0.0024	0.2832
Muscle	1	22.687	< 0.0001	0.0002	0.7751
Debone	1	5.329	0.0221	0.0097	0.0313
Muscle*Deboning	1	3.037	0.0669	0.0001	0.7915
Error	30	0.915		0.0019	
Shapiro-Wilk			0.6425		0.7178

df – Degree of freedom  
 MS – Mean Square  
 P – Probability value of F-ratio test  
 \*Interaction between main effects



The analysis of variance (ANOVA) of the dependable variable cooking loss (%) is presented in Table 5. The non-normality ( $P = 0.0005$ ) for the cooking loss (%) data was due to Kurtosis and therefore further analyses of the data were performed without transformation of the data (Glass *et al.*, 1972). There was a significant interaction between muscle and deboning ( $P < 0.001$ ), indicating that there were differences in cooking loss between the *M. gastrocnemius, pars interna* and the *M. iliofibularis* as the time *post-mortem* increased from day 1 to day 42 (Table 6). In the case of the *M. gastrocnemius, pars interna*, the hot-deboned muscles ( $37.41 \pm 3.25\%$ ) had higher ( $P < 0.05$ ) cooking loss values (%) throughout the 42-day aging period than the cold-deboned muscles ( $36.34 \pm 3.04\%$ ), while in contrast, the hot-deboned *M. iliofibularis* ( $39.81 \pm 1.95\%$ ) showed less cooking loss ( $P < 0.05$ ) than the cold-deboned *M. iliofibularis* ( $40.73 \pm 3.34\%$ ). However, the variation caused by this interaction was small (2.15%) compared to the variation caused by the main effects “ostrich carcass” (8.40%) and “muscle” (24.91%).

Similarly to muscle pH, shear force values and purge (%), there was also a large variation for cooking loss (%) between individual ostrich carcasses ( $P < 0.0001$ ). This large variation can in part be explained by the large difference in pH (Table 2) between the individual ostrich carcasses. It was also found that the cooking loss (%) was poorly, but significantly correlated ( $r = -0.1447$ ;  $P = 0.0036$ ) to muscle pH, indicating that muscle samples with a low pH showed higher cooking loss (%) than muscle samples with a higher pH.

**Table 5.** Analysis of variance (ANOVA) of the dependable variable cooking loss (%) with ostrich carcass, muscle, deboning (debone) and aging time (d) as main effects, as well as the two and three way interactions between main effects, and the Shapiro-Wilk test for non-normality.

<b>Cooking loss (%)</b>				
<b>Source</b>	<b>df</b>	<b>MS</b>	<b>P</b>	<b>% Var</b>
Ostrich carcass	10	41.828	$< 0.0001$	8.40
Muscle	1	1240.031	$< 0.0001$	24.91
Debone	1	0.469	0.8090	0.01
Aging	9	4.699	0.8088	0.85
Muscle*Debone	1	106.998	0.0003	2.15
Muscle*Aging	9	7.435	0.5015	1.34
Debone*Aging	9	5.248	0.7502	0.95
M*D*A	9	4.470	0.8318	0.81
Error	376	8.021		60.58
Shapiro-Wilk			0.0005	

df – Degree of freedom

MS – Mean Square

P – Probability value of F-ratio test

% Var – Percentage variation explained by that particular effect

\*Interaction between main effects

M\*D\*A – Interaction between main effects Muscle, Debone and Aging

Muscle had a significant effect ( $P < 0.0001$ ) on cooking loss, while in contrast to the Warner-Bratzler shear force values, pH and purge (%), aging had no significant effect ( $P = 0.8088$ ) on cooking loss. Hot-deboning also had no significant effect ( $P = 0.8090$ ) on cooking loss, and therefore the cooking loss (%) data for the hot and the cold-deboned muscles from respectively the *M. gastrocnemius, pars interna* and the *M. iliofibularis* could be pooled (Table 14), indicating that, irrespective of the interaction between muscle and

deboning, the *M. gastrocnemius, pars interna* ( $36.85 \pm 3.18\%$ ) had significantly ( $P < 0.0001$ ) less cooking loss than the *M. iliofibularis* ( $40.26 \pm 2.75\%$ ). In contrast to the results obtained in this study for the cooking loss (%) at 24 h *post-mortem* between the *M. gastrocnemius, pars interna* ( $37.57 \pm 1.90\%$ ) and the *M. iliofibularis* ( $40.30 \pm 1.97\%$ ), Sales (1994) found no significant difference in cooking loss at 24 h *post-mortem* between the *M. gastrocnemius, pars interna* ( $35.81 \pm 3.62\%$ ) and the *M. iliofibularis* ( $36.02 \pm 2.83\%$ ).

No significant correlation was found between purge (%) and pH ( $r = -0.064$ ;  $P = 0.1960$ ) for both the *M. gastrocnemius, pars interna* and the *M. iliofibularis*. There was a significant positive correlation between cooking loss and purge ( $r = 0.178$ ;  $P < 0.001$ ), showing that as the purge increased, the cooking loss also increased as the time of aging increased from day 1 to day 42. However, the pH had a larger effect on cooking loss than on purge.

**Table 6.** Mean ( $\pm$  Standard Deviation) cooking loss (%) on the individual aging days (day 1, 2, 3, 5, 7, 14, 21, 28, 35 and 42 *post-mortem*) for respectively the *M. gastrocnemius, pars interna* and the *M. iliofibularis* with results from the hot-deboned and the cold-deboned muscles pooled, as well as mean values (pooled over the 42-day storage period).

Aging time (d)	<i>M. gastrocnemius, pars interna</i>	<i>M. iliofibularis</i>
1	$37.57 \pm 1.90^b$	$40.30 \pm 1.97^a$
2	$37.30 \pm 2.47^{bc}$	$39.82 \pm 1.67^a$
3	$37.61 \pm 2.55^b$	$40.03 \pm 1.45^a$
5	$37.39 \pm 2.56^{bc}$	$40.16 \pm 2.22^a$
7	$36.58 \pm 4.22^{bc}$	$41.14 \pm 3.54^a$
14	$37.27 \pm 2.67^{bc}$	$40.41 \pm 1.97^a$
21	$36.56 \pm 2.56^{bc}$	$39.76 \pm 3.93^a$
28	$36.20 \pm 3.80^{bc}$	$40.50 \pm 2.24^a$
35	$35.76 \pm 4.34^c$	$40.26 \pm 3.04^a$
42	$36.28 \pm 3.73^{bc}$	$40.22 \pm 4.07^a$
Mean	$36.85 \pm 3.18^A$	$40.26 \pm 2.75^B$

<sup>abc</sup> Different superscripts differ at  $P < 0.05$ .

<sup>AB</sup> Different superscripts differ at  $P < 0.05$  for the mean cooking loss (%).

In this study it was found that, as the purge (%) increased, there was a general increase in the tenderness ( $r = -0.144$ ;  $P = 0.0029$ ) as the time of aging *post-mortem* increased from day 1 to day 42. In contrast, cooking loss showed no significant correlation with tenderness ( $r = -0.088$ ;  $P = 0.0731$ ). In contrast to Sales (1994), who found no significant differences in moisture content or in cooking loss between the *M. gastrocnemius, pars interna* and *M. iliofibularis*, results from this study showed a significant higher purge (%) and cooking loss (%) for the *M. iliofibularis* compared to the *M. gastrocnemius, pars interna*. The lower water holding capacity of the *M. iliofibularis* can be explained by the larger areas of exposed surface in the pre-packaged cuts (Lawrie, 1998), since the *M. iliofibularis* in the ostrich leg is the muscle with the largest individual mass (Sales, 1996), resulting in this muscle to lose more moisture during storage *post-mortem* than the *M. gastrocnemius, pars interna*. Sales (1996) reported an individual mass of  $1.16 \pm 0.28$  kg for the *M. iliofibularis*, compared to  $0.70 \pm 0.19$  kg for the *M. gastrocnemius, pars interna*. Similarly, results from this study also indicated that the *M. iliofibularis* ( $1.66 \pm 0.19$  kg) had a higher ( $P < 0.05$ ) individual mass than the *M. gastrocnemius, pars interna* ( $1.11 \pm 0.14$  kg).

The analysis of variance (ANOVA) of the dependable colour variable  $L^*$  is presented in Table 7. The non-normality ( $P = 0.0402$ ) for the data for the  $L^*$ -values was assigned to the occurrence of Kurtosis (0.3210), therefore, further analyses of the data were performed without transformation of the data (Glass *et al.*, 1972). The significant interaction ( $P = 0.0001$ ) between muscle and deboning indicate that hot-deboning caused the *M. gastrocnemius, pars interna* ( $30.48 \pm 1.98$ ) to be significantly ( $P < 0.05$ ) darker in colour than the cold-deboned muscles ( $31.44 \pm 1.80$ ), while in contrast, hot-deboning had no effect on the *M. iliofibularis*, where the hot-deboned ( $32.03 \pm 1.91$ ) and cold-deboned ( $31.90 \pm 1.66$ ) *M. iliofibularis* did not differ significantly ( $P > 0.05$ ) in  $L^*$ -values throughout the 42-day aging period. However, this interaction caused only 2.01% of the variation, while the main effects “ostrich carcass”, “muscle”, and “aging” caused respectively 33.18%, 6.85% and 2.89% of the variation in  $L^*$ -values, and therefore are discussed further. Although deboning had a significant effect ( $P = 0.0032$ ) on the  $L^*$ -values, causing the hot-deboned muscles from both the *M. gastrocnemius, pars interna* and the *M. iliofibularis* to be darker (lower  $L^*$ -values) in colour than the cold-deboned muscles, deboning explained only 1.14% of the percentage of variance and is therefore not discussed further.

Individual ostrich carcasses differed significantly with regards to  $L^*$ -values ( $P < 0.0001$ ). This variation in  $L^*$ -values between the individual ostrich carcasses can probably be assigned to the large difference in muscle pH (Table 2) between the ostrich carcasses. The relationship between lightness and pH is negative, where raw meat with a low pH

generally have higher measured L\*-values (becomes paler in colour) than meat with a high pH (Swatland, 2004; Offer, 1991).

In terms of the main effect “muscle”, both the hot-deboned and the cold-deboned *M. iliofibularis* were generally lighter (higher L\*-values) than the hot-deboned and the cold-deboned *M. gastrocnemius, pars interna* ( $P < 0.0001$ ). Aging also had a significant effect ( $P = 0.0095$ ) on the L\*-values, resulting in an increase in the L\*-values as aging time increased from day 1 to day 42 ( $r = 0.104$ ;  $P < 0.05$ ) for respectively the hot-deboned and the cold-deboned *M. gastrocnemius, pars interna* and *M. iliofibularis* (Table 13).

**Table 7.** Analysis of variance (ANOVA) of the dependable colour variable L\* with ostrich carcass, muscle, deboning (debone), and aging time (d) as main effects, as well as the two and three way interactions between main effects, and the Shapiro-Wilk test for non-normality.

Colour L*				
Source	df	MS	P	% Var
Ostrich carcass	10	54.799	< 0.0001	33.48
Muscle	1	112.076	< 0.0001	6.85
Debone	1	18.612	0.0032	1.14
Aging	9	5.250	0.0095	2.89
Muscle*Debone	1	32.822	0.0001	2.01
Muscle*Aging	9	3.507	0.0992	1.93
Debone*Aging	9	1.376	0.7561	0.76
M*D*A	9	1.108	0.8588	0.61
Error	388	2.124		50.35
Shapiro-Wilk			0.0402	

df – Degree of freedom

MS – Mean Square

P – Probability value of F-ratio test

% Var – Percentage variation explained by that particular effect

\*Interaction between main effects

M\*D\*A – Interaction between main effects Muscle, Debone and Aging

The analysis of variance (ANOVA) of the dependable colour variable a\* is presented in Table 8. The non-normality ( $P = 0.0061$ ) for the data for a\*-values was due to Kurtosis (0.8460) and therefore further analyses of the data were performed without transformation of the data (Glass *et al.*, 1972). The only significant interaction for a\* was observed between deboning and aging ( $P = 0.0141$ ), showing that the hot and the cold-

deboned muscles differed in the change in  $a^*$ -values as the aging period increased from day 1 to day 42 (Table 9). The main effects “muscle” and “aging” had no significant effect ( $P = 0.1418$  and  $P = 0.0613$  respectively) on the  $a^*$ -values, and therefore the data from the *M. gastrocnemius, pars interna* and the *M. iliofibularis* could be pooled (Table 9). The pooled data indicated that the hot-deboned muscles decreased in red colour from day 1 ( $13.53 \pm 1.65$ ) to day 42 ( $12.94 \pm 1.84$ ), while on the other hand, the cold-deboned muscles increased in red colour from day 1 ( $12.92 \pm 1.25$ ) to day 42 ( $13.52 \pm 1.42$ ). In contrast to the result obtained in this study, Otremba *et al.* (1999) reported that the red colour of raw ostrich meat (ground and cold-deboned whole muscles) did not change significantly over time during *post-mortem* storage. Similarly to the  $L^*$ -values, there was a significant difference in  $a^*$ -values between the individual ostrich carcasses ( $P < 0.0001$ ), with the most percentage of variance (23.51%) explained by this main effect.

**Table 8.** Analysis of variance (ANOVA) of the dependable colour variable  $a^*$  with ostrich carcass, muscle, deboning (debone) and aging time (d) as main effects, as well as the two and three way interactions between main effects, and the Shapiro-Wilk test for non-normality.

Colour $a^*$				
Source	df	MS	P	% Var
Ostrich carcass	10	28.743	< 0.0001	23.51
Muscle	1	4.425	0.1418	0.36
Debone	1	37.124	< 0.0001	3.04
Aging	9	3.738	0.0613	2.75
Muscle*Debone	1	0.541	0.6069	0.04
Muscle*Aging	9	1.903	0.4971	1.40
Debone*Aging	9	4.781	0.0141	3.52
M*D*A	9	0.555	0.9891	0.41
Error	389	2.042		64.97
Shapiro-Wilk			0.0061	

df – Degree of freedom

MS – Mean Square

P – Probability value of F-ratio test

% Var – Percentage variation explained by that particular effect

\*Interaction between main effects

M\*D\*A – Interaction between main effects Muscle, Debone and Aging



**Table 9.** Mean ( $\pm$  Standard Deviation)  $a^*$ -values on the individual aging days (day 1, 2, 3, 5, 7, 14, 21, 28, 35 and 42 *post-mortem*) for respectively the hot-deboned and the cold-deboned muscles with data from the *M. iliofibularis* and the *M. gastrocnemius, pars interna* pooled, as well as mean values (pooled over the 42-day storage period).

