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Coláiste na hOllscoile Corcaigh, Éire
University College Cork, Ireland

Composition and eating quality of beef from young male dairy cattle derived from different production systems assessed by standard and rapid spectroscopic methods

Thesis presented by
Yingqun Nian, BSc, MSc

To obtain the degree of
Doctor of Philosophy – PhD in Food Science and Technology

University Supervisor
Prof. Joseph P. Kerry
School of Food and Nutritional Sciences, University College Cork, Cork

Research Supervisor
Dr. Paul Allen
Department of Food Quality and Sensory Science, Teagasc Food Research Centre, Ashtown, Dublin 15, Dublin

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Declaration of authenticity

I declare that this thesis is my own work and effort, and that it has not been submitted for any other degree, neither at Univerisity College Cork, UCC, Ireland nor elsewhere. Where other sources of information have been used, they have been acknowledged.

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Signature

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Date

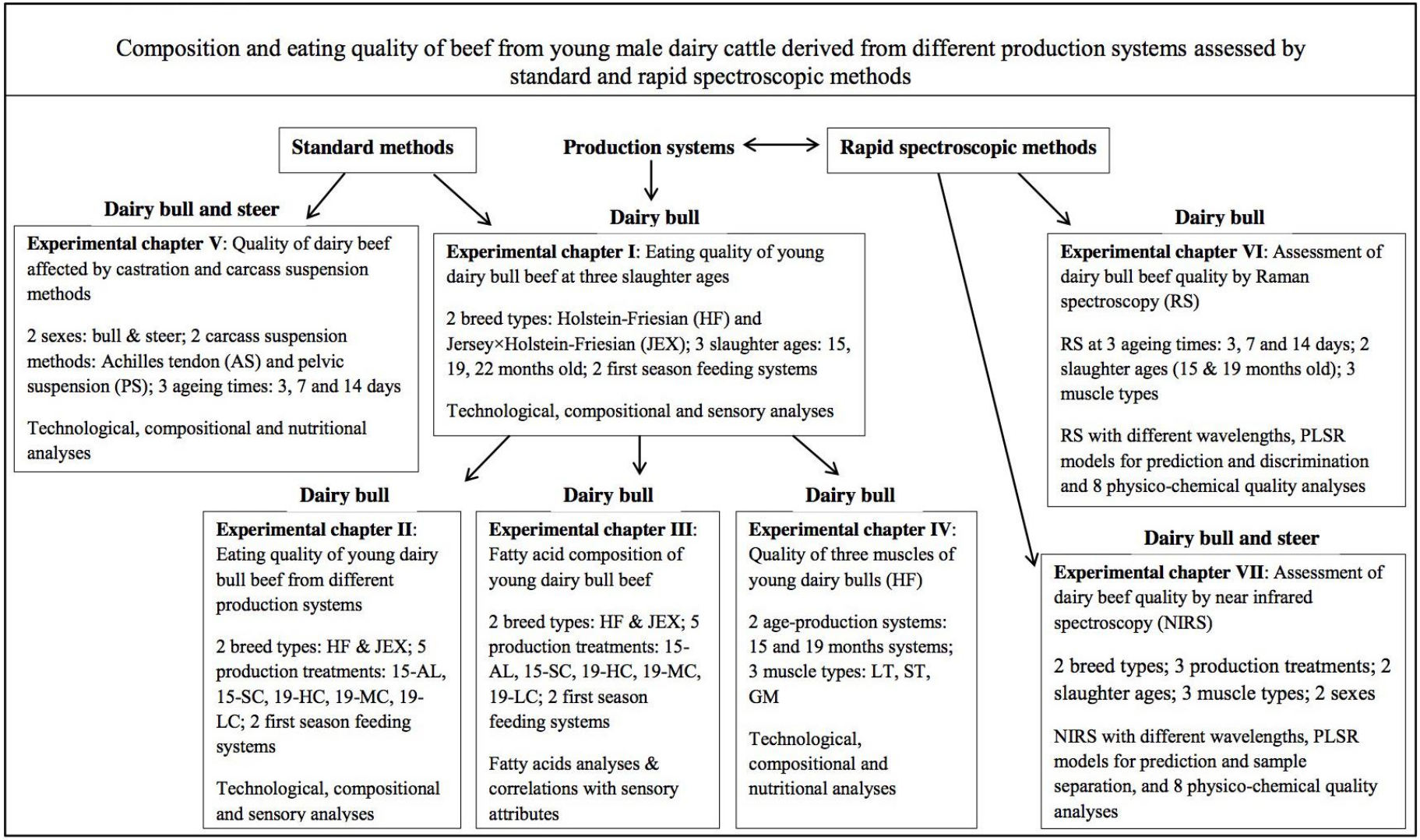
Abstract

The objective of this thesis was to assess eating quality characteristics of beef from young male Holstein-Friesian (HF) and Jersey×Holstein-Friesian (JEX) dairy cattle derived from different production systems. Dairy bulls were slaughtered at 15, 19 and 22 months-old, after finishing on diets differing in energy content. Such diets were also offered at pasture during first and second grazing seasons. *Longissimus thoracis* (LT), *Semitendinosus* (ST) and *Gluteus medius* (GM) muscles were assessed from experimental cattle in these studies, which were primarily bulls. Effects of carcass suspension method and ageing time on eating quality were also investigated. Standard eating quality variables assessed were post-mortem pH-temperature window, ultimate pH (pHu), colour, Warner-Bratzler variables (WBSF, WB-slope, WB-area), cooking loss, intramuscular fat (IMF), moisture and protein content, collagen characteristics, fatty acid (FA) composition and sensory attributes. Moreover, Raman spectroscopy (RS) and near infrared spectroscopy (NIRS) were explored as rapid methods for eating quality characteristics. Results showed that JEX breed type had superior eating quality to that of the HF breed type, expressed as less cooking loss, higher IMF content and higher beef flavour, juiciness and texture related scores as determined by sensory evaluation, while their FA profiles were similar. With increased slaughter age, bull beef held longer residual flavour length and had higher insoluble and total collagen contents, while collagen solubility reduced with age. Higher energy diets produced beef with relatively better eating quality, such as enhanced IMF content, beef flavour score and lower WB-variable values, cooking loss and moisture, but a less healthy FA profile due to increased PUFA proportion, PUFA/SFA ratio and decreased SFA proportion, n-6/n-3 PUFA ratio in beef offered a higher forage diet. However, first or second grazing season feeding systems had limited effects on meat quality traits. Quality of young dairy bull beef varied between muscles and the interaction between muscle and production system had significant effects on IMF content and FA profiles. Eating quality of LT muscle was considered superior owing to lower WB-variables, cooking loss and higher IMF content, but FA profile was inferior compared to GM and ST muscles. Castration had a marked effect on quality traits for young dairy cattle and consequently steer beef would likely have superior eating quality when compared to bull beef. When compared to Achilles tendon suspension (AS), pelvic suspension

(PS) increased redness and chroma after 24 h blooming; PS improved tenderness up to 7 days of ageing and accelerated ageing processes. Both RS (1300-2800 cm^{-1}) and NIRS (400-1900 nm) showed considerable potential in assessing a wide range of physical and chemical beef quality traits from young male dairy cattle, with RS demonstrating enhanced prediction performance. Consistent and significant correlations were also obtained among technological, compositional, sensorial and nutritional quality traits for dairy beef.

Keywords: Physico-chemical quality traits, Sensory evaluation, Dairy breeds, Young bulls, Beef, Production system, Tenderness, Eating quality, Fatty acids, Muscle type, Castration, Pelvic suspension, Raman spectroscopy, Near infrared spectroscopy

Work flow


Composition and eating quality of beef from young male dairy cattle derived from different production systems assessed by standard and rapid spectroscopic methods
Standard methods
Production systems
Rapid spectroscopic methods
Dairy bull and steer
Experimental chapter V: Quality of dairy beef affected by castration and carcass suspension methods
2 sexes: bull & steer; 2 carcass suspension methods: Achilles tendon (AS) and pelvic suspension (PS); 3 ageing times: 3, 7 and 14 days
Technological, compositional and nutritional analyses
Dairy bull
Experimental chapter I: Eating quality of young dairy bull beef at three slaughter ages
2 breed types: Holstein-Friesian (HF) and Jersey×Holstein-Friesian (JEX); 3 slaughter ages: 15, 19, 22 months old; 2 first season feeding systems
Technological, compositional and sensory analyses
Dairy bull
Experimental chapter II: Eating quality of young dairy bull beef from different production systems
2 breed types: HF & JEX; 5 production treatments: 15-AL, 15-SC, 19-HC, 19-MC, 19-LC; 2 first season feeding systems
Technological, compositional and sensory analyses
Dairy bull
Experimental chapter III: Fatty acid composition of young dairy bull beef
2 breed types: HF & JEX; 5 production treatments: 15-AL, 15-SC, 19-HC, 19-MC, 19-LC; 2 first season feeding systems
Fatty acids analyses & correlations with sensory attributes
Dairy bull
Experimental chapter IV: Quality of three muscles of young dairy bulls (HF)
2 age-production systems: 15 and 19 months systems; 3 muscle types: LT, ST, GM
Technological, compositional and nutritional analyses
Dairy bull
Experimental chapter VI: Assessment of dairy bull beef quality by Raman spectroscopy (RS)
RS at 3 ageing times: 3, 7 and 14 days; 2 slaughter ages (15 & 19 months old); 3 muscle types
RS with different wavelengths, PLSR models for prediction and discrimination and 8 physico-chemical quality analyses
Dairy bull and steer
Experimental chapter VII: Assessment of dairy beef quality by near infrared spectroscopy (NIRS)
2 breed types; 3 production treatments; 2 slaughter ages; 3 muscle types; 2 sexes
NIRS with different wavelengths, PLSR models for prediction and sample separation, and 8 physico-chemical quality analyses

Publications and presentations

Original Publications

1) **Yingqun Nian**, Joseph P. Kerry, Robert Prendiville, Paul Allen (2017). The eating quality of beef from young dairy bulls derived from two breed types at three ages from two different production systems. *Irish Journal of Agricultural and Food Research*, 56, 31-44.

2) **Yingqun Nian**, Ming Zhao, Colm P. O'Donnell, Gerard Downey, Joseph P. Kerry, Paul Allen (2017). Assessment of physico-chemical traits related to eating quality of young dairy bull beef at different ageing times using Raman spectroscopy and chemometrics. *Food Research International, 99,* 778-789.

3) **Yingqun Nian**, Paul Allen, Robert Prendiville, Joseph P. Kerry (2017). Physico-chemical and sensory characteristics of young dairy bull beef derived from two breed types across five production systems employing two first season feeding regimes. Accepted in *Journal of the Science of Food and Agriculture*.

4) **Yingqun Nian**, Paul Allen, Sabine M. Harrison, Robert Prendiville, Joseph P. Kerry (2017). Meat quality and fatty acid composition of three muscles from two age groupings of young Holstein-Friesian bulls. Revision submission to *Animal Production Science*.

5) **Yingqun Nian**, Paul Allen, Sabine M. Harrison, Joseph P. Kerry (2017). Effect of castration and carcass suspension method on the quality and fatty acid profile of beef from male dairy cattle. Submitted to *Journal of the Science of Food and Agriculture*.

6) **Yingqun Nian**, Paul Allen, Sabine M. Harrison, Nigel P. Brunton, Robert Prendiville, Joseph P. Kerry (2017). Fatty acid composition of young dairy bull beef as affected by breed type, production treatment and relationship to sensory characteristics. Submitted to *Animal Production Science*.

7) Cristina Botinestean, Carolina Gomez, **Yingqun Nian**, Mark A.E. Auty, Joseph P. Kerry, Ruth M. Hamill (2017). Possibilities to develop texture-modified beef steaks suitable for elderly consumers using fruit-derived proteolytic enzymes. Submitted to *Journal of Texture Studies*.

8) Ming Zhao, **Yingqun Nian**, Paul Allen, Gerard Downey, Joseph P. Kerry, Colm P. O'Donnell (2017). Application of Raman spectroscopy and multivariate data analysis to assess sensory characteristic of young dairy bull beef derived from two breed types at two ages. (In preparation for *Food Research International*)

Poster Presentations (Conference processings/Abstracts)

1) **Yingqun Nian**, Paul Allen, Robert Prendiville, Joseph P. Kerry (2013). Quality of beef from young dairy bulls from two breeds at three ages. Proceedings: 42th Annual Food Research Conference, Teagasc Food Research Centre, Ashtown, Dublin, Ireland.

2) A. Ferragina, **Y. Nian**, R. Hamill, A. Cecchinato, A. Cromie, G. Bittante, P. Allen (2013). Prediction of beef quality traits by Vis/NIRS: preliminary results of a TEAGASC Dublin–DAFNAE Padova joint research project fostered by a FAIM STSM. Proceedings: 2th Annual Conference on Carcass Evaluation, Meat Quality, Software and Traceability, Kaposvár, Hungary.

3) **Yingqun Nian**, Paul Allen, Robert Prendiville, Joseph P. Kerry (2014). Tenderness, cook loss and sensory characteristics of young Holstein-Friesian bull beef affected by feeding treatment. Proceedings: 43rd Annual Food Research Conference, University College Dublin, Dublin, Ireland.

4) **Yingqun Nian**, Sabine M. Harrison, Nigel P. Brunton, Joseph P. Kerry, Paul Allen (2015). Fatty acid composition of young dairy bull beef is affected by feeding

treatment. Proceedings: 44th Annual Food Research Conference, Teagasc Food Research Centre, Moorepark, Ireland.

5) **Yingqun Nian**, Paul Allen, Robert Prendiville, Joseph P. Kerry (2015). Eating quality of beef from young dairy bulls from two breeds at three ages from different production systems. Proceedings: 61st International Congress of Meat Science and Technology (ICoMST), Clermont-Ferrand, France.

6) **Yingqun Nian**, Joseph P. Kerry, Robert Prendiville, Paul Allen (2016). Quality of beef from young dairy bulls from three muscles and at two age-production systems. Proceedings: 18th World Congress of Food Science and Technology (IUFoST), Dublin, Ireland.

7) **Yingqun Nian**, Joseph P. Kerry, Robert Prendiville, Paul Allen (2016). Effect of castration and ageing time on the quality of beef from male dairy cattle. Proceedings: 62th International Congress of Meat Science and Technology (ICoMST), Bangkok, Thailand.

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Introduction

In Ireland, the livestock industry produces meat and milk products for some of the highest value and highest specification markets in the world. Meat accounts for over 40% of Ireland's gross agricultural output, dominated by beef which by far accounts for 21% of food and drink exports from Ireland. Ireland is the fifth largest beef exporter in the world and the largest exporter of beef in Europe (Enterprise Ireland, 2017). After the abolition of EU milk quotas in 2015, the dairy output volume is projected to increase by 50% by 2020 as outlined by the Food Harvest 2020 document (DAFF, 2010), which would have the effect of increasing the dairy herd markedly. As dairy cow numbers expand there will be more surplus (an extra 240,000) male dairy calves becoming available for either export or retaining to finish within the country (Murray, 2013). There are great opportunities if these dairy calves can be used for beef production as dairy beef has the advantage of being a by-product of the dairy industry and carries very little overheads compared with suckled calves who have to carry the full cost of their parents. Dairy calves for the same reason have a much lower carbon footprint as the dairy enterprise covers its parents' environmental impact (Murray, 2013).

From the meat eating quality aspects, there have been conflicting reports in the research literature on the quality of meat from dairy origin cattle. Previous research has demonstrated that *longissimus dorsi* (LD) samples from the dairy group were rated significantly more tender than that from the beef synthetic and heavily-muscled crosses both by sensory evaluation and Warner-Bratzler shear force (WBSF). In addition, the LD samples from this dairy group had a higher marbling content, but flavour and aroma scores are similar to those from all the other groups (Hawrysh & Berg, 1975). Similarly, Vatansever et al. (2000) found that beef from the Welsh Black breed was significantly tougher than Holstein-Friesian beef, but no differences in flavour were noted. However, Zeigler et al. (1971) reported that the flavour

intensity of beef from dairy-type animals was significantly lower than that of beef-type animals. Nuernberg et al. (2005) compared German Holsteins with German Simmentals and found that steaks of Holstein had a higher score for bitter taste, but overall liking was not significantly different. They also found that the colour of the lean of the dairy group was slightly darker. It has also been observed that meat from dairy-type bulls had a lower WBSF value and a higher rating for juiciness than that from Brahman-type bulls (Riley et al., 1986).

Due to higher volumes of milk production, HF is the most predomint Irish dairy breed, which accounts for approximately 56% of the dairy herd at the moment. There is currently an interest in crossbreeding Jersey with HF (JEX) as Jersey shows great potential for crossbreeding under Irish condition due to its higher intake capacity (Prendiville et al., 2011). The study conducted in Teagasc indicated that compared with either HF or Jersey pure breeds, JEX cows have a higher output of milk solids through improved milk composition and superior production efficiency as well as being more profitable (Prendiville et al., 2011). JEX progeny also possess better udder health through heterosis (Shalloo, 2007).

It is well known that bulls grow faster, have better feed conversion efficiency, higher live weight and carcass gains, higher lean meat yield and better conformation than steers (Sinclair et al., 1998; Kirkland et al., 2006), so bull beef production should be more profitable. Moreover, bulls have lower carbon emissions than steers and heifers (Dawson et al., 2010), thus bull beef production would contribute to sustainable farming and protecting the environment. Accordingly, if these male dairy calves were raised for bull beef rather than being exported as young calves it would be a significant new source of income for producers and it would improve the efficiency of processors by increasing throughput and opening up new export markets for Irish beef. However, bull beef is thought to be tougher and therefore less acceptable to consumers. There is conflicting evidence of this supposed inferior

eating quality of bull beef. Dransfield et al. (1984) found no differences in organoleptic qualities between bulls and steers and Frickh et al. (2003) reported that shear force did not differ between bulls, heifers and steers. However, some studies found that bull beef was tougher than steer beef (Purchas et al., 1993; Mach et al., 2009). Piasentier et al. (2004) found that bull beef tenderness improves with ageing at least up to 14 days. Sinclair et al. (1998) surprisingly found that age at slaughter had no significant effect on the tenderness of bull beef. However, none of this work includes the extreme dairy types.

With current market trends, issues surrounding age at slaughter, target carcass weight and discounts on bulls older than 16 months of age, finding the most profitable beef production systems for these dairy bulls is a challenge for the industry. Dairy calf-to-beef production systems are sensitive to the price of calves, concentrates and finished beef (Prendiville & Kelly, 2014). A key element of profitable dairy calf-to-beef systems is the efficient utilisation of grazed grass (Prendiville et al., 2013). Irish beef production systems are predominantly pasture-based due to the suitability of the temperate climate for growing grass, which allows farms to exploit the competitive advantages related to grass-based production systems compared with higher input systems. Grazed grass is the main source of feed for ruminants in Ireland and this is expected to increase over the next number of years because of its lower cost compared with concentrates and grass silage (O'Donovan et al., 2011). However, supplementing with concentrates is inevitable during the indoor winter period especially the finishing period to obtain more energy and sustain adequate growth rates of cattle. It is important for dairy calf to beef farmers to explore options for finishing male dairy calves and to determine the most efficient and practical production system for this type of young bull to prolong the grazing season, reduce feed costs and increase carcass output per hectare, thus to improve the profitability of the enterprise (Lawrence, 2014). Therefore, alternative finishing strategies have been investigated to reduce the concentrate input during the finishing phase, which will

greatly assist beef producers to identify the optimum production system best suited to their circumstances (Murphy & Prendiville, 2014). Moreover, the opportunity to have a second season at grass and with bulls slaughtered at 19 months of age is being investigated. Another study conducted in Teagasc indicated that the 19 month bull production system was the most profitable on a gross margin per hectare basis, mainly due to the higher output per hectare and heavy carcass weights. However, market requirements dictate that bulls are slaughtered at less than 16 months of age (Kelly et al., 2013). Eating quality aspects will continue to be the main factor influencing choice for most consumers of beef. Thus, the subsequent meat quality from bull production systems of 15 and 19 months of age with varying levels of concentrate supplementation at pasture are widely investigated in this thesis.

Meat palatability knowledge has been widely developed for the *longissimus*, whereas other major beef muscles are still not fully explored for some type of cattle, particularly for dairy breeds. Moreover, different muscles may have different response in eating quality relative to the changes in growth path, diet or production system because of their variation in structural and metabolical characteristics (Archile-Contreras et al., 2010), thus the links between production systems, muscle types and the eating quality of HF bull beef is determined in this thesis.

In addition to production factors, post-mortem processing methods have been identified having an important influence on the eating quality of beef (Thompson, 2002). Pelvic suspension (PS) is an alternative carcass hanging method to the traditional Achilles tendon (AS) method. PS aims to prevent muscle shortening and thereby contribute to the influence of myofibrillar structure on tenderness (Hostetler et al., 1970; Sørheim & Hildrum, 2002). In PS, carcasses enter rigor with the hind limb in a horizontal position, thus stretching the muscles of the outer hind limb. The vertebral column is straightened thereby stretching the loin muscle compared with the AS hanging method (Hostetler et al., 1970). Hence the stretched muscles have

longer sarcomeres, reduced overlapping between actin and myosin, smaller muscle fibre diameter, and lower WBSF compared to AS cattle (Herring et al., 1965). The effect of PS on beef quality has been mainly focused on different gender categories from beef breeds, therefore an objective of this thesis is to gain further knowledge on the effect of PS on eating quality of dairy male cattle specifically under pasture-based feeding system and to examine the effects of prolonged ageing on dairy beef from AS and PS carcasses.

It has been accepted that the terms used to infer beef quality consists of an expected (before purchase) and experienced (after consumption) quality dimension (Grunert et al., 2004). Expected eating quality is related to perceived visual appearance, i.e. colour and marbling, and some extrinsic cues i.e. origin, quality labels, brand, etc. Experienced eating quality is a combination of intrinsic cues of taste (flavour), texture and juiciness evaluations, which dominates consumers' satisfaction and purchase intentions for beef (Banovic et al., 2009). In addition to eating quality, the literature highlights the increasing human emphasis on health-related quality aspects, both expected and experienced aspects (Grunert et al., 2004). Red meats have been identified as good sources of high-quality protein and nutrients. However, they have also been related to certain illnesses such as cancer and heart disease, which may be associated with the undesirable fatty acid composition (Scollan et al., 2014). The knowledge from the present thesis provides valuable information on some expected and experienced eating qualities of the beef from young male dairy cattle and also is seeking to quantify the benefits associated with grass-based beef systems from a nutrition perspective and to determine the best way to use this information to produce beef with acceptability.

Several biophysical methods have been developed to assess meat quality traits including instrumental mechanical methods (i.e., WBSF), sensory evaluation, ultrasound, optical spectroscopy, microscopy (i.e., electron microscopy) and

magnetic resonance (i.e., NMR). These methods can either measure meat component properties directly, or through calculation and modelling indirectly to derive correlations between biophysical measurements and meat component properties. These calculations and modelling can help to improve the understanding of meat properties and hence of eating quality (Damez & Clerjon, 2008). Compared with other biophysical methods, optical spectroscopy has unique advantages such as being rapid, easy to use (no sample preparation), non-destructive, non-invasive, low cost and requiring only small sample portions. With these attributes it may well become one of the most powerful analytical techniques for the meat industry where rapid and non-invasive methods are required (Herrero, 2008). Accordingly, the most important optical spectroscopy methods, namely the application of near infrared spectroscopy (NIRS) and Raman spectroscopy (RS) for the assessment of eating quality of young dairy bull beef will be investigated in this thesis. Moreover, limited research has evaluated the application of RS for the assessment of sensory traits and chemical composition particularly for collagen characteristics of beef, thus this thesis will address this knowledge gap.

The concept of the 'pH-temperature window' is one of the initial specifications of the Meat Standards Australia (MSA) 'carcass pathways' grading scheme and is an important tool used to monitor beef eating quality, mainly for tenderness and water holding capacity (WHC) (Thompson, 2002). The 'pH-temperature window' concept can identify carcasses in danger of cold shortening (pH > 6 at temperature < 15 °C) or heat toughening (pH < 6 at temperature > 35 °C) (Pearson & Young, 1989). The slow pH fall that occurs in cold shortened carcasses results in extremely tough meat and rapid pH fall resulting in heat toughening can reduce ageing, increase drip loss and lead to unattractive retail appearance (Meat Standards Australia, 2016a). Glycolytic rate has been shown to vary widely between groups of carcasses from different production systems and slaughter ages or within groups of carcasses (Frylinck et al., 2013). Thus, in this thesis the optimal pH-temperature window of

young male dairy cattle with different production factors is determined and the individual carcasses which may be at risk of heat toughening or cold shortening is also being studied.

In summary, optimising the eating quality and related nutrition perspective of beef from young male dairy cattle through studying the effects of production systems and the optimised assessment methods will ensure there is a market for these bulls and will lead to new opportunities to raise these animals rather than exporting them live. This research will assist in devising a blueprint for Irish meat producers and processors to realise the full potential of this source of beef.

Objectives of the present study

In light of the knowledge deficits discussed in the foregoing introduction relating to various aspects of eating quality and with the aim of leading to new opportunities for producers to raise these young male dairy cattle, the principle objectives of the current study were to:

1. Evaluate the physico-chemical quality traits including post-mortem pH-temperature window, ultimate pH, colour, WBSF, cooking loss, proximate chemical composition (i.e. IMF, moisture, protein), collagen characteristics and sensory characteristics of young dairy bull beef and how this is affected by breed and slaughter age.

2. Determine the effect of production system on the physico-chemical quality traits and sensory characteristics of young dairy bull beef.

3. Investigate the fatty acid profile of young dairy bull beef and how this is affected by breed and production system and assess the relationship between fatty acid profile and sensorial properties.

4. Study the effect of muscle type and age-production system on the physico-chemical quality traits and fatty acid composition of young dairy bull beef.

5. Analyse the effect of castration, hanging method and ageing time on the physico-chemical quality traits and fatty acid composition of beef derived from young male dairy cattle.

6. Assess the physico-chemical traits related to eating quality of young dairy bull beef at different ageing times using Raman spectroscopy and multivariate data analysis.

7. Assess the beef quality traits of young male dairy cattle using near infrared spectroscopy.

8. Identify the correlation between physico-chemical quality traits, between sensory attributes, between physico-chemical quality traits and sensory attributes, between fatty acid composition and physico-chemical quality traits of beef derived from young male dairy cattle.

Chapter 1

Literature Review

1.1 The conversion of muscle to meat

1.1.1 Muscle structure

The musculature is categorized into two major types: non-striated and striated muscles, which are further categorized as either cardiac or skeletal. The meat that humans consume is recognized as skeletal muscle. Skeletal muscles are associated with the skeleton, and they either lie next to a bone or are attached through their connective tissue fascia to bones. Epimysium is the connective tissue layer surrounding the muscle. Muscle is composed by muscle fascicles (bundles of fibres) and perimysium (the main intramuscular connective tissue) which surrounds fascicles. Each fascicle bundle has a blood vessel and an axon of motor neuron located on the outer surface, with muscle fibres inside and an endomysium surrounding individual muscle fibres. Between the endomysium and the muscle fibre is a fine network of tubules, the sarcoplasmic reticulum, which is bounded by a very thin net-like membrane layer, the sarcolemma (Figure 1.1; Lawrie, 1991a).

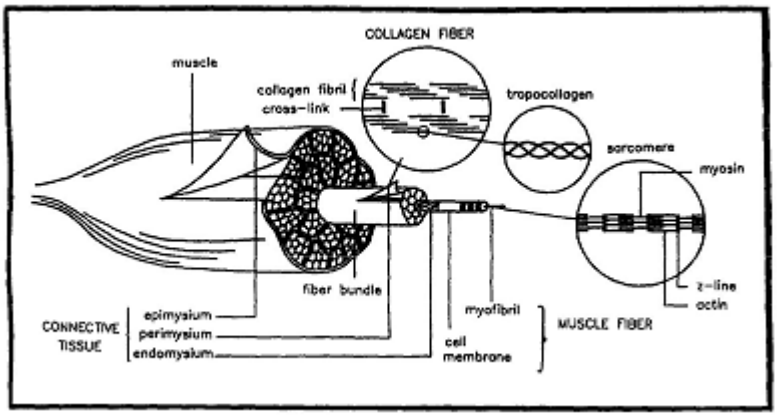


Figure 1.1. The structural hierarchy of a muscle. From Tornberg et al., 1990.

The muscle fibre is recognized as the basic unit of muscle tissue, and it is also known as the muscle cell. The sarcoplasm (cytoplasm) is a liquid structure, the intracellular substance in a muscle cell, and is composed of around 80% water with proteins, enzymes, lipids, carbohydrates, inorganic salts, and metabolic by-products. The muscle cell has the same general structure as a normal cell, consisting of a cell

membrane, cytoplasm, a nucleus, lysosomes, and mitochondria, etc. Mitochondria contain the enzymes responsible for respiration and oxidative phosphorylation, and the formation of elements of the sarcolemma and connective tissue proteins. The unique features of muscle cells are elongated myofibrils (occupy up to 90% of the volume of a cell). Myofibrils contain the major components of thick (myosin) and thin (actin) filaments. From the structure point of view, myofibrils consist of many sarcomeres. The Z-line is the vertical line separating sarcomeres. In the middle of a sarcomere, there is a bare area, called the H-Zone and the line in the middle of the H-Zone is the M-line. The thick filament area is the A-band and the remainder from the edge of the A-band to the Z-line is named the I-band. This structured hexagonal pattern of myosin and actin give the muscle fibre a striated appearance under the microscope (Lawrie, 1991a; Valin & Ouali, 1992).

1.1.2 Anaerobic glycolysis

Immediately after slaughter, the temperature of the muscles is high, however, the temperature decreases as the blood circulation has stopped and at this time no further oxygen enters the body so respiration ceases. In slaughtered muscle tissue, muscle glycogen is easily converted to glucose by the enzyme α-amylase. Then glucose is converted into pyruvate by oxidation with the help of glycolytic enzymes in the sarcoplasm of a cell under anaerobic circumstances. Furthermore, pyruvate is catalysed by the enzyme lactate dehydrogenase and is reduced to lactic acid under anaerobic conditions (Sharp, 1958).

The level of glycogen in pre-slaughter muscle tissue is around 7-11 g per kilogram and the lactic acid formed in post-mortem glycolysis is 38 molecules per molecule of glucose. Unlike the situation when an animal is still alive, the lactic acid formed cannot be transported back to the liver, so it accumulates within the muscle tissue. Thus the concentration of lactic acid steadily increases and the pH of muscle tissue declines by around 1.5-1.7 pH unit from 6.8-7.2 (in the living animals) down to 5.3-

5.4 (Feiner, 2006a).

In addition to lactic acid, a very small amount of ATP is also produced during post-mortem anaerobic glycolysis. Compared with 36 molecules of ATP obtained during aerobic glycolysis, only 3 molecules of ATP are formed from 1 molecule of glucose anaerobically. The energy obtained from this tiny ATP supports the visible fibre movement which can be observed on the carcass even though the animal is already dead. Anaerobic glycolysis generally occurs within the first 8 hours post-mortem, and pH does not decline much during rigor mortis (Feiner, 2006a).

1.1.3 Muscle contraction and rigor mortis

When an animal is alive, contraction and relaxation of muscle tissue can take place in an aerobic condition in the presence of oxygen through breathing. Immediately after exsanguination, the ATP level remains constant as it can be generated by the reaction: CP + ADP → Creatine +ATP, though the ATP generated is much less than with aerobic conditions (Lonergan et al., 2010). During this period, Ca^{2+} ions are at a very low level and no free energy is released from ATP, thus troponin does not allow the myosin head to attach with actin. Therefore, very few rigor bonds form and the muscle is still extensible, which is called the 'delay period' (Lonergan et al., 2010). A complex membranous network covering each myofibril, the sarcoplasm reticulum (SPR), has the function of maintaining a balance between Ca^{2+} and Mg^{2+} ions (release and reabsorb Ca^{2+}), and to control the levels of CP and ATP, as well as ATP-ase enzyme activity (Lawrie, 1991a).

During the first 4 hours post-slaughter, under the effect of nervous impulses, SPR starts to release large amount of Ca^{2+} ions (0.1 μM to 10 μM) obtained from the sarcoplasm, which leads to a rearrangement of the troponin and tropomyosin to interact with myosin. Ca^{2+} ions pumped from SPR also trigger the activation of the myosin-ATP-ase to improve the hydrolysis of ATP to ADP, phosphate and free

energy with the following reaction, ATP → ADP + Pi +energy (Berridge, 1986). Hence the chemical energy obtained from ATP is converted to mechanical energy to support the muscle protein movement, which contributes to a change in the configuration of the myosin head, making it bind with actin. At this moment, muscle contraction occurs, and myosin and actin are no longer presented as separate fibres, rather they are overlapped by the cross-bridge to form the actomyosin complex (Lonergan et al., 2010).

Subsequently, 4-8 h from slaughter, the level of ATP declines below 1 μmol per gram in the muscle tissue of cattle as anaerobic generation and supply of ATP ceases. At such low levels of ATP, there is loss of the ability of myosin to disassociate from actin and these two filaments bound firmly together by the established permanent 'cross-links' and are no longer able to separate (Feiner, 2006a). The same authors also show that during this phase, rigor bonds develop rapidly and muscle tissue shortening or extension is no longer possible. This is referred to as the rapid 'onset' period. This period starts when the ATP level begins to fall. This process is irreversible and makes muscle tough. Finally, when muscle ATP has been depleted, there is no further decline in extensibility and this is the 'completion' of rigor. In beef, it takes around 24-40 h to complete rigor mortis (Feiner, 2006a). When post-mortem rigor mortis completes, the pH value will have dropped to around 5.3 (Greaser, 2001).

1.1.4 pH/temperature window and cold-shortening

The pH/temperature window concept implemented in the Meat Standards Australia (MSA) grading scheme is to monitor or identify carcasses at risk of cold shortening with pH > 6 at T < 15 ˚C or heat toughening with pH < 6 at T > 35 ˚C. It describes the relationship between carcass pH and temperature from slaughter to when the ultimate pH is reached (Thompson, 2002; Meat Standards Australia, 2016a).

Cold shortening occurs when the muscle temperature is less than 15 ˚C while pH is still above 6.0. During this rigor mortis stage, the hot carcass cools too quickly, while the pH decline is too slow, remaining high while the temperature falls. The combination of high pH value and low temperature present at the same time results in damage to the SPR. The SPR loses its ability to balance the Ca^{2+} ions properly and is unable to reabsorb the Ca^{2+} ions (Locker, 1985). This situation leads to the permanent high concentration of released of Ca^{2+} ions, and is accompanied with energy obtained from ATP in an anaerobic way, leading to extreme contraction of the muscle. As a result, the meat is extremely tough and the solubility of proteins is greatly reduced as solubility depends on the numbers of cross-bridges within the muscle tissue. Light and lean carcasses may be expected to cool more rapidly and thus be more predisposed to cold shortening (Locker, 1985).

Heat shortening or rigor shortening occurs when the muscle pH is lower than 6.0 while the muscle temperature is between 35 ˚C and 40 ˚C. Contrary to cold shortening, the carcass is cooled too slowly while the pH decline is too quick resulting in the proteolytic enzyme activity becoming quickly exhausted within the muscle, thereby contributing to accelerate protein denaturation and results in the product that will not tenderize with ageing (Frylink et al., 2013). Although heat shortening will not make the meat as tough as cold-shortening, it still increases meat toughness and reduces water-holding capacity (WHC). Besides, heat toughened meat will be pale and sometimes it will be watery or PSE-like (pale, soft, exudative) (Thompson, 2002).

Therefore, the pH-temperature decline must fall through the ideal window (pH pass through 6.0 between 15 ˚C and 35 ˚C) to obtain meat of good eating quality, otherwise the eating quality will be severely compromised. Electrical stimulation is an effective intervention to accelerate the pH decline rate in order to avoid cold-shortening (Meat Standards Australia, 2016a). Chiller conditions and handling and

facilities of the abattoir also need to be considered to minimize the stress the animals experience to ensure there is sufficient muscle glycogen or energy reserved before slaughter. Even an adequate subcutaneous fat coverage can slow the chilling rate of muscle and avoid cold shortening. An increasing plane of nutrition for at least one month prior to slaughter is considered to be important to assist in even fat coverage (Meat Standards Australia, 2016a).

According to O'Halloran et al. (1997), the rate of post-mortem pH fall can play an important role in meat proteolysis and tenderness development, since post-mortem pH affects the activity of endogenous enzyme systems. Fast glycolysing *longissimus dorsi* (LD) muscles were found to be more tender in sensory analysis and texture assessment, however, slow glycolysing muscles were considered significantly tougher. Lonergan et al. (2010) have reported that beef with high and low values of 3 h postmortem pH may produce less acceptable tenderness than beef that has an intermediate 3 h postmortem pH. So a moderate pH decline rate may be beneficial to the muscle tenderness. One explanation for this phenomenon is that proteolytic enzymes in the skeletal muscle cell can be affected by pH.

1.1.5 Post-mortem proteolysis

During the post-slaughter period, beef experiences a tenderization process related to the breakdown of muscle fibres. This process predominantly results from the activity of enzymes to loosen muscle fibres and connective tissue. The variation of the rate and extent of proteolysis between muscles could be due to the difference in the enzyme content and enzyme/inhibitor ratio. The rate of post-mortem tenderization depends to a large extent on the efficiency of the endogenous peptidase systems (Sentandreu et al., 2002).

Calpain, a calcium-activated protease, is the enzyme naturally present in the sarcoplasm of meat and was believed to play a major role in the post-mortem

tenderization of meat. Members of the calpain family are classified into three different groups: ubiquitous calpains (including μ, m, and μ/m calpains), tissue specific calpains and atypical calpains (Sentandreu et al., 2002). Calpains can cut long myofibrils into smaller units by breaking down the Z-disk to disintegrate the sarcomere structure. Calpains are responsible for the specific degradation of numerous cytoskeletal proteins such as nebulin, titin, desmin and filamin. Calpastatin, as the endogenous inhibitor of the calpain system, can prevent calpains from binding to membranes and the subsequent protein degradation is also related to the rate of proteolysis (Lonergan et al., 2010).

Naturally occurring enzymes in meat, cathepsins, are cysteine proteases, which are found in the lysosomes of a muscle cell and are protected by a wall of fat, and they include 30 different enzymes. The lactic acid formation during rigor mortis destroys the walls of fat, resulting in the release of cathepsins. Cathepsins can slowly break down the bonds between myosin and actin and loosen the actomyosin complex during the maturing of meat (Feiner, 2006c).

Perimysial collagen undergoes damage caused by proteolytic attack and is partially solubilized during conditioning (Stanton & Light, 1987). Collagenases or Zn^{2+} metalloproteinases are able to breakdown the matrix components and cut the triple collagen helix (Woessner, 1991). After helix cleaving, collagen fragments are degraded in lysosomes by cathepsins, and the single α-helices are hydrolyzed into peptides and free amino acids such as hydroxyproline. Total free hydroxyproline in LD of bovine has been shown to increase from 3 to 14 days post-mortem (Feidt et al., 1996).In addition, myofibrils have also been shown to be degraded and M- and Z-lines are damaged significantly by proteasomes. The contribution of caspases and serine peptidases to muscle proteolysis should also be considered (Sentandreu et al., 2002).

However, it should be noted that proteolysis is a more complex process involving

more than one enzyme and the degradation of one or two substrate proteins. It is more likely that the combined action of multiple proteases and their target myofibrillar proteins contribute to the muscle ultrastructure change that result in beef tenderization during ageing (Lonergan & Lonergan, 2008).

1.2 Technological quality of bull beef

1.2.1 Ultimate pH

The pH is a measure of the hydrogen potential, and is based on the amount of H^+ ions present. The pH value decreases with the increase of the H^+ ion concentration. During rigor-mortis completion at around 24-40 h after slaughter, the pH of a carcass reduces from around 7.1 (live animal) to the level at which it will not fall any further, which is named the ultimate pH (pHu). The pHu has a significant effect on bull beef quality, including colour, texture, taste, shelf-life and microbiological stability of meat (Silva, 1999). The decrease in the pH of meat results from the lactic acid generated from post-mortem glycogen, hence the pHu is inversely related to the glycogen level in the muscle at slaughter.

Bulls have a greater incidence of high pH meat than steers caused by insufficient lactic acid being produced resulting from greater ante-mortem glycogen depletion. This is probably due to the more excitable temperament and the physical contests between bulls. More stress of animals immediately pre-slaughter could also induce high pH (Monin, 1990). A pHu between 5.5 and 5.8 is considered to be normal for beef, while between 5.8 and 6.2 is classified as moderate DFD (dark, firm and dry), and above 6.2 is defined as DFD by Silva (1999). DFD meat could result in a reduction in shelf-life with a coarse texture and a purple colour.

Compared with normal pH beef, high pH beef had lower L*, a*, b*, hue angle and saturation values, indicating a darker and less brown beef (Zhang et al., 2005). Yancey et al. (2005) described that steaks with higher pH exhibiting DFD

characteristics with less beef flavour, less brown-roasted flavour, and more rancid flavor than normal pH meat. Once the pHu value exceeds 6.4-6.5, sensorial spoilage takes place in meat because of the generation of high levels of ammonia (NH_3) and other metabolic by-products. At this point, slime and discolouration is observed commonly on the surface of meat (Feiner, 2006b).

There is a controversial relationship between beef pHu and tenderness. Some authors found a linear relationship (Bouton et al., 1973), whereas others found a curvilinear association between these two parameters (Jeremiah et al., 1991). The effect of pH on beef tenderness mainly results from their effect on the activity of proteases, which further affects the rate and extent of the post-mortem ageing process (Silva, 1999).

1.2.2 Colour

Colour is an important meat quality attribute affecting consumer acceptance, purchasing decisions and satisfaction and approximately 15% of retailed meats are discriminated against based on colour (Frylinck et al, 2013). A bright, cherry-red colour is mainly preferred by consumers. Colour varies and is affected by factors at all steps of production chain, such as animal breed, species, age, diet, muscle fibre type and metabolism; pre-slaughter handling, stunning and bleeding; chilling variables; packaging, distribution and other display and storage conditions, etc. (Kropf, 1993). It has been shown that dairy cattle have more unstable colour than that from beef breeds (Insausti et al., 1999).

Myoglobin is the major pigment in meat, accounting for 50-80% of the total pigment. Hemoglobin is the major colour pigment in blood which also relates to muscle pigment. There are still other pigments within muscle contributing to meat colour, such as cytochromes, catalase, and flavins, but they are present in only small amounts and have minor influence on muscle colour (Miller, 1994). The amount and chemical state of the myoglobin pigments and the superficial structure of meat are

major factors contributing to fresh meat colour (Insausti et al., 1999).

Myoglobin is a sarcoplasmic protein consisting of about 153 amino acids, containing a protein (globin) and a haem group which comprise a porphyrin ring with a central transition iron atom (Varnan & Sutherland, 1995). Myoglobin contains 8 α-helices, and there are six coordination sites available on the central iron atom, four of which are linked to pyrrole nitrogens, connecting iron to the porphyrin. The fifth coordination link is the histidine molecule attached with globin. The six coordination point is available for binding to different ligands. The nature of the sixth binding site and the oxidation state of the central iron atom play the most important role in meat colour characteristics (Mancini, 2009).

In the absence of oxygen within muscle, deoxymyoglobin (DMb) has reduced ferrous iron and no ligand at the sixth coordination point. DMb has a purple to dark-red colour. After the muscle surface is exposed to air, the DMb rapidly oxygenates to oxymyoglobin (MbO_2) This process is commonly called blooming or oxygenation. The colour quickly turns to bright red, which is considered to be fresh, good quality steak and is largely accepted by consumers. In oxymyoglobin, the state of central iron atom is the reduced ferrous iron (+2) and oxygen occupies the sixth ligand (Mancini, 2005). Further exposure to air over several days or over a shorter time at a low concentration of oxygen, both deoxymyoglobin and oxymyoglobin can react with oxygen to form the oxidized formmetmyoglobin (MMb), which has a dull, brown colour, which is unattractive to consumers. In this pigment, the heme iron is oxidized to the ferric state (+3) with H_2O present at the sixth ligand (Mancini, 2005).

The relative proportions of oxymyoglobin (bright red), deoxymyoglobin (dark red) and metmyglobin (grey brown) present in muscle are largely responsible for different meat colours. The oxymyoglobin layer forms at the meat surface, usually within the top few millimeters (Young & West, 2001). In the interior of meat, beyond the limit of penetration of oxygen, deoxymyoglobin is present (about 5 mm). As a result of a

low oxygen partial pressure at the limit of oxygen penetration, a thin layer of metmyoglobin occurs between the red oxymyoglobin layer and purple deoxymyoglobin layer. This metmyoglobin layer thickens over time and eventually becomes apparent at the surface of the meat by first darkening the translucent surface tissue and then breaking through to the surface (Young & West, 2001). To prevent or minimize metmyoglobin formation in fresh meat, oxygen must be totally excluded from the packaging atmosphere or present at saturating levels (Brandon, 2007).

When measuring meat colour, time of exposure to air needs to be considered and blooming for at least 1 h has been recommended (Honikel, 1997). Haas & Bratzler (1965) found that during the first hour of meat exposureto air, the oxygenation rate was extensive. According to Wulf & Wise (1999), stabilization of colour parameters of beef had been completed within 78 min of blooming.

1.2.3 Warner-Bratzler shear force or tenderness

Palatability of beef consists of desirable flavour, juiciness and tenderness, and it is a combination of these factors that determines the degree of eating satisfaction. However, inadequate tenderness is the most likely cause of consumer dissatisfaction and requires much attention (Verbeke et al., 2010). Three major component traits were considered to determine variation of beef tenderness: the morphology and amount of the intramuscular connective tissue (IMCT); sarcomere length; the rate and extent of postmortem proteolysis of myofibrillar proteins (Koohmaraie et al., 2002). In addition, intramuscular fat (IMF) affects tenderness indirectly (Jeremiah et al., 2003a). However, the interaction of these factors is complex and is muscle dependent.

Of course, the eating quality of meat is also highly dependent on the cooking method. The mechanism of cooked meat toughening occurs in four main phases (Figure 1.2). Upon cooking between 42 to 52 ˚C, meat exhibits a sharp increase in

shear force first and a loss of fluid because of the denaturation of the myofibrillar proteins, actin and myosin, forming a rigid gel to provide resistance to shearing. Before the temperature reaches 60 ˚C, both these proteins have undergone a transition from the gel state, to a hardened dehydrated form, initially posing little resistance to shear (McCormick, 1999). As temperature increases to 64-70 ˚C, the second phase of steeply increasing shear force and toughening occurs, relating to the thermal denaturation of collagen. Initially, collagen fibrils denature and shrink substantially, which makes the collagen triple helix contract. Consequently, compression and thermal tension is applied to muscle bundles by the squeezing together of muscle fibres accompanied by increased fluid loss released from heated myofibrils by the pressure exerted. Thus, the volume of the muscle fibres reduces, leading to increased toughness (McCormick, 1999). The degree of tension or shrinkage developed greatly depends on the proportion of heat-stable crosslinks present. In general, greater forces are generated in older animals with more mature cross-links resulting in increased meat toughness. When the temperature increases to between 80 and 90 ˚C, the collagen will eventually gelatinize and the shear force diminishes (McCormick, 1999).

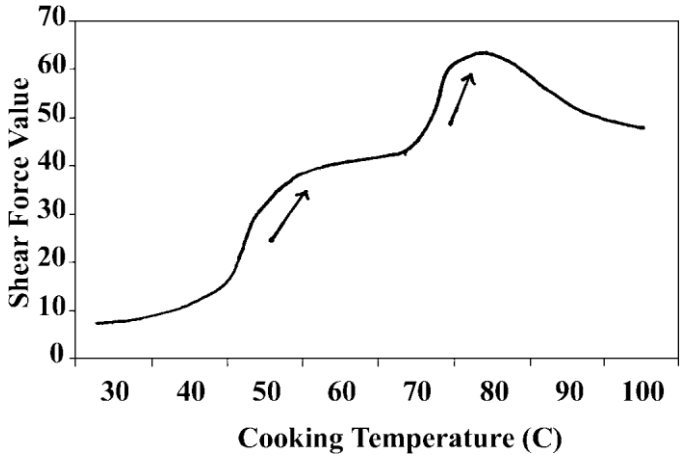


Figure 1.2. Cooking-dependent meat toughening. From Davey & Gilbert, 1974.

