Effect of Deboning time, Ageing period and Collagen Characteristics on Horse *Semimembranosus* Meat Quality

by

Mohammad Mahbubur Rahman

A thesis submitted in partial fulfilment of the requirements for the degree of

Master of Science

in

ANIMAL SCIENCE

Department of Agricultural, Food and Nutritional Science

University of Alberta

Abstract:

Horse meat is a good source of iron and unsaturated fatty acids, which make it a suitable substitute for conventional meat. To sustain its consumer acceptance in a competitive global market, ensuring that Canadian horse meat is of the highest quality is of prime importance. Currently, in Canada horse meat is harvested either through hot boning (after 2 h post mortem) of the carcass or after the carcass is completely chilled to less than 4 ºC (40 h of post mortem). Additional efficiencies in horse meat production potentially could be gained by decreasing the carcass deboning time from 40 h to 17, 26 or 30 h. To ensure that Canadian horse meat quality remains of the highest standard, modifications to the time of deboning need to be considered in light of subsequent post mortem ageing and their effect on the resultant meat quality. *Semimembranosus* muscles (n = 36) were collected over four consecutive weeks from the right sides of horse carcasses de-boned at 17, 26 and 30 h post mortem (n = 12 per week, 4 at each post mortem period) and steaks from the muscles were aged for 3, 30, 60 and 90 days (n = 36 per period). Meat L^* (lightness) decreased ($p < 0.05$) while b^*(yellowness) increased with increasing length of deboning time and ageing period. Purge loss increased with increasing ageing period ($p < 0.05$) and was highest throughout the ageing period in muscles deboned after 17 h of chilling. Warner Bratzler shear force (WBSF) decreased with length of ageing ($p < 0.05$). Muscle perimysium ($r = -0.52$, $p < 0.001$), muscle collagen ($r = -0.35$, $p < 0.05$) and intramuscular fat ($r = -0.38$, $p < 0.05$) contents were negatively correlated with WBSF. Perimysial collagen ($r = 0.47$, $p < 0.05$) and pyridinoline ($r = 0.37$, $p < 0.05$) concentrations and muscle pH ($r = 0.46$, $p < 0.05$) were positively correlated with WBSF. Muscle pH, perimysial collagen concentration and intramuscular fat collectively explained 53% of the total variation in WBSF, while 63% of the variation in collagen heat solubility was explained collectively by muscle weight, Ehrlich chromogen concentration, purge loss and intramuscular fat. Results indicated that

deboning at 26 h post mortem did not change horse *semimembranosus* quality relative to current practices, but sensory analysis is needed to ascertain the full impact of the meat quality changes observed at 30, 60, and 90 d ageing. This research confirmed that collagen contributes to the WBSF of horse meat, supporting further investigation into the effects of animal age and breed on horse meat toughness.

Preface

This thesis on horse meat quality describes original research conducted by Mohammad Mahbubur Rahman, Dr Heather Bruce, Dr Lynn McMullen, Dr Bimol C. Roy and Brian Walker. Brian Walker, Dr. McMullen, Dr. Bruce and Mahbubur Rahman contributed to the experimental design, and Brian Walker and Dr. Roy assisted Mahbubur Rahman with execution of the experiments. Animal ethics approval was not required for the purposes of this study as it was considered Category A (non-invasive) because experimentation was conducted on horse meat purchased directly from a federally-inspected slaughter facility. A portion of Chapter 2 was published in the Proceedings of the International Congress of Meat Science and Technology (ICoMST) under the title "Effects of chilling and ageing period on horse meat quality" in conjunction with a poster presented at ICoMST in Cork, Ireland, in August 2017. Brian Walker, Dr. Roy, Dr. McMullen and Dr. Bruce were co-authors of the Proceedings publication.

To my late father, Aminul Islam

Acknowledgements

First, I would like to express my sincere thanks and praise to the almighty Allah for his kind help upon which I managed to complete this study.

I would like to express my cordial thanks to my supervisor Dr. Heather Bruce for her criticism and continuous guidance, valuable suggestions, dedication of her valuable time, detailed reading and effective corrections throughout my studies. Dr. Bruce, I appreciate the great opportunity you provided me to improve my knowledge and build my career in the discipline of meat science.

I am thankful to Dr. Lynn McMullen for her generous support and guidance as a vibrant member of my supervisory committee. You impressed me with your kindness, knowledge and constructive criticism.

I would like to extend a special thanks to Brian Walker who helped me to collect the meat samples from the slaughter facility. I wish to acknowledge the co-operation and moral support from academic and non-academic staff and my colleagues in the Laboratory of Meat Science. II also wish to extend my cordial gratitude to Alberta Livestock and Meat Agency (ALMA) and the NSERC CREATE for Assuring Meat Safety and Quality for the financial support provided for my research and my stipend. I would like to acknowledge the industrial partner Bouvry Exports Calgary Ltd which also provided access to its facility and the experimental samples.

Lastly, I would like to thank my mother and my wife for their continuous encouragement and spiritual and mental support throughout my studies. Again, I would like to give thanks to all; it has been a wonderful time. May Allah bless you all.

Contents

Tables

Figures

Abbreviations

a*	Redness
ATP	Adenosine Triphosphate
*b**	Yellowness
*c**	Chroma
CFIA	Canadian Food Inspection Agency
CKL/CK	Cooking loss
CKT	Cooking time
COMb	Carboxymyoglobin
CP	Crude protein
DFD	Dark, firm and dry
DM	Dry matter
DMb	Deoxymyoglobin
EC	Ehrlich chromogen
ECMMC	Ehrlich chromogen mol per mol collagen
FAO	Food and Agricultural Organization
*h**	Hue
HPLC	High performance liquid chromatography
HSC	Heat soluble collagen
HYP	Hydroxyproline
IMCT	Intramuscular connective tissue
IMF	Intramuscular fat
*L**	Luminosity/lightness
LD	*Longissimus dorsi*
Mb	Myoglobin
MetMb	Metmyoglobin
Moi	Moisture
Moi	Moisture
Mw	Muscle weight
N	Newtons

NAD	Nicotinamide adenine dinucleotide
OMb	Oxymyoglobin
p	Probability
PC1	Principal Component 1
PC2	Principal Component 2
PCA	Principal Component Analysis
PER	Perimysium
PL	Purge loss
PSE	Pale Soft and exudative
PYR	Pyridinolines
PYRMC	Pyridinoline mole per mole collagen
R	Red
SEM	Standard error mean
SF	Shear force
SL	Sarcomere length
SM	Semimembranosus
TC	Total collagen
W	White
WBSF	Warner Brazler shear force
Wt	Weight
α	Alpha
β	Beta

Chapter 1. Introduction

1.1 Horse meat

Horse meat is characterized by its dark appearance (Price and Schweigert, 1994) and its low level of subcutaneous fat, as fat in the horse is mainly located in the abdomen surrounding the kidney (Lorenzo et al., 2014). The average horse carcass is composed of 69.6% muscle, 10.4% fat and 17.4% bone (Lorenzo et al., 2014). Horse was consumed by humans in western Europe before its domestication, as evidenced by cave paintings depicting hunts and by marks on horse bone dated to the Paleolithic era (10,000 B.C) (Edwards,1998; Hintz,1995; Peplow, 1998). It has been hypothesized that horse meat was the main source of essential fatty acids for the people of the Paleolithic era, as this was a time when the availability of plant and marine sources was limited due to recurrent glaciations (Guil-Guerrero et al., 2013). Animal domestication after 5000 B.C resulted in horse meat and milk being used as food sources and the horse itself as a draft animal in Central Asia; however, horse riding started much later from approximately 1500-1000 B.C (Brown et al., 2003; Peplow, 1998). Throughout history, horse meat consumption was interrupted due to social, cultural, or religious reasons (Fernández de Labastida, 2011). As a consequence, it was not contemplated as a popular meat and was generally associated with poor social class or famine and periods of limited food supplies. In addition, some cultures and regions considered the horse a companion animal and this perception limited its consumption in those areas.

With these local limitations, horse meat is an unconventional source of protein that is rich in unsaturated fatty acids and iron and low in saturated fatty acids and cholesterol content (Lorenzo et al., 2014). Despite cultural perceptions of the horse as a companion or work animal, some countries in Europe still have robust markets for horse meat. France, Spain, Italy and Belgium are

home to the majority of horse meat consumers; however, Canada is one of the largest horse meat exporters in the world.

The worldwide overall horse meat production from 2003 to 2016 was constant with the average amount being approximately 700 thousand tonnes per year (Figure 1.1) (FAO, 2018). Since 2003 world horse meat production gradually decreased and has fluctuated since 2010, but most of the horse meat in the world is produced by Asia countries, which average 45% of world production. China and Kazakhstan contribute largely to Asian horse meat production, and account for 30% and 10% of world production, respectively (FAO, 2018).

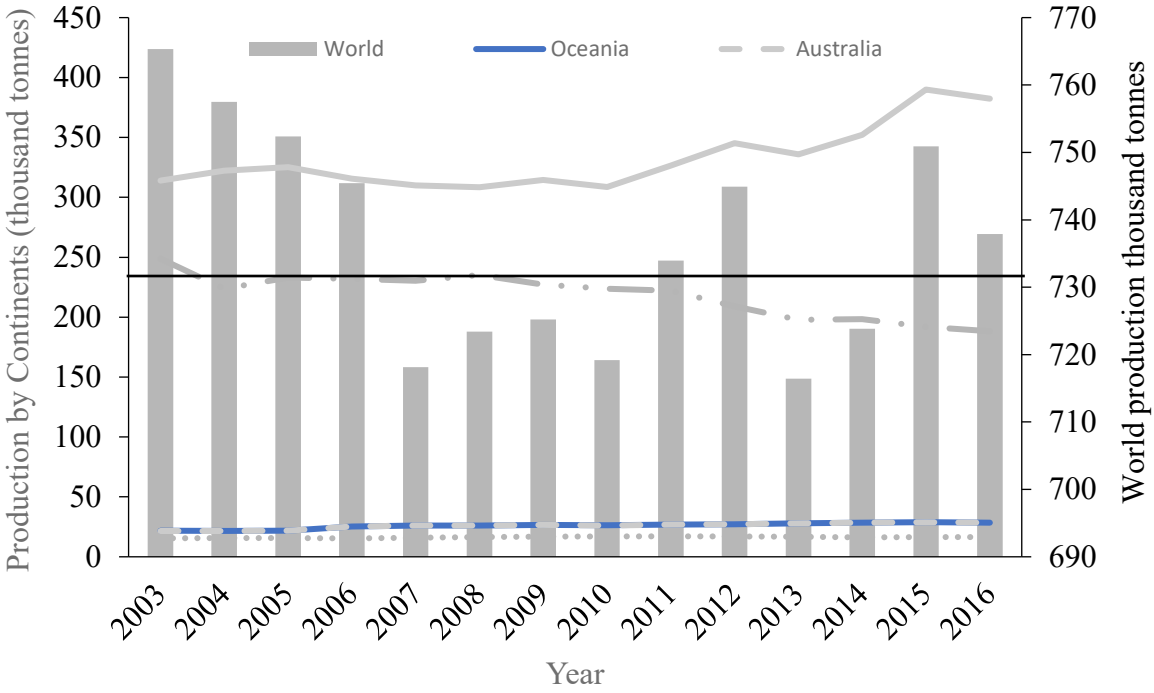

Figure 1.1 Yearly world and continental horse meat production from 2003 to 2016 [data from FAO (2018)].

However, the proportion of horse meat exported from China is small (6.38%), and Kazakhstan does not contribute to the export market (FAO, 2015). After Asia, according to FAO (2018), the

Americas (North and South), and Europe contribute 30% and 19% of world production, respectively. France, Spain, Italy, Belgium, and Japan are the major horse meat consuming countries. The average data from 2006 to 2016 (Figure 1.2) indicate that Russia, Italy, France and Belgium are the major horse meat importing countries while Argentina, Belgium and Canada are the major exporting countries (Figure 1.2).

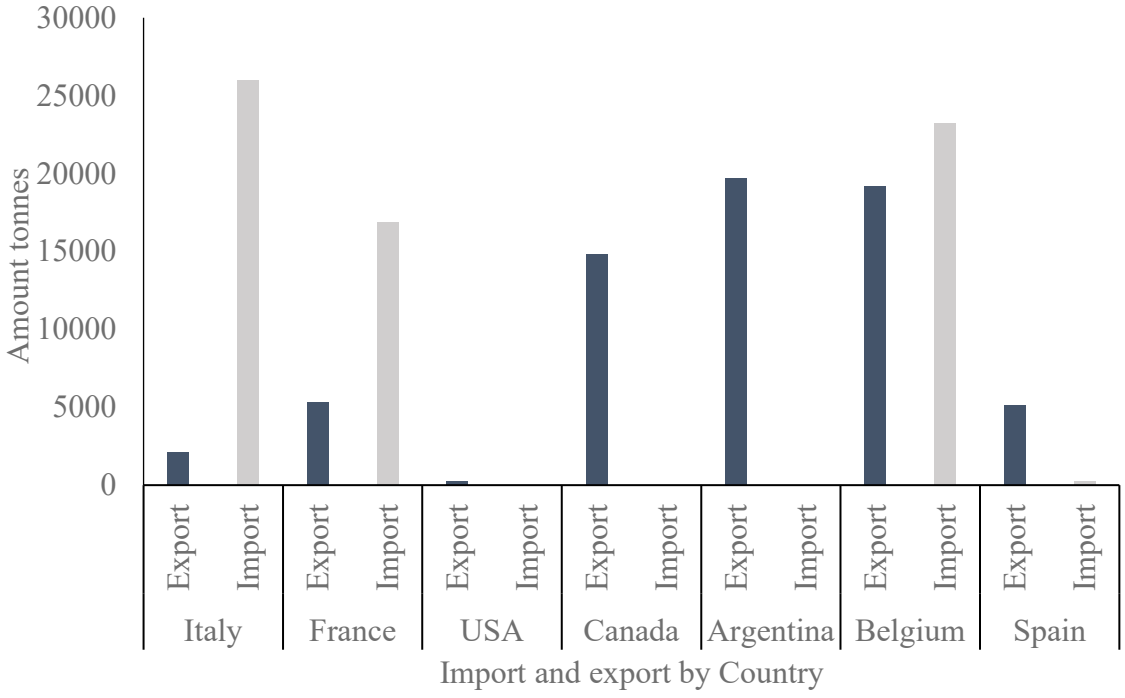

Figure 1.2 Average (2006 to 2016) yearly horse meat import and export in different countries [data from FAO (2018)].

As a result, Canada is one of the largest horse-meat exporting countries, having an average yearly export from 2006 to 2016 of around 15 thousand tonnes. After 2012 (Figure 1.3) horse meat export from Canada gradually decreased, which may have been due to a horse meat scandal

in 2015 where horse meat was sold as beef in some countries in Europe, which gradually depressed the demand for horse meat in Europe.

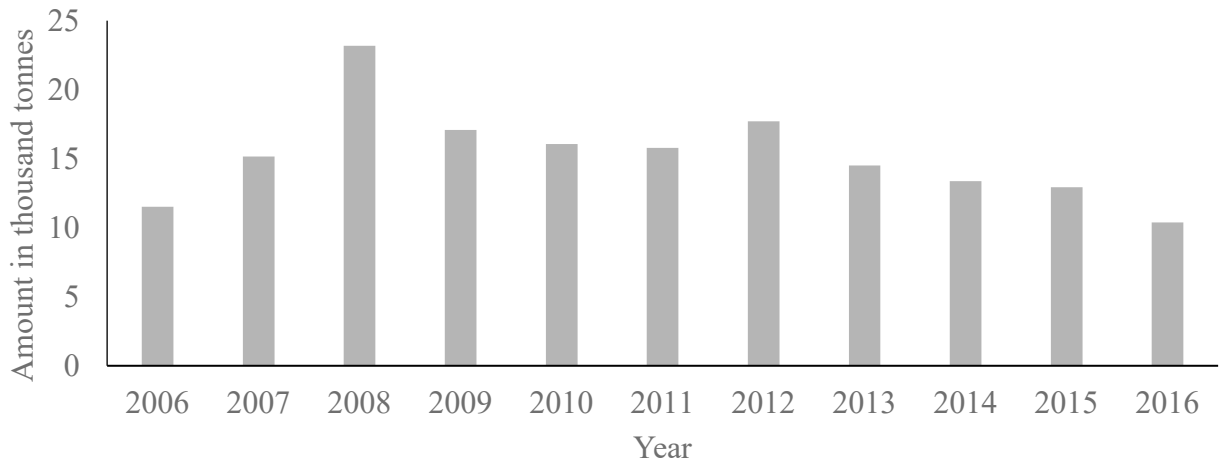

Figure 1.3 Canadian horse meat export from 2006-2016 [data from FAO (2018)]

To stay competitive in the global horse meat market, Canada needs to ensure that it produces the highest quality horse meat. Meat appearance and tenderness are the most economically important quality characteristics (Feuz et al., 2004; Carpenter et al., 2001; Ponnampalam et al., 2013), and these are influenced by animal intrinsic and extrinsic factors.

With exposure to air, the dark brown color of horse meat changes to brown or black with a bluish tinge; therefore, packaging and shipping conditions of horse meat are important for controlling the quality of horse meat destined for international markets. Indeed, post slaughter harvesting regime may affect horse meat tenderness and appearance. In order to reach these international markets, transport of the product over long distances for long periods of time (up to 90 d) is required. The effects of prolonged storage of horse meat on its quality is poorly understood, with only Gómez and Lorenzo (2012) detailing the quality and sensory acceptability of horse meat packaged under

vacuum up to 14 d post mortem. The impact of extended post mortem storage under vacuum on horse meat quality during transport to market, therefore, needs to be understood so changes in product quality during transport can be managed.

The current practice of horse meat harvest in Canada involves either hot boning (hot boned after 2 h) or chilling full carcasses (40 h after) under specific recommendations set by the Canadian Food Inspection Agency (CFIA). Rapid chilling of hot boned muscle to prevent microbial growth may cause cold shortening resulting in tough meat (Marsh and Leet, 1966). The latter method is related to storage and cost. On the other hand, no alternative approach is permitted as there are no data available to support the safety and quality of alternative deboning methods. To understand the threshold time for chilling before deboning that does not harm the quality of horse meat, that changes that occur in meat quality need to be understood before changes to processing are made. Specifically, how myofibrillar and connective tissue proteins contribute to horse meat toughness and interact with post mortem carcass management needs to be reviewed, as does how intramuscular pH and myoglobin status influence horse meat appearance.

1.2 Meat tenderness

Meat tenderness, juiciness and flavor are important factors that determined the palatability of meat and among these factors, tenderness is considered the most important characteristic (Boleman et al., 1997; Savell and Shackelford, 1992) that dominates the perception of eating quality of meat (Thompson, 2002). According to consumer statements, tenderness is the most desire attribute when eating a steak at home or in a restaurant (Huffman et al., 1996). Also, consumer willingness to pay a higher price increases when the tenderness of meat is guaranteed (Feuz et al., 2004). There are multiple factors that can impact muscle tenderness including production practices, post-slaughter handling of the carcass (Andersen et al., 2005), amount of intramuscular fat or marbling (Emerson

et al., 2013; Starkey et al., 2016; Corbin et al., 2015), total collagen content and its heat solubility, and the contractile state of the muscle fiber (Starkey et al., 2016; Hopkins and Geesink, 2009).

Structurally, muscle consists of fibrillar protein responsible for its contraction and relaxation, and the changes in myofibrillar components pre-rigor (i.e. the period between slaughter and onset of rigor) strongly influence the muscle myofibrillar toughness. Connective tissue holds the muscle and its fibers together and attaches the muscle to the skeleton. The main protein of connective tissue is collagen, which has triple helices with glycine as every third amino acid and hydroxyproline, an amino acid only found in collagen and elastin. The quantity and heat solubility of intramuscular collagen are associated with the background toughness of meat because it is assumed that the structural properties of collagen are unchanged after ageing or conditioning (Ouali, 1990). It has been reported, however, that after extended ageing damaged endomysium tubes are evident and swelled perimysium fibers increase collagen solubility in bovine muscle (Palka, 2003). Early research indicated that during ageing an ultrastructural change is observed in myofibrillar components including the disappearance of the Z-disk and disruption of the myofibrils (Gann and Merkel, 1978). Hence, muscle tenderness is determined by three major factors: the background toughness; the extent of rigor toughening; and the extent of tenderization post mortem. Toughening and tenderization take place during the post-mortem ageing period and background toughness exists during slaughter and is considered to not change with post-mortem ageing (Koohmaraie and Geesink, 2006; Hopkins and Geesink, 2009).

1.2.1 Background toughness and collagen

The background toughness of muscle is defined as "*the resistance to shearing of the unshortened muscle*" (Marsh and Leet, 1966). The background toughness depends on the amount and properties

of collagen present in muscle connective tissue (Cross et al., 1973). There are three layers of connective tissue that contribute to the structure of muscle. The first layer, the endomysium, surrounds a muscle cell, the second layer the perimysium surrounds muscle fibre bundles and the third layer the epimysium encompasses the muscle itself (Bailey, 1972). The distribution of perimysium appears to affect the background toughness of meat the most as there are correlations between the perimysium and tenderness found both in chicken and beef muscles (Strandine et al., 1949). In fact, thicker perimysium is associated with reduced tenderness (Swatland et al., 1995; Liu et al., 1996). Contrarily, a negative correlation betweem perimysium thickness and shear force has been observed in horse meat aged for 3 days (Roy et al., 2018); however, the correlation between toughness and perimysium thickness is poor (Brooks and Savell, 2004). On average the perimysium contains around 90% of the total collagen found intramuscularly (McCormick, 1994). In horse muscles collagen concentration rapidly increases between 12 to 18 months of age and then after that appears to stabilize, while the concentration of soluble collagen diminishes with animal age (Badiani et al., 1997). A recent study of horse meat aged for 14 days indicated that collagen did not contribute to changes in horse meat tenderness due to ageing (Malva et al., 2019). The literature investigating the changes in collagen characteristics during extended ageing and the impact on horse meat toughness is limited, and given the possible contribution of collagen to the background toughness of horse meat, additional research is warranted.

The basic structural unit of collagen is tropocollagen, which is composed of three alpha chain peptides that are wound around each other to form a strong triple-helical structural unit (Bailey, 1972). Collagen fibers are stabilized by both divalent and trivalent intermolecular cross links (Eyre and Wu, 2005; McCormick, 2009). In young animals the divalent aldimine-type cross links (Bailey, 1972; Shimokomaki et al., 1972) are thermolabile (Bailey and Sims, 1977). The divalent

cross links are converted to trivalent cross links with increasing animal age (McCormick, 1994). Muscle toughness increases with increasing concentration of thermostable trivalent cross links and with decreasing muscle collagen heat solubility (Hill, 1966). The trivalent cross links include the pyridinolines (PYR), specifically hydroxylysyl-pyridinoline and lysyl-pyridinoline, and the Ehrlich chromogen (EC), all of which bind two telopeptides and the helix of two or three collagen molecules together (Eyre et al., 1987). The increased concentration of the PYR cross link has been related to increased muscle toughness as well as decreased collagen heat solubility (Bosselmann et al., 1995; Steinhart et al., 1994). Hence, the concentration of trivalent cross links plays a vital role in determining the background toughness (Weston et al., 2002) and the importance of their influence increases if total collagen is constant irrespective of animal age (Smith and Judge, 1991). It is assumed that in horse meat that trivalent cross link concentration will decrease its tenderness.

Total collagen content in bovine muscle constitutes between 1 to 15% of muscle dry weight (Bendall and Voyle, 1967) and constitutes about 4-5% of horse meat muscle (Malva et al., 2019). The influence of collagen on meat eating quality is controversial as Herring et al. (1967a) and Hunsley et al. (1971) indicated that eating quality did not correlate to collagen content, while Dransfield (1977) and Nishiumi et al. (1995) stated that muscle rich in collagen was tougher than muscles with low collagen content. However, Roy et al. (2018) stated that the intramuscular total collagen content between tough and tender horse meat is not different. The relationship between collagen heat solubility and meat tenderness is also not straightforward. Some researchers have found low (Berge et al., 2003; Chambaz et al., 2003) or no (Young et al., 1994) correlations between collagen heat solubility and meat toughness while Nishiumi et al. (1995) observed a significant correlation between collagen heat solubility and toughness of pork. Earlier studies demonstrated that collagen is affected neither by time of conditioning nor temperature (Pierson

and Fox, 1976; Chizzolini et al., 1977) which indicated that during post mortem ageing collagen remains unchanged at the molecular level. However, Stanton and Light (1988, 1990) and Mikołajczak et al. (2019) reported that collagen is damaged and partially solubilized during conditioning. In addition, using differential scanning calorimetry Judge and Aberle (1982) demonstrated that the thermal shrinkage temperature of bovine intramuscular collagen decreased by 7 to 8 °C after 7 days of post-mortem ageing. Background toughness might be controlled by increasing animal growth rate (Sylvestre et al., 2002) and reducing animal slaughter age (Shimokomaki et al., 1972). In horse meat, total collagen content has been shown to increase until 18 months of age while soluble collagen content decreased from 40-50% of total collagen at 12 months of age to 22-27% at 30 months of age to 7-8% of total collagen at 10 years of age (Lorenzo et al., 2014). The decreasing heat solubility of collagen occurred concomitantly with increased shear force, which also increased with increasing animal age (Dufey, 1996; Lorenzo et al., 2014; Girard et al., 2012). This age effect on toughness is predominant in muscle that has moderate to high connective tissue (Lepetit and Culioli, 1994; Sarriés and Beriain, 2005).

1.2.2 Toughening of myofibrillar proteins

The toughening of myofibrillar proteins is caused by the shortening of sarcomere length during the development of rigor mortis (Koohmaraie, 1996). Rigor occurs or is achieved when muscle energy, adenosine triphosphate (ATP) level is depleted and muscle no longer has the ability to contract. During this stage some muscles become shorter due to the formation of a permanent cross-link between the myofibrillar proteins actin and myosin (Honikel, 2014), and this occurs due to the depletion of ATP as myosin is unable to release actin without ATP. The sarcomere length is negatively related with toughness and if the sarcomere length is less than 2 μm it can result in toughened meat (Wheeler et al., 2000). This is not always true however as in the horse

semimembranosus (SM) the sarcomere lengths for tough and tender muscles were 1.55 and 1.58 µm, respectively (Roy et al., 2018). During muscle contraction and after rigor development considerable variation in sarcomere length exists between and within muscles. For instance, after rigor the sarcomere length ranges from 0.7 to 3.7 µm in different bovine muscles (Locker, 1959). In extreme shortening, the length could be as short as 1.1 µm, which is not standard (Locker, 1960; Voyle, 1969). In extreme cases the post rigor sarcomere length could be as long as 3.7 µm due to the tension exerted on an individual muscle by its skeletal attachment (Locker, 1959). The reported sarcomere length of different muscles including the bovine *semitendinosus* is around 2.2 µm (Herring et al., 1967a). During rigor development in the bovine *longissimus* and *semitendinosus* muscles, the sarcomere length can shorten by 22 to 44% (Wheeler and Koohmaraie, 1994; Stromer et al., 1967). The muscle temperature at rigor plays an important role in rigor shortening, because if the muscle temperature is between 10 to 15 ºC during the onset of rigor the sarcomere shortening is minimized (Ertbjerg and Puolanne, 2017). A 10% decrease of muscle length was observed at a rigor temperature of 17 °C (Bendall, 1951). The rate of rigor development is variable depending on the species and size of the carcass; for instance, the rigor process is faster in lamb than in beef (Marsh and Thompson, 1958) and more rapid in pork and very fast in poultry (Ertbjerg and Puolanne, 2017). The rigor process is governed by the rate and extent of depletion of muscle glycogen concentration. Horse meat is rich in glycogen as compared to other species and has a mean glycogen concentration of about 22 mg/g (Gill, 2005). A high muscle glycogen content may prolong the rigor process for horse meat compared to other species. There is the danger, therefore, that the horse carcass muscle temperatures may decline to less than 10 °C before the onset of rigor is complete, leading to cold shortening (Marsh and Leet, 1966). Muscle attached to bone may have

some level of protection from shortening through its physical attachment, so the timing of de-boning with respect to the progress of rigor may affect the toughness of the meat.

1.2.2.1 Thaw rigor and muscle shortening

If muscles are frozen before the onset of rigor mortis it will develop massive contraction during thawing known as thaw rigor or thaw contracture (Ertbjerg and Puolanne, 2017). Thaw rigor involves the release of Ca^{2+} ions from the sarcoplasmic reticulum upon thawing, which triggers muscle contraction. During freezing ice crystals form and penetrate the sarcoplasmic reticulum where most of the cell Ca^{2+} is stored. During thawing Ca^{2+} ions are released into the sarcoplasm through the damaged sections of the sarcoplasmic reticulum and if ATP is still present this will provoke muscle contraction. The high concentration of Ca^{2+} ions in the sarcoplasm upon thawing causes a massive contraction which results in extreme shortening of the muscle. The amount of shortening will vary with thawing temperature as Cook and Langsworth (1966) showed that the shortening of lamb muscle frozen pre-rigor increased with increasing thawing temperature. Hence slow low-temperature thawing can prevent the contraction of frozen pre-rigor lamb (Davey and Gilbert, 1973). Thaw rigor in meat may increase drip loss and cause remarkable toughening during cooking. Thaw rigor can be prevented by not freezing meat until after rigor is completed.

Temperature during the early post-mortem period can have a profound effect on the progression of rigor as well. Lowering the temperature of pre-rigor meat to just below zero resulted in an increase in the rate of rigor mortis. A maximum rate of rigor development has been observed around -3 ºC, and at temperatures below this point the rate of rigor development declined, with rigor completely stopped at around -20 ºC (Behnke et al., 1973). In frozen meat ice is formed at -

1 to -2 ºC, hence rigor development around those temperature will prevent muscle contraction as well as muscle shortening.

1.2.2.2 Cold shortening and muscle toughness

Cold shortening is a consequence of muscle contraction in the early post-mortem period and occurs if muscle temperature declines to below 10 ºC before onset of rigor. (Locker, 1985; Ertbjerg and Puolanne, 2017). Cold shortening is an important factor that needs to be considered when pre-rigor muscle is exposed to rapid chilling. Locker and Hagyard (1963) characterized cold shortening when they examined the response of beef *sternomandibularis* and *longissimus* muscles to different temperatures at rigor. In their study, the least amount of shortening (10% shortening) was observed at between 10 to 19 ºC, while the maximum amount of shortening (48%) was observed at a rigor temperature of 0 ºC. During rigor, time of cooling is important as the available ATP is critical for muscle contraction, with the shortening process most rapid and dramatic during the first 1-2 hours post-mortem when there is an abundance of ATP, after which the process slows but continues until rigor-mortis is complete (Marsh and Leet, 1966). Cold shortening has been documented in all common meat animal species, including beef, lamb, pig, chicken and turkey (Ertbjerg and Puolanne, 2017). Species differences arise from differences in muscle fibre type, as cold shortening is more extensive in red muscle than in white muscle (Ertbjerg and Puolanne, 2017) so bovine muscles are more sensitive to cold shortening than porcine muscle (Fischer et al., 1980). The mechanism of cold shortening is suggested to be due to the calcium-ATPase pump in the sarcoplasmic reticulum slowing at low temperatures (Cornforth et al., 1980), but may also be due to calcium uptake by muscle mitochondria being compromised below pH 7 and at low temperatures (Whiting, 1980). Compromise of calcium uptake by mitochondria may explain the predisposition toward cold shortening by red muscles as they have high mitochondrial

concentrations (Buege and Marsh, 1975). The distance from the muscle surface is important for cold shortening as it influences the time-temperature relationship within the muscle as well as the rate of depletion of ATP and the concomitant pH decline (Puolanne and Ruusunen,1988).

Shortening of muscle can also occur when the muscle enters rigor at temperatures between 20 to 40 ºC. (Bendall, 1951; Marsh, 1954) The effect of high temperature at rigor on muscle shortening is less than that of cold shortening, possibly because timing of rigor onset differs between muscle fibre types and so the muscle fibres that have already entered rigor may serve to physically restrain the contraction of adjacent muscle fibres that have not yet entered rigor (Ertbjerg and Puolanne, 2017). Heat shortening also seems to not only reduce the sarcomere length but also decrease post mortem proteolytic activity, further compounding the toughening associated with it (Devine et al., 1999).

1.2.3 Tenderization

The tenderization of meat is a consequence of post-mortem degradation of the muscle structure and its associated proteins through enzymatic processes which include multiple proteolytic enzymes (Koohmaraie and Geesink, 2006; Ertbjerg et al., 1999; Carlson et al., 2017; HopKins, 2004). Protein degradation and oxidation have been identified as processes that modify the protein structure and meat tenderness (Huff-Lonergan et al., 2010). The complex enzymatic process involved in post-mortem tenderization is still not clearly understood (Ouali et.al., 2013). However, it is agreed that post-mortem proteolytic process involves multiple enzyme systems among which the calpain system has been identified as an enzyme system that plays a major role in the post-mortem tenderization process (Kim et. al., 2018ab). In addition, lysosomal cathepsins and the

multi-catalytic proteinase complex are two other enzymatic systems that may be involved in meat tenderization (Koohmaraie and Geesink, 2006).

The intracellular environment including temperature, pH, and Ca^{2+} concentration play vital roles in enzymatic activity during the meat tenderization process (Takahashi,1996; Huff-Lonergan et al., 2010). Normally meat aging is conducted at low temperatures (0 to 4 °C). It has been reported that massive shortening of sarcomere length can be prevented by using a freeze-thaw-refrigeration cycle which leads to more tender meat (Bowling et al., 1987). Low temperatures reduce the rate of or halt the glycolytic process by reducing enzymatic activity and pH decline (Joseph, 1996). Low pH can inhibit enzymatic activity, promote denaturation of protein, and interact with muscle temperature to cause excessive shortening of sarcomeres which results in toughness and loss of water holding capacity (Khan and Cohen, 1977). During the tenderization process sarcoplasmic Ca^{+2} ion concentration increases as the sarcoplasmic reticulum and mitochondria lose their ability to accumulate Ca^{+2} ions. This increase in cytoplasmic Ca^{2+} may lead to enhanced calpain function as the Ca^{2+} ions concentration is around 2000 times higher than that of resting skeletal muscles (Takahashi, 1996).

During the enzymatic tenderization process, the myofibrillar proteins troponin-I, troponin-T, desmin, vinculin, meta-vinculin, dystrophin, nebulin and titin are degraded (Koohmaraie, 1996). Three cytoskeletal structures are also degraded, specifically the Z-lines and their attachments to the intermediate filaments, and the Z- and M- line attachments to the sarcolemma through the costameric protein and the elastic filament protein titin (Taylor et al., 1995). Previous studies on horse meat focused on ageing time and its impact on sensory properties (Sarriés and Beriain, 2005;

Franco et al., 2011; Beldarrain et al., 2020) and did not focus on standardized ageing time for horse meat.

1.3 Meat color

Muscle color is one of the most important economic quality traits as it indicates either freshness or spoilage of meat (Carpenter et al. 2001; Ponnampalam et al., 2013) and it can accelerate the consumer purchase decision, even though color has limited or no impact on eating quality of meat (Chambaz et al., 2003). It was estimated in 2000 that the meat industry lost about one billion US dollars each year due to color disqualifications (Smith et al., 2000). Meat color is strongly associated with myoglobin (Mb) concentration and its chemical forms in the muscle (Mancini and Hunt, 2005; Suman and Joseph, 2013) because hemoglobin and cytochromes have very limited influence on muscle color (Faustman and Suman, 2017; Suman and Joseph, 2014). Not only the concentration of myoglobin but also the amount and distribution water in muscle, as well as the amount of glycogen stored in muscle prior to slaughter can affect the meat color (Offer and Knight, 1988; Lister, 1989).

1.3.1. Myoglobin and meat color

Myoglobin is a sarcoplasmic globular heme protein possessing a prosthetic group. The heme has a centrally located iron atom with six coordinated sites; four are associated with a tetrapyrollic ring structure, while the fifth and sixth are coordination sites, with the fifth connected to a histidine residue and the sixth available to bind a variety of ligands including oxygen, carbon monooxide and nitric oxide. The type of ligands that bind to the sixth coordination site determine the redox state of the iron molecule (ferrous or ferric) and the color of meat (Bekhit and Faustman, 2005; Suman and Joseph, 2013).

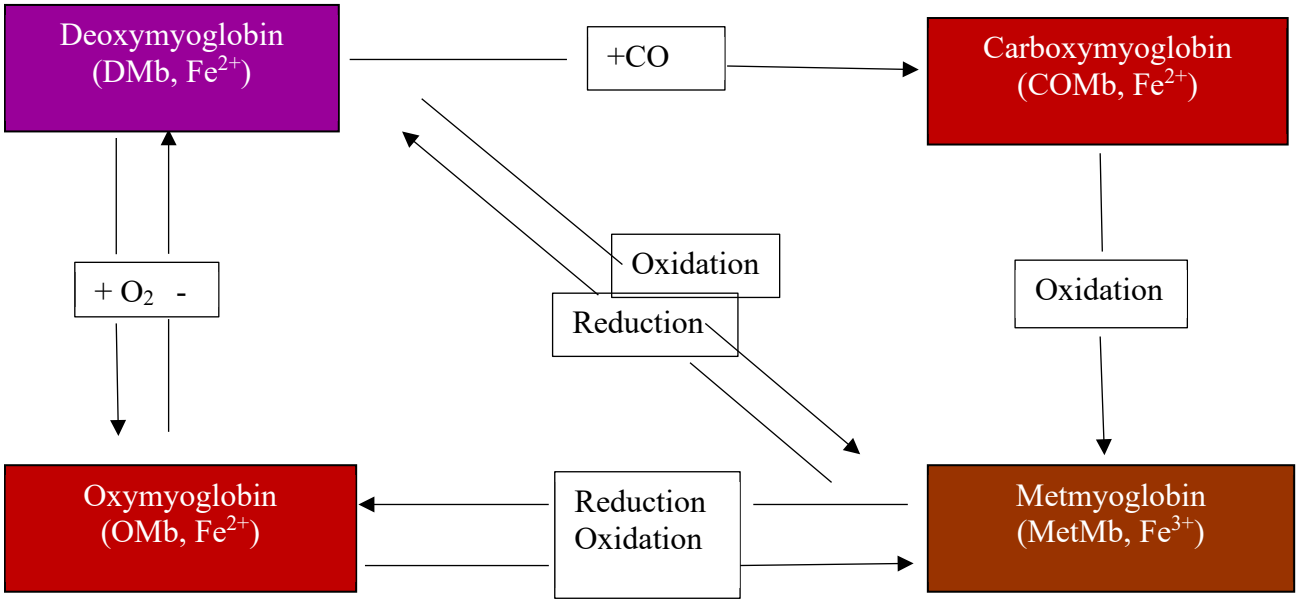

Figure 1.4 Myoglobin redox forms in fresh meats.