Aging time (d)	Hot-deboned muscles	Cold-deboned muscles
1	13.53 $\pm$ 1.65 <sup>abc</sup>	12.92 $\pm$ 1.25 <sup>cdefg</sup>
2	13.58 $\pm$ 2.04 <sup>abc</sup>	13.43 $\pm$ 1.08 <sup>abc</sup>
3	13.13 $\pm$ 1.79 <sup>bcdef</sup>	13.50 $\pm$ 1.29 <sup>abc</sup>
5	13.45 $\pm$ 1.51 <sup>abc</sup>	13.45 $\pm$ 1.25 <sup>abc</sup>
7	13.12 $\pm$ 1.91 <sup>bcdef</sup>	13.88 $\pm$ 1.48 <sup>ab</sup>
14	12.50 $\pm$ 2.27 <sup>defg</sup>	13.94 $\pm$ 1.62 <sup>ab</sup>
21	13.02 $\pm$ 1.58 <sup>cdefg</sup>	14.10 $\pm$ 1.20 <sup>a</sup>
28	12.46 $\pm$ 1.79 <sup>efg</sup>	13.23 $\pm$ 1.68 <sup>bcde</sup>
35	12.33 $\pm$ 1.68 <sup>fg</sup>	13.34 $\pm$ 1.65 <sup>abcd</sup>
42	12.27 $\pm$ 1.83 <sup>g</sup>	13.42 $\pm$ 1.48 <sup>abc</sup>
Mean	12.94 $\pm$ 1.84 <sup>A</sup>	13.52 $\pm$ 1.42 <sup>B</sup>

<sup>abcdefg</sup> Different superscripts differ at  $P < 0.05$ .

<sup>AB</sup> Different superscripts differ at  $P < 0.05$  for overall  $a^*$ -values.

The analysis of variance (ANOVA) of the dependable variable  $b^*$  is presented in Table 10. According to the Shapiro-Wilk test, the data for colour  $b^*$  was normally distributed ( $P = 0.3658$ ). The data was therefore further analysed without transformation of the data. There was a significant interaction between muscle and aging for the  $b^*$ -values ( $P < 0.05$ ), indicating that the *M. gastrocnemius, pars interna* and the *M. iliofibularis* differed in yellow colour as the time of aging increased from day 1 to day 42. The hot and the cold-deboned *M. gastrocnemius, pars interna* decreased in  $b^*$ -values from day 1 to day 42, while in contrast, the hot and the cold-deboned *M. iliofibularis* muscles increased in  $b^*$ -values from day 1 to day 42 (Table 13). A significant interaction was also observed between deboning and aging ( $P = 0.0114$ ), explaining more of the variation (3.47%) in  $b^*$ -values than the interaction between muscle and aging (2.98%). This indicated that the hot and the cold-deboned muscles differed in  $b^*$ -values at the respective aging days. However, the main effects “ostrich carcass” and “muscle” explained more of the percentage of variation in  $b^*$ -values (19.72% and 6.97% respectively) than the above mentioned two way interactions, and are therefore discussed further.

Individual ostrich carcasses showed significant differences for the  $b^*$ -values ( $P < 0.0001$ ), as was the case for the  $L^*$  and  $a^*$ -values. Deboning, nor aging had a significant effect on the  $b^*$ -values, and therefore the data from the hot and the cold-deboned muscles was pooled for respectively the *M. gastrocnemius, pars interna* and the *M. iliofibularis*, indicating that the *M. gastrocnemius, pars interna* ( $8.18 \pm 1.46$ ) were significantly ( $P < 0.05$ ) less yellow in colour than the *M. iliofibularis* ( $8.92 \pm 1.25$ ) throughout the 42-day aging period.

**Table 10.** Analysis of variance (ANOVA) of the dependable colour variable  $b^*$  with ostrich carcass, muscle, deboning (debone) and aging time (d) as main effects, as well as the two and three way interactions between main effects, and the Shapiro-Wilk test for non-normality.

Colour $b^*$				
Source	df	MS	P	% Var
Ostrich carcass	10	17.164	< 0.0001	19.72
Muscle	1	60.636	< 0.0001	6.97
Debone	1	1.008	0.3952	0.12
Aging	9	2.593	0.0559	2.68
Muscle*Debone	1	0.199	0.7054	0.02
Muscle*Aging	9	2.878	0.0314	2.98
Debone*Aging	9	3.352	0.0114	3.47
M*D*A	9	1.648	0.3034	1.70
Error	390	1.391		62.34
Shapiro-Wilk			0.3658	

df – Degree of freedom

MS – Mean Square

P – Probability value of F-ratio test

% Var – Percentage variation explained by that particular effect

\*Interaction between main effects

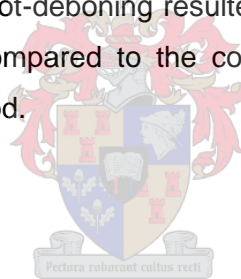
M\*D\*A – Interaction between main effects Muscle, Debone and Aging

The analysis of variance (ANOVA) of the dependable colour variable Hue angle ( $h_{ab}$ ) ( $^\circ$ ) is presented in Table 11. The non-normality ( $P = 0.0043$ ) for the Hue angle data was due to Kurtosis and therefore further analyses of the data were performed without transformation of the data (Glass *et al.*, 1972). No significant interaction ( $P > 0.05$ ) was

observed between any of the main effects and therefore the main effects “ostrich carcass”, “muscle”, “deboning” and “aging” are discussed.

In contrast to the  $L^*$ ,  $a^*$ , and  $b^*$ -values, no significant difference was found for the Hue angle values between the individual ostrich carcasses ( $P = 0.0890$ ). Although the main effects “muscle” and “deboning” were significant ( $P < 0.0001$  and  $P = 0.0134$  respectively), the main effect “aging” explained 6.11% of the percentage of variance for the Hue angle values. Aging time resulted in significant differences ( $P < 0.0005$ ) between the individual aging days, however, with the exception of the hot-deboned *M. iliofibularis*, there was no significant difference ( $P > 0.05$ ) in the Hue angle values between day 1 and day 42, for the hot and the cold-deboned *M. gastrocnemius, pars interna*, and the cold-deboned *M. iliofibularis* (Table 13).

However, there were significant differences between the *M. gastrocnemius, pars interna* and the *M. iliofibularis* ( $P < 0.0001$ ), where the *M. iliofibularis* had higher Hue angle values ( $33.79 \pm 3.39^\circ$ ) than the *M. gastrocnemius, pars interna* ( $31.96 \pm 4.67^\circ$ ). The difference in the Hue angle between these two muscles indicated that the *M. iliofibularis* were less red in colour compared to the *M. gastrocnemius, pars interna* throughout the 42-day aging period. Similarly, hot-deboning resulted in muscles to be less ( $P < 0.0134$ ) red in colour ( $33.34 \pm 3.83^\circ$ ) compared to the cold-deboned muscles ( $32.41 \pm 4.46^\circ$ ) throughout the 42-day aging period.



**Table 11.** Analysis of variance (ANOVA) of the dependable colour variable Hue angle ( $h_{ab}$ ) ( $^{\circ}$ ) with ostrich carcass, muscle, deboning (debone) and aging time (d) as main effects, as well as the two and three way interactions between main effects, and the Shapiro-Wilk test for non-normality.

Source	Hue angle ( $h_{ag}$ ) ( $^{\circ}$ )			
	df	MS	P	% Var
Ostrich carcass	10	25.336	0.0890	3.32
Muscle	1	363.737	< 0.0001	4.76
Debone	1	94.393	0.0134	1.24
Aging	9	51.841	0.0005	6.11
Muscle*Debone	1	7.276	0.4908	0.10
Muscle*Aging	9	23.652	0.1297	2.79
Debone*Aging	9	13.008	0.5699	1.53
M*D*A	9	19.763	0.2391	1.54
Error	389	15.294		77.85
Shapiro-Wilk			0.0043	

df – Degree of freedom

MS – Mean Square

P – Probability value of F-ratio test

% Var – Percentage variation explained by that particular effect

\*Interaction between main effects

M\*D\*A – Interaction between main effects Muscle, Debone and Aging

The analysis of variance (ANOVA) of the dependable colour variable Chroma ( $C^*$ ) is presented in Table 12. The results for Chroma was normally distributed ( $P > 0.05$  for the Shapiro-Wilk test for non-normality), and there were significant differences between the individual ostrich carcasses ( $P < 0.0001$ ). Similar to the  $a^*$ -values, there was only a significant interaction ( $P < 0.001$ ) between deboning and aging, which explained 4.26% of the percentage of variance for the Chroma values, indicating that the hot-deboned and the cold-deboned muscles differed in their degree of greyness ( $C^*$ -values) at the individual aging days. The hot-deboned muscles from both the *M. gastrocnemius, pars interna* and the *M. iliofibularis* decreased in brightness ( $C^*$ -values) from day 1 to day 42 (Table 13), while the cold-deboned muscles showed no significant ( $P > 0.05$ ) change in brightness from day 1 to day 42.

Aging had no significant effect ( $P = 0.1139$ ) on the Chroma values, and therefore the data for respectively the hot and the cold-deboned *M. gastrocnemius, pars interna* and *M. iliofibularis* were pooled over the 42-day aging period (Table 14), indicating that the muscles differed significantly ( $P < 0.0001$ ) in Chroma, where higher  $C^*$ -values for both the

hot-deboned ( $15.81 \pm 1.88$ ) and the cold-deboned *M. iliofibularis* ( $16.32 \pm 1.59$ ) muscles indicated that these muscles were brighter (less grey) in colour than the hot-deboned ( $15.17 \pm 2.20$ ) and the cold-deboned *M. gastrocnemius, pars interna* ( $15.82 \pm 1.54$ ).

**Table 12.** Analysis of variance (ANOVA) of the dependable colour variable Chroma (C\*) with ostrich carcass, muscle, deboning (debone) and aging time (d) as main effects, as well as the two and three way interactions between main effects, and the Shapiro-Wilk test for non-normality.

Chroma (C*)				
Source	df	MS	P	% Var
Ostrich carcass	10	43.665	< 0.0001	28.90
Muscle	1	35.685	< 0.0001	2.36
Debone	1	36.704	< 0.0001	2.43
Aging	9	3.556	0.1139	2.12
Muscle*Debone	1	0.612	0.6005	0.04
Muscle*Aging	9	3.506	0.1207	2.09
Debone*Aging	9	7.145	0.0009	4.26
M*D*A	9	1.092	0.8809	0.65
Error	388	2.226		57.16
Shapiro-Wilk			0.0548	

df – Degree of freedom

MS – Mean Square

P – Probability value of F-ratio test

% Var – Percentage variation explained by that particular effect

\*Interaction between main effects

M\*D\*A – Interaction between main effects Muscle, Debone and Aging

**Table 13.** Mean ( $\pm$  Standard Deviation) L\*-values, b\*-values, Hue angle ( $h_{ab}$ ) ( $^{\circ}$ ) and Chroma ( $C^*$ ) on day 1 (24 h *post-mortem*) and on day 42 of the 42-day aging period for respectively the hot-deboned (Hot *M. gastro*) and the cold-deboned *M. gastrocnemius, pars interna* (Cold *M. gastro*) and the hot-deboned (Hot *M. ilio*) and the cold-deboned *M. iliofibularis* (Cold *M. ilio*).

Colour variable	Day	Hot <i>M. gastro</i>	Cold <i>M. gastro</i>	Hot <i>M. ilio</i>	Cold <i>M. ilio</i>
L*-values	1	30.44 $\pm$ 2.23	31.25 $\pm$ 1.65	31.92 $\pm$ 2.06	32.00 $\pm$ 1.72
	42	30.50 $\pm$ 1.98	31.42 $\pm$ 1.55	33.23 $\pm$ 2.06	32.22 $\pm$ 1.95
b*-values	1	8.96 $\pm$ 1.74	8.82 $\pm$ 1.39	8.70 $\pm$ 1.06	8.40 $\pm$ 1.33
	42	7.78 $\pm$ 1.43	8.51 $\pm$ 1.39	9.05 $\pm$ 1.23	8.77 $\pm$ 0.99
Hue angle ( $h_{ab}$ ) ( $^{\circ}$ )	1	32.98 $\pm$ 4.05	33.74 $\pm$ 4.23	33.15 $\pm$ 2.18	31.83 $\pm$ 2.41
	42	32.30 $\pm$ 4.46	31.83 $\pm$ 4.48	36.68 $\pm$ 3.19	33.78 $\pm$ 2.62
Chroma ( $C^*$ )	1	16.46 $\pm$ 2.28	15.86 $\pm$ 1.30	15.90 $\pm$ 1.68	15.30 $\pm$ 1.25
	42	14.65 $\pm$ 2.37	16.18 $\pm$ 1.53	15.18 $\pm$ 1.72	15.81 $\pm$ 1.70

**Table 14.** Mean ( $\pm$  Standard Deviation) cooking loss (%) and Chroma ( $C^*$ ), pooled over the 42-days aging period, for respectively the hot-deboned (Hot *M. gastro*) and the cold-deboned *M. gastrocnemius, pars interna* (Cold *M. gastro*); and hot-deboned (Hot *M. ilio*) and the cold-deboned *M. iliofibularis* (Cold *M. ilio*).

Parameter	Hot <i>M. gastro</i>	Cold <i>M. gastro</i>	Hot <i>M. ilio</i>	Cold <i>M. ilio</i>
Cooking loss (%)	37.41 $\pm$ 3.25 <sup>a</sup>	36.34 $\pm$ 3.04 <sup>b</sup>	39.81 $\pm$ 1.95 <sup>c</sup>	40.73 $\pm$ 3.34 <sup>d</sup>
Chroma ( $C^*$ )	15.17 $\pm$ 2.20 <sup>c</sup>	15.82 $\pm$ 1.54 <sup>b</sup>	15.81 $\pm$ 1.88 <sup>b</sup>	16.32 $\pm$ 1.59 <sup>a</sup>

<sup>abcd</sup> Different superscripts differ at  $P < 0.05$  within rows.

### Microbiological results

The microbiological results for Aerobic Plate Counts (APC), *E. coli*, EBC (Enterobacteriaceae), and *Pseudomonas* from respectively the hot and the cold-deboned *M. gastrocnemius, pars interna* and *M. iliofibularis* at 24 h *post-mortem* and day 42 of the aging period, are summarised in Table 15. Although APC of 2 800 cfu.g<sup>-1</sup> at 24 h *post-mortem* were found for two of the hot-deboned *M. gastrocnemius, pars interna* samples, no difference in APC were found between the hot-deboned and the cold-deboned *M. gastrocnemius, pars interna* neither at 24 h *post-mortem* nor on day 42. Similarly, at 24 h *post-mortem* and at day 42 of the aging period, the *M. iliofibularis* showed no difference in

APC between the hot-deboned and the cold-deboned muscles. It can also be seen from Table 15 that there were no differences in *E. coli*, EBC, and *Pseudomonas* counts between the hot-deboned and the cold-deboned muscles, neither between *M. gastrocnemius, pars interna* and *M. iliofibularis*. With the exception of hot-deboned *M. gastrocnemius, pars interna* for *Pseudomonas*, and cold-deboned *M. iliofibularis* for APC, muscle samples showed no increase in microbial counts from 24 h *post-mortem* to day 42. Similarly, Otremba *et al.* (1999) found no significant increase in APC from initial counts up to 7 d *post-mortem* in both whole and ground ostrich muscles. Taylor *et al.* (1980-81) also indicated that hot-deboning of beef muscles had no significant effect on the initial levels of contamination for *Escherichia coli*, Enterobacteriaceae and APC. Their results also indicated little or no growth on the hot-deboned meat cuts during *post-mortem* storage. In contrast, Otremba *et al.* (1999) indicated an increase in growth from day 7 to day 28 of refrigerated storage at  $0^{\circ} \pm 2^{\circ}\text{C}$ . However, in the present study, the muscle samples were stored at lower temperatures ( $-3^{\circ}$  to  $0^{\circ}\text{C}$ ), which explains the small increase in microbial growth throughout the 42-day aging period. It was previously indicated (Lawrie, 1998) that if meat is held just above its freezing point ( $-1.5^{\circ}\text{C}$ ), it can be stored up to approximately 6 weeks (42 d).