Warner Bratzler Shear Force (WBSF) and sensory evaluation are currently used for instrumental and non-instrumental sensory determination of tenderness of bull beef,

respectively. The majority of the published research reports that WBSF and sensory tenderness are moderately correlated (e.g. Silva et al., 1999; Vestergaard et al., 2000a; Chambaz et al., 2003). It has been reported that for young bull beef, WBSF is positively correlated with age, carcass weight and cooking loss, and is negatively correlated with IMF content, sensory tenderness, juiciness, flavour, overall acceptability and myofibrillar fragmentation index (MFI) (Monteiro et al., 2013; Lucero-Borja et al., 2014). Cooking loss and juiciness have been reported as the main contributors to the tenderness differences. MFI accounted for 10% of the total WBSF variation (Monteiro et al., 2013). Reagen et al. (1976) pointed out that cattle's chronological age and total collagen content were the most important determinants in beef tenderness variation. Larger muscle fibres generally leads to tougher beef, whereas, compared with fibre size, proteolysis of myofibrillar proteins occurring during post-mortem ageing is probably more important for ultimate tenderness (Seideman & Koohmaraie, 1987).

1.2.4 Water holding capacity and cooking loss

Water holding capacity (WHC) is defined as the ability of fresh meat to retain its own water during the application of external forces, such as cutting, heating, grinding or pressing and during storage and transport. WHC can be described as drip, purge, weep, exudate or cook loss (Pearce et al., 2011; Hughes et al., 2014). The pH value and the degree of the space available between myosin and actin dominate the amount of water bound within the muscle tissue. The isoelectric point (IEP), where positive and negative charges are equal, of the actomyosin complex in meat is 5.2, (Huff-Lonergan & Lonergan, 2005). Inferior WHC leads to high drip loss and purge loss, which may affect the yield and quality of processed meat because of the significant loss of weight from carcasses and cuts. In addition, poor WHC has negative effect on the appearance of meat, which can further affect consumer willingness to purchase the product (Hughes et al., 2014).

In general, pH values below and above (more important in muscle) IEP show better WHC, and a decline in pH close to the IEP results in less WHC in meat. When the pH of muscle is at the IEP point, the same numbers of positive and negative charges attract each other and the protein molecule is tightly bound together. This results in a net charge of zero in the protein molecule, thus only a tiny amount of water can be bound within the proteins (Huff-Lonergan & Lonergan, 2005). In contrast, WHC is enhanced when there is an imbalance in the numbers of positive or negative charges. When the pH value is above or below the IEP, the repulsion force between the myosin and actin filaments increases, and creates larger gaps or spaces between these two filaments, which allows more water to be incorporated (Feiner, 2006d).

Cooking loss is the technological water lost during the cooking operation, which plays a critical role in the meat industry as it determines the yield after cooking. The water loss during cooking is from the juice expelled by the contraction of muscle structures caused by protein denaturation, which depends on the temperature of heating (Kondjoyan et al., 2013). In the temperature between 40 and 60 ˚C, transverse shrinkage occurs in the myofibrils and muscle cell, which is generally attributed to the denaturation of myosin and desmin. At 65 ˚C, a co-operative shrinkage between muscle fibres and collagen probably happens. Actin and titin denature in the region of 70-80 ˚C (Hughes et al., 2014). The shrinkage of muscle fibre and IMCT during cooking could also contribute to toughness. Accordingly, cooking loss is directly related to juiciness and indirectly associated with tenderness of beef (Lawrie, 1991a).

1.3 Chemical compositional quality of bull beef

1.3.1 Components of beef

Beef contains muscle, fat and connective tissue, which are surrounded by blood vessels and nerve tissue. The majority component of beef muscle (‘the lean’) is water, which accounts for between 70% and 75% of muscle (Feiner, 2006d).

Protein is the second most abundant component in beef muscle, accounting for around 19-22% of lean muscle composition. Muscle protein contains three main parts, 11.5-13% myofibrillar protein (salt soluble), 5.5-7% sarcoplasmic protein (water soluble or soluble at very low concentrations of salt), and around 2% stroma/connective tissue (insoluble in salt and water) (Lawrie, 1991a). Expressed as percentages of the total protein in lean muscle, myofibrillar protein is between 55-60%, sarcoplasmic protein is about 30% and connective tissue is between 10-15%. The myofibrillar protein is mainly contractile protein with myosin accounting for around 42% and actin around 16% of the total myofibrillar protein, whereas in the total muscle weight, myosin and actin only account for 7-8%. The remainder of the myofibrillar proteins are regulatory (*e.g. troponin, tropomyosin*) and cytoskeletal (*e.g. desmin, titin*) proteins (Tornberg, 1996; Feiner, 2006d).

The other components which occur in small amounts in lean muscle are lipids (fat) between 2% to 4%; carbohydrates (approximately 1%); soluble non-protein substances 2.3%; vitamins and minerals (often analyzed as ash, around 1%). The fat in beef includes subcutaneous fat, intermuscular fat, and intramuscular ('marbling') fat. All these types of fat are comprised by adipocytes (Lawrie, 1991).

1.3.2 Moisture

Water within muscle tissue exists in different ways. 'Protein-bound water' is bound firmly to protein, and accounts for around 4-6% of the water within muscle. 'Fibril-bound water' (around 55-60% of muscle water) is present between the myofibrils and is recognized as immobilized (or not freely available) water, but it binds less firmly than protein-bound water. Around 20-25% of the total water is 'free water', which is present in the sarcoplasm and is freely available. Finally, water is held outside cellular membranes in capillaries, is known as 'extracellular water' and accounts for around 8-14% (Feiner, 2006d).

As with other food products, moisture content plays a large role in the quality, taste and safety of meat. An increasing uptake of water into the intra-myofibrillar space or the release of water from bound proteins occurs during the process of ageing and proteolysis. It has been shown that a high amount of intra-myofibrillar water and low amount of extra-myofibrillar water may be related to more tender meat (Pearce et al., 2011). The most mobile myowater (extra-myofibrillar water) has been reported to be the factor affecting juiciness (Bertram et al., 2007). The mobility of the extra-myofibrillar water was suggested to play the most important role in high water release during chewing and consequently the perception of meat juiciness (Pearce et al., 2011).

1.3.3 Intramuscular fat (marbling)

Intramuscular fat (IMF) or marbling consist of triglycerides, which is the ester made from the esterification between trihydric alcohol glycerol and three fatty acids. The varying IMF content in bull beef is related to the difference in triacylglycerols but not in phospholipid content. Phospholipids are particularly rich in PUFA, whereas triacylglycerols contain much lower levels of PUFA (Costa et al., 2012). IMF or marbling is an important intrinsic factor for beef palatability and thus used as an indicator for beef quality grading (Li et al., 2006).

It has been widely accepted that a higher marbling level contributes primarily to a higher rating in sensory attributes of bull beef, i.e. juiciness, tenderness, flavour intensity, overall acceptability or liking (Sami et al., 2004; Li et al., 2006; Corbin et al., 2015). IMF dilutes the fibrous protein in muscle tissue resulting in a decrease in the resistance of the muscle to shearing (Wood et al., 1999). IMF has negative correlation with off-flavour and a positive correlation with beef flavour (Duckett et al., 2009), which suggests that IMF content affects the sensory perception of flavour. A positive correlation between IMF and water holding capacity of muscles has been reported, and a lower IMF content is associated with greater cooking loss

(Pordomingo et al., 2012).

The minimal level of IMF in beef to be described as having intense fat flavor, and for consumer acceptance and preference is approximately 3%, while higher than 7.3% may have a negative effect on health (Miller, 2002). However, consumers differ in their preferences for marbling based attributes of eating quality. In some countries, e.g. Australia, marbling is associated with the classification system (Meat Standards Australia, 2016b).

1.3.4 Collagen

Intramuscular connective tissue (IMCT) has the function to support, separate and protect the structure of organs. It provides the environment for cell growth, adjusts force development and prevents over-extension of the contractile filaments. IMCT also acts as 'background toughness' of meat during both post-mortem conditioning and cooking because of its' resistance to heat solubilization during heating (Voutila, 2009). From the structure aspect, IMCT is classified into three specific regions in muscle: epimysium, the layer sheath surrounding the entire muscle; perimysium, the connective layer separating each muscle into muscle fibre bundles or fascicles; endomysium, the innermost network surrounding individual muscle fibres (McCormick, 1994). Among these, as epimysium is easily separated from the muscle, it is not important when evaluating meat texture. On the other hand, the perimysium accounts for above 90% of IMCT and is therefore considered to play the major role in determining the variations in connective tissue related meat texture (Light et al., 1985). From the composition aspect, IMCT is composed of three proteins (collagen, elastin, reticulin), complex polysaccharides (mainly proteoglycan) and water. The three proteins are bound to polysaccharides and water, which together act as the ground substance. As elastin and reticulin are found in much smaller amounts, collagen is the main component of IMCT relating to background toughness of meat during cooking, although it only constitutes < 2% of most skeletal muscles

(Weston et al., 2002).

Collagen is the most abundant protein, constituting up to 25-30% of total protein in mammalian and avian bodies and it exists in all tissues, particularly skin, tendon and bone. Nineteen different forms of collagen have been identified (McCormick, 1999). According to Bailey (1989), these different types of collagen molecules can be divided into three categories: thick striated fibers (fibrous collagen), non-fibrous, and non-striated filamentous. The fibrous collagen I, Ⅱ, Ⅲ, V, and XI can self-assemble to form the characteristic band pattern, and they are synthetized by intramuscular fibroblasts in the endoplasmic reticulum. Their degradation (turnover) is under the control of the collagenases, matrix metalloproteinase (MMPs) and their inhibitors. Type IV, Ⅷ, X are non-fibrous collagen, and in particular, Type IV exists in basement membranes. Filamentous collagens with a loosely packed filamentous structure include the minor types, such as Ⅵ and Ⅶ. Muscle contains a combination of collagen types, Type I and Ⅲ being the major forms of intramuscular collagen, have the greatest relationship with meat texture (Bailey et al., 1979).

Tropocollagen, the basic structural unit of collagen, is a long and thin molecule with a molecular weight of 300,000 and a length of 280 nm (Marsh, 1977). Each tropocollagen molecule is a right-handed triple helix consisting of three polypeptide chains, named α-chain or procollagen, and each individual chain is composed of a repeating Gly-X-Y sequence, where X or Y is often proline or hydroxyproline. The carboxyl- (C-) and amino-terminal (N-) ends are short, non-helical regions called telopeptides. The three polypeptide chains are wound around each other and bound through hydrogen bonds to form a strong and compact molecule (McCormick, 1999). Hydroxyproline is the special amino acid in collagen, and is present as a concentration of 12.5% of collagen. It is hard to find in other proteins in animal tissues, so it is used as a tool to determine the amount of collagen (Weston, et al., 2002).

Tropocollagen molecules are bonded together with a quarter-stagger array, with each unit extending around three-quarters of the length of its neighbor by the specific bonds called intermolecular crosslinks. The intermolecular crosslink is considered to be more important to stabilize the collagen fibres than those of intramolecular within tropocollagen (Shimokomaki et al., 1972). When intermolecular crosslinks starts to form, specific lysine or hydroxylysine residues in N- and C-terminals of each α-chain are converted into peptidyl aldehydes through oxidative deamination (condensation) by lysyl oxidase enzyme (Weston et al., 2002). Due to the quarter-stagger formation of collagen molecules, the aldehyde residues of adjacent tropocollagen molecules can approach and interact with each other and form divalent bonds. As shown in Figure 1.3, the two molecules are linked together as the head of one molecule overlaps the tail of the other (Bailey 1972; McCormick, 1999). These divalent crosslinks belong to immature, reducible crosslinks, which contain Schiff base double bonds, and can be easily ruptured by pH changes, heat or denaturing agents (Weston et al., 2002). Three immature cross-links are mainly found, hydroxylysino-Δ-norleucine (ΔHLN, an aldimine) is formed between lysine aldehyde and hydroxylysine; hydroxylysino-5-ketonorleucine (oxo-imine or keto-imine) is formed between hydroxylysine aldehyde and hydroxylysine, with oxo-imine cross-links it is more heat stable than aldimine. The third minor type is a disulphide cross-link formed from the cysteine residues in the C-terminal end of the triple helix (Bailey, 1985; Voutila, 2009).

When an animal reaches physiological maturity, these reducible divalent crosslinks can no longer be detected, and are replaced by mature, thermally stable, trivalent crosslinks, to conform to more complex structures. They can link not only individual collagen molecules but also transversely crosslink adjacent microfibrils to form the three-dimensional collagen network across muscle fibre (Weston et al., 2002). There are four mature cross-links reported, with pyridinoline the predominant type including hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP) as shown in Figure 1.4. HP is formed between the reaction of oxo-imine and hydroxylysine, and

LP is derived from oxo-imine and lysine (McCormick, 1999). Ehrlich chromogen (EC) is the pyrrolic structure cross-link with two types of lysylpyrrole and hydroxylysylpyrrole, the same with HP and LP, which also has oxo-imine cross-link precursors as pyridinoline. Another two types are histidino-hydroxylysinonorleucine (HHL), which is in skin collagen and histidino-hydroxymerodesmosine (HHMD), however, these two types have gained little attention in meat quality research (Bailey, 1989; Voutila, 2009).

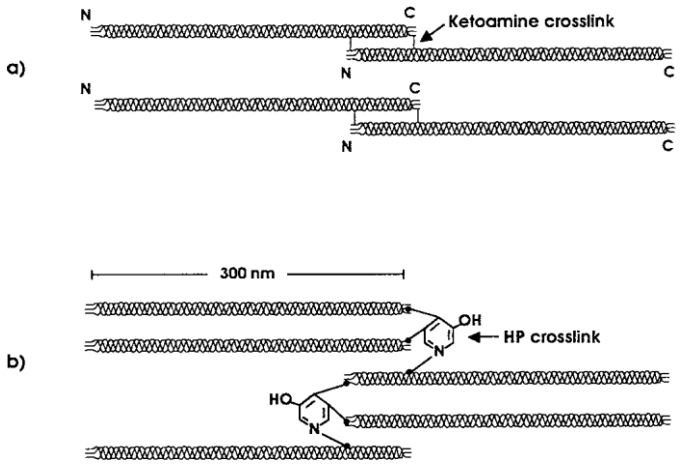


Figure 1.3. Divalent and trivalent crosslinking of fibrillar collagen. From McCormick, 1999.

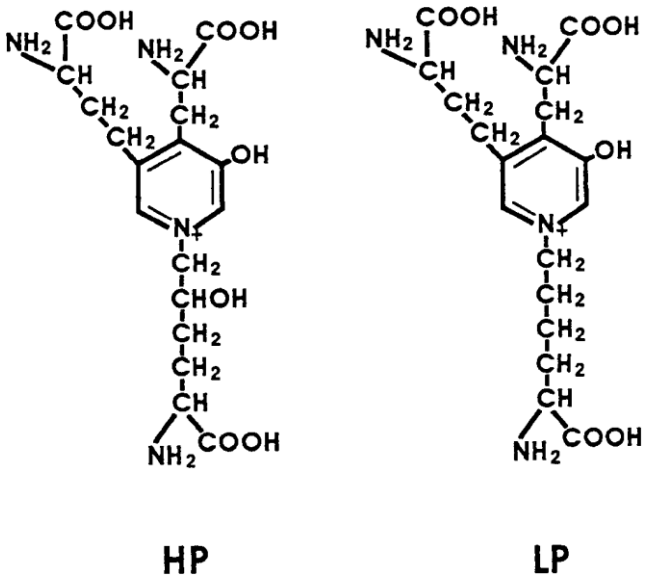


Figure 1.4. Pyridinium crosslinks. Hydroxylysylpyridinoline (HP) and its dehydroxyanalog lysylpyridinoline (LP). From McCormick, 1999.

There are conflicting findings between total amount of connective tissue and meat toughness. Although it has been reported that muscles with higher total collagen content tend to produce tougher meat (Loyd & Hiner, 1959), some studies concluded that collagen content didn't account for the variation in WBSF or taste panel tenderness (Crouse et al., 1985; Christensen et al., 2011). However, instead of mere quantity, the quality or nature of collagen in muscle plays a key role in contributing to meat texture, especially by the type and extent of the intermolecular cross-links with increasing chronological age. The amount of cross-links has been reported to have an inverse relationship with thermal solubility of collagen (McCormick, 1994). It is generally accepted that the higher proportion of thermo-stable to thermo-labile cross-links (lower thermal solubility) leads to tougher meat (Bailey, 1985).

The amount and nature of connective tissue can be affected by several production factors, including muscle, breed, diet, sex and age of the animal, which are all described in the section 5 of this review. Age and growth rate of the animal are the most important factors, which have the effect on the nature and extent of collagen cross-link (Bailey, 1989). It has been suggested that it is better to slaughter animals just after a rapid growth period to obtain the most tender meat. As during rapid a growth period, the concentrations of newly synthesized soluble collagen are greatly increased, which dilute the mature existing collagen so that there is a concomitant enhancement of its heat solubility (Etherington, 1987). With increasing maturity and consequent decreased growth rate of an animal, the collagen fibres form a more stable network via multivalent mature cross-links and generate a higher tension upon thermal shrinkage which results in tougher meat. In addition, collagen turnover (the process of structure and components degradation) rate is high during the rapid growth period or in young animals, and then reduces to a plateau by maturity. The higher the rate of turnover, the less mature, less thermally stable the collagen and the more tender the meat (Bailey, 1985). Summing up the above, animals of late-maturing breeds or with higher growth rates are likely to produce relatively more

tender meat.

1.3.5 Fatty acids

Beef is perceived having a high nutritional value as being an important source of fatty acids (FA), essential amino acids, vitamins (A, B6, B12, D) and minerals, such as iron, zinc, selenium. Fatty acids are long chains of hydrocarbon or carbonic acids with a carbon-number between 12 and 24 and a carboxyl (-COOH) and methyl (-CH_3) groups at the end. FA contains saturated and unsaturated types and saturated FA accounts for around 55-60% of total FA in beef (Feiner, 2006d).

Some fatty acids in beef have beneficial effects on human health, such as oleic acid has cholesterol-lowing effects, including reduced risk of stroke and improved blood pressure. α-linolenic acid (ALA, C18:3n-3) and the long chain *n*-3 PUFA, i.e. eicosapentaenoic acid (EPA, C20:5n-3), docosapentaenoic acid (DPA, C22:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) are also widely recognized for their beneficial effects on health in reducing the risk of cardiovascular disease (CVD), cancer, type-2 diabetes and their critical roles for proper brain function, neural and visual tissues maintenance (Scollan et al., 2014). The *n*-3 PUFA supply is recognized only being contained in three main groups of food, which are seafood, meat and eggs (Meyer et al, 2003). CLA is a mixture of conjugated isomers of linoleic acid, which provides prevention of cancer, atherosclerosis, obesity, diabetes and osteoporosis. Among these isomers, *cis*9, *trans*11-CLA accounts for about 80% of the total CLA isomers in red meat (Daley et al., 2010).

However, beef also has detrimental effects to health because of the high levels of cholesterol and saturated fatty acids, which are known to raise total and low-density lipoprotein (LDL) cholesterol levels which are related to CVD (Brugiapaglia et al., 2014). Accordingly, in Germany, it is recommended that the ratio of *n*-6/*n*-3 in the diet of humans should decrease to the level ≤ 5:1 and to reduce the intake of

saturated fatty acids (10% of the total calories) and *trans* fatty acids (less than 1%), and enhance the intake of unsaturated fatty aicds, especially *n*-3 fatty acids (Nuernberg et al., 2005). In the UK, it has been recommended that saturated fat to be decreased from 15% to 10% of total energy intake while the ratio of polyunsaturated to saturated fatty acid (P/S) should increase to above 0.4 (Department of Health, 1994).

Besides from nutritional and healthy influences, beef quality can be affected by fatty acid composition, including fat tissue firmness (hardness), shelf-life (lipid and pigment oxidation) and flavour (Wood et al., 2003). It has been shown that the fat tissue in meat is the source of the characteristic species flavour (Mottram, 1998). The flavour of beef can be derived from lipid oxidation products during cooking and the interaction between these and Maillard reaction products to form other volatiles contributing to odour and flavour (Wood et al., 2003). The alteration towards the fatty acid composition of the lipid fraction of beef could cause alteration of the amount and type of volatiles produced and subsequently its aroma and flavour (Scollan et al., 2006). Priolo et al. (2001) concluded that the particular flavour of grass-fed meat is mainly related to n-3 PUFA oxidation products. 'Green' odour from grass-based beef was associated with hexanals compounds derived from C18:1n9c and C18:3n3, and 'soapy' odour from concentrate-based beef was related with octanals derived from C18:2n6 (Lorenz et al., 2002). The sensory study conducted by Campo et al. (2003) observed that different fatty acids produce different flavour and odour properties in meat and C18:3n3 in particular produces high scores for fishy and linseed/putty or grassy. It also had been reported that C14:1, C16:1, C18:0, C18:1, C18:2 and C18:3 correlated with desirable beef flavour (Melton et al., 1982).

The effect of fatty acids on shelf-life is manly due to the oxidation of PUFA, which significantly develop sensorial abnormal flavour and rancidity as display time increases (Scollan et al., 2006). The oxidation of red oxymyoglobin to brown

metmyoglobin can cause the colour change, which generally reacts in parallel to rancidity. Lipid oxidation products and pigment oxidation can be promoted by each other (Wood et al., 2003).

Fatty acid profile shows a wide variability depending on several production factors, such as breed, sex, age and diet. The production factor effect on the overall fat content has an important impact on relative fatty acid composition of neutral lipid and phospholipid, with implications for the nutritional quality of meat (Brugiapaglia et al., 2014). It has been concluded that neutral lipid content increased markedly with increasing of total lipid content, whereas phospholipids exhibited a fairly constant level (Figure 1.5; Wood et al., 2008).

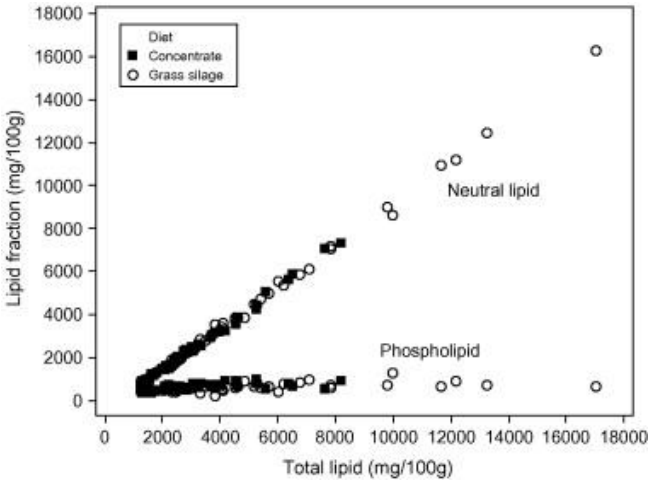


Figure 1.5. Concentrations of neutral lipid and phospholipid (mg/100g muscle) plotted against total lipid (mg/100g muscle) in *longissimus* muscle of steers given a concentrate or grass silage diet and slaughtered at 14, 19 or 24 months of age. From Warren et al., 2008.

1.4 Sensory quality of bull beef

1.4.1 Sensory evaluation process

Sensory evaluation by a trained panel is a combination of determining the tenderness, juiciness and flavour characteristics to generate a more objective and accurate tool to judge bull beef palatability (Cho et al., 2010). Descriptive analysis is the most

comprehensive, flexible and useful sensory method, which provides detailed information on the sensory properties of a product. It can detect (discriminate) and describe both qualitative and quantitative sensory characteristics (Meilgaard et al., 1991). There are several different methods of descriptive analysis reflecting various sensory philosophies and approaches, including Flavour Profile Method, Texture Profile Method, Quantitative Descriptive Analysis (QDA), the Spectrum Method, Quantitative Flavour Profiling, Free-choice Profiling and generic descriptive analysis. Of these, QDA is the most often used technique, its design is based on repeated measures and the statistical analyses is generally conducted by Analysis of Variance (Murray et al., 2001).

There are a few steps for QDA development. Firstly, a descriptive analysis panel needs to be selected. To achieve this, panellists are generally screened and then how panellists perform will be monitored and those that perform well in a variety of screening tests should be selected (Meilgaard et al., 1999). Individual interviews will be applied to assess the commitment and motivation of the panel. Several selection factors need to be considered, such as health status, allergies, verbal creativity, smoker, dietary habits, education, medication, etc. Secondly, descriptive attributes need to be generated. The training phase begins with the development of a common vocabulary which comprehensively and accurately describes the product attributes. Generally, a new panel will develop their own sensory language, and this learning process can be done with the assistance of an experienced panel leader or other members of the organization (Murray et al., 2001). Thirdly, the sensory concepts can be formed. The panels are exposed to the range of products in the category under test and are trained to use a common ‘frame of reference’ to define the product attributes and intensity (Munoz & Civille, 1998). Compared with using their own words to qualitatively describe perceptions and using their previous experiences to quantitatively rate intensities, trained sensory panels are able to use a standard language to describe sensory concepts and a common scale to rate the intensity of

attributes by acquiring a common qualitative and quantitative frame of reference. Finally, a certain level of practice is required for panels before they can confidently evaluate products, and a minimum of 10-15 h training needs to be conducted (Murray et al., 2001).

To assess the performance of a taste panel, several validation criteria should be considered: 1). Panellist consistency, i.e. the extent to an individual panel member varied in their scoring for a given trait over the period of the experiment; 2). Panellist repeatability, i.e. the consistency with which an individual panel member was able to score a trait on repeated samples from the same animal; 3). Panellist agreement, i.e. the extent to which taste panel members agreed with each other for one trait (Gill et al., 2010).

1.4.2 Texture

One of the earlier methods to place texture on a quantitative basis was the chew count, which required panelists to count the number of chews before swallowing (Harrington & Pearson, 1962). Some more descriptive terms of texture for beef products have also been developed, including hardness (defined as hardness at first bite with molars), crumbliness (sense of grittiness and dryness during chewing), and tenderness (easiness with which the meat is divided during chewing) (Lund et al., 2007). It has been well established that tenderness is a major factor contributing to consumers' perception of taste. In terms of tenderness, the following descriptive properties have been generally used to evaluate cooked steak: muscle fibre tenderness, connective tissue amount, overall tenderness (Lorenzen et al., 2003). The study conducted by Aaslyng et al. (2007) observed that compared with consumers over 30 years old, young consumers put less emphasis on tenderness of beef, instead some crumbliness was preferred. About 6% and 12% of the consumers were influenced only by flavour and texture attributes respectively in their preference, irrespective of other attributes. However, most of the consumers' preference was

affected by flavour and texture, as well as appearance.

1.4.3 Juiciness

When meat is tender, juiciness is considered to be the next quality attribute of interest for consumers (Ouali et al., 2006). The loss of juiciness to the palate is directly related to the degree of shrinkage of muscle fibres on cooking. Juiciness in cooked meat has two organoleptic components. Firstly, wetness is assessed during the first few chews, which is produced by the rapid release of meat fluid; secondly, the sensation of sustained juiciness results from the stimulatory effect of fat on salivation (Lawrie, 1991a). A strong relationship has been found between juiciness and WHC of beef. Juiciness is positively correlated with WHC of raw beef, while negatively correlated with cook loss (Hughes et al., 2014). Furthermore, juiciness is positively correlated with tenderness and flavour (Costa et al., 2012). It has been accepted that juiciness strongly associates with the movement of intracellular water towards the extracellular space. The bound water will release when the pH becomes more acidic, which is closer to the pI of myofibrillar proteins (Ouali et al., 2006).

1.4.4 Flavour

Flavour is a very complex attribute of meat palatability and it is accepted to be the most important factor affecting consumers' purchasing habits and preferences of beef products when tenderness was held constant (Killinger et al., 2004). Even small changes in the sensory rating for flavor can greatly affect the overall acceptability of steaks (Calkins & Hodgen, 2007). Off-flavour of beef is defined by the terms ammonia, bitter, gamey, liverish, metallic, old, rotten and sour. Liver flavor was found have a positive correlation with metallic and grassy aroma, sour flavor, metallic and grassy aftertaste and a negative correlation with roast beef flavor (Elmore & Mottram, 2009).

Flavour characteristics of cooked meat are derived from volatile flavor components

which generate from thermally induced reactions occurring during heat treatment via the four pathways including (1) Maillard reaction; (2) Lipid oxidation; (3) Interaction between Maillard reaction products with lipid-oxidized products; (4) Vitamin degradation during cooking (Van Ba et al, 2012). Raw meat has little flavor or aroma, and only a blood-like taste. The development of meat flavor characteristics happens during thermal condition as the result of complex interaction of precursors derived from both the lean and fat compositions (Mottram, 1998). The Maillard reaction occurs between amino acids and reducing sugars and provides compounds that give a typical meaty flavour, i.e. savoury, roast and boiled properties. Lipid degradation is responsible for the fatty aromas of cooked meat (Elmore & Mottram, 2009) and particular PUFAs significantly increase sensorial abnormal flavour and rancidity scores (Scollan et al., 2006). Lipids in meat also serve another role in flavour development, acting as a solvent for the volatile compounds developed during thermal processing (Moody, 1983).

To date, over 1000 volatile compounds have been detected and identified in cooked meat, and most of which have been identified in beef but not in other meats (Maarse & Visscher, 1996). All these volatile components are organic and with low molecular weight. They are classified into different categories by their varied chemical structures including aldehydes, ketones, hydrocarbons, pyrazines, acids, esters, alcohols, nitrogen and sulfur-containing compounds and other heterocyclic compounds (Van Ba et al, 2012).

Many factors have been found to influence the flavour of cooked beef, including pre-harvest animal genetic background, sex, age and diet, post-harvest handling and ageing, and consumers' individual preferences, etc, for example, diets have a large effect on bull beef flavour due directly to IMF content (Raes et al., 2003). These factors further can affect pH, minerals and fatty acids content of beef. Sodium and iron; medium and long chain unsaturated fatty acids have been thought to play a role

in creating the liver-like off-flavour. Metallic aroma notes are affected by the variation in content of C18:1 and C18:3 in meat from different feed sources (Calkins & Hodgen, 2007). Cooking rate and holding time are also important, and cooking at a slower rate and holding for a longer time reduces the intensity of off-flavour as the undesirable volatile compounds easily dissipate under this condition (James & Calkins, 2005). Most volatile flavour compounds increase their amounts with cooking temperature. It was also observed that the pH condition strongly affects the formation of flavour compounds, and different volatile compounds are favoured at different pH conditions, such as nitrogen-containing compounds like pyrazines are favoured at increased pH (Mottram & Madruga, 1994). The length of ageing period could be another active factor contributing to bull beef flavor development and off-flavour differences (Pordomingo et al., 2012).

1.5 Factors affecting bull beef eating quality

There are several biochemical processes affecting beef eating quality, which are during the transportation of cattle to the abattoir, the immediate pre-slaughter period, the slaughtering process and meat handling after slaughter (Muchenje et al., 2009). Production systems such as breed, slaughter age and feeding regime, handling and exercise condition have the potential to affect the physiology of muscle and therefore bull beef attributes including colour, juiciness and tenderness, etc. (Frylinck et al., 2013).

1.5.1 Pre-slaughter factors

1.5.1.1 Breed

Breed effects on tenderness can be explained by differences in proteolytic enzyme activities. For instance, increased bull beef toughness results from the increased proportion of *Bos indicus* rather than *Bos taurus* content (Frylinck et al., 2013). It was shown that above 25% *B. indicus* content will negatively affect beef palatability (Morgan et al., 1991). According to Ngapo et al. (2002), the covalent, HHMD, and

EC cross-links of intramuscular collagen were lower in double muscled than in normal cattle. Based on their function, cattle breeds can be divided into dairy type, which primarily used for milk production; beef breeds, those which are more suitable for meat production and dual-purpose type which are used for both milk and beef. There have been conflicting reports on the quality of beef from dairy-origin cattle. It has been noted that beef cattle possess a markedly greater percentage of IMF in the LD muscle than that from dairy types after the age of 18 months (Lawrie, 1991a). Holsteins have been shown to produce less stable meat than that of beef breeds (Nuernberg et al., 2005). Beef from Holstein steers is more tender compared with Charolais steers (Lively et al., 2005) or Simmental cross Angus steers (Thonney et al., 1991). However, Sinclair et al. (2001) stated that purebred Holstein steers had poorer sensory ratings in comparison to Angus steers. In addition, Christensen et al. (2011) noted that young bulls of dairy breed types such as Jersey, Holstein and Danish Red Cattle had WBSF in the middle of the range of 15 European cattle breeds. Among the 15 breed types, Simmental and Avileña-Negra Ibérica had the highest and lowest WBSF respectively.

1.5.1.2 Slaughter age

The myoglobin content in cattle muscle increases rapidly with chronological age. A darker, more intense and more saturated colour was found in older animals (Monteiro et al., 2013). Tenderness of LD muscles from young bulls on average decreases with advancing age. As during animal maturation, the divalent immature cross-links convert into mature trivalent cross-links, contributing to a decrease of collagen solubility (increase of thermal stability) upon heating, which results in tougher cooked meat (McCormick, 2009). Weston (2002) also noted that with increasing animal ages, collagen synthesis and turnover reduce, allowing time for mature crosslinks to form.

The IMF percentage was found to increase with a concomitant decrease in moisture

with animal age. Age-related changes were also found in branched-chained fatty acids with increasing age, and the ratio of linoleic to stearic acids rises, thus increasing the softness of fat (Lawrie, 1991a). Owens & Gardner (1999) mentioned that meat flavor was affected by age through increasing carcass fatness and faster growing bulls have been shown to produce beef with good flavor. Generally, the flavour intensity increases with age regardless of the type of animal. With time-on-feed, fishy and milky-oily flavours decreased linearly (Calkins & Hodgen, 2007).

1.5.1.3 Feeding system

Generally, the effects of the nutrition level during the growth of animals are reflected in the composition of individual muscles. Beef quality can be modified by both the quantity of feed energy available to the animal (plane of nutrition) and the nutrient composition of the feed (feed type) (Muir et al., 1998a). Feed costs are a major proportion of total variable costs in beef systems. Feeing systems are mainly divided by grass-based extensive system (grass, silage, hay) and grain-based intensive system (wheat, barley, soya). Grass-based diets are low energy diets, relatively high in α-linolenic acid, with low-input. They are the cheapest feedstuff in temperate climates, and are more suited to meeting the demands of naturally and animal-friendly produced beef for discerning retailers and consumers. Grain-based diets are high energy diets containing relatively high amounts of linoleic acid. Hence, compared with animals fed with grass, grain-fed animals of the same age are usually heavier with a higher percentage of body fat (French et al., 2000; Elmore & Mottram, 2009).

Muscle lipids show large differences due to diet, in particular, grass-fed animals have higher proportion of all *n*-3 fatty acids in the muscle, i.e., α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA), and conjugated linoleic acids (CLA), which have health advantages, such as the reduction in coronary heart disease (Daley et al., 2010). All *n*-6 fatty acids, such as C18:2n-6, and all long-chain *n*-6 FAs, were higher in the muscles of grain-fed

animals (Sañudo et al., 2000). Hence, the concentrate diets are unfavourable to the ratio of n-6/n-3, which is recommended to be less than 4 (Department of Health, 1994). However, it is not clear if it still holds true at the end of the winter period when typically three months have passed without consumption of fresh grass (Razminowicz et al., 2006).

Feeding management and nutritional status affect glycogen depletion and meat quality parameters, such as ultimate pH (pHu), colour, cook loss and tenderness. Frylinck et al. (2013) reported that bull beef fed with pasture has meat that is darker (lower L*), less red (a*) and has higher pHu than those fed with grain. A slow pH decline rate during post-mortem chilling resulting in cold shortening can be largely prevented by feeding grain rather than grass as the latter results in lighter carcasses and insufficient subcutaneous fat coverage (Thompson, 2002). The higher pHu of grass-fed animals may due to their greater susceptibility in pre-slaughter stress and glycogen depletion than grain-fed animals and grain-fed animals would be better accustomed to people, pens, handling and confinement whilst in the feedlot thus would be less likely to suffer glycogen depletion pre-slaughter in the factory. However, no difference in muscle pHu between grass and grain-fed cattle was reported (Muir et al., 1998a).

Grass-based fattening typically results in a darker meat colour (Priolo et al., 2001). Mancini & Hunt (2005) stated that muscle colour is affected by diets due to promoting oxidative metabolism which has affects pH, oxygen consumption and metmyoglobin reducing activity. Varnam & Sutherland (1995) speculated that there is more haem pigment, especially for muscle myoglobin in grass-fed animals due to their greater physical activity pre-slaughter than their feedlot counterparts. Higher forage levels in feedlot diets may increase antioxidant levels (vitamin E) in the lean, which could improve colour retention during retail display. One of the primary functions of vitamin E is to maintain and protect biological membranes from

oxidative damage (Rice & Kennedy, 1988).

There are controversial results for the effect of different feeding systems on tenderness. Most researchers found that grain fed cattle produces more tender beef than their pasture-fed counterparts at a similar chronological age (Bowling et al., 1977; Nuernberg et al., 2005). This probably results from higher cattle growth rates and rates of protein turnover resulting from higher energy diets, which increases the concentrations of intracellular proteolytic enzymes of the carcass at slaughter, such as the collagen degradation promoter matrix metalloproteinases (MMPs), and in consequence, both myofibrillar and connective tissue degradation increase (Archile-Contreras et al., 2010). However, some authors found that the WBSF value was lower in pasture fed beef than in conventional beef (Razminowicz et al., 2006). Moreover, no difference in WBSF was determined between beef produced from lower energy and higher energy diets (French et al., 2000; Latimori et al., 2008). In sensory tenderness, more tender and more desirable beefy flavour was produced by grain-fed beef than grass-fed beef (Larick et al., 1987). These conflicting results may be due to the interactions of such factors as feed type and composition, animal management, animal age and physical activity from different production systems in most studies.

In general, the higher energy diets produce higher concentrations of IMF and lower moisture content of the LD muscle of Simmental bulls beef than lower energy diet (Sami et al., 2004). There are also conflicting results for the relationship between collagen and diet. Generally, the collagen content of bull beef was not affected by type of diet (Dikeman et al., 1986). However, Cranwell et al. (1996) indicated that the soluble collagen content of LD muscle in mature cows increased with a higher energy ration in the finishing period. Miller et al. (1987) also reported that high energy feeding increases the newly-synthesized heat soluble collagen proportion during the faster growth period. In addition, the total collagen content of LD muscle

was found to be lower in corn-fed than in pasture-fed cattle (Archile-Contreras et al., 2010).

Forage-fed beef can experience decreased flavour acceptance due to the particular fatty acid concentration and type and the subsequent volatiles from fat oxidation and chlorophyll derivatives (Costa et al., 2012). Fish off-flavour was significantly higher in meat from grass-finished cattle with increasing unsaturated fatty acids (Wood et al., 2003). Juiciness score was higher in concentrate-fed steer beef than pasture-fed counterparts (Duckett et al., 2009). Vestergaard et al. (2000a) shown that cook loss was higher in extensively fed bulls than in the intensively fed bulls.

1.5.1.4 Muscle type

Three major types of muscle fibres have been identified by histochemical studies and their relative proportion reflects the biochemical characteristics of muscles. 'Red' fibres, type I, are slow-acting, predominantly oxidative and respiratory in metabolism; whereas 'white' fibres, type Ⅱ, are fast-acting and predominantly glycolytic in metabolism. These are subdivided into type Ⅱ A and Ⅱ B with higher oxidative capacity in type Ⅱ A (Lefaucheur, 2010). Red muscles can store less initial glycogen, thus have higher ultimate pH, and greater susceptibility to 'cold-shortening' than white muscle, and white muscles possess more active enzymes to convert glycogen to lactic acid (Lonergan et al., 2010). Red muscles have a lower content of the calcium-activated protease calpain, while white muscles have a greater ratio of calpain:calpastatin, and this may explain the observation that during conditioning red muscles tenderize less than white muscles (Ouali & Talmant, 1990). However, the diameter of white fibres is greater and they are surrounded by less mitochondria than red fibres. The protein turnover rate is two to five times faster in type I than in type Ⅱ fibres (Lawrie, 1991a).

Muscle fibre type composition is also an important source of variation in meat quality, including colour, WHC, tenderness, juiciness and flavour. It is generally

stated that total IMF level is higher in red oxidative muscles than white glycolytic muscles (Lefaucheur, 2010). An increased proportion of slow-twitch oxidative fibres has been shown to improve tenderness, juiciness and flavour of beef (Maltin et al., 1998), while a high proportion of type I fibres is prone to produce DFD beef muscle (Ozawa et al., 1999). Lightness is negatively related with type I fibres, and myoglobin content is positively related with type I fibres (Henckel et al., 1997).

McCormick (1999) ranked the tenderness of different muscles in the order of *Biceps Femoris* (BF) < *Gluteus Medius* (GM) < *Longissimus dorsi* (LD) ≤ *Psoas Major* (PM) with LD and PM being similar. Another study conducted by Rhee et al. (2004) evaluated palatability traits among 11 major beef muscles and found that PM was the most tender and was followed by *Infraspinatus* (IS) by both WBSF and sensory rating, while BF had the lowest tenderness rating. Cook loss was lowest for BF, and was followed by LD and IS, and ST had the highest value. It has been noted that a relatively wide range of collagen cross-link values exist in different muscles. In general, locomotor muscles possess more cross-links than postural muscle (Zimmerman et al., 1993). It has been shown that the *Semitendinosus* (ST) muscle contains more total collagen and lower heat soluble collagen content than the LD muscle (Archile-Contreras et al., 2010; Stolowski et al., 2006). Conversely, Berge et al. (1997) found that the ST muscle of young bulls has significantly higher shear force than the LD, but contains less total collagen and lower concentrations of cross-links. Muscle differences were noted as a factor affecting beef flavour intensity. It was reported that *Semimembranosus* (SM) has less desirable flavor than LD and GM, which both showed the similar level of flavour desirability (Wulf & Page, 2000).

1.5.1.5 Gender

From the stand point of consumer acceptance, it has been generally accepted that castration can improve beef eating quality, while the tougher texture, less desirable and dark colour and less juicy sensation are the most important disadvantages for

producing beef from bulls (Seideman et al., 1982). Mach et al. (2009) noted that Holstein steers fed a high-concentrate diet were rated more tender than those from bulls by both sensory panel and mechanical devices after ageing for 0, 7 and 14 days. The tenderness difference could be related to variation of collagen characteristics and IMF level. Total collagen content was reported to be higher in bulls than steers, and bull collagen matures and increases in thermal stability more rapidly than that of steers (Gerrard et al., 1987). Similarly, Lepetit (2007) pointed out that the mature pryridinoline cross-links HP and LP value were higher in bulls than heifers and steers, with heifers and steers being similar. Moreover, compared with Holstein bull beef fed a high-concentrate diet, pH and percentage of IMF is markedly higher and the moisture content is lower in Holstein steer beef (Marti et al., 2014). The difference of beef quality could be explained by the actions of testosterone and estradiol-17β which are the most pronounced hormonal changes related to puberty and sexual maturation in bulls (Podriguez et al., 2012). Generally, taste panel rated meat from steers had significantly more desirable flavour, was more juicy, tender and more acceptable than bulls; while difference in pH may be a possible explanation for the flavour difference (Reagan et al., 1971).

1.5.1.6 Pre-slaughter management

It is well known that insufficient glycogen available in animal at slaughter will lead to less lactic acid produced, resulting in the pH value staying high and dark cutting beef, which is unacceptable to consumers. Glycogen level can be affected by the amount and quality of feed provided to animals in the month prior to slaughter. High quality nutrition from feedlot rations or first-class pasture will be beneficial in producing a high glycogen level in the muscle. Cattle receiving restricted intake or low quality feed won't have enough glycogen, often below the critical level. Therefore, good feed quality is important for eating quality of beef (Meat Standards Australia, 2016c).

The long-term stress of animal before slaughter will deplete their energy and glycogen stored in muscle. The stress results from the animals being exposed to a new environment, with unfamiliar sounds and new animals outside the social group which can aggravates their stress, thus the glycogen stored in muscle is rapidly mobilized to enable the animal to either run or to attack. Hence maintaining animals in their social groups is a good option (Meat Standards Australia, 2016c). Particularly, the physical contests and excitable temperament of bulls makes them more prone to lead to glycogen depletion (Lawrie, 1991a).

Too high densities, lengthy transport and high temperatures also are detrimental to the well-being of animals, which can result in injuries, fatigue and exhaustion, leading to poor carcass quality, too low pH value or DFD beef (Hartung et al., 2009). Tenderness and drip loss could also be affected by lengthy transport. It has been reported that calves from shorter transport produce more tender beef compared with longer transport (Fernandez et al., 1996). According to Honkavaara et al. (2003), drip loss was lower in beef from bulls transported over short distances than long distance transports.

1.5.2 Post-slaughter factor

1.5.2.1 Pelvic suspension

Pelvic or aitch bone hanging, sometimes called tender-stretch, can be applied in beef tenderization. This involves suspending the carcass halves by the hip or pelvic bone straight after slaughter, leading to a carcass position more typical of the animals' natural state. It increases tension on the major leg and loin muscles before they pass through rigor (Hostetler et al., 1970). Compared to the conventional hanging, vertically from the Achilles tendon, this pelvic hanging reduces muscle contraction during rigor mortis and minimizes shortening by the gravity pull down both ends of the carcass. Because of this counter-force, the number of cross-links between myosin and actin are significantly reduced, sarcomere length increases, muscle fibre

diameter decreases and thus meat tenderness is improved (Sørheim & Hildrum, 2002). Moreover, another mechanism has been suggested to be responsible for the tenderness improvement with tenderstretching, which is possibly related to a more rapid degradation of the structural protein at Z-line and intermyofibre filaments (Thompson, 2002). Pelvic hanging has also been shown to affect other quality traits of bull beef, including cook loss, colour and marbling (Ahnström et al., 2012a). A lower amount of visible marbling was reported in pelvic-suspended beef, which maybe the result of a stretching of muscle fibres in the adipose tissue, contributing to less obvious visible marbling (Ahnström et al., 2012b). Reports of cook loss affected by pelvic hanging vary widely. Cook loss of Ankole bull beef decreased by pelvic suspension has been stated by Kamatara et al. (2014), who explained that this may be related to the less contracted muscles with less overlap between thick and thin filaments and thereby there is larger interfilament space and room for water storage. Nevertheless, some authors didn't find a difference (Ahnström et al., 2012b; Hou et al., 2014) or reduced (Barnier & Smulders, 1994) cook loss in pelvic suspended muscle.

1.5.2.2 Ageing

It is well known that meat can be tenderized during post-mortem ageing (conditioning) by proteolysis which breaks down both muscle fibres and connective tissue. Generally, it is recommended to age beef for at least 2 weeks between 0 to 4 ˚C in order to obtain a piece of tender beef (Farouk et al., 2009). Although meat continues to tenderize to 21 days, Ruiz de Huidobro et al. (2003) reported that a more significant reduction of shear force occurs during the first 6 days for bull beef, and extended ageing time decreased the difference of texture parameters between bulls and heifers. It also has been shown that pH value, moisture content and WHC remain stable and redness decreases during ageing in bulls, however, WHC increases significantly during ageing in heifers (Ruiz de Huidobro et al., 2003). Moreover, the different optimized ageing time of bull beef also depends on breed and muscle.

Monsón et al. (2005) observed that the highest acceptability values evaluated by a sensory panel for the Limousin breed (meat type) is at 14 days of ageing, for the Holstein breed (dairy type) it is at 21 days and for the Blonde d'Aquitaine breed (high muscularity type) it is at 35 days.