In meat, Mb can be present as any of the four redox forms (Figure 1). Myoglobin can exist as deoxymyoglobin (DMb), oxymyoglobin (OMb), carboxy-myoglobin (COMb), and metmyoglobin (MetMb). In OMb and COMb, iron is present in a ferrous state and therefore both of these ligands produce a cherry red meat color (Mancini and Hunt, 2005; Cornforth and Hunt, 2008). In OMb and COMb, oxygen and carbon monoxide associate with the sixth coordinate site of iron, respectively. However, there is no ligand associated with the sixth coordinate site of iron in DMb, which forms a purplish-red color in meat. On the other hand, the oxidation of OMb and DMb into MetMb forms an undesirable brown color on the surface of the meat (Suman and Joseph, 2013). In MetMb, ferrous heme iron is oxidized into ferric iron and the ligand coordinate site of the heme iron is occupied by water (Faustman and Suman, 2017). Myoglobins are sensitive to prolonged exposure to light, heat, oxygen, microbial growth or freezing which can oxidize Mb into MetMb (McMillin, 2008). In horse heart, *longissimus dorsi*, diaphragm and *psoas* the concentrations of Mb have been reported to be 32.5, 46.5, 61.0 and 70.5 mg/g muscle, respectively (Lawrie, 1950).

Although meat color is primarily related to myoglobin concentration and its chemical forms, its stability is influenced by multiple intrinsic and extrinsic factors. Exploring the scientific mechanisms behind those factors helps to develop strategies for pre-harvest and post-harvest handling of animals and their carcasses, respectively (Suman and Joseph, 2013). The major factors that affect meat appearance are muscle ultimate pH, muscle fiber type, lipid oxidation, mitochondrial activity, and the absence or presence of antioxidants, and these in turn can be affected by animal species, age, sex, breed and feeding system (Mancini and Hunt, 2005; Neethling et al., 2017).

1.3.2 Muscle fibre type and meat color

Muscle fiber type is related to the functional characteristics and metabolic activities of muscles and differs depending on the locomotion of the muscle the animal species, and the age of animal (Astruc, 2014). Muscle fibers are classified into types I, IIa, IIx and IIb based on their specific metabolic activity. Type I muscle fibres are defined as slow twitch muscle and they have a high capacity for oxidative metabolism. Type IIb muscle fibres are fast twitch with high glycolytic metabolism while Type IIa fibers are fast twitch with both oxidative and glycolytic capabilities (Klont et al., 1998; Rosser et al., 1992; Hintz et al., 1984; Lowry et al., 1978). Fast twitch muscle fibers are also referred to as alpha (α) fibers, and slow twitch muscle fibers as beta (β) fibers. Muscle fibres with aerobic metabolic activity are also classified as red (R) and white (W) fiber (Ashmore and Doerr, 1971). Hence, under this system fast white, slow red and intermediate fibers are designated as αW, βR and αR respectively. Meat myoglobin concentration, which varies due to muscle fiber composition (Khan, 1976) significantly contributes to muscle color (Joo et al., 2013). For instance, due to high concentration of myoglobin, type I and type IIa fibers are red while type IIb muscle fibers which have limited myoglobin appear white or pale. High affinity of

myoglobin to oxygen makes muscle color unstable; therefore, muscle with a higher proportion of type I muscle fibers usually exhibits rapid discoloration (Jeong et al., 2009). Muscle with higher βR and αR fibers possesses greater cytochrome oxidase and succinate dehydrogenase enzyme activities which increase uptake of oxygen by myoglobin and enhance metmyoglobin formation; hence, bovine psoas major muscles tend to form metmyoglobin faster than the longissimus thoracis lumborum when exposed to air (Joseph et al., 2012; Canto et al., 2016). Rapid browning of bison longissimus muscles (Pietrasik et al., 2006) results from the high proportion of intermediate muscle fibers in this species.

The deleterious pale, soft and exudative (PSE) condition is also related to fiber type composition. PSE commonly develops in pork when post-mortem muscle pH drops rapidly in combination with high muscle temperature prompting protein to undergo denaturation leading to the precipitation of sarcoplasmic protein onto myofibrillar protein which alters the biophysical properties of the meat (Offer, 1991; Klont et al., 1998; Karlsson et al., 1999). In addition to pale color, PSE meat has poor water holding capacity and is soft. A pale color can occur in beef due to insufficient cooling rate rather than fast glycolysis (Aalhus et al., 1998). In pork, PSE meat can arise from a genetic defect called porcine stress syndrome that originates from a leaky ryanodine receptor in the sarcoplasmic reticulum that is a consequence of genetic selection for rapid lean muscle growth.

Muscle glycolytic potential, which is the capacity of anaerobic metabolism substrates available to be converted into lactic acid, is related to muscle types and animal species (Gentry et al., 2004). *Longissimus thoracis et lumborum* muscle lightness is moderately correlated with muscle glycolytic potential (Hamilton et al., 2003). As a result, the color of the *longissimus* muscle is inversely related to glycolytic potential and muscle free glucose (Mancini and Hunt, 2005). However, horse *semimembranosus* (*SM*) muscle is dominated by type IIa and type IIb muscle

fibers, which account for approximately 52% and 28%, respectively, of the total muscle fibers (Roy et al., 2018). Horse meat becomes very dark post mortem on surfaces exposed to air, suggesting that the glycolytic potential of horse muscle is not positively correlated to muscle lightness.

1.3.3 Meat ultimate pH and color

Meat pH determines multiple functional properties in meat specifically meat color and color stability. Intramuscular pH influences muscle protein denaturation (Warriss et al., 1995), water holding capacity (Qu et al., 2017), and post-mortem enzymatic activity. For example, protein denaturation is high and water holding capacity is low at the isoelectric pH (5) of muscle; therefore, meat appears light in color such as when it is pale, soft, and exudative (PSE). Contrarily, high pH meat appears darker due to its high water holding capacity and an extreme example of this is dark, firm and dry (DFD) meat. The rate of post-mortem glycolysis, pH decline and ultimate pH critically affect meat color (Holdstock et al., 2014). Similarly, Hughes et al. (2014) demonstrated that muscle glycogen and pH are linked to each other. In the early post-mortem period, during muscle conversion to meat, muscle pH normally declines to between 5.5 and 5.7 (Lawrie and Ledward, 2006). If the muscle pH is above this range it is considered to be DFD meat (McGilchrist et al., 2012) although there is controversy regarding the exact pH range for DFD meat (Page et al., 2001; Ferguson et al., 2001). Post-mortem muscle glycogen content also affects the rate of muscle pH decline. For instance, for muscle pH to decline to a pH value within 5.5 to 5.7, muscles must have at least 57 μmol of available glycogen/g of meat (Tarrant, 1989). Depletion of muscle glycogen is related to several factors including poor nutrition, stress, age of animal, environmental temperature, hormone implantation, lairage time before slaughter and subclinical disease (Scanga

et al., 1998; Schneider et al., 2007; Mach et al., 2008). On the other hand, when muscle pH declines to 5.7- 6 45 minutes after slaughter it is considered to be PSE meat (O'Neill et al., 2003). In pork, PSE meat is the consequence of pre-slaughter stress, which accelerates the glycolysis process and the rapid decline of pH. The rapid decline of pH with high carcass temperature can cause denaturation of muscle proteins and reduce meat water holding capacity (Warriss et al., 1995), resulting in meat that is pale. Horse muscle contains high levels of glycogen averaging about 22 mg/g compared to bovine muscle which has less than 10 mg/g (Lawrie, 1998). Muscle pH of horse meat also ranges from 5.8 to 5.67 depending on the type of muscle (Ley, 1996; Litwinczuk et al., 2008). Gill (2005) reported that PSE or DFD have not been recorded in horse meat and speculated that this may be due to horses being selected predominantly for use as work animals rather than for meat production.

1.3.4 Effect of age and sex on meat color

The muscle color variation can occur due to animal age, sex and anatomical location of the muscle (Badiani and Manfredin, 1994; Gagaoua et al., 2018). It may be due to variation in myoglobin concentration due to age differences. For example, in horse muscle, the myoglobin concentration increases during the first two years of life and then decreases the following ten years (Badiani and Manfredini, 1994). Meat from six month old foals is lighter in color than that from 18 month old horses (De Palo et al., 2012). A similar result was reported in horse meat by Domínguez et al. (2015). Muscle physiochemical properties also differ due to sex (Gagaoua et al., 2016; Page et al., 2001) as bovine females appear to be more susceptible to stress, thus increasing muscle metabolic activity and reducing its stored glycogen and darkening their muscle compared to steer muscle. Mahmood et al. (2019) reported that heifers are more susceptible to producing a dark cutter carcass

as compared to steers. Similarly, in female horses, muscles of 16 month old fillies showed darker meat color as compared to muscles from a similarly aged group of colts (Sarriés and Beriain, 2005 and 2006).

1.3.5 Effect of ageing on meat color

Meat color and its stability during retail display are influenced by post-mortem ageing (Vitale at al., 2014). In aged muscles the mitochondrial enzymatic activities cease or are decreased and as a consequence, the competition of mitochondrial enzymes for oxygen decreases and increases the availability of oxygen to myoglobin, resulting in aged muscle having increased red color intensity compared to that of fresh aged muscle (Seideman et al., 1984). Contrarily, prolonged ageing may negatively impact meat color as lipid oxidative stability decreases with time which accelerates meat surface discoloration. Also, lipid oxidation can produce an off flavour when meat is repackaged and displayed under retail light condition (Kim et al., 2011; Kim et al., 2012; Kim et al., 2018ab). The accelerated discoloration associated with prolonged ageing of meat may be due to a reduction in metmyoglobin-reducing activity combined with depletion or alteration of mitochondrial structure and functionality (Kim et al., 2011). Meat discoloration and darkening therefore appears to result from metmyoglobin formation and surface dehydration, respectively, potentially increasing economic losses due to a perceived decrease in quality by consumers (Smith et al., 2000; Mancini and Hunt, 2005). The high myoglobin concentration and high oxygen consumption ability of horse meat accelerates the formation of metmyoglobin and reduces its shelf life (Badiani and Manfredini, 1994; Lorenzo et al., 2014). It is assumed that prolonged ageing provokes the accumulation of pro-oxidants (e.g, heme and nonheme iron) or depletes endogenous reducing compounds or antioxidants (Bekhit et al., 2013; Kim et al., 2012). For instance, NAD^+

concentration and metmyoglobin reductase activity decreased in beef *psoas* and *longissimus lumborum* after 21 days of ageing (Madhavi and Carpenter, 1993) and the reducing compounds α-tocopherol and β-carotene were depleted in Argentina buffalo meat aged for 25 days (Descalzo et al., 2008). The quantities of metabolites with antioxidant properties like NAD/NADH, acyl carnitines, nucleotides, nucleosides and glucuronides were varied depending on the muscle types after ageing (Ma et al., 2017). However, the scientific information about antioxidant properties and its changes during prolong storage of horse meat is limited.

1.3.6 Effect of feeding system on meat color

Feeding system can affect muscle glycogen content and the accumulation of fat and antioxidants in muscle, which strongly influence meat intrinsic factors associated with meat color including pH, oxygen consumption and metmyoglobin reducing activity (Mancini and Hunt, 2005). Dietary associations with meat color are commonly observed in beef (Vestergaard et al., 2000), pork, lamb and horse meat (Sarriès and Beriain, 2005; Lanza et al., 2009). Meat from animals fed in a pasture-based system usually produce meat with a dark red colour because, while on pasture, animals exhibit increased physical activity as compared to a stall-feeding system. It is assumed that high physical activity increases the formation and proportion of slow contracting muscle fibers with higher oxidative metabolic potential and a darker appearance as compared to an intensive stall-feeding system where physical activity is limited (Vestergaard et al., 2000). For instance, lambs with pasture-based feeding system produced meat with a darker color than that of concentrate-fed lambs (Vestergaard et al., 2000). Color differences in subcutaneous fat were also observed between pasture and concentrate-fed lambs (Díaz et al., 2002) and similar differences were observed in beef subcutaneous fat for cattle fed on concentrate and forage diets (Neethling et al., 2017). Forage-

based diets appear to lead to darker (lower lightness/*L** values) beef than concentrate-based diets, although redness (*a**) did not differ due to feeding system while pasture diets resulted in higher yellowness (*b**) values than concentrate diets (Cooke et al., 2004; Avilés et al., 2015). Similar results were reported in horse meat, with horses fed higher proportions of fodder producing meat exhibiting increased luminosity and yellowness (Franco et al., 2013). In a pasture-based feeding system green forage contains high concentrations of carotene, which accumulates in subcutaneous and intramuscular fat and casting a yellow hue compared to that from animals receiving grain concentrate diets. The pasture-based green forage also contains high proportions of antioxidants which also improve meat color stability and this was observed in meat from pasture-based lambs (Faustman et al., 2010). Similar findings were reported for venison (Wiklund et al., 2010), lamb (Díaz et al, 2002, Perlo et al., 2008) and beef (Lanari et al., 2002). However, there is no scientific data available as to how pasture-based feeding systems affect horse meat color quality relative to that of grain-based feeding systems.

1.4 Water Holding capacity

Water loss as purge or drip is an important economic factor associated with quality and value of meat (Huff-Lonergan and Lonergan, 2005). Muscle water can be classified as free, bound and entrapped water. The movement of free water is not restricted, and it is held in muscle by weak surface forces while the bound water is held in the muscle bound to non-aqueous constituents like protein. Bound water is resistant to freezing but can be driven off by conventional heating (Fennema, 1985) and very little changes happen to it during the post rigor process (Offer and Knight, 1988). The entrapped or immobilized water is the portion of water most affected during the rigor process as this water is held in muscle through either a steric effect or by the attraction to

the bound water. Through alteration of muscle structure, usually by it being cut and lowering of the muscle pH during the post rigor period, this water can escape from muscle as purge (Offer and Knight, 1988). Hence, water loss is inevitable during the process when muscles are converted to meat. It has been reported that for more than 50% of pork produced the level of purge or drip loss (Stetzer and McKeith, 2003) is highly unacceptable, and accounts for around 1-3% in retail weight (Offer and Knight, 1988). Additionally, this amount can be as high as 10% for PSE meat (Melody et al., 2004). In horse meat after 30 days of ageing a maximum 6.8% and a minimum of 0.99% drip loss was observed in sirloin and shoulder chuck roll respectively (Seong et al., 2016). However, purge loss not only results in the loss of water but also in a significant amount of soluble sarcoplasmic protein, which is on average 112 mg per millilitre of fluid (Savage et al., 1990). The major proportion (around 85%) of muscle water is present between the myofilaments with the rest between myofibrils, between muscle fibers and between muscle fiber bundles (Huff-Lonergan and Lonergan, 2005; Liu et al., 2016). During conversion of muscles to meat, the myofibrils shrink laterally and reduce the cross-sectional area of each muscle fiber and as a consequence sarcoplasmic fluid moves to the extracellular space, adding to the purge or drip from the muscle. In early post-mortem enlarged extracellular spaces between muscle fibers and muscle fiber bundles observed using electron microscopic imaging (Offer and Cousins, 1992) may be due to myofibrillar shrinkage and movement of intracellular fluid to the extracellular space. An improvement in water holding capacity is sometimes observed during extended ageing and is considered to be due to proteolysis of structural and cytoskeleton proteins including desmin, titin, nebulin and integrin (Lawson, 2004; Melody et al., 2004; Zhang et al., 2006). The degradation of myofibrillar linkages during post-mortem ageing may reduce myofibrillar shrinkage and increase intracellular spaces that can retain water (Huff-Lonergan and Lonergan, 2005). Furthermore,

during prolonged ageing, the breakdown of myofibrillar protein is assumed to interrupt drip channels and as a result increase the ability of the muscle cells to hold water (Farouk et al., 2012). In PSE meat protein denaturation is induced by low muscle pH (< 6.0) at high muscle temperatures (> 35°C) early post mortem, which causes poor water holding capacity post-rigor (Liu et al., 2016). Kim et al. (2014) explained how protein denaturation of both soluble and structural protein induced poor water holding capacity of PSE meat. In horse meat the normal ultimate pH range is 5.48-5.89, which is similar to that of other species and therefore unlikely to cause species-related differences. However, prolonged post-mortem ageing not only increases the extent of proteolysis but also increases the susceptibility of protein to oxidation by exposing meat to different oxidative conditions. During ageing, carbonyl and sulfhydryl groups commonly produced by protein oxidation are a result of the loss of amino acid functional groups. The loss of these groups prompts the formation of intra- and inter-protein disulphide cross links which significantly impair muscle protein functionality and negatively impact water holding capacity (Li et al., 2018; Xiong, 2000). Furthermore, protein denaturation causes loss of tertiary protein structure which leads to poor water holding capacity of the protein (Choi et al., 2010).

Despite the effects of post mortem ageing on meat water holding capacity being well characterized in other species, the effect of prolonged ageing on the water holding capacity of horse meat is not yet clear. In addition, deboning time may influence muscle shortening which may also interfere with muscle water holding capacity. Additionally, previous studies of horse meat focused mainly on meat ageing and its sensory properties (Sarriés and Beriain, 2005; Franco et al., 2011; Beldarrain et al., 2020) after a short period of ageing (24 h to 14 days) and did not focus on the impact of extended ageing on horse meat water holding capacity. Because horse meat from Canada is often aged for long periods during its transit to international markets, this warrants investigation.

1.5 Objectives of the study

In Canada, the horse meat industry is oriented toward export with Europe as the major market. To reach these long-distance customers, the process of harvesting, storage and transportation to market needs to be as short and as fast as possible. During this process it is also essential to make sure that the quality, which is gauged by customer preference, is not compromised. Currently, in Canada horse carcasses are de-boned or fabricated while hot (deboned from the carcass after two hours of slaughter) or after complete carcass chilling (deboned 40 h after slaughter). Hot boning helps to reduce the storage cost and process time to reach the customer. Contrarily, deboning before onset of rigor may lead to tough meat because of muscle shortening. It is assumed that tenderization by ageing helps to minimize the rigor toughness. However, the duration of this process for horse meat scientifically is not yet defined. In addition, collagen quantity and its chemical properties can impact meat toughness, but most of the research on collagen and its impact on meat toughness has been conducted on beef, pork and lamb (Berge et al., 2003; Chambaz et al., 2003; Schilling et al., 2003; Roy et al., 2015; Starkey et al., 2017) and not horse meat. The previous few studies on horse meat focussed on horse meat nutritional profile and sensory properties after short term ageing (up to 14 d) (Sarriés and Beriain, 2005; Franco et al., 2011; Beldarrain et al., 2020). Also, there has been no study that has focussed on the deboning process and the impact of extended post mortem ageing on horse meat quality. Therefore, an objective of this research was to examine the effect of de-boning of the *semimembranosus* muscle, a high connective tissue muscle, at various times early post mortem on horse meat quality, and the changes if any on horse meat quality after extended post mortem ageing. A second objective of this research was to characterize the contribution of collagen to the toughness of horse meat, testing the hypothesis that

the amount of collagen does not contribute significantly to horse meat toughness but the collagen heat solubility does and it increases with post mortem ageing.

References

Aalhus, J. L., Best, D. R., Murray, A. C and Jones, S. M. D. (1998). A comparison of the quality characteristics of pale, soft and exudative beef and pork. Journal of Muscle Foods, 9: 267-280.

Andersen, H. J., Oksbierg, N., Young, J. F. and Therkildsen, M. (2005). Feeding and meat quality a future approach: A review. Meat Science, 70: 543-554.

Ashmore, C. R. and Doerr, L. (1971). Comparative aspects of muscle fiber types in different species. Experimental Neurology, 31: 408-418.

Astruc, T. (2014). Muscle fiber types and meat quality. Encyclopedia of Meat Sciences (Second Edition), Academic Press, pp. 442-448.

Avilés, C., Martinez, A. L., Domenech, V. and Pena, F. (2015). Effect of feeding system and breed on growth performance, and carcass and meat quality traits in two continental beef breeds. Meat Science, 107: 94-103.

Badiani, A. and Manfredini, M. (1994). La produzione della carne di caballo. Zootecnicae Nutrizione Animale, 20: 5-43.

Badiani, A., Nanni, N., Gatta, P.P., Tolomelli, B. and Manfredini, M. (1997). Nutrient profile of horse meat. Journal of Food Composition and Analysis.10: 254-269.

Bailey, A. J. (1972). The basis of meat texture. Journal of Food and Agriculture, 23: 995-1007.

Bailey, A. J. and Sims, T. J. (1977). Meat tenderness: distribution of molecular species of collagen in bovine muscle. Journal of the Science of Food and Agriculture, 28: 565-570.

Behnke, J. R., Fennema, O., and Cassens, R. G. (1973). Rates of postmortem metabolism in frozen animal tissues. Journal of Agricultural and Food Chemistry, 21: 5-11.

Bekhit A. E. D. A., Hopkins, D. L., Fahri, F. T. and Ponnampalam, E. N. (2013). Oxidative processes in muscle systems and fresh meat: Sources, markers, and remedies. Comprehensive Reviews of Food Science and Food Safety, 12: 565-597.

Bekhit, A. E. D. and Faustman, C. (2005). Meat myoglobin reducing activity. Meat Science, 71: 407-439.

Belaunzaran, X., Bessa, R. J. B., Lavín, P., Mantecón, A. R., Kramer, J. K. G. and Aldai, N. (2015). Horse-meat for human consumption-Current research and future opportunities: Review. Meat Science, 108: 75-81.

Beldarrain, L. R., Etaio, I., Moran, L., Sentandreu, M. A. (2020). Effect of ageing time on consumer preference and sensory description of foal meat. Food Research International, 129: 108871.

Bendall, J. R. (1951). The shortening of rabbit muscles during rigor mortis: its relation to the breakdown of adenosine triphosphate and creatine phosphate and to muscular contraction. Journal of Physiology, 114: 71-88.

Bendall, J. R. (1975). Cold-contracture and ATP-turnover in the red and white musculature of the pig post-mortem. Journal of the Science of Food and Agriculture, 26: 55-71.

Bendall, J. R. and Voyle, C. A. (1967). A study of the histological changes in the growing muscles of beef animals. Journal of Food Technology, 2: 259-283.

Berge, P., Saudo, C., Sanchez, A., Alfonso, M., Stamataris, C. and Thorkelsson, C., et al. (2003). Comparison of muscle composition and meat quality traits in diverse commercial lamb types. Journal of Muscle Foods, 14: 281-300.

Boleman, S. J., Boleman, S. L., Miller R. K., Taylor, J. F., Cross, H. R., Wheeler T. L., Koohmaraie, M., Shackelford, S. D., Miller, M. F., West, R. L., Johnson, D. D. and Savell, J. W. (1997). Consumer evaluation of beef of known categories of tenderness. Journal of Animal Science, 75: 1521-1524.

Bosselmann, A., Möller, C., Steinhart, H., Kirchgessner, M. and Schwarz, F. Z. (1995). Pyridinoline cross-links in bovine muscle collagen. Journal of Food Science, 60: 953-958.

Bowling, R. A., Dutson, T. R., Smith, G. C. and Savell, J. W. (1987). Effects of cryogenic chilling on beef carcass grade, shrinkage and palatability characteristics. Meat Science, 21: 67-72.

Brooks, J. C., Savell, J. W. (2004). Perimysium thickness as an indicator of beef tenderness. Meat Science, 67: 329-334.

Brown, J. H., Pilliner, S. and Davies, Z. (2003). Horse and stable management (4th edn.). Oxford: Blackwell Publishing.

Buege, D. R., Marsh and B. B. (1975). Mitochondrial calcium and post-mortem muscle shortening. Biochemical and Biophysical Research Communications, 65: 478-482.

Canto, A. C. V. C. S., Costa-Lima, B. R. C., Suman, S. P., Monteiro, M. L.G., Viana, F. M., and Salim, A. P. A. A. (2016). Color Attributes and oxidative stability longissimus lumborum psoas major muscles from Nellore bulls. Meat Science, 121: 19-26.

Carlson, K. B., Prusa, K. J., Fedler, C. A., Steadham, E. M., Outhouse, A. C., King, D. A. and Lonergan, S. M. (2017). Postmortem protein degradation is a key contributor to fresh pork loin tenderness. Journal of Animal Science, 95: 1574-1586.

Carpenter, C. E., Cornforth, D. P., Whittier, D. (2001). Consumer preference for beef color packaging did not affect eating satisfaction. Meat Science, 57: 359-363.

Chambaz A., Scheeder, M. R. L., Kreuzer, M. and Dufey, P. A. (2003). Meat quality of Angus, Simmental, Charolais and Limousin steers compared at the same intramuscular fat content. Meat Science, 63**:** 491-500

Chizzolini, R., Ledward, D. A., Lawrie, R. A. (1977). Note on the effect of ageing on the neutral salt and acid soluble collagen from the intramuscular connective tissue of various species. Meat Science, 1: 111-117.

Choi, Y. M., Lee, S. H., Choe, J. H., Rhee, M. S., Lee, S. K., Joo, S. T. and Kim, B. C. (2010). Protein solubility is related to myosin isoforms, muscle fiber types, meat quality traits, and postmortem protein changes in porcine longissimus dorsi muscle. Livestock Science, 127: 183-191.

Cook, C. F. and Langsworth, R. F. (1966). The effect of pre-slaughter environmental temperature and post-mortem treatment upon some characteristics of ovine muscle. I. Shortening and pH. Journal of Food Science, 31: 497-503.

Cooke, D. W. I., F Monahan, F. J., Brophy, P. O. and Boland, M. P. (2004). Comparison of concentrates or concentrates plus for-ages in a total mixed ration or discrete ingredient format: Effects on beef production parameters and on beef composition, colour, texture and fatty acid profile. Irish Journal of Agricultural and Food Research, 43: 201-216.

Corbin, C. H. and Miller, M. F. (2015). Sensory evaluation of tender beef strip loin steaks of varying marbling levels and quality treatments. Meat Science, 100: 24-31.

Cornforth D and Hunt M. (2008). Low-oxygen packaging of fresh meat with carbon monoxide: meat quality, microbiology and safety. In *AMSA White Paper Series: No. 2*, pp 1-12. Savoy, IL: American Meat Science Association

Cornforth, D. P., Pearson, A. M. and Merkel, R. A. (1980). Relationship of mitochondria and sarcoplasmic reticulum to cold shortening. Meat Science, 4: 103-121.

Cross, H. R., Carpenter, Z. L. and Smith, G. C. (1973). Effects of intramuscular collagen and elastin on bovine muscle tenderness. Journal of Food Science, 38: 998-1003.

Davey, C. L. and Gilbert, K. V. (1973). The effect of carcass posture on cold, heat and thaw shortening in lamb. Journal of Food Technology, 8: 445-451.

Descalzo, A. M., Rossetti, L., Sancho, A. M., García, P. T., Biolatto, A., Carduza, F. and Grigioni, G. M. (2008). Antioxidant consumption and development of oxidation during ageing of buffalo meat produced in Argentina. Meat Science, 79: 582-588.

Devine, C. E., Wahlgren, N. M., Tornberg, E. (1999). Effect of rigor temperature on muscle shortening tenderization of restrained and unrestrained beef *m*. *longissimus thoracicus et lumborum*. Meat Science, 51: 61-72.

Díaz, M. T., Susana, V., Cañeque, V., Lauzurica, S., Ruiz de Huidobro, F., Pérez, C., González, J., and Manzanares, C. (2002). Use of concentrate or pasture for fattening lambs and its effect on carcass and meat quality. Small Ruminant Research, 43: 257-268.

Domínguez, R., Crecente, S., Borrajo, P., Agregan, R., Lorenzo, J. M. (2015). Effect of slaughter age on foal carcass traits and meat quality. Animal, 9: 1713-1720.

Dransfield, E. (1977). Intramuscular composition and texture of beef muscles. Journal of the Science of Food and Agriculture, 28: 833-842.

Dufey, P.A. (1996). Sensory and physico-chemical properties of meat from horse of different age groups. Proceeding of 42nd International congress of Meat Science and Technology (pp. 556-557).

Edwards, E. H. (1998). La enciclopedia del caballo (1st ed.). Barcelona (Spain): Blume.

Emerson, M. R., Woerner, D. R., Belk, K. E. and Tatum, J. D. (2013). Effectiveness of USDA instrument-based marbling measurements for categorizing beef carcasses according to differences in longissimus muscle sensory attributes. Journal of Animal Science, 91: 1024-1034.

Ertbjerg, P. and Puolanne, E. (2017). Muscle structure, sarcomere length and influence on meat quality: A review. Meat Science, 132: 139-152.

Ertbjerg, P., Henckel, P., Karlsson, A. H., Larsen, L. M. and Moller, A. J. (1999). Combined effect of epinephrine and exercise on calpain/calpastatin and cathepsin B and L activity in porcine longissimus muscle. Journal of Animal Science, 77: 2428-2436.

Eyre, D. R. and Wu, J. J. (2005). Collagen cross-links. Topics in Current Chemistry, 247: 207-229.

Eyre, D. R., Apon, S., Wu, J. J., Ericsson, L. H. and Walsh, K. A. (1987). Collagen type-IX-Evidence for covalent linkages to type-II collagen in cartilage. FEBS Lett, 220: 337-341.

FAO (2015). Food and Agriculture Organization of the United Nations. Faostat (http:// faostat3.fao.org/home/index.html#DOWNLOAD (2015/04/29)).

FAO (2018). Food and Agriculture Organization of the United Nations. Faostat (http:// faostat3.fao.org/home/index.html#DOWNLOAD (2018/04/29)).

Farouk, M., Mustafa, N. M., Wu, G. and Krsinic. G. (2012). The "sponge effect" hypothesis: An alternative explanation of the improvement in the water holding capacity of meat with ageing. Meat Science, 90: 670-677.

Faustman, C. and Suman, S. P. (2017). The eating quality of meat: I-Color. In Woodhead Publishing Series in Food Science, Technology and Nutrition, Lawrie´s Meat Science (Eighth Edition), pp. 329-356 (Chapter 11).

Faustman, C., Sun, Q., Mancini, R., and Suman S. P. (2010). Myoglobin and lipid oxidation interactions: Mechanistic bases and control. Meat Science, 86: 86-94.

Fennema, O. R. (1985). Water and ice. In O. R. Fennema (Ed.), Food chemistry. New York: Marcel Dekker Inc.

Ferdous, Z., Wei, V. M., Iozzo, R., Hook, M. and Grande-Allen, K. J. (2007). Decorin-transforming growth factor interaction regulates matrix organization and mechanical characteristics of three-dimensional collagen matrices. Journal of Biological Chemistry, 282: 35887-35898.

Ferguson, D. M., Bruce, H. L., Thompson, J. M., Egan, A. F., Perry, D. and Shorthose, W. R. (2001). Factor affecting beef palatability: Farmgate to chilled carcass. Australian Journal of Experimental agriculture, 41: 879-891.

Fernández de Labastida, I. (2011). Caballos de monte y carne de potro. (PhD Thesis) Análisis antropológico deun proceso contemporáneo de construcción identitaria, cultural y económica en la montaña Alavesa. Spain: University of the Basque Country.

Feuz, D. M., Umberger W. J., Calkins C. R. and Si B. (2004). U.S. Consumers willing to pay for flavor and tenderness in steaks as determined with experimental auction. Journal of Agricultural Resource Economics, 29: 501-516.

Fischer, C., Honikel, K. O. and Hamm, R. (1980). Über das Auftreten der Kälteverkürzung "cold shortening" in dunkler und heller Muskulatur bei Rind und Schwein (cold shortening in the dark and light musculature of cattle and pigs). Fleischwirtschaft, 60: 263-265.

Franco, D., Crecente, S., Vazquez, J. N., Gomez, M., Lorenzo, J. M. (2013). Effect of cross breeding and amount of finish diet on growth parameters, carcass and meat composition of foal slaughtered 15 months of age. Meat Science, 93: 547-556.

Franco, D., Rodríguez, E., Purriños, L., Crecente, S., Bermúdez, R. and Lorenzo, J. M. (2011). Meat quality of "Galician Mountain" foals breed. Effect of sex, slaughter age and livestock production system. Meat Science, 88: 292-298.

Gagaoua, M., Picard, B. and Monteils, V. (2018). Associations among animal, carcass, muscle characteristics, and fresh meat color traits in Charolais cattle. Meat Science, 40: 145-156.

Gagaoua, M., Terlouw, E. M. C., Micol, D., Hocquette, J. F., Moloney, A. P., Nuernberg, K. and Picard, B. (2016). Sensory quality of meat from eight different types of cattle in relation with their biochemical characteristics. Journal of Integrative Agriculture, 15: 1550-1563.

Gann, G. L., and Merkel, R. A. (1978). Ultra structural changes in bovine longissimus muscle during post mortem ageing. Meat Science 2: 129-144.

Gentry, J. G., McGlone, J. J., Miller, M. F. and Blanton, J. R, (2004). Environmental effects on pig performance, meat quality, and muscle characteristics. Journal of Animal Science, 82: 209-217.

Gill, C.O. (2005). Safety and storage stability of horse meat for human consumption. Meat Science, 71: 506-513.

Girard, I., Bruce, H. L., Basarab, J. A., Larsen, I. L. and Aalhus, J. L. (2012). Contribution of myofibrillar and connective tissue components to the Warner-Bratzler shear force of cooked beef. Meat Science, 92: 775-782.

Gómez, M., and Lorenzo, J. M. (2012). Effect of packaging conditions on shelf-life fresh foal meat. Meat Science, 91: 513-520.

Guil-Guerrero, J. L., Rincón-Cervera, M. A., Venegas-Venegas, C. E., Ramos-Bueno, R. P. and Suárez-Medina, M. D. (2013). Highly bioavailable α-linolenic acid from the subcuta- neous fat of the Palaeolithic Relict "Galician horse". International Food Research Journal, 20: 3249-3258.

Guil-Guerrero, J. L., Tikhonov, A., Rodríguez-García, I., Protopopov, A., Grigoriev, S., Ramos-Bueno, R. P. (2013). The fat from frozen mammals reveals sources of essential fatty acids suitable for Palaeolithic and Neolithic humans. PLoS ONE, 9: 84480.

Hamilton, D. N., Miller, K. D., Ellis, M., McKeith, F. K. and Wilson, E. R. (2003). Relationships between longissimus glycolytic potential and swine growth performance, carcass traits, and pork quality. Journal of Animal Science, 81: 2206-2212.

He, M. L., Ishikawa, S. and Hidari, H. (2005). Fatty acid profiles of various muscles and adipose tissues from fattening horses in comparison with beef cattle and pigs. Asian-Australasian Journal of Animal Sciences, 18: 1655-1661.

Herring, H. K., Cassens, R. G., Suess, C. G., Brungardt, V. H., Briskey, E. J. (1967). Tenderness and associated characteristics of stretched and contracted bovine muscles. Journal of Food Science, 32: 317-323.

Hill, F. (1966). The solubility of intramuscular collagen in meat animals various ages. Journal of Food Science, 31: 161-166.

Hintz C. S., Coyle, E. F., Kaiser, K. K, Chi M. M. Y. and Lowry, O.H. (1984). Comparison of muscle fiber typing by quantitative enzyme assays and by myosin ATPase staining. Journal of Histochemistry and Cytochemistry, 32: 655-660.

Hintz, H. F. (1995). Thoughts about the history of horses. Journal of Equine Veterinary Science, 15: 336-339.

Holdstock, J., Aalhus, J. L., Uttaro, B. A., López-Campos, Ó, Larsen, I. L. and Bruce, H. L. (2014). The impact of ultimate pH on muscle characteristics and sensory attributes of the longissimus thoracis within the dark cutting (Canada B4) beef carcass grade. Meat Science, 98: 842-849.

Honikel, K. O. (2014). Conversion of muscle to meat. In Carrick, D.E, Michael, D. (Eds.). Encyclopedia of meat sciences, pp. 353-357. (2nd ed.). Oxford: Academic Press.

Hopkins, D. L. (2004). Tenderizing mechanisms - mechanical. In J. Werner Klinth (Ed.). Encyclopedia of Meat Sciences (pp. 1355-1363). Oxford: Elsevier.

Hopkins, D. L. and Geesink G. H. (2009). Protein degradation postmortem and tenderization. Applied Muscle Biology and Meat Science,149-173.

Huff-Lonergan, E., Zhang, W., Lonergan, S.M. (2010). Biochemistry of post-mortem muscle-lessons on mechanisms of meat tenderization. Meat Science, 86:184-195.

Huff-Lonergan, E. and Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Science, 71: 194-204.

Huffman, K. L., Miller, M. F., Hoover, L.C., Wu, C. K., Britting, H. C. and Ramsey, C.B. (1996). Effect of beef tenderness on consumer satisfaction with steaks consumed in the home and restaurant. Journal of Animal Science, 74: 91-97.

Hughes, J. M., Oiseth, S. K., Purslow, P. P. and Warner, R. D. (2014). A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. Meat Science, 98: 520-532.

Hunsley, R. E., Vetter, R. L. and Kline, E. A., Burroughs, W. (1971). Effects of age and sex on quality, tenderness and collagen content of bovine Longissimus muscle. Journal of Animal Science, 33: 933-938.

Jeong, J. Y., Hur, S. J., Yang, H. S., Moon, S. H., Hwang, Y. H., Park, G. B. and Joo, S. T. (2009). Discoloration characteristics of 3 major muscles from cattle during cold storage. Journal of Food Science, 74: 1-5.

Joo, S. T., Kim, G. D., Hwang, Y. H. and Ryu, Y. C. (2013). Review: Control of fresh meat quality through manipulation of muscle fiber characteristics. Meat Science, 95: 828-836.

Joseph, P., Suman, S. P., Rentfrow, G., Li, S. and Beach C. M. (2012). Proteomics of muscle specific beef color stability. Journal of Agricultural and Food Chemistry, 60: 3196-3203.

Joseph, R. L. (1996). Very fast chilling of beef and tenderness a report from an EU concerted action. Meat Science, 43: 217-227.

Juárez, M., Polvillo, O., Gómez, M. D., Alcalde, M. J., Romero, F. and Valera, M. (2009). Breed effect on carcass and meat quality of foals slaughtered at 24 months of age. Meat Science, 83: 224-228.

Juárez, M., Polvillo, O., Gómez, M. D., Alcalde, M. J., Romero, F. and Varela, M. (2009). Breed effect on carcass and meat quality of foals slaughtered at 24 months of age. Meat Science, 83: 224-228.