**Table 15.** Microbiological counts (colony forming units per gram sample, i.e.  $\text{cfu.g}^{-1}$ ) for Aerobic Plate Counts (APC), *E. coli*, EBC (Enterobacteriaceae), and *Pseudomonas* from respectively the hot-deboned (Hot *M. gastro*) and the cold-deboned *M. gastrocnemius, pars interna* (Cold *M. gastro*) and the hot-deboned (Hot *M. ilio*) and the cold-deboned *M. iliofibularis* (Cold *M. ilio*) at 24 h *post-mortem* and day 42 of the 42-day aging period.

<b>24 h <i>post-mortem</i></b>	<b>Hot <i>M. gastro</i></b>	<b>Cold <i>M. gastro</i></b>	<b>Hot <i>M. ilio</i></b>	<b>Cold <i>M. ilio</i></b>
APC ( $\text{cfu.g}^{-1}$ )	< 1000	< 1000	< 2000	< 500
<i>E. coli</i> ( $\text{cfu.g}^{-1}$ )	< $10^1$	< $10^1$	< $10^1$	< $10^1$
EBC ( $\text{cfu.g}^{-1}$ )	< $10^1$	< $10^1$	< $10^1$	< $10^1$
<i>Pseudomonas</i> ( $\text{cfu.g}^{-1}$ )	< 1000	< 100	< 100	< 100
<b>42 d <i>post-mortem</i></b>				
APC ( $\text{cfu.g}^{-1}$ )	< 1000	< 1000	< 1000	< 1000
<i>E. coli</i> ( $\text{cfu.g}^{-1}$ )	< $10^1$	< $10^1$	< $10^1$	< $10^1$
EBC ( $\text{cfu.g}^{-1}$ )	< $10^1$	< $10^1$	< $10^1$	< $10^1$
<i>Pseudomonas</i> ( $\text{cfu.g}^{-1}$ )	< 100	< 100	< 100	< 100

All the muscle samples from this study were within the South African Standards for the microbiological monitoring of meat (Quantum Analytical Service (Pty) Ltd., 12 Voortrekker Road, Malmesbury 7300, South Africa) for refrigerated ( $< 10\ 000\ \text{cfu.g}^{-1}$ ) export as determined by APC. The *E. coli* counts from all the muscles were also below the South African Standards for the microbiological monitoring of refrigerated meat ( $< 10\ \text{cfu.g}^{-1}$ ).

## CONCLUSIONS

Hot-deboning did not have the same effect on the *M. iliofibularis* as on the *M. gastrocnemius, pars interna* in terms of tenderness as determined by Warner-Bratzler shear force values (N.  $12.7\ \text{mm}^{-1}$  diameter). Hot-deboning caused the *M. gastrocnemius, pars interna* to be significantly tougher than the cold-deboned muscles. There were no significant differences in shear force values between the hot-deboned and the cold-deboned *M. iliofibularis*. Irrespective of deboning procedures, the *M. gastrocnemius, pars interna* were tougher than the *M. iliofibularis*. However, after 42 d of aging, all muscles were similar in tenderness. Deboning at 1 h *post-mortem* caused both the *M. gastrocnemius, pars interna* and the *M. iliofibularis* to have significantly lower pH values throughout the 42-day storage period compared to the cold-deboned muscles. With the lower pH values for the hot-deboned muscles, hot-deboned *M. gastrocnemius, pars interna* and hot-deboned *M. iliofibularis*, both had more purge (%) throughout the 42-day aging period than the cold-deboned *M. gastrocnemius, pars interna* and cold-deboned *M. iliofibularis*. In terms of the raw meat colour, hot-deboning resulted in the muscles being significantly darker and less red in colour than the cold-deboned muscles. Investigation of the difference in early *post-mortem* (first 24 h *post-mortem*) pH changes within the *M. gastrocnemius, pars interna* and *M. iliofibularis* would help elucidate the differences in the physical meat quality attributes found for different ostrich muscles.

In this study no evidence was found that hot-deboning at 1 h *post-mortem* caused an increase in bacterial contamination prior to vacuum-packaging. However, higher temperatures or longer conditioning periods prior to vacuum-packaging and chilling may show adverse effects on microbiological quality, and therefore hot-deboning and vacuum packaging during slaughter practices require careful control. It is concluded from the present study that, although ostrich muscles attain a higher pH throughout *post-mortem* storage and may possibly have a greater risk of microbiological spoilage, the microbial results indicated that both the hot-deboned and the cold-deboned ostrich muscles were suitable for export after aging for 42 d, as APC counts were below the South African Standards for the microbiological monitoring of meat (Quantum Analytical Service (Pty)



Ltd., 12 Voortrekker Road, Malmesbury 7300, South Africa) for refrigerated (< 10 000 cfu.g<sup>-1</sup>) export; as well as the counts for *E. coli* (refrigerated: < 10 cfu.g<sup>-1</sup>).

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## Chapter 6

### Muscle pH and temperature changes in hot and cold-deboned ostrich (*Struthio camelus var. domesticus*) *Muscularis gastrocnemius, pars interna* and *Muscularis iliofibularis* during the first 23 hours *post-mortem*

#### ABSTRACT

Cold-shortening is the response when muscles are exposed to temperatures below 10°C with a pH > 6.20. The decline in muscle temperature and the course of pH within hot-deboned and intact ostrich *M. gastrocnemius, pars interna* and *M. iliofibularis* were followed for the first 23-24 h *post-mortem* to investigate the course of change in pH as well as to determine the point of minimum pH for ostrich muscles. The hot-deboned *M. gastrocnemius, pars interna* and the hot-deboned *M. iliofibularis* took longer ( $6.20 \pm 0.45$  h and  $6.00 \pm 0.00$  h *post-mortem* respectively) to reach the point of minimum pH compared to the intact *M. gastrocnemius, pars interna* ( $3.50 \pm 0.84$  h *post-mortem*) and the intact *M. iliofibularis* ( $2.50 \pm 0.58$  h *post-mortem*). However, there was no significant ( $P = 0.4508$ ) difference in the minimum pH ( $5.91 \pm 0.26$ ) between the hot-deboned and the intact *M. gastrocnemius, pars interna*, nor between the hot-deboned and the intact *M. iliofibularis*. At the point of minimum pH, the intact *M. gastrocnemius, pars interna* muscles was at a lower ( $P < 0.0001$ ) temperature ( $16.50^\circ \pm 5.86^\circ\text{C}$ ) than the intact *M. iliofibularis* ( $22.90^\circ \pm 10.44^\circ\text{C}$ ). The hot-deboned muscles were also ( $13.98^\circ \pm 5.60^\circ\text{C}$ ) at lower temperatures than the intact muscles ( $24.78^\circ \pm 7.83^\circ\text{C}$ ). It is concluded that both the *M. gastrocnemius, pars interna* and the *M. iliofibularis* reached a pH < 6.20 early *post-mortem* with muscle temperatures above 10°C; and therefore showed no risk of cold-shortening if these muscles were to be hot-deboned 2-4 h *post-mortem*.

**Keywords:** minimum pH, temperature decline, pH decline, hot-deboning, ostrich

## INTRODUCTION

It is well known that when *pre-rigor* muscle attains a temperature of below 10° to 15°C while the pH is above 6.0 to 6.4 and ATP levels are still high enough for muscle contraction to occur, there is a risk of cold-shortening and consequent toughening of the meat when cooked (Honikel *et al.*, 1983; Pearson & Young, 1989; Lawrie, 1998). The rapid attainment of a low muscle pH would be beneficial towards meat tenderness, not only due to its prevention of cold-shortening, but also because it may cause the release of lysosomal enzymes and the activation of cathepsins (Marsh *et al.*, 1980-81; O'Halloran *et al.*, 1997).

Changes in pH *post-mortem* in ostrich muscles have been noted to differ considerably from that of other red muscled animals resulting in ostrich muscles to be characterized as an intermediate meat type between normal (pH < 5.8) and extreme DFD (dark, firm and dry) (pH > 6.2) meat (Sales & Mellett, 1996). The normal muscle pH profile of most red meat animals show a gradual decrease until an asymptotic minimum of about 5.4-5.5 has been reached, normally occurring over a 24 h period (Lawrie, 1998). In contrast to beef and lamb muscles, Morris *et al.* (1995) found the lowest *post-mortem* pH value for ostrich *M. iliofibularis* and *M. gastrocnemius, pars interna* occurred within 30 min after slaughter. Sales & Mellett (1996) and Sales (1994) found ostrich *M. iliofibularis* to have a very rapid pH decline until 2 h *post-mortem*, after which the pH increased. The apparent minimum pH was reached rapidly at 2 h *post-mortem* in the *M. iliofibularis* (6.00 ± 0.09), and at 6 h *post-mortem* in the *M. gastrocnemius, pars interna* (6.12 ± 0.06) (Sales & Mellett, 1996). Therefore, it was suggested by Sales & Mellett (1996) that there is a risk of cold-shortening in the *M. gastrocnemius, pars interna* if this muscle would be separated from the carcass and cooled at 30-45 min *post-mortem* but not in the *M. iliofibularis*, since the *M. iliofibularis* reached a pH = 6.20 at approximately 30 min after slaughter.

The aims of the present study were to investigate the effects of hot-deboning at 5 to 6 h *post-mortem* on the temperature (°C) and pH profiles during the first 23 to 24 h *post-mortem* of ostrich *M. gastrocnemius, pars interna* and *M. iliofibularis*. Furthermore, it was of interest to quantify the decline of muscle temperature (°C), as well as the course of the change in muscle pH during the first 23 h *post-mortem* in intact ostrich *M. gastrocnemius, pars interna* and *M. iliofibularis*, to determine the minimum pH within ostrich muscles, as well as the time in hours *post-mortem* it takes for these ostrich muscles to reach the minimum pH.

With knowledge about the time (h) it takes for ostrich muscles to reach a minimum pH early *post-mortem*, as well as the temperature of intact ostrich muscles at the point of minimum pH, it can be concluded whether there is a risk of cold-shortening in these muscles if hot-deboning is to be performed early *post-mortem*. Consequently, recommendations can then be made for guidance towards future ostrich processing technologies.

## MATERIALS AND METHODS

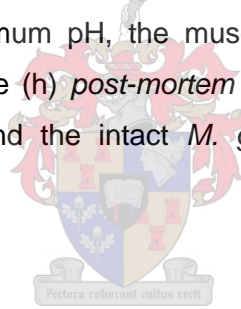
### Ostriches and muscle samples

Twelve randomly selected, well rested (12-h lairage) ostriches (*Struthio camelus var. domesticus*) were slaughtered, as described by Wotton & Sparrey (2002), over a period of 5 d during December 2003 at an EU approved abattoir at Malmesbury, South Africa. The left leg *M. gastrocnemius, pars interna* from six of the 12 ostrich carcasses were excised at approximately 2 h *post-mortem*, while the left leg *M. iliofibularis* from the other six ostrich carcasses of the 12 ostrich carcasses (carcass weight of  $43 \pm 3.95$  kg) were also excised at approximately 2 h *post-mortem*, to obtain both hot-deboned *M. gastrocnemius, pars interna* and *M. iliofibularis* before the ostrich carcasses were moved into the abattoir's cooler room ( $< 4^{\circ}\text{C}$ ). The hot-deboned muscles were then vacuum packaged and stored, next to the intact *M. gastrocnemius, pars interna* and *M. iliofibularis* (i.e. the carcasses from which the hot-deboned muscles were excised) in the abattoir's cooler room at an average temperature of  $< 4^{\circ}\text{C}$  from approximately 3-4 h *post-mortem*. From approximately 5-6 h *post-mortem*, pH and temperature SenTix 41 probes (Germany), connected to a portable pH meter 340i (WTW GmbH & Co. KG, Weilheim, Germany), were inserted at a depth of 3 cm into the hot-deboned *M. gastrocnemius, pars interna* and hot-deboned *M. iliofibularis*, thereby, breaking the vacuum seal. Muscle pH and temperature ( $^{\circ}\text{C}$ ) were thus measured and recorded continuously every 10 min for the first 23 h *post-mortem* in the six hot-deboned *M. gastrocnemius, pars interna* and in the six hot-deboned *M. iliofibularis*.

The right leg *M. gastrocnemius, pars interna* of the six ostrich carcasses from which the hot-deboned *M. gastrocnemius, pars interna* were excised, as well as the right leg *M. iliofibularis* of the other six ostrich carcasses from which the hot-deboned *M. iliofibularis* were excised, were left intact on the carcass and stored in the abattoir's cooler room from approximately 2 h *post-mortem*. The muscle pH and temperature ( $^{\circ}\text{C}$ ) of the intact *M. gastrocnemius, pars interna* and the intact *M. iliofibularis* were measured and recorded on the carcasses, from 2 h *post-mortem* prior to hot-deboning of the left leg *M.*

*gastrocnemius, pars interna* and *M. iliofibularis*, for the first 23 h *post-mortem* by inserting SenTix 41 probes (Germany), connected to portable pH meters 340i (WTW GmbH & Co. KG, Weilheim, Germany), into the respective six intact *M. gastrocnemius, pars interna* and the respective six intact *M. iliofibularis* at a dept of approximately 4 to 5 cm. The carcasses were stored in the abattoir's cooler room at < 4°C for the entire 23 to 24-hour measuring period.

The temperature (°C) and pH data from the hot-deboned and the intact *M. gastrocnemius, pars interna* and *M. iliofibularis* were then used to construct figures in Excel in an attempt to quantify the hourly changes in *post-mortem* muscle temperature (°C) and pH for the first 23 h *post-mortem*. Due to technical problems with the pH meters, not all the data from all 12 ostrich carcasses could be presented in the graphs, and therefore, the temperature and pH data for the hot-deboned and the intact *M. gastrocnemius, pars interna* from four ostrich carcasses, that are representative of the group, are presented in Fig. 1 and Fig. 3 respectively, while the temperature and pH data for the hot-deboned and intact *M. iliofibularis* from three ostrich carcasses are presented in Fig. 2 and Fig. 4 respectively. The temperature (°C) and pH data were also used to obtain and investigate the minimum pH, the muscle temperature (°C) at the point of minimum pH, as well as the time (h) *post-mortem* it took to reach the minimum pH for respectively the hot-deboned and intact *M. gastrocnemius, pars interna* and *M. iliofibularis*.