The process of ageing of can also contribute to the development of flavour precursors, which had a positive effect on the organoleptic characteristics of bull beef. In particular, ageing of beef for 10-14 days was characterized by increased desirable flavour intensity than those stored for 3 and 7 days. However, it also has been suggested that the flavour profile of meat can be altered by the microbial and enzymatic changes during ageing (Elmore & Mottram, 2009). Jeremiah et al. (2003b) stated that beef can be aged for up to four weeks without affecting the overall property of beef flavour significantly. An increased intensity of undesirable 'livery' aromatic and aftertaste occurred when beef was stored beyond 28 days.

1.6 Indirect methods to assess and predict eating quality of bull beef

1.6.1 Near infrared (NIR) spectroscopy

NIR spectroscopy is a rapid-response analytical tool which was one of the first to be applied for quantitative analysis in agricultural food samples in the 1970s by the group headed by Norris (Blanco & Villarroya, 2002). The electromagnetic spectrum below shows the ultra violet light has a wavelength range (in nanometers) 1–400 nm, visible light in 400–750 nm and the infrared region in 750–10^6 nm. The latter is subdivided into the near-infrared (NIR) region: 750–2500 nm, the mid-infrared (MIR) region: 2500–16,000 nm and the far-infrared (FIR): 16,000–10^6 nm. Within the NIR region, the wavelength of 750-1100 nm is the transmission band and 1100-2500 nm is the reflection band (Figure 1.6).

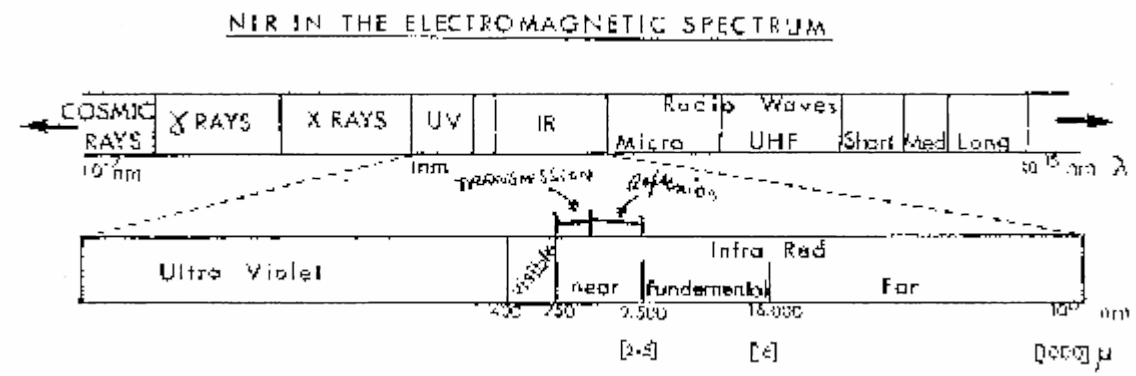


Figure 1.6. NIR in the electromagnetic spectrum. From Davis, 2000.

NIR wavenumbers are at about 14.000-3.500 cm^{-1}. Chemical bonds as weak springs in substance hold two or more atoms together, and these springs will vibrate naturally and when energy is added to the system they will vibrate more energetically. Different chemical bonds, such as O-H, C-H, and N-H vary in strength and hence different amounts of energy for the bond vibration are required. This variation in energy is seen in a spectrum as a series of absorptions at different wavelengths. By observation of the spectrum, we can deduce what vibrations are occurring and hence work out the structure of the molecule or groups of atoms present. NIR absorptions originate in molecular vibrations and are generated from fundamental vibrations by two processes: overtones and combinations. Overtones can be thought of as harmonics. Combinations arise from the sharing of NIR energy between two or more fundamental absorptions (Davis, 2000).

There are a few advantages for NIR application, as it can use reflected energy, hence NIR analysis can be done with simplicity in sample preparation. Analysis time is reduced from hours to minutes. Furthermore, not like the conventional analysis, different techniques are required for different analyses, while several analytical results can be obtained from the same NIR data. In addition, it is non-destructive and more sensitive (Prieto et al., 2009).

Over the past three decades, NIR has been approved to be one of the most efficient and advanced tools for the estimation of quality attributes in meat and meat products.

It has been shown that NIR has potential to predict the chemical composition (protein, intramuscular fat, moisture, ash, myoglobin and collagen), technological parameters (pH value; L*, a*, b* colour values, WBSF and water holding capacity) and sensory attributes of bull beef (Marchi et al., 2007; Andres et al., 2008; Ripoll et al., 2008). Moreover, NIR is used to classify meat quality grades, and beef product adulteration. It has been noted that NIR has been successfully applied to predict major meat chemical constituents and to categorize meat into quality classes. However, less reliability has been shown for predicting technological and sensory attributes (Prieto et al., 2009). For meat products, in NIR, the specific absorbance of O-H bonds is at 1400 and 1900 nm, which is used for water identification (Barlocco et al., 2006). The good performance of prediction of protein content is from the absorption of the N-H bonds at 1460-1570 nm and 2000-2180 nm. The absorption of the C-H bonds at 1300-1400 nm contributes to IMF content prediction (Prieto et al., 2008).

1.6.2 Raman spectroscopy

In contrast to NIR and Mid-IR spectroscopy, instead of being based on light absorption, Raman spectroscopy (RS) is an optic technology relying on inelastic scattering of monochromatic light which occurs when a laser light interacts with molecules of matter. The general process is that the incident light excites molecular vibrations in the material resulting in a red-shift of the scattered light which is analyzed. Thus, RS is fundamentally a vibrational spectrum and is able to provide ‘chemical fingerprint’ information for qualitative and quantitative molecular composition and structure based on the energy transfer between the excitation laser and the chemical bonds present in a sample (Das & Agrawal, 2011; Schmidt et al., 2013). Subsequently, RS is able to detect small amounts of substances in a variety of sample types rapidly with almost no sample preparation, and non-destructively, which is also potentially suited to online measurement of meat quality parameters. It

gives similar but complementary information to infrared spectroscopy (Damez & Clerjon, 2008). In comparison with NIR and Mid-infrared Fourier Transform (FT-IR) spectroscopy, especially FT-IR, RS has a distinct advantage as it is relatively insensitive to varying water content. Since meat commonly contains ≥ 75% water, while it doesn't suffer from water interference. In addition, RS yields higher resolution and is capable of providing more detailed spectral information about the chemical and physical composition of the sample than NIR (Wang et al., 2012).

The multivariate-analysis (chemometrics) method is used to construct models capable of accurately predicting the properties and characteristics of unknown samples. In the multivariate-analysis methods, for quantitative analysis, partial least-squares (PLS) regression, principal component regression (PCR), multiple linear regression (MLR) are mainly used. Principle component analysis (PCA) and cluster analysis are applied in qualitative analysis. In order to develop more simple and robust models, some of the more frequent pretreatments (mathematical treatments) are required, including normalization; derivatives (usually first or second); the multiplicative scatter correction (MSC); the standard normal variate (SNV); de-trending (DT); or a combination of them (Blanco & Villarroya, 2002).

It has been shown that RS can relate to the results obtained from conventional quality methods to evaluate muscle food quality because RS can provide structural information on the changes of proteins, water and lipids (Herrero, 2008). Previous research has demonstrated the potential for RS to predict meat quality traits. It has been shown that shear force and sensory attributes in cooked (roasted) beef can be predicted by RS with high potential (Beattie et al., 2004). RS has also exhibited the potential to predict shear force of wet aged, raw beef samples from young bulls (Bauer et al., 2016). Fowler et al. (2015a) reported RS can successfully predict pHu and colour for lamb semimembranosus (topside). Schmidt et al. (2013) found the usefulness of RS to predict shear force and cook loss of LT and LL muscle of lamb.

Although RS has been indicated as a promising tool to predict the concentrations of major fatty acid groups of lamb LL muscle (Fowler et al., 2015b), the prediction ability of RS for chemical composition of beef has not been widely investigated. In addition, RS has also been used to differentiate and classify pork loins into quality grades in terms of sensory traits (Wang et al., 2012). Classification of tough and tender beef GM muscle has also been performed by PLSR discrimination analysis with high accuracy > 70% by RS (Bauer et al., 2016).

1.6.3 Image analysis

There are multifactorial contributions of the muscle component to beef quality. Apart from quantitative properties of muscle fibres and IMCT composition, histological characteristics of them also play an important role in beef quality determination. Significant correlations between some histological measures and sensory parameters of beef like tenderness have been reported (Allingham et al., 2009). Image processing methods have also been developed to predict beef quality traits. For example, the IMCT image parameters of bovine SM muscle acquired under visible and ultraviolet lighting were reported to be good predictors of beef tenderness (R^2 = 0.89), collagen (R^2 = 0.82) and lipid content (R^2 = 0.91) (Jabri et al., 2010). The muscle bundle (fascicles) size or diameter (‘‘grain size’’) has a positive correlation with shear force (r = 0.39, $P < 0.01$) and a negative correlation with taste panel tenderness (r = -0.41, $P < 0.01$) and overall acceptability (r = -0.30, $P < 0.05$) (Cooper et al., 1968). Perimysium thickness is another active histological trait associated with beef tenderness, being positively correlated with shear force value (Brooks and Savell, 2004). Computer vision technology and digital imaging systems have also been shown to have high accuracy in predicting sensory overall acceptability (r^2 = 0.95) (Jackman et al., 2010), flavour (r^2 = 0.84), juiciness (r^2 = 0.71) and tenderness (r^2 = 0.64) of beef (Jackman et al., 2009).

The morphology of muscle structure varies tremendously between animal production

factors, such as muscle, breed, species, and animal age. Muscle bundle size was found to increase greatly with animal age in bovine LD muscle (Cooper et al., 1968). Perimysium and endomysium of LD were thinner, less ramified than in *biceps* muscle. Perimysium of Blond d'Aquitaine young bull beef occupied less area, was more ramified and muscles contained less collagen, cross links and more proteoglycans than Angus young bulls; Limousin was intermediate (Dubost et al., 2013).

Chapter 2

The eating quality of beef from young dairy bulls derived from two breed types at three ages from two different production systems

2.1 Abstract

Expansion of the Irish dairy herd has led to more dairy breed male calves being available for beef production. This study investigated the physico-chemical and sensory characteristics of beef from Holstein-Friesian (HF) and Jersey×Holstein-Friesian (JEX) young bulls fed pasture only or pasture plus 2 kg concentrate during their first grazing season and slaughtered at 15, 19 or 22 months of age. *Longissimus thoracis* (LT) muscles were collected from 67 carcasses. Post-mortem pH, ultimate pH (pHu), meat colour, chemical composition, collagen content and solubility were evaluated. After ageing for 21 days, Warner-Bratzler shear force, cooking loss and trained sensory panel assessments were determined. Meat from older animals was darker. The pHu, moisture and ash contents decreased, while residual roast beef flavour length and 'Eat-greasy' scores increased with age. However, increasing age to slaughter did not negatively influence tenderness. JEX beef had lower cooking loss, was darker, redder and had higher sensory scores for initial tenderness, fattiness, juiciness, 'Eat-pulpy' and residual pulpy than HF beef. Warner-Bratzler variables were positively correlated with cooking loss and chewiness, and negatively correlated with intramuscular fat (IMF) content, soluble collagen, and initial tenderness. In summary, most young dairy bull beef samples were acceptably tender after 21 days ageing and half of them had acceptable IMF content. Slaughter age affected beef colour, pHu, chemical composition, flavour length and greasiness. The eating quality of meat from the JEX breed type was considered to be superior to that of the HF breed type. Diet during the first season had no effect on meat quality traits.

Keywords: Dairy breeds, Meat quality, Sensory attributes, Tenderness, Young bulls

2.2 Introduction

Meat quality is a complex concept that involves intrinsic quality cues including safety, shelf-life, nutritional value and eating quality and extrinsic quality cues, such as brand, quality label and convenience of the product (Hocquette et al., 2012). Eating quality is generally considered the most important meat quality trait for consumer satisfaction, with juiciness, tenderness and flavour being the major attributes (Troy & Kerry, 2010). Marbling is another important intrinsic factor which contributes to beef palatability and hence is used as an indicator for beef quality grading (Li et al., 2006).

The ending of milk quotas in 2015, has led to increased dairy output in Ireland. Food Harvest 2020 predicted an increase in the dairy herd from 1.15 to 1.43 million by 2020 (DAFM, 2014). The number of male calves from the dairy herd is therefore rising. Raising these calves as steers is not a viable option due to their poor conformation, while rearing them as young bulls may be a more viable option due to their improved growth rate and feed conversion efficiency and subsequent lower carbon emissions compared to steers and may generate viable financial returns (Seideman et al., 1982). Such an approach would provide a significant new source of income for producers, increase beef supplies and potentially open up new export markets for Irish beef. However, although young bull beef production has increased in the last decade, it still only accounted for 19% of overall Irish male cattle slaughtered in 2015, down from 22% in 2014, 25% in 2013 and 30% in 2012 (DAFM, 2015). There is conflicting evidence about the palatability of bull beef, and there is a view in the industry that it is tougher than steer beef and therefore, less acceptable to consumers. There is little recent information on the palatability of beef from young dairy bulls and how this is affected by the production system utilised. This study addresses that knowledge gap.

Meat attributes such as colour, water holding capacity (WHC) and tenderness can be affected by production systems, including breed, feeding regime, slaughter age, handling and exercise conditions (Frylinck et al., 2013). Holstein-Friesian (HF) is the predominant Irish dairy breed, however, there is currently interest in the Jersey breed as these have shown potential for crossbreeding under Irish conditions, due to their improved reproductive efficiency, intake capacity and increased milk solids yields (Prendiville et al., 2011). Growth rate, carcass traits and performance of HF and Jersey × HF (JEX) bulls were recently reported (McNamee et al., 2015), however palatability has not been well investigated and there are concerns about the eating quality of bull beef in general. Moreover, cattle age is known to closely correlate with the physico-chemical characteristics of meat and is an important factor in determining meat tenderness and palatability (Schönfeldt & Strydom, 2011). Beef quality from pasture- or grain-based finishing systems has been extensively researched (French et al., 2000; Avilés et al., 2015). It has been shown that the high energy diet produced higher concentration of IMF and flavour acceptance of beef compared with the low energy diet (Corbin et al., 2015), while the effect of first season (grazing phase) on beef quality has not been explored. It is hypothesised that beef produced from a higher energy diet during the first grazing season could exhibit higher potential for marbling deposition than that from a lower energy diet.

This study aims to determine the physico-chemical and sensory characteristics of beef from young bulls of two dairy breed types, slaughtered at three ages from two different production systems. A greater understanding of how breed types, slaughter age and first season feeding affect dairy bull production efficiency and beef quality attributes would assist in decision making on how meat from dairy bulls should be produced and marketed.

2.3 Materials and methods

2.3.1 Source of materials

This project was submitted to the Teagasc Animal Ethics Committee who advised that provided best husbandry practice was followed no ethical issues would arise. A total of 300 (mainly HF & JEX) weaned spring-born male dairy breed type calves (10 to 12 weeks of age) were sourced and transported to Teagasc, Johnstown Castle Research Centre in 2010. Then they were assigned to one of two production systems (grass only: PO vs grass plus 2 kg concentrate: PC) during the first grazing season according to breed type, date of birth, body weight on arrival and farm origin. The concentrates were offered per head daily was composed of 80% *Hordeum vulgare* (ground barley), 14% *Glycine max (L.) Merr* (soya bean meal), 4% *black treacle* (molasses) and 2% minerals. Bulls were slaughtered at 15, 19 and 22 months of age, respectively. The experiment was set up as a 3 (slaughter age) × 2 (breed type) × 2 (first season feeding) factorial design resulting in 12 treatment groups.

Permanent grassland sward of predominantly perennial ryegrass (*Lolium perenne*) was used for rotational grazing systems. Animals assigned to PO or PC (excluding 15-month old bulls) were housed together during the winter period within their production system respectively. Before finishing, 19- and 22-month old bulls were also offered the second grazing season with a grass only diet. During the finishing period, animals were penned within their own treatment group and offered on an *ad-libitum* concentrate diet. The duration of each feeding treatment was shown in Figure 2.1.

2.3.2 Sampling and sample preparation

At a commercial abattoir, bulls were stunned by captive-bolt and exsanguinated within 30 s. Electrical stimulation was not applied. Each carcass was conventionally hung, dressed and centrally-split into two sides. The pH and temperature of the

longissimus thoracis (LT) muscle at the 10th rib on the left side of each carcass were measured hourly for up to 8 h. Carcasses were chilled at 4 °C. A subsample of each batch was selected for meat quality analysis. As there were many more HF than JEX, all suitable (normal pH and not detained for veterinary inspection) JEX and up to ten HF carcasses per treatment group were selected. In total 67 bulls were sampled; 33 from PO and 34 from PC; 39 were HF and 28 were JEX; 29 were slaughtered at 15 months, 19 at 19 months and 19 at 22 months.

The LT muscle was removed from the cube roll (ribs 6 to 10) from the left side of each selected carcass at 48 h post mortem. After holding until 72 h post mortem, the ultimate pH (pHu) of the LT samples was measured, and the muscle was cut into individual slices (25 mm thick). The fresh cut surface of the first slice from the 10th rib end was used for colour measurement, and the rest of the slices were vacuum-packed. Steaks for chemical composition and collagen determination were stored at -20 °C immediately, while samples for Warner-Bratzler shear force (WBSF), cook loss and sensory analysis were aged for 21 days at 4 °C and then frozen at -20 °C for further analysis.

2.3.3 Post-mortem pH, temperature and ultimate pH

A portable pH meter model 420A (Orion, Germany) and an Amagruss pH electrode EC-2010-11 (Refex sensors Ltd., Westport, Co. Mayo, Ireland) were calibrated using standard buffer solutions (pH 4.0 and pH 7.0). The temperature probe (Digitron 2046T, Instrument Technology Ltd, Ireland) and pH electrode were inserted approximately 50 mm into the LT muscle near the 10th rib following a scalpel incision. The electrode was rinsed thoroughly with distilled water between measurements. The pH/temperature profiles of each carcass were made using the pH and temperature values measured up to 8 h post-mortem. The individual pH values at 15 °C and 35 °C of each carcass were then read from the pH/temperature curves directly.

2.3.4 Meat colour

Freshly cut samples were wrapped with an oxygen-permeable polyvinylchloride film (oxygen permeability of 580 $mL \cdot m^{-2} \cdot h^{-1}$ at standard temperature and pressure) and left to bloom at 4 °C for 2 h and 24 h. A dual beam spectrometer (UltraScan XE, Hunter Lab., VA, USA) with a wavelength range from 360 to 750 nm and a wavelength interval of 5 nm was used and a light trap and a white tile were applied for standardization. Illumination was matched to daylight (D65, 10°) with an 8° viewing angle and a 25 mm port size. Sample readings were taken through the cling film at five locations on each muscle and averaged. CIE L* (Lightness, black-white), a* (Redness, + to − from red to green) and b* (Yellowness, + to − from yellow to blue) values were provided by the CIE Lab System. Hue angle $(\tan^{-1}(b^*/a^*))*57.29$ and Chroma index $(a^2 + b^2)^{1/2}$ were calculated.

2.3.5 Warner-Bratzler shear force and cooking loss

Trimmed steaks (150 – 180 g) were thawed in a circulating water bath at 10 to 15 °C (approximately 45 mins). Excess moisture was removed by patting surfaces of steaks with tissue paper before weighing. The steaks were cooked in open bags suspended in a water bath (TC120, Grant Instruments Ltd, England) at 72 °C until the temperature in the centre of the steak reached 70 °C. When the steaks had cooled to room temperature, they were again patted with tissue paper and weighed. Cooking loss was determined as the difference between cooked and raw weights expressed as a percentage of the raw weight.

After tempering overnight at 4 °C, seven 12.5 mm diameter cores per steak were cut parallel to the longitudinal orientation of the muscle fibres. When cores reached room temperature (approximately 20 °C), they were sheared perpendicular to the muscle fibre orientation using the Warner-Bratzler (WB) shear blade attached to an Instron Universal Testing Machine (Model 5543, Instron (UK) ltd., High Wycombe,

UK) with a 500 N load cell using a crosshead speed 50 mm/min. Bluehill software was used and the average maximum shear force (WBSF) was calculated by excluding the two extreme values. Warner-Bratzler-Slope (WB-slope) was recorded from a line drawn from 20% to 80% of the WBSF curve and expressed as 'Shear-Firmness' in Mpa (Brady & Hunecke, 1985), and Warner-Bratzler-Area (WB-area) was calculated by the whole energy used during shearing and expressed as Joules (J).

2.3.6 Chemical composition

Frozen samples trimmed of all external fat and connective tissue were thawed at 4 °C overnight. The lean meat and exudate were homogenised using a blender (R301 Ultra, Robot Coupe SA, France). Moisture and IMF concentrations of thawed minced beef samples were measured using the Smart System 5 microwave moisture drying oven and NMR Smart Trac rapid Fat Analyser (CEM Corporation, USA) using AOAC Official Method 985.14 (AOAC, 1991). Protein concentration was determined using a LECO FP328 (LECO Corp., MI, USA) protein analyser based on the Dumas method and according to AOAC method 992.15 (AOAC, 1992). Approximately 2 to 3 g of homogenised samples were weighed in a crucible dish which was placed on the electric hot plate until charred. The crucible dish was then transferred to a muffle furnace (515 to 540 °C) and left overnight to ash. Samples were removed from the furnace, cooled to room temperature and reweighed to determine the ash percentage. All composition tests were carried out as two determinations per sample with the coefficient of variation (CV) between replicates of moisture content below 1.0%; of IMF content below 10%; of protein content below 1.5%.

2.3.7 Collagen content and solubility

According to a combination of the methods of Voutila et al. (2007); Kolar (1990) and Nordic Committee on Food Science (2002), 5 g of homogenized meat were heated in

a water bath at 77 °C for 65 min in 12 mL of buffer solution with pH of 6.0 (30 g citric acid monohydrate, 15 g NaOH and 90 g sodium acetate trihydrate dissolved in 290 mL 1-propanol and diluted to I L with water) and centrifuged for 10 min at 3,990 x *g* (MSE Mistral 3000i, UK) at room temperature. The supernatant was collected and 8 mL of buffer solution was mixed with the precipitate and centrifuged again for another 10 min. The precipitate and supernatant from the two centrifugations were combined. Each fraction was individually hydrolysed in 30 mL 7 N H_2SO_4 in an oven at 105 °C for 17 h. The hot hydrolysate was transferred to a 250 mL volumetric flask with the aid of water and the solution was neutralised with 4.37 mL of 1 M NaOH, diluted to volume with water and mixed. Part of the solution was passed through filter paper into a 100 mL Erlenmeyer flask. A total of 5 mL of the filtrate was diluted to 100 mL. To 2 mL of the final dilution, 1 mL of oxidant solution (1.41 g chloramine T reagent in 100 mL buffer solution) was added and left to stand for approximately 20 min at room temperature. Then 1 mL of colour reagent (10 g 4-dimethylaminobenzaldehyde dissolved in 35 mL perchloric acid (60%w/w) and 65 mL 2-propanol) was added and the tubes were immediately placed in a water bath at 60 ± 0.5 °C for 15 min. The tubes were cooled and dried and the absorbance was measured at 558 ± 2 nm with a UV-Vis Spectrophotometer UV-1700 (Columbia, USA). Soluble and insoluble collagen contents were calculated by the hydroxyproline amount multiplied by a factor of 7.52 and 7.25, respectively. Total collagen (mg collagen/g meat) was defined as the sum of soluble collagen and insoluble collagen, and percentage solubility was calculated as soluble collagen as a percentage of total collagen. Each sample was analysed in duplicate and the CV value between duplicates was below 10%.

2.3.8 Trained sensory panel evaluation

Frozen vacuum-packed 25 mm thick steaks were thawed in a circulating water bath at 10 to 15 ºC (approximately 45 mins). Steaks were cooked on a electric grill (Velox

CG-3, Velox Grills, UK) set at 230 °C, to an internal temperature of 70 °C, according to the AMSA Guidelines (AMSA, 1978). Temperature was monitored with a probe (Eurolec TH103TC, Technology House, Ireland) inserted into the centre of each steak. Steaks were grilled for 1 min on one side, turned over for 1 min, and turned twice more for 2 min followed by continuous turning each minute until done. Cooked samples were then trimmed of all external fat and major connective tissue, cut into pieces (20 × 15 × 25 mm) and wrapped in aluminium foil for resting for about 3 min and served to an eight member trained sensory panel seated in individual booths with red fluorescent light. Each panel member, trained according to AMSA (1995) standards, received six samples in randomized order (each panellist tasted the steak samples in a different order within each session) separated by two sessions (3 by 3) at approximately 3 min intervals between sessions. Panel members were provided with salt-free crackers and water for cleansing the palate between samples.

Panellists scored each sample for 16 attributes, defined and rated during different phases of eating (Table 2.1). Roast beef aroma intensity was evaluated before eating, while the initial tenderness was the texture of the first bite. During further mastication, juiciness, cohesiveness, ease of disintegration, chewiness, fattiness/greasiness, stringiness, astringency and the flavour terms roast beef flavour, metallic, stale/rancid/aged were evaluated. Residual-roast beef flavour length, residual-metallic flavour, residual-fattiness/greasiness and residual-dryness were the sensation left in the mouth 12 s after swallowing the sample thus they were described as residual or after-effects. Each attribute was rated through ‘Compusense® five’ sensory evaluation software (Guelph, Ontario, Canada) on station computers, by a 100 mm unstructured line scale with 0 mm being equivalent to no intensity and 100 mm being equivalent to the highest intensity of the attribute.

Sensory analysis was also conducted in University of Bristol, UK (Bristol analysis). The panel assessed some overall attributes of ‘Texture’, ‘Juiciness’, ‘Beef Flavor’,

'Abnormal Beef Flavour', 'Flavour Like' and 'Overall Acceptability' using an 8 point hedonic scale, where 1 and 8 were equivalent to no attribute intensity and the highest intensity of the attribute, respectively. Other detailed attributes shown in Table 2.2 were scored on an unstructured 100 mm line scale (0 = extremely low, 100 = extremely high). Among those attributes, 'Ease of Cutting' and 'Cleanness of Cut' were described during cutting the sample before eating, and the terms with the 'Bite-' prefix were evaluated just on the initial chewing of the sample with the first bite. There were a few terms rated during eating, which had the prefix 'Eat-'. The attributes evaluated after swallowing, which means residual or after effects sensation left in the mouth were given the prefix 'Res-'.

2.3.9 Statistical analysis

The data were analysed by analysis of variance (ANOVA) using the general linear model (GLM) procedure of Statistical Analysis System (SAS) Version 9.3 (Cary, NC: SAS Institute, 2002). The experimental unit was the individual animal for all variables to keep the unit consistent throughout the whole feeding period. The model included the fixed effects of breed type, age, first season feeding and their interactions. As the effects of interactions were all non-signifiant, the corresponding results were not listed in tables. Multiple comparisons were adjusted by the Tukey-Kramer test with a significance level of $P < 0.05$, whereas differences of $P > 0.05$ to $P \leq 0.10$ were considered as trends. Pearson correlation coefficients between variables were calculated using the CORR procedure of SAS (2002).

2.4 Results and discussion

2.4.1 Post-mortem pH-temp decline and ultimate pH

The pH/temperature window concept implemented in the Meat Standards Australia (MSA) grading scheme is used to monitor or identify carcasses at risk of cold shortening with pH > 6 at temperature < 15 °C or heat toughening with pH < 6 at

temperature > 35 °C. Cold shortening occurs while ATP is available for muscle contraction, whereas heat toughening occurs when the activity of proteolytic enzymes is reduced within the muscle, thereby reducing the ageing potential (Thompson, 2002). In this study, the only group of carcasses falling inside the cold-shortening window were from 15-month old dairy bulls (2 JEX and 1 HF) and no groups were inside the heat-shortening window (Figure 2.2). This was probably due to faster chilling of the lighter carcasses of 15-month old bulls and particularly JEX bulls.

The pH at 15 °C and at 35 °C was highest for 15-month old bulls ($P < 0.001$; Table 2.3). Likewise, at each hour post-mortem younger animals had higher pH values than older ones, probably due to their faster chilling rate resulting from insufficient subcutaneous fat cover. Early post-mortem pH contributes to meat tenderness as it affects the activity of endogenous enzymes with a pH_{3h} of 6.0-6.1 reflecting the optimum glycolytic rate to give the most tender meat (Chambaz et al., 2003), while individual carcass pH_{3h} values in this study ranged from 5.87 to 6.42, suggesting that tenderness would be variable.

The pHu was higher for 15-month old bulls ($P < 0.001$), while 19- and 22-month old bulls were similar (Table 2.3). The pHu did not differ between breed types or first season feeding ($P > 0.05$). Mean pHu values for all groups ranged from 5.53 to 5.74, which is within the range considered normal for beef of $5.4 \leq pHu \leq 5.7$ (Tarrant, 1989). No DFD (dark, firm, dry, $pH_u > 6.0$) meat was observed.

2.4.2 Meat colour

CIE L^* after both 2 h ($P < 0.01$) and 24 h ($P < 0.001$) blooming decreased with slaughter age (Table 2.3), in agreement with others (Page et al., 2001; Serra et al., 2004), indicating that meat from older animals was darker than meat from younger animals. Gil et al. (2001) found that muscle pigment content increased with age,

which is consistent with meat from older animals being darker. JEX beef was darker than HF after both 2 h ($P < 0.01$) and 24 h ($P < 0.05$) blooming. The paler appearance of HF beef was probably due to increased light scattering due to the larger muscle fibre gap relating to their higher cooking loss. Water loss during cooking is mainly from the juice expelled by myofibrillar lattice shrinkage caused by protein denaturation. Higher extent of muscle fibre shrinkage also creates larger gaps between fibres which could allow for an increased light scattering (Hughes et al., 2014). JEX bull beef was redder after 2 h blooming ($P < 0.05$). Likewise, the greater hue angle after both 2 h ($P < 0.001$) and 24 h ($P < 0.01$) blooming in HF compared to JEX beef indicates a less pure red colour in HF. The metmyoglobin reducing activity, or the amount of its essential cofactor NADH, can cause differences in redness development in meat between breeds (Bekhit & Faustman, 2005).

2.4.3 Warner-Bratzler shear force

Generally, it is recommended to age steak for at least 14 days to ensure tender beef (Farouk et al., 2009). Monsón et al. (2005) suggested that longer ageing periods (21 days) would be needed for Holstein beef to attain optimum acceptability. Hence, WB-variables and sensory traits of steaks aged for 21 days were investigated.

WB-slope and area are two other instrumental variables relating to sensory texture attributes. WB-slope or modulus was calculated to express ‘Shear-Firmness’, with higher values corresponding to lower elasticity (Brady & Hunecke, 1985). Total energy, corresponding to the total area under the WBSF curve, was used to describe the total energy consumed to chew the meat until it could be swallowed. It is hypothesized that the initial yield force occurring before maximum peak force is probably associated with the myofibrillar component and the final yield after maximum shear force corresponded to the connective tissue component (Moller, 1981). Consequently, the first peak of the WB curve is related to the contribution of the myofibrillar structure to toughness.

WBSF, WB-slope, WB-area and WB-first peak force were unaffected by breed type, age and first season feeding ($P > 0.05$; Table 2.4). The 15-month old dairy bulls did not produce more tender meat than the 19- and/or 22-month old bulls, in agreement with Sinclair et al. (1998). However, this is in contrast to Dransfield et al. (2003) who found that beef from 15-month old bulls was more tender than beef from 19-month or 24-month old bulls, which had similar tenderness levels. It is likely that, in the present study, cold-shortening occurred in some of the 15-month old bulls and increased the mean WBSF of that group. The relatively narrow range in WBSF values may be due to the extended post-mortem ageing of 21 days, which may also have contributed to removing any age effect. It was shown by Jurie et al. (2005) that differences in steak WBSF values between breeds disappeared after 14 days of ageing.

WBSF varied from 17.37 to 46.08 N for LT steaks aged for 21 days. Shackelford et al. (1991) categorized muscle groups into 'very tender' (WBSF < 31.36 N), 'tender' (31.36 < WBSF < 38.22 N), 'intermediate' (38.22 < WBSF < 45.08 N), and 'tough' (WBSF > 45.08 N). Based on this classification, only 4 animals could be considered 'intermediate' tough, and only 1 animal could be considered 'tough'. All other samples were in the 'very tender' and 'tender' categories. Likewise, Tatum et al. (1999) set the WBSF limit of 44.5 N as unacceptably tender beef. In addition, a 98% acceptability rating by American consumers would correspond to a WBSF value equal to, or below 40.18 N (Huffman et al., 1996). The mean WBSF value for the 12 groups investigated in this study ranged from 25.76 N (22-month old JEX bulls from PO) to 33.09 N (15-month old JEX bulls from PO). Therefore, LT steaks from HF and JEX bulls were considered tender after 21 days ageing.

2.4.4 Cooking loss

Cooking loss was affected only by breed type, with JEX bull beef having lower cooking loss than HF ($P < 0.01$; Table 2.4). The mean values of individual groups

varied between 26.6% and 30.9%. Pordomingo et al. (2012) found that muscles with a higher IMF content have lower cooking loss. Even though there was no difference between the breed types in IMF content in the present study, in the sensory evaluation beef from JEX bulls was found to be more fatty/greasy than that from HF ($P < 0.05$; Table 2.5).

2.4.5 Chemical composition

Moisture and ash contents were higher in beef from 15- than 22-month old bulls ($P < 0.05$), while IMF and protein content were unaffected by slaughter age ($P > 0.05$; Table 2.4). Pflanzer & Felício (2011) also found that beef from lower maturity animals had more moisture than those from more mature animals. Chemical composition parameters were similar for HF and JEX and for the two first season treatments ($P > 0.05$; Table 2.4). Mean IMF content in all groups ranged from 2.02 to 4.04%, which was within the range from 0.45% to 6.65% indicated in Waritthitham et al. (2010a) for IMF content among beef breeds. The average IMF content of LT muscle observed here was low (< 5%) and this finding is in agreement with others (O'Neill et al., 2004; Riley et al., 2005; Serra et al., 2008). An IMF level of approximately 3.25% was defined as a grade 'slight degree of marbling', and was reported to be preferred by US consumers on visual quality (Killinger et al., 2000). Most (47%) Swiss consumers preferred beef with 3 to 4% IMF, however, 27% selected beef with no visible marbling (Chambaz et al., 2003). According to these authors, IMF of below 3% was considered to result in tougher, drier, and less flavourful meat by most consumers. Approximately one half (n = 32) of the samples had IMF values above 3%, and therefore would be in the acceptable range for most consumers.

2.4.6 Collagen content and solubility

The total amount and chemical composition of collagen is believed to primarily determine the "background" toughness of beef after prolonged ageing. It is generally accepted that higher levels of intramuscular connective tissue, particularly the more mature cross-links are associated with reduced beef tenderness (Jeremiah et al., 2003a). Collagen content and solubility were unaffected by breed type, slaughter age and first season feeding ($P > 0.05$; Table 2.4). Collagen solubility during heat treatment depends on the number and extent of multivalent mature cross-links present between tropocollagens (Bailey, 1985). The accumulation of intermolecular cross-links accelerates with age, decreasing collagen thermal solubility (Weston et al., 2002). The relatively narrow range of animal age in this study may have been insufficient to result in differences in collagen characteristics. Dransfield (1977) reported the strong relationship between collagen content and cooked meat tenderness is mainly from samples with large variation in collagen content, e.g. inter-muscle comparisons. Shorthose & Harris (1990) pointed out that collagen-rich muscles were more likely to show age-associated toughness of beef. It is important to note that in the current study only the LT, a relatively tender muscle was used, which further reduced the potential for variation in collagen content and solubility. Similarly, no effect of age on collagen content of LD muscle from bulls and steers slaughtered at four ages was reported by Dikeman et al. (1986). Schönfeldt & Strydom (2011) also showed no age effect on collagen content of South African cattle. The lack of a diet effect on collagen is in accordance with Dikeman et al. (1986).

Mean total and insoluble collagen contents observed in all groups ranged from 6.06 to 7.09 mg/g wet tissue and from 5.16 to 6.21 mg/g, respectively, while collagen solubility ranged from 13.09% to 15.83%. A previous study showed that LT muscle from Jersey and Holstein bulls of 13 to 16 months old had 4.07 and 3.86 mg/g wet

tissue of total collagen, 2.96 and 3.02 mg/g of insoluble collagen and collagen solubility of 27.3% and 21.7%, respectively (Christensen et al., 2011). The higher contents of both total and insoluble collagen and the lower collagen solubility determined in the current study probably resulted from the different collagen determination method applied. Christensen et al. (2011) concluded that among most cattle breeds in Europe, the dairy breeds Jersey, Holstein and Danish Red have the highest total and insoluble collagen content while the meat breeds Piedmontese, Limousin and Asturiana de los Valles have the lowest values. However, these authors also determined that the percentage of heat-soluble collagen was highest in Jersey but lowest in Danish Red and Holstein beef when compared to all breeds investigated within their study. This may explain why in the current study HF beef was judged as relatively tougher than JEX beef by the sensory panel.

2.4.7 Sensory evaluation

2.4.7.1 Teagasc sensory evaluation

Residual roast beef flavour length was higher in 22- in comparison to 15-month old bulls ($P < 0.05$; Table 2.5). This was expected as Lawrie (1991b) and Dransfield et al. (2003) reported that flavour intensity increased with animal age. Intensity of fattiness/greasiness was higher in JEX beef ($P < 0.05$), in line with the finding that Jersey cattle tend to produce a highly-marbled product (Albertí et al., 2008), even though there was no difference in IMF content in the present study. Riley et al. (1986) found no differences in WBSF and overall palatability between Jersey-type and Holstein-type bull beef, while in this study JEX beef tended to have higher initial tenderness than HF beef ($P = 0.10$), which may be related to a higher WHC represented by less cooking loss. The difference in tenderness could also be due to variation in calpain and calpastatin activity contributing to variation in the rate and extent of muscle proteolysis during the post-mortem ageing period (Lawrie, 1991c).

Rancidity is an off-flavour resulting from enzymatic degradation processes and lipid peroxidation of unsaturated fatty acids which can occur in meat during ageing (Wood et al., 2003). The rancid flavour score in our study is surprisingly low (< 5) given the long ageing period adopted (21 days) and was probably due to storing beef samples under vacuum, thus reducing the rate of lipid oxidation (Resconi et al., 2010). The mean overall score for beef flavour and juiciness were 56 and 47 respectively in this study, in agreement with the sensory scores of young Friesian bulls reported by Partida et al. (2007). Initial tenderness score was higher (around 70) in this study, indicating that tender beef can be produced from dairy bulls after ageing for 21 days.

2.4.7.2 Bristol sensory evaluation

The intensity of greasiness during eating ('Eat-Greasy') was higher in 22- than in 15- month old bulls ($P < 0.05$; Table 2.6). As the samples were trimmed before tasting by the panel, greasiness or fattiness intensity scores from beef samples originated from IMF. Compared with muscle growth, fat is deposited at a slower rate during the first period of postnatal life, however, when animals get older, a greater rate of fat deposition occurs. Therefore, the fat concentration within muscle (i.e., IMF content) will inevitably increase later in an animal's life (Pflanzer & de Felício, 2011). A higher deposition of IMF will occur at more advanced stages of maturity, even though the longer *ad-lib* concentrate diet was used in the 15- compared with the 22-month system.

Beef from the JEX breed was given higher scores for 'Juiciness', 'Eat-Pulpy' and 'Res-Pulpy' ($P < 0.05$; Table 2.6). Pulpy is related to a soft and soggy sensation, which is inversely correlated with dryness and firmness. Therefore, samples scoring higher for pulpy related attributes also had higher scores for juiciness and tenderness. The higher juiciness scores of JEX beef could be explained by their lower cooking loss. In addition, JEX beef tended to have higher sensory scores for 'Ease of Cutting' and 'Eat-Dissolubility' than HF beef ($P < 0.10$). Dissolubility

determined the degree of melting or disintegration of samples and the ease with which meat breaks down into particles in the mouth, which is related to the texture of the beef.

Considering the primary sensory parameters, the panel rated the two first season treatments similarly. However, the panel rated HF beef (22 months old) as having the highest score for 'Overall Acceptability' (5.26), and HF beef (19 months old) with the lowest 'Overall Acceptability' (4.85) among all groups examined.

2.4.7.3 Summary for two sensory studies

There were considerable agreements between both sensory panels. Firstly, both panels determined that with increasing animal age, the intensity of 'Res-Roast Beef Flavour Length' or 'Eat-Greasy' sensation increased, and it is reasonable that a higher IMF level can increase the flavour acceptability of beef (Corbin et al., 2015). Secondly, both panels rated JEX beef texture better than HF beef. Additionally, in both sensory studies, first season feeding had no effect on sensory parameters, which concurs with the finding from Bidner et al. (1986) that diet did not affect WBSF or sensory tenderness of beef *longissimus* muscle. Nevertheless, Schroeder et al. (1980) found a lower tenderness rating for pasture-finished steers over grain-finished steers. Bennett et al. (1995) conversely found that LT steaks from pasture fed steers were rated more tender by panelists than steaks from feedlot steers. The conflicting results for the effect of feeding system on beef tenderness probably derive from differences in the amount, length of feeding time and type of concentrate fed to animals.

2.4.8 Correlations between variables

Both pH (15 °C) ($P < 0.05$) and pH (35 °C) ($P < 0.01$) were negatively correlated with WB-first peak force (Table 2.7) confirming that rate of pH fall has an effect on myofibrillar tenderness, but not with other WB-variables. Likewise, pH (35 °C) was positively correlated with sensory scores for initial tenderness and ease of

disintegration ($P < 0.05$), which indicated the potential that heat-shortening increases meat toughness although no heat-shortening occurs in this study. L* was positively correlated with cooking loss ($P < 0.01$), in agreement with Frylinck et al. (2013) who found that lightness was negatively correlated with WHC. WB-slope was positively correlated with cooking loss ($P < 0.05$), which agreed with Monteiro et al. (2013) and inversely correlated with IMF and soluble collagen content ($P < 0.05$). Samples with higher cooking loss had higher moisture content ($P < 0.05$), which is in line with Chambaz et al. (2003).

WB-variables were positively correlated with cohesiveness ($P < 0.001$), chewiness, stringiness ($P < 0.05$) and negatively correlated with initial tenderness and ease of disintegration ($P < 0.05$; Table 2.8). The correlations between WBSF and sensory tenderness are in agreement with others (Chambaz et al., 2003; Schönfeldt & Strydom, 2011; Monteiro et al., 2013). IMF content was positively correlated with initial tenderness ($P < 0.01$) and negatively correlated with cohesiveness ($P < 0.05$), in agreement with several reports that meat tenderness can be improved by IMF content (Savell & Cross, 1988; Sami et al., 2004; Corbin et al., 2015) as IMF dilutes the fibrous protein in muscle tissue resulting in a decrease in the muscle resistance to shearing (Wood et al., 1999).

Soluble collagen content was positively correlated with initial tenderness ($P < 0.01$), ease of disintegration ($P < 0.05$) and negatively correlated with stringiness ($P < 0.01$; Table 2.8). Stringiness means fibrous with long, thin string-like pieces that are hard to chew, thus it relates to chewiness. However, in this study, no correlations between total collagen content and WB variables or sensory tenderness parameters were found ($P > 0.05$). This corresponds with other findings that while total collagen content is not directly associated with objective and sensory texture characteristics, percentage of soluble collagen was the main determinant of sensory panel tenderness and ease of fibre fragmentation (Bailey, 1985; Schönfeldt & Strydom, 2011).

Juiciness was positively correlated with initial tenderness ($P < 0.01$) and negatively correlated with chewiness ($P < 0.05$; Table 2.8), which is in agreement with others (e.g., Serra et al., 2008; Monteiro et al., 2013). This was probably a result of juices being released more quickly through chewing more tender meat, thereby contributing to a juicer meat sensation (Savell & Cross, 1988). Roast beef flavour was positively correlated with juiciness ($P < 0.01$), in agreement with Monteiro et al. (2013). A positive correlation was also observed between roast beef flavour and initial tenderness score ($P < 0.05$), which was in accordance with the finding by Gill et al. (2010). This effect could be due to the 'halo effect', that is when only a few attributes of a product are evaluated, the ratings will tend to influence each other (Meilaard et al., 1999). Likewise, initial tenderness had high positive relationship with ease of disintegration and negative relationship with cohesiveness, chewiness and stringiness ($P < 0.001$). It seems that when a panellist rated a piece of tender meat, he/she was also prone to giving higher score to other traits, particularly the unrelated ones, such as flavour (Gill et al., 2010).

2.5 Conclusions

The eating quality of beef from these young dairy bulls was generally good after 21 days ageing. Only three of the samples had a WBSF score above 40 N, indicating that most beef samples were acceptably tender. Some eating quality attributes were affected by breed and age at slaughter, but first season feeding had no effect. With age at slaughter increasing from 15 to 22 months, beef became darker, moisture decreased and the meat was judged to hold a longer beef flavour length and more greasy during eating during sensory evaluation. JEX beef had lower cooking loss, was more greasy, juicy and relatively tender than HF beef. Therefore, these data suggest that crossbreeding the Jersey breed with HF might improve the beef quality of young dairy bulls though a larger scale study would be needed to establish definitively the relative merits of the two breeds. Variation in cooking loss, IMF and

soluble collagen content could explain the differences determined in cooked meat texture. Higher WB-variables (WB-slope, WB-area and WB-first peak force) were associated with higher sensory cohesiveness and chewiness scores, and lower scores for initial tenderness and ease of disintegration. The effects on colour could be important in terms of selecting carcasses for different markets. Marbling level had positive influence on sensory texture parameters of young dairy bull beef. It can be concluded that good quality beef can be produced from young dairy bulls of different breed types.

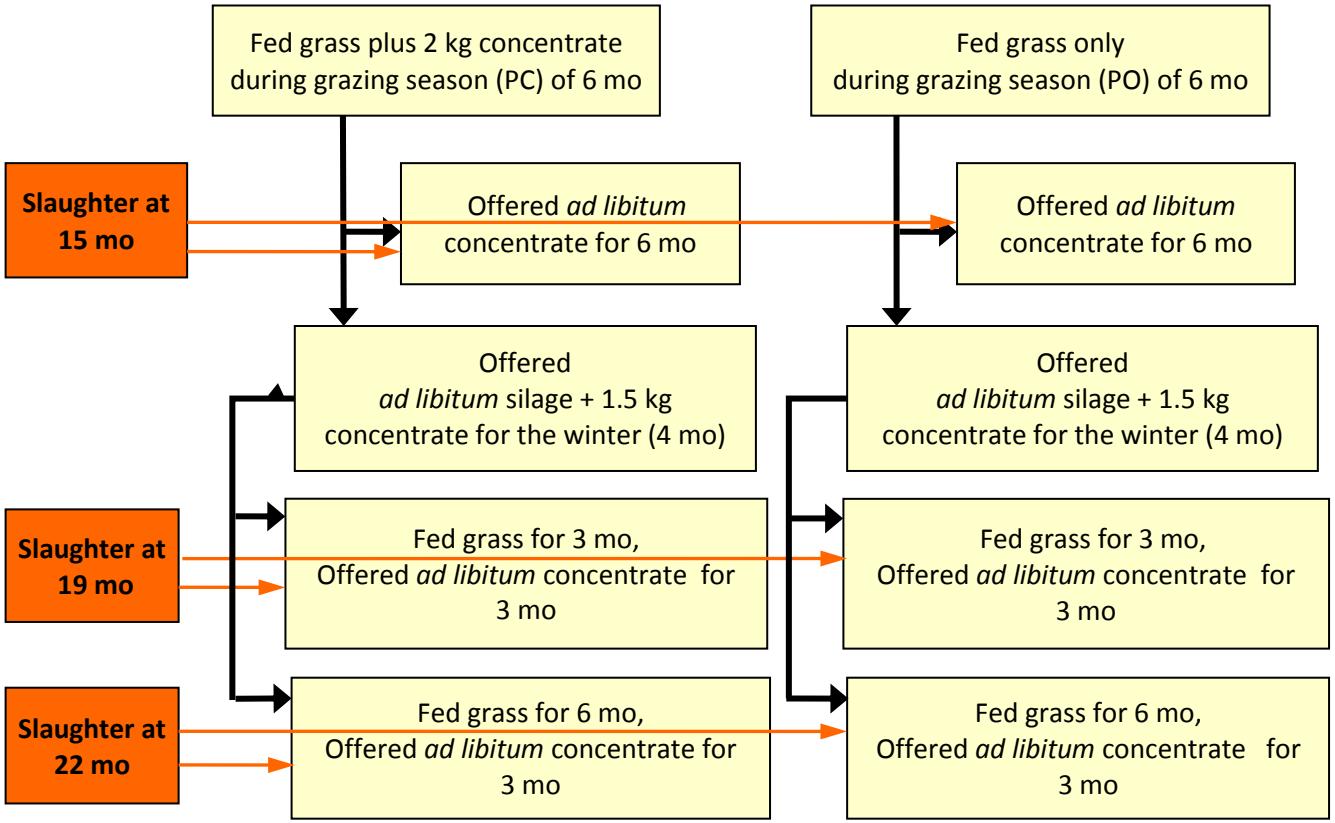


Figure 2.1. Production treatments for young dairy bulls.