Judge, M. D. and Aberle, E. D. (1982). Effects of chronological age and postmortem aging on thermal shrinkage temperature of bovine intramuscular collagen. Journal of Animal Science, 54: 68-71.

Karlsson, A. H., Klont, R. E. and Fernandez, X. (1999). Skeletal muscle fibers as factors for pork quality. Livestock Production Science, 60: 255-269.

Khan, A. W. and Cohen, D. C. (1977). Rapid estimation of muscle proteins in beef vegetable protein mixtures. Journal of Agricultural Food Chemistry, 25: 236-238.

Khan, M. A. (1976). Histochemical characteristics of vertebrate striated muscle: A review. Progress in Histochemistry and Cytochemistry, 8: 1-48.

Kim, H. W., Kim, J. H., Seo, J. K., Setyabrata, D. and Kim, Y. H. B. (2018). Effects of aging/freezing sequence and freezing rate on meat quality and oxidative stability of pork loins. Meat Science, 139: 162-170.

Kim, Y. H. B, Ma, D., Setyabrata, D., Farouk, M. M., Lonergan, S. M., Huff-Lonergan, E. and Hunt, M. C. (2018). Review: understanding postmortem biochemical processes and post-harvest ageing factors to develop novel smart-aging strategies. Meat Science, 144: 74-90.

Kim, Y. H. B., Frandsen, M. and Rosenvold, K. (2011). Effect of ageing prior to freezing on colour stability of ovine longissimus muscle. Meat Science, 88: 332-337.

Kim, Y. H. B., Stuart, A., Black, C. and Rosenvold, K. (2012). Effect of lamb age and retail packaging types on the quality of long-term chilled lamb loins. Meat Science, 90: 962-966.

Kim, Y. H. B., Warner, R. D. and Rosenvold, K. (2014). Influence of high pre-rigor temperature and fast pH fall on muscle proteins and meat quality: a review. Animal Production Science, 54: 375-395.

Klont, R. E., Brocks, L. and Eikelenboom, G. (1998). Muscle fiber type and meat quality. Meat Science, 49: 219-229.

Koohmaraie, M. (1996). Biochemical factors regulating the toughening and tenderization processes of meat. Meat Science, 43: 193-201.

Koohmaraie, M., and Geesink, G. H. (2006). Contribution of post-mortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. Meat Science, 74: 34-43.

Lanari, M. C., Brewster, M., Yang, A. and Tume, R. K. (2002). Pasture and grain finishing affect the color stability of beef. Journal of Food Science, 67: 2467-2473.

Lanza, M., Landi, C., Scerra, M., Galofaro, V. and Pennisi, P. (2009). Meat quality and intramuscular fatty acid composition of Sanfratellano and Haflinger foals. Meat Science, 81: 142-147.

Lawrie, R. A. (1950). Some observation on factors affecting myoglobin concentrations in muscle. Journal of Agricultural Science, 40: 356-366.

Lawrie, R. A. (1998). Meat Science, 6th Ed. Woodhead Publishing Cambridge, UK. Pp. 58-95.

Lawrie, R. A., and D. A. Ledward. (2006). Lawrie's meat science. 7th ed. Woodhead Publishing Ltd., Cambridge, UK. doi:10.1533/9781845691615

Lawson, M. A. (2004). The role of integrin degradation in post-mortem drip loss in pork. Meat Science, 68: 559-566.

Lepetit, J. and Culioli, J. (1994). Mechanical properties of meat. Meat Science, 36: 203-237.

Ley, T. (1996). Untersuchungen zu postmortalen Veränderungen in Pferdschlachttierkörpern. Fleischwirtschaft, 76: 172-175.

Li, X., Wei, X., Wang, H., Zhang, C. H., and Mehmood, W. (2018). Relationship between protein denaturation and Water holding capacity of pork during postmortem ageing. Food Biophysics, 13: 18–24

Lister D. (1989). Muscle metabolism and animal physiology in the dark cutting condition. In: Fabiansson S. U., Shorthose W. R., Warner R. D., editors. Proceedings of an Australian Workshop, AMLRDC. p 19-25.

Litwinczuk, A., Florek, M., Skalecki, P. and Litwinczuk, Z. (2008). Chemical composition and physicochemical properties of horse meat from the Longissimus Lumborum and Semitendinosus muscle. Journal of Muscle and Foods, 19: 223-236.

Liu, A., Nishimura, T., Takahashi, K. (1996). Relationship between structural properties of intramuscular connective tissue and toughness of various chicken skeletal muscles. Meat Science, 43: 43-49.

Liu, J., Arner, A., Puolanne, E. and Ertbjerg, P. (2016). On the water-holding of myofibrils: effect of sarcoplasmic protein denaturation. Meat science, 119: 32-40.

Locker, R. H. (1959). Striation patterns of ox muscle in rigor mortis. Journal of Biophysical and Biochemical Cytology, 6: 419-422.

Locker, R. H. (1960). Degree of muscular contraction as a factor in the tenderness of beef. Food Research, 25: 304-307.

Locker, R. H. (1985). Cold-induced toughness of meat. In A. M. Pearson, T. R. Dutson (Vol. Eds.), Electrical stimulation.Vol. 1. Advances in meat research (pp. 1-44). Westport, Connecticut: AVI Publishing Co.

Locker, R. H. and Hagyard, C. J. (1963). A cold shortening effect in beef muscle. Journal of the Science of Food and Agriculture, 14: 787-793.

Lorenzo, J. M., and Pateiro, M. (2013a). Influence of type of muscles on nutritional value of foal meat. Meat Science, 93: 630-638.

Lorenzo, J. M., Fuciños, C., Purriños, L. and Franco, D. (2010). Intramuscular fatty acid composition of "Galician Mountain" foals breed: Effect of sex, slaughtered age and livestock production system. Meat Science, 86: 825-831.

Lorenzo, J. M., Sarriés, M. V., and Franco, D. (2013b). Sex effect on meat quality and carcass traits of foals slaughtered at 15 months of age. Animal, 7: 1199-1207.

Lorenzo, J. M., Sarriés, M. V., Tateo, A., Polidori, P., Franco, D. and Lanza, M. (2014). Carcass characteristics, meat quality and nutritional value of horsemeat: A review, Meat Science, 96: 1478-1488.

Lowry, C. V., Kimmey, J. S., Felder, S., Chi, M. M. Y., Kaiser, K. K, Passonneau, P. N., Kirk, K. A. and Lowry, O. H. (1978). Enzyme pat- terns in single human muscle fibres. Journal of Biological Chemistry, 253: 8269-8277.

Ma, R., Qiu, S., Jiang, Q., Sun, H., Xue, T., Cai, G., et al. (2017). AI-2 quorum sensing negatively regulates rbf expression and biofilm formation in *Staphylococcus aureus*. International Journal of Medical Microbiology, 307:257-267.

Mach, N., Bach, A., Velarde, A. and Devant, M. (2008). Association between animal, transportation, abattoir practices, and meat pH in beef. Meat Science, 78: 232-238.

Madhavi D. L. and Carpenter, C. (1993). Aging and processing affect color, metmyoglobin reductase and oxygen consumption of beef muscles. Journal of Food Science, 58: 939 - 942.

Mahmood, S., Dixon, W. T., Bruce, H. L. (2019). Cattle production practices and incidence of dark cutting beef. Meat Science, 157: 107873.

Malva, A. D., Palo, P. D., Lorenz, J. M., Maggiolino, A., Albenzio, M. and Marino, R. (2019). Application of proteomic to investigate the post-mortem tenderization rate of different horse muscles. Meat Science, 157: 107885.

Mancini, R. A. and Hunt, M. C. (2005). Review: Current research in meat color. Meat Science, 71: 100-121.

Marsh, B. B. (1954). Rigor mortis in beef. Journal of the Science of Food and Agriculture, 5: 70-75.

Marsh, B. B. and Leet, N. G. (1966). Studies in meat tenderness. 3. The effects of cold shortening on tenderness. Journal of Food Science, 3: 450-459.

Marsh, B. B. and Thompson, J. F., (1958). Rigor mortis and thaw rigor in lamb. Journal of the Science of Food and Agriculture, 9: 417-424.

McCormick, R. J. (1994). The flexibility of the collagen compartment of muscle. Meat Science. 36: 79-91.

McCormick, R. J. (2009). Collagen. Applied Muscle Biology and Meat Science. CRC press, Taylor and Francis group, USA. Pp.129-148

McGilchrist, P., Alston, C. L., Gardner, G. E., Thomson, K. L. and Pethick, D.W. (2012). Beef carcasses with larger eye muscle areas, lower ossification scores and improved nutrition have a lower incidence of dark cutting. Meat Science, 92: 474-480.

McMillin K. W. (2008). Where is MAP going? A review and future potential of modified atmosphere packaging for meat. Meat Science, 80: 43-65.

Melody, J. L., Lonergan, S. M., Rowe, L. J., Huiatt, T. W., Mayes, M. S. and Huff-Lonergan, E. (2004). Early postmortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles. Journal of Animal Science, 82: 1195-1205.

Mikołajczak, B., Iwańska, E., Spychaj, A., Danyluk, B., Montowska, M., Grześ, B., Banach, J. K., Żywica, R., Pospiech, E. (2019) An analysis of the influence of various tenderising treatments on the tenderness of meat from Polish Holstein-Friesian bulls and the course of changes in collagen. Meat Science, 158: 107906

Neethling, N. E., Suman, S. P., Sigge, G. O., Hoffman, L. C. and Hunt, M. C. (2017). Exogenous and endogenous factors influencing color of fresh meat from ungulates. Meat and Muscle Biology, 1: 253–275.

Nishiumi, T., Kunishima, R., Nishimura, T. and Yoshida, S. (1995). Intramuscular connective tissue components contributing to raw meat toughness in various porcine muscles. Animal Science and. Technology, 66: 341-348.

O'Neill, D. J., Lynch, P. B., Troy, D. J., Buckley, D. J. and Kerry, J. P. (2003). Influence of the time of year on the incidence of PSE and DFD in Irish pig meat. Meat Science, 64: 105-111.

Offer G and Knight P. (1988). The structural basis of water-holding in meat. Part 2: Drip losses. In: Developments in meat science. Vol 4. New York: Elsevier Science Publishers. p 173-243.

Offer, G. (1991). Modelling of the formation of pale, soft and exudative meat: effects of chilling regime and rate and extent of glycolysis. Meat Science, 30: 157-184.

Offer, G. and Cousins, T. (1992). The mechanism of drip production: Formation of two compartments of extracellular space in muscle post-mortem. Journal of the Science of Food and Agriculture, 58: 107-116.

Offer, G. and Knight, P. (1988). The structural basis of water-holding capacity in meat. Part 2: drip losses. In R. Lawrie (Ed.). Developments in meat science, 4: 173-243. London: Elsevier Science Publications.

Ouali, A. (1990). Meat Tenderization: Possible causes and Mechanism: A review, Journal of Muscle Foods, 1: 129-165.

Ouali, A., Gagaoua, M., Boudida, Y., Becila, S., Boudjellal, A., Herrera-Mendez, C. H. and Sentandreu, M. A. (2013). Biomarkers of meat tenderness: Present knowledge and perspectives inregards to our current understanding of the mechanisms involved. Meat Science, 95: 854-870.

Page, J. K., Wulf, D. M. and Schwotzer, T. R. (2001). A survey of beef muscle color and pH. Journal of Animal Science, 79: 678-687.

Palka, K. (2003). The influence of post-mortem ageing and roasting on the microstructure, texture and collagen solubility of bovine semitendimosus muscle. Meat science, 64: 191-198.

De Palo, P., Maggiolino, A., Centoducati, P. and Tateo, A. (2012). Color changes in meat Foals as affected by slaughtering age and post-thawing time. Asian Australian journal of Animal Science, 25: 1775-1779.

Peplow, E. (1998). Encyclopedia of the horse (1st ed.). London: Hamlyn.

Perlo, F., Bonato, P., Teira, G., Tisocco, O., Vicentin, J., Pueyo, J. and Mansilla, A. (2008). Meat quality of lambs produced in the Mesopotamia region of Argentina finished on different diets. Meat Science, 79: 576-581.

Pierson, C. J and Fox, J. D. (1976). Effect of postmortem aging time and temperature on pH, tenderness and soluble collagen fractions in bovine longissimus muscle. Journal of Animal Science 43: 1206–1210.

Pietrasik, Z., Dhanda, J. S., Shand, P. J. and Pegg, R. B. (2006). Influence of injection, packaging, and storage conditions on the quality of beef and bison steaks. Journal of Food Science, 71: 110-118.

Pikul, J., Wójtowski, J., Danków, R., Kuczyńska, B. and Łojek, J. (2008). Fat content and fatty acids profile of colostrum and milk of primitive Konik horses (Equus caballus gmelini Ant.) during six months of lactation. Journal of Dairy Research, 75: 302-309.

Ponnampalam, E. N., Butler, K. L., Burnett, V. F., McDonagh, M. B., Joe, L., Jacobs J. L. and Hopkins D. L. (2013). Aged vacuum packaged lamb cuts are less brown than fresh muscle cuts under simulated retail display. Food and Nutrition Science, 4: 147-153.

Price, J. F. and Schweigert, B. S. (1994). Ciencia de la carne y de los productos cárnicos. Zaragoza: Editorial Acribia.

Puolanne, E. and Ruusunen, M. (1988). Reducing the incidence of dark-cutting beef in Finland – A systematic approach. In S. U. Fabiansson, W. R. Shorthose, R. D. Warner (Eds.), Proceedings of an Australian workshop. Sydney: AMLRDC. (pp. 32-37).

Qu, D., Zhou, X., Yang, F., Tian, S. Zhang, X., Ma, L. and Han, J. (2017). Development of carcass model based on blood biochemical parameters as a diagnostic tool of PSE meat. Meat Science, 128: 24-29.

Rosser, B. W. C., Norris, B. J. and Nemeth, P. M. (1992). Metabolic capacity of individual fibers from different anatomic locations. Journal of Histochemistry and Cytochemistry, 40: 819-825.

Roy, B. C., Walker, B., Rahman, M. M., Bruce, H. L. and McMullen, L. (2018). Role of myofibers, perimysium and adipocytes in horse meat toughness. Meat Science, 146: 109-121.

Roy, B. C., Sedgewick, G., Aalhus, J. L., Basarab, J. A. and Bruce, H. L. (2015). Modification of mature non-reducible collagen cross-link concentration in bovine m. *gluteus medius* and *semitendinosus* with steer age at slaughter, breed cross and growth promotants. Meat science, 110:109-117.

Sarriés, M. V. and Beriain, M. J. (2005). Carcass characteristics and meat quality of male and female foals. Meat Science, 70: 141-152.

Sarriés, M. V. and Beriain, M. J. (2006). Colour and texture characteristics in meat of male and female foals, Meat Science, 74: 738-745.

Savage, A. W. J., Warriss, P. D. and Jolley, P. D. (1990). The amount and composition of the proteins in drip from stored pig meat. Meat Science, 27: 289-303.

Savell, J. W. and Shackelford, S. D. (1992). Significance of tenderness to the meat industry. In Proceedings of 45th annual reciprocal meat conference, Colorado, USA (pp. 43-46).

Scanga, J. A., Belk, K. E., Tatum, J. D., Grandin, T. and Smith, G. C. (1998). Factors contributing to the incidence of dark cutting beef. Journal of Animal Science, 76: 2040-2047.

Schilling, M. W., Mink, L. E., Gochenour, P. S., Marriott, N. G. and Alvarado, C. Z. (2003). Utilization of pork collagen for functionality improvement of boneless cured ham manufactured from pale, soft, and exudative pork. Meat Science, 65: 547-553.

Schneider, B. A., Tatum, J. D., Engle, T. E. and Bryant, T. C. (2007). Effects of heifer finishing implants on beef carcass traits and longissimus tenderness. Journal of Animal Science, 85: 2019-2030.

Seideman, S. C., Cross, H. R., Smith, G. C. and Durland, P. R. (1984). Factor associated with fresh meat color: Review. Journal of Food Quality, 6: 211-237.

Seong, P. N, Park, K. M., Kang, G. H., Cho, S. H., Park, B. Y., Chae, S. H., Ba, H. V. (2016). The differences in chemical composition, physical quality traits and nutritional values of horse meat as affected by various retail cut types. Asian Australas Journal of Animal Science, 29: 89-99.

Shimokomaki, M., Elsden, D. F. and Bailey, A. J. (1972). Meat tenderness: Age related changes in bovine intramuscular collagen. Journal of Food Science, 37: 892-896.

Shorthose, W. R. and Harris, P. V. (1990). Effect of animal age on the tenderness of selected beef muscles. Journal of Food Science, 55: 1-8.

Smith, G. C., Belk, K. E., Sofos, J. N., Tatum, J. D. and Williams, S. N. (2000). Economic implications of improved color stability in beef. In Decker E.A., Faustman, C., Lopez- Bote, C.J. (Eds.), Antioxidants in Muscle Foods: Nutritional Strategies to Improve Quality. New York: Wiley Inter science. pp. 397- 426.

Smith, S. H. and Judge, M. D. (1991). Relationship between pyridinoline concentration and thermal stability of bovine intramuscular collagen. Journal of Animal Science, 69: 1989-1993.

Stanton, C. and Light, N. (1990). The effects of conditioning on meat collagen: part 3 evidence for proteolytic damage to endomysial collagen after conditioning. Meat Science, 27**:** 41-54.

Stanton, C. and Light, N. (1988). The effects of conditioning on meat collagen: part 2 - direct biochemical evidence for proteolytic damage in insoluble perimysial collagen after conditioning. Meat Science, 23: 179-199.

Starkey, C. P. and Hopkins, D. L. (2016). Do sarcomere length, collagen content, pH, intramuscular fat and desmin degradation explain variation in the tenderness of three ovine muscles? Meat Science, 113: 51-58.

Starkey, C. P., Geesink, G. H., Ven, R. V. D. and Hopkins, D. L. (2017). The relationship between shear force, compression, collagen characteristics, desmin degradation and sarcomere length in lamb biceps femoris. Meat Science, 126:18-21.

Steinhart, H., Bosselmann, A. and Möller, C. (1994). Determination of pyridinoline in bovine collagenous tissue. Journal of Agricultural Food Chemistry, 42: 1943-1947.

Stetzer, A. J. and McKeith, F. K. (2003). Benchmarking value in the pork supply chain: Quantitative strategies and opportunities to improve quality Phase 1. Savoy (IL): American Meat Science Association.

Strandine, E. J., Koonz, C. H. and Ramsbottom, J. M. (1949). A study of variations in muscle of beef and chicken. Journal of Animal Science, 8: 483-494.

Stromer, M. H., Goll, D. E. and Roth, L. E. (1967). Morphology of rigor-shortened bovine muscle and the effect of trypsin on pre- and post-rigor myofibrils. Journal of Cell Biology, 34: 431-445.

Suman, S. P. and Joseph, P. (2013). Myoglobin chemistry and meat color. Annual Review Food Science and Technology, 4: 79-99.

Suman, S. P. and Joseph, P. (2014). Chemical and physical characteristics of meat: color and pigment. In: Dikeman, M., Devine, C. (Eds.), Encyclopedia of Meat Sciences, second ed., Elsevier, Oxford, United Kingdom, 3: 244-251.

Swatland, H. J., Gullett, E., Hore, T., Buttenham, S. (1995). UV fiber-optic probe measurements of connective tissue in beef correlated with taste panel scores for chewiness. Food Research International, 28:23-30.

Sylvestre, M. N., Balcerzak, D., Feidt, C., Baracos, V. E., and Bellut, J. B. (2002). Elevated rate of collagen solubilization and postmortem degradation in muscles of lambs with high growth rates: possible relationship with activity of matrix metalloproteinases. Journal of Animal Science, 80: 1871-1878.

Takahashi, K. (1996). Structural weakening of skeletal muscle tissue during post-mortem ageing of meat: the non-enzymatic mechanism of meat tenderization. Meat Science, 43: 67-80.

Tarrant, P. V. (1989). Animal behaviour and environment in the dark cutting condition in beef-A review. Irish Journal of Food Science and Technology, 113: 1-21.

Tateo, A., De Palo, P., Ceci, E. and Centoducati, P. (2008). Physicochemical properties of meat of Italian Heavy Draft horses slaughtered at the age of eleven months. Journal of Animal Science, 86: 1205-1214.

Taylor, R. G., Geesink, G. H., Thompson, V. F., Koohmaraie, M. and Goll, D. E. (1995). Is Z-disk degradation responsible for post-mortem tenderization? Journal of Animal Science., 21: 1351-1367.

Thompson, J. (2002). Managing meat tenderness. Meat Science, 62: 295-308.

Tonial, I. B., Aguiar, A. C., Oliveira, C. C., Bonnafé, E. G., Visentainer, J. V. and Souza, N. E. (2009). Fatty acid and cholesterol content, chemical composition and sensory evaluation of horsemeat. South African Journal of Animal Science, 39: 328-332.

USDA (2013). National nutrient database for standard reference, Release 20. http://fnic.nal.usda.gov/food-composition (consulted: 19/02/2013).

Vestergaard, M., Therkildsen, M. Henckel, P., Jensen, L. R., Andersen, H. R. and Sejrsen, K. (2000). Influence of feeding intensity, grazing and finishing feeding on meat and eating quality of young bulls and the relationship between muscle fibre characteristics, fibre fragmentation and meat tenderness. Meat Science, 54 : 187-195.

Vitale, M., Pérez-Juan, M., Lloret, E., Arnau, J., Realini, C. E. (2014). Effect of aging time in vacuum on tenderness, and color and lipid stability of beef from mature cows during display in high oxygen atmosphere package**.** Meat Science, 96: 270-277.

Voyle, C. A. (1969). Some observations on the histology of cold-shortened muscle. Journal of Food Technology, 4: 275-281.

Warriss, P. D, Brown, S, N., Nute, G. R., Knowles, T. G., Edwards, J. E., Perry, A. M., Johnson, S. P. (1995). Potential interactions between the effects of preslaughter stress and post-mortem electrical stimulation of the carcasses on meat quality in pigs. Meat Science, 41: 55-68.

Weston, A. R., Rogers, R. W., Althen, T. G. (2002). Review: The role of collagen in meat tenderness. The Professional Animal Scientist, 18: 107-111.

Wheeler, T. L., Koohmaraie, M. (1994). Prerigor and postrigor changes in tenderness of ovine longissimus muscle. Journal of Animal Science, 72: 1232-1238.

Wheeler, T. L., Shackelford, S. D., and Koohmaraie, M. (2000). Variation in proteolysis, sarcomere length, collagen content, and tenderness among major pork muscles. Journal of Animal Science, 78: 958-965.

Whiting, R. C. (1980). Calcium uptake by bovine muscle mitochondria and sarcoplasmic reticulum. Journal of Food Science, 45:288-292.

Wiklund, E., Dobbie, P., Stuart, A., and Littlejohn, R. P. (2010). Seasonal variation in red deer (Cervus elaphus) venison (M. longissimus dorsi) drip loss, calpain activity, colour and tenderness. Meat Science, 86:720-727.

Xiong, Y. L. (2000). Protein oxidation and implications for muscle food quality. In Decker, E. A., Faustman, C. and Lopez-Bote, J. L. (Eds.) Antioxidants in muscle foods: Nutritional strategies to improve quality (pp. 85-90). New York: Wiley

Young, O. A., Braggins, T. J., Barker, G. J. (1994). Pyridinoline in ovine intramuscular collagen. Meat Science, 37**:** 297-303.

Zhang, W. G., Lonergan, S. M., Gardner, M. A. and Huff-Lonergan, E. (2006). Contribution of postmortem changes of integrin, desmin and mu-calpain to variation in water holding capacity of pork. Meat Science, 74: 578-585.

Chapter 2. Effect of post mortem ageing on horse meat quality from carcasses deboned at different times

Abstract:

Horse meat is a good source of iron and unsaturated fatty acids, which make it a suitable substitute for meat from conventional meat species. Canada is one of the largest horse meat exporters in the world; therefore, for Canada to stay competitive in the world horse meat market it must ensure that horse meat of the highest quality is supplied. Suitable deboning and ageing methods to ensure the superior quality of horse meat were developed using 36 horse carcasses to investigate the effect of three deboning time (17, 26 and 30 h post slaughter, n = 12 per period). *Semimembranosus* muscles, an economically valuable muscle from the horse carcass, were removed and aged for either 3, 30, 60 and 90 days (n = 36 per period). Meat *L** (lightness) decreased ($p < 0.05$) while *b**(yellowness) increased with increasing length of deboning time and ageing period. The purge loss increased with increasing ageing period ($p < 0.05$) with meat from carcasses chilled for 17 h having consistently higher means over the ageing period while Warner Bratzler shear force decreased with ageing time. The deboning time of 26 h and ageing period 30 days were suitable quality horse meat.

2.1 Introduction

Low fat and cholesterol, and high unsaturated fatty acid and iron contents are characteristics of horse meat (Franco and Lorenzo, 2014) and make it a good substitute for that of conventional meat species (e.g., beef, pork and chicken). Several countries in Western Europe and Japan are the primary consumers of horse meat (FAO, 2015). Tenderness of meat is considered the most important indicators of meat quality (Glitsh, 2000) as consumers are willing to pay increased prices if the tenderness of meat is ensured (Lyford et al., 2010). Every year the consumption of horse

meat increases and along with it are increased production volumes and competition in international markets. Canada is one of the largest exporters of horse meat in the world and Alberta contributes significantly to its export supply. To remain competitive, Canada must ensure the highest quality of horse meat, even after the up to 3 months post mortem necessary for transport of horse meat to its international markets.

Although muscle appearance has little effect on its eating quality (Chambaz et al., 2003), it plays a vital role in consumer purchasing decisions as it is taken as an indicator of the spoilage or freshness of meat (Carpenter et al., 2001). Meat colour is strongly associated with myoglobin (Mb) concentration and its chemical forms [e.g. deoxymyoglobin (DMb), oxymyoglobin (OMb) and metmyoglobin (MetMb)] (Suman and Joseph, 2013). Myoglobin is a monomeric globular haem protein with a prosthetic group. The haem has a centrally located iron atom with six coordinated sites; four are associated with a tetrapyrollic ring structure, one is connected with a histidine residue and the other is available to bind to a variety of ligands. The type of ligands that bind with the sixth coordination site determine the redox state of the iron molecule (ferrous or ferric) and the color of the meat (Bekhit and Faustman, 2005).

The myofibrillar toughness associated with sarcomere length can be minimized by controlling the post slaughter deboning time and ageing of the carcass (Young et al., 2005). If the sarcomere length is long, the muscles most likely will be tender but if the sarcomere length is short, the muscle most likely will be tough. If carcass muscle temperature is less than 10 °C before the onset of rigor or if the muscle is removed from the carcass (hot boning) in the pre-rigor phase there is a chance of myofibrillar shortening (Klont et al., 2000). An early carcass muscle temperature below 10 °C before 10 h post-mortem can slow or stop the glycolytic process, leaving energy available to the muscle in the form of adenosine triphosphate (ATP) and a higher ultimate pH (Locker and

Hagyard, 1963; Marsh and Leet, 1966; Ertbjerg and Poulanne, 2017). In low carcass muscle temperatures, the concentration of Ca^{+2} is increased in the sarcoplasm and causes massive contraction of muscles fiber, resulting in cold shortening (Honikel, 2014). In the hot deboning process, the muscles may go through more contraction as compared to the muscles that remain on the carcass because their ability to shorten is no longer limited by attachments within the carcass. In this case the risk of myofibrillar shortening is high (Lonergan et al, 2010; Rees et al., 2003).

When muscle ATP is depleted and muscles no longer have the ability to contract, the sarcomere length is set by the formation of a permanent cross-link between the myofibrillar proteins actin and myosin (Honikel, 2014) to produce rigor. However, this toughness associated with rigor is counteracted by tenderization during the ageing process (Sørheim and Hildrum, 2002). During the ageing process, myofibrillar proteins present in the I bands on both sides of the Z-disc are degraded by proteolytic enzymes (Taylor et al., 1995).

Currently, in Canada, horse meat is harvested by either hot boning (after 2 h post-mortem) of the carcass or after the carcass is completely chilled to less than 4 °C (40 h post-mortem) (Gill and Landers, 2005). Completely chilling the carcass requires a large chilled storage capacity, and significant amounts of energy and time which increases the cost of carcass processing. The hypothesis of this study was that chilling the carcass for a shortened period of time post-mortem prior to de-boning will not change horse meat quality if it is aged post-mortem. Therefore, the objectives of the study were to investigate different deboning and ageing periods and identify those that produced the highest technological quality horse meat.

2.2 Materials and methods

The experiment was conducted with 36 *Semimembranosus* (*SM*) muscles collected from a horse slaughter facility in Alberta over four consecutive weeks (visits), with nine muscles collected at

each visit. The muscles were taken from the right sides of horse carcasses randomly selected from those with weights greater than 179 kg and information regarding animal age, sex, breed, transportation conditions before slaughter, and diet was not available. However, to ensure muscles were representative of the population and the variation in carcass weight considered, three muscles were randomly distributed among deboning treatments at every visit and the carcass right side weight was used as a covariate in statistical models. During chilling, intramuscular temperature and pH were measured in the *SM* muscle as described in Section 2.2.1 and were recorded hourly over the first three hours. The *SM* muscles were deboned from right sides after 17, 26 and 30 h of chilling post-mortem.

After three days of ageing at 0 ± 0.5 °C, muscles were sliced into five portions (Figure 2.1), with two steaks in each portion and each portion was randomly distributed to 3, 30, 60 and 90 d of ageing periods with the exception of the middle portion which was frozen and stored at -18 °C at day 3 post mortem for study of intra muscular connective tissue (IMCT).

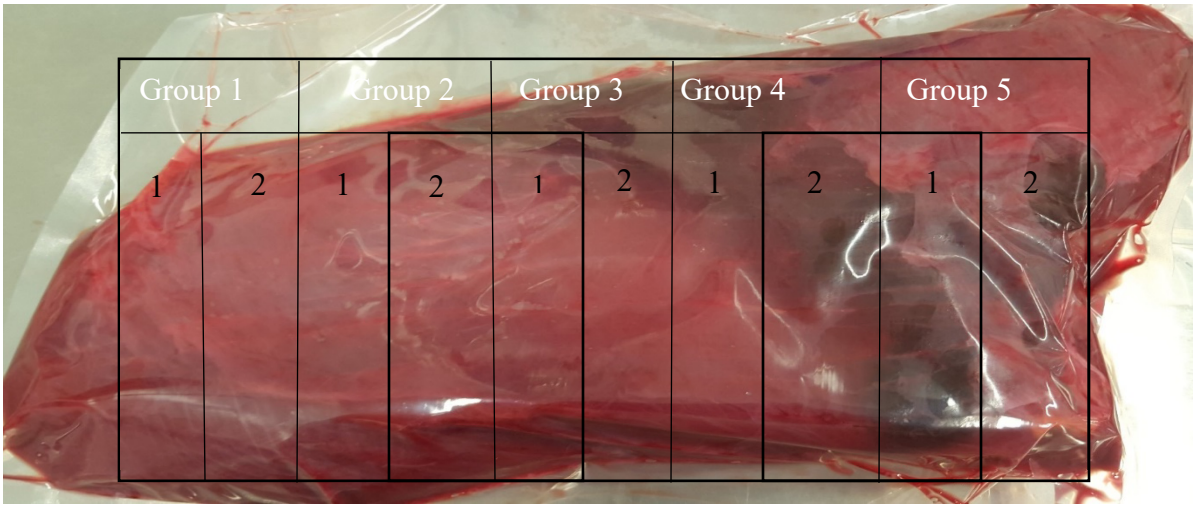

Figure 2.1 Distribution of muscle steak among different ageing periods

The steaks within each portion were weighed and then packaged under vacuum for ageing at 0 ± 0.5 °C. After each ageing period and within each portion, one steak was used for purge loss, cooking loss and Warner Bratzler shear force (WBSF) and one steak was used for proximate analysis. Objective color and pH were measured on both steaks within each portion as described in Section 2.2.1

2.2.1 Purge loss, pH and color measurement

After each ageing period steaks were weighed in the vacuum package and then the steaks were removed from the vacuum package. The steak and the cleaned dried package were weighed separately to determine the purge loss. The purge loss was calculated using the following equation

$$Purge\ loss\ \% = \frac{(\text{Steak including purge and bag weight} - \text{Aged steak including bag weight})\text{x}100}{\text{Aged steak weight} + \text{Purge weight}}$$

The initial pH and temperature of the *SM* was measured in the right side of each carcass at one and three hours post slaughter and ultimate pH was measured at the conclusion of each ageing period. The ultimate pH was measured in both steaks within each portion and readings were taken in three different places of each steak using a portable pH meter (Accumet AP71, Fisher Scientific, US). Before pH readings, the pH meter was calibrated with pH 4, 7 and 10 commercial standards (Fisher Scientific, US).

The color luminosity (L^*), redness (a^*), yellowness (b^*), chroma (c^*) and hue (h^*) of the lean muscle were measured in three different places on every aged steak after 20 minutes of blooming using a Chroma meter Minolta CR400 (Konica Minolta, Japan). Before taking measurements, the

chroma meter was calibrated with a white calibration tile supplied by the manufacturer with coordinate values of Y= 91.59, X =0.3167 and y= 0.3331.

2.2.2 Cooking loss and Warner Bratzler shear force (WBSF) measurement

The steaks were trimmed to 150 to 250 g and the length, width, height and weight of each steak was measured. A stainless steel temperature probe linked to a data logger (Tinytag View 2 TV-2040, Gemini Data Loggers Ltd, West Sussex, UK) was inserted in the geometric centre of each steak. Each steak was then placed in a polypropylene bag and cooked in a water bath as previously described by Franco et al. (2011). Briefly, steaks were cooked at 73 °C water temperature until the core temperature reached 71 °C. The cooking time of each steak was recorded. After cooking the water lost from the steak during cooking was decanted from the bag and the steak was cooled in ice water for 20 minutes before being stored overnight at 4 °C. Following overnight the steaks were removed from the bags, blotted dry with paper towel, and weighed to measure the cooking loss. The cooking loss was calculated as:

$$\text{Cooking loss } \% = \frac{(\text{Raw steak weight} - \text{cooked steak weight}) \text{ x } 100}{\text{Raw steak weight}}$$

The WBSF was measured in cooked meat according to Roy et al. (2018) where six cores (1.27 cm diameter) were removed from each steak parallel to the fibre direction. Peak shear force was measured by shearing across the muscle fibre direction using a Warner-Bratzler-like blade affixed to a material testing machine (Lloyd Instrument LRX plus, AMETEK™, Digital Measurement Metrology Inc. Brampton, ON). Steak cores were sheared at a crosshead speed of 200 mm/min (AMSA, 1995) and WBSF was reported in Newtons (N).

2.2.3 Measurement of Sarcomere length

Sarcomere length was measured by the technique described by Cross et al. (1981), where the frozen muscles were thawed at 4 °C until slightly soften and then a 5 g sample from the middle of

each muscle was removed and homogenized with 25 mL 0.25 M sucrose solution for two minutes using a laboratory blender (Waring, Fisher Scientific). A small drop of homogenized sample was placed on a glass slide, covered with a glass slide cover and observed under a phase contrast using a microscope (Axio Scope A1, Carl Zeiss Microscopy, Thornwood, NY, USA) fitted with a camera (Axiocam MRm, Carl Zeiss Microscopy, Thornwood, NY, USA). Ten myofibrils from each sample and five sarcomeres (Figure 2.2) for each myofibril were measured. The average of the five sarcomere lengths was taken as the average length of each observation and the average of ten observations was considered the sample sarcomere length.

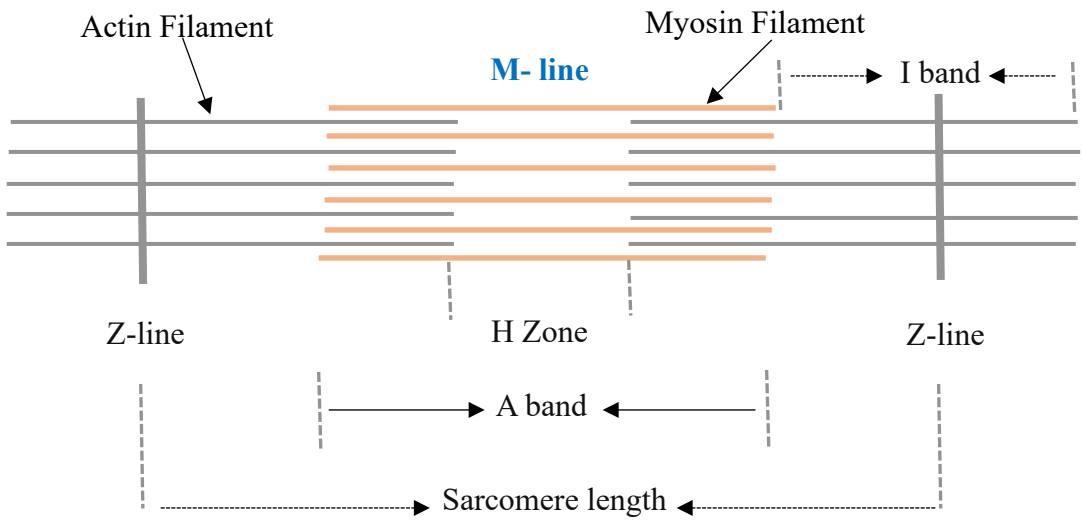

Figure 2.2 Structural composition of muscle fiber and sarcomere length

2.2.4 Quantification of myoglobin

Myoglobin (Mb) quantification was performed according to Tang et al. (2004) and Warriss (1979) using 10 ± 0.5 g of muscle tissue. Muscle tissue was taken from the middle of each steak, thawed at 4 °C for 3 h, and trimmed of outer fat and connective tissue. The samples were homogenized for 40 second with 50 mL ice cold phosphate buffer (0.04 M, pH 6.8). Homogenized samples were stored at 4°C for an hour before centrifugation for 10 min at 6500 g at 4 °C. The supernatants were

filtered with No. 4 Whatman ™ Filter paper (GE Healthcare Life Science, Mississauga, ON). The filtered supernatant absorbance was then measured at 503, 525, 557, 582 and 730 nm against a blank of phosphate buffer (0.04 M, pH 6.8) using a spectrophotometer (Evolution, 60s, UV-Visible Spectrophotometer, Thermo Scientific, USA). The proportions of oxy-myoglobin (OMb), deoxy-myoglobin (DMb) and Met-myoglobin (MMb) were calculated according to the equations of Tang et al. (2004). The summation of OMb, DMb and MMb proportion was considered as 1 ± 0.01 and total myoglobin contents were measured using the following formula:

Mb (mg/g) = (Absorbance at 525 nm-Absorbance at 730 nm) x 2.2303 x (dilution factor).