### Statistical analyses

The results for the minimum pH, muscle temperature (°C) at the point of minimum pH and the time (h) *post-mortem* it took to reach the minimum pH were part of a complete randomised block design, performed with two treatments (hot-deboning and intact muscles) replicated in 6 blocks (ostrich carcasses) for respectively the *M. gastrocnemius, pars interna* and *M. iliofibularis*. The data were subjected to factorial analysis of variance (ANOVA) using SAS version 8.2 statistical software (SAS, 1999). Shapiro-Wilk tests were performed for testing non-normality (Shapiro & Wilk, 1965). The results from the factorial analysis of variance (ANOVA) and Shapiro-Wilk tests for the minimum pH values, the temperature (°C) at which the minimum pH was reached and the time (h) *post-mortem* at which the minimum pH was reached with muscle (*M. gastrocnemius, pars interna* and *M. iliofibularis*) and deboning (hot-deboned and intact) as main effects, as well as interaction between main effects, are summarised in Table 1.

## RESULTS AND DISCUSSION

The analysis of variance (ANOVA) of the dependable variables: minimum pH, the temperature (°C) at which the minimum pH was reached and the time (h) *post-mortem* at which the minimum pH was reached in respectively the hot-deboned and intact *M. gastrocnemius, pars interna* and *M. iliofibularis* are presented in Table 1. The data for the temperature (°C) at minimum pH was normally distributed ( $P = 0.2179$ ), while the data for the minimum pH and the data for the time (h) *post-mortem* at which the minimum pH was reached were not normally distributed ( $P < 0.05$ ) due to Kurtosis (value not equal to 0). However, the latter does not have a significant effect on the normality of the data (Glass *et al.*, 1972), therefore, the data was analysed without transformation.

There was no significant interaction between muscle and deboning for any of the dependable variables ( $P > 0.05$ ). There were also no significant differences between muscles (Table 2) for the minimum pH ( $P = 0.4508$ ), nor for the time (h) *post-mortem* to reach the minimum pH ( $P = 0.2904$ ). However, deboning had a significant effect ( $P = 0.0003$ ) on the temperature (°C) when the minimum pH was reached, causing respectively the hot-deboned *M. gastrocnemius, pars interna* ( $11.54^{\circ} \pm 1.16^{\circ}\text{C}$ ) and the hot-deboned *M. iliofibularis* ( $16.42^{\circ} \pm 7.37^{\circ}\text{C}$ ) to attain lower temperatures than the intact *M. gastrocnemius, pars interna* ( $20.63^{\circ} \pm 4.73^{\circ}\text{C}$ ) and the intact *M. iliofibularis* ( $31.00^{\circ} \pm 7.78^{\circ}\text{C}$ ).

**Table 1.** Analysis of variance (ANOVA) of the dependable variables: minimum pH values, the temperature (°C) at which the minimum pH was reached and the time (h) *post-mortem* at which the minimum pH was reached with muscle and deboning as main effects, two way interaction between muscle and deboning, as well as the Shapiro-Wilk test for non-normality.

Source	df	Minimum pH		Temperature (°C) at minimum pH		Time (h) of minimum pH	
		MS	P	MS	P	MS	P
Muscle	1	0.040	0.4508	202.752	0.0234	0.396	0.2904
Deboning	1	0.049	0.4088	661.045	0.0003	46.320	< 0.0001
Muscle*Deboning	1	0.155	0.1490	36.861	0.3011	0.784	0.1436
Error	16	0.067		32.278		0.331	
Shapiro-Wilk			0.0038		0.2179		0.0264

df – Degree of freedom

MS – Mean Square

P – Probability value of F-ratio test

\*Interaction between main effects



**Table 2.** Mean ( $\pm$  Standard Deviation) minimum pH, the temperature ( $^{\circ}\text{C}$ ) at which the minimum pH was reached and the time (h) *post-mortem* at which the minimum pH was reached for the hot-deboned and the intact *M. gastrocnemius, pars interna* and the hot-deboned and the intact *M. iliofibularis*, respectively.

Muscle	Minimum pH	Temperature ( $^{\circ}\text{C}$ ) at minimum pH	Time (h) to reach minimum pH
<i>M. gastrocnemius, pars interna</i>			
Hot-deboned	5.81 $\pm$ 0.17 <sup>a</sup>	11.54 $\pm$ 1.16 <sup>c</sup>	6.20 $\pm$ 0.45 <sup>a</sup>
Intact	6.07 $\pm$ 0.41 <sup>a</sup>	20.63 $\pm$ 4.73 <sup>b</sup>	3.50 $\pm$ 0.84 <sup>b</sup>
<i>M. iliofibularis</i>			
Hot-deboned	5.90 $\pm$ 0.16 <sup>a</sup>	16.42 $\pm$ 7.37 <sup>b</sup>	6.00 $\pm$ 0.00 <sup>a</sup>
Intact	5.81 $\pm$ 0.07 <sup>a</sup>	31.00 $\pm$ 7.78 <sup>a</sup>	2.50 $\pm$ 0.58 <sup>c</sup>

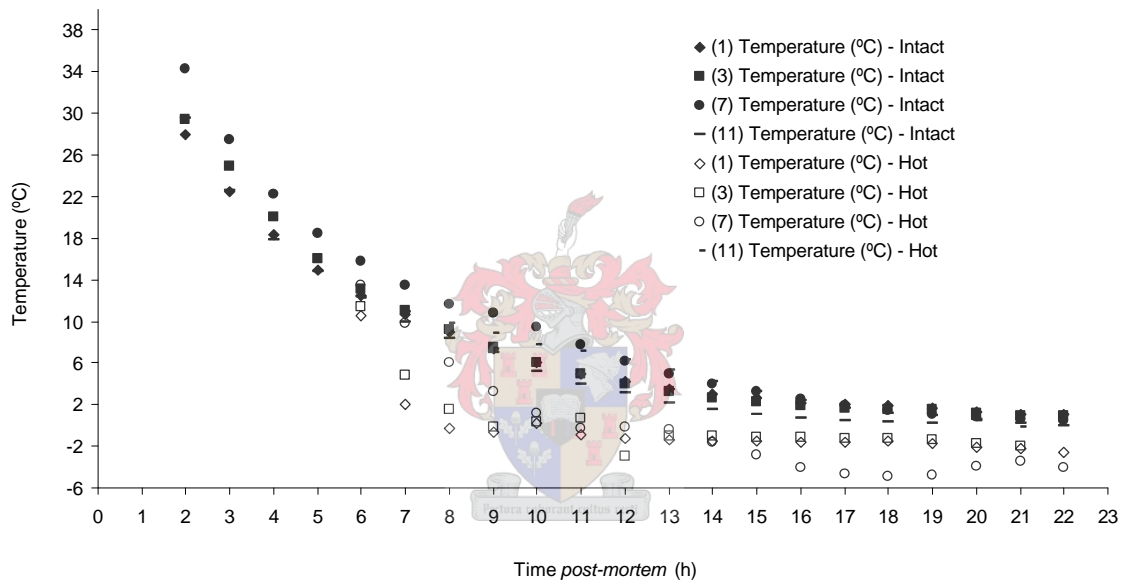
<sup>ab</sup> Different superscripts within columns differ at  $P < 0.05$ .

From the data in Fig. 1 and Fig. 2 it can be seen that the mean temperature at 2 h *post-mortem* for the intact *M. gastrocnemius, pars interna* ( $29.33^{\circ} \pm 5.19^{\circ}\text{C}$ ) was lower than for the intact *M. iliofibularis* ( $34.50^{\circ} \pm 3.07^{\circ}\text{C}$ ). This difference in muscle temperature at 2 h *post-mortem* between these two muscle types can be explained by the anatomical location of the muscles in the ostrich leg. The *M. gastrocnemius, pars interna* is a superficial muscle (Fig. 3) in the ostrich leg (pelvic limb), while the *M. iliofibularis* is located deeper within the ostrich leg (Fig. 4) (Mellett, 1994), therefore remaining at higher temperatures *in vivo* during the first hours *post-mortem* compared to the *M. gastrocnemius, pars interna*. None the less, after refrigeration at  $< 4^{\circ}\text{C}$  for 20 h, the intact *M. gastrocnemius, pars interna* ( $0.45^{\circ} \pm 0.48^{\circ}\text{C}$ ) and the intact *M. iliofibularis* ( $0.47^{\circ} \pm 0.38^{\circ}\text{C}$ ) were at similar temperatures.

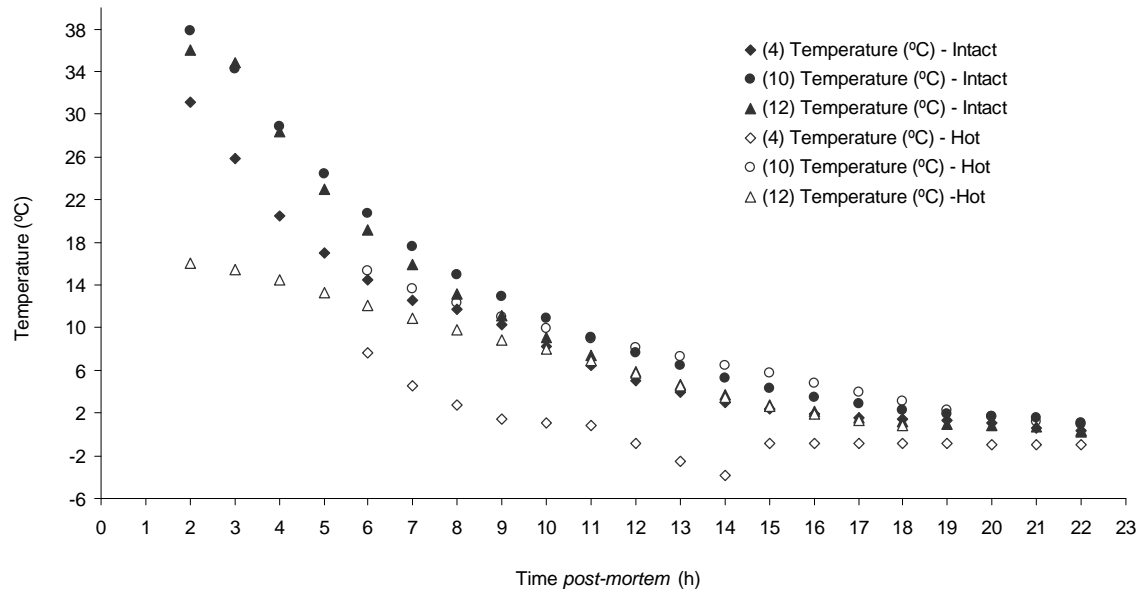
What is also evident from the data in Fig. 1 and Fig. 2 is that the muscle temperature of some of the hot-deboned *M. gastrocnemius, pars interna* and hot-deboned *M. iliofibularis* declined below  $0^{\circ}\text{C}$  during the 20 h measuring period, while other hot-deboned muscles followed a similar trend of decline in muscle temperature to that of the intact muscles, also reaching a temperature of just above  $0^{\circ}\text{C}$  at 22 h *post-mortem*. Although the temperature of the cold room at the abattoir was set at  $4^{\circ}\text{C}$ , due to the prolonged shelf-life (42 d) required by the ostrich industry, it is common practice for the abattoir to allow the temperature to run a cycle resulting in ambient temperatures below  $0^{\circ}\text{C}$ . However, care is always taken to ensure that the meat does not freeze as this would result in the meat losing its “fresh” status. It may be hypothesised that the lower muscle temperature for some of the hot-deboned muscles was due to the location of the muscles

in the abattoir's cooler room, being closer to the fan of the cooling system, or closer to the floor of the cooler room, causing the temperature of these muscles to decrease more rapidly and to a lower temperature compared to the cold-deboned muscles placed at a different location in the cooler room.

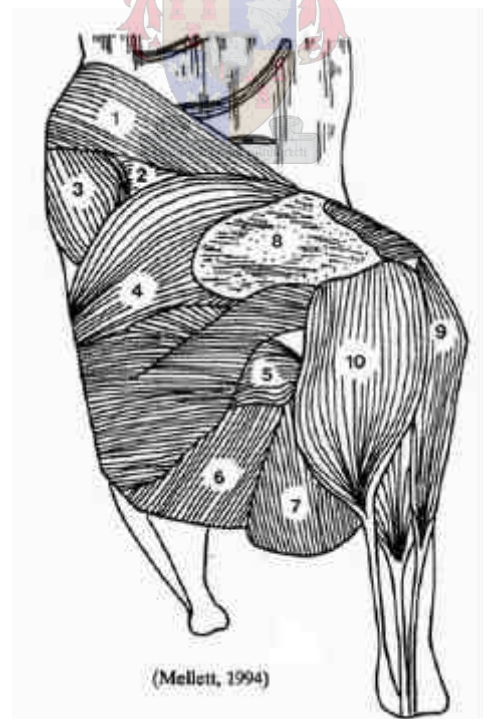
It is well known that the rates of cooling are more rapid in hot-deboned vacuum-packaged meat cuts compared to muscles left on the carcass, and therefore cold-shortening and consequent toughening of the meat would be more readily induced in hot-deboned muscles than in muscles left on the carcass, refrigerated at the same temperature (Lawrie, 1998).



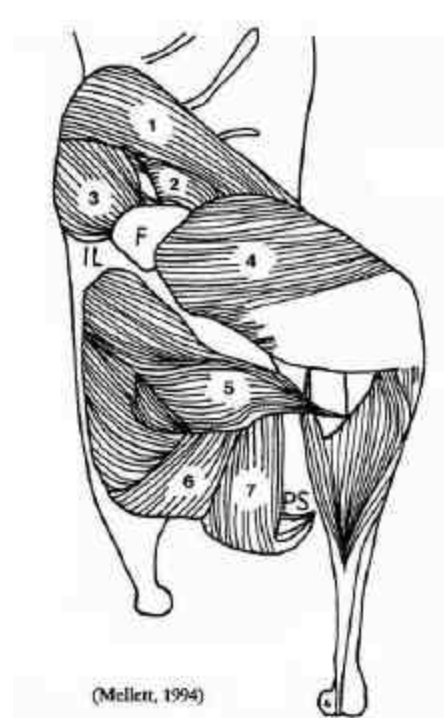
**Figure 1.** The decline in muscle temperature ( $^{\circ}\text{C}$ ) during the first 22 h *post-mortem* from respectively 2 h *post-mortem* for the intact *M. gastrocnemius, pars interna* (Intact) and from 6 h *post-mortem* for the hot-deboned *M. gastrocnemius, pars interna* (Hot) from four individual ostrich carcasses cooled at  $< 4^{\circ}\text{C}$ . (The numbers 1, 3, 7 and 11 in parenthesis represents the identity of the individual ostrich carcasses).