Table 2.1. Profile description of sensory attributes of LT muscles of young dairy bulls by 'Teagasc study'.

Attribute	Definition	Scale anchors[1]
Aroma		
Roast Beef Aroma	Intensity of roast beef aroma	Weak-Strong
	Cut beef sample into bite sized pieces. Put a bite sized piece into the mouth and assess the resistance to the teeth (molars)	
Texture		
Initial Tenderness	The resistance to teeth biting through the sample	Not-Very
During Eating		
Juiciness	In the first 3-4 chews, the moisture release from sample	Not-Very
Cohesiveness	In the first 3-4 chews, how well the sample holds its structure	
Ease of Disintegration	After 6-7 chews, the ease by which the meat breaks down into particles in the mouth	Not easily-Easily
Chewiness	Number of chews/the force required to break down sample to swallow	Not-Very
Fattiness/Greasiness	Fatty film on the inside of the mouth during mastication	Not-Very
Just before swallowing		
Stringiness	Sensation of strings during mastication	Not-Very
Astringent	Drying effect in the mouth during mastication	Not-Very
	Using a new bite sized piece of beef-assess the flavour and after effects attributes	
Flavour		
Roast Beef Flavour	Intensity of roast beef flavour during mastication	Low-High
Metallic	Flavour associated with iron or blood	Not-Very
Stale/Rancid/Aged	Flavour of stale, aged, gone off beef	Not-Very
	Swallow the beef and wait for 12 seconds before scoring after effects below	
After effect		
Roast Beef Flavour Length	Intensity of beef flavour left 12 seconds after swallowing the sample	Not-Very
Metallic	Flavour associated with iron or blood after swallowing	Not-Very
Fattiness/Greasiness	Fatty mouth coating after swallowing the sample	Not-Very
Dryness	Dry mouthfeel after swallowing the sample	Not-Very

[1] First term anchors left end of scale-second term anchors right.

Table 2.2. Profile description of sensory attributes of LT muscles of young dairy bulls by 'Bristol study'.

Attribute	Description	Scale anchors[1]
On Cutting		
Ease of Cutting	Ease with which sample is cut through by knife	Hard-Easy
Cleanness of Cut	Appearance of sample on cutting with knife	Jagged fibres- Very clean cutting
Initial Chewing (Bite)		
Bite-Tough	Amount of resistance to teeth on initial chewing	Low-High
Bite-Crunchy	Amount of perceived crispness in the sample on initial chewing	Nil- Extreme
Bite-Juiciness	Amount of moisture in the sample on initial chewing	Dry- Juicy
Bite-Sponginess	Amount of springiness in the sample, bounce back to bite	Nil- Extreme
On Eating		
Eat-Tough	Toughness on eating	Low-High
Eat-Moisture	The perceived moisture content in the sample during eating	Dry- Wet
Eat-Chewiness	The total perceived effort required to prepare the sample to a state ready for swallowing	Nil- Extreme
Eat-Greasy	Amount of perceived oil or fatty matter in the sample on eating	Not-Very
Eat-Fibres	Amount of perceived fibres in the sample on eating	Nil- Extreme
Eat-Gristle	Amount of gristle in the sample	Nil- Extreme
Eat-Pulpy	Pulpiness in the sample on eating	Dry- Soft, soggy
Eat-Dissolubility	Degree to which it melts or disintegrates in mouth	None- Dissolves or disintegrates
Residue		
Res-Greasy Mouthfeel	Amount of greasy coating in the mouth	Nil- Extreme
Res-Ease of Swallow	Degree to which the residue is easy to swallow	Not-Very
Res-Pulpy	Pulpiness in the residue	Dry- Soft, soggy
Res-Particles	Fine particles in residue	Not-Lots
Res-Mouthfeel at end	Sensation in the mouth after chewing	Dry- Wet

[1] First term anchors left end of scale-second term anchors right.

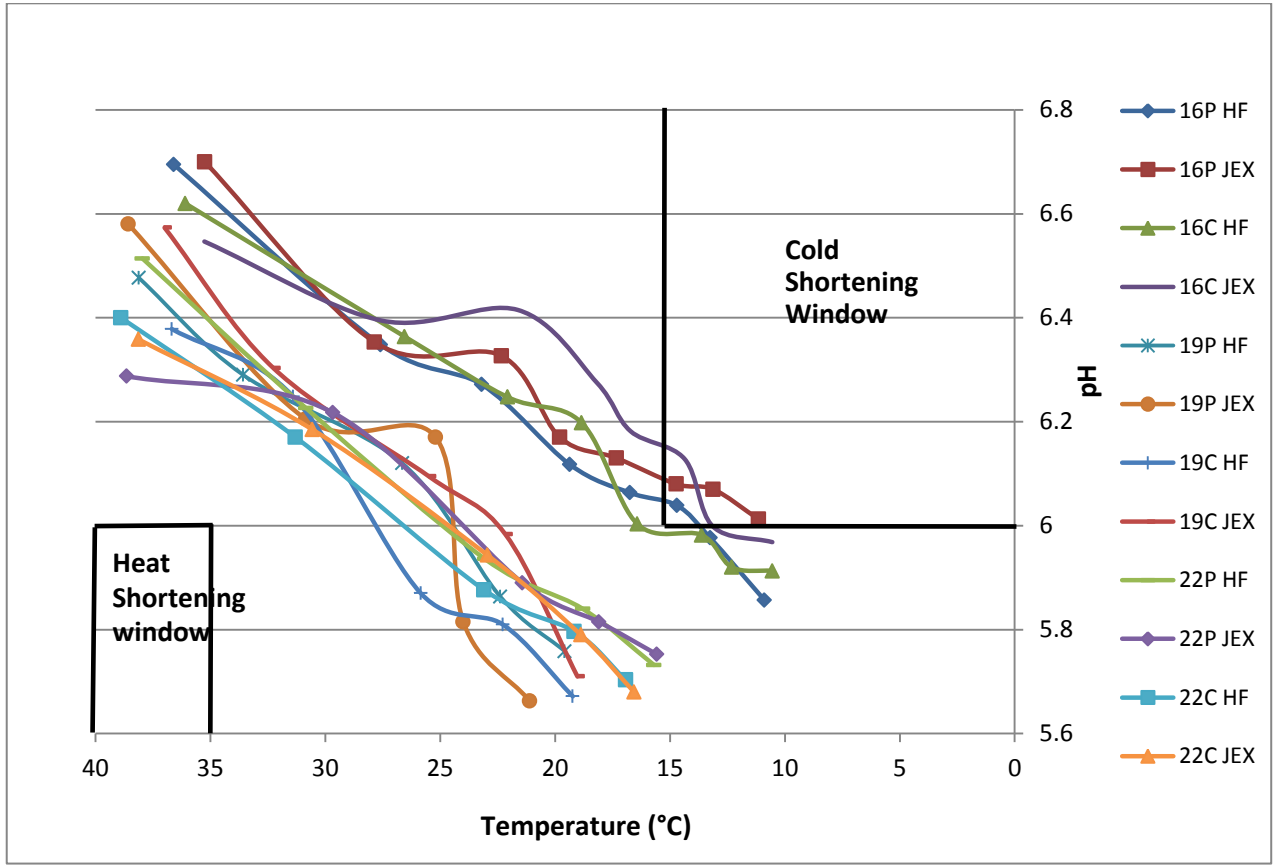


Figure 2.2. Post-mortem pH and temperature decline of young dairy bulls up to 8 hours after slaughter (average for the animals in each production group). 15, 19, 22 = slaughter age (months). PO = grass only during first grazing season; PC = grass plus 2 kg concentrate during first grazing season. HF = Holstein-Friesian; JEX = Jersey × Holstein-Friesian. Cold and heat shortening windows were according to the review of Thompson (2002).

Table 2.3. pH at 15 and 35 °C, pHu, and colour after 2 h and 24 h blooming of LT muscles of young dairy bulls.

	Age (months) (A)						Breed (B)				First season (F)				*P*-value		
	15		19		22		HF		JEX		Concentrates		Pasture		A	B	F
	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM			
pH (15°C)[1]	6.05[a]	0.04	5.48[c]	0.04	5.69[b]	0.04	5.73	0.03	5.74	0.04	5.75	0.03	5.73	0.03	<.001	0.798	0.766
pH (35°C)[2]	6.65[a]	0.05	6.40[b]	0.06	6.30[b]	0.06	6.44	0.04	6.46	0.05	6.46	0.04	6.44	0.04	<.001	0.835	0.660
pHu[3]	5.73[a]	0.02	5.55[b]	0.03	5.57[b]	0.02	5.62	0.02	5.61	0.02	5.60	0.02	5.62	0.02	<.001	0.749	0.471
L* 2h	45.1[a]	0.60	42.9[b]	0.62	42.1[b]	0.61	44.4[a]	0.46	42.4[b]	0.53	43.0	0.50	43.7	0.49	0.003	0.006	0.342
a* 2h	16.4	0.37	15.9	0.38	16.2	0.37	15.7[b]	0.28	16.7[a]	0.32	16.4	0.31	16.0	0.30	0.700	0.030	0.448
b* 2h	13.2	0.31	12.6	0.32	12.6	0.31	13.1	0.24	12.4	0.27	12.7	0.26	12.9	0.25	0.229	0.058	0.556
Hue angle 2h	38.9	0.64	38.4	0.66	37.7	0.64	39.9[a]	0.49	36.7[b]	0.56	37.8	0.54	38.9	0.52	0.429	<.001	0.153
Saturation 2h	21.1	0.42	20.3	0.44	20.5	0.43	20.5	0.33	20.8	0.37	20.7	0.35	20.6	0.34	0.450	0.540	0.821
L* 24h	45.2[a]	0.47	43.4[b]	0.49	42.0[b]	0.47	44.3[a]	0.36	42.8[b]	0.41	43.0	0.39	44.1	0.38	<.001	0.011	0.067
a* 24h	17.6[a]	0.47	15.7[b]	0.49	17.5[a]	0.47	16.7	0.36	17.2	0.41	17.2	0.39	16.7	0.38	0.010	0.395	0.329
b* 24h	14.0[a]	0.31	12.1[b]	0.32	13.4[a]	0.32	13.5	0.24	12.8	0.28	13.0	0.26	13.3	0.25	0.001	0.073	0.552
Hue angle 24h	38.6	0.59	37.7	0.62	37.4	0.60	39.0[a]	0.46	36.8[b]	0.52	37.2	0.50	38.5	0.48	0.330	0.003	0.067
Saturation 24h	22.5[a]	0.52	19.8[b]	0.54	22.1[a]	0.52	21.5	0.40	21.4	0.46	21.6	0.44	21.3	0.42	0.002	0.939	0.645

LSM = least square means; SEM = standard error of LSM.

[a-c] Means within a row within a main effect with different superscripts significantly differ ($P < 0.05$).

[1] pH (15°C) = pH at the temperature was 15°C.

[2] pH (35°C) = pH at the temperature was 35°C.

[3] pHu = Ultimate pH.

Table 2.4. WB-variables, cooking loss, chemical composition and collagen characteristics of LT muscles of young dairy bulls.

	Age (months) (A)						Breed (B)				First season (F)				*P*-value		
	15		19		22		HF		JEX		Concentrates		Pasture		A	B	F
	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM			
WBSF[1] (N)	29.6	1.15	29.8	1.39	27.7	1.38	28.4	1.01	29.6	1.13	28.9	1.09	29.1	1.05	0.495	0.406	0.880
WB-slope (Mpa)	0.56	0.03	0.56	0.03	0.50	0.03	0.54	0.02	0.54	0.03	0.55	0.03	0.53	0.02	0.263	0.914	0.499
WB-area (J)	0.28	0.01	0.28	0.01	0.27	0.01	0.27	0.01	0.29	0.01	0.28	0.01	0.28	0.01	0.738	0.380	0.955
WB-first peak force (N)	23.6	0.84	26.0	1.02	24.6	1.01	24.5	0.74	24.9	0.83	24.8	0.80	24.7	0.77	0.209	0.724	0.929
Cooking loss (%)	29.3	0.49	28.7	0.59	28.8	0.59	29.9[a]	0.43	27.9[b]	0.48	29.3	0.46	28.5	0.45	0.632	0.003	0.233
Moisture (%)	73.3[a]	0.20	72.6[ab]	0.24	72.4[b]	0.24	72.9	0.17	72.7	0.19	72.7	0.19	72.9	0.18	0.013	0.633	0.357
IMF[2] (%)	2.76	0.26	3.34	0.31	3.26	0.31	3.08	0.23	3.15	0.25	3.28	0.24	2.95	0.24	0.287	0.834	0.337
Protein (%)	22.6	0.16	22.3	0.20	22.7	0.19	22.5	0.14	22.6	0.16	22.5	0.15	22.6	0.15	0.442	0.713	0.775
Ash (%)	1.07[a]	0.01	1.05[ab]	0.01	1.03[b]	0.01	1.05	0.01	1.05	0.01	1.05	0.01	1.05	0.01	0.049	0.630	0.943
Soluble collagen (mg/g)	0.95	0.03	0.92	0.04	0.88	0.04	0.92	0.03	0.92	0.03	0.91	0.03	0.92	0.03	0.437	0.951	0.774
Insoluble collagen (mg/g)	5.84	0.31	5.52	0.39	5.63	0.40	5.53	0.28	5.80	0.32	5.67	0.30	5.66	0.31	0.808	0.537	0.979
Total collagen (mg/g)	6.75	0.34	6.41	0.42	6.48	0.43	6.41	0.30	6.68	0.35	6.55	0.32	6.55	0.33	0.787	0.560	0.998
Collagen solubility (%)	14.4	0.54	15.1	0.68	13.9	0.69	14.7	0.48	14.2	0.56	14.3	0.52	14.5	0.53	0.487	0.530	0.794

LSM = least square means; SEM = standard error of LSM.

[a,b] Means within a row within a main effect with different superscripts significantly differ ($P < 0.05$).

[1]WBSF = Warner-Bratzler Shear Force.

[2]IMF = Intramuscular fat.

Table 2.5. Teagasc sensory evaluation of LT muscles of young dairy bulls.

	Age (months) (A)						Breed (B)				First season (F)				*P*-value		
	15		19		22		HF		JEX		Concentrates		Pasture		A	B	F
	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM			
Roast Beef Aroma	56.5	1.70	63.7	2.70	60.0	2.24	58.5	1.61	61.7	2.04	61.9	2.01	58.3	1.64	0.080	0.220	0.175
Initial Tenderness	72.7	2.43	69.4	3.84	69.5	3.19	67.4	2.29	73.6	2.91	71.4	2.86	69.6	2.34	0.649	0.100	0.643
Juiciness	51.0	2.90	48.8	4.59	41.0	3.81	44.3	2.74	49.5	3.47	49.7	3.42	44.1	2.80	0.122	0.244	0.215
Cohesiveness	53.4	2.50	56.2	3.95	52.7	3.28	55.1	2.36	53.1	2.99	53.6	2.95	54.6	2.41	0.774	0.592	0.784
Ease of Disintegration	74.9	2.27	66.9	3.60	70.7	2.99	69.5	2.15	72.1	2.72	69.9	2.68	71.8	2.2	0.159	0.462	0.597
Chewiness	29.5	2.67	34.4	4.22	31.1	3.50	32.6	2.52	30.7	3.19	31.4	3.15	32.0	2.57	0.624	0.641	0.880
Fattiness/Greasiness	15.1	0.97	14.5	1.53	14.7	1.27	13.0[b]	0.91	16.5[a]	1.16	15.2	1.14	14.3	0.93	0.935	0.022	0.549
Stringiness	11.5	1.51	15.0	2.39	15.0	1.98	15.5	1.43	12.2	1.81	15.8	1.78	11.9	1.46	0.272	0.152	0.102
Astringent	16.0	1.60	17.3	2.54	20.7	2.10	17.7	1.51	18.2	1.92	18.0	1.89	18.0	1.55	0.217	0.838	0.981
Roast Beef Flavour	54.0	1.91	57.0	3.02	56.6	2.51	54.2	1.80	57.5	2.29	53.5	2.25	58.2	1.84	0.597	0.271	0.114
Metallic	12.8	1.88	17.5	2.98	16.3	2.47	13.0	1.78	18.1	2.25	16.2	2.22	14.9	1.81	0.320	0.086	0.670
Stale/Rancid/Aged	3.45	0.63	4.94	1.00	4.13	0.83	3.55	0.60	4.80	0.76	4.98	0.75	3.37	0.61	0.443	0.202	0.103
Res[1]-RBFL[2]	47.9[b]	1.71	50.7[ab]	2.70	55.1[a]	2.24	49.9	1.61	52.6	2.04	49.4	2.01	53.0	1.65	0.048	0.300	0.181
Res-Metallic	12.3	1.85	17.3	2.92	17.9	2.42	14.3	1.74	17.4	2.21	17.6	2.18	14.0	1.78	0.140	0.288	0.207
Res-Fattiness/Greasiness	16.7	1.13	17.5	1.80	16.4	1.49	16.1	1.07	17.6	1.36	17.8	1.34	15.9	1.09	0.889	0.388	0.283
Res-Dryness	16.7	1.62	17.6	2.56	17.7	2.13	18.3	1.53	16.4	1.94	16.4	1.91	18.2	1.56	0.907	0.461	0.469

LSM = least square means; SEM = standard error of LSM.

[a,b] Means within a row within a main effect with different superscripts significantly differ ($P < 0.05$).

[1]'Res-' = Residual (after effects).

[2]Res-RBFL = Res-Roast Beef Flavour Length.

Table 2.6. Bristol sensory evaluation of LT muscles of young dairy bulls.

	Age (months) (A)						Breed (B)				First Season (F)				*P*-value		
	15		19		22		HF		JEX		Concentrates		Pasture		A	B	F
	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM			
Texture	4.84	0.17	4.90	0.17	5.21	0.18	4.82	0.14	5.15	0.14	5.04	0.15	4.93	0.14	0.302	0.109	0.594
Juiciness	5.11	0.10	5.15	0.10	5.19	0.11	5.00^{b}	0.08	5.31^{a}	0.09	5.09	0.09	5.21	0.08	0.883	0.013	0.349
Beef Flavour	4.53	0.07	4.61	0.07	4.51	0.07	4.57	0.06	4.54	0.06	4.57	0.06	4.54	0.06	0.594	0.701	0.738
Abnormal Beef Flavour	2.37	0.07	2.37	0.07	2.42	0.07	2.33	0.05	2.44	0.06	2.35	0.06	2.42	0.05	0.839	0.147	0.361
Flavour Like	5.30	0.08	5.34	0.08	5.22	0.08	5.31	0.07	5.26	0.07	5.29	0.07	5.27	0.07	0.623	0.587	0.828
Overall Acceptability	5.00	0.09	4.99	0.09	5.08	0.09	5.00	0.07	5.05	0.07	5.02	0.07	5.03	0.07	0.711	0.639	0.963
Ease of Cutting	55.0	2.54	55.1	2.58	59.9	2.64	54.0	2.10	59.3	2.13	57.0	2.15	56.3	2.07	0.335	0.084	0.815
Cleanness of Cut	56.0	2.06	55.9	2.09	59.5	2.14	56.0	1.70	58.2	1.73	57.4	1.74	56.9	1.68	0.388	0.372	0.841
Bite[1]-Toughness	42.6	2.80	41.8	2.85	37.4	2.92	43.0	2.31	38.2	2.35	40.5	2.38	40.7	2.29	0.399	0.155	0.962
Bite-Crunchy	24.4	1.13	24.0	1.15	24.4	1.18	24.3	0.94	24.3	0.95	24.7	0.96	23.9	0.93	0.960	0.966	0.553
Bite-Juiciness	49.3	1.26	50.5	1.28	50.3	1.32	48.8	1.04	51.3	1.06	49.3	1.07	50.7	1.03	0.750	0.105	0.365
Bite-Sponginess	24.8	1.03	25.6	1.05	25.2	1.08	25.4	0.85	25.0	0.87	25.6	0.88	24.8	0.84	0.868	0.748	0.559
Eat[2]-Toughness	42.2	2.78	41.5	2.82	37.2	2.89	42.7	2.29	37.9	2.33	40.4	2.36	40.2	2.27	0.411	0.153	0.959
Eat-Moisture	51.0	1.39	52.8	1.41	52.5	1.44	50.9	1.14	53.4	1.16	51.9	1.18	52.3	1.13	0.627	0.130	0.813
Eat-Chewiness	37.3	2.79	36.3	2.84	33.7	2.90	38.4	2.30	33.1	2.34	36.7	2.37	34.9	2.28	0.654	0.112	0.586
Eat-Greasy	16.0^{b}	1.07	17.9^{ab}	1.09	20.3^{a}	1.12	17.7	0.89	18.4	0.90	18.6	0.91	17.5	0.88	0.035	0.564	0.408
Eat-Fibres	42.0	1.65	40.9	1.67	41.7	1.71	42.9	1.36	40.2	1.38	41.3	1.40	41.7	1.34	0.894	0.166	0.832
Eat-Gristle	7.58	1.01	9.06	1.03	7.70	1.06	8.44	0.84	7.79	0.85	8.01	0.86	8.21	0.83	0.534	0.587	0.866
Eat-Pulpy	56.8	1.22	56.2	1.24	57.5	1.27	55.2^{b}	1.01	58.5^{a}	1.02	56.8	1.03	56.9	1.00	0.776	0.026	0.970
Eat-Dissolubility	44.9	1.99	44.6	2.02	49.2	2.07	44.1	1.64	48.4	1.67	47.0	1.69	45.5	1.63	0.228	0.075	0.548
Res[3]-Greasy Mouthfeel	16.6	0.97	17.6	0.99	18.9	1.01	18.1	0.80	17.3	0.81	18.1	0.82	17.3	0.79	0.244	0.511	0.468
Res-Ease of Swallow	60.1	1.85	60.2	1.88	61.8	1.92	59.9	1.52	61.5	1.55	62.0	1.56	59.3	1.51	0.762	0.451	0.222
Res-Pulpy	57.3	1.27	56.9	1.29	58.6	1.32	55.9^{b}	1.05	59.4^{a}	1.07	57.7	1.08	57.5	1.04	0.645	0.024	0.908
Res-Particles	48.6	1.18	47.8	1.20	50.9	1.23	49.4	0.97	48.9	0.99	49.2	1.00	49.0	0.96	0.181	0.717	0.869
Res-Mouthfeel at End	58.0	1.29	57.5	1.31	58.3	1.34	56.9	1.06	58.9	1.08	57.5	1.09	58.3	1.05	0.911	0.198	0.567

LSM-least square means; SEM-standard error of LSM.

[a,b] Means within a row within a main effect with different superscripts significantly differ ($P < 0.05$).

[1]'Bite-'=First bite; [2]'Eat-'=During eating; [3]'Res-'=Residual (after effects).

Table 2.7. Pearson correlation coefficients between physico-chemical traits of LT muscles of young dairy bulls.

	WBSF	WB-slope	WB-area	WB-first peak force	Cooking loss	IMF	Moisture	Soluble collagen
pH (15°C)	-0.13	-0.01	-0.10	-0.26*	0.19	0.14	0.25*	0.10
pH (35°C)	-0.13	-0.09	-0.07	-0.34**	0.04	0.09	0.06	0.06
L*2h	0.12	0.10	0.12	-0.16	0.45**	-0.05	0.13	-0.08
L*24h	0.13	0.15	0.13	-0.02	0.45**	-0.11	0.22	0.06
WBSF[1]		0.82***	0.92***	0.66***	0.19	-0.20	0.16	-0.23
WB-slope			0.66***	0.61***	0.25*	-0.32*	0.30*	-0.31*
WB-area				0.54***	0.23	-0.16	0.14	-0.24
Cooking loss						-0.23	0.25*	-0.11
IMF[2]							-0.90***	0.19

Significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.
[1]WBSF = Warner-Bratzler shear force.
[2] IMF = Intramuscular fat.

Table 2.8. Pearson correlation coefficients between physico-chemical and sensory traits of LT muscles of young dairy bulls.

	Initial Tenderness	Ease of Disintegration	Cohesiveness	Chewiness	Stringiness	Juiciness
WBSF[1]	-0.42**	-0.41**	0.52***	0.47**	0.20	-0.02
WB-slope	-0.54***	-0.52***	0.56***	0.53***	0.37*	-0.03
WB-area	-0.26	-0.29*	0.45**	0.40**	0.07	-0.02
WB-first peak force	-0.41**	-0.40**	0.48***	0.37**	0.15	0.05
IMF[2]	0.37**	0.29*	-0.29*	-0.26	-0.20	0.01
Soluble collagen	0.31*	0.29*	-0.20	-0.21	-0.37*	0.23
Initial Tenderness	1.00	0.73***	-0.55***	-0.69***	0.63***	0.38**
Roast Beef Flavour	0.25*	0.12	-0.13	-0.15	-0.26	0.32*
Chewiness	-0.69***	-0.77***	0.59***	1.00	0.60***	-0.32*

Significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.
[1]WBSF = Warner-Bratzler shear force.
[2]IMF = Intramuscular fat.

Chapter 3

Physico-chemical and sensory characteristics of young dairy bull beef derived from two breed types across five production systems employing two first season feeding regimes

3.1 Abstract

The study objective was to assess the physico-chemical and sensory characteristics of beef from young dairy bulls; Holstein-Friesian (HF) and Jersey×Holstein-Friesian (JEX). Bulls were finished either on silage with 5 kg concentrate or, *ad-libitum* concentrate and slaughtered at 15-months old, while 19-month old bulls differed in energy consumption during a second grazing season (*ad-libitum* pasture versus pasture with 5 kg concentrate) and finishing period (pasture with 5 kg concentrate versus *ad-libitum* concentrate). Either 1 or 2 kg of concentrate was offered to all bulls at pasture during the first grazing season. *Longissimus thoracis* (LT) muscle was removed from 112 cube rolls and evaluated. Post-mortem pH, ultimate pH, chemical composition, collagen content and solubility were measured. Meat colour, Warner-Bratzler (WB) variables, thawing loss, cooking loss, and trained sensory panel evaluation were determined following ageing for 21 days. Insoluble and total collagen contents increased with slaughter age, while collagen solubility and hue angle reduced with age. Beef from 19-month old bulls fed a higher concentrate finishing diet possessed a longer beef flavor as determined by sensory evaluation. Intramuscular fat content (IMF%) and beef flavour score were enhanced, and moisture content (%) reduced, by higher concentrate intake in combination with second season and finishing period. Beef from a higher forage diet displayed a more intense red colour (higher a* and saturation), had higher thawing loss and an increased astringent taste. However, there was no independent effect of silage finishing or second season feeding on quality parameters. Similarly, first season diet had limited effects on meat quality traits. Beef from JEX breed type had higher IMF, lower moisture, higher beef flavour, juiciness and texture-related scores than HF beef. WB-slope was positively correlated with thawing loss and negatively correlated with IMF. WB-area was negatively correlated with soluble collagen content and collagen solubility. WBSF was positively correlated with chewiness and stringiness, but negatively correlated with initial tenderness, juiciness and beef flavour scores.

Keywords: Dairy breed, Feeding regime, Meat quality, Sensory analysis, Tenderness, Young bulls

3.2 Introduction

The number of male calves from the Irish dairy herd has markedly increased following the abolition of EU milk quotas in 2015. This is a potential new resource for the industry if they can be reared economically to produce meat of acceptable eating quality. Raising them as bulls may be the most viable option due to their improved growth rate and feed conversion efficiency compared with steers. A review of studies carried out in Ireland showed that, on average, bulls have an 8.4% higher live weight gain, 9.5% heavier carcass weight and 20% greater lean meat yield than steers reared in the same way (Fallon et al., 2001). Moreover, the lower carbon emissions from bull production systems compared with steers will benefit sustainable farming and the environment (Dawson, 2010). Although the number of bulls reared increased in the last decade, any further growth in bull beef production is constrained by the reluctance of processors to purchase bulls arising from concerns pertaining to acceptability of bull beef.

Holstein-Friesian (HF) is the predominant Irish dairy breed. However, there is interest in using Jersey cross cows due to their improved reproductive efficiency, intake capacity and concentration of milk solids (Prendiville et al., 2011). Growth rate, carcass traits and performance of HF and Jersey×Holstein-Friesian (JEX) bulls were recently studied (McNamee et al., 2015), however, eating quality has not been extensively investigated.

The grass utilization advantage of Ireland resulting from the temperate climate allows Irish farms to exploit the natural comparative advantages related to grass-based feeding instead of high input production systems (O'Donovan et al., 2010). Supplementation with concentrates is inevitable to sustain adequate growth rates of cattle, particularly during the winter. A higher energy level of feed, rather than the diet *per se,* was suggested to benefit cattle growth rate and beef quality (Muir et al., 1998a). Therefore, optimizing the contribution of grazed grass, combined with an optimum quantity of concentrates would be the prerequisite for economic sustainability, production efficiency and beef quality under Irish conditions. The beef industry has great potential to increase grass utilization particularly in late autumn (first grazing season) and early spring (second grazing season). Turning cattle out to grass in early spring can substantially reduce the overall feed budget (concentrate

and grass silage), reduce labour input and slurry accumulation, and increase animal performance (O'Donovan et al., 2011). Thereby, the efficient utilization of grass during the grazing season is an important factor for sustainable production. The effects of finishing systems on beef quality have been widely researched, little information is available about the effect of grazing season on beef quality.

Another study conducted in Teagasc indicated that the 19-month old bull system was more profitable than the 15-month system, largely owing to the high output per hectare and heavy carcass weight of the former. However, with current market trends, bulls are typically slaughtered not older than 16 months of age (Kelly et al., 2013). Hence, it is worthwhile to assess beef quality at these two ages to evaluate the integrated effectiveness of age-related production systems.

Consequently, this study evaluated the physico-chemical and sensory characteristics of beef from young dairy bulls from different production systems. Moreover, a greater understanding of how breed-type, age, feeding system (first and second grazing seasons and finishing period) affect dairy bull beef palatability would assist in developing the most efficient and economic approach to bringing dairy-bred bulls to beef to meet market specifications.

3.3 Materials and methods

3.3.1 Animals and Diets

This project was submitted to the Teagasc Animal Ethics Committee who advised that provided best husbandry practice was followed no ethical issues would arise. A total of 112 male dairy breed-type calves (HF and JEX), born in early spring, 2011, were identified on commercial farms. After weaning (10 to 12 weeks of age), they were sourced and transported to Teagasc, Johnstown Castle Research Centre. They were assigned according to breed type, date of birth, body weight on arrival and farm origin one, of two, production systems; pasture plus 1 kg concentrate per head daily (PC1) or plus 2 kg concentrate (PC2) during the first grazing season). A permanent grassland sward of predominantly perennial ryegrass (*Lolium perenne*) was used for rotational grazing. The concentrate mix consisted of 58% *Hordeum vulgare* (ground barley), 26% beet pulp, 10% *Glycine max (L.) Merr* soya bean meal, 4% *black treacle* molasses and 2% minerals. After the first grazing period both PC1 and PC2

were assigned to five different feeding treatments (Table 3.1). Bulls from 15-AL and 15-SC were slaughtered at 15 months of age from different finishing systems. Bulls from 19-HC, 19-MC and 19-LC were housed during the winter period within their production system (PC1 or PC2) and were slaughtered at 19 months of age and differed in second grazing season and finishing system. During the finishing period (excluding treatment 19-LC), all bulls were penned within their own final treatment group and offered a concentrate diet. The experiment was set up as a 5 (treatment) × 2 (breed type) × 2 (first season feeding) factorial design, resulting in 20 treatment groups, with on average, 5 or 6 animals as replicates per group.

3.3.2 Slaughter, Post-mortem pH/temperature and Sampling

Bulls were transported 133 km to a commercial abattoir, stunned by captive-bolt, conventionally hung, exsanguinated within 30 s, centrally-split into two sides and weighed. The carcasses were chilled at 4 °C. The pH (model 420A, Orion, Germany fitted with a glass pH electrode EC-2010-11 (Refex sensors Ltd., Westport, Co. Mayo, Ireland, calibrated using pH 4.0 and pH 7.0 buffers) and temperature (Digitron 2046T, Instrument Technology Ltd, Ireland) were recorded in the *longissimus thoracis* (LT) muscle at the 10^{th} rib on the left side of each carcass hourly from 1 to 5 h post mortem. At 48 h post mortem, the LT muscle was removed from the cube roll (ribs 6 to 10) from the left-hand side of each carcass. The pH/temperature profile for each carcass was plotted and the pH values at 15 °C and 35 °C were read from the curves. After chilling for 72 h at 4 °C, ultimate pH (pHu) was measured at the 10^{th} rib end, muscles were cut into individual slices (25 mm thick) and vacuum-packed. Samples for proximate chemical composition and collagen determinations were stored at -20 °C immediately, while samples for pH_{21d}, colour, Warner-Bratzler (WB) variables, thawing loss, cooking loss, and sensory analysis were aged for 21 days at 4 °C then frozen at -20 °C until required.

3.3.3 Meat Colour

The method was described in Chapter 2.

3.3.4 Warner-Bratzler Shear Force (WBSF) and Thawing Loss, Cooking Loss

Steaks were thawed in a circulating water bath at 10 to 15 °C (approximately 45 mins) and cooked in open bags suspended in a water bath at 72 °C until the

temperature in the center of the steak reached 70 °C. Cooking loss was determined as a percentage of the initial weight ([(initial weight−final weight)/initial weight] ×100). Similarly, thawing loss was expressed as a percentage of the initial weight. The method of WBSF was described in Chapter 2.

3.3.5 Proximate Chemical Composition

The method was described in Chapter 2.

3.3.6 Collagen Content and Solubility

Samples were freeze-dried and then milled to a fine homogenate. Approximately 4 g muscle homogenate was defatted overnight using 20 mL of diethyl ether. The extract was removed by centrifugation at 3,990 × *g* at room temperature for 5 mins and the sample was re-dried for 48 h. Heat-soluble collagen was extracted as described by Hill (1966) with slight modifications. Briefly, 2.5 g of fat-free dry (FFD) muscle hydrolysate was heated in a water bath for 2 h at 90 °C with 15 mL of Ringer's solution. The solution was centrifuged (LYNX 6000, Thermo Scientific) at 3,990 × *g* for 10 min at room temperature twice. The supernatant from the two centrifugations were combined and the soluble part was filtered using Whatman 41 filtration paper. Then 100 µL of the final supernatant and 3 mg of FFD (total collagen) of each muscle (triplicate) were hydrolysed using 2 mL of 6 M HCl under nitrogen in sealed vials at 110 °C overnight. Following hydrolysis, the vials were cooled and 1 mL of hydrolysate was centrifuged (5174C/R, Eppendorf, UK) at 18,187 × *g* for 1 min at room temperature to remove particulate matter.

Quantitative analysis of hydroxyproline in FFD muscle hydrolysates was carried out using LC-MS/MS with slight modification of the methods previously reported (Colgrave et al., 2008). Briefly, 100 µL aliquots of the hydrolysates were dried under nitrogen and reconstituted in 1 mL of 0.1% formic acid. The sample solution was then filtered using a 5 mL syringe and 0.45 µL Econofiltr PTFE filter. To 100 µL of the reconstituted filtered samples, 100 µL of 0.1% formic acid was added. A 5 µL aliquot of the final reconstituted sample was injected into a Waters Acquity UPLC system with an ACQUITY UPLC@BEH C18 (50 mm × 2.1 mm, particle size 1.7 µm) column coupled to tandem mass spectrometry (Waters Corp, MA, USA). The isocratic flow rate was 0.5 mL/min using the mobile phase 95% solvent A (0.1%

formic acid in HPLC water) and 5% solvent B (0.1% formic acid in acetonitrile). Data acquisition and processing were performed using the Target Lynx Software (Waters Corp, MA, USA). The MS detection was performed with electrospray ionization source in positive ion mode. The parameters of the mass spectrometer were as follows: ion source temperature 150 ºC, capillary voltage 3 KV; cone voltage 40 V, extractor voltage 3 V; desolvation temperature 350 ºC; high purity nitrogen as the desolvent and cone gases with flow rates of 800 L/h and 50 L/h, respectively.

Rat tail (α-1 (1) chain) (Enzo Life Sciences, Farmingdale, NY, EEUU) was used as the quality control collagen standard for validation. Aliquots of 100 µL of rat tail solution were hydrolysed and reconstituted using the same procedure as above. It was then then diluted with 0.1% formic acid to obtain three different standards in the high, medium and low levels of hydroxyproline. The concentration of hydroxyproline (nmol/L) was determined from integration of the area under the curve against a standard curve with a linear range from 100 to 5000 nmol/L (r^2 = 0.999) (dilution with 0.1% formic acid). The conversion of these data to mass of collagen was as previously described (Colgrave et al., 2008). Percentage solubility was calculated as soluble hydroxyproline as a percentage of total hydroxyproline. All collagen properties were determined in triplicate for each sample and were then averaged.

3.3.7 Trained Sensory Panel Evaluation

The method was described in Chapter 2.

3.3.8 Imaging

Beef samples (2 × 2 × 1 cm^3) were flash frozen in liquid nitrogen and stored at −80 °C. Sections were cut (20 µm) using a Leica CM1950 cryostat (Leica Biosystems, Nussloch, Germany) after equilibration to specimen chamber temperature (−25 °C). Confocal scanning laser microscopy (CSLM) was used in conjunction with differential staining to visualize bull beef ultrastructure. Sections were stained with Fast Green (FCF) and Nile Red stains and examined under a Leica SP5 confocal microscope (Leica Microsystems GmBH, Mannheim, Germany).

3.3.9 Statistical Analysis

Data were subjected to ANOVA with treatment, breed type, first season and their interactions as sources of variation, using the GLM procedure of SAS Version 9.3 (Cary, NC: SAS Institute, 2002). The experimental unit was the individual animal for all variables to keep the unit consistent throughout the whole feeding period. Tukey-Kramer test was used to compare mean values with a significance level of $P \leq 0.05$, whereas differences of $P > 0.05$ to $P \leq 0.10$ were considered as trends. As there were very few significant results from the effect of first season feeding, the least square means of first season determinations and the interactions with other factors were excluded in the results table except collagen variables. Pearson correlation coefficients were calculated using the CORR procedure of SAS (2002).

Sensory analysis results were analysed by Partial Least Squares Regression (PLSR) to achieve an overview of the correlations between variables and to visualize the contribution of feeding treatment and breed type to the variation. The corresponding PLSR correlation loading plot and standardized regression coefficients were performed using Unscrambler Software, Version 10.3 (CAMO ASA, Oslo, Norway).

3.4 Results and discussion

3.4.1 Post-mortem pH-temperature decline

The post-mortem pH-temperature window concept implemented by Meat Standards Australia (MSA) aims to identify carcasses at risk of cold shortening and heat toughening during rigor mortis. Cold shortening occurs when the carcass temperature falls below 15 ºC while the pH remains above 6.0. The combination of high pH and low temperature decreases the ability of the sarcoplasmic reticulum and mitochondria to retain calcium, thereby resulting in a high concentration of Ca^{2+} ions being uncontrollably released. This in combination with available ATP leads to excessive contraction of the muscle fibres, resulting in tough meat (Locker, 1985). Conversely, when the pH falls rapidly, so that it is below 6.0 while the temperature is above 35 ºC heat toughening can occur. The combination of high temperature and low pH reduces the proteolytic enzyme activity, and accelerates protein denaturation, thereby the meat is incapable of ageing (Thompson, 2002). Only the 19-HC of both breed types avoided the cold shortening window, while no group fell within the heat

toughening window (Figure 3.1). The chilling regime in operation in the commercial abattoir may have been too severe for the very lean young bull carcasses, thereby increasing the risk of cold shortening (Moloney et al., 2008). Bulls from 19-HC were the heaviest and fattest carcasses (Appendix I), thus they were expected to chill more slowly. To avoid the risk of cold shortening, young bulls should either be finished on a high energy diet to provide sufficient subcutaneous fat cover or chilling regimes will have to be modified for leaner carcasses coming from 15-month old bulls or 19-month old bulls fed pasture only during a second pasturing season.

Carcasses from 19-LC had higher pH values from 1 h to 5 h post-mortem, pH (15 ºC) and pH (35 ºC) compared with 19-HC ($P < 0.05$; Table 3.2). This is also probably due to the faster chilling rate resulting from insufficient subcutaneous fat cover of 19-LC bulls. Alternatively, it is possible that forage-fed animals are more susceptible to pre-slaughter stress, thereby leading to the greater depletion of glycogen, thus insufficient glycogen is available for lactic acid production (Muir et al., 1998a). In contrast, grain-fed animals would be better accustomed to people, pens, handling and confinement whilst in the feedlot, thus, would be less likely to suffer glycogen depletion pre-slaughter. In addition, Vestergaard et al. (2000b) stated that the lower energy intake resulting from extensive diets also contributed to the lower glycogen levels stored in muscle.

3.4.2 Ultimate pH

Despite the differences in the rate of pH fall, pHu did not differ between treatments and breeds ($P > 0.05$, Table 3.2), suggesting that pre-mortem depletion of glycogen was not an issue, even in the extensively reared bulls. Similarly, Muir et al. (1998a) reported that the pHu of beef did not differ between intensive and extensive feeding. Mean pHu for all 20 groups in this study ranged from 5.70 to 5.96, while according to Tarrant (1989), the ideal pHu range for beef is $5.4 \leq$ pHu ≤ 5.7. There were 15 out of 112 bulls (13.4%) with pH > 5.90 which could be considered as DFD (dark, firm, dry) meat (Pethick et al., 2000). It has been reported that the DFD incidence is high in young bulls due to the physical contests and excitability of temperament arising from their male status leading to glycogen depletion (Lawrie, 1991c). Moreover, unfavourable ante-mortem environmental factors, such as extreme weather, stressful handling, transportation, or mustering can also contribute to DFD meat (Meat

Standards Australia, 2016c). The pH of all groups declined after 21 days ageing and did not differ between treatments or breeds (Table 3.2). The reason for the change of pH after ageing merits investigation.

3.4.3 Meat Colour

The appearance strongly influences the decision of consumers to purchase beef with a bright red or pink colour being more desirable than darker or discolored beef (Morrissey et al., 1994). Generally, beef is recommended to be aged for up to 14 days between 2 to 4 ºC in order to obtain tender beef (Farouk et al., 2009). A 21 day ageing period was used in the present study as Monsón et al. (2005) suggested that longer ageing periods (21 days) would be needed for Holstein breed beef to obtain their optimum acceptability values.

Animal age as a factor did not affect the L*, a* and b* of LT muscle after 21 days of ageing ($P > 0.05$; Table 3.2), which is in agreement with previous observations reported by Pflanzer & Felício (2011). However, it has been hypothesized that older cattle produce darker beef than younger cattle (Page et al., 2001; Serra et al., 2004). The lack of difference in colour parameters in the present study is probably due to the small age difference between animals (merely 4 months). However, hue angle after 2 h ($P < 0.001$) and 24 h blooming ($P < 0.001$) were both higher in beef from 15-month old bulls than that of 19-month old bulls, indicating that the beef of younger bulls had a less pure red colour.

Beef from 19-LC had higher a* ($P < 0.001$) and chroma ($P < 0.05$) values than 15-AL beef after both 2 h and 24 h blooming (Table 3.2), which could partly be associated with more muscle myoglobin in grazing animals due to increased physical exercise pre-slaughter than their feedlot counterparts (Varnam & Sutherland, 1995; Vestergaard et al., 2000b). In general, the degree of pigmentation is positively correlated with a* value (Gil et al., 2001). Yellowness differed significantly between treatment groups ($P = 0.03$), even though individual means were not different as determined by the Tukey-Kramer test. However, there was a trend within both age groups for the b* to increase following a lower level of concentrate feeding. This is in contrast to Duckett et al. (2007) who reported a higher b* value for concentrate than pasture finished steers. L* value did not differ between feeding treatments ($P > 0.05$), in accordance with French et al. (2000), but differed from the general

observation that forage-fed animals mostly produce darker beef than grain-fed animals (Vestergaard et al., 2000b). The pHu has been suggested to largely affect meat darkness (Muir et al., 1998a) and the lack of L* difference in this study probably was due to the similar pHu between treatments.

In a previous study, where JEX beef was compared with HF beef, HF beef was determined to be lighter, less red and had higher yellowness at day 3 post mortem (Nian et al., 2017). However, after 21 days, colour parameters did not differ between breeds ($P > 0.05$) where both breeds had lower a* values due to a decline in the oxygen consumption rate (OCR) during post-mortem (Lanari & Cassens, 1991).

3.4.4 Warner-Bratzler Shear Force

The mean WBSF value for the ten groups ranged from 25.4 N (JEX from 19-HC) to 32.6 N (HF from 15-AL). According to Shackelford et al. (1991), muscle can be categorized into several tenderness groups; < 31.36 N 'very tender', 31.36-38.22 N 'tender', 38.22-45.08 N 'intermediate', and > 45.08 N 'tough'. The individual WBSF value for samples varied from 15.63 N to 64.14 N. Based on the above classification system, 10 samples can be defined as 'intermediate' (WBSF between 38.22 N and 45.08 N), and only 3 samples would be regarded as tough (WBSF above 45.08 N). The remaining 99 of the 112 LT muscles (88.4%) would conform to 'very tender' or 'tender' categories. Likewise, Tatum et al. (1999) set the WBSF limit of 44.5 N as unacceptably tender beef. Consequently, the majority of the beef from HF and JEX young bulls from the current production systems would be considered tender after ageing for 21 days.

Two other parameters, WB-slope and WB-area relating to meat instrumental and sensory texture attributes were determined. WB-slope or Modulus was calculated as a line drawn from the origin of the shear force curve to its peak, and was expressed 'Shear-Firmness' (Brady & Hunecke, 1985). According to Bouton & Harris (1978), the initial yield before the maximum peak force relates to the myofibrillar component, thus a higher WB-slope value is associated with the toughness derived from the myofibrillar component. WB-area, expressed as total area of the shear force graph, corresponds to the total energy consumed to chew the meat until it can be swallowed (Moller, 1981). As WB-area correlated negatively with soluble collagen

and collagen solubility in this study ($P < 0.01$; Table 3.5), it was considered to relate to insoluble material of connective tissue and background toughness.

WB-slope was higher in beef from treatment 15-SC than 19-MC ($P < 0.05$; Table 3.3). It is reasonable to assume that cold shortening occurred in some carcasses from these treatments, especially in younger bulls, so that the WB-slope related myofibrillar toughness increased due to increased contraction of the muscle fibres. However, no significant difference was observed in WB-variables between potentially cold shortened and normal beef, which was not expected. In contrast, WB-area was higher in 19-MC beef compared to 15-SC beef ($P < 0.01$), indicating that connective tissue related background tenderness decreased with slaughter age as would be expected.