The dilution factor was computed as the ratio of buffer volume (mL) and the mass weight (g) of the meat sample used.

2.2.5 Proximate analysis

After each ageing period, 100 ± 10 g of trimmed steak were chopped into small cubes, placed in a pre-weighed aluminium tray, weighed and stored at -20 °C until freeze-dried. Samples were dried in a freeze drier and then powdered in a laboratory stainless steel blender (Waring, Fisher Scientific) with a pellet of dry ice. The powdered samples were stored at -20 °C in Nasco Whirl-Pak bags until further analysis.

Crude fat analysis was conducted using petroleum ether extraction (Method 960.39; Association of Official Analytical Chemist (AOAC) 1995) in a Foss Soxtech System (Model 2050). For each sample 2 ± 0.003 g of powdered meat in duplicate were used. For crude protein, 100 ± 0.02 mg freeze dried powdered meat sample in duplicate were analyzed for total nitrogen content (Method 992.15; AOAC, 1995) using a TruSpec™ carbon/nitrogen analyzer (LECO ™ Corporation, St. Joseph, MI, USA). For the measurement of total moisture content, 2 ± 0.02 g of powdered muscle were dried in a glass vial at 105°C in oven for 20 h and then allowed to return to room temperature

under vacuum in a desiccator before being weighed. After this, total ash content of the oven dried samples was measured by burning each sample at 495 °C in a muffle furnace for 24 h. Before weighing the ash weight, the glass vials with ash were removed from the muffle furnace and returned into room temperature in desiccator under vacuum. The weight of ash was calculated based on fresh muscle weight according to the following equation.

$$Ash\ \% = \frac{(\text{Ash including bottle weight} - \text{bottle weight}) \text{ x DM \% in fresh meat}}{\text{Weight of dry meat used for ash}}$$

2.2.6 Statistical analysis

The data were analyzed using R (version 3.1.1) with the package nlme as a mixed model (split plot design) where deboning (main plot) and ageing (split plot) and their interaction were fixed effects and visit was a random effect. Carcass weight was included as covariate in the model. When the interaction effects were not significant ($p > 0.05$) the final statistical analysis was performed without the interaction term. Differences between least squares means were determined when effects were significant ($p < 0.05$) using least squares mean differences. The carcass pH and temperature data were analysed as randomized bock design using the package nlme where deboning was fixed effect, visit was random effect and carcass weight was covariate. The Pearson correlation analysis between the measurements was performed using the linear model package of R (package lm).

2.3 Results

2.3.1 Post slaughter carcass temperature and pH early post mortem

The means of horse *Semimembranosus* intramuscular pH and temperature during cooling by deboning time (17, 26, and 30 h) are presented in Table 2.1. The intramuscular pH value differed between the deboning groups at both 1 and 3 hours post slaughter. At 1 h post mortem, muscles

in carcasses deboned at 17 h had a lower mean pH value than those deboned at 26 and 30 h. At 3 h post mortem, muscles deboned from the carcass at 17 h had a lower mean pH than those deboned at 30 h, but the pH was similar to that of muscles deboned at 26 h. Mean muscle temperatures did not differ among the deboning treatments at either 1 or 3 h post mortem.

2.3.2 Effect of deboning time and ageing period on color, pH and purge loss of horse meat

Horse *Semimembranosus* muscle mean *L** value was lower when *SM* muscles were deboned at 30 h than at 17 and 26 h post mortem. *SM* mean *b** value was greater when deboned at 17 h than at 30 h post mortem (Table 2.2). *SM* mean *a**, chroma, hue and intramuscular ultimate pH values were unaffected by deboning time, although purge loss was highest when deboned at 17 h and lowest when deboned at 26 and 30 h, where there was no difference (Table 2.2).

2.3.3 Effect of deboning time and ageing period on cooking loss and cooking time and WBSF of horse meat

Cooking loss and cooking time (Table 2.2) were unaffected by deboning time. Cooking loss was also not affected by post mortem ageing time (Table 2.2) although mean cooking time was greater at 3 d than at 90 d post mortem (Table 2.2). Deboning time did not affect WBSF values (Table 2.2) while as the ageing period increased the WBSF value decreased (Table 2.2), with the highest and lowest WBSF values observed at 3 and 90 d of ageing respectively.

2.3.4 Effect of deboning time on myoglobin concentration and sarcomere length

Mean myoglobin concentration and sarcomere length of horse meat did not change with increasing the deboning time (Table 2.3); however, the greatest proportions of OMb and MMb were observed when carcasses were deboned at 30 and 17 h, respectively, with no effect observed on DMb (Table

2.3). Carcass weight did not influence the significance of these measurements as it was not a significant covariate (Table 2.3, $p > 0.05$).

2.3.5 Effect of deboning time and ageing period on proximate composition of horse meat

The proximate composition of horse meat is presented in Table 2.4. Horse meat dry matter (DM) was highest when carcasses were deboned at 30 h relative to those deboned at 17 and 26 h (Table 2.4), while moisture was lowest in meat from carcasses deboned at 30 h relative to that deboned from carcasses at 17 and 26 h. De-boning time did not affect the percentage of crude protein (CP) and intramuscular fat, while the ash content was lower in meat from carcasses deboned at 17 h than that from carcasses deboned at 26 and 30 h.

Percentage dry matter decreased with time post mortem (Table 2.4), being lower at 60 and 90 days than at 3 days post mortem. Percentage moisture was lower at 3 days than at 90 days post mortem, while the percentage CP was lower at 90 d post mortem than all other ageing periods (Table 2.4). Ash content was higher at 3 d post mortem than at all other ageing periods and intramuscular fat percentage was unaffected by post mortem ageing (Table 2.4).

2.3.6 Pearson correlation coefficients between carcass and meat quality measurements in different ageing periods

Pearson correlation coefficients of horse meat quality measures for the ageing times of 3, 30, 60 and 90 d are presented in Tables 2.5, 2.6, 2.7 and 2.8, respectively. At day 3 post mortem (Table 2.5), carcass weight was positively correlated to *Semimembranosus* L^* ($r = -0.40$, $p \leq 0.05$) and intramuscular fat percentage ($r = 0.61$, $p \leq 0.001$). Meat L^* values were positively correlated with b^* values ($r = 0.38$, $p \leq 0.05$) and hue ($r = 0.47$, $p \leq 0.01$), and negatively with ash content ($r =$ -

0.42, $p \leq 0.05$). Meat *b** values were positively correlated with chroma ($r = 0.77$, $p \leq 0.001$) and hue ($r = 0.96$, $p \leq 0.001$). Chroma was positively correlated with hue ($r = 0.60$, $p \leq 0.001$). Meat pH was correlated with cooking loss ($r = 0.43$, $p \leq 0.01$) and WBSF ($r = 0.46$, $p \leq 0.01$). Cooking loss was positively correlated with cooking time ($r = 0.64$, $p \leq 0.001$), while WBSF was positively correlated with percentage moisture ($r = 0.46$, $p \leq 0.05$) and negatively correlated with intramuscular fat percentage ($r = -0.38$, $p \leq 0.05$). Meat moisture percentage was negatively correlated with percentage of intramuscular fat ($r = -0.75$, $p \leq 0.001$), as was ash ($r = -0.34$, $p \leq 0.05$). Intramuscular fat percentage was negatively correlated with percentage crude protein ($r = -0.42$, $p \leq 0.05$).

At day 30 post mortem (Table 2.6), carcass weight was positively correlated to IMF percentage ($r = 0.51$, $p \leq 0.01$). Meat *L** values were positively correlated with *b** ($r = 0.68$), hue ($r = 0.78$, $p \leq 0.001$), and pH ($r = 0.49$, $p \leq 0.01$), and negatively correlated with ash content ($r = -0.44$, $p \leq 0.01$). Meat *a** values were positively correlated with *b** values ($r = 0.51$, $p \leq 0.01$), chroma ($r = 0.99$, $p \leq 0.001$), and pH ($r = 0.38$, $p \leq 0.05$). Meat *b** values were positively correlated with chroma ($r = 0.59$), hue ($r = 0.88$, $p \leq 0.001$) and pH ($r = 0.48$, $p \leq 0.05$). Chroma and hue were positively correlated with pH ($r = 0.41$ and 0.35, respectively, $p \leq 0.05$). Meat pH was positively correlated with percentage moisture ($r = 0.43$, $p < 0.01$) and ash ($r = 0.37$, $p \leq 0.05$). Purge loss was positively correlated with percentage moisture ($r = 0.60$, $p \leq 0.001$) and negatively correlated with crude protein percentage ($r = -0.38$, $p \leq 0.05$). Cooking loss was negatively correlated with crude protein percentage ($r = -0.43$, $p \leq 0.01$), and moisture was negatively correlated with percentage of IMF ($r = -0.73$, $p \leq 0.001$).

At day 60 post mortem (Table 2.7), carcass weight was positively correlated with meat *L** ($r = 0.54$), *b** ($r = 0.55$), hue ($r = 0.58$) and IMF ($r = 0.57$, $p \leq 0.001$), and negatively correlated with

cooking loss (r = -0.42), moisture (r = -0.33) and ash (r = -0.39, $p \leq 0.05$). Meat *L** was positively correlated with *b** (r = 0.85, $p \leq 0.001$), hue (r = 0.88), purge loss (r = 0.43)($p \leq 0.01$), and IMF (r = 0.56, $p \leq 0.0001$), and negatively correlated with ash (r = -0.52) and crude protein percentage (r = -0.48)($p \leq 0.01$). Meat *a** values were positively correlated with *b** (r = 0.34, $p \leq 0.05$), chroma (r = 0.99, $p \leq 0.001$) and intramuscular pH (r = 0.43, $p \leq 0.01$). Meat *b** values were positively correlated with chroma (r = 0.44, $p \leq 0.01$), hue (r = 0.91, $p \leq 0.001$), purge loss (r = 0.42, $p \leq 0.05$), and IMF (r = 0.58, $p \leq 0.001$) and negatively correlated with ash (r = -0.43) and crude protein (r = -0.38, $p \leq 0.05$). Meat chroma was correlated to intramuscular pH (r = 0.43, $p \leq 0.05$). Meat hue was positively correlated with purge loss (r = 0.50, $p \leq 0.01$), IMF (r = 0.59, $p \leq 0.001$) and negatively correlated with percentage ash (r = - 0.36, $p \leq 0.05$) and crude protein (r = -0.52, $p \leq 0.01$). Intramuscular pH was correlated with cooking loss (r = -0.45) and WBSF (r = 0.43, $p \leq 0.01$), and purge loss was correlated with percentage moisture (r = 0.51) and crude protein (r = -0.48, $p \leq 0.01$). Cooking time was positively correlated with crude protein (r = 0.45, $p \leq 0.01$). Meat moisture content was negatively correlated with IMF (r = -0.50) and crude protein (r = -0.43) ($p \leq 0.01$). Percentage IMF was negatively correlated with percentage crude protein (r = -0.36, $p \leq 0.05$).

At 90 d post mortem (Table 2.8), carcass weight was correlated with *b** values (r = 0.36), hue (r = 0.36) ($p < 0.05$) and IMF (r = 0.47, $p < 0.01$). Meat *L** values were correlated with *a** values (r = 0.42, $p < 0.05$), *b** values (r = 0.77, $p < 0.001$), chroma (r = 0.49, $p < 0.01$), hue (r = 0.72, $p < 0.001$), WBSF (r = 0.37) and crude protein (r = - 0.36)($p < 0.05$). Meat *a** values were correlated with *b** values (r = 0.34, $p < 0.05$), chroma (r = 0.99, $p < 0.001$), and WBSF (r = 0.38, $p < 0.05$). Meat *b** values were correlated with chroma values (r = 0.44, $p < 0.01$), hue values (r = 0.94, $p < 0.001$), IMF (r = 0.41) and percentage crude protein (r = -0.35, $p < 0.05$). Meat chroma values

were correlated with WBSF ($r = 0.37$, $p < 0.05$). Meat hue values were correlated with IMF ($r = 0.42$) and crude protein percentage ($r = -0.39$, $p < 0.05$). Purge loss was correlated with cooking loss ($r = 0.37$), and cooking loss was correlated with cooking time ($r = -0.35$, $p < 0.05$). Percentage moisture was negatively correlated with crude protein ($r = -0.87$, $p < 0.001$).

2.4 Discussion

The impacts of alterations made to the post mortem processing of food animal carcasses needs to be characterized so that meat quality is not jeopardized by institution of procedures that optimize food safety or fabrication efficiency. Changes to early post mortem processing procedures are of particular concern because post-mortem muscle fibers have the potential to contract until the muscle ATP is depleted and rigor onset is complete. To minimize the amount of contraction that can occur, the attachment of the muscle by connective tissue to the carcass is usually maintained until after rigor onset is complete. However, if muscle is separated from the carcass before onset of rigor, such as during hot carcass deboning, there is a chance of massive contraction of the sarcomeres during rigor which would shorten the muscle and reduce the intra-myofibrillar spaces. The reduction in intra-myofibrillar spaces would force free water out of the sarcomere and into the extra-myofibrillar and extra-fascicular spaces. Shortened sarcomere length can in this way lead to increased shear force values (Hughes et al., 2014), which may indicate increased cooked product sensory toughness.

In this research, the effect of deboning the semimembranosus muscle from the horse carcass at 17, 26 and 30 h post mortem on its meat quality characteristics was examined. Early fabrication of the horse carcass can increase the throughput of horse slaughter facilities and reduce time required to transport product to an overseas market. Also examined in this research was the effect of post mortem ageing of the SM muscle for 3, 30, 60 and 90 d under vacuum, and the data were

investigated for an interaction with deboning time. Thirty hours is the normal de-boning time for horse carcasses is Canada, as it allows for the carcass to be cooled to approximately 4 °C prior to fabrication. Advancing carcass fabrication time even by 4 hours (the 26 h treatment) would provide efficiency advantages and hence was included as a treatment in this study. Similarly, export of horse meat to European markets can take up to 90 d post mortem, and the deterioration of horse meat quality, if any, within that time has not been fully investigated and was therefore examined in this research as well.

Results indicated that fabrication of the horse carcass at 17 h post mortem increased the proportion of metmyoglobin in the SM, which can increase the yellowness of muscle (Mancini and Hunt, 2005). Horse meat is a rich source of iron and unsaturated fatty acids (Franco and Lorenzo, 2014) which together accelerate the oxidation of Mb (Papuc et al., 2017) by forming free radicals. The lightness (L^*) of the muscle was also increased, which may have been due to increased cut surface moisture (Purslow et al., 2020) arising from the higher purge loss and increased moisture content from this muscle when harvested at 17 h. The changes in lightness and yellowness were extremely small (< 2 units) but were statistically significant and likely discernable by consumers as Lorenzo et al. (2013) found that horse meat consumers could discern differences in L^*, a^* and b^* of less than 1 unit.

Post mortem ageing time in vacuum packaging had the greatest effect on horse meat quality. With increasing ageing time, the value of L^* gradually decreased indicating that the cut surface of the horse meat darkened. The variation in the L^* value can be explained by meat pH differences and purge loss. The low pH (pH < 5.8) (Li et al., 2014; Wu et al., 2014) of post mortem muscle accelerates the denaturation of myofibrillar and sarcoplasmic protein and reduces the water holding capacity, resulting in movement of intracellular water and Mb into intrafibrillar spaces

(Warriss, 2000). As a consequence, muscle structure become soft and loose which scatters light and makes the muscle surface appear pale (Warriss, 2000). In addition, the average pH value of horse meat in this thesis was 5.49 which is lower than the normal range of horse meat pH of 5.58 to 6.1 (Franco et al., 2011; Lorenzo et al., 2013ab; Seog et al., 2016).

The value of *b** (Table 2.2) fluctuated with post mortem ageing period. Why in the mid stage of ageing *b** gradually reached its peak and then decreased is unclear, and the redox forms of Mb were not measured. Extended ageing and its associated purge loss can remove cytoplasm-soluble proteins including MMb in the later stage of ageing period. Therefore, the value of *b** at 90 d post mortem may have been due to the high level of purge loss, but if this was the case then a low *b** value should also have been observed at 60 d post mortem as well. Gómez and Lorenzo (2012) also found that *L** decreased with refrigerated storage of foal m. *longissimus* muscle under vacuum at 2 °C, and found that *b** values also increased and then decreased with 14 d ageing. The difference in the length of time of colour stability between the horse meat used in this research and that of Gómez and Lorenzo (2012) may have been due to the age of the horses, with those of Gómez and Lorenzo (2012) being 15 months, and the horses studied in this research being more than 2 years old. It may also have been due to the refrigerated storage temperature difference, with Gómez and Lorenzo (2012) storing their product at 2 °C, while the horse meat used in this thesis was stored at 0 ± 0.5 °C. Given that these declines in colour coordinate values were also accompanied by a decline in sensory consumer acceptability by Gómez and Lorenzo (2012), further research on the chemistry and stability of horse meat colour during refrigerated storage is warranted.

Retention of water within meat is of the utmost important because it makes up about 75% of meat. The purge loss increased with prolonged ageing time with the greatest loss observed over ageing

when the *SM* was removed at 17 h post mortem. Muscle water consists of immobilized and free water. Free water can be lost due to low intramuscular pH and physical forces like myofibrillar shrinkage (Hughes et al., 2014). Immobilized water comprises 85% of the total muscle water located within the thick filament, and is located between the thick and thin filaments of the myofibrils (Honikel et al., 1986). This immobilized water stays within the muscle either by hydrogen bonds with myofibrillar protein or other macromolecules or by steric attraction between the myofilaments (Pearce et al., 2011). During ageing enzymatic activity degrades muscle fibres and they lose their native structure, and this is exacerbated by low muscle pH which promotes the denaturation of protein structure. Denatured protein water holding capacity is minimal. In this study the average pH value of horse meat was 5.49 which is close to the muscle protein iso-electric pH (5-5.4). Therefore, with extended ageing time, muscle degradation along with denaturation compromises meat water holding capacity and as a consequence water is lost as purge. Why there was increased purge associated with the 17 h fabrication time is unclear, but it was not due to sarcomere length differences as there was no difference in mean sarcomere length between the three fabrication times. The results of this study did indicate however that there is little increase in purge loss from day 60 to 90 post mortem, suggesting that there is a point at which there is no further increase in purge loss, and that point may be before or at 60 d post mortem.

Warner-Bratzler shear force of the cooked horse meat declined significantly with post mortem ageing, indicating that the horse meat became more tender with length of ageing time. Meat tenderization is the process of post-mortem fragmentation of muscle structure and associated proteins by endogenous enzymes throughout the ageing period (Carlson et al., 2017; Hopkins, 2004; Kemp et al., 2010; Kim et al., 2014). It is well established that meat toughness is reduced by post slaughter ageing of the muscle (Sarrie´s and Beriain 2006; Lonergan et al., 2001). Animal

age (Roth et al., 1995; Segato et al., 1999) and sex might have effects on muscle toughness but after a certain period of ageing (e.g., 14 d in beef muscle) the sex effect may not predominates as was shown in beef muscle (Lonergan et al., 2001). The effect of animal age may not disappear with ageing, however, as when the age differences among the animals at slaughter is large, muscle toughness differences can be considerable (Roth et al., 1995; Segato et al., 1999; Sarrie´s and Beriain 2006). Animal age can affect the protein degradation and the nature of the interaction between actin and myosin cross bridge (Goll et al., 1997). However, irrespective of animal species, sex and age, post slaughter ageing can reduce the toughness of meat to a finite level (Roth et al., 1995; Segato et al., 1999; Sarrie´s and Beriain 2006,). The maximum WBSF value was observed for the horse *SM* muscle at 3 d post mortem while the lowest WBSF value was observed at aged 90 days. A negative correlation was observed between WBSF and IMF ($r = -0.38$, $p < 0.05$) at day 3 of ageing with this relationship diminished with further ageing. A similar relationship between horse meat WBSF and IMF approaching significance ($r = -0.44$, $p < 0.1$) was reported by Roy et al. (2018). In later stages of ageing the WBSF is manipulated by other factors like enzymatic degradation and denaturation of myofibrillar protein, which is supported by the reduced intact protein (e.g., myosin heavy chain, troponin T) and accumulation of poly peptide after 14 d of ageing horse *SM* muscle (Malva et al., 2019).

Conclusions

Results indicated that deboning the *SM* from the horse carcass at 17 h post mortem did not profoundly affect resulting meat quality with the exception of increased purge loss. The decrease in colour lightness and redness associated with ageing of horse meat to 90 d post mortem should be examined with respect to consumer acceptability to truly gauge the impact of these changes on

product quality. In light of these results, fabrication of the horse carcass at 17 h post mortem will not substantially affect meat quality of the *SM*, particularly after prolonged ageing.

Table. 2.1 Intramuscular pH and temperature of horse *Semimembranosus* early post mortem as affected by deboning time

	Deboning group			SEM[1]	P value covariate[2]	P value
	17h	26h	30h			
n	12	12	12			
pH						
Hour 1	6.60[b]	6.85[a]	6.89[a]	0.081	0.748	0.006
Hour 3	6.47[b]	6.65[ab]	6.78[a]	0.077	0.773	0.003
Temperature						
Hour 1	31.6	32.2	30.8	0.901	0.045	0.983
Hour 3	19.1	19.3	18.9	1.54	0.373	0.983

[1]SEM, standard error of the mean.
[2]Probability of the F test of carcass weight as the covariate.
[a,b] Means in the same row with different superscripts are significantly different from each other ($p \leq 0.05$) according to least squares means differences tests.

Table 2.2 Effect of deboning time and ageing period on horse meat SM muscle color (*L**, *a**, *b**, *c**, hue), purge loss (PL), pH, cooking loss (CKL), cooking time (CKT) and Warner Bratzler shear force (WBSF)

	P value covariate[1]	Deboning (hours)			P value	SEM[2]	Ageing (days)				P value	SEM[2]
		17	26	30			3	30	60	90		
n		12	12	12			36	36	36	36		
Color[3]												
*L**	0.006	28.95^{a}	28.44^{a}	27.48^{b}	<0.001	0.325	29.80^{a}	29.62^{a}	27.65^{b}	26.09^{c}	<0.001	0.354
*a**	0.282	19.91	19.94	20.02	0.99	0.310	20.04^{ab}	20.82^{a}	20.02^{ab}	18.95^{b}	<0.001	0.348
*b**	0.001	3.89^{a}	3.79^{ab}	3.62^{b}	0.04	0.206	3.22^{b}	4.74^{a}	4.21^{a}	2.89^{b}	<0.001	0.221
*c**	0.165	20.38	20.34	20.40	0.96	0.317	20.34^{ab}	21.37^{a}	20.51^{ab}	19.27^{b}	<0.001	0.358
Hue	0.001	10.73	10.42	10.08	0.99	0.548	8.77^{b}	12.71^{a}	11.79^{a}	8.38^{b}	<0.001	0.583
PL (%)	0.016	3.87^{a}	2.87^{b}	2.64^{b}	<0.001	0.423	0.51^{c}	2.99^{b}	4.33^{a}	4.68^{a}	<0.001	0.436
pH	0.700	5.48	5.49	5.52	0.44	0.031	5.43^{b}	5.57^{a}	5.62^{a}	5.35^{b}	<0.001	0.034
CKL%	0.182	26.59	26.20	25.76	0.46	0.454	26.19	26.34	26.13	26.05	0.95	0.485
CKT (sec/g)	0.4779	17.15	16.91	17.15	0.95	0.757	18.83^{a}	17.01^{ab}	17.52^{a}	14.93^{b}	<0.01	0.806
WBS (N)	0.659	47.34	45.61	43.91	0.21	2.022	57.53^{a}	47.99^{b}	40.24^{c}	36.73^{c}	<0.001	2.139

[1]Probability of the F test for the covariate carcass weight.
[2]SEM, standard error of the mean;
[3]*L**, lightness; *a**, redness; *b**, yellowness; *c**, chroma;
[a,b,c] Means with different superscripts in the same row are significantly different (p<0.05) according to least squares means differences.

Table 2.3. Effects deboning time on horse meat sarcomere length (SL, µm), myoglobin (Mb), deoxy-myoglobin (DMb), oxymyglobin (OMb) and metmyoglobin (MMb) concentrations

	Covariate p value[1]	Deboning time (h)			P value	SEM[2]
		17	26	30		
n		12	12	12		
Mb (mg/g meat)	0.631	6.08	5.92	6.45	0.722	0.399
DMb	0.713	0.092	0.097	0.050	0.191	0.018
OMb	0.815	0.659[a]	0.717[ab]	0.796[b]	0.013	0.032
MMb	0.538	0.248[a]	0.185[b]	0.152[b]	<0.001	0.017
SL (µm)	0.460	1.75	1.73	1.83	0.318	0.091

[1]Probability of the F test for carcass weight as the covariate.
[2]SEM, standard error of the mean.
[a,b] Means with different superscripts in the same row are significantly differ ($p \leq 0.05$) from each other according to least squares means differences.

Table 2.4. Proximate composition of horse *Semimembranosus* at different deboning and ageing times

	Covariate p value[1]	Deboning time (hours)			P value	SEM[2]	Ageing time (days)				P value	SEM[2]
		17	26	30			3	30	60	90		
n		12	12	12			36	36	36	36		
DM%[3]	<0.001	24.41[a]	24.41[a]	25.41[b]	0.021	0.161	25.49[a]	24.94[ab]	24.49[bc]	24.04[c]	<0.001	0.181
Moisture%	<0.001	75.59[a]	75.59[a]	74.59[b]	0.021	0.161	74.51[a]	75.06[ab]	75.51[ab]	75.96[b]	<0.001	0.181
CP (%)[4]	0.128	22.56	22.45	22.40	0.845	0.286	22.95[a]	22.45[a]	22.87[a]	21.61[b]	<0.001	0.245
IMF%[5]	0.128	0.90	0.96	1.54	0.264	0.112	1.12	1.17	1.08	1.16	0.927	0.109
Ash%	0.014	1.31[a]	1.76[b]	1.83[b]	0.011	0.111	2.03[a]	1.60[b]	1.59[b]	1.33[b]	<0.001	0.114

[1]Probability of the F test for the covariate carcass weight.
[2]SEM, standard error of the mean.
[3]DM, dry matter.
[4]CP, crude protein.
[5]IMF, intramuscular fat.
[a,b,c] Means with different superscripts in the same row are significantly differ ($p \leq 0.05$) from each other according to least squares means differences.

Table 2.5 Pearson correlation coefficients between meat quality measurements of horse SM (*Semimembranosus*) at 3 days post mortem

	*L**	*a**	*b**	Chr.	Hue	pH	PL	CK	CKT	WBSF	Mois	Ash	IMF	CP
Carcass wt.	0.40*	0.11	0.21	0.13	0.23	0.14	-0.12	0.22	0.25	-0.13	-0.38	-0.26	0.61***	-0.14
*L**		-0.09	0.38*	-0.04	0.47**	0.18	0.18	0.07	0.01	0.09	0.16	-0.42*	0.08	0.07
*a**			0.72***	1***	0.54***	-0.03	-0.14	0.05	0.00	-0.15	-0.28	0.19	0.07	0.10
*b**				0.77***	0.96***	0.04	0.16	-0.01	-0.09	0.01	-0.01	-0.05	0.06	-0.04
Chr.					0.60***	-0.02	-0.11	0.06	0.00	-0.12	-0.25	0.15	0.07	0.09
Hue						0.03	0.26	-0.06	-0.11	0.02	0.04	-0.10	0.11	-0.12
pH							-0.27	0.43**	0.11	0.46**	0.10	-0.21	-0.01	0.15
PL								-0.09	-0.16	0.09	0.34*	-0.04	-0.21	-0.22
CK									0.64***	0.13	0.18	0.01	-0.06	0.00
CKT										0.08	-0.13	-0.22	0.18	-0.05
WBSF											0.46**	-0.29	-0.38*	0.27
Mois												-0.01	-0.75***	0.05
Ash													-0.34*	0.24
IMF														-0.42*

Carcass wt, carcass weight; *L**, lightness; *a**, redness; *b**, yellowness; chr, chroma; PL, purge loss; CK, cooking loss; CKT, cooking time; WBSF, Warner Bratzler shear force; Mois, Moisture; IMF, Intramuscular fat; CP, Crude protein; *, $p = \leq 0.05$; **, $p = \leq 0.01$; ***, $p \leq 0.001$

Table 2.6 Pearson correlation coefficients between meat quality measurements of horse SM (*Semimembranosus*) at 30 days post mortem

	*L**	*a**	*b**	Chr.	Hue	pH	PL	CK	CKT	WBSF	Mois	Ash	IMF	CP
Cwt.	0.33	0.15	0.36	0.18	0.33	-0.02	-0.07	-0.06	0.29	-0.13	-0.35*	-0.20	0.51**	-0.03
*L**		0.01	0.68***	0.09	0.78***	0.49**	0.17	-0.10	0.15	0.19	0.29	-0.44**	0.08	-0.33
*a**			0.51**	0.99***	0.05	0.38*	0.12	-0.08	-0.03	0.22	0.31	0.17	-0.20	0.14
*b**				0.59**	0.88***	0.48**	0.02	00	0.09	0.35*	0.22	-0.17	0.12	-0.21
Chr.					0.16	0.41*	0.13	-0.10	-0.02	0.25	0.32	0.15	-0.16	0.12
Hue						0.35*	-0.03	0.01	0.10	0.29	0.10	-0.28	0.26	-0.32
pH							0.18	-0.18	0.08	0.28	0.43**	-0.37*	-0.12	-0.15
PL								0.11	-0.06	-0.02	0.60***	0.05	-0.27	-0.38*
CK									0.24	0.03	0.16	-0.19	-0.15	-0.43**
CKT										-0.26	-0.27	-0.07	0.12	0.14
WBSF											0.31	-0.22	-0.21	0.08
Mois												-0.16	-0.73**	0.22
Ash													0.01	0.09
IMF														-0.33

Carcass wt, carcass weight; *L**, lightness; *a**, redness; *b**, yellowness; chr., chromo; PL, purge loss; CK, cooking loss; CKT, cooking time; WBSF, Warner Bratzler shear force;Mois, Moisture; IMF, Intramuscular fat; CP, Crude protein; *, $p = \leq 0.05$, **; $p = \leq 0.01$; ***$p \leq 0.001$

Table 2.7 Pearson correlation coefficients between meat quality measurements of horse SM (*Semimembranosus*) at 60 days post mortem

	*L**	*a**	*b**	Chr	Hue	pH	PL	CK	CKT	WBS	Mois	Ash	IMF	CP
Cwt.	0.54***	0.02	0.55***	0.09	0.58***	0.14	0.25	-0.42*	-0.20	0.17	-0.33*	-0.39*	0.57***	-0.12
*L**		0.04	0.85***	0.14	0.88**	0.15	0.43**	-0.27	-0.18	0.01	0.17	-0.52**	0.56***	-0.48**
*a**			0.34*	0.99***	-.06	0.43**	-0.13	-0.20	0.03	0.14	-0.21	-0.10	0.04	0.30
*b**				0.44**	0.91***	0.18	0.42*	-0.27	-0.25	-0.10	0.05	-0.43*	0.58***	-0.38*
Chr					0.05	0.43**	-0.08	-0.22	0.00	0.12	-0.20	0.15	0.11	0.24
Hue						0.02	0.50**	-0.24	-0.27	-0.15	0.15	-0.36*	0.59***	-0.52**
pH							-0.05	-0.45**	-0.20	0.43**	-0.19	0.24	0.04	0.23
PL								-0.03	-0.31	0.04	0.51**	-0.16	0.02	-0.48**
CK									0.30	-0.21	0.31	0.13	-0.32	-0.26
CKT										0.12	-0.19	0.27	-0.14	0.45**
WBSF											-0.06	-0.14	-0.10	0.29
Mois												-0.06	-0.50**	-0.43**
Ash													-0.28	0.20
IMF														-0.36*

Carcass wt, carcass weight; *L**, color lightness; *a**, color redness; *b**, color yellowness; chr, color chromo; PL, purge loss; CK, cooking loss; CKT, cooking time; WBSF, Warner Bratzler shear force; Mois, Moisture; IMF; Intramuscular fat; CP, Crude protein; *, $p = \leq 0.05$, **; $p = \leq 0.01$; ***$p \leq 0.001$

Table 2.8 Pearson correlation coefficients between meat quality measurements of horse SM (*Semimembranosus*) aged 90 days post mortem

	L*	a*	b*	Ch.	Hue	pH	PL	CK	CKT	WBSF	Mois	Ash	IMF	CP
Carcass wt.	0.25	0.03	0.36*	0.08	0.36*	0.15	-0.13	-0.13	-0.07	0.11	0.08	-0.03	0.47**	-0.24
L*		0.42*	0.77***	0.49**	0.72***	0.13	0.23	0.13	-0.32	0.37*	0.23	0.03	0.27	-0.36*
a*			0.34*	0.99***	0.07	-0.26	-0.01	0.05	-0.01	0.38*	0.01	-0.08	0.10	-
b*				0.44 **	0.94***	0.05	0.16	0.23	-0.15	0.26	0.16	-	0.41*	-0.35*
Chr.					0.18	-0.24	-0.01	0.07	-0.03	0.37*	-	-0.07	0.18	-0.04
Hue						0.07	0.23	0.19	-0.16	0.17	0.19	0.04	0.42*	-0.39*
pH							0.01	-0.03	-0.27	-0.01	-0.10	-0.16	0.19	-0.01
PL								0.37*	-0.16	0.02	0.22	0.08	-0.09	-0.22
CK									-0.35*	-0.11	0.04	0.25	0.01	-0.21
CKT										-0.24	0.20	0.01	0.11	-0.18
WBSF											-0.05	-0.24	-0.25	0.24
Mois.												-0.20	-0.14	-0.87***
Ash													0.11	-
IMF														-0.29

Carcass wt., carcass weight; *L**, color lightness; *a**, color redness; *b**, color yellowness; chr, color chromo; PL, purge loss; CK, cooking loss; CKT, cooking time; WBSF, Warner Bratzler shear force; Mois., Moisture; IMF; Intramuscular fat; CP, Crude protein; *, p = ≤ 0.05, **; p = ≤0.01; ***p ≤ 0.001

References:

Astruc, T. (2014). Muscle fiber types and meat quality. Editors: Michael Dikeman, Carrick Devine. Encyclopedia of meat sciences (Second edition). Academic press, pp 442-448.

Bekhit, A. E. D. and Faustman, C. (2005). Meat Myoglobin reducing activity. Meat science, 71: 407-439.

Bendall, J. R., Restall, D. J. (1983). The cooking of single myofibres, small myofibre bundles and muscle strips from beef M. psoas and M. *sternomandibularis* muscles at varying heating rates and temperatures. Meat Science, 8: 93-117.

Bouton, P. E., Harris, P. V., Shorthose, W. R., and Baxter, R. I. (1973). A comparison of the effects of ageing, conditioning and skeletal restraint on the tenderness of mutton. Journal of Food Science, 38: 932-937.

Carlson, K. B., Prusa, K. J., Fedler, C. A., Steadham, E. M., Outhouse, A. C., King, D. A., Lonergan, S.M. (2017). Postmortem protein degradation is a key contributor to fresh pork loin tenderness123. Journal of Animal Science, 95: 1574-1586.

Carpenter, C. E., Cornforth, D. P., Whittier, D. (2001). Consumer preference for beef color packaging did not affect eating satisfaction. 57: 359-363.

Chambaz A., Scheeder, M. R. L., Kreuzer, M. and Dufey, P. A. (2003). Meat quality of Angus, Simmental, Charolais and Limousin steers compared at the same intramuscular fat content. Meat Science, 63**:** 491-500.

Colle, M. J., Richard, R. P., Killinger, K. M., Bohlscheid, J. C., Gray, A. R., Loucks, W. I., Day, R. N., Cochran, A. S., Nasados, J. A., Doumit, M. E. (2015). Influence of extended aging on beef quality characteristics and sensory perception of steaks from the gluteus medius and longissimus lumborum. Meat Science, 110: 32-39.

Corbin, C. H., O'Quinn, T. G., Garmyn, A. J., Legako, J. F., Hunt, M. R., Dinh, T. T. N., Rathmann, R. J., Brooks, J. C., Miller, M. F. (2015). Sensory evaluation of tender beef strip loin steaks of varying marbling levels and quality treatments. Meat Science, 100: 24-31.

Cross, H. R., West, R. L., and Dutson, T. R. (1981). Comparison of methods for measuring sarcomere length in beef semitendinosus muscle. Meat Science, 5, 261-266.

Domínguez R, Crecente S, Borrajo P, Agregán R and Lorenzo JM 2015. Effect of slaughter age on foal carcass traits and meat quality. Animal 9: 1713-1720.

Ertbjerg, P. and Puolanne, E. (2017). Muscle structure, sarcomere length and influences on meat quality: A review, Meat Science, 132: 139-152.

FAO (2015). Food and Agriculture Organization of the United Nations. Faostat (http:// faostat3.fao.org/home/index.html#DOWNLOAD (2015/04/29)).

Franco, D., and Lorenzo, J. M. (2014). Effect of muscle and intensity of finishing diet on meat quality of foals slaughtered at 15 months. Meat Science, 96: 327-334.

Franco, D., Rodríguez, E., Purriños, L., Crecente, S., Bermúdez, R., Lorenzo, J. M. (2011). Meat quality of "Galician Mountain" foals breed. Effect of sex, slaughter age and livestock production system. Meat Science, 88: 292-298.

Gill, C. O. (2005). Safety and storage stability of horse meat for human consumption. Meat Science, 71: 506-513.

Gill, C. O. Landers, C. (2005). Microbiological condition of horse meat prepared at a North American packing plant, and control of the temperatures of product air freighted to Europe. Meat Science, 69: 501-507.

Glitsh, K. (2000). Consumer perceptions of fresh meat quality: Cross-national comparison. British Food Journal. 120: 177-194.

Goll, D. E., Boehm, M. L., Geesink, G. H., and Thompson, V. F. (1997). What causes tenderization? InProceedings of the 50th annual reciprocal meat conference of the American Meat Science Association (pp. 60-67).

Gómez, M., Lorenzo, J. M. (2012). Effect of packaging conditions on shelf-life of fresh foal meat. Meat Science, 91:513-520.

Honikel, K. O. (2014). Conversion of muscle to meat. In Carrick, D. E, Micheal, D. (Eds.). Encyclopedia of meat sciences, pp. 353-357. (2nd ed.). Oxford: Academic Press.