**Figure 2.** The decline in muscle temperature (°C) during the first 22 h *post-mortem* from respectively 2 h *post-mortem* for the intact *M. iliofibularis* (Intact) and from 6 h *post-mortem* for the hot-deboned *M. iliofibularis* (Hot) from three individual ostrich carcasses cooled at < 4°C. (The numbers 2, 10 and 12 in parenthesis represents the identity of the individual ostrich carcasses).



**Figure 3.** Lateral view of the ostrich leg, illustrating the muscles of the superficial layer of the pelvic limb, showing the anatomical location of the *M. gastrocnemius* (number 10; Mellett, 1994).



**Figure 4.** Lateral view of the ostrich leg, illustrating the muscles of the second layer of the pelvic limb, showing the anatomical location of the *M. iliofibularis* (Number 5; Mellett, 1994).

The variation in muscle pH between the individual ostrich carcasses for respectively the *M. gastrocnemius, pars interna* (Fig. 5) and the *M. iliofibularis* (Fig. 6) can partly be explained by the intrinsic variation found naturally between ostriches, as well as different levels of pre-slaughter stress and consequent differences in the levels of *post-mortem* muscle glycogen, leading therefore to different pH values between the individual ostrich carcasses. In addition, all the ostrich carcasses used for the present study were not slaughtered on the same day and were thus not from the same producer or from the same farm, factors that could therefore also explain the assumed differences in energy levels between the individual ostriches.

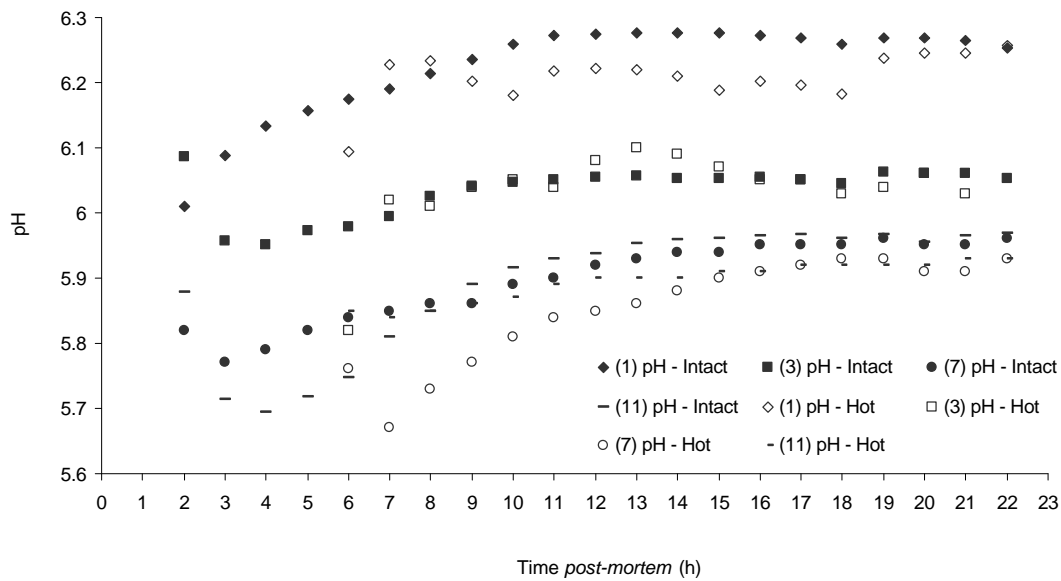
From the data in Fig. 5 it can be seen that all the intact *M. gastrocnemius, pars interna* showed a decrease in pH from 2 h *post-mortem* to approximately 4 h *post-mortem*, reaching a mean minimum pH of  $6.07 \pm 0.41$  at approximately  $3.50 \pm 0.84$  h *post-mortem* (Tables 1 and 2). After reaching the minimum pH, the pH then increased to a mean value of  $6.26 \pm 0.41$  at 22 h *post-mortem*. For the intact *M. iliofibularis* (Fig. 6) only some of the muscles showed a similar decline in pH from 2 h *post-mortem* to approximately 3 h *post-mortem*, while all the intact *M. iliofibularis* increased in pH to a mean value of  $6.09 \pm 0.06$  at 22 h *post-mortem*. This indicated that the initial decline in pH during the first 2-4 h *post-*

*mortem* was not measured and recorded for all the intact *M. iliofibularis*. However, for these muscles, it was assumed that the pH values measured at 2 h *post-mortem* was the minimum pH as was the case for the intact *M. gastrocnemius, pars interna*. This also indicated that the rate of pH decline was faster in the intact *M. iliofibularis* than in the intact *M. gastrocnemius, pars interna*, reaching a mean minimum pH of  $5.81 \pm 0.07$  at approximately  $2.50 \pm 0.58$  h *post-mortem*.

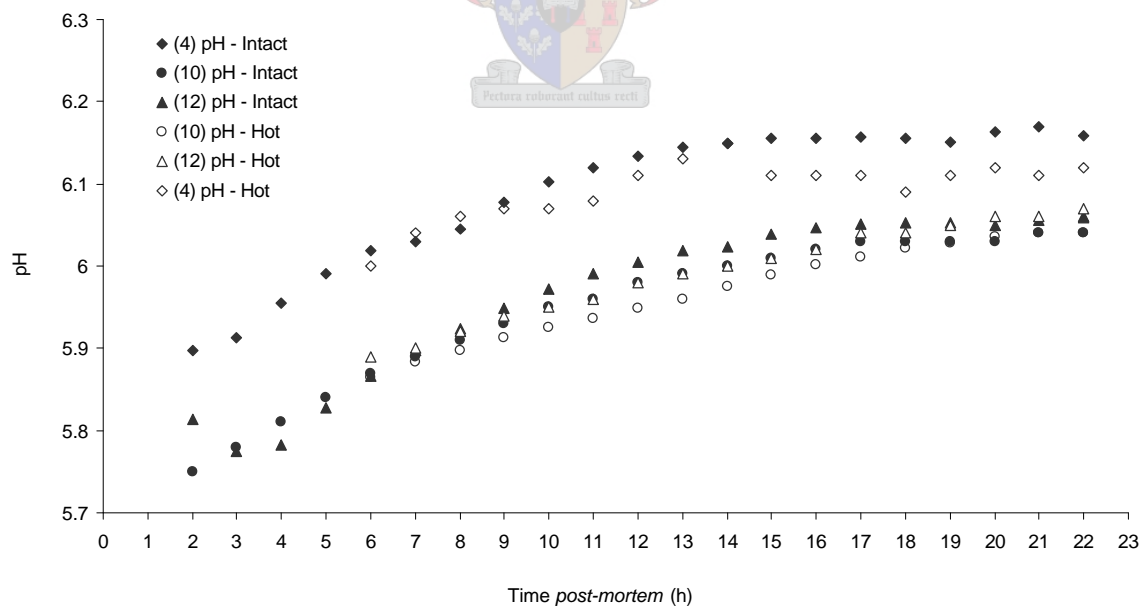
Similar to these results, Sales & Mellett (1996) also found a rapid decline in pH during the first 2 h *post-mortem* for intact *M. iliofibularis*, after which the pH increased to a value of  $6.13 \pm 0.10$  at 24 h *post-mortem*. The increase in the pH is difficult to explain, but it can be hypothesised that the fast decline in pH early *post-mortem* enhanced the release of cathepsins from the lysosomes, and in addition, calpain I was uninhibited by calpastatin under the influence of the low pH (O'Halloran *et al.*, 1997). This early *post-mortem* proteolysis leads to the production of amino-acids and the change in ion-protein relationships, thereby causing a rise in muscle pH earlier *post-mortem* than what is commonly observed for beef, lamb and pork muscles (Lawrie, 1998).

In contrast to the present study, Sales & Mellett (1996) indicated that intact *M. gastrocnemius, pars interna* reached a minimum pH at 6 h *post-mortem* and reported no subsequent increase in pH. These authors also concluded that there is no risk of cold-shortening if the *M. iliofibularis* is to be separated from the carcass early *post-mortem*, while there is a risk of cold-shortening if the *M. gastrocnemius, pars interna* is to be excised within 2 to 3 h *post-mortem*. However, results from this study indicated that the cold-deboned *M. gastrocnemius, pars interna* reached a pH value less than 6.20 within 2 to 4 h *post-mortem*. There should therefore be no risk of cold-shortening if this muscle is to be excised early *post-mortem*.

Investigating the effects of hot-deboning at 6 h *post-mortem* on the pH profile of respectively *M. gastrocnemius, pars interna* and *M. iliofibularis*, it can be seen from the data in Fig. 5 and Fig. 6 that, although the pH of the hot-deboned *M. gastrocnemius, pars interna* and *M. iliofibularis* (from 6 h *post-mortem* to 22 h *post-mortem*) were lower than that of the intact muscles, these differences in pH between the hot-deboned and the intact *M. gastrocnemius, pars interna* and *M. iliofibularis* were not significant (Table 1); and the change in pH for the hot-deboned muscles followed the same trend as in the intact muscles. However, the lower pH values for the hot-deboned *M. gastrocnemius, pars interna* and *M. iliofibularis* could be explained by the lower muscle temperatures attained by the hot-deboned muscles compared to the intact muscles, since low temperatures slow the rate of *post-mortem* reactions within muscles (Lawrie, 1998). This indicated that temperature had a significant effect on the *post-mortem* proteolysis, which most probably caused the subsequent increase in the pH.



**Figure 5.** The course of pH change during the first 22 h *post-mortem* from 2 h *post-mortem* for the intact *M. gastrocnemius, pars interna* (Intact) and from 6 h *post-mortem* for the hot-deboned *M. gastrocnemius, pars interna* (Hot) from four individual ostrich carcasses cooled at  $< 4^{\circ}\text{C}$ . (The numbers 1, 3, 7 and 11 in parenthesis represents the identity of the individual ostrich carcasses).



**Figure 6.** The course of pH change during the first 22 h *post-mortem* from 2 h *post-mortem* for the intact *M. iliofibularis* (Intact) and from 6 h *post-mortem* for the hot-deboned *M. iliofibularis* (Hot) from three individual ostrich carcasses cooled at  $< 4^{\circ}\text{C}$ . (The numbers 4, 10 and 12 in parenthesis represents the identity of the individual ostrich carcasses).

## CONCLUSIONS

The results from this study indicate that ostrich *M. gastrocnemius, pars interna* and *M. iliofibularis* have a rapid pH decline *post-mortem*, reaching a minimum pH < 6.20 within 2-4 h *post-mortem* while the muscle temperatures are still > 10°C; and therefore there is no great risk of cold-shortening if these muscles are to be excised early *post-mortem*. An aspect that warrants further investigation is the cause(s) for the increase in the pH after the minimum pH had been reached.

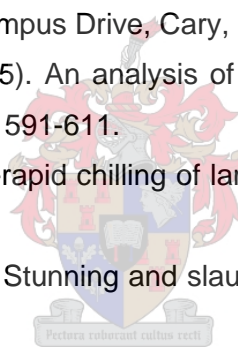
## ACKNOWLEDGEMENTS

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## Chapter 7

### The effect of *post-mortem* temperature on isometric tension, shortening and pH in ostrich (*Struthio camelus var. domesticus*) *Muscularis gastrocnemius, pars interna*

#### ABSTRACT

Fully developed rigor in muscles is characterised by the maximum loss of extensibility. The course of *post-mortem* changes in ostrich muscle were registered by following isometric tension, shortening and the change in pH during the first 23 to 24 h *post-mortem* within muscle strips from the *M. gastrocnemius, pars interna* at constant temperatures of 7° and 37°C. Maximum tension was ( $P = 0.1321$ ) higher at 7°C ( $347.48 \pm 41.53 \text{ mN}\cdot\text{mm}^{-2}$ ) than at 37°C ( $284.83 \pm 82.44 \text{ mN}\cdot\text{mm}^{-2}$ ), while maximum shortening was significantly ( $P < 0.0001$ ) higher at 37°C ( $33.39 \pm 3.57\%$ ) compared to 7°C ( $10.69 \pm 2.63\%$ ). The rate of rigor development was temperature dependent, reaching a maximum tension at  $4.08 \pm 3.89 \text{ h post-mortem}$  in muscle strips at 37°C; while at 7°C maximum tension was reached at  $10.50 \pm 6.47 \text{ h post-mortem}$ . Maximum shortening was also reached earlier in strips at 37°C ( $5.59 \pm 1.53 \text{ h}$ ) than at 7°C ( $23.00 \pm 0.45 \text{ h}$ ). Intact muscles reached a minimum pH ( $5.85 \pm 0.22$ ) at approximately 2 h *post-mortem*, while muscle strips at 37°C reached a minimum pH ( $5.76 \pm 0.13$ ) within  $4.83 \pm 3.82 \text{ h post-mortem}$ , and muscle strips at 7°C reached a minimum pH ( $5.94 \pm 0.21$ ) at  $6.42 \pm 4.51 \text{ h post-mortem}$ . It was concluded that the change in the *post-mortem* pH, as well as the development of *rigor mortis* was highly temperature dependent. It was also concluded that the completion of rigor occurred at the point of minimum pH. It is suggested that hot-deboning of ostrich muscles should occur after 3-4 h *post-mortem* to avoid the early occurrence of rigor shortening at high muscle temperatures or the occurrence of super contraction and cold-shortening under the influence of cold temperatures.

**Keywords:** *rigor mortis*, isometric tension, shortening, ostrich, rigor shortening, cold-shortening, hot-deboning, rate of pH decline

## INTRODUCTION

During the development of *rigor mortis*, muscles become inextensible due to the sum of each muscle fibre going into full rigor, with irreversible cross bridge formation (actomyosin) of the contractile components, actin and myosin (Hwang *et al.*, 2003). Bendall (1973) defined the onset of *rigor mortis* to be the beginning of the decrease in extensibility of the muscle, while completion of rigor is seen as the maximum loss of extensibility (Honikel *et al.*, 1983). The rigor process consists in the first place of a delay period, when the level of ATP (adenosine triphosphate) is constant; CP (creatine phosphate) is falling rapidly and there is a slow production of lactate and no onset of rigor development. This is then followed by a rapid phase when CP is low enough to initiate a rapid decline in ATP concentration, which is accompanied by a decreasing extensibility of the muscle due to irreversible cross bridge formation of actin and myosin (Bendall, 1973). Shortening of muscles should take place before the onset of *rigor mortis* since contraction requires a minimum ATP concentration and an increase in the level of  $\text{Ca}^{2+}$  ions around the myofibrils (Honikel *et al.*, 1983).