Interestingly, beef from 19-MC had a higher WB-area than 19-LC beef ($P < 0.01$), suggesting that beef produced from the higher energy finishing system had higher background toughness. This finding supported the data reported previously by Razminowicz et al. (2006) that intensively produced beef had higher total shear energy than grass-based beef. Conversely, others reported that cattle finished on high energy diets produced more tender beef than cattle finished on low energy diets at a similar chronological age (Bowling et al., 1977; Mitchell et al., 1991; Jenschke et al., 2008). Moreover, in many studies, no WBSF effect pertaining to dietary energy level has been reported (French et al., 2001; Latimori et al., 2008; Pordomingo et al., 2012). French et al. (2000) concluded that supplementing grass with low levels of concentrate produced the most tender beef at 2 days post-mortem, however, all treatment effects on palatability of beef were eliminated by further ageing. Grazing season or silage system had no effect on WB-variables. HF beef tended to have higher WB-area value than JEX beef ($P = 0.051$), which indicated that beef from HF breed exhibited higher connective tissue related toughness than that determined for JEX breed.

3.4.5 Thawing Loss and Cooking Loss

Thawing and cook losses are the important attributes related to water holding capacity (WHC) of beef muscles, and water losses during thawing and cooking affect the overall acceptability of beef (Hughes et al., 2014). The mean cook losses from individual treatment groups varied between 24.6% and 28.9%, while thawing loss

was between 2.2% and 4.8%. Cooking loss was not affected by either diet or breed type ($P > 0.05$; Table 3.3), and is in agreement with French et al. (2001) who showed that cooking loss did not differ between dietary treatments, but only differed with postmortem ageing time. However, difference in thawing loss for muscles derived from different nutritional planes was detected; with 19-LC having higher values compared to other treatments ($P = 0.001$).

A similar trend was reported by Sapp et al. (1999) who found that total cook losses from steer beef was not affected by feeding pasture, pasture with concentrate or concentrate alone, however, these authors showed that thawing loss decreased as the pasture content in the diet decreased, thereby indicating that pasture-based diets increased the moisture content in steer beef. Fatter meat cuts have also been shown to sustain WHC to a greater degree than leaner meat cuts (Savell et al., 1986). Thus, the marked higher pasture intensity, accompanied with lower IMF content in 19-LC beef compared to other treatments, could explain the higher thawing loss obtained in this study. According to Jeremiah et al. (2003c), total cook losses was positively correlated with total and insoluble hydroxyproline on a dry, defatted basis, while thaw-drip loss was negatively associated with total and soluble hydroxyproline, although no difference in collagen contents were found between diets ($P > 0.05$) in the present study. There was no grazing seasons or silage system effect on thawing and cooking loss.

3.4.6 Proximate Chemical Composition

A higher moisture content for beef from 15-SC than from 19-HC ($P < 0.01$; Table 3.3) indicated a decreased moisture content in heavier carcasses (slaughter weight: 451 kg for 15-SC vs 587 kg for 19-HC; carcass weight: 228 kg for 15-SC vs 305 kg for 19-HC). Similarly, Keane & Allen (2011) reported that moisture concentration declined with slaughter weight or age, and conversely, higher deposition of lipid occurred with heavier carcasses.

Beef from treatment 19-LC had a higher moisture content than 19-HC ($P < 0.01$) with a concomitant increase in IMF content for 19-HC ($P < 0.01$; Table 3.3). These differences are likely due to the combined effects of second grazing season and finishing system, instead of grazing seasons alone or utilization of a silage system. These results are supported by Sami et al. (2004) who observed that a higher energy

diet increased IMF content and reduced moisture content compared to beef derived from a lower energy diet. Vestergaard et al. (2000a) also found that the IMF content was higher in intensively reared rather than in extensively reared Friesian calves. In terms of biological mechanisms, the higher net energy glucose supply from grain feeding might increase IMF deposition because the glucose delivery to muscle, not only increases intramuscular adipocyte promotion directly, but also increases circulating insulin level, which is known to stimulate lipogenesis (Pethick et al., 2004).

JEX beef had higher IMF and lower moisture ($P < 0.05$) contents than HF beef. This finding is in agreement with Albertí et al. (2008) who stated that Jersey cattle demonstrated the potential to produce a highly-marbled product. Marbling fat content has been shown to have high genetic variability. The balance in activity between fatty acid elongase enzyme and synthase may be associated with differences between animals in their propensity to deposit marbling fat (Kazala et al., 1999). IMF differences have also been shown to be associated with muscle fibre composition variation. For example, IMF is positively correlated with 'red' or oxidative fibre proportions, while negatively correlated with 'white' or glycolytic fibres in beef (Hwang et al., 2010). For protein content, the interaction of treatment and breed type was significant ($P < 0.05$); groups HF/15-SC and JEX/15-SC having the highest and lowest values of 22.8% and 21.9%, respectively.

Mean IMF content in all groups ranged from 1.1% (HF from 19-LC) to 4.9% (JEX & 19-HC) which was within the range from 0.5% to 6.7% among beef breeds (Waritthitham et al., 2010a). Killinger et al. (2000) reported that approximately 3.3% IMF corresponds to the grade 'slight degree of marbling' of beef which was preferred by US consumers. Although fat content preference is market specific, IMF between 3 and 7.3% of meat is generally acceptable for both eating experience and health concerns (Miller, 2002; Duckett et al., 2007). The IMF level from groups JEX /15-AL, HF/19-HC, JEX/19-HC, and JEX/19-MC ranged from 3.0% to 4.0%, and therefore was in the acceptable beef range. Other groups had lower IMF contents between 1.0% and 3.0%. It is possible that beef from higher forage intake treatments 15-SC and 19-LC accompanied by HF breed type were unable to deposit sufficient lipid within muscle.

3.4.7 Collagen Content and Solubility

Mean insoluble and total collagen content observed in all groups ranged from 1.76 to 2.90 mg/g wet tissue and 2.58 to 3.62 mg/g, respectively, while collagen solubility was between 17.2% and 34.8%.

Collagen characteristics were affected by slaughter age (Table 3.3). Both insoluble ($P < 0.001$) and total collagen ($P < 0.001$) contents were higher in 19- than 15-month old bull beef, and inversely, collagen solubility ($P < 0.001$) decreased with chronological age. This supports the theory that the older the animal, the higher the proportion of insoluble cross-links, with a consequent reduction in collagen solubility (McCormick, 2009). Likewise, Bailey (1989) described that with increasing age to maturity, the divalent immature heat-labile cross-links in connective tissue greatly converts into mature trivalent heat-stable cross-links. The formation of the mature multivalent transverse cross-links contributes to a dramatic increase in the tension generated upon heating, resulting in a decline in meat tenderness. Weston et al. (2002) also noted that as animals mature, collagen synthesis and turnover reduce, thereby allowing time for mature cross-links to form.

Across diets collagen characteristics were only affected by first season feeding. Both insoluble and total collagen contents were higher in 2 kg compared with 1 kg concentrate at pasture during first season feeding ($P < 0.05$; Figure 3.2), indicating increased collagen portion in beef receiving a higher energy diet. Contrary to the present result, Aberle et al. (1981) concluded that a high energy diet accelerated newly-synthesized heat-liable collagen proportions in cattle during the period of rapid protein synthesis. Moreover, Archile-Contreras et al. (2010) found that the total collagen content of LD muscle was greater in pasture-fed than in corn-fed cattle. Nonetheless, Dikeman et al. (1986) reported that the collagen content of Angus male calves was not affected by high or low energy diets. Therefore, some uncertainty and debate surrounds this particular dietary effect and further studies may be required to clarify the true situation.

Among most cattle breeds in Europe, the dairy breeds Jersey, Holstein and Danish Red Cattle have the highest total and insoluble collagen contents, while meat producing breeds like Piedmontese, Limousin and Asturiana de los Valles have the lowest values (Christensen et al., 2011). No differences in collagen content and

solubility were detected between breeds ($P > 0.05$) in this study and this is in contrast with findings by Christensen et al. (2011) who found that Jersey young bulls had the highest percentages of heat-soluble collagen, while Danish Red Cattle and Holstein had the lowest levels of soluble collagen among 15 European breeds. This may explain the present findings which clearly demonstrated that JEX beef was more tender than HF breed type when assessed by sensory panels.

3.4.8 Trained Sensory Panel Evaluation

Sensory attributes did not differ between cattle ages ($P > 0.05$). Dransfield et al. (2003) found that flavour and juiciness are highest in meat from older animals. This may be explained by the narrow age range (4 months) employed in the current study compared to a seven month range (15 vs 22 months) which resulted in a significant difference in residual roast beef flavour length determined in another study reported by the same authors (Nian et al., 2017).

Genetics and environment are identified as main contributors to meat flavour, from which feed source is the most important environmental factor (Melton, 1990). Roast beef flavour and residual roast beef flavour length ($P < 0.05$) both increased with increased concentrate intake as shown by the highest score for treatment 19-HC, followed by 19-MC and 19-LC (Table 3.4). Similarly, previous studies have reported a more acceptable or intense flavour in beef from high-energy grain diets compared with low-energy forage or grass diets (Schaake et al., 1993; Duckett et al., 2007). Generally, cattle finished off grass produced beef which had higher ‘bloody’, ‘fishy’ or ‘liver-like’ notes (Nuernberg et al., 2005). This could be explained by the corresponding IMF content of each group. The amount and composition of IMF are considered as the main reasons for the increase in flavour intensity in beef because the characteristic meaty volatile compounds and flavour-related fatty acid composition are mostly derived from the lipid fraction (Muir et al., 1998a; Monsón et al., 2005). It was concluded that n-3 and n-6 PUFA are primarily responsible for the particular flavour of grass- and grain-fed meat, respectively (Wood et al., 2003). The ‘green’ flavour from grass-fed cooked meat is mainly related to compounds such as hexanals derived from oleic acid (C18:1n9c) and α-linolenic acid (C18:3n3) which are found in grass. In contrast, the ‘soapy’ flavour of grain-fed beef is mainly associated with octanals from linoleic acid (C18:2n6c) (Lorenz et al., 2002). In

addition, the less desirable flavour in pasture-fed beef was also probably due to the high level of PUFAs, especially n-3 PUFA, which produce higher concentrations of lipid oxidation products, including aldehydes, alcohols and ketones, which are responsible for undesirable flavour in beef (Wood et al., 2003). Roast beef flavour was affected by the combination of second season and finishing system, while residual roast beef flavour length was only affected by finishing system. Grazing seasons and silage system had no effect on sensory characteristics.

Cohesiveness was higher in 19-MC than 19-LC ($P < 0.05$), suggesting a tougher texture and confirming the trend observed in WB-area (Table 3.4). However, a decline in tenderness score in pasture-fed beef has been previously reported (Pordomingo et al., 2012). Juiciness was not affected by diet ($P > 0.05$), as previously observed by French et al. (2000). Similarly, Schroeder et al. (1980) found that LD muscle from grain-fed steers had more marbling, but juiciness did not differ. Stelzleni (2006) also found no conclusive evidence that meat juiciness is affected by forage or concentrate feeding directly. However, juiciness is affected by indirect factors, such as IMF content or pH. Nevertheless, greater juiciness scores in concentrate-fed rather than pasture-fed steer beef was reported by Vestergaard et al. (2000a).

The higher scores for initial tenderness and ease of disintegration ($P < 0.05$), and lower scores for chewiness ($P < 0.05$), cohesiveness ($P = 0.10$) and stringiness ($P = 0.075$) for JEX beef compared with HF beef indicated that JEX produced more tender beef with a superior texture than HF breed type. In addition, JEX beef exhibited higher roast beef flavour intensity than HF ($P < 0.05$). These results can be explained by the higher IMF content in JEX beef ($P < 0.05$; Table 3.3), due to its positive role in both flavour and tenderness of cooked beef at the time of consumption (Costa et al., 2012). The higher collagen solubility of Jersey compared to Holstein bulls found by Christensen et al. (2011) could also contribute to the tougher HF beef produced. Furthermore, the difference in tenderness could be associated with variations in muscle fibre size, total number and (or) fibre composition (Penand et al., 2001).

Surprisingly, the off-flavour traits recorded after a long ageing period (21 d), while low, were described as metallic < 10 and rancid < 5. This was probably due to the

maintenance of beef samples under stored vacuum, thus reducing the rate of lipid oxidation (Resconi et al., 2010). The score for beef flavour was around 50 and juiciness was around 45 for all of the treatments, in agreement with the sensory scores of young Friesian bulls reported by Partida et al. (2007).

3.4.9 Correlations

3.4.9.1 Correlations between Physico-chemical Quality Variables

The pH (15 ºC) ($P < 0.01$, $r^2 = -0.28$) and pH (35 ºC) ($P < 0.001$ $r^2 = -0.42$) were both negatively correlated with fat score, which confirmed that insufficient subcutaneous fat coverage lead to the rapid chilling of muscle and slower pH fall, thus risking cold-shortening. However, pH (15 ºC) showed no correlation with WB-variables ($P > 0.05$), which was not expected. The pH (35 ºC) was negatively correlated with WB-slope and WB-area ($P < 0.05$; Table 3.5), even though no carcasses were within the heat-toughening window. The pHu values at 3 and 21 days were both negatively correlated with colour parameters including L*, a*, b* and chroma after both 2 and 24 h blooming ($P < 0.001$; $r^2 = -0.58$-0.84), which supports the previous finding by Purchas et al. (2002). L* was positively correlated with cooking loss ($P < 0.001$), which is in agreement with Frylinck et al. (2013) who noted that lightness had a negative relationship with WHC. The paler colour of muscle can be explained by the increased light scattering during blooming caused by the increased extracellular space as suggested by the lower WHC (Schäfer et al., 2000). The observed positive correlation of lightness with IMF content ($P < 0.01$) agreed with Muir et al. (1998b) who showed that a more marbled concentration contributes to lighter muscle (higher reflectance values).

WB-slope had a positive correlation with thawing loss ($P < 0.01$) and a negative correlation with IMF content ($P < 0.05$), in line with other researchers who showed that IMF can improve meat tenderness (Sami et al., 2004; Costa et al., 2012) through diluting muscle fibrous protein, thereby contributing to a decrease in muscle resistance under shearing (Wood et al., 1999). A higher WB-area was associated with a lower soluble collagen content and reduced collagen solubility ($P < 0.01$). This finding was supported by several other reports which demonstrated that collagen solubility, instead of total collagen content, was the critical factor contributing to meat tenderness (Bailey, 1985; Young & Braggins, 1993; Schönfeldt & Strydom,

2011). A higher thawing loss was related to a higher moisture concentration ($P<0.01$).

3.4.9.2 Correlations between Physico-chemical and Sensory Quality Variables

WBSF was positively correlated with chewiness and stringiness scores and negatively correlated with initial tenderness, juiciness, ease of disintegration and roast beef flavour ($P < 0.001$; Table 3.6). The correlations between WBSF and sensory tenderness parameters were in agreement with others (Schönfeldt & Strydom, 2011; Monteiro et al., 2013). Samples with higher thawing loss released less juice during eating (therefore, had lower juiciness score) ($P < 0.01$). Similarly, Hughes et al. (2014) reported that juiciness was positively associated with WHC of raw meat. Steaks with higher cook losses had higher cohesiveness score ($P < 0.05$), in agreement with Monteiro et al. (2013), who reported that cooking loss had a negative effect on young bull beef tenderness. IMF content was positively correlated with initial tenderness ($P < 0.01$), ease of disintegration ($P < 0.01$), juiciness ($P < 0.001$) and roast beef flavour ($P < 0.01$), while negatively correlated with chewiness ($P < 0.05$). These results are consistent with the general finding that IMF is primarily responsible for increasing beef flavour, juiciness (Sami et al., 2004; Pordomingo et al., 2012) and tenderness (Partida et al., 2007). Adipocytes may provide a lubricating effect during chewing owing to weakening the strength of connective tissue, thus improving texture parameters (Renand et al., 2001). Sustained juiciness has been reported to result from the stimulatory effect of fat on salivation (Lawrie, 1991a). IMF content was also positively correlated with fattiness/greasiness score ($P < 0.05$; $r^2 = 0.27$).

3.4.9.3 Correlations between Sensory Quality Variables

Steaks with higher juiciness scores had higher initial tenderness and ease of disintegration scores ($P < 0.001$), but lower chewiness ($P < 0.001$) and stringiness scores ($P < 0.05$), indicating that juiciness is related to tender beef, a finding that is in broad agreement with other researchers (Destefanis et al., 2000; Serra et al., 2008). The quicker release of juices during chewing may result from the degradation of muscle and a reduction in myofibrillar strength (Partida et al., 2007). Juiciness was positively correlated with roast beef flavour ($P < 0.001$; Table 3.6), which is

consistent with findings by Monteiro et al. (2013). Ease of disintegration was also positively correlated with roast beef flavour ($P < 0.05$).

3.4.9.4 Sensory Attributes Analysed by PLSR

The two-dimensional PLSR correlation loading plot (Figure 3.3) identified correlations between sensory attributes and investigated which production factors were involved in this discrimination through factor 1 versus 2, since these two components summarize the main cross-validated variance (47% for X matrix and 9% for Y matrix). The texture terms ease of disintegration and initial tenderness had a high positive correlation on both factor 1 and factor 2, and they were negatively correlated to scores for cohesiveness, chewiness, residual dryness, stringiness and astringent; two groups shown on the opposite quadrant of the plot. Furthermore, the desirable roast beef flavour terms, including; aroma, flavour, residual roast beef flavour length, fattiness and residual fattiness scores were positively associated with each other, but were negatively correlated with rancid, metallic and residual metallic scores which belong to off-flavour attributes. The outer ellipse and inner ellipse indicate 100% and 50% explained variance, respectively. Seven sensory terms were placed between the inner and outer ellipses, $r^2 = 0.5$ and 1.0, respectively, indicating they were well explained by the PLSR model.

The PLSR model identified the production factors (treatment and breed type) that contributed positively and negatively to the attributes of young dairy bull beef (Figure 3.4). The columns containing sensory terms that were located on the positive portions of the Y axis (or standardized coefficients) are considered to be positively correlated with production factors, while the columns that were on the negative portion of the Y axis represent the attributes that were negatively correlated with production factors. The size of the columns represents the extent of influence (both positive and negative), thus larger columns indicate a greater influence (regression coefficients) of the production factors on sensory attributes. In addition, the jack-knife uncertainty test was applied to inspect the significant variables of sensory attributes by calculating estimated regression coefficients. When the vertical line that represents the estimated uncertainty limits of 95% confidence interval crosses the X axis (non-shadow column), the corresponding production factor does not have significant influence on the attribute.

No sensory attributes were significantly (at the 5% level) affected by the treatments 15-AL, 15-SC and 19-MC (Figure 3.4). Ease of disintegration, roast beef flavour and residual roast beef flavour length were positively correlated with 19-HC ($P < 0.05$), while astringent score was positively related to 19-LC ($P < 0.05$), indicating that higher energy diet combined with second season and finishing improved texture and desirable beef flavour, while the pungent and drying taste was higher in lower energy diet. This result supports that an off-flavour taste commonly existed in ruminants slaughtered directly off pasture or forage feeding. This taste probably results from the level of degradation products of chlorophyll such as terpenes from grass and a higher content of n-3 PUFA (Vestergaard et al., 2000a). Roast beef aroma, flavour, residual beef flavour and juiciness scores were positively correlated with JEX beef, however, they were negatively correlated with HF breed type ($P < 0.05$).

3.5 Conclusions

Good quality beef can be produced from young dairy bulls, and most samples were acceptably tender after 21 days ageing. Insoluble and total collagen increased, while collagen solubility decreased at the higher (19 month) slaughter age. Moisture content and hue angle were both higher in younger (15 month) bulls.

The higher energy diet during finishing combined with the second grazing season period increased the level of IMF and beef flavour intensity, decreased moisture content and protected cattle from potential glycogen depletion, reducing the risk of cold shortening. Young dairy bulls slaughtered directly from pasture had shorter beef flavour length than those directly from concentrate. Higher forage diet produced inferior WHC, but more intense red colour of raw beef and increased astringent taste. Although WB-area and cohesiveness score were higher in the higher energy finishing system of 19-month old bulls, the PLSR model suggested beef produced from a higher energy diet combined with finishing and second season had better texture. The IMF level depended on the diet. However, different levels of concentrate feeding in the first and second grazing seasons or silage mixed concentrate diet during finishing period had very limit effect on beef quality traits.

Crossbreeding Jersey with HF can improve IMF content, sensory texture, flavour and juiciness scores and reduce instrumental WB-area value of young dairy bulls. The effects on colour could be important in terms of selecting carcasses for different

markets. Variation in thawing loss, IMF, soluble collagen content and collagen solubility could explain the differences determined in cooked beef texture. Higher WBSF was correlated with higher sensory scores of chewiness and stringiness, and lower scores of initial tenderness, ease of disintegration, juiciness and roast beef flavour.

In conclusion, eating quality of beef from young dairy bulls was generally good and most samples were acceptably tender after 21 days. Slaughter age and the energy level of diet had obvious effects on quality characteristics. Crossbreeding Jersey with HF breed type can improve the beef quality of young dairy bulls.

Table 3.1. Production treatment design of young dairy bulls.

Age (month)	Treatment	First season (May-November)	Finishing period (November-May)
15	15-AL	PC1	*Ad-lib* concentrate
		PC2	
	15-SC	PC1	*Ad-lib* silage + 5 kg concentrate
		PC2	

Age (month)	Treatment	First season (May-November)	Winter period (November-March)	Second season (March-June)	Finishing period (June-September)
19	19-HC	PC1	*Ad-lib* silage + 1.5 kg concentrate	*Ad-lib* pasture + 5 kg concentrate	*Ad-lib* concentrate
		PC2			
	19-MC	PC1	*Ad-lib* silage + 1.5 kg concentrate	*Ad-lib* pasture only	*Ad-lib* concentrate
		PC2			
	19-LC	PC1	*Ad-lib* silage + 1.5 kg concentrate	*Ad-lib* pasture only	*Ad-lib* pasture + 5 kg concentrate
		PC2			

PC1 = 1 kg concentrate at pasture; PC2 = 2 kg concentrate at pasture. *Ad-lib = Ad-libitum.*

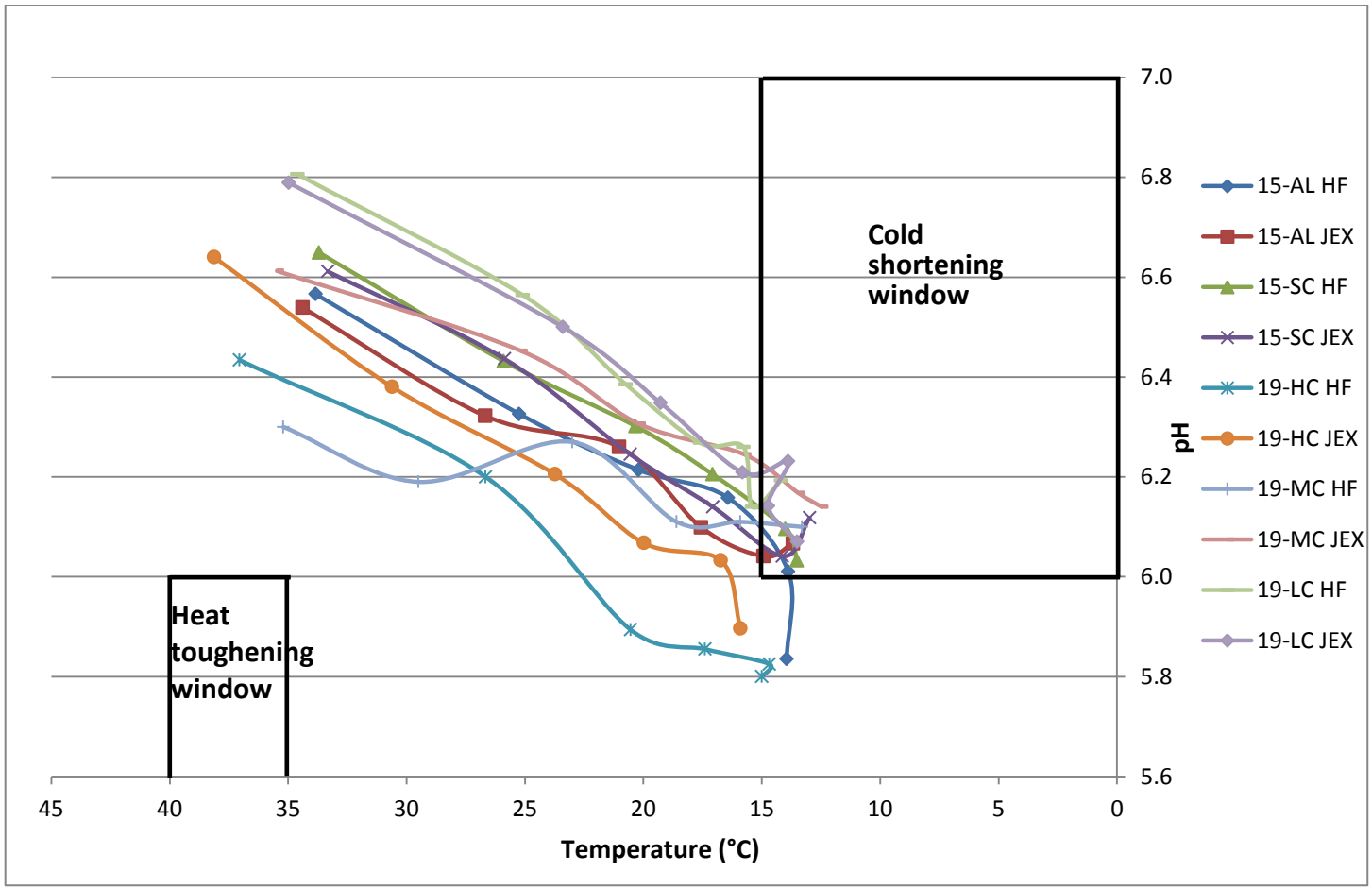


Figure 3.1. Post-mortem pH and temperature decline of young dairy bulls (mean value). 15-AL – 19-LC-Refer to the treatment design in Table 3.1.

Table 3.2. Post-mortem pH values and colour after 2 hour and 24 hour blooming in 21 day aged LT muscles of young dairy bulls.

	Treatment (T)										Breed (B)				*P*-value		
	15-AL		15-SC		19-HC		19-MC		19-LC		HF		JEX		T	B	T×B
	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM			
pH, 15°C	6.11^{ab}	0.07	6.11^{ab}	0.05	5.94^{b}	0.07	6.10^{ab}	0.07	6.24^{a}	0.05	6.06	0.04	6.14	0.04	0.019	0.137	0.681
pH, 35°C	6.65^{abc}	0.06	6.70^{ab}	0.04	6.42^{c}	0.06	6.52^{bc}	0.06	6.80^{a}	0.04	6.65	0.04	6.59	0.03	<0.001	0.182	0.531
pH1h	6.59^{b}	0.053	6.63^{b}	0.037	6.53^{b}	0.056	6.57^{b}	0.052	6.81^{a}	0.037	6.64	0.031	6.62	0.029	<0.001	0.616	0.793
pH2h	6.35^{ab}	0.059	6.43^{ab}	0.041	6.27^{b}	0.062	6.43^{ab}	0.057	6.54^{a}	0.041	6.40	0.035	6.41	0.032	0.006	0.880	0.740
pH3h	6.24^{ab}	0.060	6.28^{a}	0.043	6.05^{b}	0.064	6.25^{ab}	0.059	6.36^{a}	0.042	6.20	0.036	6.27	0.033	0.005	0.126	0.231
pH4h	6.15^{ab}	0.061	6.17^{ab}	0.043	5.98^{b}	0.064	6.16^{ab}	0.059	6.26^{a}	0.042	6.12	0.036	6.16	0.033	0.014	0.378	0.077
pH5h	6.04^{ab}	0.067	6.07^{b}	0.047	5.97^{b}	0.071	6.08^{ab}	0.066	6.27^{a}	0.046	6.05	0.040	6.12	0.037	0.003	0.153	0.280
pHu[1]	5.85	0.07	5.78	0.06	5.90	0.07	5.87	0.07	5.74	0.05	5.84	0.04	5.81	0.04	0.301	0.664	0.509
pH21d	5.76	0.09	5.63	0.09	5.79	0.08	5.76	0.07	5.65	0.05	5.73	0.04	5.71	0.06	0.419	0.818	0.320
L* 2h21d	43.3	0.58	43.5	0.58	42.2	1.02	42.3	0.94	42.0	0.67	42.7	0.48	42.7	0.51	0.384	0.993	0.872
a* 2h21d	11.4^{b}	0.51	12.9^{ab}	0.51	13.0^{ab}	0.89	12.8^{ab}	0.82	14.5^{a}	0.58	12.5	0.42	13.3	0.45	0.005	0.156	0.476
b* 2h21d	10.8	0.47	11.7	0.47	10.5	0.83	10.9	0.77	12.2	0.54	11.0	0.39	11.4	0.42	0.203	0.471	0.720
Chroma 2h21d	15.8^{b}	0.67	17.4^{ab}	0.67	16.7^{ab}	1.19	16.8^{ab}	1.09	19.0^{a}	0.77	16.7	0.55	17.6	0.59	0.043	0.257	0.581
Hue angle 2h21d	43.7^{a}	0.61	42.3^{ab}	0.61	39.1^{b}	1.07	40.4^{b}	0.99	40.1^{b}	0.70	41.6	0.50	40.6	0.54	<0.001	0.156	0.616
L* 24h21d	43.7	0.61	43.9	0.62	42.0	1.09	41.8	1.01	42.1	0.71	43.1	0.51	42.3	0.54	0.147	0.316	0.406
a* 24h21d	12.9^{b}	0.42	14.2^{ab}	0.43	13.7^{ab}	0.76	13.5^{ab}	0.70	15.3^{a}	0.50	13.6	0.35	14.2	0.38	0.010	0.213	0.073
b* 24h21d	11.9	0.37	12.9	0.38	11.0	0.67	11.1	0.62	12.7	0.44	11.8	0.31	12.1	0.34	0.030	0.578	0.243
Chroma 24h21d	17.6^{b}	0.54	19.2^{ab}	0.55	17.6^{ab}	0.97	17.5^{ab}	0.90	19.9^{a}	0.63	18.0	0.45	18.7	0.49	0.028	0.322	0.106
Hue angle 24h21d	42.8^{a}	0.53	42.3^{ab}	0.55	38.9^{c}	0.96	39.7^{bc}	0.89	39.7^{c}	0.63	41.1	0.45	40.2	0.48	<0.001	0.183	0.825

LSM = least square means; SEM = standard error of LSM. $^{a, b, c}$ Means within a row within a main effect with different superscripts significantly differ ($P < 0.05$).

15-AL – 19-LC-Refer to the treatment design in Table 3.1.

[1]pHu = ultimate pH.

Table 3.3. WB-variables, water holding capacity, chemical composition and collagen characteristics of LT muscles of young dairy bulls.

	Treatment (T)										Breed (B)				*P*-value		
	15-AL		15-SC		19-HC		19-MC		19-LC		HF		JEX		T	B	T×B
	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM			
WBSF[1] (N)	30.8	1.52	29.3	1.56	26.1	2.74	27.9	2.53	26.9	1.79	29.2	1.27	27.2	1.37	0.415	0.292	0.960
WB-slope (Mpa)	0.57^{ab}	0.03	0.58^{a}	0.03	0.45^{ab}	0.06	0.40^{b}	0.05	0.57^{ab}	0.04	0.50	0.03	0.53	0.03	0.021	0.547	0.288
WB-area (J)	0.24^{ab}	0.02	0.22^{b}	0.02	0.27^{ab}	0.03	0.33^{a}	0.03	0.20^{b}	0.02	0.27	0.02	0.23	0.02	0.006	0.051	0.315
Thawing loss (%)	3.06^{b}	0.32	2.82^{b}	0.33	2.43^{b}	0.56	2.62^{b}	0.52	4.67^{a}	0.37	3.15	0.26	3.09	0.28	0.001	0.893	0.925
Cooking loss (%)	27.2	0.46	28.5	0.47	26.2	0.83	28.0	0.76	27.2	0.54	27.8	0.39	27.1	0.41	0.094	0.208	0.181
Moisture (%)	73.9^{ab}	0.28	74.5^{a}	0.29	72.7^{b}	0.50	73.6^{ab}	0.47	75.0^{a}	0.33	74.3^{a}	0.24	73.6^{b}	0.25	0.002	0.049	0.722
IMF[2] (%)	2.84^{ab}	0.33	2.24^{ab}	0.33	4.01^{a}	0.59	3.01^{ab}	0.54	1.29^{b}	0.38	2.17^{b}	0.27	3.19^{a}	0.29	0.002	0.013	0.847
Protein (%)	22.5	0.13	22.3	0.13	22.3	0.23	22.5	0.22	22.3	0.15	22.5	0.11	22.3	0.12	0.757	0.321	0.024
Soluble collagen (mg/g)	0.75	0.07	0.80	0.07	0.67	0.11	0.59	0.10	0.68	0.08	0.67	0.06	0.72	0.05	0.457	0.491	0.688
Insoluble collagen (mg/g)	1.92^{b}	0.14	1.88^{b}	0.14	2.87^{a}	0.20	2.37^{ab}	0.18	2.88^{a}	0.14	2.39	0.10	2.37	0.10	<0.001	0.895	0.537
Total collagen (mg/g)	2.67^{b}	0.17	2.68^{b}	0.17	3.54^{a}	0.24	2.96^{ab}	0.22	3.55^{a}	0.17	3.06	0.12	3.10	0.12	<0.001	0.850	0.625
Collagen solubility (%)	28.5^{a}	2.06	30.4^{a}	2.06	19.1^{bc}	2.98	19.4^{bc}	2.69	18.4^{c}	2.15	22.3	1.55	24.0	1.51	<0.001	0.410	0.423

LSM = least square means; SEM = standard error of LSM. ^{a} ,^{b,} ^{c} Means within a row within a main effect with different superscripts significantly differ ($P < 0.05$).

15-AL – 19-LC-Refer to the treatment design in Table 3.1.

[1]WBSF = Warner-Bratzler Shear Force; [2]IMF = Intramuscular fat.

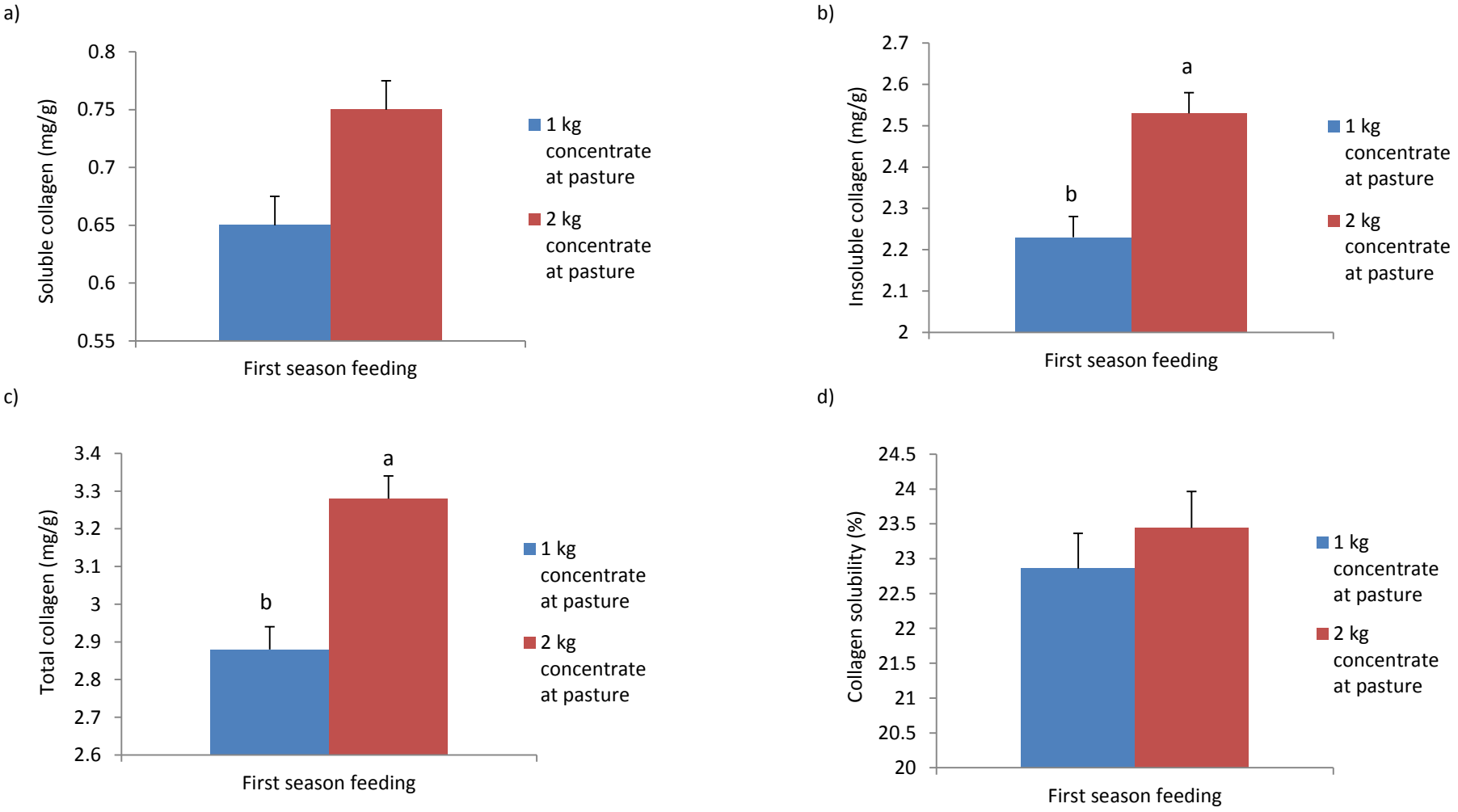


Figure 3.2. Collagen characteristics of LT muscles of young dairy bulls from two different first season feeding systems.

Table 3.4. Sensory evaluation of LT muscles of young dairy bulls.

		Treatment (T)										Breed (B)				*P*-value		
		15-AL		15-SC		19-HC		19-MC		19-LC		HF		JEX		T	B	T×B
		LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM			
1	Roast Beef Aroma	53.6	2.88	53.2	2.88	55.0	4.16	54.5	3.76	46.2	3.00	51.1	2.17	53.9	2.10	0.277	0.368	0.446
2	Initial Tenderness	68.6	3.10	65.2	3.10	71.9	4.47	59.4	4.04	65.9	3.23	62.1^{b}	2.33	70.3^{a}	2.26	0.287	0.014	0.226
3	Juiciness	42.1	2.46	41.2	2.46	51.4	3.55	45.6	3.20	40.3	2.56	42.5	1.85	45.7	1.79	0.105	0.224	0.473
4	Cohesiveness	56.9^{ab}	1.88	62.5^{ab}	1.88	57.8^{ab}	2.71	64.9^{a}	2.45	55.5^{b}	1.96	61.2	1.41	57.9	1.37	0.014	0.100	0.340
5	Ease of Disintegration	61.3	2.75	59.5	2.75	65.9	3.97	59.6	3.59	59.0	2.87	57.8^{b}	2.07	64.3^{a}	2.01	0.660	0.027	0.101
6	Chewiness	31.6	3.36	34.7	3.36	26.9	4.85	40.6	4.38	34.7	3.50	38.1^{a}	2.53	29.3^{b}	2.45	0.298	0.015	0.274
7	Fattiness/Greasiness	9.91	1.07	10.1	1.07	9.36	1.54	9.28	1.39	8.93	1.11	9.09	0.80	9.95	0.78	0.944	0.445	0.338
8	Stringiness	7.57	1.01	6.81	1.01	5.70	1.45	9.43	1.31	7.86	1.05	8.43	0.76	6.52	0.73	0.380	0.075	0.540
9	Astringent	9.81	1.07	11.2	1.07	6.93	1.54	10.3	1.40	10.0	1.11	10.4	0.80	8.98	0.78	0.269	0.225	0.805
10	Roast Beef Flavour	43.4^{ab}	2.67	46.5^{ab}	2.67	53.4^{a}	3.86	50.5^{ab}	3.49	38.5^{b}	2.78	43.3^{b}	2.01	49.7^{a}	1.95	0.017	0.027	0.927
11	Metallic	5.47	0.57	6.14	0.57	6.29	0.83	6.66	0.75	7.58	0.60	6.51	0.43	6.34	0.42	0.159	0.779	0.939
12	Stale/Rancid/Aged	3.30	0.40	2.05	0.40	2.36	0.58	1.48	0.52	1.95	0.42	2.17	0.30	2.29	0.29	0.057	0.782	0.442
13	Res[1]-RBFL[2]	38.8^{ab}	2.87	41.0^{ab}	2.87	49.1^{a}	4.14	47.0^{a}	3.74	33.4^{b}	2.99	39.8	2.16	43.9	2.10	0.017	0.181	0.734
14	Res-Metallic	7.72	0.66	7.67	0.66	7.88	0.96	9.11	0.86	8.87	0.69	8.26	0.50	8.24	0.48	0.509	0.972	0.199
15	Res-Fattiness/Greasiness	9.13	0.91	8.96	0.91	9.29	1.31	8.75	1.18	7.79	0.94	8.44	0.68	9.12	0.66	0.837	0.478	0.931
16	Res-Dryness	9.72	0.73	10.6	0.73	7.96	1.06	8.84	0.96	8.86	0.76	9.32	0.55	9.06	0.54	0.268	0.739	0.851

LSM = least square means; SEM = standard error of LSM. [a, b, c] Means within a row within a main effect with different superscripts significantly differ ($P < 0.05$).
15-AL – 19-LC-Refer to the treatment design in Table 3.1.
[1]'Res-' = Residual (after-effects); [2]Res-RBFL = Res-Roast Beef Flavour Length.

Table 3.5. Pearson correlation coefficients between physico-chemical traits of LT muscles of young dairy bulls.

	WBSF	WB-slope	WB-area	Thawing loss	Cooking loss	Moisture	IMF[2]	Soluble collagen	Collagen solubility
pH, 15°C	0.17	0.14	-0.09	0.07	-0.05	0.27*	-0.21	-0.02	-0.002
pH, 35°C	0.07	-0.24*	-0.27*	0.44***	0.02	0.35***	-0.35***	-0.03	0.09
L*2h21d	-0.25**	-0.02	-0.13	-0.09	0.38***	-0.28**	0.27**	0.23	0.22
L*24h21d	-0.27**	-0.05	-0.14	-0.03	0.51***	-0.27**	0.26*	0.30*	0.34**
WBSF[1]		0.68***	0.46***	0.01	-0.03	0.18	-0.15	-0.13	-0.11
WB-slope			-0.09	0.26**	0.03	0.24*	-0.22*	0.10	0.11
WB-area				-0.15	0.08	-0.003	-0.002	-0.33**	-0.32**
Thawing loss					-0.09	0.26**	-0.23*	0.08	0.07

Significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.
[1]WBSF = Warner-Bratzler Shear Force; [2]IMF = Intramuscular fat.

Table 3.6. Pearson correlation coefficients between physico-chemical and sensory traits of LT muscles of young dairy bulls.

	Initial Tenderness	Juiciness	Cohesiveness	Ease of Disintegration	Chewiness	Stringiness	Roast Beef Flavour
WBSF[1]	-0.46***	-0.41***	0.08	-0.39***	0.29*	0.24*	-0.41***
WB-slope	-0.36**	-0.42***	0.08	-0.37**	0.22	0.18	-0.31**
WB-area	-0.35**	-0.12	0.07	-0.19	0.24*	0.10	-0.06
Thawing loss	-0.11	-0.38**	-0.12	-0.22	0.17	-0.03	-0.33**
Cooking loss	-0.18	0.02	0.25*	-0.09	0.09	-0.03	0.37**
IMF[2]	0.36**	0.44***	-0.02	0.34**	-0.27*	-0.21	0.34**
Initial Tenderness		0.54***	-0.58***	0.82***	-0.78***	-0.44***	0.14
Juiciness			-0.06	0.49***	-0.44***	-0.28*	0.55***
Cohesiveness				-0.60***	0.66***	0.34**	0.22
Ease of Disintegration					-0.86***	-0.42***	0.25*
Chewiness						0.59***	-0.13

Significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.
[1]WBSF = Warner-Bratzler Shear Force; [2]IMF = Intramuscular fat.

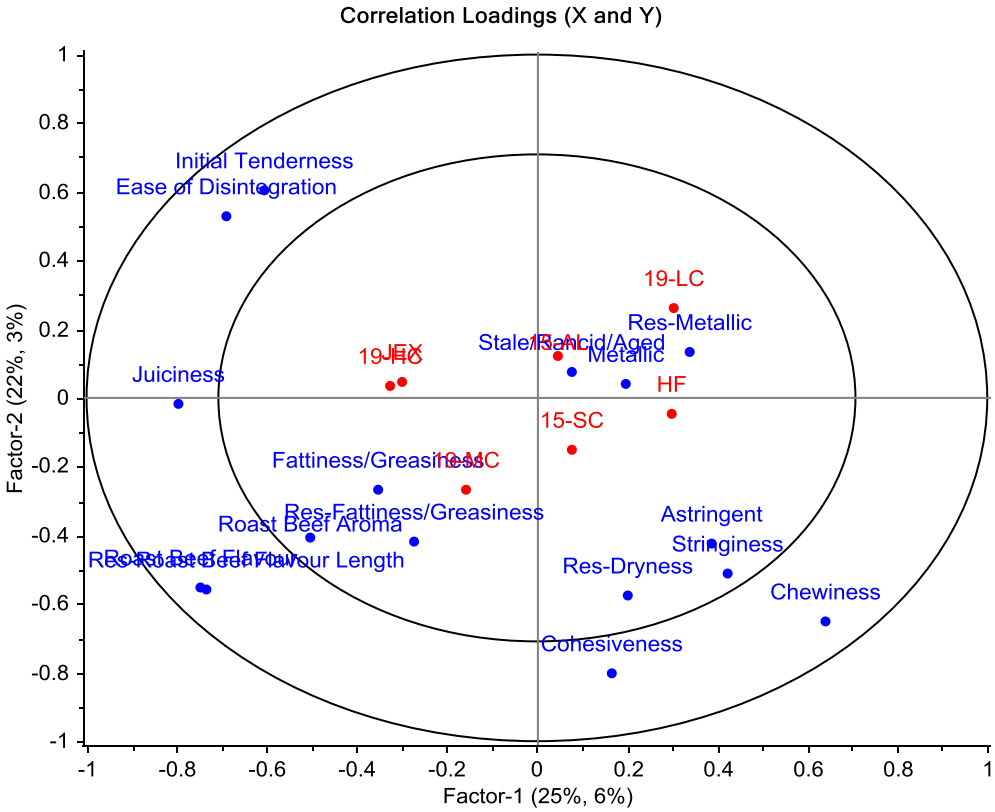


Figure 3.3. Partial least square regression (PLSR) correlation loadings plot of Factor1 versus Factor2. The model was derived from the sensory terms in the X-matrix and treatments and breed types in the Y-matrix. HF – Holstein-Friesian, JEX – Jersey × Holstein-Friesian. 15-AL – 19-LC-Refer to the treatment design in Table 3.1. 'Res-' = Residual (after-effects).