Honikel, K., Kim, C., and Hamm, R. (1986). Sarcomere shortening of prerigor muscles and its influence on drip loss. Meat Science, 16: 267-282.

Hopkins, D. L. (2004). Tenderizing mechanisms - mechanical. In J. Werner Klinth (Ed.). Encyclopedia of Meat Sciences (pp. 1355-1363). Oxford: Elsevier

Hughes, J. M., Oiseth, S. K., Purslow, P. P. and Warner, R. D. (2014). A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. Meat Science, 98: 520-532.

Immonen, K., Ruusunen, M., Hissa, K., and Puolanne, E. (2000). Bovine muscle glycogen concentration in relation to finishing diet, slaughter and ultimate pH. Meat Science, 55: 25-31.

Kemp, C. M., Sensky, P. L., Bardsley, R. G., Buttery, P. J., Parr, T. (2010). Tenderness - An enzymatic view. Meat Science, 84: 248-256.

Khan, A. W., Lentz, C. P. (1973). Influence of antemortem glycolysis and dephosphorilation on high energy phosphates on beef ageing and tenderness. Journal of Food Science, 38:56-58.

Kim, G. D., Jeong, J. Y., Hur, S. J., Yang, H. S., Jeon, J. T., Joo, S. T. (2010). The relationship between meat color (CIE L* and a*), myoglobin content and their influence on muscle fiber

characteristics and pork quality, Korean Journal of Food Science and Animal Resource, 30: 626-633.

Kim, Y. H. B., Warner, R. D., Rosenvold, K. (2014). Influence of high pre-rigor temperature and fast pH fall on muscle proteins and meat quality: a review. Animal Production Science, 54: 375-395.

Klont, R. E., Barnier, V. M. H, Dijk, A. V., Smulders, F. J. M., Hoving-Bolink, A. H., Hulsegge, B. and Eikelenboom, G. (2000). Effects of pH fall, time of deboning, aging period, and their interaction on veal quality characteristics. Journal of Animal Science, 78: 1845-1851.

Kubelka, P., Munk, F. (1931). Ein Beitrag Zur Optik Der Farbanstriche. Zeitschrift für Technische Physik, 12: 593-601.

Lawrie R. A., Ledward D. A. (2006). Chapter 4-chemical and biochemical constitution of muscle. In: Lawrie R.A., Ledward D. A., editors. Lawrie's meat science. 7th ed. England, Cambridge: Woodhead Publishing. p 75-127.

Lawrie, R. (1950). Some observations on factors affecting myoglobin concentrations in muscle. The journal of Agricultural Science, 40: 356-366.

Li, P. Wang, T. Mao, Y., Zhang, Y., Niu, L., Liang, R., Zhu, L., and Luo, X. (2014). Effect of Ultimate pH on postmortem myofibrillar protein degradation and meat quality characteristics Chinese yellow crossbreed cattle. The Science World Journal, 1-8.

Locker, R. H. and Hagyard, C. J. (1963). A cold shortening effect in beef muscles. Journal of Food Science and Agriculture, 14: 787-793.

Lonergan, E. H., Zhang, W. and Lonergan, S. M. (2010). Biochemistry of postmortem muscle-lessons on mechanisms of meat tenderization. Meat Science. 86: 184-195.

Lonergan, S. M., Huff-Lonergan, E., Wiegand, B. R., Kriese-Anderson, L. A. (2001). Post mortem proteolysis and tenderization of top loin steaks from Brangus cattle**.** Journal of Muscle Foods, 12: 121-136.

Lorenzo, J.M, Pateiro, M. and Franco, D. (2013). Influence of muscle type on physiochemical and sensory properties of foal meat. Meat Science, 94: 77-83.

Lyford, C., Thompson, J.M., Polkinghorne, R., Miller, M., Nishimura, T., Neath, K., Allen, P. and Belasco, E. (2010). Is willingness to pay (WTP) for beef quality grade affected by consumer demographics and meat consumption habits. Australian Agribusiness Review, 18: 1-17.

Mach, N., Bach, A., Velarde, A., and Devant, M. (2008). Association between animal, transportation, slaughterhouse practices, and meat pH in beef. Meat Science, 78: 232-238.

Marsh, B. B. and Leet, N. G. (1966). Studies in meat tenderness. III. The effect of cold shortening on tenderness. Journal of Food Science, 31: 450-459.

Matarneh, S. K., England, E. M., Scheffler, T. L., Gerrard D. E. (2017). The conversion of Muscle to meat. Lawrie's Meat Science. http://dx.doi.org/10.1016/B978-0-08-100694-8.00005-4: 159-185.

Oillic, S., Lemoine, E., Gros, J. B., Kondioyan, A. (2011). Kinetic analysis of cooking losses from beef and other animal muscles heated in a water bath -Effect of sample dimensions and prior freezing and ageing. Meat Science, 88: 338-346.

Papuc, C., Goran, G. V., Predescu, C. N. and Nicorescu, V. (2017). Mechanisms of oxidative processes in Meat and Toxicity induced by postprandial degradation of products: A review. Comprehensive Reviews in Food Science and Food Safety, 16: 96-123.

Pearce, K. L., Rosenvold, K., Andersen, H. J., Hopkins, D. L. (2011). Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes-A review. Meat Science, 89: 111-124.

Pethick, D.W., Rowe, J.B., Tudor, G. (1995). Glycogen metabolism and meat quality. Recent Advances in Animal Nutrition in Australia, 13: 97-103.

Purslow, P. P., Warner, R. D., Clarke, F.M., Hughes, J. M. (2020). Variations in meat colour due to factors other than myoglobin chemistry; a synthesis of recent findings (invited review). Meat Science, 159: 107941

Rees, M. P., Trout, G. R., and Warner, R. D. (2003). The influence of the rate of pH decline on the rate of ageing for pork. II: Interaction with chilling temperature. Meat Science, 65: 805-818.

Robbins, K., Jensen, J., Ryan, K. J., Homco-Ryan, C., McKeith, F. K., Brewer, M. S. (2007). Enhancement effects on sensory and retail display characteristics of beef rounds. Journal of Muscle Foods, 13: 279-288.

Roth, D. M., Brewer, M. S., Bechtel, P. J., Kline, K. H., & Mckeith, F. K. (1995). Sensory, color and composition characteristics of young and mature chevaline. Journal of Muscle Food, 6: 83–89

Sarrie´s, M. V. and Beriain, M. J. (2005). Carcass characteristics and meat quality of male and female foals. Meat Science, 70: 141-152.

Segato, S., Cozzi, G., Andrighettto, I. (1999). Effect of animal morphotype, sex and age on quality of horse meat imported from Poland. In Recent Progress in Animal Production Science: Progress, XIII Congress (pp. 674-676). Piacenza.

Seog, P., Park, K. M., Cho, S., Kang, G. H., Chae, H. S., Park, B. Y. and Ba, H. V. (2016). Effect of cut type and post-mortem ageing on the technological quality, textural profile and sensory characteristics of horse meat. Animal Production Science, 56: 1551-1559.

Sørheim, O. and Hildrum, K. I. (2002). Muscle stretching techniques for improving meat. Trends in Food Science and Technology, 13: 127-135.

Suman, S. P. and Joseph, P. (2013). Myoglobin chemistry and meat color. Annual review Food Science and Technology, 4: 79-99.

Swatland, H. J. (2002). Optical dispersion through muscle fibers isolated from pork. Food Research International, 35: 559-564.

Swatland, H. J. (2004). Progress in understanding the paleness of meat with a low pH. South African Journal of Animal Science, 34: 1-7

Tang, J, Faustman, C., Hoagland, T. A., Mancini, R. A., Seyfert, M., Hunt, M. C. (2005). Postmortem oxygen consumption by mitochondria and its effects on myoglobin form and stability. Journal of Agricultural and Food Chemistry, 53: 1223-1230.

Tang, J., Faustman, C., and Hoagland, T. A. (2004). Krzwicki revisited: equation for spectrophotometric determination of myoglobin redox form in aqueous meat extracts. Journal of Food Science, 69: 717-720.

Taylor, R. G., Geesink, G. H., Thompson, V. F., Koohmaraie, M. and Goll, D. E. (1995). Is Z-disk degradation responsible for post-mortem tenderization? Journal of Animal Science., 21: 1351-1367.

Warriss, P. D. (1979). The extraction of haem pigments from fresh meat. Journal of food Technology, 14:75-80

Warriss, P. D. (1990). The handling of cattle pre-slaughter and its effects on carcass and meat quality. Applied Animal Behaviour Science, 28: 171-186.

Warriss, P. D. (2000). Meat Science: An introductory text. Journal of the South African Veterinary Association, 71: 247-248.

Wu, G., Farouka, M. M., Clerens, S. and Rosenvold, K. (2014). Effect of beef ultimate pH and large structure protein changes with ageing of meat tenderness, Meat science, 98: 637-645.

Xiong, Y. L. (2014). Chemical and physical characteristics of meat-protein functionality. In: Dikeman M, Devine C, editors. Encyclopedia of meat sciences. 2nd ed. Oxford: Academic Press. p 267-73.

Young O. A., Zhang S. X., Farouk M. M., Podmore, C. (2005). Effects of pH adjustment with phosphates on attributes and functionalities of normal and high pH beef. Meat Science, 70: 133-9.

Yu, T. Y., Morton, J. D., Clerens, S., Dyer, I. M. (2016). Cooking -Induced Protein Modifications in Meat. Comprehensive Review in Food Science and Food Safety, 16: 141-159.

Chapter 3: The variation in shear force explained by horse *Semimembranosus* muscle collagen characteristics

Abstract

Tenderness is one of the most valuable characteristics of meat quality. To sustain relevance in the competitive global market, ensuring the highest quality of horse meat is of prime importance. The *Semimembranosus* muscle (n = 36) collected over four consecutive weeks from the right side of horse carcasses after deboning at either 17, 26 and 30 h post mortem was used to study the impact of collagen characteristics on toughness as measured by Warner Bratzler shear force (WBSF). Muscle perimysium ($r = -0.52$, $p < 0.001$), muscle total collagen ($r = -0.35$, $p < 0.05$) and intramuscular fat ($r = -0.38$, $p < 0.05$) were negatively correlated with WBSF. Perimysial collagen ($r = 0.47$, $p < 0.05$) and pyridinoline ($r = 0.37$, $p < 0.05$) concentrations and muscle pH ($r = 0.46$, $p < 0.05$) were positively correlated with WBSF. Muscle pH, perimysial collagen concentration and intramuscular fat collectively explained 53% of the total variation in WBSF. About 63% of collagen heat solubility variation was explained by muscle weight, Ehrlich chromogen, purge loss and intramuscular fat. Results indicated that collagen contributes to horse *SM* toughness, but further study is required to characterize the impact of horse age on muscle collagen properties and WBSF.

3.1 Introduction:

Meat quality is an integrated term that incorporates meat appearance, tenderness, juiciness and flavour (Miller et al., 2001). After meat appearance, tenderness is considered the second most important quality trait, as it enhances the eating quality, consumer acceptability and consumer further purchase decision-making process (Grunert et al., 2004; Mennecke et al., 2007). Consumer willingness to pay studies have shown that consumers are willing to pay more for guaranteed

tender meat (Miller et al., 2001). In general, skeletal muscle toughness is associated with collagen characteristics, sarcomere length, pH, and the amount of intramuscular fat (Koohmaraie et al., 2002; Li et al., 2014; Wu et al., 2014; Starkey et al., 2016). In muscle, the amount of perimysium collagen is greater than that of the endomysium collagen (McCormick, 1999) and intramuscular variation of toughness is therefore reflected better by the perimysium collagen concentration rather than by that of the endomysium (Light et al., 1985; Purslow, 1999). Therefore, perimysium collagen is used to represent intramuscular connective tissue in general (IMCT, Roy et al., 2015) to determine the influence of collagen on muscle toughness. The muscle toughness attributed to connective tissue is called background toughness (Veiseth et al., 2004) and is influenced by the collagen concentration (Cross et al., 1973), heat solubility (Hill, 1966) and cross links (Bailey and Shimokomaki, 1972).

Collagen heat solubility changes with animal age (Young and Braggins, 1993), sex and muscle types (Wheeler et al., 2000). To explain the variation of muscle toughness, total collagen is considered limiting as compared to soluble collagen (Warner et al., 2010). However, total collagen and collagen heat stability both have an additive effect on meat toughness (McCormick, 1999). Collagen fibers are linked together by both divalent and trivalent cross links which provide its stability (Bailey and Sims, 1976). Divalent cross links are heat labile while trivalent cross links are heat stable. In young animals, newly synthesized collagen is rich in divalent heat labile cross links that are converted to trivalent heat stable cross links with increasing the animal age (Eyre and Wu, 2005). Therefore, with increasing animal age collagen heat solubility decreases and muscle toughness increases. The trivalent cross links include the pyridinolines (PYR), specifically hydroxylysilpyridinoline and lysidpyridinoline, and Ehrlich chromogen (EC). Each trivalent cross link combines two telopeptides and the helix of at least one other collagen molecule (Eyre, 1987).

There is limited information on how collagen affects the toughness of horse meat. Seong et al. (2019) found that horse m. *longissimus* contained 1.59 ± 0.06% collagen but did not characterize its trivalent cross links. Similarly, Perez-Chambala et al. (2005) found that horse *biceps femoris* contained 35.32 ± 1.55%, and did not characterize its trivalent cross links either. Given the large variation in total collagen content in the few horse muscles characterized, and the lack of characterization of the biochemistry of collagen cross-links, further research to understand the contribution of collagen to horse meat toughness is warranted. For this reason, the hypothesis that increased collagen solubility is related to decreased cooked horse meat WBSF was tested, and the relationships between horse meat quality and its collagen characteristics explored. Therefore, the objective of this study was to examine the relationships between collagen characteristics and other quality measurements and horse *SM* muscle tenderness as measured by shear force.

3.2. Materials and Methods

3.2.1 Experimental design

The experiment was conducted with 36 *Semimembranosus* (*SM*) muscles that were collected from a horse slaughter facility in Alberta over four consecutive weeks (visits), with nine muscles collected at each visit. During chilling, carcass temperature and pH values were recorded hourly for the first three hours as described in Section 2.2. The *SM* muscles were deboned from the right side of the randomly selected horse carcasses after 17, 26 and 30 h of chilling post-mortem. Three muscles were included in each deboning period at every visit. After three days of ageing at 0 ± 0.5 °C the *SM* was portioned as described in Section 2.2, with the middle portion of the muscle frozen and stored at -18 °C for intramuscular connective tissue (IMCT) and sarcomere length (SL) analysis. Another two steaks taken randomly within the muscle balanced for position within the

muscle were used for Warner Bratzler Shear force (WBSF) and proximate analysis, respectively, with pH measured on both steaks in three different places on each steak. Lyophilized and powdered muscle from steaks aged for 3 and 30 d post mortem that was used for proximate analysis (Section 2.2.5) was also used for measurement of collagen heat solubility. The average value of the six pH and WBSF readings from Section 2 were used for statistical analysis with the new collagen characteristics data generated in this chapter. The detailed procedure for analyses of intramuscular fat (IMF), WBSF, pH, purge loss and SL were explained in Sections 2.2.1 to 2.2.5 in the previous chapter.

3.2.2 Isolation of intramuscular connective tissue (IMCT)

To isolate the perimysium connective tissue, about 100 g of cubed, thawed (4 °C, overnight) muscle steak aged to 3 days post mortem were blended with 5 volume (w/v) deionized water (4 °C) 10s at low speed and 10s at high speed by a laboratory blender. The homogenate was filtered through a metal sieve (pore size 1mm^2) and the retained residues on the sieve were deemed IMCT. The IMCT was re-blended in 4 °C deionized water and filtered through metal sieve again and this step was repeated twice to ensure that the perimysium was visual free of myofibres. The IMCT was then blotted dry with Whatman No.4 filter paper (Fisher Scientific, Mississauga, Ontario). Visible blood vessels were removed from the retained IMCT prior to blotting with filter paper. The wet IMCT was weighed prior to storage at -70 °C. IMCT fat was extracted by immersing the IMCT into a chloroform: methanol, 2:1 (v/v) solution volume at 20 x mass of the IMCT and allow it to extract for an hour. After an hour, the chloroform/methanol solution was drained and the IMCT dried in a fume hood before the weight was recorded. The dried purified IMCT was then weighed and stored at -20 °C until analysis.

3.2.3 Ehrlich chromogen (EC) cross links concentration

Ehrlich chromogen (EC, pyrrole) cross-link concentrations in the finely diced perimysium IMCT were determined for each muscle according to the method of Horgan et al. (1990). Briefly, 100 mg IMCT were suspended in 5 mL of 50 mM Tris HCl containing 1mM calcium chloride (pH 7.5) at a concentration of 20 mg/mL and stored for 24 h at 4 °C. The suspension was denatured at 65 °C for 1h and then cooled to 37 °C in 65 °C and 37 °C water bath respectively. Trypsin was added at 0.5 mg trypsin /mL suspension (Ngapo et al., 2002) and the IMCT incubated at 37 °C for 4 h, with mixing by vortex every 15 min. After 4 h incubation, the IMCT suspension was heated at 65 °C for 20 minutes to stop denature the trypsin and halt proteolysis. The suspension was then cooled to room temperature and centrifuged at 28000 g for 30 minutes. After centrifugation, the supernatant was filtered through a 0.45 µm nylon filter. From the filtrate, 1 mL of supernatant was taken and added to 200µL of 5% (w/v) P-dimethyl amino benzaldehyde in 4N perchloric acid containing 0.01% (w/v) mercuric chloride, followed by centrifugation for 2 minutes to remove turbidity. The supernatant absorbance was measured at 572 and 640nm as soon as possible to prevent degradation of the chromogen formed. The pyrrole cross link concentration was expressed as mol/mol collagen and nmol/g wet muscle using the molar extinction coefficient of 25000 (Kemp and Scott, 1988). The extinction coefficient was used to calculate the molar concentration of EC in the tryptic digest from its absorbance using A/ε = molar concentration, where A = absorbance, and ε = molar extinction coefficient. The remaining trypsin digest was stored at -20 ºC for determination of hydroxyproline (Bergman and Loxley, 1963) as an estimate of total collagen content in the tryptic digest. Assays were performed in duplicate.

3.2.4 Pyridinoline (PYR) quantification

For quantification of PYR, approximately 200 ± 2 mg purified perimysial connective tissue was hydrolyzed with 6M hydrochloric acid (6mL for 20h at 110 ºC) in a Teflon^{R} capped glass tube flushed with nitrogen gas. After hydrolysis the samples were cooled with ice water and the hydrolysate was filtered through Whatman No. 4 filter paper (Fisher scientific, Mississauga, Ontario). From the total filtrate (8 mL) a 2 mL aliquot was taken for total collagen quantification by hydroxyproline (HYP) estimation (Bergman and Loxley, 1963), with the remaining filtrate 6 mL used for PYR quantification. Pyridinoline content was determined using the methods of Lamprecht (1997) ensuring minimal exposure of purified perimysium and PYR hydrolysates to light (Sakura et al., 1982). The acid hydrolysate was evaporated to dryness and reconstituted to 1.5 mL with 10% acetic acid (v/v). The PYR elution was performed using 10% acetic acid buffer and fractions containing PYR were detected by PYR natural fluorescence (Robins et al., 1996) (λex = 295 nm, λem = 395 nm). Fractions containing PYR were then filtered through Bio Gel P-2 media (from Bio Rad USA) Pac column (20 mL bed volumes, 1.5 cm x12 cm Bio rad USA). The pooled PYR fractions were evaporated to dryness and reconstitutes with 1.5 mL 0.1 M HCl which was then passed through a cation exchange chromatography column (20 mL bed volume, 1.5 cm x12 cm Bio rad USA) filled with cellulose phosphate (Sigma Chemical Co., St. Louis). The reconstituted PYR was further purified during cation exchange chromatography which washed out remaining fluorophores and amino acids by rinsing first with 0.1M HCl (3 times the volume of the column) (Fujimoto and Moriguchi, 1978; Yamauchi et al. 1987) prior to elution of PYR with 1M HCl. PYR fraction were pooled, evaporated to dryness and reconstituted to 1.5 mL with 1% aqueous heptafluorobutyric acid (Sigma-Aldrich Canada Ltd) for quantification using reverse

phase high performance liquid chromatography (HPLC). Reversed phase HPLC separation of PYR cross link and quantification was perform according to Roy et al. (2015).

3.2.5 Total collagen quantification

For the quantification of total collagen, a 2 mL aliquot of 6 M HCl hydrolysate for PYR quantification was evaporated and neutralized with NaOH, reconstituted to 5 mL with deionized water and stored at -20 °C until used. Prior to HYP analysis, neutralized reconstituted sample were thawed at room temperature and 50 µL duplicate aliquot were taken and 950 µL deionized water was added to each sample. Total collagen was quantified by determination of HYP with a modified version of Bergman and Loxley (1963) using standard generated dilution of stock solution of trans-4-hydroxy-L-proline (Sigma Aldrich Canada ltd. Oakville, Ontario) to 2.5, 5.0, 10, 20 and 40 µg/mL and the absorbance of the final experimental sample were measured at 558 nm against a water blank. Sample HYP concentrations were derived from a HYP standard regression equation plotting absorbances against standard concentrations. Collagen content was derived by multiplication of the HYP concentration in IMCT by 7.14, assuming that HYP constitute 14% percent of collagen (Etherington and Sims, 1981).

The HYP concentration of the trypsin digest was obtained using the method of Neuman and Logan (1950). From the trypsin digest, a 1mL aliquot was hydrolyzed with 6M HCl at 110 °C for 20 h in a Teflon^{R} capped glass tube flushed with nitrogen gas. The hydrolysate was evaporate and neutralized with NaOH and reconstituted to 5 mL with deionized water and stored at -20 °C. Prior to HYP analysis, the samples were thawed at room temperature and a 100 µL aliquot of reconstituted sample was added to 900 µL deionized water for each sample to determine the HYP concentration (Neuman and Logan, 1950). Similarly, absorbance of the final samples was measured at 558 nm with a water blank and HYP concentration was derived using a standard

regression curve which consisted of trans -4-hydroxy-L-proline (Sigma-Aldrich Canada Ltd., Oakville, Ontario) 2.5, 5, 10, 20, 40 µg/mL aqueous standards. Similarly, total collagen content was derived by multiplying HYP concentration on trypsin digest by 7.14 (Etherington and Sims, 1981). All analysis was performed in duplicate. The molar concentrations of total collagen in the trypsin digest and IMCT hydrolysate were converted to moles assuming the molecular weight of 3 x 10^5 g/mol for collagen (Eyre et al., 1984). The molar concentrations of collagen in IMCT were used to express EC, PYR on a mol EC or PYR/mol collagen basis.

3.2.6 Quantification of soluble collagen

The soluble collagen was extracted according to the modified method of Hill (1966) and Palka and Daun (1999). One gram of lyophilized powdered meat samples (day 3 and 30 post mortem) was gently homogenised by vortex in duplicate with 12 mL ¼ Ringer solution (consisting of 2.25 g sodium chloride, 0.105 g potassium chloride and 0.159 g calcium chloride per L solution, pH 7.4). Homogenates were heated at 77 °C for 63 min and mixed by hand every 10 min. After heating the samples were cooled in ice for 15 min and centrifuged at 3500 g for 10 min at 4 °C. The supernatant was retained and the residue mixed with a further 8mL Ringer solution before centrifugation again at 3500 g for 10 minutes at 4 °C. The second supernatant was retained and combined with the first supernatant. The pooled supernatants were then stored at -20 °C and then thawed at 4°C overnight prior to use.

For the determination of hydroxyproline, 3 mL of supernatant were hydrolyzed with 3 mL of 12 M HCl for 20 h at 110 °C. The hydrolysate was evaporated and neutralized with NaOH and reconstituted to 5 mL with deionized water and stored at -20 °C. Prior to HYP analysis, the samples were thawed at room temperature and duplicate 1 mL aliquots of reconstituted sample used to

determine sample HYP concentration (Neuman and Logan, 1950). Similarly, absorbances of the final samples were measured at 558 nm against a water blank and HYP concentration was derived by the standard regression curve which consisted of trans -4-hydroxy-L-proline (Sigma-Aldrich Canada Ltd., Oakville, Ontario) 2.5, 5, 10, 20, 40 µg/mL aqueous standards. Similarly, soluble collagen content was derived by multiplying HYP concentrations of the hydrolysates by 7.14 (Etherington and Sims, 1981). All analyses were performed in duplicate.

3.2.7 Statistical analysis

The data were analyzed using R (version 3.5.1) using mixed models. The data from measurements that were collected at day 3 only were analyzed with a randomized complete block design using a mixed model with deboning time as the sole fixed effect. The data from measurements made on different experimental units over time were analyzed with a split plot design built within the package nlme where deboning time was the sole source of variation in the main plot and ageing and its interaction with deboning time were fixed effects in the split plot. Visit was a random effect and carcass weight was included as a covariate in both models. The mean values of the deboning times were compared by least squares mean differences using the R package lsmeans. Pearson correlation analysis was performed using the linear model (package lm). Multivariate linear regression was performed using package lm and the model finalized using the "step" function in which a "direction" argument was indicated in the algorithm using the terms "forward", "backward" and "both". Polynomial regression was performed using the package lm. Principal Component Analysis (PCA) was conducted used the "prcomp" and "ggbiplot" packages. Prior to the actual PCA, data were normalized to each other by using the centre and scale option within the statistical package.

3.3 Results

3.3.1 Effects of deboning time on muscle and perimysial collagen characteristics

Horse meat *SM* muscle average weight did not differ among the deboning time treatment groups (Table 3.1). The amount of connective tissue separated as perimysium as wet and dry percentages of raw muscle did differ between the deboning time treatments however (Table 3.1), with higher perimysium wet and dry weights observed at 30 h deboning as compared to the 17 and 26 h deboning times. Moisture percentage of perimysium did not differ between deboning times but the trypsin digest perimysium and raw meat collagen concentrations differed between deboning times (Table 3.1).

The EC density in collagen differed among the treatment groups as well with a higher density of EC in collagen observed in muscles deboned at 17 h than in those deboned at 26 and 30 h. The concentration of heat soluble collagen (HSC) mean values was compared within ageing period day 3 and day 30 and between deboning times. No differences in heat soluble collagen (HSC) concentrations were observed between the 3 and 30 d ageing periods. However, mean HSC concentrations from muscles deboned at 17 h were higher than those deboned at 30 h on day 3 and both 26 and 30 h on day 30 when compared as a concentration in raw meat but no differences were observed when HSC was expressed as a proportion of total collagen (Table 3.1).

Differences in total collagen concentration derived during PYR analysis did not reflected those observed during the EC crosslink analysis although that derived from muscles harvested at 30 h post mortem did have a higher collagen concentration than those at 17 h post mortem (Table 3.2). Pyridinoline cross link concentration was greater in the muscles deboned at 30 h than in those deboned at the other times (Table 3.2).

3.3.2 Relationships between collagen characteristics and other quality measurements

Pearson correlations were observed between the measurements (Table 3.3). Meat shear force (SF) was negatively correlated with IMF, heat soluble collagen and EC and perimysium, meat total collagen and positively correlated with intramuscular collagen concentration, moisture and purge loss. IMF was positively correlated with EC and HSC, perimysium and muscle total collagen amounts. WBSF was strongly correlated with muscle pH, IMF and perimysium, total collagen and PYR cross link, while collagen heat solubility was strongly correlated with EC and intramuscular fat contents (Table 3.3).

Principal component analysis (PCA) was performed using all the perimysial collagen characteristics from this chapter, and the shear force and other meat quality measurements from Section 2 (Figure 3.1). The first and second principal components (PC1 and PC2, respectively) explained 24.7 and 23.5% of the total variation, respectively. The PCA biplot in Figure 3.1 indicated that muscles with increased concentrations of PYR also had increased total collagen, shear force and moisture, while those with increased EC and decreased PYR densities had increased HSC.

3.3.3 Multiple regression predictive model for WBSF and HSC

The prediction model of WBSF (Table 3.4) showed that muscle pH, perimysial collagen and intramuscular fat significantly impacted cooked muscle shear force values and collectively explained 53% of the variation in horse meat *SM* muscle shear force. Horse *SM* muscle heat soluble collagen variation was influenced by muscle weight (Mw), raw meat EC concentration, purge loss and IMF, which collectively explained 63% of collagen solubility variation observed (Table 3.5).

3.4 Discussion

3.4.1 Deboning time and muscle collagen characteristics

Collagen is the major protein of connective tissues consisting of at least one triple helical structure and it is considered to determine muscle background toughness (Blanco et al., 2013; Koohmaraie et al., 2002). The helical structure is stabilized by divalent and trivalent cross links. In young animals, total collagen content is higher due to the development of connective tissue at an early stage and it tends to remain high until maturity is reached (Hill, 1966; Vognarova et al., 1968; Boccard et al., 1979). During early life, due to high collagen turnover, the proportion of soluble collagen is higher than that of a mature animal, and with increased maturity the density of trivalent collagen cross links also increases and the proportion of soluble collagen decreases (Herring et al.1967; Goll et al., 1962) despite the total collagen concentration remaining the same. Goll et al. (1962) observed in the *biceps femoris* muscle of mature cow, young cow, steer and veal muscles that with increased animal age the frequency of stronger collagen cross links increased as the muscle collagen became increasingly heat insoluble. Therefore, meat tenderness has been related with collagen solubility (Cross et al., 1973; Renand et al., 2001; Blanco et al., 2013). Hence, the quality of collagen as determined by its cross linking is considered more important than the quantity of collagen for the purposes of determining its contribution to muscle toughness. In addition, the variation of collagen quality depends on animal age, sex, breed, muscle type and preslaughter management of the animal (Boccard et al., 1979; Blanco et al., 2013) and that also is considered.

In this current study, muscle perimysium, total collagen, and EC concentrations differed between the deboning time treatments (Table 3.1) which was unexpected. The variation of collagen and its density of EC is expected to be related with animal age, sex and breed (Horgan et al., 1991; Cross

et al. 1984) but not with deboning time suggesting that in this study there was significant variation in muscle collagen in the animal cohort that was not evenly distributed among the treatments. PYR cross link density in raw meat (Table 3.2) and soluble collagen in the connective tissue (Table 3.1) varied similarly between treatments without a real biological reason being apparent. Matrix metalloproteinases are the enzyme family considered to cause proteolytic damage to collagen post mortem as they are extracellular and therefore co-located near their collagen substrates (Sylvestre et al., 2002; Christensen and Purslow, 2016). Matrix metalloproteinase activity is considered to be slow because collagen turnover is usually slow (Rucklidge et al., 1992); thus, the impact of time of deboning on collagen characteristics would not be anticipated to be observed by day 3 post mortem, although this was not confirmed in the present research. These results therefore most likely indicate variation in the collagen content and composition between the muscles used from the various animals at the different fabrication times. Not knowing the ages of the animals from which the carcasses were obtained did not allow for age due to variation to be distributed across the treatments.

The mature cross link PYR is thermostable and its increasing density in muscle collagen has been linked to the thermostability of collagen and decreased collagen solubility (Goll et al., 1962; Bailey and Light, 1989; McCormick, 1994). In this study the highest density of PYR and the lowest proportion of soluble collagen were found in the same treatment, but no significant correlation was noted. Contrarily, EC and collagen solubility showed a positive correlation ($r = 67$, $p < 0.001$). On average total collagen content was 0.25 to 0.40% which was close to the total collagen value (0.44%) of horse meat *SM* muscle reported by Tateo et al. (2008), but lower than the horse meat *longissimus dorsi* muscle collagen value (0.47-0.64) obtained by Sarriés and Beriain (2005). The observed collagen solubility value in this thesis was much lower at 0.012% than that of the 0.39%

reported for horse meat *SM* muscle collagen solubility previously (Tateo et al., 2005; Tateo et al., 2008). In the current study, the low value of collagen solubility observed may be due to the muscle used in this work being obtained from horses older than 24 months old as compared to the 0.39% value obtained from foals from 11-24 months of age (Sarriés and Beriain, 2005; Tateo et al., 2008). It may also have stemmed from a different type of horse being used, with the horses in this research likely including light horses and not only draft crosses. The predictive model for heat solubility conducted by multiple regression (Table 3.4) indicated that muscle weight, EC, purge, and IMF strongly correlated with collagen heat solubility and explained 63% of the variation in collagen heat solubility. Draft crosses tend to have large muscles and be young and actively growing at slaughter weight, while those of light horses tend to be smaller due to physical maturity at slaughter. The regression equation indicated that increased HSC was most strongly related to increased EC density, suggesting that the muscles with increased HSC were from the youngest horses in this research.

3.4.2 Effect of collagen characteristics on WBSF

Warner Bratzler shear force was used to estimate cooked meat toughness, with high forces indicating a tough muscle. Muscle shear force value is directly or indirectly influenced by muscle total collagen content as well collagen heat solubility (Lepetit, 2008; Fang et al., 1999; Kopp and Bonnet, 1982). Total collagen can partially explain the variation in muscle toughness between different muscles in the same animal (Dransfield, 1977; Jeremiah et.al., 2003; Purslow, 2018). The variation of collagen cross links is assumed to play a vital role in the contribution of collagen to muscle toughness (McCormick, 1994). Intramolecular crosslinks provide mechanical strength to collagen fibers with divalent cross links replaced by mature cross links through condensation reactions between divalent cross links (Eyre and Wu, 2005). A consequence of this is compromised

collagen solubility and increased cooked meat toughness (Bailey and Light, 1989). In this study, a negative correlation was observed between shear force and perimysium concentration, total collagen concentration in raw meat, density of the EC in collagen, and collagen heat solubility in the loading plot generated by PCA. The collagen characteristics among them showed strong positive relationships. The Pearson correlation values indicated that WBSF was negatively correlated with the proportions of perimysium ($r = -0.52$, $p < 0.001$) and total muscle collagen ($r = -0.35$) and positively correlated with PYR ($r = 0.37$, $p < 0.05$) and total intramuscular collagen ($r = 0.47$, $p < 0.001$). Interestingly, shear force values were not correlated with collagen solubility. This may be because a very minor proportion of collagen is soluble in the muscle and therefore, the cross links of which the heat-stable collagen consists is more important. A strong positive correlation between total collagen and shear force ($r = 0.47$ $p < 0.001$) supports this conclusion. The negative correlation between perimysium and shear force appears to arise from the interrelationship between IMF, shear force and perimysium. Shear force was negatively correlated with both perimysium ($r= -0.52$, $p < 0.001$) and IMF ($r = -0.38$, $p < 0.05$) content and perimysium and IMF were both positively correlated to each other ($r = 0.56$, $p < 0.001$). The positive correlation between the perimysium and IMF contents indicated that perimysium content was enhanced by muscle IMF deposition which is supported by Roy et al (2018). In Roy et al. (2018), scanning electron microscope images of horse meat *SM* muscle showed an abundance of fat deposition in perimysium in tender muscles as compared to tough muscles. The IMF content depends upon the formation of large flecks of marbling or an increase in the number of fat flecks (Albrecht et al., 2006), both of which require an increase in the number and size of adipocytes (Albrecht et al. 2017). Generally, most muscle fat is deposited as triacylglycerols within adipocytes while a negligible amount fat is deposited within muscle fibers as phospholipids (Hocquette et al., 2012).

Furthermore, Roy et al. (2018) also reported that fat was negatively associated with WBSF, which is consistent with the current study as well as previous studies (Mortimer et al., 2014, Starkey et al., 2016). Furthermore, fat deposition in perimysium may reduce the strength of the collagen by disruption of its physical structure (Roy et al., 2018) and thus decreases the shear force value which is reflected in this thesis by their negative correlation. Hence, it can be assumed that the negative correlation between perimysium content and shear force is enhanced by the degree of new perimysium deposition associated with the formation of fat.

3.4.3 Effect of muscle pH, purge loss, intramuscular fat, sarcomere length, cooking loss, moisture content on WBSF

The relationship between intramuscular pH and meat tenderness is contradictory, as some researchers state that meat ultimate pH and tenderness are linearly related (Guignot et al., 1994, Silva et al., 1999, Wu et al., 2014), while others stated that a low pH (< 5.8) and a high ultimate pH (pH > 6.2) produces more tender meat as compared to an intermediate pH (pH = 5.8-6.2) (Li et al., 2014; Wu et al., 2014). A recent study reported that tough muscle is associated with intermediate pH values due to retarded enzymatic activity (Jeleníková et al., 2008; Pulford et al., 2008). For instance, beef muscle with a pH range of 5.79-5.83 produced toughest meat (Voisenet et al., 1997; Holdstock et al., 2014; Mahmood et al., 2017), while Li et al. (2014) reported that beef *longissimus dorsi* (*LD)* muscle with high pH produced beef that was more tender after one day slaughter and remained tender over the ageing period as compared to beef with low and intermediate pH values. Similar results have also been reported in beef by others (Yu and Lee, 1986; Knox et al., 2008). In the current study, a positive relation ($r = 0.46$, $p < 0.05$) was observed between WBSF and pH in *SM* muscle (Table 3.3) within the pH range of 5.32-5.55. In this study

the observed pH range was within the low ultimate pH range (Li et al., 2014; Wu et al., 2014). Bouton et a1. (1973) found a curvilinear relationship between bovine intramuscular ultimate pH and WBSF through quadratic linear regression and a similar curvilinear relationship was demonstrated for sheep SM (Bouton et al., 1972). In the current study, a curvilinear relationship between horse *SM* pH and WBSF was observed which explained 23% of variations of total WBSF (Figure 3.2), indicating that the effect of pH was low.

Every year the meat industry loses approximately one billion dollars (USD) in drip or purge loss (Smith et al., 2000). Hence, understanding the mechanism of purge loss is important to prevent undesirable water loss from muscle during processing. Muscle consists of more than 75% water (Huff-Lonergan and Lonergan, 2005), of which 85% of myo-water is trapped between the myofilaments and 15% is trapped in extracellular space, which includes between myofibers and myofiber bundles (Huff-Lonergan and Lonergan, 2005; Liu et al., 2016). After slaughter, due to the onset of rigor, the muscle fiber cross section and the space between the myofilaments is reduced, which is called lattice space. As a consequence, intracellular water moves and accumulates between myofiber and myofiber bundles which further is then lost as drip or purge (Pearce et al., 2011). In addition, during the conversion of muscle to meat, proteolytic enzymes breakdown muscle fiber bonds and further compromise the water holding capacity of muscle fiber. It is a well accepted concept that muscle pH plays a vital role in controlling purge loss by influencing protein structure and enzymatic activity (Bendall and Swatland, 1988). In this study, there was no correlation between WBSF and purge loss, although purge loss was negatively correlated with perimysium ($r = -045$, $p < 0.05$) and total muscle collagen ($r = -0.41$, $p < 0.05$). It is assumed that increased muscle collagen concentration reduces the apparent purge loss of muscle, giving the appearance of increased water holding capacity of muscle (Gariepy et al., 1999).