Cold-shortening occurs when muscles are exposed to low temperatures (below 10° to 15°C) early *post-mortem*, when ATP and pH (above 6.20) levels are still high (Nuss & Wolfe, 1980-81; Lawrie, 1998). On the other hand, rigor tension occurs much later and at any temperature between 0° and 37°C, reaching maximum values when ATP levels have been depleted and pH is at a minimum value (Nuss & Wolfe, 1980-81). The stiffness characteristic of *rigor mortis* is then maintained by continuous tension exerted by the cross-bridges between myosin and actin filaments (Pearson & Young, 1989).

In both pork and beef muscles, shortening is a major determinant of tenderness when the tissue has not yet entered *rigor mortis* and when the rate of muscle pH decline is rapid (Møller & Vestergaard, 1987; Smulders *et al.*, 1990). It has been reported by Tornberg (1996) that a more shortened beef muscle has a higher cooking loss and higher numbers of fibres per unit cross-area, leading to higher Warner-Bratzler peak shear force values and greater toughness. Marsh & Leet (1966) and Locker & Daines (1975) also reported shortened beef muscles to be tougher, while Powell (1978) and Honikel *et al.* (1980) reported higher drip losses in contracted than in non-shortened muscles. Hot-deboned (1 h *post-mortem*) ostrich *M. gastrocnemius, pars interna* had shorter sarcomere lengths at 24 h *post-mortem* than cold-deboned muscles (Chapter 4 of this thesis). Also, a higher percentage purge was found in hot-deboned vacuum-packaged ostrich *M. gastrocnemius, pars interna* during *post-mortem* storage of 21 and 42 d than in cold-deboned muscles (Chapters 5 and 6 of this thesis).

Knowledge of the course of *rigor mortis* and *post-mortem* pH changes, as well as the influence of temperature on the onset of *rigor mortis* might help to reduce purge and initial toughness in hot-deboned ostrich muscles. Therefore the aim of this study was to investigate the development of isometric tension (developing tension while the muscle is prevented from contracting) and shortening in ostrich *M. gastrocnemius, pars interna* during *rigor mortis* at respectively 7° and 37°C in an attempt to determine the time course of rigor, pH decline, and degree and extent of shortening (occurrence of cold-shortening and/or rigor shortening). From this investigation, recommendations can be made towards the hot-deboning and vacuum packaging of whole ostrich muscles as soon as possible after bleeding without detrimental effects on the quality of the meat, and therefore provide guidance towards future ostrich processing technologies.

## MATERIALS AND METHODS

### Ostriches and muscle samples

Ten rested (12 h of lairage) ostriches (*Struthio camelus var. domesticus*) from different farms were slaughtered as described by Wotton & Sparrey (2002), over a 3-month period (February to April 2004) at the same EU approved ostrich abattoir at Malmesbury, South Africa. The right leg *M. gastrocnemius, pars interna* were removed from the ostrich carcasses within 20 min to 1 h after stunning and exsanguination. Two muscle strips were carefully cut, parallel to the fibre axis, from each muscle sample. The length of the strips was 30 mm, with dimensions of 10 x 10 x 30 mm, weighing between 1.5 and 3 g. The cross-sectional area of each muscle strip was calculated with the use of Rigotech® software (RigoTech version 3.0, ©ReoLogica, 1999) by using the length and the weight of the strip together with the density of the muscle (1.06 g. cm<sup>-3</sup>). The muscle strips were glued onto the aluminium discs of the rigometer with cyanoacrylate glue (Lynlim Superglue, Norway). To provide an anaerobic environment and to minimise dehydration, the strips were covered with a mixture of paraffin oil and petroleum jelly. Two separate rigometers (Rigotech®) at constant temperatures of 7° and 37°C respectively were used to record isometric tension and muscle shortening during *rigor mortis* every 15 min for the first 23 to 24 h *post-mortem*. Isometric tension was expressed as force per unit area (mN.mm<sup>-2</sup>) and shortening was expressed as the percentage decrease in length relative to the initial length of the muscle strip.

Muscle pH was measured by using SenTix 41 probes (Germany), connected to portable pH meters 340i (WTW, GmbH & Co. KG, Weilheim, Germany). The pH probes were placed directly into larger portions of the muscles. These larger portions of the

muscle were also covered with a mixture of paraffin oil and petroleum jelly to minimise dehydration and placed into the rigometers at 7° and 37°C respectively, for continuous measurement of pH every 10 min during the rigor process.

The change in pH and temperature (°C) were also measured continuously every 10 min for the first 23 to 24 h *post-mortem* in the intact left leg *M. gastrocnemius, pars interna* from the same ostrich carcasses by inserting pH and temperature probes (SenTix 41 probes, Germany), connected to portable pH meters 340i (WTW, GmbH & Co. KG, Weilheim, Germany) at a dept of approximately 4 to 5 cm into the intact *M. gastrocnemius, pars interna* at 1 h *post-mortem*. At approximately 2 h *post-mortem*, these carcasses (with the pH-meters inserted in the intact left leg *M. gastrocnemius, pars interna*) were moved to the refrigerator (< 4°C) for 24 h at the abattoir.

### Statistical analyses

The results for the isometric tension and muscle shortening were part of a complete randomised block design, performed with two treatments (muscle strips at 7° and 37°C, respectively) replicated in ten blocks (ostrich carcasses). The data were subjected to factorial analysis of variance (ANOVA) using SAS version 8.2 statistical software (SAS, 1999). Shapiro-Wilk tests were performed for testing non-normality (Shapiro & Wilk, 1965). Results from the factorial analysis of variance (ANOVA) and Shapiro-Wilk tests for dependable variables: maximum tension (mN.mm<sup>-2</sup>), pH at maximum tension, maximum shortening (%), pH at maximum shortening, time (h) *post-mortem* to reach maximum tension and time (h) *post-mortem* to reach maximum shortening with ostrich and temperature (°C) as main effects, as well as the Shapiro-Wilk test for non-normality, are presented in Tables 1, and 2.

The results from the continuous pH measurements were also part of a complete randomised block design, performed with three treatments, replicated in ten blocks (ostrich carcasses). The three treatments included the intact *M. gastrocnemius, pars interna* refrigerated for 24 h at < 4°C, excised (between 20 min and 1 h *post-mortem*) *M. gastrocnemius, pars interna* from which muscle strips were kept at respectively 7° and 37°C in two separate Rigometers. The data were subjected to factorial analysis of variance (ANOVA) using SAS version 8.2 statistical software (SAS, 1999). Shapiro-Wilk tests were performed for testing non-normality (Shapiro & Wilk, 1965). Results from the factorial analysis of variance (ANOVA) and Shapiro-Wilk tests for dependable variables: minimum pH and the time (h) *post-mortem* at which the minimum pH was reached with ostrich and deboning [intact *M. gastrocnemius, pars interna* refrigerated for 24 h at < 4°C, excised (20 min to 1 h *post-mortem*) *M. gastrocnemius, pars interna* from which muscle

strips were kept at respectively 7° and 37°C in two separate rigometers] as main effects, as well as the Shapiro-Wilk test for non-normality are presented in Table 3.

## RESULTS AND DISCUSSION

### Tension and Shortening

The analysis of variance (ANOVA) of the dependable variables: maximum tension (mN.mm<sup>-2</sup>), the pH at maximum tension, as well as the time (h) *post-mortem* to reach maximum tension are presented in Table 1. The data for the maximum tension ( $P = 0.9941$ ), the pH values at maximum tension ( $P = 0.9163$ ) and the time (h) at which maximum tension was reached ( $P = 0.9032$ ), were normally distributed, therefore the data were analysed without transformation thereof. No significant differences were found between the individual ostrich carcasses for the maximum tension ( $P = 0.3783$ ), for the pH values at maximum tension ( $P = 0.3501$ ), nor for the time (h) *post-mortem* to reach maximum tension ( $P = 0.8490$ ). Temperature (°C) also had no significant effect ( $P = 0.1321$ ) on the maximum tension. Nevertheless, the maximum tension at 7°C ( $347.48 \pm 41.53$  mN.mm<sup>-2</sup>) was higher than the maximum tension ( $284.83 \pm 82.44$  mN.mm<sup>-2</sup>) at 37°C. Similarly, the pH value at the time of maximum tension was higher for muscle strips at 7°C ( $6.07 \pm 0.30$ ) compared to muscle strips at 37°C ( $5.87 \pm 0.13$ ), but again this was insignificant ( $P = 0.2475$ ).

Although not significant ( $P = 0.1454$ ), the rate of tension development and the time it took to reach maximum tension differed between 7° and 37°C. Muscle strips going into rigor at 37°C reached maximum tension within  $4.08 \pm 3.89$  h *post-mortem*, while at 7°C, muscle strips took a longer time ( $10.50 \pm 6.47$  h) to reach maximum tension, indicating a slower rate of tension development at 7°C. Nuss & Wolfe (1980-81) also indicated that the time to reach maximum tension in beef muscles decreased with an increase in the temperature from 5° to 37°C. Devine *et al.* (1999) also demonstrated that the time for bovine muscle to reach maximum tension was highly negatively correlated with temperature.

The analysis of variance (ANOVA) of the dependable variables: maximum shortening (%), the pH at maximum shortening and the time (h) *post-mortem* it took to reach maximum shortening are presented in Table 2. The data for the percentage maximum shortening ( $P = 0.8620$ ) and the data for the pH values at maximum shortening ( $P = 0.6608$ ) were normally distributed; therefore analyses were performed without transformation of the data. However, the data for the time (h) to reach maximum shortening was not normally distributed ( $P = 0.0012$ ) due to the occurrence of Kurtosis.

As the latter does not have a significant effect on the normality of the data (Glass *et al.*, 1972); the data was analysed without transformation thereof. The individual ostrich carcasses showed no significant difference for maximum shortening ( $P = 0.5398$ ), nor for the pH values at maximum shortening ( $P = 0.1059$ ). There was also no significant difference in the time (h) *post-mortem* to reach maximum shortening between the individual ostrich carcasses ( $P = 0.5561$ ).

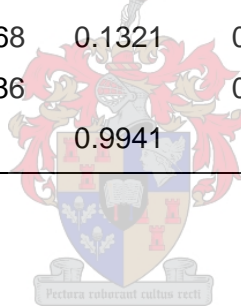
**Table 1.** Analysis of variance (ANOVA) of the dependable variables: maximum tension ( $\text{mN}\cdot\text{mm}^{-2}$ ), pH at maximum tension and the time (h) *post-mortem* to reach maximum tension with ostrich carcass and temperature ( $^{\circ}\text{C}$ ) as main effects, as well as the Shapiro-Wilk test for non-normality.

Source	df	Maximum tension ( $\text{mN}\cdot\text{mm}^{-2}$ )		pH at Maximum tension		Time (h) to reach maximum tension	
		MS	P	MS	P	MS	P
Ostrich carcass	5	4877.631	0.3783	0.061	0.3501	15.471	0.8490
Temperature	1	11775.068	0.1321	0.072	0.2475	123.521	0.1454
Error	5	3642.686		0.039		41.571	
Shapiro-Wilk			0.9941		0.9163		0.9032

df – Degree of freedom

MS – Mean Square

P – Probability value of F-ratio test



Maximum shortening was significantly ( $P < 0.0001$ ) higher at  $37^{\circ}\text{C}$  ( $33.39 \pm 3.57\%$ ) than at  $7^{\circ}\text{C}$  ( $10.69 \pm 2.63\%$ ), while the pH values at the time of maximum shortening did not differ significantly ( $P = 0.7539$ ) for respectively  $7^{\circ}\text{C}$  ( $6.11 \pm 0.25$ ) and  $37^{\circ}\text{C}$  ( $6.09 \pm 0.37$ ). This indicated that the muscle strips at  $7^{\circ}$  and  $37^{\circ}\text{C}$  from the same individual ostrich carcass were, as expected, at the same initial energy levels (lactate and ATP concentrations) early *post-mortem* when the muscle strips were placed into the rigometers at  $7^{\circ}$  and  $37^{\circ}\text{C}$ , respectively, leading therefore to similar pH values at the point of maximum tension development. The significant difference ( $P = 0.0006$ ) in the time to reach maximum shortening for the main effect “temperature”, indicated that the maximum shortening was reached sooner in the muscle strips at  $37^{\circ}\text{C}$  ( $5.59 \pm 3.65$  h) than the muscle strips at  $7^{\circ}\text{C}$  ( $23.00 \pm 0.45$  h). This also indicated that the rate of muscle shortening was therefore faster at  $37^{\circ}$  than at  $7^{\circ}\text{C}$ .

**Table 2.** Analysis of variance (ANOVA) of the dependable variables: maximum shortening (%), pH at maximum shortening and the time (h) *post-mortem* to reach maximum shortening with ostrich carcass and temperature (°C) as main effects, as well as the Shapiro-Wilk test for non-normality.

Source	df	Maximum shortening (%)		pH at Maximum shortening		Time (h) to reach maximum shortening		
		MS	P	MS	P	df	MS	P
Ostrich carcass	5	9.384	0.5398	0.153	0.1059	5	18.458	0.2096
Temperature	1	1546.780	< 0.0001	0.004	0.7539	1	757.422	0.0006
Error	5	10.309		0.039		4	7.726	
Shapiro-Wilk			0.8620		0.6608			0.9110

df – Degree of freedom

MS – Mean Square

P – Probability value of F-ratio test

The occurrence of respectively a higher maximum tension ( $347.48 \pm 41.53$  mN.mm<sup>-2</sup>) and less shortening ( $10.69 \pm 2.63\%$ ) at 7°C, compared to 37°C (maximum tension of  $284.83 \pm 82.44$  mN.mm<sup>-2</sup>, and maximum shortening of  $33.39 \pm 3.57\%$ ), could partly be explained by the release of calcium ions (Ca<sup>2+</sup>) from the sarcotubular system at low temperatures (Cornforth *et al.*, 1980; Whiting, 1980; Lawrie, 1998). It was demonstrated by Cornforth *et al.* (1980) and Whiting (1980) that the amount of Ca<sup>2+</sup> released from bovine sarcoplasmic reticulum was least at a pH of 6.6 when the temperature declined from 38° to 0°C, however, as the pH declined, the sarcoplasmic reticulum released more and more Ca<sup>2+</sup> (Kanda *et al.*, 1977) with essentially no activity near pH 5.0 (Whiting 1980). These findings indicated that both cold temperatures and low pH values decrease the ability to accumulate Ca<sup>2+</sup> and increases the release of Ca<sup>2+</sup> from the sarcoplasmic reticulum. In the case of bovine mitochondria, the maximum Ca<sup>2+</sup> uptake was at a pH of 7.2, its activity decreasing rapidly at a pH of 6.5 and was very low at a pH value of 5.5 (Whiting 1980). Whiting (1980) thus concluded that the mitochondria would be the first to lose their ability to sequester Ca<sup>2+</sup> as the *post-mortem* pH declines from 6.5 to 6.0. Low temperatures below 15°C also stimulate the release of Ca<sup>2+</sup> ions from the sarcotubular system, enhancing the contractile actomyosin ATP-ase (Lawrie, 1998). With the increase in Ca<sup>2+</sup> concentrations, actin and myosin interconnect and under normal circumstances where there is enough ATP, the contractile ATP-ase provide the energy for the actin filament to be pulled inwards, towards the centre of the sarcomere, causing the sarcomere to shorten.