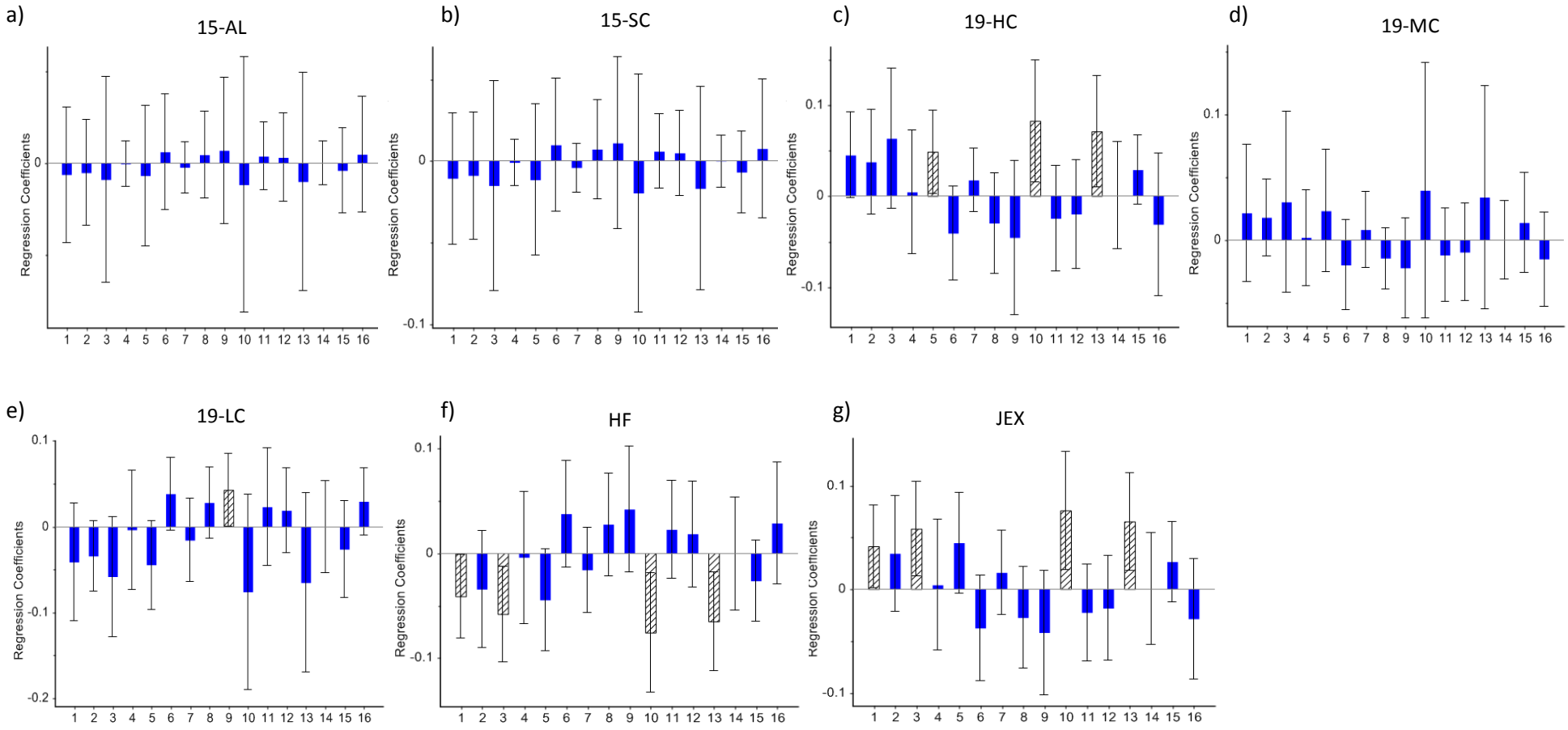


Figure 3.4. Standardized regression coefficients and significance indications from PLSR prediction models for treatments and breed types from the X-matrix - sensory attributes. Sensory attributes of 1-16 correspond to the code attributes in Table 3.4. HF − Holstein-Friesian, JEX − Jersey × Holstein-Friesian. 15-AL – 19-LC-Refer to the treatment design in Table 3.1. Columns with shadow represent the significant effect ($P < 0.05$).

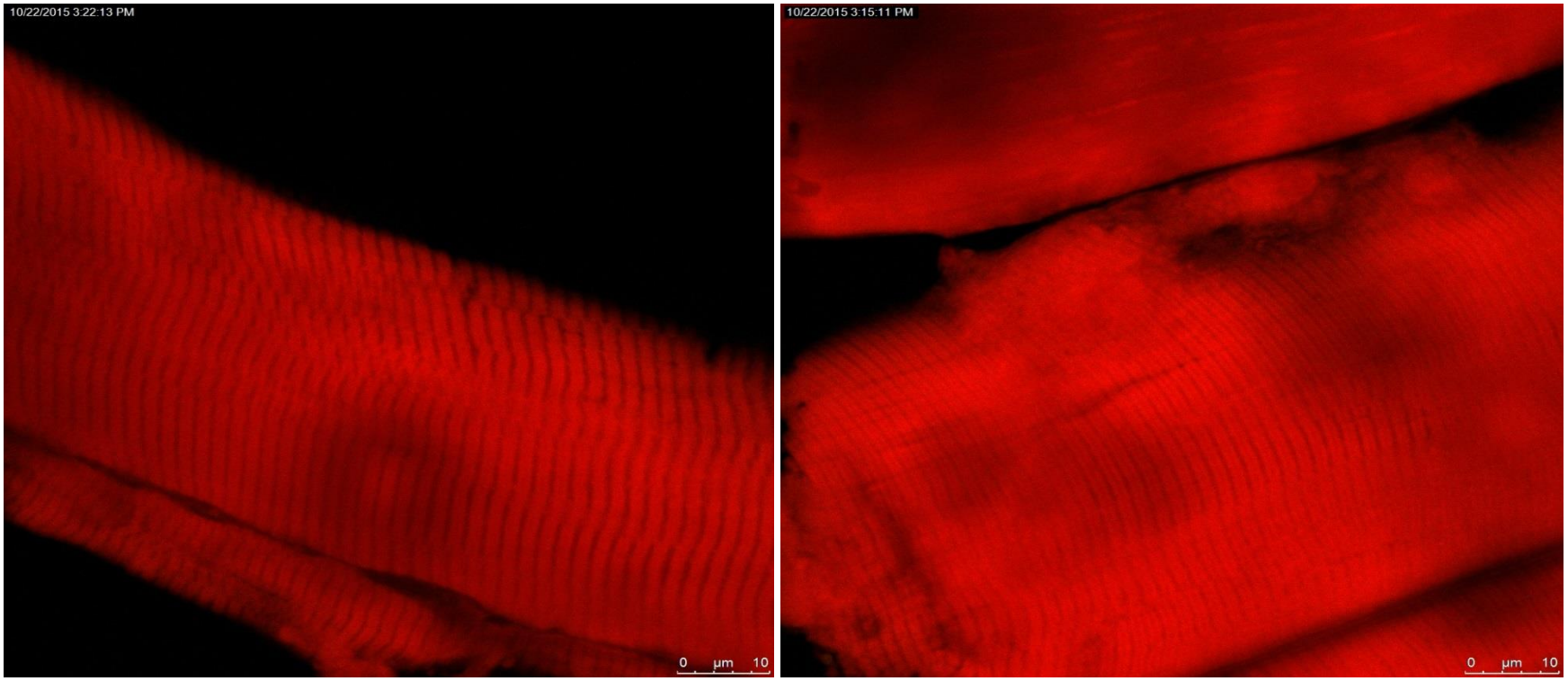


Figure 3.5. Image of LT muscle at 21 ageing days of 19-month old Holstein-Friesian (HF) bulls (Confocal Scanning Laser Microscopy of cryostat sections).

Chapter 4

Fatty acid composition of young dairy bull beef as affected by breed type, production treatment and relationship to sensory characteristics

4.1 Abstract

The effects of breed and feeding treatment on the fatty acid (FA) profile of the *longissimus thoracis* (LT) muscles of young dairy bulls (n = 69)−Holstein-Friesian (HF) and Jersey×Holstein-Friesian (JEX)−were evaluated. The relationship between FA composition and sensory characteristics was also investigated. Bulls were offered either 1 or 2 kg concentrates at pasture during the first grazing season. Bulls finished on silage with 5 kg concentrates or on *ad-lib* concentrates were slaughtered at 15 months of age, while bulls slaughtered at 19-months were fed either *ad-lib* pasture or pasture with 5 kg concentrates during a second grazing season and finished on pasture with 5 kg concentrates or *ad-lib* concentrates. Pasture finished 19-month old bulls had higher relative proportions of C18:0, C20:5n3 (EPA), C18:2n6c, C20:4n6, total n-3 and n-6 PUFA, but reduced C14:0, C16:0, C14:1, C16:1 and total MUFA proportions compared to other production treatments. The silage-based finishing system of 15-month old bulls increased the relative proportions of C15:0, C18:0 and total SFA, but decreased the proportions of C20:3n6 and total unsaturated FA (UFA) compared to the *ad-lib* concentrates finishing system. Thus forage-based beef had a higher nutritional quality relative to concentrate-based beef. However, the first or second grazing season feeding system had limited effects on the FA profile. JEX breed type had higher relative proportions of C14:1 and C16:1, but lower C15:0, C17:0 and C17:1 proportions than HF beef. Slaughter age did not affect the FA profile. MUFA, C14:0 and C16:0 proportions were positively correlated, but PUFA proportion was negatively correlated with IMF content, sensory roast beef aroma, flavour, flavour length, juiciness, initial tenderness and ease of disintegration scores. Finishing system and slaughter weight were well discriminated based on FA relative proportions by canonical discriminant analysis.

Keywords: Dairy breeds, Fatty acids, Feeding treatment, Intramuscular fat, Sensory attributes, Young bulls

4.2 Introduction

The ending of milk quotas in April 2015 significantly increased the potential for dairy output in Ireland, leading to a substantial increase in the number of male calves from the dairy herd. Raising these calves as steers may not be a viable option due to their poor conformation, but since bulls grow faster and have better feed efficiency than steers (Fallon et al., 2001) leaving them intact may generate viable financial returns.

The eating quality of beef from 15- and 19-month old dairy bulls has been shown to be very acceptable (Nian et al., 2017), but the nutritional status has not been determined. The potential effect of beef on human health and nutrition is a concern for consumers. Beef has been recognised as an important dietary source of fatty acids (FA) with beneficial effects on human health. Polyunsaturated FAs (PUFA), especially the n-3 series, particularly eicosapentaenoic acid (EPA), docasahexaenoic acid (DHA), and a lower ratio of n-6/n-3 PUFA are recommended due to the beneficial properties for disease prevention, i.e. reduce risk of cardiovascular disease, cancer, depression and type-2 diabetes. Moreover, they also play critical roles in the maintenance of neural, visual tissues and proper brain function (Scollan et al., 2014), while the saturated FAs (SFA), n-6 FA and *trans* FAs can raise plasma cholesterol levels which are related to cardio-vascular diseases (Brugiapaglia et al., 2014).

Fat in beef is classified into membrane fat (phospholipid), marbling (IMF), intermuscular fat and subcutaneous fat. Although fat content varies widely depending on the cut and degree of trimming, lean beef with a low IMF content, normally 2-5% is accepted in many countries as ‘low in fat’ (Scollan et al., 2006). Marbling fat is also considered to affect beef palatability because of a positive relationship with tenderness, juiciness and flavour of beef. FA composition has also been suggested to affect meat quality characteristics including fat tissue firmness (hardness), shelf life and flavour (Wood et al., 2003). PUFAs are susceptible to oxidation, and lipid peroxidation leads to colour deterioration and undesirable flavour development, thus reducing product shelf life (Scollan et al., 2006). Furthermore, some FAs are identified as the precursor of volatile compounds responsible for aroma and flavour characteristics of beef during cooking. For example, the ‘grassy’ and ‘bloody’ odour of grass-based cooked meat was associated with hexanals derived from oleic and α-

linolenic acid (Daley et al., 2010). Also, meat aromas are generated due to the interactions between Maillard reaction products and FAs (Wood et al., 2003).

However, there is considerable variation in the FA composition of beef due to numerous production and management factors, such as breed, diet, age/body weight and sex, etc. (Nürnberg et al., 1998). Genetics plays an active role in lipid metabolism contributing to the significant breed effects. Holstein-Friesian (HF) is the predominant breed type in the Irish dairy cow population due to its high milk yield. Some dairy farmers raise Jersey crosses, due to their improved reproductive performance, increased milk solids yields, longevity and improved profitability under Irish conditions (Prendiville et al., 2011). Therefore, the proportion of Jersey × Holstein-Friesian (JEX) bull calves may increase over time. Growth rate, carcass traits and performance of HF and JEX bulls have been reported (McNamee et al., 2015), and eating quality was reported by Nian et al. (2017). The FA composition of these two breed types has not been extensively studied.

Diet is the sole source of the essential FAs linoleic acid and α-linolenic acid and also affects the activity of lipogenic enzymes (Smith et al., 2009). Beef from pasture-finished cattle is generally leaner, containing greater proportions of n-3 PUFA and conjugated linoleic acid (CLA), and less to the cholesterol rising SFAs (myristc and palmitic acid) than cattle finished on a concentrate-based diet at a similar age range (Daley et al., 2010). Though grass and concentrate feeding systems have been extensively studied, especially during the finishing period, there is little information available on how the FA profile is affected by the grazing season.

Increased slaughter weights or older slaughter ages have been reported to improve the carcass quality traits of HF bulls (Węglarz, 2010). However, the increased carcass fatness at higher weights leads to a consequent increase in the proportion of MUFAs and a reduced PUFA concentration (Moreno et al., 2008). Thus the optimum time on feed to produce palatable beef with a desired FA profile needs to be identified.

The aim of this study was to investigate the FA profile of young dairy bull beef and to determine how it is affected by specific production factors- breed type and feeding regimes, including finishing and grazing seasons. A further objective was to establish the relationship between FA composition and sensory quality traits as this would help

in the understanding of how FAs contribute to the palatability characteristics of young dairy bull beef.

4.3 Materials and methods

4.3.1 Animals and diets

A subsample of each batch was selected from Chapter 3 for FA composition analysis. A total of 69 male dairy breed type bulls were selected: 35 HF and 34 JEX; 35 from PC1 and 34 from PC2; 15 from 15-AL, 16 from 15-SC; 10 from 19-HC, 13 from 19-MC, 15 from 19-LC. The experiment was set up as a 5 (treatment) × 2 (breed type) × 2 (first season feeding) factorial design, resulting in 20 groups with on average 3 or 4 animals as replications per group. The detailed production design was described in Table 3.1 of Chapter 3.

4.3.2 Slaughter and Sampling

On reaching the target slaughter age (15 or 19 months old), the bulls were transported to a commercial slaughterhouse. They were stunned by captive-bolt, conventionally hung, exsanguinated within 30 s, centrally-split into two sides and weighed. The carcasses were chilled at 4 °C under factory conditions. At 48 h post mortem, the LT muscle was removed from the cube roll (ribs 6 to 10) from the left-hand side of each carcass. After chilling for 72 h at 4 °C, muscles were cut into individual slices (25 mm in thickness) and vacuum-packed. Samples for intramuscular fat (IMF) concentration and FA analysis were stored at -20 °C immediately, while samples for sensory evaluation were aged for 21 days at 4 °C and then frozen at -20 °C for further analysis.

4.3.3 IMF measurement

The method was described in Chapter 2.

4.3.4 Trained sensory evaluation

The method was described in Chapter 2.

4.3.5 Fatty acid analysis

Fatty acid methyl esters (FAME) were generated by microwave assisted preparation and quantified by gas chromatography-flame ionisation detector (GC-FID) analysis according to Brunton et al. (2015) with slight modification. Briefly, 1 g of wet minced beef sample was added to a PFA 55 mL reaction vessel containing a 10 mm stirring bar. To this 10 mL of potassium hydroxide in methanol (2.5%) with 100 µL of internal standard (C23:0) was added. The reaction vessel was heated in the MARS 6 Express 40 position Microwave Reaction System (CEM Corporation, Matthews, NC, USA) to 130 ºC over 4 min and held at this temperature for 4 min to conduct the saponification process. The reaction vessel was then removed from the spindle wheel and cooled on ice for 5 min. Esterification was then carried out by adding 15 mL of 5% acetyl chloride in MeOH solution and heating to 120 ºC over 4 min and holding at this temperature for 2 min. The reaction tubes were removed again and cooled on ice for 5 min until they reached room temperature. Ten mL of pentane was added to the cooled tubes which were mixed gently while holding a bung over the top. Following this, 20 mL of a saturated salt solution was added, and the solution was mixed again as described above. After the solution was separated, the top pentane layer was removed and aliquoted into amber GC vials (1.5 mL) containing sodium sulphate and stored at -20 ºC until analysis. Triplicate assays were carried out for each sample.

A PerkinElmer Clarus 580 Gas Chromatograph (PerkinElmer, Waltham, MA, USA) fitted with a flame ionisation detector (FID) was used for FAME quantification. All analytes were separated using a Zebron ZB−5MS (Phenomenex, Torrance, CA, USA) fused silica capillary column (30 m × 0.25 mm ID, 0.25 µm film thickness). The oven was initially held at 50 °C for 5 min and was then heated at 5 °/min to 240 °C, where it remained for 20 min. The temperature of the injector was 200 °C, and the injection volume was 1 µm with the split set to 5:1. Hydrogen was used as the carrier gas at a flow rate of 1 mL/min. The flame ionisation detector temperature was maintained at 260 °C. Compounds were identified by comparing their retention times with those of authentic standards from the Supelco 37 FAME. Peak area analysis was performed using TotalChrom 6.3.2 software (PerkinElmer, Waltham, MA, USA). The concentration of each FA was expressed as the relative proportion (percentage of total FAs) and as absolute concentration (mg/100 g of wet minced meat), calculated

the content of each FA according to the following equation (ISTD: internal standard C23:0):

(Peak Area of Sample / Peak Area of ISTD) × (Weight of ISTD / Weight of Sample) × ISTD Purity × 10 × 0.96 × 100 = Content

4.3.6 Statistical analysis

Data were analysed using ANOVA with treatment, breed, first season feeding and their interactions as fixed effects using the GLM procedure of SAS Version 9.3 (Cary, NC: SAS Institute, 2002). The experimental unit was the individual animal for all variables to keep the unit consistent throughout the whole feeding period. The Tukey-Kramer test was applied for multiple comparisons among least square means, considering $P < 0.05$ as significant. As there were very few significant effects of the first season feeding, the least square means of first season determinations and their interactions with other factors were excluded in the results table. Pearson correlation coefficients were calculated using the CORR procedure of SAS (2002).

Canonical discriminant analysis was applied to FA composition in order to classify and distinguish different treatments. Variables with major discriminant ability were selected by the stepwise discriminant analysis (STEPDISC Procedure). The correct classifications and cross-validation methods were conducted using DISCRIM Procedure with the linear discriminant functions from SAS (2002). The efficiency of the discriminant power of the models selected was assessed by the Wilks' λ value test. The variance was explained by each canonical likelihood and by the analysis of the standardized canonical coefficients

4.4 Results and discussion

4.4.1 Breed effect

JEX beef had higher relative proportions of myristoleic (C14:1) and palmitoleic (C16:1) acids ($P < 0.05$), but less pentadecanoic (C15:0) ($P < 0.01$), heptadecanoic (C17:0) ($P < 0.001$) and cis-10-heptadecenoic (C17:1) acids proportions ($P < 0.01$) than HF beef (Table 4.2). The higher level of C14:1 and C16:1 of JEX beef is probably due to an elevated activity of Δ^9 desaturase in the JEX breed type bulls, even though it wasn't measured in this study. As stated by Zembayashi et al. (1995),

the major lipogenic enzyme Δ^9 desaturase is responsible for the conversion of SFA-like C14:0, C16:0, and C18:0 to their respective n-9 monounsaturated counterparts, and Δ^9 desaturase is encoded by the stearoyl-CoA desaturase (SCD) gene. According to Taniguchi et al. (2004a and b), the higher MUFA percentage in IMF of Holstein Japanese Black cattle resulted from elevated stearoyl-CoA desaturase mRNA expression and a single nucleotide polymorphism (SNP). In general, compared to dietary factors, genetic factors have less influence on the FA composition of beef. However, a small effect can still reflect differences in gene expression or enzyme activities in FA synthesis and modification (Scollan et al., 2014). Bartoň et al. (2010) also demonstrated that MUFA content in beef breeds can be affected by the polymorphism of the gene encoding for Δ^9 desaturase resulting from the effect of genetic factors. IMF content in the present study tended to be higher in the JEX than in the HF breed type (P = 0.088; Table 4.1) and Brugiapaglia et al. (2014) showed that fatter animals have an elevated activity of Δ^9 desaturase. Breed type did not affect the content of any PUFA in this study (P > 0.05). The absolute concentration of all FAs was not affected by breed type (Table 4.3, P > 0.05).

4.4.2 Production treatment effect

Beef from treatment 19-LC had lower proportions of myristic (C14:0), palmitic (C16:0), myristoleic (C14:1), palmitoleic (C16:1) acids, total MUFA and higher proportions of stearic (C18:0), eicosapentaenoic (C20:5n3, EPA) acids and total n-3 PUFA than other treatments (P < 0.001; Table 4.2). Similarly, linoleic (C18:2n6c) (P < 0.05), arachidonic (C20:4n6) acids (P < 0.01) and total n-6 PUFA (P < 0.05) proportions were higher in beef from 19-LC in comparison to other treatments, even though the difference between 19-LC and 16-AL was not statistically significant. Total PUFA percentage was higher in 19-LC than in 19-HC (P = 0.01). Pentadecanoic acid (C15:0) and total SFA percentages were higher in beef from treatments of 15-SC and 19-LC than beef from 15-AL, while total unsaturated FA (UFA) percentage was higher in 15-AL than 15-SC and 19-LC (P < 0.01). In addition, beef from 15-SC had higher C18:0 (P < 0.001) and lower cis8,11,14-Eicosatrienoic acid (C20:3n6) proportions compared with 15-AL (P < 0.05). Proportions of heptadecanoic (C17:0), cis-10-heptadecenoic (C17:1) and elaidic (C18:1n9t) acid were not affected by production treatment (P > 0.05). These results reflected the influence of dietary energy level on fatty acid profile of dairy bull beef;

in this study treatment 19-LC had the highest dietary forage content among all treatments while 15-SC also had relatively more dietary forage than the treatments finished on *ad-lib* concentrates (Table 3.1).

These results are in agreement with others who found that cattle fed primarily grass have higher proportions of PUFA, EPA and C18:0 and lower proportions of C14:0, C16:0, C14:1 and C16:1 (Alfaia et al., 2009; Daley et al., 2010). C14:0 and C16:0 have detrimental effects on health due to their cholesterol raising risk (Daley et al., 2010). The higher percentage of PUFA in the 19-LC system could be explained by the high pasture feeding because the presence of secondary plant metabolites in fresh grass may inhibit ruminal biohydrogenation relative to that of grain or silage (Lourenço et al., 2008). However, in contrast to our results, Leheska et al. (2008) found no feeding system effect on the percentages of n-6 and total PUFA of beef.

Moreover, it was shown that the decreased ruminal biohydrogenation reaction of FAs upon grain feeding, due to the reduced ruminal pH, could depress the population or activity of ruminal microorganisms that hydrogenate oleic acid (C18:1n9c) from the diet to stearic acid (C18:0) (Duckett et al., 1993). Accordingly, a relatively higher deposition of C18:1n9c and a lower deposition of C18:0 was found in grain-fed beef compared with pasture-fed beef. This could explain the relatively higher C18:0 proportion in beef resulting from high pasture feeding in treatments 15-SC and 19-LC, even though C18:1n9c was not separated and analysed in the present study as a result of co-elution with C18:3n3 and C18:2n6t. Even though the C18:0 percentage was higher in 15-SC and 19-LC beef, it has a neutral effect on plasma cholesterol with no hypercholesterolemic effect like other SFAs (Pavan & Duckett, 2013). Thus, it should be noted that the negative effect of increased SFAs on human health of beef from forage-based cattle in the present study has been attenuated because of the fact that the increased C18:0 accounted for the higher proportionate increase in total SFAs. In addition, the high level of long-chain products, such as C20:5n3 and C20:4n6 were synthesised from α-linolenic acid (C18:3n3) and linoleic acid (C18:2n6c) respectively by the elongase enzyme and Δ^5 and Δ^6 desaturase (Wood et al., 2008).

The results were also in accordance with Leheska et al. (2008) who reported that pasture-fed beef had lower a percentage of MUFAs but higher a percentage of SFAs

in comparison to grain-produced beef. This could be explained by the previous finding that the stearoly-CoA desaturase (SCD) gene was highly expressed in the IMF adipose tissue of high-concentrate fed steers, but undetectable in pasture-fed steers (Smith et al., 2009). The increased Δ^9 desaturase activity which converts SFAs to their respective MUFAs in high grain diets is probably mediated by a higher production of insulin (Daniel et al., 2004). Therefore, because of the depressed SCD activity, even though pasture feeding increases the PUFA proportion in beef, it also increases SFAs at the expense of MUFAs (Smith et al., 2009). In contrast, Noci et al. (2005) reported lower proportions of SFAs in grass-fed beef than grain-fed beef. In addition, according to Wood et al. (2008), MUFAs are located in neutral lipids which consist of triacylglycerols, they are mainly deposited at the fat drop inside the cell and increase markedly during fattening, whereas the phospholipid content (mg/100g muscle) remains constant. Thus the relative proportion of MUFAs increases with increased total lipid content, and consequently the relative percentage of PUFAs decreases. Accordingly, the lower proportion of total MUFAs and the higher proportion of total PUFA in beef from 19-LC compared with 19-HC could be due to their lower IMF content as shown in Table 4.1 ($P < 0.01$). Similarly, Indurain et al. (2006) also reported that fatter carcasses had higher MUFA and lower PUFA percentage in IMF.

For absolute concentration (mg/100g meat), only EPA was higher in beef from 19-LC compared to other treatments ($P < 0.001$; Table 4.3), which showed the same trend with the relative proportion in total FA. All other FAs were higher in beef produced from higher energy diet 19-HC, and the difference between 19-HC and 19-LC was more marked, which could be explained by their corresponding IMF content (Table 4.1). With the increasing of total lipid level in *longissimus* muscle, content of individual FA increased, especially those with greater proportion in the neutral lipid fraction, such as SFA and MUFA (Pavan & Duckett, 2013). Therefore, even though the enhanced FA profile in beef from higher pasture diet, daily intake of desirable FA such as MUFA could be greater when consuming beef from concentrate-fed cattle due to its greater total fat content (Smith et al., 2009; Daley et al., 2010). Thus the challenge is to maximize intake of desirable FAs while reducing intake of total fat and non-desirable FAs (Pavan & Duckett, 2013).

Slight difference in the concentrate level in pasture during the first grazing season had little effect on the relative proportions and absolute concentration of FAs. Only the proportion and absolute concentration of cis-10-heptadecenoic acid (C17:1) were higher in beef from bulls fed 1 kg than in those fed 2 kg of concentrate at pasture ($P < 0.05$).

4.4.3 Discriminant analysis

Canonical discriminant analysis was applied to the FA profiles in order to classify and discriminate the treatment groups used in this study, resulting in two discriminant functions. A stepwise forward discriminant analysis was initially applied in order to select the most relevant variables for classification. The following eight variables C18:0, C20:5n3, C20:4n6, C20:3n6, C16:0, total UFA, C15:0, and C17:0 were selected as the highest discriminatory power. The standardised discriminant coefficients obtained for each variable are presented in Table 4.4 with a higher coefficient corresponding to a greater contribution of the respective variable to the discrimination between groups. Thus the most important variables were C15:0, C18:0, C20:3n6 and C20:5n3 in the first canonical variable, C16:0, C18:0 and total UFA in the second canonical variable. Groups were discriminated with high accuracy with a total of 95.09% of the variance explained (Figure 4.1). The model is highly significant ($P < 0.001$) with a Wilks' Lambda value of 0.038.

The first function (canonical 1) discriminated treatments by feeding systems. Treatments finished with *ad-lib* concentrate (15-AL, 19-HC, and 19-MC) had negative loadings and treatments finished off grass *ad-lib* with 5 kg concentrate (19-LC) had positive loadings, while the treatment finished off silage *ad-lib* with 5 kg concentrate (15-SC) was located close to the origin (Figure 4.1). As *ad-lib* concentrate was applied for 6 months in 15-AL, while only 3 months for 19-HC and 19-MC, the result indicated that dietary energy level decreased from the negative to positive side of the plot. Moreover, according to canonical 2, the 19-HC and 19-MC treatments with higher slaughter weights mainly had positive scores, while most of the samples in treatments 15-AL, 15-SC and 19-LC with lighter carcasses had negative scores. This indicated that slaughter weight could be discriminated by canonical 2.

The differences between treatment groups can be evaluated by the values of squared Mahalanobis distance (D^2). In line with this, the distance was smallest between 19-HC and 19-MC (1.46), indicating similar FA profiles for different energy levels during the second grazing season, in agreement with the previous results in this study. However, larger differences were found between 15-AL and 15-SC (D2 = 10.50), between 19-MC and 19-LC ($D^2 = 27.82$), and between 19-HC and 19-LC (D^2 = 39.36), indicating that under the same age, finishing period or finishing system combined with second grazing season feeding can be better discriminated by two functions. The largest difference was between 15-AL and 19-LC with a D^2 of 56.24 suggesting the most apparent separation.

This methodology has been previously applied by Alfaia et al. (2009) to the discrimination of bull beef production systems: pasture, pasture supplemented with two levels of concentrate, and feedlot. Their result indicated that the meat FA profile enables the bulls to be allocated to one of the four feeding systems with good accuracy. Amorim et al. (2016) applied this method to discriminate chicken groups and found FA profile is an accurate tool to discriminate and distinguish different meat from different breeds and sexes. Furthermore, Monteiro et al. (2012) used discriminant analysis to discriminate veal and beef from different breeds based on FA composition. Dias et al. (2008) pointed out that FA profile as an origin discriminator is an effective tool to differentiate raw bovine meat based on production system and breed.

4.4.4 Correlations between fatty acid proportion and IMF content

IMF content in LD muscle has been recognized as the parameter having the closest relationship with intramuscular FA composition (Indurain et al., 2006) and likewise, strong correlations between the relative proportions of FAs and total lipid content were found in the current study. The relative proportion of total MUFA was positively correlated, while total PUFA, total n-6 and n-3 PUFA proportions were negatively correlated with IMF content ($P < 0.001$; Table 4.5), in agreement with Oka et al. (2002). According to Scollan et al. (2006), SFA and MUFA as neutral lipids increased markedly as total lipid increased, nevertheless the phospholipid FAs, mainly PUFA decreased with increasing fatness.

C20:4n6 was negatively associated with IMF content ($P < 0.001$), in line with Indurain et al. (2006). The relative proportions of C14:0 ($P < 0.001$), C16:0 ($P < 0.05$) and C16:1 acids ($P < 0.001$) showed a general increase, and linoleic acid (C18:2n6c) ($P < 0.001$) decreased with increasing IMF content, which has been reported by Kazala et al. (1999). Similar to our study, Skelley et al. (1973) reported a weak negative relationship between stearic acid (C18:0) percentage and marbling score of LD muscle, while in contrast, they found that C14:0 had a negative relationship with marbling score. Linoleic acid (C18:2n6c), as the predominant PUFA in bovine tissues, is most prevalent in the phospholipid fraction. The negative relationship between linoleic acid and marbling may be due to a dilution of the contribution of phospholipids to the total lipid with increasing amounts of triacylglycerol (Kazala et al., 1999).

In MUFA, apart from C16:1, the relative proportions of C14:1 ($P < 0.001$) and C17:1 ($P < 0.05$) had positive correlations, while C18:1n9t ($P < 0.05$) had a negative correlation with IMF content. In PUFA, apart from C20:4n6 and C18:2n6c, the relative proportions of C20:3n6 ($P < 0.05$) and C20:5n3 (EPA) ($P < 0.001$) were negatively corrected, but C20:3n3 ($P < 0.05$) was positively corrected with IMF content.

4.4.5 Correlations between fatty acid proportion and sensory attributes

The relative proportion of total MUFA was positively correlated with sensory flavour attributes including roast beef aroma, roast beef flavour, residual roast beef flavour length (Table 4.5), initial tenderness, ease of disintegration and juiciness ($P < 0.05$; Table 4.6), and was negatively correlated with chewiness ($P < 0.05$) and stringiness ($P < 0.01$; Table 4.6). Total PUFA, total n-6 and n-3 PUFA proportions showed the opposite trend to MUFA for the above sensory attributes ($P < 0.05$; Table 4.5 & 4.6). Similarly to the present results, Duckett et al. (1993) reported that PUFA was negatively correlated with tenderness (r = -0.49) and flavour (r = -0.35) ratings from taste panel evaluation. The contribution of MUFAs to tenderness, juiciness and flavour of cooked meat reflected the role of marbling fat in meat palatability as MUFAs were considered as the most important component in IMF being composed mainly of neutral lipids (Wood et al., 2008). The neutral lipids are present within the perimysium in fat cells which separates muscle fibre bundles and open the muscle

structure, leading to the positive effect on tenderness of meat (Wood et al., 2003). The breakdown products from FAs are the primary source of different flavour volatiles. A high PUFA amount in phospholipids produced a higher concentration of lipid oxidation products including aldehydes, alcohols and ketones, which are responsible for undesirable flavours in red meats, such as rancidity (Wood et al., 2003). It should be noted that even though the rancidity is low (< 5.0) in this study because of vacuum packing during ageing, lipid oxidation still occurred to a small degree probably due to oxidation occurred during sample preparation.

Accordingly, the varied MUFA and PUFA content between different diets affected the sensory flavour profile, texture and juiciness of beef to an important extent. This could be explained by the fact that panels gave higher scores of roast beef flavour and residual roast beef flavour length ($P < 0.05$) and tended to a higher juiciness score for beef from 19-HC ($P < 0.10$; Table 4.1) which had higher total MUFA proportion and gave the lower above sensory scores to beef from 19-LC which had high total PUFA, total n-6 and n-3 PUFA proportions. As bulls from 19-LC were fed higher dietary grass than bulls from 19-HC, it is in agreement with the previous study that grass-fed beef had a 'grassy' flavour and was less palatable than conventional beef (Bjorklund et al., 2014).

For individual MUFAs, the relative proportion of C16:1 was positively correlated with sensory flavour attributes (aroma, $P < 0.05$; flavour, $P < 0.001$; residual beef flavour length, $P < 0.01$; Table 4.5), initial tenderness ($P < 0.05$), ease of disintegration ($P < 0.01$) and juiciness ($P < 0.05$), and was negatively correlated with chewiness and stringiness scores ($P < 0.01$; Table 4.6). Following the same trend, C14:1 was also positively correlated with the three flavour attributes and juiciness score ($P < 0.05$). It is hypothesized that the higher percentages of C16:1 and C14:1 in JEX beef compared with HF beef could explain the more tender texture and higher flavour intensity in JEX breed expressed as the higher initial tenderness, ease of disintegration, roast beef flavour scores and lower chewiness scores ($P < 0.05$; Table 4.1).

For individual PUFAs, the relative proportions of C18:2n6c, C20:3n6, C20:4n6 and C20:5n3 (EPA) contributed to the less desirable beef flavour, lower juiciness and tougher texture expressed as the negative correlation with the three flavour attributes,

ease of disintegration, juiciness and the positive correlation with chewiness score. In particular, steaks with a higher C20:3n6 percentage had a higher rancidity score ($P < 0.05$; Table 4.5). Similarly, it has been noted that the susceptibility of n-6 PUFA to oxidation is high, which can easily generate a liver flavour (Partida et al., 2007). In addition, C20:2 was negatively correlated with metallic and residual metallic scores ($P < 0.05$; Table 4.5).

It should be noted that although the improvement of PUFA content, especially n-3 PUFA in meat is beneficial to nutritional value, it also produces an oxidative challenge. Thus the need to increase PUFA should be balanced by the role of PUFA in beef off-flavour generation. Even though higher amounts antioxidants in grass causes higher tissue levels of vitamin E, α-tocopherol, in forage-based animals with benefits for lower lipid oxidation, more off-flavours may still be present in forage-based beef compared with concentrate-based beef (Wood & Enser, 1997). The vitamin E content in many animals' muscles is less than 3.0-3.5 IU, the recommended value for optimum stability (Liu et al., 1996). Hence, usage of antioxidant supplements in the diet may overcome this problem.

The total SFA relative proportion showed no relationship with taste panel ratings, while David & Hedrick (1979) reported that total SFA percentage was negatively associated with beef flavour, but not significantly correlated with tenderness and juiciness scores. The different flavour scores between feeding treatments could also be explained by the C18:0 content which had a positive correlation with metallic score ($P < 0.05$; Table 4.5). Accordingly, the higher C18:0 content in beef from grass-finished cattle (19-LC) could have contributed to their relatively higher off-flavour score in comparison to beef from concentrate-finished cattle (19-HC), and thereby indirectly reduced the sensation of the desirable beef flavour intensity as shown in Table 4.1. This result is in accordance with the 'bloody' note of beef from grass-finished cattle reported by Nuernberg et al. (2005). The negative relationship between C18:0 and desirable beef flavour has also been reported by Duckett et al. (1993). On the contrary, C14:0 and C16:0 were positively correlated with the three flavour attributes ($P < 0.001$; Table 4.5), juiciness ($P < 0.05$) and ease of disintegration ($P < 0.05$), and were negatively correlated with stringiness score ($P < 0.01$; Table 4.6). Thereby, the lower C14:0 and C16:0 proportions in beef from 19-LC could also have contributed to their lower beef flavour intensity, beef flavour

length ($P < 0.05$) and juiciness ($P < 0.10$; Table 4.1). However, sensory attributes of fattiness/greasiness, residual fattiness/greasiness, cohesiveness, astringent and residual dryness had no significant correlation with FA composition ($P > 0.05$; Table 4.5 & 4.6).

4.5 Conclusions

Different dietary regimes markedly affect fatty acid composition of LT muscle of young dairy bulls. Beef produced from a forage-based diet had relatively better nutritional characteristics than that from the concentrate-based diet. The leaner muscle of cattle from a pasture-based finishing system contributes to an enhanced healthier fatty acid profile shown by more desirable SFAs (more C18:0 cholesterol neutral SFA and less C14:0 and C16:0 cholesterol elevating SFAs) and increased total n-3 PUFA and EPA relative proportions compared with other treatments. Moreover, beef from a pasture-based finishing diet had higher total PUFA proportion than the higher energy diet during finishing combined with the second grazing season period. A silage-based finishing system increased total SFA, C18:0 and C15:0 proportions but decreased total UFA and C20:3n6 proportions of 15-month old bulls. However, first and second grazing season feeding systems had very limited effect on fatty acid profile and for the absolute concentration, FAs from higher energy diet 19-HC was greater, except for EPA. Only slight effects of breed were observed, with JEX beef having higher C14:1 and C16:1 but lower C15:0, C17:0 and C17:1 proportions than HF beef. Slaughter age had no effect on fatty acid profile.

Fatty acid composition had a marked influence on beef palatability. The different beef fatty acid profile resulting from different diets could have contributed to the variation in sensory properties of beef between diets. However, even though nutritionally beneficial PUFAs, especially n-3 PUFA, were enhanced in grass fed beef they had negative effect on eating quality expressed as the negative correlation with IMF content, beef flavour intensity, tenderness and juiciness scores. There appears to be considerable scope to change fatty acid profile to make it more acceptable from both healthiness and taste perspectives. Finally, beef fatty acid profile seems to be an efficient chemical marker to discriminate production systems including slaughter weight and feeding systems with different energy level,

suggesting that this approach might be useful for the development of a practical discrimination tool for the young dairy bull beef industry.

Table 4.1. IMF content, sensory evaluation, slaughter weight and fat score of LT muscles of young dairy bulls.

	Treatment (T)										Breed (B)				*P*-value		
	15-AL		15-SC		19-HC		19-MC		19-LC		HF		JEX		T	B	T×B
	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM			
IMF (%)	2.6^{ab}	0.4	2.8^{ab}	0.39	4.2^{a}	0.56	2.6^{ab}	0.52	1.3^{b}	0.40	2.30	0.29	3.0	0.29	0.003	0.088	0.765
Slaughter weight (kg)	449.9^{b}	13.1	454.6^{b}	12.6	596.6^{a}	18.2	571.5^{a}	16.9	478.6^{b}	13.1	544.9^{a}	9.49	475.6^{b}	9.45	<0.001	<0.001	0.071
Fat score	5.38^{b}	0.36	5.19^{b}	0.34	7.85^{a}	0.49	7.06^{a}	0.46	4.42^{b}	0.36	5.87	0.26	6.09	0.26	<0.001	0.538	0.252
Roast Beef Aroma	53.3	3.04	53.2	2.92	55.0	4.22	54.0	3.90	46.2	3.04	51.1	2.20	53.5	2.19	0.324	0.447	0.487
Initial Tenderness	67.6	3.13	65.2	3.01	71.9	4.34	57.7	4.02	65.9	3.13	62.1^{b}	2.26	69.2^{a}	2.25	0.187	0.029	0.153
Juiciness	41.5	2.45	41.2	2.35	51.4	3.40	43.9	3.14	40.3	2.45	42.5	1.77	44.8	1.76	0.096	0.367	0.500
Cohesiveness	56.7^{ab}	2.01	62.3^{ab}	2.01	57.8^{ab}	2.79	64.8^{a}	2.58	55.5^{b}	2.01	61.1	1.47	57.7	1.45	0.025	0.114	0.475
Ease of Disintegration	61.0	2.88	59.5	2.77	65.9	3.99	58.7	3.70	59.0	2.88	57.8^{b}	2.08	63.9^{a}	2.07	0.640	0.043	0.120
Chewiness	31.3	3.52	34.7	3.38	26.9	4.88	41.7	4.51	34.7	3.52	38.1^{a}	2.54	29.6^{b}	2.53	0.238	0.021	0.402
Fattiness/Greasiness	9.61	1.02	10.6	1.02	9.36	1.42	8.47	1.31	8.93	1.02	9.28	0.75	9.50	0.74	0.719	0.832	0.398
Stringiness	7.57	1.05	7.06	1.05	5.70	1.45	9.87	1.34	7.86	1.05	8.53	0.76	6.70	0.75	0.312	0.093	0.474
Astringent	9.73	1.14	11.2	1.14	6.93	1.58	10.6	1.46	10.0	1.14	10.3	0.83	9.04	0.82	0.305	0.278	0.825
Roast Beef Flavour	43.2^{ab}	2.8	46.5^{ab}	2.69	53.4^{a}	3.89	49.6^{ab}	3.59	38.5^{b}	2.80	43.3^{b}	2.02	49.2^{a}	2.01	0.023	0.043	0.938
Metallic	5.56	0.60	6.14	0.58	6.29	0.83	6.52	0.77	7.58	0.60	6.51	0.43	6.32	0.43	0.215	0.757	0.888
Stale/Rancid/Aged	3.31	0.42	2.05	0.41	2.36	0.59	1.51	0.54	1.95	0.42	2.17	0.31	2.30	0.30	0.082	0.758	0.482
Res[1]-RBFL[2]	38.5^{ab}	3.02	41.0^{ab}	2.9	49.1^{a}	4.19	46.3^{ab}	3.88	33.4^{b}	3.02	39.8	2.18	43.5	2.17	0.023	0.239	0.751
Res-Metallic	7.86	0.70	7.70	0.7	7.88	0.98	9.23	0.90	8.87	0.70	6.47	0.44	6.32	0.43	0.563	0.933	0.218
Res-Fattiness/Greasiness	9.02	0.95	9.32	0.95	9.29	1.32	8.86	1.22	7.79	0.95	8.59	0.69	9.12	0.68	0.804	0.587	0.942
Res-Dryness	9.60	0.77	10.7	0.77	7.96	1.06	9.11	0.99	8.86	0.77	9.38	0.56	9.12	0.55	0.260	0.734	0.819

LSM = least square means; SEM = standard error of LSM. $^{a, b}$ Means within a row within a main effect with different superscripts significantly differ ($P < 0.05$).

15-AL–19-LC-Refer to the treatment design in Table 3.1 in Chapter 3.

[1]'Res-' = Residual (after effects); [2]Res-RBFL = Res-Roast Beef Flavour Length.

Table 4.2. Fatty acid proportion (g/100 g of total fatty acids) of LT muscles of young dairy bulls.

	Treatment (T)										Breed (B)				*P*-value		
	15-AL		15-SC		19-HC		19-MC		19-LC		HF		JEX		T	B	T×B
	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM			
C14:0	2.19^{a}	0.10	2.38^{a}	0.09	2.48^{a}	0.14	2.37^{a}	0.13	1.69^{b}	0.10	2.13	0.07	2.31	0.07	<.001	0.068	0.174
C15:0	0.24^{b}	0.02	0.34^{a}	0.02	0.27^{ab}	0.03	0.29^{ab}	0.03	0.35^{a}	0.02	0.33^{a}	0.01	0.27^{b}	0.01	0.002	0.005	0.212
C16:0	26.2^{a}	0.46	26.9^{a}	0.45	26.1^{a}	0.64	26.4^{a}	0.60	23.2^{b}	0.46	25.7	0.34	25.8	0.33	<.001	0.892	0.871
C17:0	0.96	0.05	1.07	0.05	1.07	0.07	1.16	0.07	1.13	0.05	1.21^{a}	0.04	0.95^{b}	0.04	0.111	<.001	0.025
C18:0	16.2^{c}	0.68	20.1^{b}	0.65	17.5^{bc}	0.94	18.6^{bc}	0.87	23.7^{a}	0.68	19.2	0.49	19.3	0.49	<.001	0.827	0.347
C14:1	0.39^{a}	0.03	0.34^{a}	0.03	0.45^{a}	0.05	0.37^{a}	0.04	0.18^{b}	0.04	0.30^{b}	0.02	0.39^{a}	0.02	<.001	0.014	0.731
C16:1	2.85^{a}	0.11	2.62^{a}	0.11	3.03^{a}	0.16	2.64^{a}	0.15	1.65^{b}	0.11	2.40^{b}	0.08	2.71^{a}	0.08	<.001	0.012	0.716
C17:1	0.71	0.04	0.75	0.04	0.81	0.05	0.80	0.05	0.68	0.04	0.81^{a}	0.03	0.70^{b}	0.03	0.181	0.006	0.008
C18:1n9t	1.49	0.13	1.45	0.13	1.22	0.18	1.43	0.17	1.77	0.13	1.50	0.09	1.44	0.09	0.159	0.697	0.541
C18:2n6c	6.71^{ab}	0.71	5.21^{b}	0.68	4.79^{b}	0.98	5.16^{b}	0.91	8.07^{a}	0.71	6.40	0.51	5.58	0.51	0.019	0.262	0.657
C20:3n6	0.43^{a}	0.05	0.23^{b}	0.05	0.26^{ab}	0.07	0.24^{ab}	0.06	0.35^{ab}	0.05	0.35	0.03	0.26	0.03	0.030	0.096	0.711
Coelution	39.4^{ab}	0.69	36.9^{bc}	0.66	40.5^{a}	0.96	38.9^{ab}	0.89	34.2^{c}	0.69	37.5	0.50	38.4	0.50	<.001	0.207	0.026
C20:4n6	1.86^{ab}	0.22	1.24^{b}	0.21	0.98^{b}	0.30	1.10^{b}	0.28	2.15^{a}	0.22	1.61	0.16	1.32	0.16	0.004	0.206	0.652
C20:5n3	0.29^{b}	0.06	0.34^{b}	0.06	0.26^{b}	0.09	0.32^{b}	0.08	0.88^{a}	0.06	0.47	0.05	0.37	0.05	<.001	0.120	0.177
∑ SFA[1]	45.8^{b}	0.92	50.9^{a}	0.89	47.5^{ab}	1.28	48.9^{ab}	1.19	50.1^{a}	0.92	48.6	0.67	48.7	0.66	0.002	0.912	0.250
∑ UFA[2]	54.3^{a}	0.92	49.1^{b}	0.89	52.5^{ab}	1.28	51.1^{ab}	1.18	49.9^{b}	0.92	51.4	0.67	51.3	0.66	0.002	0.897	0.245
∑ MUFA[3]	5.44^{a}	0.19	5.16^{a}	0.18	5.51^{a}	0.27	5.24^{a}	0.25	4.25^{b}	0.19	5.01	0.14	5.23	0.14	<.001	0.255	0.673
∑ PUFA[4]	9.42^{ab}	1.01	7.09^{ab}	0.97	6.53^{bc}	1.40	6.94^{ab}	1.29	11.5^{a}	1.01	8.91	0.73	7.66	0.72	0.010	0.229	0.630
∑ n-6 PUFA	9.01^{ab}	0.96	6.68^{b}	0.92	6.03^{b}	1.33	6.51^{b}	1.23	10.6^{a}	0.96	8.36	0.69	7.17	0.69	0.013	0.229	0.666
∑ n-3 PUFA	0.37^{b}	0.06	0.39^{b}	0.06	0.43^{b}	0.09	0.41^{b}	0.08	0.89^{a}	0.06	0.53	0.05	0.47	0.05	<.001	0.328	0.077

LSM = least square means; SEM = standard error of LSM. ^{a}, ^{b}, ^{c} Means within a row within a main effect with different superscripts significantly differ ($P < 0.05$).