The degree of marbling plays an important role in muscle tenderness (Starkey et al., 2016). Muscle intramuscular fat is associated with increased tenderness and decreased shear force and that relationship was observed in Japanese black cattle where fat deposition was attributed to the disorganisation of intramuscular connective tissue and weakened mechanical structure of IMCT (Fang et al., 1999). In this current study IMF was negatively correlated with shear force ($r = -38$, $p < 0.05$). The correlation showed that for every 1% intramuscular fat increase there was a 1.4 N shear force decrease in horse *SM* muscle. This result suggests that increased marbling may enhanced muscle tenderness by remodeling of connective tissue and dilutes the concentration of the mature cross links PYR and EC (Christensen and Purslow 2016). Similar correlations between shear force and IMF were reported in bovine muscle by Wang et al. (2016) and Starkey et al. (2016). In addition, IMF in this study had a positive correlation with perimysium ($r = 0.56$, $p < 0.001$), total collagen ($r = 0.62$, $p < 0.001$), heat soluble collagen ($r = 0.58$, $p < 0.001$) and EC ($r = 0.42$, $p < 0.05$) which is consistent with the relationship between that of bovine collagen and IMF reported by Nishimura et al. (1999).

The length of the sarcomere depends on the contractile status of myofiber. It is observed that shortened sarcomere length is associated with tough meat as compare to normal meat (Bouton et al., 1974). However, fiber shortening is not only responsible for meat toughness but it changes the orientation and waviness of collagen fibers also responsible for meat toughness (Rowe,1977; Purslow and Trotter, 1994; Lepetit, 2008). Bouton et al. (1974) showed that myofibrillar length was responsible for cold shortened meat toughness when the meat was cooked to 60 ºC. Rowe (1977) and Purslow and Trotter (1994) suggested that the orientation of muscle collagen fibers during cold shortening should be considered in the model of muscle toughness. Muscle sarcomere length has been negatively correlated with muscle shear force (Starkey et al., 2016), but in this

research sarcomere length did not affect shear force as there was no significant Pearson correlation between shear force and sarcomere length. Roy et al. (2018) did not find any differences in sarcomere length between tough and tender *SM* muscle of horse meat as measured by transmission electron microscope. The results indicated that the differences in deboning time did not affect muscle sarcomere lengths and thus did not affect cooked meat toughness.

In this study WBSF and muscle moisture content were positively correlated ($r = 46$, $p < 0.05$). Moisture content of raw meat is important, however, and the retention of moisture after processing and cooking of meat is more important as it may determine the meat juiciness (Christensen et al., 2012). A major part of meat water is lost during cooking; hence, cooking loss may be more important than raw meat moisture content. Cooking loss is a complex phenomenon which is influenced by several factor including cooking conditions, animal species, age, sex and muscle, meat storage conditions, and extent of post mortem ageing (Oillic et al., 2011). Major parts of muscle water are trapped between myofilaments, and when myofibrillar protein is denatured during cooking at temperatures from 50 °C to 90 °C (Oillic et al., 2011) water holding capacity and water is lost as cooking loss. The properties of collagen fiber and shrinkage of waviness exert force on myofibers in that cooking loss depends on the myofibrillar resistance to the force exerted by the shrinkage of collagen fibers during cooking (Palka and Daun, 1999; Tornberg, 2005). Muscle pH in this research was positively correlated with cooking loss but polynomial regression (Figure 3.3) between cooking loss and pH explained only 20% cooking loss variation meaning that other factors affected cooking loss. In this current study, no correlation was observed between cooking loss and WBSF of horse meat *SM* muscle. To predict the collective effect of variables which are independent of each other we conducted a multiple regression analysis using collagen characteristics and uncorrelated meat quality measurements. The predicted model shows that pH

and total collagen positively and muscle IMF negatively correlated with shear force, and explained 53% of total variation.

3.5 Conclusion

The study showed that collagen concentration along with muscle pH and intramuscular fat contributed to horse *SM* shear force, indicating that total collagen concentration and EC were more important than PYR concentrations and heat soluble collagen in determining horse SM shear force. From these results, it would be possible to develop a predictive model for horse SM shear force through measurement of muscle pH, IMF, and collagen contents. Validation of such a shear force model in future study should include consideration of animal age as well, given its relationship to collagen characteristics.

Table 3.1 Effect of deboning time on Semimembranosus muscle and perimysium weights, perimysial collagen content, Ehrlich chromogen (EC) content and collagen heat solubility means

	Deboning (hours)			SEM[1]	P-value[2]	Covariate P-value[3]
	17	26	30			
n	12	12	12			
Muscle weight (kg)	4.90	4.51	4.39	0.19	0.106	0.063
Perimysium wet weight (% raw meat)	2.15[a]	2.43[a]	3.21[b]	0.23	0.005	0.025
Perimysium dry weight (mg dry/100g raw meat)	0.611[a]	0.660[a]	0.854[b]	0.041	0.001	0.003
Perimysium moisture %	71.2	72.5	73.0	1.61	0.112	0.993
Collagen (g/100g perimysium)	41.0[a]	49.5[b]	46.1[ab]	3.09	0.023	0.776
Collagen (g/100 g raw meat)	0.25[a]	0.33[b]	0.40[b]	0.031	0.001	0.001
EC[4] (nmol/g raw meat)	2.04	1.64	2.37	0.27	0.167	0.029
EC (mol/mol collagen	0.251[a]	0.162[b]	0.192[b]	0.021	0.004	0.854
Ageing day 03						
HSC[5] (µg/g raw meat)	189	176	198	20.93	0.875	0.009
HSC (µg/ 100 µg total collagen)	8.19[a]	6.05[ab]	5.41[b]	0.746	0.012	0.603
Ageing day 30						
HSC (µg/g raw meat)	187	167	190	18.86	0.509	0.354
HSC (µg/ 100 µg total collagen)	8.17[a]	5.62[b]	5.11[b]	0.546	0.002	0.032

[1] Standard error of the mean.
[2] Probability of the null hypothesis being accepted, with rejection of the null hypothesis at $p < 0.05$.
[3] Probability of the F test for the covariate carcass weight.
[4]EC, Ehrlich chromogen.
[5]HSC, heat soluble collagen
[a,b] Means having different superscripts in the same row indicates that they were significantly different ($p \leq 0.05$) from each other according to the least squares mean difference test.

Table 3.2 Effects of deboning time on Semimembranosus muscles total collagen and pyridinoline (PYR) cross link content

	Deboning time (hours)			SEM[1]	P-value[2]	Covariate p-value[3]
	17	26	30			
n	12	12	12			
Collagen (g/100g perimysium)	42.6	44.2	42.3	2.40	0.253	0.902
Collagen (g/100g raw meat)	0.259[a]	0.290[a]	0.355[b]	0.016	0.003	0.010
PYR (nmol/ g raw meat)	1.24[a]	1.42[a]	1.98[b]	0.157	0.002	0.626
PYR (µg/g perimysium)	88.3	94.6	100.6	9.47	0.217	0.252
PYR (mg/g collagen)	0.206	0.214	0.238	0.0169	0.127	0.172
PYR (mol/mol collagen)	0.144	0.150	0.166	0.0110	0.125	0.167

[1] Standard error of the mean.
[2] Probability of the null hypothesis being accepted, with rejection of the null hypothesis at $p < 0.05$.
[3] Covariate was carcass weight.
[a, b] Means having different superscripts in the same row indicate that they are significantly different ($p \leq 0.05$) from each other according to the least squares mean difference test. SEM, standard error of the mean; covariate, carcass weight

Table 3.3 Pearson correlation coefficients between collagen and meat quality characteristics of horse SM

	PER	TCI	TCR	PYRI	PYRR	PYRC	PYRMC	HSM	HSC	EC	EC1	pH	PL	SF	IMF	SL	Moi	Mb	CK
MW[1]	-0.02	-0.2	-0.12	-0.22	-0.21	-0.17	-0.17	0.49**	0.35*	0.16	0.09	0.18	0.02	-0.04	0.11	-0.09	0.04	-0.01	0.35*
PER		-0.41*	0.88***	-0.14	0.55***	0.04	0.04	0.45*	-0.28	0.74***	0.05	-0.01	-0.45*	-0.52**	0.56**	0.25	-0.58***	0.37*	0.14
TCI			0.07	0.46*	0.06	0.07	0.07	-0.19	0.07	-0.37*	-0.13	0.02	0.13	0.47**	0.01	0.11	0.19	-0.1	-0.3
TCR				0.07	0.63***	0.07	0.07	0.39*	-0.28	0.6	-0.03	0	-0.41*	-0.35*	0.62***	0.34*	-0.55***	0.38*	-0.02
PYRI					0.73***	0.92***	0.92**	-0.13	-0.05	-0.18	-0.13	-0.01	-0.04	0.37*	-0.1	-0.07	0.08	0.17	-0.33*
PYRR						0.81***	0.81***	0.18	-0.24	0.34*	-0.08	0	-0.32	-0.07	0.28	0.13	-0.32	0.43*	-0.19
PYRC							1***	-0.07	-0.11	-0.02	-0.08	-0.02	-0.1	0.2	-0.11	-0.12	0.02	0.24	-0.24
PYRMC								0.07	-0.11	-0.02	-0.08	-0.02	-0.1	0.2	-0.11	-0.12	0.02	0.24	-0.24
HSM									0.52**	0.67***	0.27	0.19	-0.34*	-0.22	0.58***	0.21	-0.41*	0.15	0.28
HSC										0.23	0.7	-0.03	0.08	0.06	0.02	0.15	0.08	-0.19	0.14
EC											0.6***	0.1	-0.34*	-0.27	0.42*	0.32	-0.34*	0.19	0.41*
EC1												-0.11	0.11	-0.03	-0.06	0.25	0.1	-0.14	0.27
pH													-0.27	0.46*	-0.01	0.11	0.1	-0.06	0.43*
PL														0.09	-0.2	-0.12	0.34*	-0.27	-0.09
SF															-0.38*	-0.11	0.46*	-0.18	0.13
IMF																0.27	-0.74***	0.27	-0.06
SL																	-0.28	0.17	0.3
Moi																		-0.49**	0.18
Mb																			-0.07
Ageing day 30																			
HSM												0.09	-0.53	-0.26	0.13		-0.44		0.02
HSC												0.10	-0.05	-0.08	-0.46		-0.17		-0.28

[1]MW, muscle weight kg; PER, perimysium % raw meat; TCI, total collagen, g/100g intramuscular connective tissue; TCR, total collagen% raw meat; PYRI, pyridinoline µg/g intramuscular connective tissue; PYRR, pyridinoline nmol/g raw meat; PYRC, pyridinoline mg/g collagen; PYRMC, pyridinoline mol/mol collagen; HSM, heat soluble collagen µg/g raw meat; HSC, heat soluble collagen µg/100g total collagen; EC, Ehrlich chromogen nmol/g raw meat; EC1, Ehrlich chromogen mol/mol collagen; PL, purge loss %; SF, sear force N; IMF, intramuscular fat%; SL, sarcomere length µm; Moi, moisture%; Mb, myoglobin; ck, cooking loss%.

* p ≤ 05; ** ≤0.001, *** p≤0.0001.

Table 3.4 Multiple regression predictive model for heat soluble collagen (raw meat) of horse SM muscle

	Coefficient	SEM[1]	P value[3]
Intercept	-191.13	75.41	0.0165
Muscle weight (kg)	61.87	15.40	0.0003
ECMMC[3]	388.45	136.03	0.0076
Purge	-88.05	32.39	0.0106
Intramuscular fat (%)	16.11	3.401	0.00004

[1] Standard error.
[2] Probability of the null hypothesis being accepted, with rejection of the null hypothesis at $p < 0.05$.
[3] ECMMC Ehrlich chromogen mol/mol collagen,
Adjusted R^2, 0.63.

Table 3.5. Multiple regression predictive model for WRSF of horse meat SM muscle

	Coefficient	SE[1]	P value[2]
Intercept	-437.93	118.10	0.0007
pH	84.24	21.67	0.0004
Collagen (g/100g perimysium)	1.01	0.253	0.0003
Intramuscular Fat (%)	-1.39	0.423	0.002

[1] Standard error.
[2] Probability of the null hypothesis being accepted, with rejection of the null hypothesis at $p < 0.05$.
Adjusted R^2 value of the model 0.53.

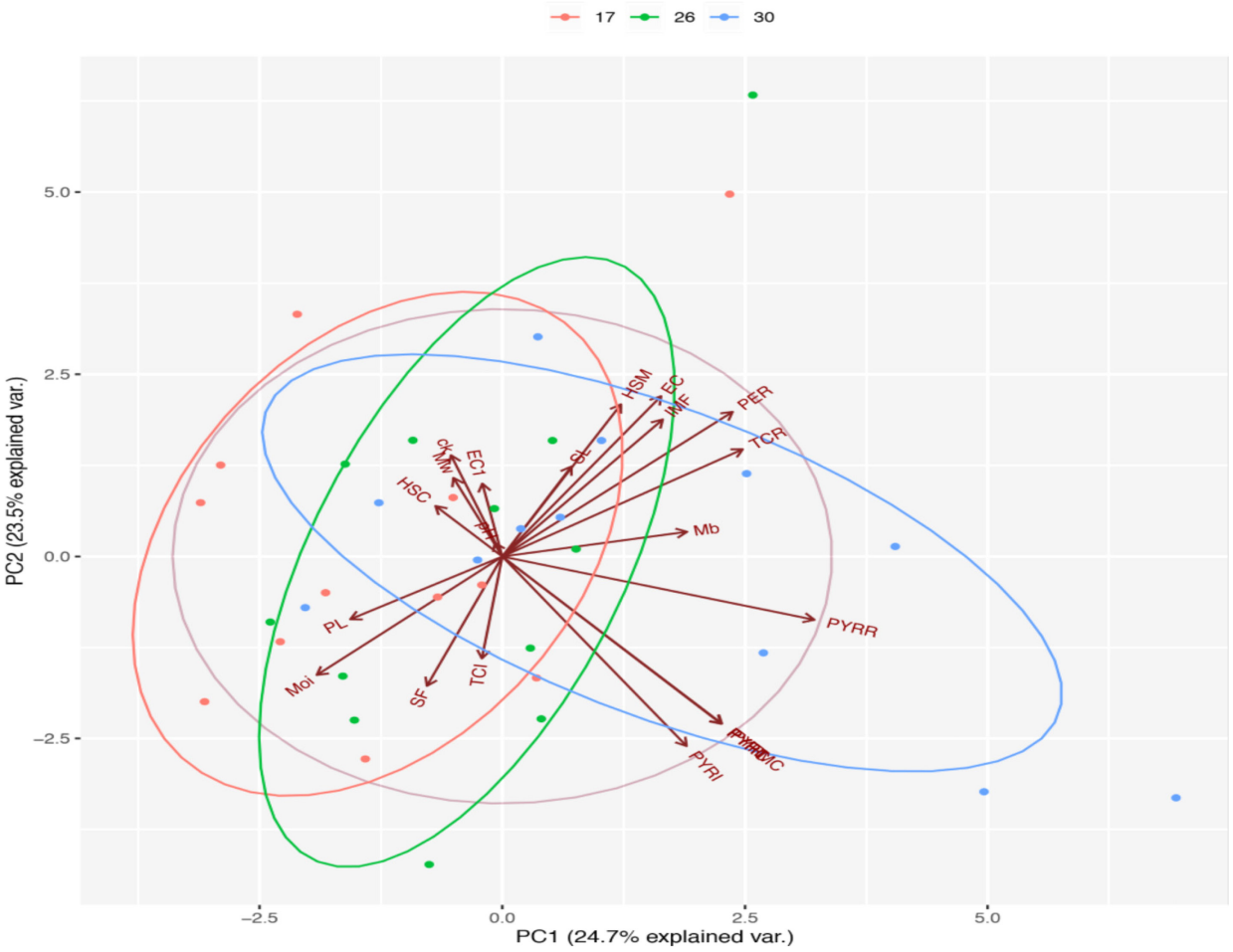

Figure 3.1. Loadings plot for the first two principal components from the principal component analysis showing generalized coefficients for Mw (muscle weight kg), TCR (total collagen% raw meat), PER (perimysium % raw meat), EC (Ehrlich chromogen nmol/g raw meat), EC1 (Ehrlich chromogen mol/mol collagen), HSM (heat soluble collagen µg/g raw meat), HSC (heat soluble collagen µg/100g total collagen), IMF(intramuscular fat%), SL (sarcomere length, µm), pH, TCI (total collagen, g/100g intramuscular connective tissue), SF (sear force N), PYRI (pyridinoline µg/g intramuscular connective tissue), PYRT (Pyridinoline mg/g collagen); PYRMC (Pyridinoline mol/mol collagen), PYRR (pyridinoline nmol/g raw meat); PYRC(Pyridinoline mg/g collagen), PL (purge loss %), Moi (moisture%), Mb(myoglobin), ck(cooking loss%)

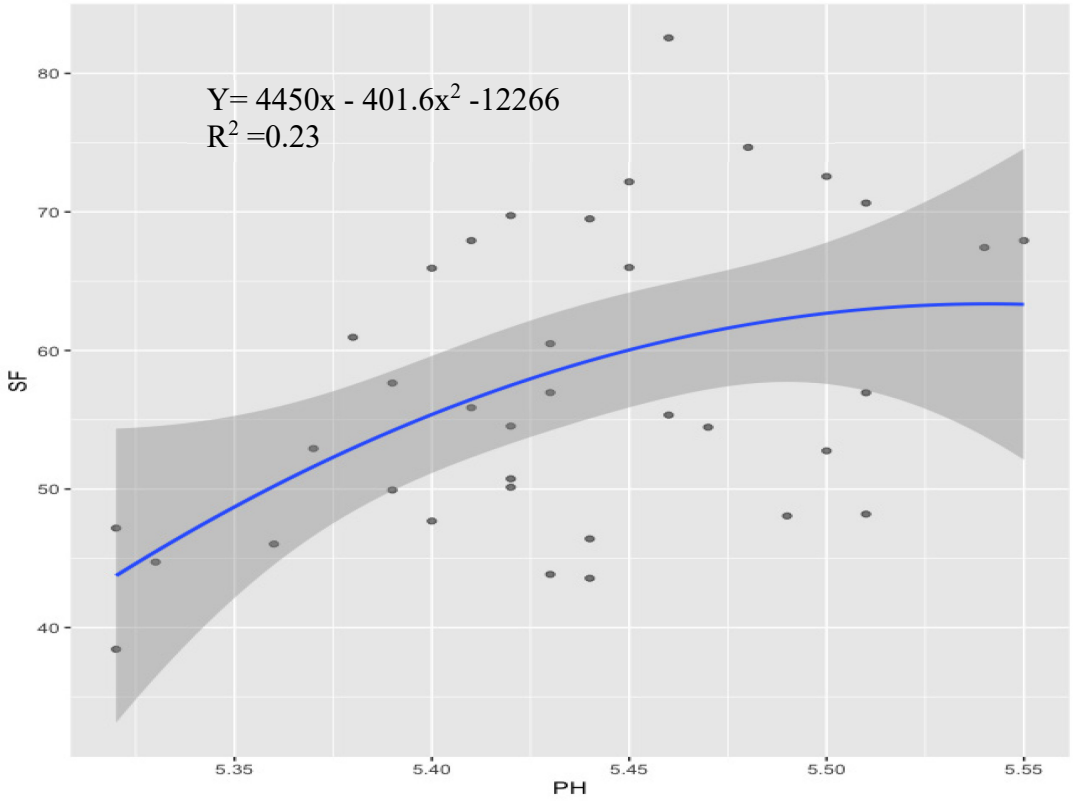

Figure 3.2 Polynomial relationship of *SM* muscle pH and Warner Brazler shear force (WBSF)

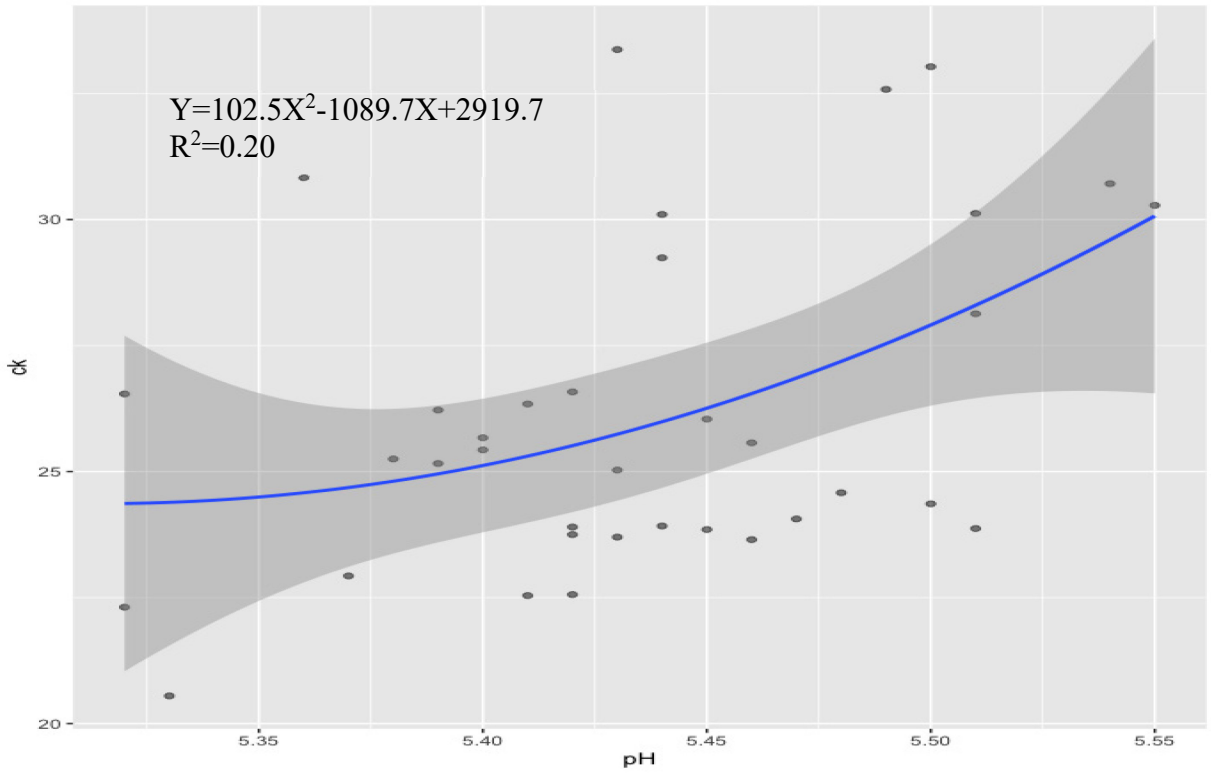

Figure 3.3. Polynomial relationship between horse *SM* muscle pH and cooking loss (CK)

References

Albrecht, E., Schering, L., Liu, Y., Komolka, K., Kühn, C., Wimmers, K. and Maak, S. (2017). Factors influencing bovine intramuscular adipose tissue development and cellularity. Journal of Animal Science, 95: 2244-2254.

Albrecht, E., Teuscher, F., Ender, K. and Wegner, J. (2006). Growth and breed-related changes of marbling characteristics in cattle. Journal of Animal Science, 84:1067-1075.

Bailey, A. J., and Light, N. D. (1989). Connective tissue in meat and meat products. London: Elsevier Applied Science

Bailey, A. J., and Shimokomaki, M. S. (1972). Age related changes in the reducible cross-links of collagen. FEBS Letters, 16: 86-88.

Bailey, A. J., and Sims, T. J. (1976). Chemistry of the collagen cross-links. Biochemical Journal, 153: 211-215.

Bendall, J. R., and Swatland, H. J. (1988). A review of the relationships of pH with physical aspects of pork quality. Meat Science 24: 85-126

Bergman, I., and Loxley, R. (1963). Two improved and simplified methods for the spectrophotometric determination of hydroxyproline. Analytical Chemistry, 35: 1961-196

Blanco, M., Jurie, C., Micol, D., Agabriel, J., Picard, B. and Garcia-Launay, F. (2013). Impact of animal management factors on collagen characteristics in beef: a meta-analysis approach. Animal, 7: 1208-1218.

Boccard, R. L., Naude, R. T., Cronje, D. E., Smith, M. C., Venter, H. J. and Rossouw, E. J. (1979). The influence of age, sex and breed of cattle on their muscle characteristics. Meat Science, 3: 261-280.

Bouton, P. E., Carrol, F.D., Fisher, A., Harris, P. V. and Shorthose, W. R. (1973). Effect of altering ultimate pH on bovine muscle tenderness. Journal of Food Science, 38: 816-820.

Bouton, P. E., Harris, P. V. and Shorthose, W. R. (1972). The effect of ultimate pH on bovine muscle: Mechanical properties. Journal of food Science, 37: 356-360.

Bouton, P. E., Harris, P. V., Shorthose, W. R. and Ratcliff, D. (1974). Changes in the mechanical properties of veal muscles produced by myofibrillar contraction state, cooking temperature and cooking time. Journal of Food Science, 39: 869-875.

Buchter, L. (1972). The influence of rate of chilling on development of rigor and cold shortening. In Proc. Meat Research Institute. Symposium no. 2, Langford, Bristol, U.K. pp. 4-51.

Christensen, L., Gunvig, A., Tørngren, M.A., Aaslyng, M.D., Knøchel, S., and Christensen, M. (2012). Sensory characteristics of meat cooked for prolonged times at low temperature. Meat Science, 90: 485-489.

Christensen, S. and Purslow, P.P. (2016). The role of matrix metalloproteinases in muscle and adipose tissue development and meat quality: A review. Meat Science, 119: 138-146.

Cross, H. R., Carpenter, Z. L., Smith, G. C. (1973). Effects of intramuscular collagen and elastin on bovine muscle tenderness. Journal of Food Science, 38: 998-1003.

Dransfield, E. (1977). Intramuscular composition and texture of beef muscles. Journal of the Science of Food and Agriculture, 28: 833-842.

Eikelenboom, G., and F. J. M. Smulders. (1987). Early and accelerated processing: Meat quality aspects. In: A. Romita, C. Valin, and A. A. Taylor (Ed.) Accelerated Processing of Meat. pp 161-169. Elsevier Applied Science, London.

Etherington, D. J., and Sims, T. J. (1981). Detection and estimation of collagen. Journal of the Science of Food and Agriculture, 32: 539-546.

Eyer, D. R. and Wu, J. J. (2005). Collagen cross-links. Topics in Current Chemistry, 247: 207-229.

Eyre, D. (1987). Collagen cross-linking amino acids. Methods in Enzymology, 144: 115-139.

Fang, S. H., Nishimura, T. and Takahashi, K. (1999). Relationship between development of intramuscular connective tissue and toughness of pork during growth of pigs. Journal of Animal Science, 77: 120-130.

Fujimoto, D. and Moriguchi, T. (1978). Pyridinoline, a non-reducible crosslink of collagen. Journal of Biochemistry, 83: 863-867.

Gariepy, C., Seoane, J. R., Cloteau, C., Martin, J. F. and Roy, G. L. (1999). The use of double-muscled cattle breeds in terminal crosses: Meat quality. Canadian Journal of Animal Science, 79: 301-308

Goll. D. E., Hoekstra. W. G. and Bray, R. W. (1962). Changes in the physicochemical properties of bovine connective tissue with chronological age. Journal of Animal Science 21: 981-982.

Grunert, K. G., Bredahl, L. and Brunsø, K. (2004). Consumer perception of meat quality and implications for product development in the meat sectorda review. Meat Science, 66: 259-272.

Guignot, F., Touraille, C., Ouali, A., Renerre, M., and Monin, G. (1994). Relationships between post-mortem pH changes and some traits of sensory quality in veal. Meat Science, 37: 315-325.

Herring, H. K., Cassens, R. G. and Briskey, E. J. (1967). Factors affecting collagen solubility in bovine muscle. Journal of Food Science, 32: 534-538.

Hill, F. (1966). The solubility of intramuscular collagen in meat animals of various ages. Journal of Food Science. 31: 161-166

Hocquette, J. F., Cassar-Malek, I., Jurie, C., Bauchart, D., Picard, B., and Renand, G. (2012). Relationships between muscle growth potential, intramuscular fat content and different indicators of muscle fibre types in young Charolais bulls. Animal Science Journal, 83: 750-758.

Holdstock, J., Aalhus, J. L., Uttaro, B. A., López-Campos, Ó., Larsen, I. L., and Bruce, H. L. (2014) The impact of ultimate pH on muscle characteristics and sensory attributes of the longissimus thoracis within the dark cutting (Canada B4) beef carcass grade. Meat Science, 98: 842-849.

Horgan, D. J., King N. L., Kurth, L. B. and Kuypers, R. (1990). Collagen cross links and their relationship to the thermal properties of calf tendons. Archives of Biochemistry and Biophysics, 28: 21-26.

Huff-Lonergan, E. and Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Science, 71: 194-204.

Jeleníková, J., Pipek, P., and Staruch, L. (2008). The influence of ante-mortem treatment on relationship between pH and tenderness of beef. Meat Science, 80: 870-874.

Jeremiah, L. E., Dugan, M. E. R., Aalhus, J. L. and Gibson, L. L. (2003). Assessment of the relationship between chemical components and palatability of major beef muscles and muscle groups. Meat Science, 65: 1013-1019.

Kemp, P. D. and Scott, J. E. (1988). Ehrlich chromogens probable cross-links in elastin and collagen. Biochemistry Journal. 252: 387-393.

Klont, R. E., Barnier, V. M. H, Dijk, A. V., Smulders, F. J. M., Hoving-Bolink, A. H., Hulsegge, B. and Eikelenboom, G. (2000). Effects of pH fall, time of deboning, aging period, and their interaction on veal quality characteristics. Journal of Animal Science. 78: 1845-1851.

Knox, B. L., Van Laack, R. L., and Davidson, P. M., (2008). Relationships between ultimate pH and microbial, chemical, and physical characteristics of vacuum-packaged pork loins. Journal of Food Science, 73: 104-110.

Koohmaraie, M., Kent, M. P., Steven, S. D., Veiseth, E. and Wheeler, T. L. (2002). Meat tenderness and muscle growth is there any relationship? Meat science, 62: 345-352.

Kopp, J., and Bonnet, M. (1982). Dureté de la Viande et Résistance au Cisaillement des Fibres de Collagène. Sciences des Aliments, 2: 127-132.

Lamprecht, G. (1997) Rapid method for the isolation of hydroxylysylpyridinoline and lysylpyridinoline from concentrated bone hydrolysates by liquid chromatographic techniques. Journal of Chromatograph B: Biomedical Science and Applications, 691: 297-304.

Lepetit, J. (2008). Collagen contribution to meat toughness: Theoretical aspects: A review. Meat Science, 80: 960-967.

Li, P. Wang, T. Mao, Y., Zhang, Y., Niu, L., Liang, R., Zhu, L., and Luo, X. (2014). Effect of Ultimate pH on postmortem myofibrillar protein degradation and meat quality characteristics Chinese yellow crossbreed cattle. The Science World Journal. 1-8.

Light, N., Champion, A. E., Voyle, C. and Bailey, A. J. (1985). The role of epimysial, perimysial and endomysial collagen in determining texture in six bovine muscles. Meat Science, 13: 137-149.

Liu, J., Arner, A., Puolanne, E. and Ertbjerg, P., (2016) On the water-holding of myofibrils: Effect of sarcoplasmic protein denaturation. Meat Science, 119: 32-40.

Lorenzo, J. M., Pateiro, M., and Franco, D. (2013). Influence of muscle type on physicochemical and sensory properties of foal meat. Meat Science, 94: 77-83.

Mahmood, S., Roy, B. C., Larsen, I. L., Aalhus, J. L., Dixon, W. T. and Bruce, H. L, (2017). Understanding the quality of typical and atypical dark cutting beef from heifers and steers. Meat Science, 133: 75-85.

McCormick R. J. (1994). The flexibility of the collagen compartment of muscle. Meat Science 36: 79-91.

McCormick, R. J. (1999). Extracellular modifications to muscle collagen: implications for meat quality. Poultry Science, 78: 785-791.

Mennecke, B. E., Townsend, A. M., Hayes, D. J. and Lonergan, S. M. (2007). A study of the factors that influence consumer attitudes toward beef products using the conjoint market analysis tool. Journal of Animal Science 85: 2639-2659.

Miller, M. F., Carr, M. A., Ramsey, C. B., Crockett, K. L. and Hoover, L. C. (2001). Consumer thresholds for establishing the value of beef tenderness. Journal of Animal Science 79: 3062-3068.

Mortimer, S. I., van Derwerf, J. H. J., Jacob, R. H., Hopkins, D. L., Pannier, L., Pearce, K. L. and Pethick, D. W. (2014). Genetic parameters for meat quality traits of Australian lamb meat. Meat Science, 96: 1016-1024.

Neuman, R. E. and Logan, M. A. (1950). The determination of hydroxyproline. Journal of Biological Chemistry, 184: 299-306.

Ngapo, T. M., Berge, P., Culioli, J. and Smet, S. De (2002). Perimysial collagen crosslinking in Belgian blue double muscle cattle. Food Chemistry, 77: 15-26.

Nishimura, T., A. Hattori, and. Takahashi, K. (1999). Structural changes in intramuscular connective tissue during the fattening of Japanese Black cattle: Effect of marbling on beef tenderization. Journal of Animal Science, 77: 93-104.

Oillic, S., Lemoine, E., Gros, J. B. and Kondjoyan, A. (2011). Kinetic analysis of cooking losses from beef and other animal muscles heated in a water bath -Effect of sample dimensions and prior freezing and ageing. Meat Science, 88: 338-346.

Palka, K., and Daun, H. (1999). Changes in texture, cooking losses, and myofibrillar structure of bovine M-semitendinosus during heating. Meat Science, 51: 237-243.

Pearce, K. L., Rosenvold, K., Andersen, H. J. and Hopkins, D. L. (2011). Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes- A review. Meat Science, 89: 111-124.

Pulford, D. J., Fraga Vazquez, S., Frost, D. F., Fraser-Smith, E., Dobbie, P. and Rosenvold, K. (2008). The intracellular distribution of small heat shock proteins in post-mortem beef is determined by ultimate pH. Meat Science, 79: 623-630.

Purslow, P. P. (1999). The intramuscular connective tissue matrix and cell matrix interactions in relation to meat toughness. In: Proceedings of the 45th international congress of meat science and technology, Yokohama, Japan, pp. 210-219.

Purslow, P. P. (2018). Review: Contribution of collagen and connective tissue to cooked meat toughness; some paradigms reviewed. Meat Science, 144: 127-134.

Purslow, P. P., and Trotter, J. A. (1994). The morphology and mechanical properties of endomysium in series-fibred muscles: Variations with muscle length. Journal of Muscle Research and Cell Motility, 15: 299-308.

Renand, G., Picard, B., Touraille, C., Berge, P. and Lepetit, J. (2001). Relationships between muscle characteristics and meat quality traits of young Charolais bulls. Meat Science 59: 49-60.

Robins, S. P., Duncan, A., Wilson, N. and Evans, B. J. (1996). Standardization of pyridinium crosslinks, pyridinoline and deoxypyridinoline, for use as biochemical markers of collagen degradation. Clinical Chemistry, 42: 1621-1626.

Rowe, R. W. D. (1977). The influence of the collagen fibre network of muscle on the compliance and tensile strength of muscle samples subjected to loads at right angles to the muscle fibre axis. Meat Science, 1: 135-148.

Roy, B. C., Sedgewick, G., Aalhus, J. L., Basarab, J. A. and Bruce, H. L. (2015) Modification of mature non-reducible collagen cross-link concentrations in bovine m. gluteus medius and semitendinosus with steer age at slaughter, breed cross and growth promotants. Meat Science 110: 109-117.

Roy, B. C., Walker, B., Rahman, M. M., Bruce, H. L. and McMullen, L. (2018). Role of myofibers, perimysium and adipocytes in horse meat toughness. Meat Science, 146: 109-121.

Sakura, S., Fujimoto, D., Sakamoto, K., Mizuno, A., and Motegi, K. (1982). Photolysis of pyridinoline, a cross-linking amino acid of collagen, by ultraviolet light. Canadian Journal of Biochemistry, 60: 525-529.

Sarries, M. V., and Beriain, M. J. (2005). Carcass characteristics and meat quality of male and female foals. Meat Science, 70: 141-152.

Silva, J. A., Patarata, L., and Martins, C. (1999). Influence of ultimate pH on bovine meat tenderness during ageing. Meat Science, 52: 453-459.

Starkey, C. P., Geesink, G. H., Collins, D., Oddy, V. H. and Hopkins, D. L. (2016). Do sarcomere length, collagen content, pH, intramuscular fat and desmin degradation explain variation in the tenderness of three ovine muscles? Meat Science, 113: 51-58.

Sylvestre, M.N., Balcerzak, D., Feidt, C., Baracos, V.E., Bellut, J.B. (2002). Elevated rate of collagen solubilization and post mortem degradation in muscle of lambs with high growth rates: possible relation ship with activity of matrix metalloproteinases. Journal of Animal Science, 80: 1871-1878.

Tateo, A., De Palo, P., Ceci, E. and Centoducati, P. (2008). Physicochemical properties of meat of Italian Heavy Draft horses slaughtered at the age of eleven months. Journal of Animal Science, 86: 1205-1214.

Tateo, A., De Palo, P., Padalino, B. and Centoducati, P. (2005). Quality of carcasses in IHDH foals reared in the province of Bari (Italy). Italian Journal of Animal Science, 4: 418-420.

Tornberg, E. (2005). Effects of heat on meat proteins - Implications on structure and quality of meat products. Meat Science, 70: 493-508.

Van Laack, R. L. J. M., and F. J. M. Smulders. (1994). Postmortem glycolysis in veal carcasses; influence on meat quality. In: Proc. 40th Int. Congress of Meat Science and Technology., The Hague, Netherlands. Manuscript S-IVB-04.

Veiseth, E., Shackelford, S., Wheeler, T. and Koohmaraie, M. (2004). Factors regulating lamb longissimus tenderness are affected by age at slaughter. Meat Science, 68: 635-640.

Vognarova, J., Dvorak, Z. and Bohm, R. (1968). Collagen and elastine in different cuts of veal and beef. Journal of Food Science, 33: 339-343.

Voisenet, B. D., Grandin, T., Tatum, J. D., O'Connor, S.F., and Struthers, J. J. (1997). Feedlot cattle with calm temperaments have higher average daily gain than cattle with excitable temperaments. Journal of Animal Science, 75: 892-896.