For this study it can therefore be hypothesised that since muscle strips from the same ostrich were placed in two separate rigometers at respectively 7° and 37°C, the muscle strips had similar energy levels (as indicated earlier). However, as illustrated by data in Fig. 1, showing the development of maximum tension and maximum shortening at 7°C and 37°C, respectively, within muscle strips from an individual ostrich, the influence of the low temperature of 7°C slowed the rate of muscle contraction and therefore maximum tension and maximum shortening were reached sooner in muscle strips at 37°C than in muscle strips at 7°C. It can also be hypothesised that the low temperature of 7°C stimulated the release of Ca<sup>2+</sup> ions from the sarcotubular system, while slowing the rate of anaerobic glycolysis and therefore the production of ATP and lactic acid, leading to lower levels of ATP than in the muscle strips at 37°C during the early hours *post-mortem*. With the lack of sufficient levels of ATP at low temperatures, actin and myosin bind with each other due to the Ca<sup>2+</sup>, but without the “row-action” of actin being pulled toward the centre of the sarcomere. There is thus tension due to the binding between actin and myosin, but very little shortening since there is not enough energy to cause the sarcomeres to shorten. In the absence of ATP, the continuous tension is exerted by the cross-bridges formed between actin and myosin filaments (Pearson & Young, 1989).

The higher maximum shortening (%) at the higher temperature of 37°C in this study could, in its turn, be explained by ATP levels (Hertzman *et al.*, 1993) in conjunction with the influence of the high temperature on the sarcotubular system (Whiting, 1980). Hertzman and co-workers (1993) found a high correlation between maximum shortening and the ATP level at the onset of the shortening rapid phase and explained the higher shortening at 37°C compared to 15°C due to the higher ATP level at the higher temperature. Whiting (1980) showed that the Ca<sup>2+</sup> sequestering ability of the mitochondria (from beef muscles) at pH 7.2 decreased rapidly at temperatures higher than 20°C and was practically non-existent after 30 min at 37°C. On the other hand, the Ca<sup>2+</sup> sequestering activity of the sarcoplasmic reticulum remained relatively constant at temperatures up to 37°C at pH 7.2, where after its ability to sequester Ca<sup>2+</sup> decreased rapidly at temperatures higher than 37°C. Therefore, it could be hypothesised that the decreased stability of the Ca<sup>2+</sup> uptake ability of especially mitochondria, and also that of the sarcoplasmic reticulum, together with a faster decline in pH at 37°C compared to 7°C (Fig. 2), might initiate rigor shortening at higher ATP levels, resulting in the higher maximum shortening (%) observed at 37°C than at 7°C in the present study. However, from the data in Fig. 1, illustrating the development of maximum tension and maximum shortening at respectively 7° and 37°C within muscle strips from an individual ostrich, it can be seen that the maximum shortening was reached very rapidly in the muscle strip at 37°C (5.59 ± 1.53 h), while the shortening in the muscle strip at 7°C increased at a slow



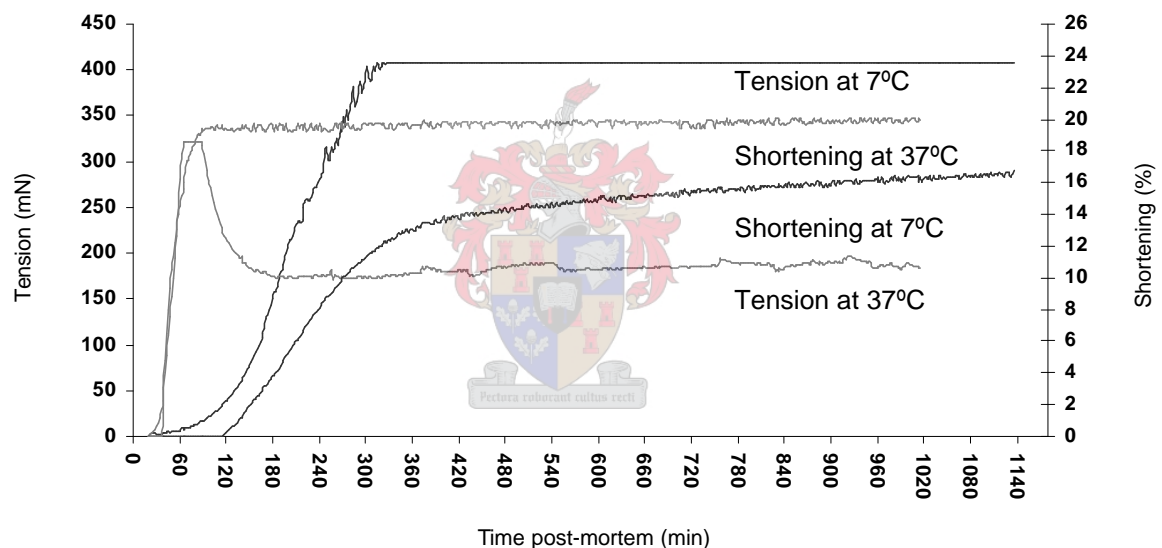
rate, tending towards an asymptotic value, although not yet reaching a constant value as was observed for the muscle strip at 37°C. It can therefore be hypothesised that, since the muscle strips at respectively 7° and 37°C had similar initial energy levels, the muscle strips at 7°C would eventually (beyond 24 h *post-mortem*) have reached a similar degree of muscle shortening than the muscle strips at 37°C.

Isometric tension results for beef muscles, obtained by Hertzman *et al.* (1993), were characterised by a delay phase preceding the development of tension, where this delay was shorter at 37°C than at 15°C. In the case of shortening, these authors found a delay period at 15°C, while at 37°C shortening started immediately. From the data in Fig. 1, showing an illustration of the development of muscle isometric tension and shortening from the time (20 min *post-mortem*) the muscle strips from an individual ostrich were placed in the rigometers, maintained at 7° and 37°C, respectively, it can be seen that, similar to the findings of Hertzman *et al.* (1993), there was a short delay period at both 7° and 37°C before the onset of tension development, with the delay period being shorter at 37° than at 7°C. The development of shortening started almost immediately in muscle strips at 37°C; while at 7°C there was a delay of approximately 100 min before the onset of shortening.

Honikel *et al.* (1983) found the onset of rigor to start at a pH value of 6.15 when beef neck muscles were incubated at 38°C, while the shortening started at a pH value of 6.30. These authors therefore found rigor shortening to begin just before the onset of rigor (tension development). Similar to the findings of Honikel *et al.* (1983), rigor shortening started before the onset of tension in muscle strips at 37°C (Fig. 1), while at 7°C, shortening started after the onset of tension development. Fully developed rigor, which was characterised by Hertzman *et al.* (1993) to be at constant shortening, constant tension and constant pH, were reached within approximately the same time region as indicted by their results. It was explained by these authors that it is natural for shortening to take place before the development of isometric tension: shortening occurs when myosin starts to attach to actin, but the muscle can still be extensible as long as there is enough ATP available. When tension starts to develop, extensibility of the muscle is being lost and the binding of myosin with actin becomes irreversible leading to force development within the muscle. However, it was concluded by Hertzman *et al.* (1993) that the rapid development of force in the isometric tension does not start until all CP (creatine phosphate) is depleted.

What was also noteworthy is that the tension peak for the muscle strips at 37°C was followed by a fall off in tension; while at 7°C the maximum tension remained constant. The constant tension at 7°C, as depicted in Fig. 1, was attributed to an unexplained technical failure of the rigometer. However, when the rigometer did not show any

technical failure, the tension data obtained from the muscle strips from the other ostriches, did in fact follow a constant maximum tension at 7°C from the time the muscle strips were placed in the rigometer to 22 h *post-mortem*. The rigometer, whose temperature was set at a constant temperature of 37°C, functioned without technical problems. The fall in tension at 37°C could be explained by proteolysis or to the initial ageing process of the muscle after fully developed rigor at high temperatures. The rate of tenderisation early *post-mortem* would be enhanced in muscles which are at higher temperatures since the extent of proteolysis is temperature dependent, being greater at 37°C than at 5°C (Lawrie, 1998). It may be hypothesised that the muscle strips at 7°C would eventually also have shown this fall off in tension, but at some later period (h) compared to the muscle strips at 37°C, since the low temperature of 7°C slowed the rate of proteolysis.



**Figure 1.** Development of muscle isometric tension and shortening from the time (20 min *post-mortem*) the muscle strips from an individual ostrich were placed in the rigometers, maintained at 7°C and 37°C, respectively.

### Muscle pH

The analysis of variance (ANOVA) of the dependable variables: minimum pH and the time (h) *post-mortem* at which the minimum pH was reached are presented in Table 3. Analyses were performed without transformation of the data since the data for the minimum pH values ( $P = 0.2353$ ) and the data for the time (h) *post-mortem* to reach the minimum pH ( $P = 0.0820$ ) were normally distributed. There were no significant

differences between individual ostrich carcasses for respectively the minimum pH values ( $P = 0.2812$ ) and the time (h) *post-mortem* to reach the minimum pH values ( $P = 0.1547$ ).

Although not significant ( $P = 0.2512$ ), muscle strips at 37°C reached a lower mean minimum pH ( $5.76 \pm 0.13$ ) than the muscle strips at 7°C ( $5.94 \pm 0.21$ ). The mean minimum pH value within the intact muscles ( $5.85 \pm 0.22$ ) was also lower compared to the mean minimum pH values for muscles strips at 7°C ( $5.94 \pm 0.21$ ). Temperature had an effect on the rate of pH decline, being faster in muscle strips at 37°C than in muscle strips at 7°C (Fig. 2). The intact muscles reached a minimum pH value ( $5.85 \pm 0.22$ ) within 2 h after slaughter (Table 4), while muscle strips at 7°C reached a minimum pH of  $5.90 \pm 0.21$  at  $6.41 \pm 4.51$  h (between 300 and 600 min) *post-mortem* ( $P < 0.05$ ). This indicated that *post-mortem* glycolysis and anaerobic production of ATP were faster in muscles with higher muscle temperatures during the rigor process, which in turn could explain the higher percentage of maximum shortening obtained at 37°C ( $33.39 \pm 3.57\%$ ) compared to 7°C ( $10.69 \pm 2.63\%$ ), as described earlier.

**Table 3.** Analysis of variance (ANOVA) of the dependable variables: minimum pH and the time (h) *post-mortem* at which the minimum pH was reached with ostrich and deboning [intact *M. gastrocnemius, pars interna* refrigerated for 24 h at 4°C, excised (20 min to 1 h *post-mortem*) *M. gastrocnemius, pars interna* from which muscle strips were kept at respectively 7°C and 37°C in two separate rigometers] as main effects, as well as the Shapiro-Wilk test for non-normality.

Source	df	Minimum pH		Time (h) of minimum pH	
		MS	P	MS	P
Ostrich carcass	5	0.047	0.2812	17.530	0.1547
Deboning	2	0.504	0.2512	33.160	0.0569
Error	9	0.031		8.275	
Shapiro-Wilk			0.2353		0.0820

df – Degree of freedom

MS – Mean Square

P – Probability value of F-ratio test

**Table 4.** Mean ( $\pm$  Standard Deviation) minimum pH and time (h) *post-mortem* at which the minimum pH values was reached, respectively within cold-deboned (excised at 24 h *post-mortem*) muscles, muscles strips from hot-deboned (20 min to 1 h *post-mortem*) muscles at 7°C, and muscles strips from hot-deboned (20 min to 1 h *post-mortem*) muscles at 37°C.

Treatment	Minimum pH	Time (h) to reach minimum pH	Temperature (°C) at minimum pH
Intact muscle	5.85 $\pm$ 0.22	2.00 $\pm$ 0.00 <sup>a</sup>	32.08 $\pm$ 4.29
Muscle strips at 7°C	5.94 $\pm$ 0.21	6.42 $\pm$ 4.51 <sup>b</sup>	7.00 $\pm$ 0.50
Muscle strips at 37°C	5.76 $\pm$ 0.13	4.83 $\pm$ 3.82 <sup>ab</sup>	37.00 $\pm$ 0.06

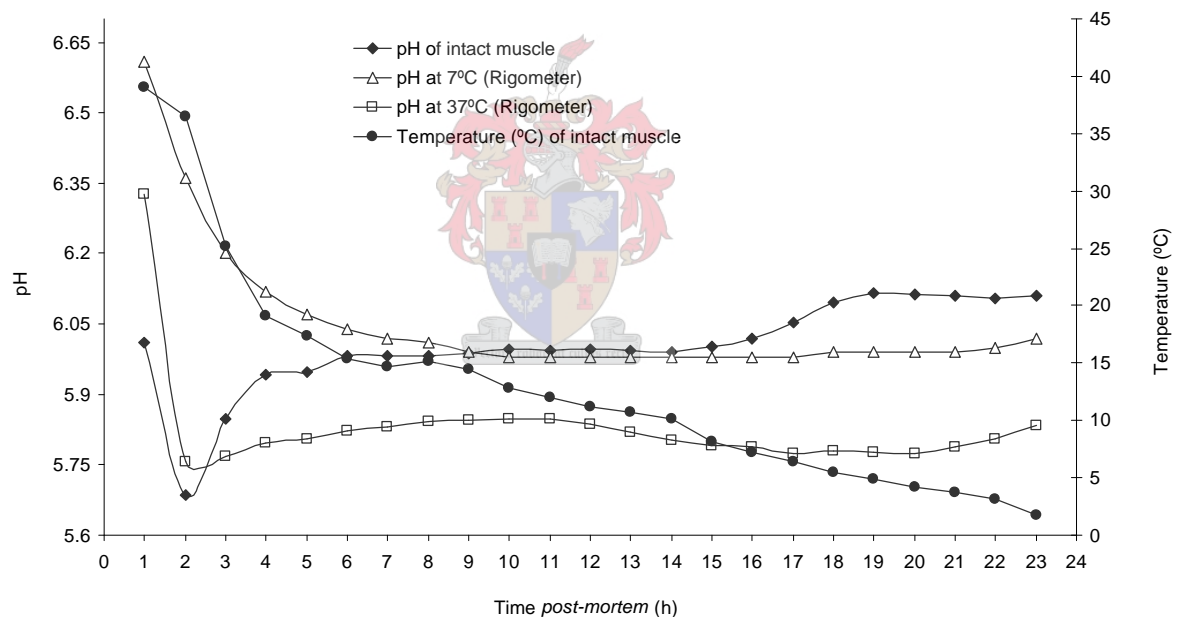
<sup>ab</sup> Different superscripts within columns differ at  $P < 0.05$ .