15-AL–19-LC-Refer to the treatment design in Table 3.1 in Chapter 3.

Coelution = Sum of C18:1n9c + C18:2n6t + C18:3n3.

[1]∑ SFA = Sum of saturated fatty acids.

[2]∑ UFA = Sum of unsaturated fatty acids.

[3]∑ MUFA = Sum of monounstaturated fatty acids (excluding co-eluting peaks).

[4]∑ PUFA = Sum of polyunsaturated fatty acids (excluding co-eluting peaks).

∑ n-6 PUFA = Sum of n-6 polyunsaturated fatty acids (excluding co-eluting peaks).

∑ n-3 PUFA = Sum of n-3 polyunsaturated fatty acids (excluding co-eluting peaks, included C20:3n3).

Table 4.3. Fatty acid concentration (mg/100g meat) of LT muscles of young dairy bulls.

	Treatment (T)										Breed (B)				*P*-value		
	15-AL		15-SC		19-HC		19-MC		19-LC		HF		JEX		T	B	T×B
	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM			
C14:0	40.6^{bc}	5.83	46.8^{ab}	5.60	71.6^{a}	8.09	49.5^{ab}	7.48	21.3^{c}	5.83	41.8	4.21	50.1	4.19	<.001	0.168	0.921
C15:0	4.37	0.84	6.59	0.81	7.81	1.17	6.15	1.08	4.27	0.84	6.22	0.61	5.46	0.61	0.056	0.384	0.354
C16:0	473.7^{ab}	55.6	523.9^{a}	53.5	734.7^{a}	77.1	551.0^{a}	71.4	274.4^{b}	55.6	481.8	40.2	541.3	40.0	<.001	0.299	0.961
C17:0	17.5^{ab}	2.9	21.0^{ab}	2.79	31.0^{a}	4.02	24.6^{ab}	3.72	13.6^{b}	2.9	23.3	2.09	19.8	2.08	0.011	0.249	0.289
C18:0	298.8^{ab}	42.8	400.6^{ab}	41.1	502.9^{a}	59.3	390.5^{ab}	54.9	280.1^{b}	42.8	351.7	30.9	397.5	30.8	0.022	0.299	0.736
C14:1	7.54^{ab}	1.3	6.51^{b}	1.25	12.8^{a}	1.8	7.77^{ab}	1.66	2.40^{b}	1.39	6.12	0.95	8.68	0.94	0.001	0.062	0.932
C16:1	52.4^{b}	6.91	50.9^{b}	6.64	86.6^{a}	9.59	55.3^{ab}	8.87	20.2^{c}	6.91	46.7	4.99	59.4	4.97	<.001	0.078	0.956
C17:1	12.6^{b}	2.03	14.6^{ab}	1.95	23.2^{a}	2.82	17.1^{ab}	2.61	8.16^{b}	2.03	15.6	1.47	14.6	1.46	0.001	0.621	0.436
C18:1n9t	23.9	3.41	27	3.28	35.8	4.73	29.2	4.37	19.3	3.41	24.8	2.46	29.3	2.45	0.073	0.206	0.861
C18:2n6c	104.9^{a}	6.35	92.5^{ab}	6.1	121.7^{a}	8.8	104.5^{ab}	8.14	78.1^{b}	6.35	99.6	4.59	101.1	4.56	0.002	0.821	0.697
C20:3n6	6.51^{a}	0.34	3.92^{c}	0.33	6.09^{ab}	0.47	4.72^{bc}	0.44	3.38^{c}	0.34	5.12	0.25	4.73	0.24	<.001	0.26	0.416
Coelution	717.9^{bc}	86.5	721.5^{bc}	83.1	1149.7^{a}	119.9	822.3^{ab}	111	402.8^{c}	86.5	704	62.5	821.7	62.2	<.001	0.188	0.964
C20:4n6	27.3^{a}	0.96	21.0^{b}	0.93	23.4^{ab}	1.34	21.3^{b}	1.24	20.1^{b}	0.96	22.6	0.7	22.6	0.69	<.001	0.996	0.602
C20:5n3	4.12^{c}	0.4	5.77^{b}	0.39	5.97^{bc}	0.56	6.11^{b}	0.52	8.38^{a}	0.4	5.99	0.29	6.15	0.29	<.001	0.689	0.669
∑ SFA[1]	835.4^{b}	106.1	1001.0^{ab}	101.9	1350.9^{a}	147.1	1023.1^{ab}	136.1	595.1^{b}	106.1	906.1	76.6	1016.1	76.3	0.002	0.314	0.887
∑ UFA[2]	960.0^{bc}	102.5	944.7^{bc}	98.5	1471.8^{a}	142.2	1070.7^{ab}	131.5	562.8^{c}	102.5	932.8	74.1	1071.2	73.7	<.001	0.191	0.975
∑ TFA[3]	1795.4^{b}	206.3	1945.7^{ab}	198.2	2822.8^{a}	286.1	2093.8^{ab}	264.7	1157.8^{b}	206.3	1838.9	149	2087.3	148.3	0.001	0.243	0.948
∑ MUFA[4]	96.5^{bc}	12.3	98.9^{b}	11.8	158.4^{a}	17	109.4^{ab}	15.7	49.7^{c}	12.3	93.3	8.86	111.9	8.82	<.001	0.143	0.972
∑ PUFA[5]	145.6^{ab}	7.57	124.3^{bc}	7.27	163.7^{a}	10.5	139.0^{abc}	9.71	110.3^{c}	7.57	135.5	5.46	137.7	5.44	0.001	0.773	0.624
∑ n-6 PUFA	138.7^{ab}	7.04	117.5^{bc}	6.76	151.1^{a}	9.76	130.5^{abc}	9.03	101.7^{c}	7.04	127.4	5.08	128.4	5.06	0.001	0.882	0.645
∑ n-3 PUFA	6.01^{b}	0.69	6.49^{b}	0.66	10.9^{a}	0.96	8.10^{ab}	0.88	8.59^{ab}	0.69	7.41	0.5	8.65	0.5	0.001	0.083	0.07

LSM = least square means; SEM = standard error of LSM. $^{a, b, c}$ Means within a row within a main effect with different superscripts significantly differ ($P < 0.05$).

15-AL–19-LC-Refer to the treatment design in Table 3.1 in Chapter 3.

Coelution = Sum of C18:1n9c + C18:2n6t + C18:3n3.

[1]∑ SFA = Sum of saturated fatty acids.

[2]∑ UFA = Sum of unsaturated fatty acids.

[3]∑ TFA = Total fatty acids.

[4]∑ MUFA = Sum of monounstaturated fatty acids (excluding co-eluting peaks).

[5]∑ PUFA = Sum of polyunsaturated fatty acids (excluding co-eluting peaks).

∑ n-6 PUFA = Sum of n-6 polyunsaturated fatty acids (excluding co-eluting peaks).

∑ n-3 PUFA = Sum of n-3 polyunsaturated fatty acids (excluding co-eluting peaks, included C20:3n3).

Table 4.4. F values of fatty acid proportion (g/100 g of total fatty acids) variables used in the discriminant analysis and standardized canonical coefficients of predictor variables in discriminant functions (canonical 1 and 2).

Variable	*F* ratio	*P*-value	Canonical 1	Canonical 2
C15:0	3.40	0.015	0.641	-2.251
C16:0	2.84	0.032	-0.420	-8.661
C17:0	4.13	0.005	-0.393	0.938
C18:0	20.4	<.0001	0.703	-14.137
C20:3n6	3.83	0.008	-1.228	0.529
C20:4n6	14.0	<.0001	-0.272	-1.963
C20:5n3	18.6	<.0001	2.473	2.212
∑ UFA[1]	2.33	0.067	-0.240	-14.798

[1]∑ UFA = Sum of unsaturated fatty acids.

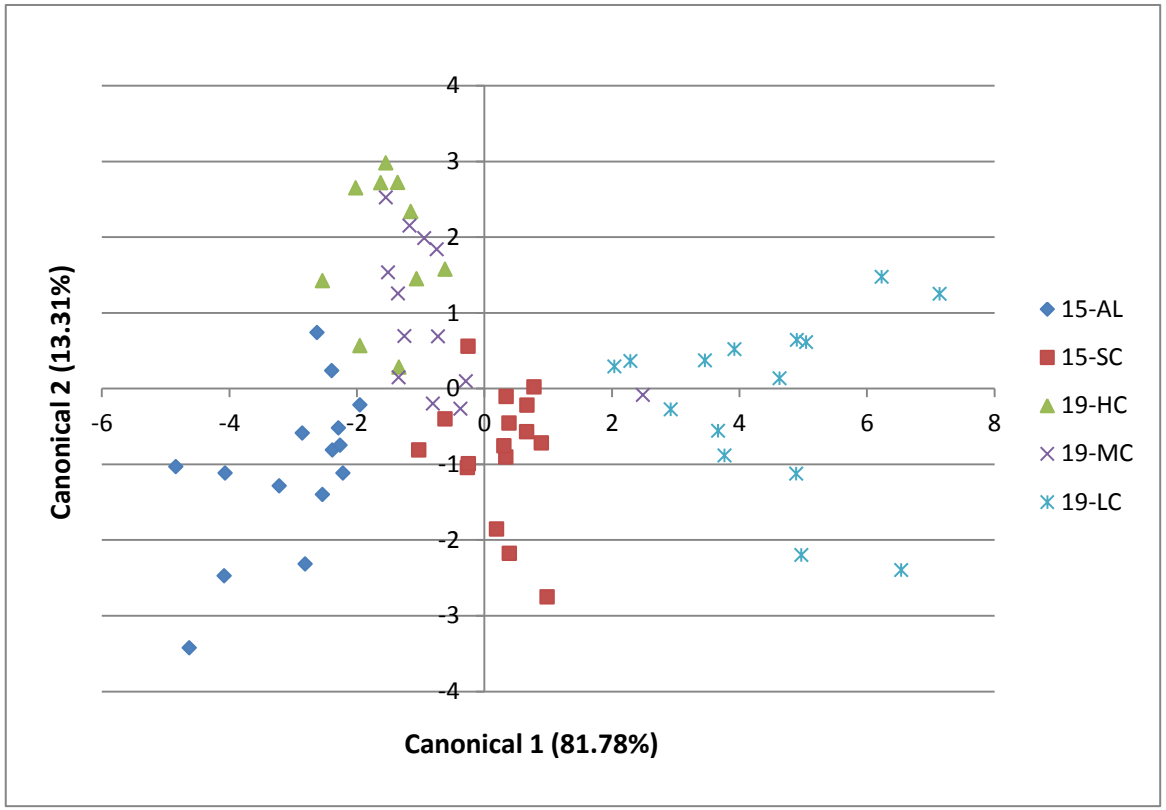


Figure 4.1. Scatter plot of the first two canonical variables of LT muscles of young dairy bulls from five treatments considered.

Table 4.5. Pearson correlation coefficients between fatty acid proportion (g/100 g of total fatty acids) and IMF content, sensory flavour attributes of LT muscles of young dairy bulls.

	IMF	Roast Beef Aroma	Roast Beef Flavour	Res[5]-Roast Beef Flavour Length	Metallic	Res-Metallic	Stale/Rancid /Aged	Fattiness/ Greasiness	Res-Fattiness/ Greasiness
C14:0	0.44***	0.41***	0.50***	0.49***	-0.14	-0.17	-0.07	0.16	0.12
C15:0	-0.15	-0.05	-0.05	-0.01	0.01	-0.07	-0.05	0.06	0.05
C16:0	0.29*	0.34**	0.43***	0.41***	-0.16	-0.17	-0.01	0.15	0.11
C17:0	-0.08	-0.10	-0.02	0.04	0.03	-0.02	-0.04	-0.02	0.07
C18:0	-0.31*	-0.14	-0.18	-0.16	0.29*	0.002	-0.13	0.12	-0.02
C20:0	-0.30	-0.08	-0.24	-0.20	0.08	-0.06	-0.04	0.20	0.08
C14:1	0.45***	0.30*	0.39***	0.35**	-0.19	0.04	-0.02	-0.10	0.04
C16:1	0.57***	0.32*	0.38***	0.34**	-0.20	-0.05	0.00	0.01	0.01
C17:1	0.24*	-0.04	0.08	0.08	-0.13	-0.17	-0.13	-0.12	0.14
C18:1n9t	-0.24*	-0.07	-0.16	-0.10	0.02	-0.05	-0.05	0.14	-0.07
C18:2n6c	-0.42***	-0.28*	-0.34**	-0.30*	0.004	0.12	0.23	-0.01	0.02
C20:2	-0.27	0.11	0.05	0.12	-0.64*	-0.54*	0.19	0.36	0.12
C20:3n6	-0.25*	-0.16	-0.26*	-0.23*	-0.11	0.11	0.27*	-0.04	0.003
C20:3n3	0.44*	0.13	0.22	0.05	-0.06	0.29	0.13	0.15	-0.28
Coelution	0.48***	0.14	0.19	0.14	-0.14	-0.01	-0.08	-0.22	-0.06
C20:4n6	-0.41***	-0.20	-0.28*	-0.27*	-0.03	0.15	0.19	-0.04	-0.02
C20:5n3	-0.43***	-0.31*	-0.37**	-0.38**	0.14	0.19	-0.05	-0.13	-0.16
∑ SFA[1]	-0.09	0.09	0.11	0.13	0.17	-0.11	-0.14	0.21	0.06
∑ UFA[2]	0.09	-0.09	-0.12	-0.13	-0.17	0.11	0.14	-0.21	-0.06
∑ MUFA[3]	0.43***	0.26*	0.27*	0.27*	-0.23	-0.11	-0.06	0.05	0.01
∑ PUFA[4]	-0.42***	-0.27*	-0.33*	-0.31*	-0.002	0.14	0.21	-0.03	-0.01
∑ n-6 PUFA	-0.42***	-0.26*	-0.33**	-0.30*	-0.01	0.13	0.23	-0.02	0.01
∑ n-3 PUFA	-0.37**	-0.30*	-0.34**	-0.37**	0.12	0.21	-0.04	-0.17	-0.21

Significance: *, P <0.05; **, P <0.01; ***, P <0.001.

Coelution = Sum of C18:1n9c + C18:3n3 + C18:2n6t.

[1]∑ SFA = Sum of saturated fatty acids.

[2]∑ UFA = Sum of unsaturated fatty acids.

[3]∑ MUFA = Sum of monounstaturated fatty acids (excluding co-eluting peaks).

[4]∑ PUFA = Sum of polyunsaturated fatty acids (excluding co-eluting peaks).

[5]'Res-' = Residual (after effects).

Table 4.6. Pearson correlation coefficients between fatty acid proportion (g/100 g of total fatty acids) and sensory juiciness and texture attributes of LT muscles of young dairy bulls.

	Initial Tenderness	Cohesiveness	Ease of Disintegration	Chewiness	Stringiness	Juiciness	Astringent	Dryness
C14:0	0.22	0.08	0.36**	-0.30*	-0.32**	0.33*	-0.09	-0.07
C15:0	-0.03	0.05	0.01	0.03	0.02	-0.03	0.002	0.15
C16:0	0.19	0.11	0.30*	-0.23	-0.31*	0.27*	0.02	-0.02
C17:0	-0.07	0.11	0.01	0.08	0.19	0.02	-0.05	0.09
C18:0	-0.11	0.01	-0.11	0.10	0.17	-0.16	0.11	0.08
C20:0	0.13	-0.14	-0.07	-0.08	-0.01	-0.07	-0.06	0.11
C14:1	0.16	-0.05	0.24	-0.24	-0.22	0.27*	-0.14	-0.05
C16:1	0.29*	-0.07	0.35**	-0.34**	-0.37**	0.31*	-0.14	-0.14
C17:1	0.05	-0.08	0.12	-0.06	-0.02	0.13	-0.21	0.05
C18:1n9t	-0.06	-0.07	-0.19	0.11	0.02	-0.06	-0.01	0.01
C18:2n6c	-0.19	-0.04	-0.36**	0.28*	0.27*	-0.23	0.08	0.19
C20:2	-0.36	0.33	-0.42	0.54*	0.17	-0.30	0.28	0.46
C20:3n6	-0.13	-0.08	-0.27*	0.20	0.15	-0.22	0.06	0.12
C20:3n3	0.24	-0.14	0.25	-0.17	-0.20	0.07	-0.36	-0.30
Coelution	0.14	-0.003	0.25*	-0.19	-0.16	0.18	-0.16	-0.25
C20:4n6	-0.20	-0.04	-0.35**	0.26*	0.18	-0.26*	0.08	0.17
C20:5n3	-0.24	-0.06	-0.39**	0.28*	0.14	-0.26*	0.13	0.05
∑ SFA[1]	0.02	0.08	0.10	-0.06	-0.03	0.04	0.10	0.06
∑ UFA[2]	-0.02	-0.08	-0.10	0.06	0.04	-0.04	-0.09	-0.06
∑ MUFA[3]	0.24*	-0.12	0.24*	-0.27*	-0.32**	0.27*	-0.19	-0.10
∑ PUFA[4]	-0.20*	-0.05	-0.37**	0.28*	0.24*	-0.25*	0.08	0.18
∑ n-6 PUFA	-0.19	-0.05	-0.36**	0.28*	0.24*	-0.24*	0.08	0.19
∑ n-3 PUFA	-0.21	-0.06	-0.36**	0.28*	0.13	-0.27*	0.12	0.03

Significance: *, P <0.05; **, P <0.01; ***, P <0.001.

Coelution = Sum of C18:1n9c + C18:3n3 + C18:2n6t.

[1]∑ SFA = Sum of saturated fatty acids.

[2]∑ UFA = Sum of unsaturated fatty acids.

[3]∑ MUFA = Sum of monounstaturated fatty acids (excluding co-eluting peaks).

[4]∑ PUFA = Sum of polyunsaturated fatty acids (excluding co-eluting peaks).

Chapter 5

Meat quality and fatty acid composition of three muscles from two age groupings of young Holstein-Friesian bulls

5.1 Abstract

This study investigated the physico-chemical characteristics of beef from Holstein-Friesian (HF) bulls slaughtered at 15 and 19 months of age. *Longissimus thoracis* (LT), *Semitendinosus* (ST) and *Gluteus medius* (GM) muscles were collected from 30 carcasses. Post-mortem pH decline, ultimate pH (pHu), meat colour, proximate chemical composition, collagen characteristics and fatty acid (FA) composition were evaluated. After ageing for 14 days, Warner-Bratzler variables (WBSF, WB-slope, WB-area) and cooking loss were measured. pHu was higher in 19-month old bulls, while redness, yellowness and saturation at 24 h post-blooming were lower. WB-variables, cooking loss, insoluble and total collagen content increased with slaughter age, and collagen solubility decreased with age. The 15-month feeding system employing a higher energy diet increased beef intramuscular fat (IMF) content, SFA proportion and n-6/n-3 PUFA ratio, while the 19-month feeding system increased moisture content, PUFA proportion and PUFA/SFA ratio. GM had the darkest colour, followed by LT and ST. Yellowness, saturation and hue angle were higher in ST. LT had lower WB-variables, cooking loss and moisture, but had a higher IMF content than ST and GM. PUFA proportion and PUFA/SFA ratio were highest in ST, followed by GM and LT, while LT had higher SFA proportion. Consequently, the eating quality of LT muscle of HF bulls was considered superior, but FA profile was inferior to the other muscles. Based on this production system, 15-month old bulls produced beef with relatively better eating quality, but with a less healthy FA profile than 19-month old bulls. The interaction between muscle and age-production system had marked effect on IMF content and total FA and SFA concentration. IMF content, cooking loss and collagen solubility primarily contributed to tenderness variation in LT, ST and GM muscles, respectively. FA composition correlated with IMF in LT, and with WBSF and cooking loss in ST.

Keywords: Dairy bulls, Fatty acids, Meat quality, Muscle, Production system, Tenderness

5.2 Introduction

As a result of the abolition of EU milk quotas in 2015, the Irish national dairy herd is expected to increase from 1.15 to 1.43 million by 2020 (DAFM, 2014). Undoubtedly, this will lead to a substantial increase in the supply of male calves from the dairy herd. Finding the most feasible and profitable beef production systems for these surplus male dairy calves is a challenge for the industry. Raising them as steers is not a viable option due to their poor conformation and lower economic returns. Leaving these males intact to develop as bulls may be more profitable due to their improved growth rate and feed conversion efficiency. A review of studies carried out in Ireland showed that, on average, bulls grow 8.4% faster, have 9.5% heavier carcasses with 20% greater lean meat yield than steers reared in the same way (Fallon et al., 2001). Moreover, bull-based production systems have a carbon footprint of only 52% of that of steer-based systems, which could contribute significantly to more sustainable farming practices and in terms of environmental impact (Dawson, 2010). Compared with the important suckler beef production systems in Ireland, dairy beef can be produced at a lower economic and environmental cost as the overhead cost of the cow is borne by the dairy industry (Murray, 2013). Therefore, rearing male dairy calves for beef production would provide a significant new source of income and opportunity for producers and processers, increase alternative meat supplies and open up new export markets for Irish beef. For the last number of years, there is renewed interest in bull beef production, however, young bulls constituted only 10.1% of the national total slaughtering figures in 2015 (DAFM, 2015). Furthermore, growth in bull beef production is constrained by the reluctance of processors to purchase bulls arising from concerns about acceptability of bull beef. Holstein-Friesian (HF) is the predominant Irish dairy breed due to their high milk yield and they represent approximately 56% of the current Irish calves produced from the national dairy herd.

Consumers are becoming more aware of the nutritional value of beef. Fatty acid (FA) composition not only plays an important role in human health, but also in meat palatability and shelf-life (Wood et al., 2008). The intrinsic meat quality attributes such as colour, water holding capacity (WHC), tenderness and FA composition have been shown to be affected by extrinsic ‘on-farm’ factors, such as; sex, breed, diet, age, anatomical location, muscle handling and exercise conditions (Nürnberg et al., 1998; Frylinck et al., 2013).

Generally, meat tenderness decreases with slaughter age due to the accumulation of mature trivalent cross-links of intramuscular connective tissue as animals mature (Bailey, 1985). Pigment, lipid and dry matter contents increase with animal age (Renand et al., 2001). It is recognised that increasing slaughter weight *per se* can alter fatty acid composition in cattle (Moreno et al., 2008). The grass utilization advantage of Ireland, resulting from the temperate climate, allows Irish farms to exploit the natural competitive advantages related to grass-based production systems. However, supplementation with concentrates is inevitable during indoor winter periods, to obtain more energy to sustain adequate growth rates, and to finish cattle prior to slaughter. Another study conducted within Teagasc demonstrated that the 19-month bull system was more profitable than the 15-month system, largely owing to its' high output per hectare and heavy carcass weight (Kelly et al., 2013). However, with current market requirements, bulls need to be slaughtered not older than 16 months of age. Palatability aspects will continue to be the main factor influencing choice for most consumers of beef. Thus, it is worthwhile to assess the eating quality of beef from these male, dairy-derived calves to identify the most effective production system with the optimum combination of slaughter age and feeding treatment.

The eating quality of different muscles varies due to intrinsic factors such as connective tissue content, intramuscular fat percentage and fibre type, but also due to differential rates of post mortem proteolysis, and their response to extrinsic factors, such as; electrical stimulation, hanging method and ageing time (Rhee et al., 2004). Structural and metabolical differences between muscles may also result in the different reaction to changes in growth path, diet or production system (Archile-Contreras et al., 2010). Meat palatability (Purchas & Zou, 2008) and FA profile (Lengyel et al., 2003) from Friesian bulls have been previously researched, whereas, the eye of round (*semitendinosus*, ST) and top sirloin butt eye (*gluteus medius*, GM) of young HF bulls have not been comprehensively investigated to date. Moreover, the links between production systems, muscle types and the eating quality of HF bull beef is not fully understood.

The objective of this study was to investigate the physico-chemical characteristics and fatty acid profile in the three major muscles of young dairy bulls affected by 15-month and 19-month production systems. The interrelationships between physico-

chemical traits; between physico-chemical properties and FA composition within each muscle were also studied.

5.3 Materials and methods

5.3.1 Animals and diets

A total of 30 weaned spring-born male HF calves (10 to 12 weeks of age) were sourced and transported to Teagasc, Johnstown Castle Research Centre in 2013. Calves were turned out to pasture predominantly comprised of perennial ryegrass (*Lolium perenne*) and supplemented with 1.5 kg concentrate (80% *Hordeum vulgare* (ground barley), 14% *Glycine max (L.) Merr* (soya bean meal), 4% *black treacle* (molasses) and 2% minerals) per head daily during the first grazing season for 6 months (May-Nov). Then they were assigned to two production strategies. Fifteen calves were selected at random to be housed indoors and fed *ad-libitum* concentrate during the winter and finishing periods for 6 months and were slaughtered at the target age of 15 months old (in May 2014). Indoor accommodation consisted of a slatted floor shed in groups of 5 animals per pen, thereby giving rise to 3 pens (accounting for 3 experimental replicates). The remaining 15 bulls were housed and offered grass silage plus 2 kg concentrate during the winter period for 4 months until March 2014. These bulls were then turned out to pasture again for a second grazing season of 3 months. They were housed again in June and adapted to an *ad libitum* concentrate finishing diet for 3 months and were slaughtered in September at the target age of 19 months old. During indoor periods, these bulls were penned in groups of 5 animals, again giving rise to 3 pens of animals (again accounting for 3 experimental replicates).

5.3.2 Slaughter, Post-mortem pH/temperature and sampling

At a commercial abattoir, cattle were stunned by captive-bolt, conventionally hung, exsanguinated within 30 s, centrally-split into two sides and weighed. The pH (model 420A, Orion, Germany fitted with a glass pH electrode EC-2010-11 (Refex sensors Ltd., Westport, Co. Mayo, Ireland, calibrated using pH 4.0 and pH 7.0 buffers) and temperature (Digitron 2046T, Instrument Technology Ltd, Ireland) were recorded in the *longissimus thoracis* (LT) muscle at the 10th rib on the left side of each carcass hourly from 1 to 6 h post mortem. At 48 h post mortem, the LT from ribs 6 to 10, ST

and GM muscles were excised from the left-hand side of each carcass. The pH/temperature profile for each carcass was plotted and the pH values at 15 °C and 35 °C were read from the curves. After chilling for 72 h at 4°C, ultimate pH (pHu) of the three muscles were measured, and they were cut into individual slices (25 mm thick). The fresh cut surface of the first slice from the 10th rib end of LT and the anterior end of the ST and GM were used for colour measurement, and the rest of the slices were vacuum-packed. Steaks for proximate chemical composition, collagen and FA determinations were stored at -20°C immediately, while samples for Warner-Bratzler shear force (WBSF) and cooking loss were aged for 14 days at 4°C then frozen at -20°C until required.

5.3.3 Meat colour

The method was described in Chapter 2.

5.3.4 Warner-Bratzler shear force and cooking loss

The method was described in Chapter 2.

5.3.5 Proximate chemical composition

The method was described in Chapter 2.

5.3.6 Collagen content and solubility

The method was described in Chapter 3.

5.3.7 Fatty acid analysis

Fatty acid methyl esters (FAME) were generated by microwave assisted preparation and quantified by gas chromatography flame ionisation detector (GC-FID) analysis according to Brunton et al. (2015) with slight modification. Briefly, 1 g of wet minced beef was added to a 55 mL PFA reaction vessel containing a 10 mm stir bar. To this, 10 mL of potassium hydroxide in methanol (2.5%) with 100 µL internal standard (C23:0) was added. The reaction vessel was heated in the MARS 6 Express 40 position Microwave Reaction System (CEM Corporation, Matthews, NC, USA) to 130 ºC for 4 min and held at this temperature for 4 min to complete the saponification process. The reaction vessel was then removed from the spindle wheel and cooled on ice for 5 min. Esterification was then carried out by adding 15mL of

5% acetyl chloride in MeOH solution heating to 120 ºC for 4 min and holding at this temperature for 2 min. The reaction tube was removed and cooled on ice for 5 min and to reach room temperature. Pentane (10 mL) was added into the reaction vessels which were mixed gently by holding bung over the top. Following this step, 20 mL of saturated salt solution was added, and the solution was mixed again as described above. After the solution was separated, the top pentane layer was removed and aliquoted into amber GC vials (1.5 mL) containing sodium sulphate and stored at -20 ºC until analysis. Three replicates were carried out for each sample.

A PerkinElmer Clarus 580 Gas Chromatograph (PerkinElmer, Waltham, MA, USA) fitted with a flame ionisation detector (FID) was used for FAME quantification. All analytes were separated using a CP-Sil 88 capillary 100 m x 0.25 mm (Internal diameter) x 0.2 µm (film thickness) column (Agilent, USA). The oven was heated at 6.2 °C/min from 80 °C to 220 °C held for 3.2 min, then heated at 6.3 °C/min from 220 °C to 240 °C and held for 6.5 min. The temperature of the injector was 250 °C, and the injection volume was 0.5 µm with the split set to 10:1. Hydrogen was used as the carrier gas at a flow rate of 1.25 mL/min. The temperature of the flame ionisation detector was maintained at 270 °C. Compounds were identified by comparing their retention times with those of authentic standards from the Supelco 37 FAME. Peak areas analysis was performed using TotalChrom 6.3.2 software (PerkinElmer, Waltham, MA, USA). The fatty acid composition was expressed both as relative proportion (percentage of total fatty acids) and absolute concentration (mg/100 g of wet minced meat) according to the following equation (ISTD: internal standard C23:0):

(Peak Area of Sample / Peak Area of ISTD) × (Weight of ISTD / Weight of Sample) × ISTD Purity × 10 × 0.96 × 100 = Content

5.3.8 Imaging

Beef samples (2 × 2 × 1 cm^3) were flash frozen in liquid nitrogen and stored at −80 °C. Sections were cut (20 µm) using a Leica CM1950 cryostat (Leica Biosystems, Nussloch, Germany) after equilibration to the specimen chamber temperature (−25 °C). Light microscopy sections were stained with fast green and iodine (ratio 10:1) and examined using a Leica DMLB light microscope (Leica Microsystems AG, Wetzlar, Germany).

5.3.9 Statistical analysis

Data were analysed in a two-way analysis of variance (ANOVA) with type Ⅲ sums of squares using the GLM (General Linear Model) procedure of SAS Version 9.3 (Cary, NC: SAS Institute, 2002); muscle, age-production system and their interaction were fixed effects. Pen was used as the experimental unit for all variables. The Tukey-Kramer test was applied to compare least square mean values with a significance level of $P < 0.05$. Pearson's correlation coefficients were calculated using the CORR procedure of SAS (SAS, 2002). The correlations between physico-chemical traits; between physico-chemical traits and muscle and age-production system were also analysed by Partial Least Squares Regression (PLSR) using Unscrambler Software, Version 10.3 (CAMO ASA, Oslo, Norway).

5.4 Results and discussion

5.4.1 Post-mortem pH-temp decline

The post-mortem pH/temperature window concept implemented by Meat Standards Australia (MSA) grading scheme aims to identify or monitor shortening conditions of carcasses during rigor mortis. Cold shortening occurs when the temperature of a carcass falls below 15 ºC while the pH remains above 6.0. The combination of quick temperature decline and slow pH fall leads to the decreased ability of sarcoplasmic reticulum and mitochondria to retain calcium, which contributes to the uncontrollable and permanent release of a high concentration of free Ca^{2+} ions into the sarcoplasm. In the presence of ATP, the muscle fibres contract excessively, thereby causing tough meat to be produced (Locker, 1985). Conversely, when carcass pH falls below 6.0 while the temperature remains above 35 ºC, rigor or heat toughening can occur. The combination of high temperature and low pH results in more acid conditions which diminishes the proteolytic activity and accelerates protein denaturation, therefore the product is incapable of ageing (Thompson, 2002).

In the present study, 7 out of 15x 15-month old bull carcasses were located within the cold shortening window, although only marginally, which may have caused increased shortening of the sarcomeres as described by Marsh & Carse (1974); while no carcass was determined to be within the heat toughening window (Figure 5.1a). In contrast, two 19-month old bull carcasses were determined to be within the heat

toughening window, although again, only marginally, while no carcass was determined to exist within the cold shortening window (Figure 5.1b). The potential risk of cold shortening in younger bulls probably results from faster chilling due to their relatively lighter and leaner carcasses compared with their older counterparts ($P < 0.05$). The carcass weight and fat scores for 15- and 19-month old bulls were 277.6 kg, 5.47 and 290.9 kg, 6.73, respectively. The higher pH values at 15 ºC and 35 ºC for 15-month compared with 19-month bulls ($P < 0.05$; Table 5.1) were also probably due to their faster chilling rate.

5.4.2 Ultimate pH

The pHu was higher in 19-month old bulls compared to 15-month old bulls ($P < 0.05$; Table 5.1). Mellor et al. (1991) observed that temperament and stress level increase with age, thus pHu also increases with age. Dunne et al. (2004) also reported that the pH of *longissimus dorsi* (LD) was higher in the heavier carcass weight group. In addition, the longer grazing period of the 19-month system may also have contributed to the higher pHu, as forage-fed animals are more susceptible to pre-slaughter stress, leading to glycogen depletion (Muir et al., 1998a). LT and ST had higher pHu than GM ($P < 0.05$; Table 5.1). Seggern et al. (2005) found that GM had the lowest pH (5.45) among 39 beef cuts, with the pHu of the LD being 5.76. The variation in muscle pH could also be explained by proximity to bone as the pH may rise after neutralization of lactic acid by calcium carbonate in the bone (Callow, 1939).

Mean pHu for all groups ranged from 5.49 to 5.73, which is within the range considered normal for beef of $5.4 \leq pHu \leq 5.7$ (Tarrant, 1989). However, one LT and two ST muscles of 15-month bulls and one LT of 19-month bulls were likely to be DFD (dark, firm, dry, pHu > 5.9) meat (4.44% frequency). It is reasonable to assume that insufficient lactic acid generation caused by glycogen depletion may have occurred in those bulls due to physical contests and excitability of temperament (Monin, 1990). Moreover, unfavourable ante-mortem environmental factors, such as stressful handling, transportation, fasting or mixing can also induce DFD meat (Dunne et al., 2004).

5.4.3 Meat colour

The 15-month production system produced beef that possessed higher redness ($P < 0.001$), yellowness ($P < 0.05$) and chroma ($P = 0.001$) values after 24 h blooming (Table 5.1). The higher hue angle for meat derived from 19-month old bulls indicated a less pure red colour. This is consistent with Frylinck et al. (2013) who observed that younger bulls fed more concentrates had higher yellowness and chroma, and lower hue angle compared with older bulls fed a pasture based diet. Nevertheless, it is widely recognised that muscle pigment content increases with age (Gil et al., 2001). Additionally, beef derived from grazing animals contain more haem pigment than their feedlot counterparts (Vestergaard et al., 2000a). Even with older animal age and higher pasture intensity employed in the 19-month production system, it is proposed that in this study, pH played an important role in colour development. Redness has been reported to be negatively correlated with pHu (Guignot et al., 1993), thus, this might also explain why meat from 19-month old bulls, which had higher pHu levels, were less red in colour. The higher yellowness valuess for beef derived from 15-month old bulls is likely due to the higher meat IMF content, which correlates to the b* value (Waritthitham et al., 2010a). In addition, the rate of pH decline during rigor development may also account for colour differences detected between muscles (Bayraktaroglu & Kahraman, 2011). Colour differences between two ages were found after 24 h rather than after 2 h blooming, which may indicate that blooming of muscles from young HF bulls is incomplete after 2 h of exposure.

ST had higher lightness, yellowness, chroma and hue angle values than LT and GM muscles, which were similar ($P < 0.05$; Table 5.1); indicating a paler but more yellow and intense colour in ST. Similarly, Cho et al. (2016) found that ST had the highest lightness values among 12 muscles of Hanwoo bulls. Hunt & Hedrick (1977) reported a highest total pigment content (including both myoglobin and haemoglobin) in GM among the three muscles studied, followed by LD and ST, which is in accordance with the highest lightness values in ST, but which conflicts with other colour parameters results in the present study.Differences in muscle colour could be partly related to the biological and biochemical properties of muscles investigated (Seggern et al., 2005).

5.4.4 Warner-Bratzler shear force

WB-variables were all higher in beef from 19-month old bulls compared with that derived from 15-month old bulls, regardless of muscle type ($P < 0.001$; Table 5.2). This finding is in agreement with another study which reported a significant decrease in meat tenderness from 15- to 19-months old Charolais bull calves (Renand et al., 2001). The same outcome was also determined for sensory tenderness scores in beef from 15-month old animals compared with that derived from 19- or 24-month old French breed bulls (Dransfield et al., 2003). It is well established that two major components determine the ultimate tenderness of muscle; the myofibrillar component and the content, composition and structure of connective tissue (Jeremiah et al., 2003a).

The decreased tenderness with increasing age finding in this study was probably a reflection of changes in the connective tissue characteristics of muscle as the insoluble and total collagen content increased, but collagen solubility decreased with age ($P < 0.05$; Table 5.2). The 'background toughness' of meat is primarily related to the degree of multivalent cross-links between collagen molecules. When fewer intermolecular cross-links occur, collagen is easily converted to gelatin during cooking, which improves tenderness. In contrast, more moisture is squeezed out of muscle containing higher amounts of heat-stable intermolecular cross-links, consequently reducing tenderness (Ledward, 1984). Collagen, either with or without cross-links, can be separated based on their solubility. Cross-linked collagen is more heat stable than non-cross-linked collagen, and therefore, is referred to as the insoluble collagen component (Hill, 1966).

In terms of the myofibrillar component of tenderness, WBSF difference between ages could partly be explained by the increased muscle fibre size in older bulls. According to Wegner et al. (2000), conversion of intermediate fibres to white fibres in HF bulls occurs from 2 to 6 months after birth. After 6 months of age, fibre composition is consistent, while the proportion of red fibre remains stable throughout life. In contrast, muscle fibre cross-sectional area increases continuously during animal maturity. Larger fibre area has been reported to be positively correlated with toughness of young bull beef (Renand et al., 2001, Chriki et al., 2013). Therefore, the larger fibre size in older HF bulls contributes to tougher meat. Although beef derived

from 15-month old bulls presented a higher risk of cold shortening than meat derived from 19-month old bulls, it should be noted that older bulls were also at risk of heat toughened shortening.

Juiciness and flavour can be affected by IMF directly and tenderness is affected by IMF indirectly. Jeremiah et al. (2003a) reported that 12-14% of the variation in all palatability traits is related to the amount of IMF in muscle. Accordingly, the higher muscle IMF content of 15-month old bulls also contributed to increased meat tenderness compared to 19-month old bulls. Lower cooking loss was another reason for more tender beef to have been associated with 15-month bulls, as cooking loss has been linked to meat toughness in young bull beef (Monteiro et al., 2013).

LT was determined to be the most tender muscle of the three types examined, while GM tended to have higher WBSF scores compared to ST ($P < 0.01$; Table 2). It has been noted that muscle fibre type greatly influences beef tenderness, with an increased proportion of red oxidative type I fibres and a decreased proportion of white glycolytic type Ⅱ fibres, improving tenderness (Renand et al., 2001; Hwang et al., 2010). Although the three muscles studied are all classified as white muscles, the LD has a significantly higher percentage of β-red and lower percentage of α-white fibres than GM and ST in cattle, while the LD has similar level of intermediate α-red with GM and ST. GM and ST have similar proportions of all three types of fibres (Kirchofer et al., 2002). Hunt & Hedrick (1977) also reported the highest aerobic potential in LD, followed by GM and outer ST. Therefore, tenderness values determined between muscles in this study is a good reflection of fibre typing property. Furthermore, IMF and cooking loss differences determined between the three muscles investigated in this study could also explain tenderness differences observed. Different enzyme activities could explain the variation in rate and extent of proteolysis between muscles (Feidt et al., 1996).

Based on the classification described previously, only the mean WBSF values for GM and ST muscles from 19-month old bulls were above 45.08 N, which was considered ‘tough’ and unacceptable for most consumers. LT from 19-month old bulls and GM and ST from 15-month old bulls were considered ‘intermediate tender’ ($38.22 < WBSF < 45.08$ N) while LT from 15-month old bulls would fit into the

'tender' category (31.36 < WBSF < 38.33 N); no grouping fell within the 'very tender' category (below 31.36 N) (Shackelford et al., 1991).

5.4.5 Cooking loss

Cooking loss was higher in 19-month old bull beef than 15-month old bull beef ($P < 0.01$, Table 5.2), which is in line with Maher et al. (2004) who found that heavier HF bulls had higher cook losses on different ageing days than lighter equivalents. The higher IMF content for 15-month old bull beef could explain the increase in WHC, as others found fatter muscles with less moisture sustain lower cooking loss than leaner muscles (Jeremiah et al., 2003c). Moreover, Jeremiah et al. (2003c) also found that total cooking loss was positively related to total and insoluble hydroxyproline, thus the higher cooking loss associated with beef derived from 19-month old bulls in the present study may also be associated with higher total and insoluble collagen contents. In addition, larger muscle fibre size can result in higher cook losses in bull beef (Waritthitham et al., 2010b). Thus, the increased muscle fibre size with animal maturity could be another contributor to the higher cook losses observed in older bulls (Wegner et al., 2000).

Cooking loss was higher in ST than GM and LT ($P = 0.001$, Table 5.2). Similarly, cooking loss was reported to be lower for LD than ST (Rhee et al., 2004). The difference in cooking loss could also be explained by differences in IMF content between muscles. Moreover, WHC could be affected by muscle fibre type, as increasing the proportion of fast-twitch glycolytic type Ⅱ fibres in *longissimus* has been shown to increase cooking loss in pigs (Choe et al., 2008).

5.4.6 Proximate chemical composition

IMF content was higher and moisture content lower in 15-month old bulls ($P < 0.01$, Table 5.2), which is in contrast with the general finding that IMF tends to increase with advancing age (Renand et al., 2001). However, this is not apparent in the GM and ST muscles investigated in this study. The primary reason for this finding could be associated with the feeding regime difference between the two age-production systems, since the 15-month system utilised more concentrates. It is widely reported that a higher energy diet results in higher IMF and a concomitant lower moisture content (Vestergaard et al., 2000a; Sami et al., 2004). In terms of biological

mechanisms, the higher net energy supply from grain feeding might increase IMF deposition, because the glucose delivery to muscle not only increases intramuscular adipocytes promotion directly, but also increases the circulating insulin level, which is known to stimulate lipogenesis (Pethick et al., 2004). Similarly to our study, Dunne et al. (2004) also reported that LD from heavier bulls had lower IMF and a higher moisture concentration than observed in LD from lighter bulls.

In this study, IMF content was higher and was accompanied by a lower moisture content in LT than was observed in the other two muscles, which is in accordance with McKeith et al. (1985) and Jeremiah et al. (2003c). This could be explained by the higher frequency of β-red fibres in LT because 'red' or oxidative fibres contain more IMF compared with 'white' or glycolytic fibres in cattle (Kirchofer et al., 2002; Hwang et al., 2010). There was a significant age x muscle interaction effect that the LT having significantly more IMF than the other muscles, but only for the 15-month bulls ($P < 0.001$; Table 5.2). This might indicate a higher potential for marbling deposition in response to higher dietary energy intake in the LT muscle than in GM and ST. Similarly, Lengyel et al. (2003) found that IMF content showed significant changes between age-production systems of young HF bulls in the LD muscle, but not in the *Psoas major* or ST muscles. Total protein and ash content did not differ between muscles from the different production systems ($P > 0.05$).

IMF or marbling level is considered to be an important criterion when assessing meat at retail. In general, 3 to 7% of IMF is considered as a normal range to ensure palatability and is not detrimental to human health (Miller, 2002). In the present study, only the LT muscle in 15-month bulls was within the acceptable range. This confirms that dietary energy is a critical factor affecting IMF level, but does not affect all muscles equally.

5.4.7 Collagen content and solubility

Age-production system affected collagen characteristics regardless of muscle type. Insoluble and total collagen contents were higher, but collagen solubility was lower in muscle from 19-month old bulls ($P < 0.05$; Table 5.2). Firstly, this is due to the age difference between the two production systems. It is generally accepted that the older the animal, the higher the proportion of heat-stable cross-linking. With increasing age to maturity, the divalent aldimine and keto-imine cross-links are

converted into transverse multivalent cross-links, which link the longitudinal polymers together to form a three-dimensional stable network (Bailey, 1985). This structure can further increase the residual strength of collagen fibres, generating a high tension on thermal shrinkage, thereby resulting in tougher meat. Furthermore, the turnover of collagen is rapid in younger animals, and the less mature collagen and less tough meat are produced from the higher rate of turnover (Bailey, 1985).

Secondly, it should be noted that the lower energy diet in the 19-month production system may also have contributed to the higher amount of total collagen and lower collagen solubility. Several studies have stated that a high energy diet can improve collagen solubility by accelerating newly-synthesized heat-liable collagen proportions within cattle due to the faster rate of protein turnover resulting from their potentially faster growth rates (Aberle et al., 1981; Miller et al., 1987). Archile-Contreras et al. (2010) showed that cattle fed corn contained less total collagen in LD muscle than their pasture-fed counterparts due to greater myofibrillar protein deposition diluting out the collagen. Nonetheless, Dikeman et al. (1986) pointed out that collagen content was not affected by energy level. Accordingly, our results hypothesise that the combination effect of age and diet accounted for differences in collagen characteristics between the age-production systems.

Collagen characteristics did not differ between muscles ($P > 0.05$; Table 5.2), which is in agreement with Cho et al. (2016). However, Rhee et al. (2004) reported that GM and LD had the second lowest collagen concentration among 11 beef muscles studied after *psoas major*, while the ST, *biceps femoris* and *supraspinatus* muscles had the highest collagen concentrations. McKeith et al. (1985) found a similar level of total collagen between ST and GM, with both being higher than LD. These inconsistencies between studies might be as a result of different methodologies applied to measure collagen. The variation of collagen between muscle types is most probably related to their locomotory functions (Archile-Contreras et al., 2010). In agreement with our results, LT muscle from Holstein bulls of 13 to 16 months old had 3.86 mg/g wet tissue of total collagen, 3.02 mg/g of insoluble collagen and 21.7% of collagen solubility (Christensen et al., 2011).

5.4.8 Fatty acid composition

5.4.8.1 Age-production system effect

Myristic acid (C14:0) ($P < 0.01$), palmitic acid (C16:0) ($P < 0.05$), palmitoleic acid (C16:1) ($P < 0.05$), total SFA ($P < 0.05$) proportions and n-6/n-3 PUFA ratio ($P < 0.001$) were higher in beef from 15-month old bulls than in beef from 19-month old bulls (Table 5.3). However, cis-10-Pentadecenoic acid (C15:1) ($P = 0.001$), linoleic acid (C18:2n6c) ($P < 0.05$), α-linolenic acid (C18:3n3) ($P < 0.001$), arachidonic acid (C20:4n6) ($P < 0.05$), eicosapentaenoic acid (C20:5n3, EPA) ($P < 0.001$), docosapentaenoic acid (C22:5n3, DPA) ($P = 0.001$), total UFA ($P < 0.05$), total PUFA ($P < 0.01$), total n-6 PUFA ($P < 0.05$), total n-3 PUFA ($P < 0.001$) proportions and PUFA/SFA ratio were all higher in beef from 19-month rather than in 15-month old bulls.