Wang, F., Zhang, Y., Li, J., Guo, X., Cui, B., and Peng, Z. (2016). Contribution of cross-links and proteoglycans in intramuscular connective tissue to shear force in bovine muscle with different marbling levels and maturities. LWT. Food Science and Technology, 66: 413-419.

Warner, R., Jacob, R., Hocking Edwards, J., McDonagh, M., Pearce, K., Geesink, G. and Pethick, D. (2010,). Quality of lamb meat from the information nucleus flock. Animal Production Science, 50: 1123-1134.

Wheeler, T., Shackelford, S. and Koohmaraie, M. (2000). Variation in proteolysis, sarcomere length, collagen content, and tenderness among major pork muscles. Journal of Animal Science, 78: 958-965.

Wu, G., Farouka, M. M., Clerens, S. and Rosenvold, K. (2014). Effect of beef ultimate pH and large structure protein changes with ageing of meat tenderness, Meat Science, 98: 637-645.

Yamauchi, M., London, R. E., Guenat, C., Hashimoto, F., and Mechanic, G. L. (1987). Structure and formation of stable histidine-based trifunctional cross-link in skin collagen. Journal of Biological Chemistry, 262: 11428-11434.

Young, O. A., and Braggins, T. J. (1993). Tenderness of ovine semimembranosus: Is collagen concentration or solubility the critical factor? Meat Science, 35: 213-222.

Yu L. P., and Lee, Y. B. (1986). Effects of postmortem pH and temperature on bovine muscle structure and meat tenderness. Journal of Food Science, 51: 774-780.

Eyre, D.R., Paz, M.A. and Gallop, P. M. 1984. Cross-Linking in collagen and elastin. Annual Review of Biochemistry, 53: 717-748.

Rucklidge, G. J., Milne, G., Mcgaw, B. A., Milne, E. and Robins, S. P. (1992). Turnover rates of different collagen types measured by isotope ratio mass-spectrometry. Biochimca et Biophysca Acta, 1156: 57-61.

Smith, G. C., Belk, K. E., Sofos, J. N., Tatum, J. D. and Williams, S. N. (2000). Economic implications of improved color stability in beef. In Decker E.A., Faustman, C., Lopez- Bote, C.J. (Eds.), Antioxidants in Muscle Foods: Nutritional Strategies to Improve Quality. New York: Wiley Inter science. pp. 397- 426.

Chapter 4 Summary

High unsaturated fatty acid and iron contents and low fat and cholesterol make horse meat a suitable substitute for meat from conventional meat animal species. Several countries in Western Europe and Japan are the primary consumers of horse meat, and Canada is the one of the largest exporters of horse meat in the world. With increased horse meat consumption, the competition in the world market is likely to also increase. To sustain the Canadian market share in the competitive global market, Canadian horse meat purveyors must ensure that they supply the highest quality of horse meat as possible in a manner that sustains the industry. Because most Canadian horse meat is exported, it can be in transit for up to 3 months, and this may have a detrimental effect on its quality. As a result, any changes to horse meat processing need to consider the potential for an interaction with lengthy post mortem ageing time. The lengthy time to market provides an opportunity to study the effect of post mortem ageing on horse meat collagen. Previous studies conducted on horse meat have not focused on the effect of collagen on horse meat toughness, but assumed that intramuscular connective tissue contributed. However, collagen heat solubility has been shown to increase in beef (Palka, 2003) and lamb (Starkey et al., 2015) with post mortem ageing, and therefore warrants characterization in horse as well.

Gaining processing efficiencies, no matter how slight, can assist Canadian horse meat processors with remaining competitive. The current practice of hot boning the horse carcass at 2 h post mortem provides for substantial process efficiency, but places the product at a significant potential for cold shortening. It would be most desirable therefore for the horse meat to be removed as soon after rigor is completed as possible, thus mitigating the potential for cold shortening while still gaining the reduction in time to market.

Hence, the objectives of this research were to identify suitable deboning times and gauge their interaction with possible post mortem ageing times to ensure superior quality of horse meat and also to investigate the impact of intramuscular connective tissue on horse meat toughness. This was done by exploring the factors influencing horse meat tenderness. The experiment was conducted with 36 *SM* muscles that were collected from a horse slaughter facility in Alberta over four consecutive weeks (visit), where nine muscles were collected at each visit. The *SM* muscles were deboned from the right side of horse carcasses randomly selected carcass sides above 179 kg after 17, 26 and 30 h of chilling post-mortem. Three muscles were included in each deboning period at every visit. After three days of ageing (0 ± 0.5 °C) muscles were sliced into five portions, with two steaks in each portion, and the portions from within each muscle being randomly distributed to the 3, 30, 60 and 90 days of ageing with the exception of muscle middle portion which was frozen and stored at -18 °C for IMCT analysis. The steaks within each portion were packaged under vacuum for ageing. After each ageing period within each portion, one steak was used for purge loss, cook loss and WBSF and one steak was used for proximate analysis. Color and pH were however were measured on both steaks within each portion. Separated IMCT was used for total collagen, EC and PYR cross link quantification and freeze-dried meat samples were used for heat soluble collagen analysis.

Both deboning time and ageing periods impacted meat lightness (L^*) and yellowness (b^*). Early deboning (17 h) increased the purge loss. The post mortem ageing period had the largest effect on horse meat quality, as the purge loss increased and the WBSF decreased with increasing ageing period. Also horse meat darkened with post mortem ageing time and its cooking loss decreased. What sort of impact these changes would have on the acceptability of horse meat from a consumer

aspect was unclear however and future research should address this so that the changes noted can be put into perspective for the horse meat industry.

One of the major limitations of this study was that little was known regarding the production backgrounds, sex, breed and age of the animals from which the carcasses were derived. Juárez et al. (2009) found that breed significantly affected carcass weight and shape and that maturity increased meat redness and Sarriés and Beriain (2005) indicated that horse meat became lighter, redder and yellower with animal age, and that female foals at 16 months of age produced meat that was darker than that from their male counterparts. Such information would have assisted with interpretation of the research results. Horse meat in Canada is not differentiated by breed or animal age presently, and such differentiation presents a market opportunity; therefore, future investigation into horse meat quality should investigate the impact of production factors on horse meat quality.

Notably, work reported in Chapter 3 indicated that horse meat WBSF increased as the amount of perimysium and intramuscular fat percentage in the muscle decreased. Intramuscular fat deposition appeared to promote the formation of perimysium and total collagen, having significant correlations with both ($r = 0.56$ and 0.62, respectively, Chapter 3). Aside from the work by Roy et al. (2018), which was performed in our laboratory, little research was found during review of the literature that examined the relationship between horse meat toughness and its intramuscular collagen. Tateo et al. (2008) reported that horse meat SM had a total collagen content of 0.44%, while Seong et al. (2019) found that the horse m. *longissimus dorsi* contained 1.59 ± 0.06 % collagen using near infrared spectroscopy. Perez-Chabela et al. (2005) found that the horse *biceps femoris* contained 35.32 ± 1.55 mg collagen/g muscle or 3.5.% collagen. The amount of collagen in the horse *semimembranosus* reported in this thesis was comparably very low at about 0.3%, and

closer to the values of Tateo et al. (2008) and Seong et al. (2019) and much lower than that of Perez-Chabela et al. (2005). In this research, however, unlike that of Seong et al. (2019) and Perez-Chabela et al. (2005), the amount of collagen estimated was for the perimysium only. This suggests that the endomysium, which was not harvested in the current research, may make up a larger part of the total collagen in horse muscle and play an important role in horse muscle structure, more so than in beef. With this in mind, future research on the relationships between horse intramuscular collagen and horse meat toughness should involve collection and characterization of the endomysium as well.

Despite not considering the endomysium collagen in this research, muscle pH, perimysial collagen concentration and IMF collectively explained 53% of the total variation of WBSF, while muscle weight, EC concentration, purge loss and IMF collectively explained 63% of the variation in collagen heat solubility. This is the first work to substantiate that intramuscular collagen contributes to the toughness of horse meat as measured using shear force, and indicates that IMF and muscle pH are also important factors.

These results are important to the horse meat industry because they indicated that adequately fattening and resting horses prior to slaughter can reduce the shear force of the meat they produce. In this study we also found that early deboning increased purge loss, which could reduce the saleable yield and reduce revenue per carcass. Further research on the effects of early de-boning of horse carcasses is warranted given the financial implications of this practice. There was limited interaction of de-boning time post mortem with ageing period in this study, but increasing the replication in a future study may indicate that the early purge loss observed in this study may persist throughout the ageing period, as there was a tendency for SM muscles harvested at 17 h post mortem to have higher levels of purge than those harvested at 26 and 30 h regardless of the

ageing time post mortem. What these results did indicate as well was that deboning the horse carcass at 26 h instead of 30 h would provide a 4 h throughput advantage without changing horse meat quality from that observed at 30 h post mortem fabrication. According to the data then a 26 h post mortem fabrication time could be adopted without impacting horse meat quality.

The previous bovine study (Roy et al. 2015) indicated that with increasing collagen density of PYR decreased collagen heat solubility and increased muscle toughness. However, in this current study we did not find any relationships between PYR density, collagen heat solubility and WBSF. Previous study also reported relationships among animal age, sex, PYR density and collagen heat solubility as well as muscle toughness (Boccard et al., 1979; Blanco et al., 2013). Knowing animal age, sex, and breed would have assisted with the interpretation of the results in this study as collagen characteristics in other species can be affected by animal age (Hill, 1966; Horgan et al., 1991), sex, and breed (Cross et al., 1984). Hence, future work should include information on horse age, sex and breed to provide fuller understanding of the effects of these production factors on collagen characteristics as well as overall muscle toughness of horse meat.

References

Blanco, M., Jurie, C., Micol, D., Agabriel, J., Picard, B. and Garcia-Launay, F. (2013). Impact of animal management factors on collagen characteristics in beef: a meta-analysis approach. Animal, 7: 1208-1218.

Boccard, R. L., Naude, R. T., Cronje, D. E., Smith, M. C., Venter, H. J. and Rossouw, E. J. (1979). The influence of age, sex and breed of cattle on their muscle characteristics. Meat Science, 3: 261-280.

Cross, H. R., Schanbacher, B. D., & Crouse, J. D. (1984). Sex, age and breed related changes in bovine testosterone and intramuscular collagen. Meat Science, 10, 187-195.

Hill, F. (1966). The solubility of intramuscular collagen in meat animals of various ages. Journal of Food Science, 31, 161-166. https://doi.org/10.1111/j.1365-2621.1966.tb00472.x

Horgan, D. J., Jones, P. N., King, N. L., Kurth, L. B., & Kuypers, R. (1991). The relationship between animal age and the thermal stability and cross-link content of collagen from five goat muscles. Meat Science, 29, 251–262.

Juárez, M., Polvillo, O., Gómez, M. D., Alcalde, M. J., Romero, F., and Valera, M. (2009). Breed effect on carcass and meat quality of foals slaughtered at 24 months of age. Meat Science, 83: 224-228.

Lorenzo, J. M., Pateiro, M., and Franco, D. (2013). Influence of muscle type on physicochemical and sensory properties of foal meat. Meat Science, 94: 77-83.

Palka, K. (2003). The influence of post-mortem ageing and roasting on the microstructure, texture and collagen solubility of bovine *semitendinosus* muscle. Meat Science, 64: 191-198.

Perez-Chabela, M. L., Guerrero, I., Gutierrez-Ruiz, M. C., and Betancourt-Rule, J. M. (2005). Effect of calcium chloride margination and collagen content on beef, horse, rabbit and hen meat hardness. Journal of Muscle Foods, 16: 141-154.

Roy, B. C., Sedgewick, G., Aalhus, J. L., Basarab, J. A., Bruce, H. L. (2015). Modification of mature non -reducible collagen cross link -link concentrations in bovine m. gluteus medius and semitendinosus with steer age at slaughter, breed cross, and growth promotants. Meat Science, 110:109-117.

Sarriés, M. V., and Beriain, M. J. (2006). Colour and texture characteristics in meat of male and female foals. Meat Science, 74: 738-745.

Seong, P. N., Kang, G. H., Cho, S. H., Park, B. Y., Park, N. G., Kim, J. H., and Ba, H. V. (2019). Comparative study of nutritional composition and color traits of meats obtained from the horses and Korean native black pigs raised in Jeju Island. Asian-Australasian Journal of Animal Science, 32: 249-256.

Starkey, C. P., Geesink, G. H., Oddy, H. V., and Hopkins, D. L. (2015). Explaining the variation in lamb longissimus shear force across and within ageing periods using protein degradation, sarcomere length and collagen characteristics. Meat Science, 105: 32-37.

Tateo, A., De Palo, P., Ceci, E. and Centoducati, P. (2008). Physicochemical properties of meat of Italian Heavy Draft horses slaughtered at the age of eleven months. Journal of Animal Science, 86:1205-1214.

Bibliography

Aalhus, J. L., Best, D. R., Murray, A. C and Jones, S. M. D. (1998). A comparison of the quality characteristics of pale, soft and exudative beef and pork. Journal of Muscle Foods, 9: 267-280.

Albrecht, E., Schering, L., Liu, Y., Komolka, K., Kühn, C., Wimmers, K. and Maak, S. (2017). Factors influencing bovine intramuscular adipose tissue development and cellularity. Journal of Animal Science, 95: 2244-2254.

Albrecht, E., Teuscher, F., Ender, K. and Wegner, J. (2006). Growth and breed-related changes of marbling characteristics in cattle. Journal of Animal Science, 84:1067-1075.

Andersen, H. J., Oksbierg, N., Young, J. F. and Therkildsen, M. (2005). Feeding and meat quality a future approach: A review. Meat Science, 70: 543-554.

Ashmore, C. R. and Doerr, L. (1971). Comparative aspects of muscle fiber types in different species. Experimental Neurology, 31: 408-418.

Astruc, T. (2014). Muscle fiber types and meat quality. Editors: Michael Dikeman, Carrick Devine. Encyclopedia of meat sciences (Second edition). Academic press, pp 442-448.

Avilés, C., Martinez, A. L., Domenech, V. and Pena, F. (2015). Effect of feeding system and breed on growth performance, and carcass and meat quality traits in two continental beef breeds. Meat Science, 107: 94-103.

Badiani, A. and Manfredini, M. (1994). La produzione della carne di caballo. Zootecnicae Nutrizione Animale, 20: 5-43.

Badiani, A., Nanni, N., Gatta, P. P., Tolomelli, B. and Manfredini, M. (1997). Nutrient profile of horse meat. Journal of Food Composition and Analysis.10: 254-269.

Bailey, A. J. (1972). The basis of meat texture. Journal of Food and Agriculture, 23: 995-1007.

Bailey, A. J. and Sims, T. J. (1977). Meat tenderness: distribution of molecular species of collagen in bovine muscle. Journal of the Science of Food and Agriculture, 28: 565-570.

Bailey, A. J., and Light, N. D. (1989). Connective tissue in meat and meat products. London: Elsevier Applied Science

Bailey, A. J., and Shimokomaki, M. S. (1972). Age related changes in the reducible cross-links of collagen. FEBS Letters, 16: 86-88.

Bailey, A. J., and Sims, T. J. (1976). Chemistry of the collagen cross-links. Biochemical Journal, 153: 211-215.

Behnke, J. R., Fennema, O., and Cassens, R. G. (1973). Rates of postmortem metabolism in frozen animal tissues. Journal of Agricultural and Food Chemistry, 21: 5-11.

Bekhit A. E. D. A., Hopkins, D. L., Fahri, F. T. and Ponnampalam, E. N. (2013). Oxidative processes in muscle systems and fresh meat: Sources, markers, and remedies. Comprehensive Reviews of Food Science and Food Safety, 12: 565-597.

Bekhit, A. E. D. and Faustman, C. (2005). Meat Myoglobin reducing activity. Meat science, 71: 407-439.

Beldarrain, L. R., Etaio, I., Moran, L., Sentandreu, M. A. (2020). Effect of ageing time on consumer preference and sensory description of foal meat. Food Research International, 129:108871.

Bendall, J. R. (1951). The shortening of rabbit muscles during rigor mortis: its relation to the breakdown of adenosine triphosphate and creatine phosphate and to muscular contraction. Journal of Physiology, 114: 71-88.

Bendall, J. R. and Voyle, C. A. (1967). A study of the histological changes in the growing muscles of beef animals. Journal of Food Technology, 2: 259-283.

Bendall, J. R., and Swatland, H. J. (1988). A review of the relationships of pH with physical aspects of pork quality. Meat Science 24: 85-126.

Berge, P., Saudo, C., Sanchez, A., Alfonso, M., Stamataris, C. and Thorkelsson, C., et al. (2003). Comparison of muscle composition and meat quality traits in diverse commercial lamb types. Journal of Muscle Foods, 14: 281-300.

Bergman, I., and Loxley, R. (1963). Two improved and simplified methods for the spectrophotometric determination of hydroxyproline. Analytical Chemistry, 35: 1961-196

Blanco, M., Jurie, C., Micol, D., Agabriel, J., Picard, B. and Garcia-Launay, F. (2013). Impact of animal management factors on collagen characteristics in beef: a meta-analysis approach. Animal, 7: 1208-1218.

Boccard, R. L., Naude, R. T., Cronje, D. E., Smith, M. C., Venter, H. J. and Rossouw, E. J. (1979). The influence of age, sex and breed of cattle on their muscle characteristics. Meat Science, 3: 261-280.

Boleman, S. J., Boleman, S. L., Miller R. K., Taylor, J. F., Cross, H. R., Wheeler, T. L., Koohmaraie, M., Shackelford, S. D., Miller, M. F., West, R. L., Johnson, D. D. and Savell, J. W. (1997). Consumer evaluation of beef of known categories of tenderness. Journal of Animal Science, 75: 1521-1524.

Bosselmann, A., Möller, C., Steinhart, H., Kirchgessner, M. and Schwarz, F. Z. (1995). Pyridinoline cross-links in bovine muscle collagen. Journal of Food Science, 60: 953-958.

Bouton, P. E., Carrol, F. D., Fisher, A., Harris, P. V. and Shorthose, W. R. (1973). Effect of altering ultimate pH on bovine muscle tenderness. Journal of Food Science, 38: 816-820.

Bouton, P. E., Harris, P. V. and Shorthose, W. R. (1972). The effect of ultimate pH on bovine muscle: Mechanical properties. Journal of food Science, 37: 356-360.

Bouton, P. E., Harris, P. V., Shorthose, W. R. and Ratcliff, D. (1974). Changes in the mechanical properties of veal muscles produced by myofibrillar contraction state, cooking temperature and cooking time. Journal of Food Science, 39: 869-875.

Bowling, R. A., Dutson, T. R., Smith, G. C. and Savell, J. W. (1987). Effects of cryogenic chilling on beef carcass grade, shrinkage and palatability characteristics. Meat Science, 21: 67-72.

Brooks, J. C., Savell, J. W. (2004). Perimysium thickness as an indicator of beef tenderness. Meat Science, 67: 329-334.

Brown, J. H., Pilliner, S. and Davies, Z. (2003). Horse and stable management (4th edn.). Oxford: Blackwell Publishing.

Buchter, L. (1972). The influence of rate of chilling on development of rigor and cold shortening. In Proc. Meat Research Institute. Symposium no. 2, Langford, Bristol, U.K. pp. 4-51.

Buege, D. R., Marsh and B. B. (1975). Mitochondrial calcium and post-mortem muscle shortening. Biochemical and Biophysical Research Communications, 65: 478-482.

Canto, A. C. V. C. S., Costa-Lima, B. R. C., Suman, S. P., Monteiro, M. L.G., Viana, F. M., and Salim, A. P. A. A. (2016). Color Attributes and oxidative stability longissimus lumborum psoas major muscles from Nellore bulls. Meat Science, 121: 19-26.

Carlson, K. B., Prusa, K. J., Fedler, C. A., Steadham, E. M., Outhouse, A. C., King, D. A., Lonergan, S. M. (2017). Postmortem protein degradation is a key contributor to fresh pork loin tenderness123. Journal of Animal Science, 95: 1574–1586.

Carpenter, C. E., Cornforth, D. P., Whittier, D. (2001). Consumer preference for beef color packaging did not affect eating satisfaction. Meat Sciene, 57: 359-363.

Chambaz A., Scheeder, M. R. L., Kreuzer, M. and Dufey, P. A. (2003). Meat quality of Angus, Simmental, Charolais and Limousin steers compared at the same intramuscular fat content. Meat Science, 63**:** 491-500.

Chizzolini, R., Ledward, D. A., Lawrie, R. A. (1977). Note on the effect of ageing on the neutral salt and acid soluble collagen from the intramuscular connective tissue of various species. Meat Science, 1: 111-117.

Choi, Y. M., Lee, S. H., Choe, J. H., Rhee, M. S., Lee, S. K., Joo, S. T. and Kim, B. C. (2010). Protein solubility is related to myosin isoforms, muscle fiber types, meat quality traits, and postmortem protein changes in porcine longissimus dorsi muscle. Livestock Science, 127: 183-191.

Christensen, L., Gunvig, A., Tørngren, M. A., Aaslyng, M. D., Knøchel, S., and Christensen, M. (2012). Sensory characteristics of meat cooked for prolonged times at low temperature. Meat Science, 90: 485-489.

Christensen, S. and Purslow, P. P. (2016). The role of matrix metalloproteinases in muscle and adipose tissue development and meat quality: A review. Meat Science, 119: 138-146.

Colle, M. J., Richard, R. P., Killinger, K. M., Bohlscheid, J. C., Gray, A. R., Loucks, W. I., Day, R. N., Cochran, A. S., Nasados, J. A., Doumit, M. E. (2015). Influence of extended aging on

beef quality characteristics and sensory perception of steaks from the gluteus medius and longissimus lumborum. Meat Science, 110: 32-39.

Cook, C. F. and Langsworth, R. F. (1966). The effect of pre-slaughter environmental temperature and post-mortem treatment upon some characteristics of ovine muscle. I. Shortening and pH. Journal of Food Science, 31: 497-503.

Cooke, D. W. I., Monahan, F. J., Brophy, P. O. and Boland, M. P. (2004). Comparison of concentrates or concentrates plus for-ages in a total mixed ration or discrete ingredient format: Effects on beef production parameters and on beef composition, colour, texture and fatty acid profile. Irish Journal of Agricultural and Food Research, 43: 201-216.

Corbin, C. H., O'Quinn, T. G., Garmyn, A. J., Legako, J. F., Hunt, M. R., Dinh, T. T. N., Rathmann, R. J., Brooks, J. C., Miller, M. F. (2015). Sensory evaluation of tender beef strip loin steaks of varying marbling levels and quality treatments. Meat Science, 100: 24-31.

Cornforth D and Hunt M. (2008). Low-oxygen packaging of fresh meat with carbon monoxide: meat quality, microbiology and safety. In *AMSA White Paper Series: No. 2*, pp 1-12. Savoy, IL: American Meat Science Association

Cornforth, D. P., Pearson, A. M. and Merkel, R. A. (1980). Relationship of mitochondria and sarcoplasmic reticulum to cold shortening. Meat Science, 4: 103-121.

Cross, H. R., Carpenter, Z. L., Smith, G. C. (1973). Effects of intramuscular collagen and elastin on bovine muscle tenderness. Journal of Food Science, 38: 998-1003.

Cross, H. R., Schanbacher, B. D., & Crouse, J. D. (1984). Sex, age and breed related changes in bovine testosterone and intramuscular collagen. *Meat Science, 10,* 187-195.

Cross, H. R., West, R. L., and Dutson, T. R. (1981). Comparison of methods for measuring sarcomere length in beef semitendinosus muscle. Meat Science, 5, 261-266.

Davey, C. L. and Gilbert, K. V. (1973). The effect of carcass posture on cold, heat and thaw shortening in lamb. Journal of Food Technology, 8: 445-451.

De Palo, P., Maggiolino, A., Centoducati, P. and Tateo, A. (2012). Color changes in meat Foals as affected by slaughtering age and post-thawing time. Asian Australian journal of Animal Science, 25: 1775-1779.

Descalzo, A. M., Rossetti, L., Sancho, A. M., García, P. T., Biolatto, A., Carduza, F. and Grigioni, G. M. (2008). Antioxidant consumption and development of oxidation during ageing of buffalo meat produced in Argentina. Meat Science, 79: 582-588.

Devine, C. E., Wahlgren, N. M., Tornberg, E. (1999). Effect of rigor temperature on muscle shortening tenderization of restrained and unrestrained beef *m. longissimus thoracicus et lumborum*. Meat Science, 51: 61-72.

Díaz, M. T., Susana, V., Cañeque, V., Lauzurica, S., Ruiz de Huidobro, F., Pérez, C., González, J., and Manzanares, C. (2002). Use of concentrate or pasture for fattening lambs and its effect on carcass and meat quality. Small Ruminant Research, 43: 257-268.

Domínguez R, Crecente S, Borrajo P, Agregán R and Lorenzo JM (2015). Effect of slaughter age on foal carcass traits and meat quality. Animal, 9: 1713-1720.

Dransfield, E. (1977). Intramuscular composition and texture of beef muscles. Journal of the Science of Food and Agriculture, 28: 833-842.

Dufey, P. A. (1996). Sensory and physico-chemical properties of meat from horse of different age groups. Proceeding of 42nd International congress of Meat Science and Technology (pp. 556-557).

Edwards, E. H. (1998). La enciclopedia del caballo (1st ed.). Barcelona (Spain): Blume.

Eikelenboom, G., and F. J. M. Smulders. (1987). Early and accelerated processing: Meat quality aspects. In: A. Romita, C. Valin, and A. A. Taylor (Ed.) Accelerated Processing of Meat. pp 161-169. Elsevier Applied Science, London.

Emerson, M. R., Woerner, D. R., Belk, K. E. and Tatum, J. D. (2013). Effectiveness of USDA instrument-based marbling measurements for categorizing beef carcasses according to differences in longissimus muscle sensory attributes. Journal of Animal Science, 91: 1024-1034.

Ertbjerg, P. and Puolanne, E. (2017). Muscle structure, sarcomere length and influences on meat quality: A review, Meat Science, 132: 139-152.

Ertbjerg, P., Henckel, P., Karlsson, A. H., Larsen, L. M. and Moller, A. J. (1999). Combined effect of epinephrine and exercise on calpain/calpastatin and cathepsin B and L activity in porcine longissimus muscle. Journal of Animal Science, 77: 2428-2436.

Etherington, D. J., and Sims, T. J. (1981). Detection and estimation of collagen. Journal of the Science of Food and Agriculture, 32: 539-546.

Eyer, D. R. and Wu, J. J. (2005). Collagen cross-links. Topics in Current Chemistry, 247: 207-229.

Eyre, D. (1987). Collagen cross-linking amino acids. Methods in Enzymology, 144: 115-139.

Eyre, D. R., Apon, S., Wu, J. J., Ericsson, L. H. and Walsh, K. A. (1987). Collagen type-IX-Evidence for covalent linkages to type-II collagen in cartilage. FEBS Lett, 220: 337-341.

Eyre, D. R., Paz, M. A. and Gallop, P. M. (1984). Cross-Linking in collagen and elastin. Annual Review of Biochemistry, 53: 717-748.

Fang, S. H., Nishimura, T. and Takahashi, K. (1999). Relationship between development of intramuscular connective tissue and toughness of pork during growth of pigs. Journal of Animal Science, 77: 120-130.

FAO (2015). Food and Agriculture Organization of the United Nations. Faostat (http:// faostat3.fao.org/home/index.html#DOWNLOAD (2015/04/29)).

FAO (2018). Food and Agriculture Organization of the United Nations. Faostat (http:// faostat3.fao.org/home/index.html#DOWNLOAD (2018/04/29)).

Farouk, M., Mustafa, N. M., Wu, G. and Krsinic. G. (2012). The “sponge effect” hypothesis: An alternative explanation of the improvement in the water holding capacity of meat with ageing. Meat Science, 90: 670-677.

Faustman, C. and Suman, S. P. (2017). The eating quality of meat: I-Color. In Woodhead Publishing Series in Food Science, Technology and Nutrition, Lawrie´s Meat Science (Eighth Edition), pp. 329-356 (Chapter 11).

Faustman, C., Sun, Q., Mancini, R., and Suman S. P. (2010). Myoglobin and lipid oxidation interactions: Mechanistic bases and control. Meat Science, 86: 86–94.

Fennema, O. R. (1985). Water and ice. In O. R. Fennema (Ed.), Food chemistry. New York: Marcel Dekker Inc.

Ferguson, D. M., Bruce, H. L., Thompson, J. M., Egan, A. F., Perry, D. and Shorthose, W. R. (2001). Factor affecting beef palatability: Farmgate to chilled carcass. Australian Journal of Experimental agriculture, 41: 879-891.

Fernández de Labastida, I. (2011). Caballos de monte y carne de potro. (PhD Thesis) Análisis antropológico de un proceso contemporáneo de construcción identitaria, cultural y económica en la montaña Alavesa. Spain: University of the Basque Country.

Feuz, D. M., Umberger W. J., Calkins C. R. and Si B. (2004). U.S. Consumers willing to pay for flavor and tenderness in steaks as determined with experimental auction. Journal of Agricultural Resource Economics, 29: 501-516.

Fischer, C., Honikel, K. O. and Hamm, R. (1980). Über das Auftreten der Kälteverkürzung "cold shortening" in dunkler und heller Muskulatur bei Rind und Schwein (cold shortening in the dark and light musculature of cattle and pigs). Fleischwirtschaft, 60: 263-265.

Franco, D., and Lorenzo, J. M. (2014). Effect of muscle and intensity of finishing diet on meat quality of foals slaughtered at 15 months. Meat Science, 96: 327-334.

Franco, D., Crecente, S., Vazquez, J. N., Gomez, M., Lorenzo, J. M. (2013). Effect of cross breeding and amount of finish diet on growth parameters, carcass and meat composition of foal slaughtered 15 months of age. Meat Science, 93: 547-556.

Franco, D., Rodríguez, E., Purriños, L., Crecente, S., Bermúdez, R., Lorenzo, J. M. (2011). Meat quality of "Galician Mountain" foals breed. Effect of sex, slaughter age and livestock production system. Meat Science, 88: 292-298.

Fujimoto, D. and Moriguchi, T. (1978). Pyridinoline, a non-reducible crosslink of collagen. Journal of Biochemistry, 83: 863-867.

Gagaoua, M., Picard, B. and Monteils, V. (2018). Associations among animal, carcass, muscle characteristics, and fresh meat color traits in Charolais cattle. Meat Science, 40: 145-156.

Gagaoua, M., Terlouw, E. M. C., Micol, D., Hocquette, J. F., Moloney, A. P., Nuernberg, K. and Picard, B. (2016). Sensory quality of meat from eight different types of cattle in relation with their biochemical characteristics. Journal of Integrative Agriculture, 15: 1550-1563.

Gann, G. L., and Merkel, R. A. (1978). Ultra structural changes in bovine longissimus muscle during post mortem ageing. Meat Science 2: 129-144.

Gariepy, C., Seoane, J. R., Cloteau, C., Martin, J. F. and Roy, G. L. (1999). The use of double-muscled cattle breeds in terminal crosses: Meat quality. Canadian Journal of Animal Science, 79: 301-308.

Gentry, J. G., McGlone, J. J., Miller, M. F. and Blanton, J. R, (2004). Environmental effects on pig performance, meat quality, and muscle characteristics. Journal of Animal Science, 82: 209-217.

Gill, C. O. (2005). Safety and storage stability of horse meat for human consumption. Meat Science, 71: 506-513.

Gill, C. O. Landers, C. (2005). Microbiological condition of horse meat prepared at a North American packing plant, and control of the temperatures of product air freighted to Europe. Meat Science, 69: 501-507.

Girard, I., Bruce, H. L., Basarab, J. A., Larsen, I. L. and Aalhus, J. L. (2012). Contribution of myofibrillar and connective tissue components to the Warner-Bratzler shear force of cooked beef. Meat Science, 92: 775-782.

Glitsh, K. (2000). Consumer perceptions of fresh meat quality: Cross-national comparison. British Food Journal. 120: 177-194.

Goll, D. E., Boehm, M. L., Geesink, G. H., and Thompson, V. F. (1997). What causes tenderization? InProceedings of the 50th annual reciprocal meat conference of the American Meat Science Association (pp. 60–67).

Goll. D. E., Hoekstra. W. G. and Bray, R. W. (1962). Changes in the physicochemical properties of bovine connective tissue with chronological age. Journal of Animal Science 21: 981-982.

Gómez, M., Lorenzo, J. M. (2012). Effect of packaging conditions on shelf-life of fresh foal meat. Meat Science, 91: 513-520.

Grunert, K. G., Bredahl, L. and Brunsø, K. (2004). Consumer perception of meat quality and implications for product development in the meat sectorda review. Meat Science, 66: 259-272.

Guignot, F., Touraille, C., Ouali, A., Renerre, M., and Monin, G. (1994). Relationships between post-mortem pH changes and some traits of sensory quality in veal. Meat Science, 37: 315-325.

Guil-Guerrero, J. L., Tikhonov, A., Rodríguez-García, I., Protopopov, A., Grigoriev, S., Ramos-Bueno, R. P. (2013). The fat from frozen mammals reveals sources of essential fatty acids suitable for Palaeolithic and Neolithic humans. PLoS ONE, 9: 84480.

Hamilton, D. N., Miller, K. D., Ellis, M., McKeith, F. K. and Wilson, E. R. (2003). Relationships between longissimus glycolytic potential and swine growth performance, carcass traits, and pork quality. Journal of Animal Science, 81: 2206-2212.

Herring, H. K., Cassens, R. G. and Briskey, E. J. (1967). Factors affecting collagen solubility in bovine muscle. Journal of Food Science, 32: 534-538.

Herring, H. K., Cassens, R. G., Suess, C. G., Brungardt, V. H., Briskey, E. J. (1967). Tenderness and associated characteristics of stretched and contracted bovine muscles. Journal of Food Science, 32: 317-323.

Hill, F. (1966). The solubility of intramuscular collagen in meat animals of various ages. Journal of Food Science. 31: 161-166.

Hintz C. S., Coyle, E. F., Kaiser, K. K, Chi M. M. Y. and Lowry, O.H. (1984). Comparison of muscle fiber typing by quantitative enzyme assays and by myosin ATPase staining. Journal of Histochemistry and Cytochemistry, 32: 655-660.

Hintz, H. F. (1995). Thoughts about the history of horses. Journal of Equine Veterinary Science, 15: 336-339.

Hocquette, J. F., Cassar-Malek, I., Jurie, C., Bauchart, D., Picard, B., and Renand, G. (2012). Relationships between muscle growth potential, intramuscular fat content and different indicators of muscle fibre types in young Charolais bulls. Animal Science Journal, 83: 750-758.

Holdstock, J., Aalhus, J. L., Uttaro, B. A., López-Campos, Ó., Larsen, I. L., and Bruce, H. L. (2014) The impact of ultimate pH on muscle characteristics and sensory attributes of the longissimus thoracis within the dark cutting (Canada B4) beef carcass grade. Meat Science, 98: 842-849.

Honikel, K. O. (2014). Conversion of muscle to meat. In Carrick, D. E, Micheal, D. (Eds.). Encyclopedia of meat sciences, pp. 353-357. (2nd ed.). Oxford: Academic Press.

Honikel, K., Kim, C., and Hamm, R. (1986). Sarcomere shortening of prerigor muscles and its influence on drip loss. Meat Science, 16: 267-282.

Hopkins, D. L. (2004). Tenderizing mechanisms - mechanical. In J. Werner Klinth (Ed.). Encyclopedia of Meat Sciences (pp. 1355–1363). Oxford: Elsevier.

Hopkins, D. L. and Geesink G. H. (2009). Protein degradation postmortem and tenderization. Applied Muscle Biology and Meat Science, 149-173.

Horgan, D. J., King N. L., Kurth, L. B. and Kuypers, R. (1990). Collagen cross links and their relationship to the thermal properties of calf tendons. Archives of Biochemistry and Biophysics, 28: 21-26.

Horgan, D. J., Jones, P. N., King, N. L., Kurth, L. B., & Kuypers, R. (1991). The relationship between animal age and the thermal stability and cross-link content of collagen from five goat muscles. *Meat Science, 29*, 251–262.

Huff-Lonergan, E. and Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Science, 71: 194-204.

Huff-Lonergan, E., Zhang, W., Lonergan, S. M. (2010). Biochemistry of post-mortem muscle-lessons on mechanisms of meat tenderization. Meat Science, 86: 184-195.

Huffman, K. L., Miller, M. F., Hoover, L. C., Wu, C. K., Britting, H. C. and Ramsey, C. B. (1996). Effect of beef tenderness on consumer satisfaction with steaks consumed in the home and restaurant. Journal of Animal Science, 74: 91-97.

Hughes, J. M., Oiseth, S. K., Purslow, P. P. and Warner, R. D. (2014). A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. Meat Science, 98: 520-532.

Hunsley, R. E., Vetter, R. L. and Kline, E. A., Burroughs, W. (1971). Effects of age and sex on quality, tenderness and collagen content of bovine Longissimus muscle. Journal of Animal Science, 33: 933-938.

Immonen, K., Ruusunen, M., Hissa, K., and Puolanne, E. (2000). Bovine muscle glycogen concentration in relation to finishing diet, slaughter and ultimate pH. Meat Science, 55: 25-31.

Jeleníková, J., Pipek, P., and Staruch, L. (2008). The influence of ante-mortem treatment on relationship between pH and tenderness of beef. Meat Science, 80: 870-874.

Jeong, J. Y., Hur, S. J., Yang, H. S., Moon, S. H., Hwang, Y. H., Park, G. B. and Joo, S. T. (2009). Discoloration characteristics of 3 major muscles from cattle during cold storage. Journal of Food Science, 74: 1- 5.

Jeremiah, L. E., Dugan, M. E. R., Aalhus, J. L. and Gibson, L. L. (2003). Assessment of the relationship between chemical components and palatability of major beef muscles and muscle groups. Meat Science, 65: 1013-1019.

Joo, S. T., Kim, G. D., Hwang, Y. H. and Ryu, Y. C. (2013). Review: Control of fresh meat quality through manipulation of muscle fiber characteristics. Meat Science, 95: 828-836.

Joseph, P., Suman, S. P., Rentfrow, G., Li, S. and Beach C. M. (2012). Proteomics of muscle specific beef color stability. Journal of Agricultural and Food Chemistry, 60: 3196-3203.

Joseph, R. L. (1996). Very fast chilling of beef and tenderness a report from an EU concerted action. Meat Science, 43: 217-227.