Figure 2 shows an illustration of the changes in pH and the decline in muscle temperature (°C) from an individual ostrich carcass. As illustrated by the pH and temperature (°C) data, similar to the intact muscle refrigerated  $< 4^{\circ}\text{C}$ , the change in pH for the muscle strip at 37°C showed a similar initial fall in pH, followed by an increase in pH after a minimum value had been reached. In general, the pH for intact *M. gastrocnemius, pars interna* decreased from 1 h *post-mortem* to a mean minimum value of 5.85  $\pm$  0.22 at 2 h *post-mortem*, indicating a rapid fall in pH during the first 2-3 h *post-mortem*, after which the pH then increased to a mean value of 6.14  $\pm$  0.19 at 24 h *post-mortem*. It has previously been reported by Pearson & Young (1989) that for chicken, *post-mortem* muscle glycogen levels decline to extremely low levels at approximately 2-4.5 h *post-mortem*, which indicated that the muscles were in full rigor and had reached pH values near that found at 24 h *post-mortem* in beef muscles.

The intact muscles had a mean muscle temperature of 32.08<sup>o</sup>  $\pm$  4.29<sup>o</sup>C at the time when the minimum pH was reached. It was also observed that the minimum pH in both the intact muscles and the muscle strips at 37°C was reached at approximately the same time ( $P > 0.05$ ) *post-mortem* (Table 4). The mean temperature of the left intact muscles at 1 h *post-mortem* was 42.14<sup>o</sup>  $\pm$  7.58<sup>o</sup>C, and since muscle strips were cut from the right muscles from the same ostrich carcasses and placed in the rigometers at 1 h *post-mortem*, it was assumed that the mean initial temperature (1 h *post-mortem*) of the muscle strips was  $> 35^{\circ}\text{C}$ . Thus, muscle strips at 37°C had a similar fast rate of pH decline during the first 2 h *post-mortem* than the intact muscles. Although the muscle strips at 7°C also had a mean initial temperature of  $> 35^{\circ}\text{C}$ , their surface area to volume ratio would have resulted in the muscle strips having a rapid decline in temperature at 7°C, causing the rate of pH decrease to be slowed. Therefore, muscle strips at 7°C reached a minimum pH at

$6.42 \pm 4.51$  h *post-mortem*. This clearly indicates that the change in *post-mortem* pH is highly temperature dependent.

The high initial (1 h *post-mortem*) mean muscle temperature of  $42.14^\circ \pm 7.58^\circ\text{C}$  can be explained by outliers as there were two individual ostrich carcasses in this study with a muscle temperature of  $54.7^\circ$  and  $43.9^\circ\text{C}$ , respectively. When these outliers were removed from the data, the mean muscle temperature at 1 h *post-mortem* was  $37.77^\circ \pm 0.98^\circ\text{C}$ . Blight & Hartley (1965) reported the live body temperature of ostriches to be  $38.7^\circ\text{C}$ . It is hypothesised that the high muscle temperature of these two individual ostrich carcasses at 1 h *post-mortem* resulted from the kicking action of the legs while the legs were fixed in the leg clamp during the stunning procedure (Wotton & Sparray, 2002). The high ambient temperatures in the plucking and skinning halls would also caused the muscle temperature to remain at a high value.



**Figure 2.** Hourly *post-mortem* temperature ( $^\circ\text{C}$ ) and pH values indicating the decline in temperature (●) and pH change (△) during the first 23 h *post-mortem* for intact *M. gastrocnemius, pars interna* refrigerated at  $< 4^\circ\text{C}$ , as well as for muscle strips from the *M. gastrocnemius, pars interna* maintained at  $7^\circ\text{C}$  (△) and  $37^\circ\text{C}$  (□) in the rigometers from the same ostrich carcass.

## CONCLUSIONS

In this study it was concluded that the rate of rigor development, the course of *rigor mortis*, as well as the rate of change in pH in ostrich *M. gastrocnemius, pars interna*, is temperature dependent, which is in accordance with the results of Hertzman *et al.* (1993). The degree of maximum tension and shortening were also temperature dependant, where maximum tension was higher at 7°C than at 37°C. In contrast, maximum shortening was significantly higher at 37°C than at 7°C, suggesting that muscle shortening at 7°C was not complete at 20 h *post-mortem*. Muscle strips at 37°C reached a maximum tension value (completion of rigor) within  $4.08 \pm 3.89$  h *post-mortem*, while the minimum pH was also reached within  $4.83 \pm 3.82$  h *post-mortem* in muscle strips at 37°C, indicating that full *rigor mortis* in ostrich muscles occurred at the point of minimum pH. The intact muscles reached a minimum pH at approximately 2 h *post-mortem*, while muscle temperature was still relatively high ( $32.08^\circ \pm 4.29^\circ\text{C}$ ). It is therefore suggested that ostrich muscles not be hot-deboned within the first 2 h *post-mortem*.

Cold-shortening and super contraction was shown to occur (Chapter 4 of this thesis) when ostrich *M. gastrocnemius, pars interna* was exposed to low temperatures early *post-mortem* (before the completion of *rigor mortis*) and when the pH was still high. However, the initial toughness caused by the cold-shortening and super contractions was negated when the muscles were aged at 4°C for more than 5 d *post-mortem*. With the rapid fall in pH in ostrich muscles (reaching a minimum pH within approximately 2 h *post-mortem*), as well as the early onset of *rigor mortis post-mortem*, it may be suggested that, in combination with refrigerated aging, hot-deboning of ostrich muscles as soon as 3 to 4 h *post-mortem* would be without detrimental effects on the eating quality in terms of meat tenderness. In an ostrich abattoir, 3 to 4 h would be about the time carcasses arrive at the cooler room (< 4°C) or deboning hall (< 7°C), since it takes approximately 1 h to pluck the feathers, another hour or hour and 30 min to remove the skin and complete evisceration, after which the carcasses are moved to the cooler room (< 4°C), awaiting deboning for approximately 30 min. It would therefore not be necessary to change the design of the slaughter line or the normal slaughter process in an ostrich abattoir with the implementation of hot-deboning of ostrich muscles at 4 h *post-mortem*.

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## Chapter 8

### GENERAL DISCUSSION AND CONCLUSIONS

The hot-deboning of muscles is beneficial in terms of the reduction of time, costs, space and refrigeration capacity requirements (Taylor *et al.*, 1980-81; Pollok *et al.*, 1997). However, with the performance of hot-deboning there is always the risk of cold-shortening and toughening of the meat, as well as the possibility for microbial contamination when warm muscles are removed from the carcass early *post-mortem*. On the other hand, hot-deboning is beneficial for controlling microbial spoilage (Lawrie, 1998), as the temperature decline in hot-deboned muscles is faster and more uniform than in muscles left on the carcass (Van Laack & Smulders, 1992). Currently in South Africa, it is general practice to refrigerate (< 4°C) ostrich carcasses for 24 h before the muscles are excised (cold-deboning) and vacuum packed for export. However, with the benefits of hot-deboning in terms of reduced costs, time and space, it is of great interest for the ostrich meat industry to have knowledge about the effects of hot-deboning on the physical quality characteristics of ostrich meat, as well as on the shelf-life of vacuum packed meat cuts. The objectives of this study were to investigate the effects of hot-deboning on the physical quality characteristics of two economically important ostrich muscles; the *M. gastrocnemius, pars interna* and the *M. iliofibularis*, to determine whether hot-deboning would cause cold-shortening in these muscles and whether the shelf-life of vacuum-packed meat cuts from these muscles would be negatively influenced.

In this thesis it was shown that, according to sensory evaluation and Warner-Bratzler shear force values (N. 12.7 mm<sup>-1</sup> diameter), hot-deboned (1 h *post-mortem*) *M. gastrocnemius, pars interna* were tougher and also less juicy than the cold-deboned (24 h *post-mortem*) muscles after aging for 48 h *post-mortem*. Hot-deboning caused some degree of cold-shortening and/or super contraction in the *M. gastrocnemius, pars interna*, since the hot-deboned muscles had a sarcomere length of 2.05 ± 0.29 µm at 24 h *post-mortem*, compared to the cold-deboned muscles which had a sarcomere length of 2.52 ± 0.14 µm. Sarcomere lengths of 1.77 ± 0.14 µm (Silva *et al.*, 1999) and of 1.79 ± 0.12 µm, (Smulders *et al.*, 1990) had been found for cold-deboned beef *M. longissimus thoracis et lumborum* muscles; while cold-shortened beef muscles had a sarcomere length of 1.57 ± 0.53 µm (Bouton *et al.*, 1973). This indicates that ostrich muscles, in general, have longer

sarcomeres than beef muscles and can in part explain the greater tenderness found for ostrich meat compared to beef (Paleari *et al.*, 1998; Rødbotten *et al.*, 2004).

After an aging period of only 21 d at 4°C, the toughness found for the hot-deboned *M. gastrocnemius, pars interna* was insignificant compared to the cold-deboned muscles. In contrast to the *M. gastrocnemius, pars interna*, hot-deboning did not result in the hot-deboned *M. iliofibularis* to be significantly tougher than the cold-deboned muscles, indicating that hot-deboning did not have the same effects on the *M. iliofibularis* than on the *M. gastrocnemius, pars interna* in terms of tenderness. Hot-deboning therefore, does not have similar effects on different ostrich muscles and it would be beneficial to investigate the effects of hot-deboning on other economically important ostrich muscles as part of future research. None the less, both the *M. iliofibularis* and the *M. gastrocnemius, pars interna* increased in tenderness as the aging time increased, with all muscles similar in tenderness (as determined by Warner-Bratzler shear force values) at day 42 of a 42-day aging period. This indicated that, although hot-deboning caused toughening of the *M. gastrocnemius, pars interna* early *post-mortem*, this toughness did not prevail throughout *post-mortem* aging and there is therefore no risk that consumers will buy tough ostrich meat, since consumers seldom consume meat aged for less than 7 d. While muscles decreased in toughness with an increase in aging time, it is of concern that hot-deboning resulted in the *M. gastrocnemius, pars interna* ( $2.90 \pm 1.88\%$ ) and the *M. iliofibularis* ( $3.81 \pm 2.63\%$ ) to have significantly ( $P < 0.05$ ) more purge (lower water holding capacity) than the cold-deboned *M. gastrocnemius, pars interna* ( $1.16 \pm 1.44\%$ ) and cold-deboned *M. iliofibularis* ( $3.12 \pm 2.13\%$ ) throughout *post-mortem* storage. Several researchers have documented that hot-deboning of beef muscles in combination with electrical stimulation resulted in less purge in vacuum packaged meat cuts during *post-mortem* storage, than cold-deboning alone (Cross *et al.*, 1979; Cross & Tennent, 1980; Griffin *et al.*, 1992). Therefore, further research on the effects of electrical stimulation in combination with hot-deboning is required in an attempt to minimise the amount of purge (%) during storage for ostrich muscles.

Although hot-deboning caused some degree of cold-shortening in the *M. gastrocnemius, pars interna*; the course of muscle temperature (°C) decline, the course of the change in pH, as well as the course of the development of *rigor mortis*, indicated that the *M. gastrocnemius, pars interna* and the *M. iliofibularis* can be hot-deboned without the risk of cold-shortening. In an ostrich abattoir, after death of the bird, it takes approximately 1 h to pluck the feathers, another hour to hour and 30 min to remove the skin and complete the evisceration process, after which the carcass moves to the cooler room (< 4°C), awaiting deboning, indicating that it would take approximately 3 to 4 h for carcasses to arrive at the deboning hall. The *M. gastrocnemius, pars interna* and the *M.*

*iliofibularis* both have a rapid pH decline reaching a minimum pH < 6.20 within 2 to 4 h *post-mortem* while muscle temperature is above 10°C; and therefore there is no risk of cold-shortening if these muscles are to be excised at approximately 4 h *post-mortem*. In addition, the *M. gastrocnemius, pars interna* and the *M. iliofibularis* showed an early onset of *rigor mortis*, as well as a rapid completion of rigor at approximately  $4.08 \pm 3.89$  h *post-mortem* when entering rigor at 37°C; and it was therefore concluded that hot-deboning of ostrich muscles as soon as 3-4 h *post-mortem* would be without detrimental effects on the eating quality in terms of meat tenderness. On the other hand, a high degree of rigor-shortening ( $33.39 \pm 3.57\%$ ) was observed in *M. gastrocnemius, pars interna* when entering rigor at 37°C. It is therefore suggested that hot-deboning of ostrich muscles should not be performed within the first 2 h after slaughter in an attempt to avoid rigor shortening, which could also have detrimental effects on the tenderness of the meat (Nuss & Wolfe, 1980-81; Lawrie, 1998; Devine *et al.*, 1999).

In terms of the shelf-life of ostrich meat, hot-deboning did not result in any additional microbial contamination. Hot-deboning also did not result in an increase in microbial counts during the 42-day aging period (-3° to 0°C) when compared to cold-deboned muscles. The bacterial counts were less the South African Standards for the microbiological monitoring of chilled meat (Quantum Analytical Service (Pty) Ltd., 12 Voortrekker Road, Malmesbury 7300, South Africa) destined for export, where the standards for the APC counts is < 10 000 cfu.g<sup>-1</sup> and for the *E. coli* counts is < 10 cfu.g<sup>-1</sup>.

Although regulation 36 (2) of the Red Meat Regulations (Anon, 2004), as well as regulation 40 (2) of the Ostrich Regulations (Anon, 2004) state that during the cutting of unfrozen meat, the core temperature of the meat must be maintained at or below 7°C, both these regulations make provision for hot-deboning. In regulation 34 (4) of the Red Meat Regulations (Anon, 2004) and in regulation 38 (4) of the Ostrich Regulations (Anon, 2004) it is stated that in spite of regulations 36 (2) and 40 (2), muscles may be hot-deboned if the carcass is transferred directly from the dressing room to the deboning hall in a single operation and if hot-deboning is carried out immediately after transfer. Since ostrich muscles have a rapid pH fall, as well as a rapid onset and completion of *rigor mortis*, it is finally concluded that hot-deboning of ostrich *M. gastrocnemius, pars interna* and *M. iliofibularis* may be performed as soon as 3-4 h *post-mortem* without the risk of cold-shortening, without a decrease in the shelf-life of vacuum packed meat cuts from these muscles, as well as without the abattoir having to change the slaughter line and normal slaughter process.

In terms of the slaughtering process, the implementation of hot-deboning could therefore allow the ostrich meat industry to save time, space and overall costs. However, hot-deboning may cause toughening of some of the ostrich muscles, but as indicated in

this thesis, this toughening effect can be negated with refrigerated aging for 5 d at 4°C and for 14 d at -3° to 0°C. Refrigerated storage of vacuum packed meat cuts (to reduce toughness) will require increased storage space and time. However, the ostrich meat industry has the capacity and has a well developed system for the export of vacuum packed refrigerated meat cuts. Therefore, the ostrich abattoir can implement the process of hot-deboning without having to undergo major changes. It is none the less of great importance to investigate the effects of hot-deboning on the commercial aspect of the ostrich meat industry. It is therefore recommended for future research to include a study of the economical implications of hot-deboning on the commercial aspects of the ostrich meat industry.

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