Juárez, M., Polvillo, O., Gómez, M. D., Alcalde, M. J., Romero, F. and Valera, M. (2009). Breed effect on carcass and meat quality of foals slaughtered at 24 months of age. Meat Science, 83: 224-228.

Judge, M. D. and Aberle, E. D. (1982). Effects of chronological age and postmortem aging on thermal shrinkage temperature of bovine intramuscular collagen. Journal of Animal Science, 54: 68-71.

Karlsson, A. H., Klont, R. E. and Fernandez, X. (1999). Skeletal muscle fibers as factors for pork quality. Livestock Production Science, 60: 255-269.

Kemp, C. M., Sensky, P. L., Bardsley, R. G., Buttery, P. J., Parr, T. (2010). Tenderness - An enzymatic view. Meat Science, 84: 248-256.

Kemp, P. D. and Scott, J. E. (1988). Ehrlich chromogens probable cross-links in elastin and collagen. Biochemistry Journal. 252: 387-393.

Khan, A. W. and Cohen, D. C. (1977). Rapid estimation of muscle proteins in beef vegetable protein mixtures. Journal of Agricultural Food Chemistry, 25: 236-238.

Khan, A. W., Lentz, C. P. (1973). Influence of antemortem glycolysis and dephosphorilation on high energy phosphates on beef ageing and tenderness. Journal of Food Science, 38: 56-58.

Khan, M. A. (1976). Histochemical characteristics of vertebrate striated muscle: A review. Progress in Histochemistry and Cytochemistry, 8: 1- 48.

Kim, G. D., Jeong, J. Y., Hur, S. J., Yang, H. S., Jeon, J. T., Joo, S. T. (2010). The relationship between meat color (CIE L* and a*), myoglobin content and their influence on muscle fiber characteristics and pork quality, Korean Journal of Food Science and Animal Resource, 30: 626-633.

Kim, H. W., Kim, J. H., Seo, J. K., Setyabrata, D. and Kim, Y. H. B. (2018a). Effects of aging/freezing sequence and freezing rate on meat quality and oxidative stability of pork loins. Meat Science, 139: 162 -170.

Kim, Y. H. B, Ma, D., Setyabrata, D., Farouk, M. M., Lonergan, S. M., Huff-Lonergan, E. and Hunt, M. C. (2018b). Review: understanding postmortem biochemical processes and post-harvest ageing factors to develop novel smart-aging strategies. Meat Science, 144: 74-90.

Kim, Y. H. B., Frandsen, M. and Rosenvold, K. (2011). Effect of ageing prior to freezing on colour stability of ovine longissimus muscle. Meat Science, 88: 332-337.

Kim, Y. H. B., Stuart, A., Black, C. and Rosenvold, K. (2012). Effect of lamb age and retail packaging types on the quality of long-term chilled lamb loins. Meat Science, 90: 962-966.

Kim, Y. H. B., Warner, R. D., Rosenvold, K. (2014). Influence of high pre-rigor temperature and fast pH fall on muscle proteins and meat quality: a review. Animal Production Science, 54: 375-395.

Klont, R. E., Barnier, V. M. H, Dijk, A. V., Smulders, F. J. M., Hoving-Bolink, A. H., Hulsegge, B. and Eikelenboom, G. (2000). Effects of pH fall, time of deboning, aging period, and their interaction on veal quality characteristics. Journal of Animal Science. 78: 1845-1851.

Klont, R. E., Brocks, L. and Eikelenboom, G. (1998). Muscle fiber type and meat quality. Meat Science, 49: 219-229.

Knox, B. L., Van Laack, R. L., and Davidson, P. M., (2008). Relationships between ultimate pH and microbial, chemical, and physical characteristics of vacuum-packaged pork loins. Journal of Food Science, 73: 104-110.

Koohmaraie, M. (1996). Biochemical factors regulating the toughening and tenderization processes of meat. Meat Science, 43: 193-201.

Koohmaraie, M., and Geesink, G. H. (2006). Contribution of post-mortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. Meat Science, 74: 34-43.

Koohmaraie, M., Kent, M. P., Steven, S. D., Veiseth, E. and Wheeler, T. L. (2002). Meat tenderness and muscle growth is there any relationship? Meat science, 62: 345-352.

Kopp, J., and Bonnet, M. (1982). Dureté de la Viande et Résistance au Cisaillement des Fibres de Collagène. Sciences des Aliments, 2: 127-132.

Kubelka, P., Munk, F. (1931). Ein Beitrag Zur Optik Der Farbanstriche. Zeitschrift für Technische Physik, 12: 593-601.

Lamprecht, G., (1997) Rapid method for the isolation of hydroxylysylpyridinoline and lysylpyridinoline from concentrated bone hydrolysates by liquid chromatographic techniques. Journal of Chromatograph B: Biomedical Science and Applications, 691: 297-304.

Lanari, M. C., Brewster, M., Yang, A. and Tume, R. K. (2002). Pasture and grain finishing affect the color stability of beef. Journal of Food Science, 67: 2467-2473.

Lanza, M., Landi, C., Scerra, M., Galofaro, V. and Pennisi, P. (2009). Meat quality and intramuscular fatty acid composition of Sanfratellano and Haflinger foals. Meat Science, 81: 142-147.

Lawrie R. A., Ledward D. A. (2006). Chapter 4-chemical and biochemical constitution of muscle. In: Lawrie R.A., Ledward D. A., editors. Lawrie's meat science. 7th ed. England, Cambridge: Woodhead Publishing. p 75-127.

Lawrie, R. (1950). Some observations on factors affecting myoglobin concentrations in muscle. The journal of Agricultural Science, 40: 356-366.

Lawrie, R. A. (1998). Meat Science, 6th Ed. Woodhead Publishing Cambridge, UK. Pp. 58-95.

Lawson, M. A. (2004). The role of integrin degradation in post-mortem drip loss in pork. Meat Science, 68: 559-566.

Lepetit, J. (2008). Collagen contribution to meat toughness: Theoretical aspects: A review. Meat Science, 80: 960-967.

Lepetit, J. and Culioli, J. (1994). Mechanical properties of meat. Meat Science, 36: 203-237.

Ley, T. 1996. Untersuchungen zu postmortalen Veränderungen in Pferdschlachttierkörpern. Fleischwirtschaft, 76: 172-175.

Li, P. Wang, T. Mao, Y., Zhang, Y., Niu, L., Liang, R., Zhu, L., and Luo, X. (2014). Effect of Ultimate pH on postmortem myofibrillar protein degradation and meat quality characteristics Chinese yellow crossbreed cattle. The Science World Journal. 1-8.

Li, X., Wei, X., Wang, H., Zhang, C. H., and Mehmood, W. (2018). Relationship between protein denaturation and Water holding capacity of pork during postmortem ageing. Food Biophysics, 13: 18-24.

Light, N., Champion, A. E., Voyle, C. and Bailey, A. J. (1985). The role of epimysial, perimysial and endomysial collagen in determining texture in six bovine muscles. Meat Science, 13: 137-149.

Lister D. (1989). Muscle metabolism and animal physiology in the dark cutting condition. In: Fabiansson S. U., Shorthose W. R., Warner R. D., editors. Proceedings of an Australian Workshop, AMLRDC. p 19-25.

Litwinczuk, A., Florek, M., Skalecki, P. and Litwinczuk, Z. (2008). Chemical composition and physicochemical properties of horse meat from the Longissimus Lumborum and Semitendinosus muscle. Journal of Muscle and Foods, 19: 223-236.

Liu, A., Nishimura, T., Takahashi, K. (1996). Relationship between structural properties of intramuscular connective tissue and toughness of various chicken skeletal muscles. Meat Science, 43: 43-49.

Liu, J., Arner, A., Puolanne, E. and Ertbjerg, P. (2016) On the water-holding of myofibrils: Effect of sarcoplasmic protein denaturation. Meat Science, 119: 32-40.

Locker, R. H. (1959). Striation patterns of ox muscle in rigor mortis. Journal of Biophysical and Biochemical Cytology, 6: 419-422.

Locker, R. H. (1960). Degree of muscular contraction as a factor in the tenderness of beef. Food Research, 25: 304-307.

Locker, R. H. (1985). Cold-induced toughness of meat. In A. M. Pearson, T. R. Dutson (Vol. Eds.), Electrical stimulation.Vol. 1. Advances in meat research (pp. 1-44). Westport, Connecticut: AVI Publishing Co.

Locker, R. H. and Hagyard, C. J. (1963). A cold shortening effect in beef muscles. Journal of Food Science and Agriculture, 14: 787-793.

Lonergan, E. H., Zhang, W. and Lonergan, S. M. (2010). Biochemistry of postmortem muscle-lessons on mechanisms of meat tenderization. Meat Science, 86: 184-195.

Lonergan, S. M., Huff-Lonergan, E., Wiegand, B. R., Kriese-Anderson, L. A. (2001). Post mortem proteolysis and tenderization of top loin steaks from Brangus cattle**.** Journal of Muscle Foods, 12: 121-136.

Lorenzo, J. M, Pateiro, M. and Franco, D. (2013). Influence of muscle type on physiochemical and sensory properties of foal meat. Meat Science, 94: 77-83.

Lorenzo, J. M., Fuciños, C., Purriños, L. and Franco, D. (2010). Intramuscular fatty acid composition of "Galician Mountain" foals breed: Effect of sex, slaughtered age and livestock production system. Meat Science, 86: 825-831.

Lorenzo, J. M., Sarriés, M. V., and Franco, D. (2013b). Sex effect on meat quality and carcass traits of foals slaughtered at 15 months of age. Animal, 7: 1199-1207.

Lorenzo, J. M., Sarriés, M. V., Tateo, A., Polidori, P., Franco, D. and Lanza, M. (2014). Carcass characteristics, meat quality and nutritional value of horsemeat: A review, Meat Science, 96: 1478-1488.

Lorenzo, J.M, Pateiro, M. and Franco, D. (2013a). Influence of muscle type on physiochemical and sensory properties of foal meat. Meat Science, 94: 77-83.

Lowry, C. V., Kimmey, J. S**.,** Felder, S., Chi, M. M. Y., Kaiser, K. K, Passonneau, P. N., Kirk, K. A. and Lowry, O. H. (1978). Enzyme pat- terns in single human muscle fibres. Journal of Biological Chemistry, 253: 8269-8277.

Lyford, C., Thompson, J. M., Polkinghorne, R., Miller, M., Nishimura, T., Neath, K., Allen, P. and Belasco, E. (2010). Is willingness to pay (WTP) for beef quality grade affected by consumer demographics and meat consumption habits. Australian Agribusiness Review, 18: 1-17.

Ma, R., Qiu, S., Jiang, Q., Sun, H., Xue, T., Cai, G., et al. (2017). AI-2 quorum sensing negatively regulates rbf expression and biofilm formation in *Staphylococcus aureus*. International Journal of Medical Microbiology, 307: 257-267.

Mach, N., Bach, A., Velarde, A., and Devant, M. (2008). Association between animal, transportation, slaughterhouse practices, and meat pH in beef. Meat Science, 78: 232-238.

Madhavi D. L. and Carpenter, C. (1993). Aging and processing affect color, metmyoglobin reductase and oxygen consumption of beef muscles. Journal of Food Science, 58: 939 - 942.

Mahmood, S., Dixon, W.T., Bruce, H. L. (2019). Cattle production practices and incidence of dark cutting beef. Meat Science, 157: 107873.

Mahmood, S., Roy, B. C., Larsen, I. L., Aalhus, J. L., Dixon, W. T. and Bruce, H. L. (2017). Understanding the quality of typical and atypical dark cutting beef from heifers and steers. Meat Science, 133: 75-85.

Malva, A. D., Palo, P. D., Lorenz, J. M., Maggiolino, A., Albenzio, M. and Marino, R. (2019). Application of proteomic to investigate the post-mortem tenderization rate of different horse muscles. Meat Science, 157: 107885.

Mancini, R. A. and Hunt, M. C. (2005). Review: Current research in meat color. Meat Science, 71: 100-121.

Marsh, B. B. (1954). Rigor mortis in beef. Journal of the Science of Food and Agriculture, 5: 70-75.

Marsh, B. B. and Leet, N. G. (1966). Studies in meat tenderness. III. The effect of cold shortening on tenderness. Journal of Food Science, 31: 450-459.

Marsh, B. B. and Thompson, J. F., (1958). Rigor mortis and thaw rigor in lamb. Journal of the Science of Food and Agriculture, 9: 417-424.

Matarneh, S. K., England, E. M., Scheffler, T. L., Gerrard D. E. (2017). The conversion of Muscle to meat. Lawrie's Meat Science. http://dx.doi.org/10.1016/B978-0-08-100694-8.00005-4: 159-185.

McCormick R. J. (1994). The flexibility of the collagen compartment of muscle. Meat Science 36: 79-91.

McCormick, R. J. (1999). Extracellular modifications to muscle collagen: implications for meat quality. Poultry Science, 78: 785-791.

McCormick, R. J. (2009). Collagen. Applied Muscle Biology and Meat Science. CRC press, Taylor and Francis group, USA. Pp.129-148.

McGilchrist, P., Alston, C. L., Gardner, G. E., Thomson, K. L. and Pethick, D. W. (2012). Beef carcasses with larger eye muscle areas, lower ossification scores and improved nutrition have a lower incidence of dark cutting. Meat Science, 92: 474-480.

McMillin K. W. (2008). Where is MAP going? A review and future potential of modified atmosphere packaging for meat. Meat Science, 80: 43-65.

Melody, J. L., Lonergan, S. M., Rowe, L. J., Huiatt, T. W., Mayes, M. S. and Huff-Lonergan, E. (2004). Early postmortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles. Journal of Animal Science, 82: 1195-1205.

Mennecke, B. E., Townsend, A. M., Hayes, D. J. and Lonergan, S. M. (2007). A study of the factors that influence consumer attitudes toward beef products using the conjoint market analysis tool. Journal of Animal Science, 85: 2639-2659.

Mikołajczak, B., Iwańska, E., Spychaj, A., Danyluk, B., Montowska, M., Grześ, B., Banach, J. K., Żywica, R., Pospiech, E. (2019) An analysis of the influence of various tenderising treatments on the tenderness of meat from Polish Holstein-Friesian bulls and the course of changes in collagen. Meat Science, 158: 107906.

Miller, M. F., Carr, M. A., Ramsey, C. B., Crockett, K. L. and Hoover, L. C. (2001). Consumer thresholds for establishing the value of beef tenderness. Journal of Animal Science 79: 3062-3068.

Mortimer, S. I., van Derwerf, J. H. J., Jacob, R. H., Hopkins, D. L., Pannier, L., Pearce, K. L. and Pethick, D. W. (2014). Genetic parameters for meat quality traits of Australian lamb meat. Meat Science, 96: 1016-1024.

Neethling, N. E., Suman, S. P., Sigge, G. O., Hoffman, L. C. and Hunt, M. C. (2017). Exogenous and endogenous factors influencing color of fresh meat from ungulates. Meat and Muscle Biology, 1: 253-275.

Neuman, R. E. and Logan, M. A. (1950). The determination of hydroxyproline. Journal of Biological Chemistry, 184: 299-306.

Ngapo, T. M., Berge, P., Culioli, J. and Smet, S. De (2002). Perimysial collagen crosslinking in Belgian blue double muscle cattle. Food Chemistry, 77: 15-26.

Nishimura, T., A. Hattori, and. Takahashi, K. (1999). Structural changes in intramuscular connective tissue during the fattening of Japanese Black cattle: Effect of marbling on beef tenderization. Journal of Animal Science, 77: 93-104.

Nishiumi, T., Kunishima, R., Nishimura, T. and Yoshida, S. (1995). Intramuscular connective tissue components contributing to raw meat toughness in various porcine muscles. Animal Science and. Technology, 66: 341-348.

O'Neill, D. J., Lynch, P. B., Troy, D. J., Buckley, D. J. and Kerry, J. P. (2003). Influence of the time of year on the incidence of PSE and DFD in Irish pig meat. Meat Science, 64: 105-111.

Offer G and Knight P. (1988). The structural basis of water-holding in meat. Part 2: Drip losses. In: Developments in meat science. Vol 4. New York: Elsevier Science Publishers. p 173-243.

Offer, G. (1991). Modelling of the formation of pale, soft and exudative meat: effects of chilling regime and rate and extent of glycolysis. Meat Science, 30: 157-184.

Offer, G. and Cousins, T. (1992). The mechanism of drip production: Formation of two compartments of extracellular space in muscle post-mortem. Journal of the Science of Food and Agriculture, 58: 107-116.

Offer, G. and Knight, P. (1988). The structural basis of water-holding capacity in meat. Part 2: drip losses. In R. Lawrie (Ed.). Developments in meat science, 4: 173-243. London: Elsevier Science Publications.

Oillic, S., Lemoine, E., Gros, J. B., Kondioyan, A. (2011). Kinetic analysis of cooking losses from beef and other animal muscles heated in a water bath -Effect of sample dimensions and prior freezing and ageing. Meat Science, 88: 338-346.

Ouali, A. (1990). Meat Tenderization: Possible causes and Mechanism: A review, Journal of Muscle Foods, 1: 129-165.

Ouali, A., Gagaoua, M., Boudida, Y., Becila, S., Boudjellal, A., Herrera-Mendez, C. H. and Sentandreu, M. A. (2013). Biomarkers of meat tenderness: Present knowledge and perspectives inregards to our current understanding of the mechanisms involved. Meat Science, 95: 854-870.

Page, J. K., Wulf, D. M. and Schwotzer, T. R. (2001). A survey of beef muscle color and pH. Journal of Animal Science, 79: 678-687.

Palka, K. (2003). The influence of post-mortem ageing and roasting on the microstructure, texture and collagen solubility of bovine *semitendinosus* muscle. Meat Science 64: 191-198.

Palka, K., and Daun, H. (1999). Changes in texture, cooking losses, and myofibrillar structure of bovine M-semitendinosus during heating. Meat Science, 51: 237-243.

Papuc, C., Goran, G. V., Predescu, C. N. and Nicorescu, V. (2017). Mechanisms of oxidative processes in Meat and Toxicity induced by postprandial degradation of products: A review. Comprehensive Reviews in Food Science and Food Safety, 16: 96-123.

Pearce, K. L., Rosenvold, K., Andersen, H. J. and Hopkins, D. L. (2011). Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes- A review. Meat Science, 89: 111-124.

Peplow, E. (1998). Encyclopedia of the horse (1st ed.). London: Hamlyn.

Perez-Chabela, M. L., Guerrero, I., Gutierrez-Ruiz, M. C., and Betancourt-Rule, J. M. (2005). Effect of calcium chloride margination and collagen content on beef, horse, rabbit and hen meat hardness. Journal of Muscle Foods, 16: 141-154.

Perlo, F., Bonato, P., Teira, G., Tisocco, O., Vicentin, J., Pueyo, J. and Mansilla, A. (2008). Meat quality of lambs produced in the Mesopotamia region of Argentina finished on different diets. Meat Science, 79: 576-581.

Pethick, D. W., Rowe, J. B., Tudor, G. (1995). Glycogen metabolism and meat quality. Recent Advances in Animal Nutrition in Australia, 13: 97-103.

Pierson, C. J, Fox, J. D. (1976). Effect of postmortem aging time and temperature on pH, tenderness and soluble collagen fractions in bovine longissimus muscle. Journal of Animal Science, 43: 1206-1210.

Pietrasik, Z., Dhanda, J. S., Shand, P. J. and Pegg, R. B. (2006). Influence of injection, packaging, and storage conditions on the quality of beef and bison steaks. Journal of Food Science, 71: 110-118.

Pikul, J., Wójtowski, J., Danków, R., Kuczyńska, B. and Łojek, J. (2008). Fat content and fatty acids profile of colostrum and milk of primitive Konik horses (Equus caballus gmelini Ant.) during six months of lactation. Journal of Dairy Research, 75: 302-309.

Ponnampalam, E. N., Butler, K. L., Burnett, V. F., McDonagh, M. B., Joe, L., Jacobs J. L. and Hopkins D. L. (2013). Aged vacuum packaged lamb cuts are less brown than fresh muscle cuts under simulated retail display. Food and Nutrition Science, 4: 147-153.

Price, J. F. and Schweigert, B. S. (1994). Ciencia de la carne y de los productos cárnicos. Zaragoza: Editorial Acribia.

Pulford, D. J., Fraga Vazquez, S., Frost, D. F., Fraser-Smith, E., Dobbie, P. and Rosenvold, K. (2008). The intracellular distribution of small heat shock proteins in post-mortem beef is determined by ultimate pH. Meat Science, 79: 623-630.

Puolanne, E. and Ruusunen, M. (1988). Reducing the incidence of dark-cutting beef in Finland – A systematic approach. In S. U. Fabiansson, W. R. Shorthose, R. D. Warner (Eds.), Proceedings of an Australian workshop. Sydney: AMLRDC. (pp. 32-37).

Purslow, P. P. (1999). The intramuscular connective tissue matrix and cell matrix interactions in relation to meat toughness. In: Proceedings of the 45th international congress of meat science and technology, Yokohama, Japan, pp. 210-219.

Purslow, P. P. (2018). Review: Contribution of collagen and connective tissue to cooked meat toughness; some paradigms reviewed. Meat Science, 144: 127-134.

Purslow, P. P., and Trotter, J. A. (1994). The morphology and mechanical properties of endomysium in series-fibred muscles: Variations with muscle length. Journal of Muscle Research and Cell Motility, 15: 299-308.

Purslow, P. P., Warner, R. D., Clarke, F. M., Hughes, J. M. (2020). Variations in meat colour due to factors other than myoglobin chemistry; a synthesis of recent findings (invited review). Meat Science, 159: 107941.

Qu, D., Zhou, X., Yang, F., Tian, S. Zhang, X., Ma, L. and Han, J. (2017). Development of carcass model based on blood biochemical parameters as a diagnostic tool of PSE meat. Meat Science, 128: 24-29.

Rees, M. P., Trout, G. R., and Warner, R. D. (2003). The influence of the rate of pH decline on the rate of ageing for pork. II: Interaction with chilling temperature. Meat Science, 65: 805-818.

Renand, G., Picard, B., Touraille, C., Berge, P. and Lepetit, J. (2001). Relationships between muscle characteristics and meat quality traits of young Charolais bulls. Meat Science 59: 49-60.

Robbins, K., Jensen, J., Ryan, K. J., Homco-Ryan, C., McKeith, F. K., Brewer, M. S. (2007). Enhancement effects on sensory and retail display characteristics of beef rounds. Journal of Muscle Foods, 13: 279-288.

Robins, S. P., Duncan, A., Wilson, N. and Evans, B. J. (1996). Standardization of pyridinium crosslinks, pyridinoline and deoxypyridinoline, for use as biochemical markers of collagen degradation. Clinical Chemistry, 42: 1621-1626.

Rosser, B. W. C., Norris, B. J. and Nemeth, P. M. (1992). Metabolic capacity of individual fibers from different anatomic locations. Journal of Histochemistry and Cytochemistry, 40: 819-825.

Roth, D. M., Brewer, M. S., Bechtel, P. J., Kline, K. H., & Mckeith, F. K. (1995). Sensory, color and composition characteristics of young and mature chevaline. Journal of Muscle Food, 6: 83-89

Rowe, R. W. D. (1977). The influence of the collagen fibre network of muscle on the compliance and tensile strength of muscle samples subjected to loads at right angles to the muscle fibre axis. Meat Science, 1: 135-148.

Roy, B. C., Sedgewick, G., Aalhus, J. L., Basarab, J. A. and Bruce, H. L. (2015) Modification of mature non-reducible collagen cross-link concentrations in bovine m. gluteus medius and semitendinosus with steer age at slaughter, breed cross and growth promotants. Meat Science, 110: 109-117.

Roy, B. C., Walker, B., Rahman, M. M., Bruce, H. L. and McMullen, L. (2018). Role of myofibers, perimysium and adipocytes in horse meat toughness. Meat Science, 146: 109-121.

Rucklidge, G. J., Milne, G., Mcgaw, B. A., Milne, E. and Robins, S. P. (1992). Turnover rates of different collagen types measured by isotope ratio mass-spectrometry. Biochimca et Biophysca Acta, 1156: 57-61.

Sakura, S., Fujimoto, D., Sakamoto, K., Mizuno, A., and Motegi, K. (1982). Photolysis of pyridinoline, a cross-linking amino acid of collagen, by ultraviolet light. Canadian Journal of Biochemistry, 60: 525-529.

Sarriés, M. V. and Beriain, M. J. (2005). Carcass characteristics and meat quality of male and female foals. Meat Science, 70: 141-152.

Sarriés, M. V. and Beriain, M. J. (2006). Colour and texture characteristics in meat of male and female foals, Meat Science, 74: 738-745.

Savage, A. W. J., Warriss, P. D. and Jolley, P. D. (1990). The amount and composition of the proteins in drip from stored pig meat. Meat Science, 27: 289-303.

Savell, J. W. and Shackelford, S. D. (1992). Significance of tenderness to the meat industry. In Proceedings of 45th annual reciprocal meat conference, Colorado, USA (pp. 43-46).

Scanga, J. A., Belk, K. E., Tatum, J. D., Grandin, T. and Smith, G. C. (1998). Factors contributing to the incidence of dark cutting beef. Journal of Animal Science, 76: 2040-2047.

Schilling, M. W., Mink, L. E., Gochenour, P. S., Marriott, N. G. and Alvarado, C. Z. (2003). Utilization of pork collagen for functionality improvement of boneless cured ham manufactured from pale, soft, and exudative pork. Meat Science, 65: 547-553.

Schneider, B. A., Tatum, J. D., Engle, T. E. and Bryant, T. C. (2007). Effects of heifer finishing implants on beef carcass traits and longissimus tenderness. Journal of Animal Science, 85: 2019-2030.

Segato, S., Cozzi, G., Andrighettto, I. (1999). Effect of animal morphotype, sex and age on quality of horse meat imported from Poland. In Recent Progress in Animal Production Science: Progress, XIII Congress (pp. 674-676). Piacenza.

Seideman, S. C., Cross, H. R., Smith, G. C. and Durland, P. R. (1984). Factor associated with fresh meat color: Review. Journal of Food Quality, 6: 211-237.

Seog, P., Park, K. M., Cho, S., Kang, G. H., Chae, H. S., Park, B. Y. and Ba, H. V. (2016). Effect of cut type and post-mortem ageing on the technological quality, textural profile and sensory characteristics of horse meat. Animal Production Science, 56: 1551-1559.

Seong, P. N., Kang, G. H., Cho, S. H., Park, B. Y., Park, N. G., Kim, J. H., and Ba, H. V. (2019). Comparative study of nutritional composition and color traits of meats obtained from the horses and Korean native black pigs raised in Jeju Island. Asian-Australasian Journal of Animal Science, 32: 249-256.

Shimokomaki, M., Elsden, D. F. and Bailey, A. J. (1972). Meat tenderness: Age related changes in bovine intramuscular collagen. Journal of Sood Science, 37: 892-896.

Shorthose, W. R. and Harris, P. V. (1990). Effect of animal age on the tenderness of selected beef muscles. Journal of Food Science, 55: 1-8.

Silva, J. A., Patarata, L., and Martins, C. (1999). Influence of ultimate pH on bovine meat tenderness during ageing. Meat Science, 52: 453-459.

Smith, G. C., Belk, K. E., Sofos, J. N., Tatum, J. D. and Williams, S. N. (2000). Economic implications of improved color stability in beef. In Decker E. A., Faustman, C., Lopez-Bote, C. J. (Eds.), Antioxidants in Muscle Foods: Nutritional Strategies to Improve Quality. New York: Wiley Inter science. pp. 397- 426.

Smith, S. H. and Judge, M. D. (1991). Relationship between pyridinoline concentration and thermal stability of bovine intramuscular collagen. Journal of Animal Science, 69: 1989-1993.

Sørheim, O. and Hildrum, K. I. (2002). Muscle stretching techniques for improving meat. Trends in Food Science and Technology, 13: 127-135.

Stanton, C. and Light, N. (1988). The effects of conditioning on meat collagen: part 2 - direct biochemical evidence for proteolytic damage in insoluble perimysial collagen after conditioning. Meat Science, 23: 179-199.

Stanton, C. and Light, N. (1990). The effects of conditioning on meat collagen: part 3 evidence for proteolytic damage to endomysial collagen after conditioning. Meat Science, 27**:** 41-54.

Starkey, C. P., Geesink, G. H., Collins, D., Oddy, V. H. and Hopkins, D. L. (2016). Do sarcomere length, collagen content, pH, intramuscular fat and desmin degradation explain variation in the tenderness of three ovine muscles? Meat Science, 113: 51-58.

Starkey, C. P., Geesink, G. H., Oddy, H. V., and Hopkins, D. L. (2015). Explaining the variation in lamb longissimus shear force across and within ageing periods using protein degradation, sarcomere length and collagen characteristics. Meat Science, 105: 32-37.

Starkey, C. P., Geesink, G. H., Ven, R. V. D. and Hopkins, D. L. (2017). The relationship between shear force, compression, collagen characteristics, desmin degradation and sarcomere length in lamb biceps femoris. Meat Science, 126: 18-21.

Steinhart, H., Bosselmann, A. and Möller, C. (1994). Determination of pyridinoline in bovine collagenous tissue. Journal of Agricultural Food Chemistry, 42: 1943-1947.

Stetzer, A. J. and McKeith, F. K. (2003). Benchmarking value in the pork supply chain: Quantitative strategies and opportunities to improve quality Phase 1. Savoy (IL): American Meat Science Association.

Strandine, E. J., Koonz, C. H. and Ramsbottom, J. M. (1949). A study of variations in muscle of beef and chicken. Journal of Animal Science, 8: 483-494.

Stromer, M. H., Goll, D. E. and Roth, L. E. (1967). Morphology of rigor-shortened bovine muscle and the effect of trypsin on pre- and post-rigor myofibrils. Journal of Cell Biology, 34: 431-445.

Suman, S. P. and Joseph, P. (2013). Myoglobin chemistry and meat color. Annual review Food Science and Technology, 4: 79-99.

Suman, S. P. and Joseph, P. (2014). Chemical and physical characteristics of meat: color and pigment. In: Dikeman, M., Devine, C. (Eds.), Encyclopedia of Meat Sciences, second ed., Elsevier, Oxford, United Kingdom, 3: 244-251.

Swatland, H. J. (2002). Optical dispersion through muscle fibers isolated from pork. Food Research International, 35: 559-564.

Swatland, H. J. (2004). Progress in understanding the paleness of meat with a low pH. South African Journal of Animal Science, 34: 1-7.

Swatland, H. J., Gullett, E., Hore, T., Buttenham, S. (1995). UV fiber-optic probe measurements of connective tissue in beef correlated with taste panel scores for chewiness. Food Research International, 28: 23-30.

Sylvestre, M. N., Balcerzak, D., Feidt, C., Baracos, V. E., Bellut, J. B. (2002). Elevated rate of collagen solubilization and post mortem degradation in muscle of lambs with high growth rates: possible relation ship with activity of matrix metalloproteinases. Journal of Animal Science, 80: 1871-1878.

Takahashi, K. (1996). Structural weakening of skeletal muscle tissue during post-mortem ageing of meat: the non-enzymatic mechanism of meat tenderization. Meat Science, 43: 67-80.

Tang, J, Faustman, C., Hoagland, T. A., Mancini, R. A., Seyfert, M., Hunt, M. C. (2005). Postmortem oxygen consumption by mitochondria and its effects on myoglobin form and stability. Journal of Agricultural and Food Chemistry, 53: 1223-1230.

Tang, J., Faustman, C., and Hoagland, T. A. (2004). Krzwicki revisited: equation for spectrophotometric determination of myoglobin redox form in aqueous meat extracts. Journal of Food Science, 69: 717-720.

Tarrant, P. V. (1989). Animal behaviour and environment in the dark cutting condition in beef-A review. Irish Journal of Food Science and Technology, 113: 1-21.

Tateo, A., De Palo, P., Ceci, E. and Centoducati, P. (2008). Physicochemical properties of meat of Italian Heavy Draft horses slaughtered at the age of eleven months. Journal of Animal Science, 86: 1205-1214.

Tateo, A., De Palo, P., Padalino, B. and Centoducati, P. (2005). Quality of carcasses in IHDH foals reared in the province of Bari (Italy). Italian Journal of Animal Science, 4: 418-420.

Taylor, R. G., Geesink, G. H., Thompson, V. F., Koohmaraie, M. and Goll, D. E. (1995). Is Z-disk degradation responsible for post-mortem tenderization? Journal of Animal Science., 21: 1351-1367.

Thompson, J. (2002). Managing meat tenderness. Meat Science, 62: 295-308.

Tornberg, E. (2005). Effects of heat on meat proteins - Implications on structure and quality of meat products. Meat Science, 70: 493-508.

USDA (2013). National nutrient database for standard reference, Release 20. http://fnic. nal.usda.gov/food-composition (consulted: 19/02/2013).

Van Laack, R. L. J. M., and F. J. M. Smulders. (1994). Postmortem glycolysis in veal carcasses; influence on meat quality. In: Proc. 40th Int. Congress of Meat Science and Technology., The Hague, Netherlands. Manuscript S-IVB-04.

Veiseth, E., Shackelford, S., Wheeler, T. and Koohmaraie, M. (2004). Factors regulating lamb longissimus tenderness are affected by age at slaughter. Meat Science, 68: 635-640.

Vestergaard, M., Therkildsen, M., Henckel, P., Jensen, L. R., Andersen, H. R. and Sejrsen, K. (2000). Influence of feeding intensity, grazing and finishing feeding on meat and eating

quality of young bulls and the relationship between muscle fibre characteristics, fibre fragmentation and meat tenderness. Meat Science, 54: 187-195.

Vitale, M., Pérez-Juan, M., Lloret, E., Arnau, J., Realini, C. E. (2014). Effect of aging time in vacuum on tenderness, and color and lipid stability of beef from mature cows during display in high oxygen atmosphere package**.** Meat Science, 96: 270-277.

Vognarova, J., Dvorak, Z. and Bohm, R. (1968). Collagen and elastine in different cuts of veal and beef. Journal of Food Science, 33: 339-343.

Voisenet, B. D., Grandin, T., Tatum, J. D., O'Connor, S. F., and Struthers, J. J. (1997). Feedlot cattle with calm temperaments have higher average daily gain than cattle with excitable temperaments. Journal of Animal Science, 75: 892-896.

Voyle, C. A. (1969). Some observations on the histology of cold-shortened muscle. Journal of Food Technology, 4: 275-281.

Wang, F., Zhang, Y., Li, J., Guo, X., Cui, B., and Peng, Z. (2016). Contribution of cross-links and proteoglycans in intramuscular connective tissue to shear force in bovine muscle with different marbling levels and maturities. LWT. Food science and Technology, 66: 413-419.

Warner, R., Jacob, R., Hocking Edwards, J., McDonagh, M., Pearce, K., Geesink, G. and Pethick, D. (2010,). Quality of lamb meat from the information nucleus flock. Animal Production Science, 50: 1123-1134.

Warriss, P. D, Brown, S, N., Nute, G. R., Knowles, T. G., Edwards, J. E., Perry, A. M., Johnson, S. P. (1995). Potential interactions between the effects of preslaughter stress and post-mortem electrical stimulation of the carcasses on meat quality in pigs. Meat Science, 41: 55-68.

Warriss, P. D. (1979). The extraction of haem pigments from fresh meat. Journal of food Technology, 14: 75-80

Warriss, P. D. (1990). The handling of cattle pre-slaughter and its effects on carcass and meat quality. Applied Animal Behaviour Science 28: 171-186.

Warriss, P. D. (2000). Meat Science: An introductory text. Journal of the South African Veterinary Association, 71: 247-248.

Weston, A. R., Rogers, R. W., Althen, T. G. (2002). Review: The role of collagen in meat tenderness. The Professional Animal Scientist, 18: 107-111.

Wheeler, T. L., Koohmaraie, M. (1994). Prerigor and postrigor changes in tenderness of ovine longissimus muscle. Journal of Animal Science, 72: 1232-1238.

Wheeler, T., Shackelford, S. and Koohmaraie, M. (2000). Variation in proteolysis, sarcomere length, collagen content, and tenderness among major pork muscles. Journal of Animal Science, 78: 958-965.

Whiting, R. C. (1980). Calcium uptake by bovine muscle mitochondria and sarcoplasmic reticulum. Journal of Food Science, 45: 288-292.

Wiklund, E., Dobbie, P., Stuart, A., and Littlejohn, R. P. (2010). Seasonal variation in red deer (Cervus elaphus) venison (M. longissimus dorsi) drip loss, calpain activity, colour and tenderness. Meat Science, 86: 720-727.

Wu, G., Farouka, M. M., Clerens, S. and Rosenvold, K. (2014). Effect of beef ultimate pH and large structure protein changes with ageing of meat tenderness, Meat science, 98: 637-645.

Xiong, Y. L. (2000). Protein oxidation and implications for muscle food quality. In Decker, E.A., Faustman, C. and Lopez-Bote, J. L. (Eds.) Antioxidants in muscle foods: Nutritional strategies to improve quality (pp. 85-90). New York: Wiley

Xiong, Y. L. (2014). Chemical and physical characteristics of meat-protein functionality. In: Dikeman M, Devine C, editors. Encyclopedia of meat sciences. 2nd ed. Oxford: Academic Press. p 267-73.

Yamauchi, M., London, R. E., Guenat, C., Hashimoto, F., and Mechanic, G. L. (1987). Structure and formation of stable histidine-based trifunctional cross-link in skin collagen. Journal of Biological Chemistry, 262: 11428-11434.

Young O. A., Zhang S. X., Farouk M. M., Podmore, C. (2005). Effects of pH adjustment with phosphates on attributes and functionalities of normal and high pH beef. Meat Science, 70: 133-139.

Young, O. A., and Braggins, T. J. (1993). Tenderness of ovine semimembranosus: Is collagen concentration or solubility the critical factor? Meat Science, 35: 213-222.

Young, O. A., Braggins, T. J., Barker, G. J. (1994). Pyridinoline in ovine intramuscular collagen. Meat Science, 37**:** 297-303.

Yu L. P., and Lee, Y. B. (1986). Effects of postmortem pH and temperature on bovine muscle structure and meat tenderness. Journal of Food Science, 51: 774-780.

Yu, T. Y., Morton, J. D., Clerens, S., Dyer, I. M. (2016). Cooking -Induced Protein Modifications in Meat. Comprehensive Review in Food Science and Food Safety, 16: 141-159.

Zhang, W. G., Lonergan, S. M., Gardner, M. A. and Huff-Lonergan, E. (2006). Contribution of postmortem changes of integrin, desmin and mu-calpain to variation in water holding capacity of pork. Meat Science, 74: 578-585.