In accordance with our results, C14:0 percentage decreased with age in Angus, Hereford and Brahman cattle, while C18:0 percentage also decreased with age, which was not observed in our study (Malau-Aduli et al., 1998). Duckett et al. (1993) noted that oleic acid (C18:1n9c) percentage increased linearly, but PUFA percentage in polar lipid (PL) decreased with time on feed, which is not in agreement with the results of our study. Weglarz (2010) reported that the percentage of total SFA decreased and total UFA increased with slaughter age in Polish HF bulls, which agrees with our results, whereas they also found that the percentage of C18:1n9c increased, but EPA and total PUFA n-3 decreased with slaughter age, which differs with our results. Likewise, with the exception of total SFA, which is in line with our results, the opposite trend for FA percentages between slaughter ages was observed by Moreno et al. (2008).

This conflicting result can be explained by diet-related differences in fatness. In general, older animals are fatter, so the differences in FA content reflect differences in fat content rather than age differences (Monteiro et al., 2012). However, in the present study, the younger animals were fatter due to a higher plane of nutrition. With equal IMF content between ages, Monteiro et al. (2012) found higher percentages of C16:1, C18:1n9c and a lower percentage of C18:2n6c in younger bulls. Nevertheless, it has also been reported that for young HF bulls, the more

significant effect of slaughter age on FA composition is between 7 and 14 months of age (Lengyel et al., 2003).

According to Duckett et al. (1993), the higher IMF content in beef results from an enlargement of adipocyte cells with storage of triacylgrycerols rather than an increase in adipocyte cell number as the structural membrane components of the cell (phospholipids) remain constant regardless of age condition. Therefore, the enlargement of the adipocyte diluted the contribution of membrane PL to the total lipids, thus meat from 15-month old bulls exhibited a relatively lower proportion of PUFA, as PL is a rich of source of PUFA. In addition, the higher percentage of meat PUFA from the 19-month production system could also be explained by pasture feeding during the second grazing season because the presence of secondary plant metabolites in fresh grass may inhibit ruminal biohydrogenation relative to that of grain or silage (Lourenço et al., 2008). Jiang et al. (2013) also stated that the lower energy diet increased total PUFA percentage in Jersey steers. Similarly, Daley et al. (2010) found that grass-based beef tends toward a lower proportion of cholesterol-elevating C14:0 and C16:0. Generally, MUFA (predominately oleic acid, C18:1n9c) proportion exhibits an exponential relationship with fatness (Moreno et al., 2008). Surprisingly, MUFA did not differ between production systems in the present study.

Absolute concentrations of C14:0 ($P < 0.01$), pentadecanoic (C15:0) ($P < 0.05$), C16:0 ($P < 0.01$), palmitoleic (C16:1) ($P < 0.01$), heptadecanoic (C17:0) ($P < 0.05$), stearic (C18:0) ($P < 0.01$), elaidic (C18:1n9t) ($P < 0.05$), oleic (C18:1n9c) ($P = 0.01$), vaccenic (C18:1n7) ($P < 0.01$), C18:2n6c ($P < 0.01$), cis8,11,14-eicosatrienoic (C20:3n6) ($P < 0.001$) acids, total SFA ($P < 0.01$), total UFA ($P < 0.05$), total MUFA ($P < 0.05$), total n-6 PUFA ($P < 0.01$), and total FA (TFA) ($P < 0.01$) were higher in beef from 15-month old bulls, while C18:3n3, C20:5n3 (EPA), C22:5n3 (DPA), total n-3 PUFA concentrations were higher in beef from 19-month old bulls ($P < 0.001$; Table 5.4).

Differences in FA absolute concentration between age-production systems mainly reflected differences in the size of the neutral lipid (NL) fraction. In contrast to our results, Moreno et al. (2008) reported that an increasing slaughter age exhibited a tendency to increase total SFA, MUFA, PUFA and TFA concentrations. According to Duckett et al. (1993), throughout time on feed, total lipid content (IMF or marbling)

increases because NL increases proportionately, while PL remains constant. This conflicting result compared to the above studies reflects the opposite tendency of IMF content with age in our study. The content of NL with its high proportions of SFA and MUFA had a positive relationship with total lipid content, while the content of phospholipid FAs, mainly PUFA, did not differ with total lipid content (Wood et al., 2008). This could explain the higher absolute concentrations of individual and total SFA and MUFA in the 15-month old system with higher IMF content, while no difference was found for total PUFA absolute concentration between age-production systems.

Our results agree with the general finding that concentrate-fed beef has more C18:1n9c (the major MUFA) and C18:2n6c (which are major FAs in cereal grains) in its composition, while an elevated content of C18:3n3 was found in grass-fed beef because green pastures are a good source of C18:3n3 (Wood & Enser, 1997; Alfaia et al., 2009). Consequently, the precursor of the n-6 and n-3 series, C18:2n6c and C18:3n3 can synthesise other long chain (C20-22) n-6 and n-3 PUFAs, respectively, through an elongation-desaturation pathway (Wood et al., 2008), which increased the n-6 PUFAs in the 15-month production system and n-3 PUFAs in the 19-month production system. Similarly, Sañudo et al. (2000) also stated that muscles from grass-fed animals contain greater concentrations of n-3 PUFA and concentrate-fed animals have higher concentrations of n-6 PUFA.

From a human nutrition perspective, the 19-month production system increased the n-3 series (Table 5.4), which are widely recognized for their beneficial effects on health, such as in the prevention of atherosclerosis, heart attack, depression and cancer and reducing inflammation due to rheumatoid arthritis (Brugiapaglia et al., 2014). Moreover, the higher amounts of SFA, especially C14:0 and C16:0, in beef from 15-month old bulls have negative health implications, which are associated with coronary health risk due to their total and low-density lipoprotein (LDL) cholesterol raising effect (Daley et al., 2010). Nevertheless, it should be noted that the higher concentration of oleic acid (18:1n9c) determined in15-month old bull beef has cholesterol-lowering effects and other healthy properties, including decreased risk of stroke and beneficial influences on blood pressure (Costa et al., 2008; Daley et al., 2010).

5.4.8.2 Muscle effect

For individual SFA, including C14:0 ($P < 0.001$), C15:0 ($P < 0.01$), C16:0 ($P < 0.01$), C18:0 ($P < 0.001$) and total SFA ($P < 0.001$), proportions were higher in LT than detected in GM and ST (Table 5.3). In contrast, total UFA proportion and MUFA/SFA ratio were higher in GM and ST than LT ($P < 0.001$). ST had the highest C15:1, individual n-6 PUFAs (C18:2n6c, C20:3n6, C20:4n6), total PUFA, total n-6 PUFA proportions and PUFA/SFA (P/S) ratio, followed by GM and LT ($P < 0.001$). For individual n-3 PUFAs (C18:3n3, C20:5n3, C22:5n3) and total n-3 PUFA proportions, ST had higher values than GM and LT ($P < 0.001$). C17:0 and SFA/UFA ratio were higher in LT than ST, and conversely, C18:1n7 was higher in ST than LT; C18:1n9c percentage was higher in GM than ST ($P < 0.05$). C14:1, C16:1, C18:1n9t, C22:0 and total MUFA proportions were not affected by muscle ($P > 0.05$).

Similarly, Costa et al. (2008) reported that LD had higher proportions of SFA and MUFA but less PUFA, n-6 PUFA, n-3 PUFA, P/S proportions than ST muscle in Mertolenga young bulls. In line with our results, Lengyel et al. (2003) reported a higher proportion of PUFA and lower proportions of SFA and MUFA in ST muscle in comparison to LT in young HF bulls. Eichhorn et al. (1985) also pointed out that *longissimus* muscle from beef breeds contained about 6% less PUFA than ST. These results are probably due to a relationship between lipid content and muscle fibre metabolic and contractile characteristics. A higher proportion or relative area of oxidative type Ⅰ fibres was associated with a greater amount of TFA and NL due to its higher lipid droplet content as the oxidative capacity of type Ⅰ fibers uses fatty acids as an energy source (Dyck et al., 1997). Glycolytic type ⅡB fibres proportion or relative area had a positive relationship with PL content (Costa et al., 2008). Thereby, the greater oxidative and glycolytic potential of LT and ST, respectively, expressed their corresponding influence on FA profile. In addition, the higher PUFA proportion in ST and greater SFA proportion in LT could reflect differences in IMF content. Muscles with higher IMF content like LT contain a higher proportion of triglycerides, accompanied by a decrease in the proportion of phospholipids in total lipids (Marmer et al., 1984). In accordance with our result, Pavan & Duckett (2013) stated that leaner retail cuts had better n-3 PUFA profiles. Besides, there may be some other factors which have biological relevance that affect FA composition in muscles (Costa et al., 2008). The difference in C18:1n9c percentage was probably

due to the differences in stearoyl-CoA desaturase expression between muscles (Monteiro et al., 2012). However, in disagreement with our results, Pavan & Duckett (2013) observed that GM had similar total SFA, PUFA, n-6 PUFA to LT, while LT had a higher total MUFA percentage than GM in grass-fed Aberdeen Angus steers.

The absolute muscle FA concentration is shown (Table 5.4). LT had higher C16:1 ($P = 0.001$), individual SFA (C14:0, C15:0, C16:0, C17:0) and total SFA concentrations than GM and ST ($P < 0.001$). For C18:1n9c ($P = 0.001$), C18:1n7, C18:2n6c ($P < 0.001$), total UFA, MUFA, PUFA and n-6 PUFA ($P < 0.01$), LT and GM had similar absolute values, and both were higher than ST. LT had the highest concentration of C18:0 and TFA, followed by GM and ST ($P < 0.001$). C14:1 ($P < 0.05$), C18:1n9c and C18:3n3 ($P < 0.01$) were higher in LT than in ST muscle. GM had higher C20:3n6 and C20:4n6 concentrations than LT and ST ($P < 0.001$), GM and ST had a higher C20:5n3 concentration than LT ($P < 0.05$), and C22:5n3 concentration was higher in GM than LT ($P < 0.05$).

The highest concentrations of most individual FAs in the LT muscle and the lowest concentrations in ST (except the long chain (LC) PUFAs) generally followed trends in IMF levels. The content of individual fatty acids increased with increasing muscle total lipid content, particularly for those that had greater proportions in the NL fraction, such as SFA and MUFA (Pavan & Duckett, 2013). The different absolute concentration of total PUFA between muscles could suggest that the membrane phospholipid content varied among muscles.

From the human nutrition perspective, ST had the most enhanced FA profile, LT had the least healthy profile and GM lay between both of these, which was expressed as the higher proportions of non-desirable FAs, such as SFA in LT and the greater proportions of desirable PUFA, especially n-3 PUFA in ST. Despite the enhanced FA profile in ST muscle, daily intake of desirable fatty acids including MUFA, PUFA would be greater when consuming LT or GM muscles due to their greater total IMF content (Daley et al., 2010). Thus, the challenge is to maximize the intake of desirable FAs, while reducing intake of total fat and non-desirable FAs (Pavan & Duckett, 2013).

5.4.8.3 Interaction effect between age-production system and muscle and nutritional value

Regardless of age-production system and muscle, C18:1n9c (32.3-36.7%) was the most abundant FA in young HF bulls, followed by C16:0 (23.6-30.1%) and C18:0 (12-17%). These three FAs accounted for 76.2-83.6% of the FAs in LT, 73.2-75.4% in GM and 68.4-70.8% in ST muscles. C18:2n6c (4.77-11.8%) and C20:4n6 (1.02-4.11%) were the predominant PUFA (Table 5.3). Similar results were reported previously for dairy and beef breed (Moreno et al., 2008; Alfaia et al., 2009). Only the LT had a higher content of total SFA than total UFA, while GM and ST both had greater total UFA content in relation to total SFA content (Table 5.3 & 5.4).

There was an age-production and muscle-type interaction effect on the absolute concentrations of C14:0, C15:0, C16:0, C16:1, C17:0, C18:0, C18:1n9t, C18:1n9c, C18:1n7, total SFA and TFA all being higher in LT from the 15-month bulls but not for the 19-month old bulls ($P < 0.05$; Table 5.4). This result is in accordance with the interaction effect on IMF content between groups as shown in Table 5.2, which suggested that the NL FAs including SFA and MUFA deposited markedly more in the LT muscle rather than in either GM or ST under a high energy diet. This supports the previous finding that although the trend (increase or decrease) of response to dietary treatment was the same in all muscles, the magnitude of the response was different (Pavan & Duckett, 2013). It also suggests that leaner retail cuts or cuts from grass-fed bulls could be selected in order to get a relatively enhanced dietary fatty acid profile, because leaner cuts or grass-fed beef would provide lower total fat and SFA intake, and proportionally more PUFA, whereas the intake of MUFA also can be reduced, which is not desirable.

The P/S and n-6/n-3 PUFA ratios have been identified as crucial parameters when evaluating the nutritional effect of foods on human health. High intake of SFA and n-6 PUFA have been implicated as risk factors associated with coronary heart disease and cancer, while increasing the intake of PUFA, especially n-3 series is encouraged (Scollan et al., 2006; Costa et al., 2008). ST had the greatest P/S ratio, followed by GM and LT ($P < 0.001$). P/S ratio in ST of both ages are in line with the ideal dietary guidelines (> 0.4) (Department of Health, 1994), however, LT and GM at both ages showed a less favourable ratio, below nutritional recommendations. Generally, the P/S ratio in beef is unfavourably low due to rumen biohydrogenation of dietary

USFA (Costa et al., 2008). P/S increased in the 19-month system ($P < 0.01$), while the n-6/n-3 PUFA ratio was higher in the 15-month system ($P < 0.001$). This agrees with the general consensus that cereal-based diets are rich in linoleic acid and all other n-6 FAs, leading to an undesirably higher n-6/n-3 ratio than forage-based beef (Scollan et al., 2006). The PUFA profile of pasture-fed beef was enhanced as indicated by increasing P/S and decreasing n-6/n-3 ratios (Alfaia et al., 2009). Only ST from the 19-month production system had a n-6/n-3 PUFA ratio within the ideal dietary guidelines (< 4) (Department of Health, 1994). For the 15-month production system, the n-6/n-3 PUFA ratio lay between 7.41 and 10.1, which largely exceeds nutritional recommendations. Compared with beef breeds, dairy breeds have been reported to have higher n-6/n-3 PUFA ratios (Moreno et al., 2008).

According to the recommended daily nutrient intakes for humans, the three muscles from young HF bulls in the present study were classified as a low fat food, as the fat content was below 5% (Scientific Review Committee, 1990). Based on 2700 Kcal/day for human adults (25-49 years old) a minimum of 1.5 g of n-3 PUFA, 9 g of n-6 PUFA and approximately 350 mg of long chain (LC) n-3 series PUFA are recommended for daily intake (Scientific Review Committee, 1990). If we consider a common serving of beef as being 100 g, young HF bulls would provide 15.2-31.2 mg of n-3 PUFA, 99.6-149.9 mg of n-6 PUFA and 13.32-21.43 mg n-3 LC PUFA (sum of C20:5n3 and C22:5n3), which are still far below the recommended guidelines.

5.4.9 Correlations

5.4.9.1 Correlations between physico-chemical quality variables

For the LT, WB-slope was positively correlated with moisture and negatively correlated with IMF content ($P < 0.05$, Table 5.5). The increased level of IMF correlated with a decrease in WBSF values and this finding is in agreement with previous findings (Starkey et al., 2016). It is well documented that IMF has a positive influence on beef tenderness, because IMF is deposited between fasciculi, separates perimysial fibres and disrupts the endomysium honeycomb structure, thus decreasing muscle resistance under shearing (Jeremiah et al., 2003a). Sarcomere length and desmin degradation are other significant factors that explain shear force variation in *longissimus* from lamb (Starkey et al., 2016). In the present study, pH at 35 ˚C was negatively correlated with WBSF and WB-slope ($P < 0.001$), indicating that heat

toughening may have occurred. L* values after 2 h ($P < 0.05$) and 24 h ($P < 0.001$) blooming were positively correlated with cooking loss, in line with Frylinck et al. (2013) who reported that beef with lower WHC appeared brighter in appearance. pHu was negatively correlated with cooking loss and IMF content ($P < 0.05$), in accordance with Purchas et al. (2002) who reported that a higher ultimate pH was associated with higher WHC. pHu was also negatively correlated with all the colour parameters after both 2 and 24 h blooming ($P < 0.01$; $r^2 = -(0.50\text{-}0.75)$), which again is consistent with findings reported by Purchas et al. (2002). Beef samples with lower WBSF and higher IMF content had higher redness, yellowness and chroma after 24 h blooming ($P < 0.05$).

For the ST, cooking loss was positively correlated with WB-variables ($P < 0.01$; Table 5.6), which supports the finding that cooking loss was the main contributor to the tenderness differences observed for young bull beef (Monteiro et al., 2013). Moreover, total and insoluble collagen content, LDH (lactate dehydrogenase) activity and fast-glycolytic muscle fibre proportion also have been reported to explain 6%, 6%, 4% and 5% of the variability in shear force, respectively, in ST muscle from cattle (Chriki et al., 2013). pHu was positively correlated with moisture and negatively correlated with IMF content, similar to LT. The opposite tendency for LT was found for ST muscle in that beef with higher values of redness, yellowness and chroma after 2 h blooming had higher cooking loss ($P < 0.05$). pHu was negatively correlated with L*, a*, b* values and chroma after both 2 and 24 h blooming ($P < 0.05$; $r^2 = -(0.40\text{-}0.64)$), similar to LT. Samples with higher cooking loss had higher moisture contents ($P < 0.05$), which concurs with findings reported by Chambaz et al. (2003).

For GM, pHu was positively correlated with WB-variables ($P < 0.05$), which differed from LT and ST (Table 5.7). pHu value was negatively correlated with a* values and chroma after both 2 and 24 h blooming ($P < 0.05$; $r^2 = -(0.42\text{-}0.50)$). Redness, yellowness and chroma after 24 h blooming were negatively correlated with WBSF ($P < 0.05$), similar to LT, but not found in ST. Collagen solubility was negatively correlated with WB-slope and WB-area ($P < 0.05$), while no correlation was found between total collagen content and WB-variables. This result supports the conclusion that solubility (based on the amount of mature collagen cross-links) of

collagen rather than total amount of collagen is the main determinant of objective or subjective texture of beef (Crouse et al., 1985; Jeremiah et al., 2003a).

5.4.9.2 Correlations between fatty acid composition and other quality variables

In LT, IMF content was positively correlated with the proportions of C14:0, C18:0, C18:1n9c, and total SFA ($P < 0.05$) and negatively correlated with C15:1, C18:2n6c, C18:3n3, C20:3n6, C20:4n6, C20:5n3, C22:5n3, total UFA, PUFA, n-6 PUFA and n-3 PUFA ($P < 0.001$; Table 5.8). These correlations are in accordance with Brugiapaglia et al. (2014) for Piemontese, Limousin and Friesian breeds. Several researchers found the relative proportion of C18:2n6c in lipid decreased as marbling increased in *longissimus* (Dryden & Marchello, 1970; Kazala et al., 1999; Lengyel et al., 2003). Linoleic acid (C18:2n6c), which is the predominant PUFA in bovine muscle, is a major component of the membrane phospholipids fraction. A dilution of total membrane lipids with increasing amounts of triacylglycerol may explain the negative correlation observed between C18:2n6c and extracted lipid (Kazala et al., 1999). A positive association between the relative proportion of C14:0 and marbling score in *longissimus* has been reported previously (Dryden & Marchello, 1970), and which supports our result. However, the proportions of C18:0 and C18:1n9c tended to decrease with increasing lipid content, which is in contrast with our results (Kazala et al., 1999). Additionally, a negative relationship between C15:0 and total lipid has been reported by Dryden & Marchello (1970). No correlation between fatty acid percentage and WBSF was found in this study, while C18:1n9t and C22:0 were negatively and positively correlated with cooking loss, respectively ($P < 0.05$).

In ST, WBSF and cooking loss were positively correlated with C18:1n7, C18:2n6c, total UFA, PUFA and n-6 PUFA percentages ($P < 0.05$), while negatively correlated with C16:0 and total SFA percentages ($P < 0.05$). This result supports the previous finding that PUFA is positively correlated with WBSF ($r = 0.66$) and negatively correlated with sensory tenderness (Duckett et al., 1993). However, in contrast to our findings, the same authors reported that the SFA content was not correlated with WBSF while the major MUFA oleic acid (C18:1n9c) was negatively associated with WBSF (Duckett et al., 1993). In addition, a negative association between C17:0 and WBSF has been previously reported (Dryden & Marchello, 1970).

In GM, IMF content was positively correlated with C14:0 and C17:0, and negatively correlated with C18:1n7 proportion ($P < 0.05$). GM muscle with higher C18:1n9t percentage had greater WBSF values ($P < 0.05$). Cooking loss had a positive relationship with C18:1n9c, and a negative relationship with C22:0 percentage ($P < 0.05$).

5.4.9.3 Correlations analysed by PLSR

Partial least squares regression (PLSR) analysis was used to visualize the correlations between physico-chemical quality traits and to identify the variables that contributed to the variation between age-production systems or muscles. Factors 1 and 2 totally explained 62% of the variance of the X matrix and 29% of the variance of Y matrix (Figure 5.2). WB-variables, insoluble collagen, total collagen, moisture content and cooking loss were located in the same quadrant of the plot and were therefore positively correlated with each other. They were negatively correlated with IMF content and collagen solubility, which were located in the diagonally opposed quadrant. Likewise, colour parameters and soluble collagen were positively correlated with each other, and they were negatively correlated with pHu, protein and ash content. The outer ellipse and inner ellipse indicate 100% and 50% explained variance, respectively. WB-variables and most colour parameters were placed between these ellipses, indicating that they were well explained by the PLSR model.

Cooking loss, hue angle, lightness and yellowness were positively linked to ST muscle while pHu, total protein and ash content were found to be positively linked to GM muscle and IMF and collagen solubility was found to be positively linked to LT muscle. Two age-production systems were placed in the opposite diagonal line, indicating their opposite relationship effect on quality traits. WB-variables, insoluble and total collagen content and moisture content were determined to be positively linked to the 19-month system, while IMF and collagen solubility was determined to be positively linked to the 15-month bull production system.

5.5 Conclusions

Results showed that when optimising production systems, the effects of production factors such as age at slaughter and level of concentrate feeding on the eating quality of Holstein-Friesian bull beef need to be considered. Moreover, these effects may

vary for individual muscles. In particular, IMF content, total saturated fatty acid and total fatty acid concentrations increased, but only in LT muscle when bulls were finished at 15 months utilising a high energy diet. Total collagen and insoluble collagen increased with age and would be expected to negatively affect eating quality. Beef from 15-month old bulls had more redness and intense colour and was more tender, had higher WHC than beef from 19-month old bulls. Even though beef from 15-month old bulls contained higher marbling, it was still more predisposed to cold shortening because of lighter carcasses weights produced from the production system compared with 19-month old bulls. This could be addressed by modifying the chilling regime for this type of carcass. Most physico-chemical quality traits vary between muscles. LT was consistently superior in tenderness, cooking loss and marbling. ST showed the highest cooking loss, moisture content and lightest muscle, but had more intense colour. With the exception of GM and ST muscles from bulls produced from the 19-month production system, Hostein-Friesian bulls can produce acceptable and tender beef after ageing for 14 days.

The changes observed in FA composition between age-production systems reflected mainly dietary influences rather than slaughter age. Muscles varied in most individual and total FA. The leaner ST muscle or meat derived from the 19-month production system had enhanced FA profiles, expressed as more desirable n-3 PUFA, PUFA/SFA ratio and less non-desirable SFA, n-6/n-3 PUFA ratio. IMF content, collagen solubility and cooking loss could explain the variations in Warner-Bratzler variables of LT, GM and ST muscles, respectively. FA composition, which is beneficial to human health, has a detrimental effect on eating quality across three muscles. For example, beef with higher SFA proportions contained higher IMF contents and lower cooking loss and was more tender. However, PUFA contributed to the lower IMF levels and tougher beef with higher cooking loss. The PLSR model concurred with ANOVA analysis and Pearson correlations.

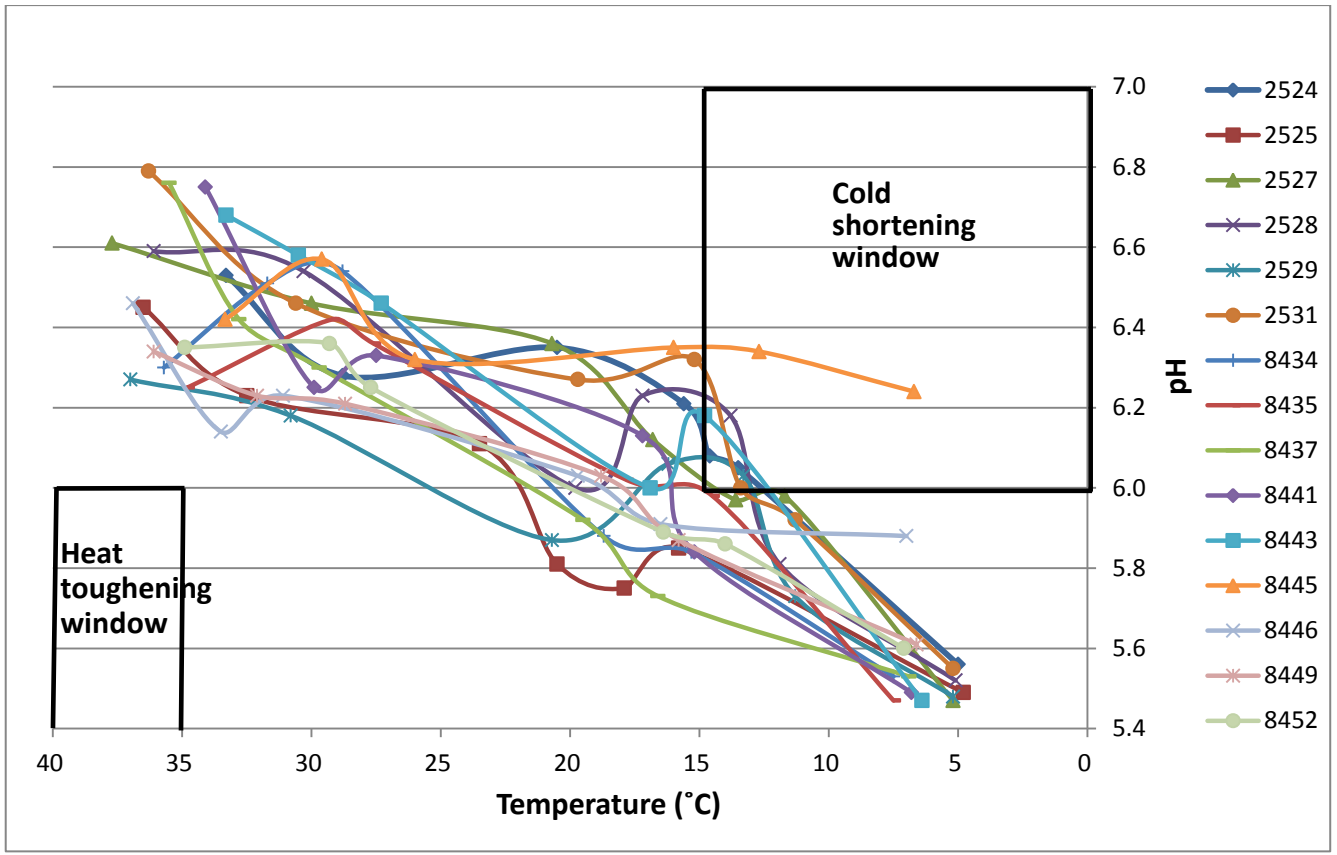
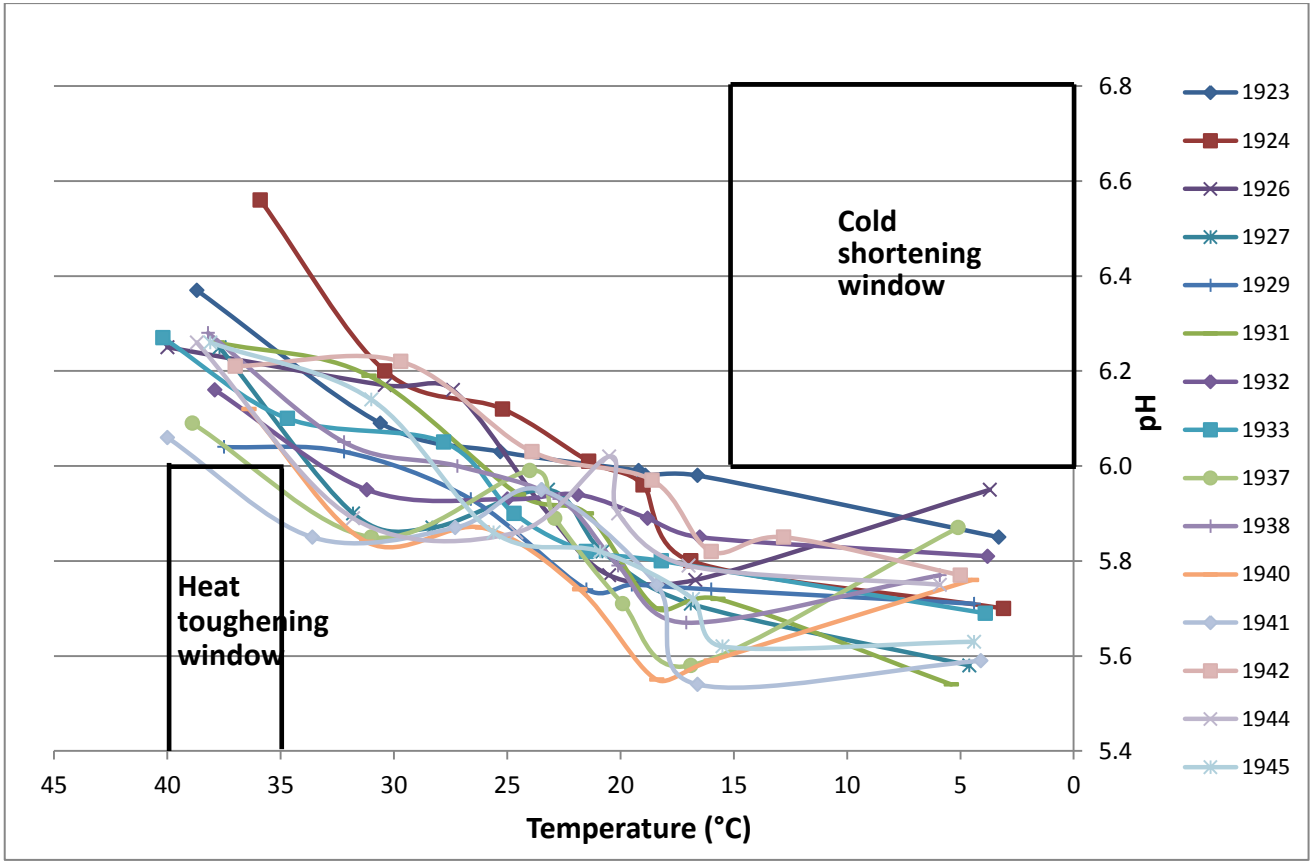


Figure 5.1. a). Post-mortem pH-temperature decline of 15-month old Holstein-Friesian bulls.



b). Post-mortem pH-temperature decline of 19-month old Holstein-Friesian bulls.

Table 5.1. Ultimate pH and colour after 2 and 24 h blooming of three muscles of young Holstein-Friesian bulls from two age-production systems.

	15 month system			19 month system			SEM	*P*-value		
	LT	GM	ST	LT	GM	ST		A	M	A×M
pHu[1]	5.61^{bA}	5.49^{bB}	5.60^{bAB}	5.73^{aA}	5.57^{aB}	5.66^{aAB}	0.04	0.023	0.013	0.741
pH (15°C)	6.00	-	-	5.73	-	-	0.03	0.003	-	-
pH (35°C)	6.48	-	-	6.13	-	-	0.04	0.002	-	-
L* 2h	43.9^{B}	42.5^{B}	47.0^{A}	43.9^{B}	42.7^{B}	46.1^{A}	0.59	0.632	<.001	0.646
a* 2h	15.4	15.6	15.4	13.3	15.0	15.3	0.57	0.076	0.206	0.208
b* 2h	11.9^{B}	11.1^{B}	12.8^{A}	10.2^{B}	10.9^{B}	13.5^{A}	0.64	0.488	0.008	0.206
Chroma 2h	19.5	19.2	20.0	16.8	18.6	20.5	0.82	0.184	0.073	0.194
Hue angle 2h	37.4^{B}	35.3^{B}	39.9^{A}	37.5^{B}	36.0^{B}	41.5^{A}	0.79	0.250	<.001	0.632
L* 24h	44.3^{B}	42.5^{C}	46.8^{A}	45.5^{B}	42.6^{C}	47.8^{A}	0.69	0.184	<.001	0.679
a* 24h	18.2^{a}	18.0^{a}	18.6^{a}	15.5^{b}	15.7^{b}	16.3^{b}	0.58	<.001	0.482	0.905
b* 24h	13.5^{aB}	13.0^{aB}	15.6^{aA}	12.3^{bB}	12.2^{bB}	15.0^{bA}	0.43	0.032	<.001	0.785
Chroma 24h	22.7^{aB}	22.2^{aB}	24.3^{aA}	19.8^{bB}	19.9^{bB}	22.2^{bA}	0.71	0.001	0.016	0.843
Hue angle 24h	36.7^{bB}	36.1^{bB}	40.0^{bA}	38.3^{aB}	38.1^{aB}	42.7^{aA}	0.42	<.001	<.001	0.507

SEM = standard error of least square means.

A = age-production system; M = muscle; A×M = interaction of A×M.

$^{a, b, c}$ = $P < 0.05$ (age-production effect); $^{A, B, C}$ = $P < 0.05$ (muscle type effect).

LT = *Longissimus thoracis*; ST = *Semitendinosus*; GM = *Gluteus medius*.

[1]pHu = ultimate pH.

Table 5.2. WB-variables and cooking loss, chemical composition and collagen characteristics of three muscles of young Holstein-Friesian bulls from two age-production systems.

	15 month system			19 month system			SEM	*P*-value		
	LT	GM	ST	LT	GM	ST		A	M	A×M
WBSF[1] (N)	32.8^{bB}	44.5^{bA}	42.6^{bA}	44.0^{aB}	56.2^{aA}	52.1^{aA}	2.61	<.001	0.002	0.903
WB-slope (Mpa)	0.67^{bB}	0.93^{bA}	0.90^{bA}	0.93^{aB}	1.16^{aA}	1.10^{aA}	0.04	<.001	<.001	0.738
WB-area (J)	0.29^{bB}	0.36^{bA}	0.36^{bA}	0.36^{aB}	0.45^{aA}	0.45^{aA}	0.02	<.001	0.003	0.974
Cooking loss (%)	30.2^{bB}	31.2^{bB}	34.0^{bA}	32.6^{aB}	33.1^{aB}	35.1^{aA}	0.60	0.003	0.001	0.573
Moisture (%)	72.9^{bC}	74.8^{bB}	75.7^{bA}	74.4^{aC}	75.0^{aB}	76.0^{aA}	0.35	0.041	<.001	0.171
IMF[2] (%)	4.15^{aA}	1.39^{aB}	1.10^{aB}	1.78^{bA}	1.54^{bB}	0.86^{bB}	0.26	0.002	<.001	0.001
Protein (%)	22.8	22.7	22.4	23.2	22.5	22.8	0.24	0.347	0.271	0.421
Ash (%)	1.09	1.11	1.09	1.10	1.11	1.10	0.03	0.911	0.852	0.942
Soluble collagen (mg/g)	0.71	0.80	0.95	0.71	1.05	0.92	0.11	0.431	0.103	0.410
Insoluble collagen (mg/g)	2.38^{b}	1.87^{b}	2.48^{b}	3.35^{a}	3.32^{a}	3.21^{a}	0.50	0.023	0.834	0.763
Total collagen (mg/g)	3.00^{b}	2.67b	3.43^{b}	3.99^{a}	4.37^{a}	4.23^{a}	0.55	0.024	0.805	0.697
Collagen solubility (%)	25.4^{a}	31.0^{a}	29.8^{a}	18.8^{b}	25.2^{b}	22.8^{b}	3.62	0.049	0.274	0.987

SEM = standard error of least square means.

A = age-production system; M = muscle; A×M = interaction of A×M.

$^{a, b, c}$ = $P < 0.05$ (age-production effect); $^{A, B, C}$ = $P < 0.05$ (muscle type effect).

LT = *Longissimus thoracis*; ST = *Semitendinosus*; GM = *Gluteus medius*.

[1]WBSF = Warner-Bratzler Shear Force; [2]IMF = Intramuscular fat.

Table 5.3. Fatty acid proportion (g/100 g of total fatty acids) of three muscles of young Holstein-Friesian bulls from two age-production systems.

	15 month system			19 month system			SEM	*P*-value		
	LT	GM	ST	LT	GM	ST		A	M	A×M
C14:0	3.51^{aA}	2.24^{aB}	1.98^{aB}	2.59^{bA}	1.87^{bB}	1.56^{bB}	0.22	0.009	<.001	0.409
C14:1	0.60	0.40	0.52	0.50	0.42	0.51	0.08	0.673	0.241	0.747
C15:0	0.50^{A}	0.37^{B}	0.37^{B}	0.42^{A}	0.37^{B}	0.37^{B}	0.03	0.262	0.007	0.243
C15:1	0.07^{bC}	0.17^{bB}	0.24^{bA}	0.17^{aC}	0.22^{aB}	0.34^{aA}	0.02	0.001	<.001	0.470
C16:0	30.1^{aA}	24.9^{aB}	24.5^{aB}	26.6^{bA}	23.9^{bB}	23.6^{bB}	1.01	0.048	0.002	0.403
C16:1	3.76^{a}	3.20^{a}	3.83^{a}	3.13^{b}	3.14^{b}	3.09^{b}	0.20	0.013	0.307	0.235
C17:0	1.52^{A}	1.24^{AB}	1.05^{B}	1.35^{A}	1.19^{AB}	1.07^{B}	0.09	0.401	0.005	0.607
C18:0	17.0^{A}	13.8^{B}	12.0^{B}	14.6^{A}	13.5^{B}	12.5^{B}	0.54	0.122	<.001	0.065
C18:1n9t	3.16	2.39	2.12	2.07	2.06	1.84	0.51	0.210	0.504	0.695
C18:1n9c	36.5^{AB}	36.7^{A}	34.3^{B}	35.0^{AB}	35.8^{A}	32.3^{B}	1.01	0.103	0.027	0.866
C18:1n7	2.40^{B}	2.59^{AB}	2.78^{A}	2.32^{B}	2.65^{AB}	2.85^{A}	0.11	0.843	0.004	0.752
C18:2n6c	4.77^{bC}	8.46^{bB}	10.3^{bA}	7.43^{aC}	9.18^{aB}	11.8^{aA}	0.83	0.034	<.001	0.517
C18:3n3	0.30^{bB}	0.37^{bB}	0.57^{bA}	0.93^{aB}	0.96^{aB}	1.38^{aA}	0.08	<.001	0.002	0.334
C22:0	0.06	0.12	0.23	0.11	0.15	0.21	0.04	0.633	0.058	0.739
C20:3n6	0.26^{C}	0.64^{B}	0.90^{A}	0.46^{C}	0.66^{B}	0.96^{A}	0.08	0.140	<.001	0.488
C20:4n6	1.02^{bC}	2.50^{bB}	3.66^{bA}	2.03^{aC}	2.98^{aB}	4.11^{aA}	0.27	0.014	<.001	0.527
C20:5n3	0.10^{bB}	0.35^{bB}	0.62^{bA}	0.62^{aB}	0.86^{aB}	1.45^{aA}	0.10	<.001	<.001	0.251
C22:5n3	0.26^{bB}	0.67^{bB}	1.19^{bA}	0.90^{aB}	1.17^{aB}	1.96^{aA}	0.16	0.001	<.001	0.718
∑ SFA[1]	52.4^{aA}	42.1^{aB}	39.6^{aB}	45.7^{bA}	40.7^{bB}	38.0^{bB}	1.53	0.023	<.001	0.194
∑ UFA[2]	47.6^{bB}	57.9^{bA}	60.4^{bA}	54.3^{aB}	59.3^{aA}	62.0^{aA}	1.53	0.023	<.001	0.194
∑ MUFA[3]	40.9	44.9	43.2	42.1	43.6	40.4	1.94	0.557	0.329	0.600
∑ PUFA[4]	6.76^{bC}	13.0^{bB}	17.2^{bA}	12.3^{aC}	15.7^{aB}	21.6^{aA}	1.47	0.004	<.001	0.658
∑ n-6 PUFA	6.13^{bC}	11.6^{bB}	14.9^{bA}	9.93^{aC}	12.8^{aB}	16.9^{aA}	1.15	0.028	<.001	0.534
∑ n-3 PUFA	0.63^{bB}	1.37^{bB}	2.30^{bA}	2.33^{aB}	2.90^{aB}	4.69^{aA}	0.39	<.001	<.001	0.531

SEM = standard error of least square means.

A = age-production system; M = muscle; A×M = interaction of A×M.

$^{a, b, c}$ = $P < 0.05$ (age-production effect); $^{A, B, C}$ = $P < 0.05$ (muscle type effect).

LT = *Longissimus thoracis*; ST = *Semitendinosus*; GM = *Gluteus medius*.

[1]∑ SFA = Sum of saturated fatty acids.

[2]∑ UFA = Sum of unsaturated fatty acids.

[3]∑ MUFA = Sum of monounstaturated fatty acids.

[4]∑ PUFA = Sum of polyunsaturated fatty acids.

∑ n-6 PUFA = Sum of n-6 polyunsaturated fatty acids.

∑ n-3 PUFA = Sum of n-3 polyunsaturated fatty acids.

Table 5.4. Fatty acid concentration (mg/100g meat) of three muscles of young Holstein-Friesian bulls from two age-production systems.

	15 month system			19 month system			SEM	*P*-value		
	LT	GM	ST	LT	GM	ST		A	M	A×M
C14:0	92.7^{aA}	37.8^{aB}	17.2^{aB}	35.1^{bA}	21.5^{bB}	11.4^{bB}	9.75	0.006	0.001	0.045
C14:1	16.0^{A}	6.20^{AB}	4.27^{B}	7.11^{A}	6.03^{AB}	4.29^{B}	2.35	0.142	0.024	0.134
C15:0	12.8^{aA}	5.53^{aB}	3.04^{aB}	5.60^{bA}	4.82^{bB}	2.90^{bB}	1.19	0.018	0.001	0.021
C15:1	1.78	2.22	1.80	1.93	2.36	1.97	0.17	0.277	0.040	0.995
C16:0	775.4^{aA}	365.5^{aB}	197.2^{aB}	354.6^{bA}	300.0^{bB}	164.8^{bB}	65.4	0.007	<.001	0.021
C16:1	96.7^{aA}	49.5^{aB}	30.8^{aB}	41.2^{bA}	40.8^{bB}	22.7^{bB}	8.30	0.004	0.001	0.022
C17:0	39.3^{aA}	18.6^{aB}	8.67^{aB}	18.6^{bA}	15.3^{bB}	7.98^{bB}	3.88	0.023	0.001	0.049
C18:0	435.4^{aA}	199.1^{aB}	96.1^{aC}	197.2^{bA}	167.1^{bB}	83.5^{bC}	34.9	0.006	<.001	0.013
C18:1n9t	84.0^{aA}	36.4^{aAB}	18.1^{aB}	30.8^{bA}	31.9^{bAB}	15.6^{bB}	9.17	0.027	0.006	0.042
C18:1n9c	992.3^{aA}	549.6^{aA}	273.7^{aB}	474.5^{bA}	460.0^{bA}	233.1^{bB}	86.9	0.010	0.001	0.034
C18:1n7	60.6^{aA}	37.8^{aA}	21.8^{aB}	29.3^{bA}	32.1^{bA}	18.1^{bB}	4.26	0.002	<.001	0.012
C18:2n6c	116.1^{aA}	109.1^{aA}	77.4^{aB}	85.6^{bA}	99.9^{bA}	69.9^{bB}	5.98	0.007	<.001	0.145
C18:3n3	7.48^{bA}	5.17^{bAB}	4.32^{bB}	10.5^{aA}	10.5^{aAB}	7.81^{aB}	0.70	<.001	0.005	0.262
C22:0	1.49	1.79	1.61	1.32	1.60	1.33	0.30	0.435	0.611	0.979
C20:3n6	6.37^{aB}	8.24^{aA}	6.64^{aB}	5.40^{bB}	6.84^{bA}	5.55^{bB}	0.19	<.001	<.001	0.532
C20:4n6	24.4^{B}	32.5^{A}	27.1^{B}	23.1^{B}	31.4^{A}	24.1^{B}	1.35	0.124	<.001	0.754
C20:5n3	2.11^{bB}	4.50^{bA}	4.54^{bA}	6.94^{aB}	8.93^{aA}	8.37^{aA}	0.61	<.001	0.013	0.750
C22:5n3	6.37^{bB}	8.82^{bA}	8.84^{bAB}	10.2^{aB}	12.5^{aA}	11.3^{aAB}	0.89	0.001	0.049	0.721
∑ SFA[1]	1350.8^{aA}	618.9^{aB}	319.0^{aB}	612.0^{bA}	508.0^{bB}	265.7^{bB}	112.6	0.007	<.001	0.018
∑ UFA[2]	1238.1^{aA}	841.6^{aA}	474.2^{aB}	711.3^{bA}	733.1^{bA}	418.7^{bB}	107.6	0.022	0.001	0.095
∑ MUFA[3]	1074.1^{aA}	673.5^{aA}	345.6^{aB}	570.8^{bA}	563.3^{bA}	291.9^{bB}	104.6	0.023	0.002	0.105
∑ PUFA[4]	163.9^{A}	168.1^{A}	128.5^{B}	140.5^{A}	169.8^{A}	126.7^{B}	9.10	0.313	0.002	0.359
∑ n-6 PUFA	148.7^{aA}	149.9^{aA}	111.1^{aB}	114.3^{bA}	138.7^{bA}	99.6^{bB}	7.31	0.008	0.001	0.234
∑ n-3 PUFA	15.2^{b}	18.2^{b}	17.4^{b}	26.2^{a}	31.2^{a}	27.1^{a}	2.24	<.001	0.238	0.771
∑ TFA[5]	2750.2^{aA}	1462.6^{aB}	796.2^{aC}	1327.0^{bA}	1244.1^{bB}	691.5^{bC}	223.7	0.008	<.001	0.022
MUFA/SFA	0.84^{B}	1.07^{A}	1.09^{A}	0.92^{B}	1.07^{A}	1.06^{A}	0.05	0.620	0.002	0.536
PUFA/SFA	0.13^{bC}	0.31^{bB}	0.44^{bA}	0.27^{aC}	0.39^{aB}	0.57^{aA}	0.04	0.003	<.001	0.705
n-6/n-3 PUFA	10.1^{a}	9.28^{a}	7.41^{a}	4.51^{b}	4.53^{b}	3.69^{b}	1.15	<.001	0.307	0.716
SFA/UFA	1.36^{A}	0.73^{AB}	0.66^{B}	0.84^{A}	0.69^{AB}	0.62^{B}	0.15	0.134	0.022	0.244

SEM = standard error of least square means.

A = age-production system; M = muscle; A×M = interaction of A×M.

$^{a, b, c}$ = $P < 0.05$ (age-production effect); $^{A, B, C}$ = $P < 0.05$ (muscle type effect).

LT = *Longissimus thoracis*; ST = *Semitendinosus*; GM = *Gluteus medius*.

[1]∑ SFA = Sum of saturated fatty acids.
[2]∑ UFA = Sum of unsaturated fatty acids.
[3]∑ MUFA = Sum of monounstaturated fatty acids.
[4]∑ PUFA = Sum of polyunsaturated fatty acids.
∑ n-6 PUFA = Sum of n-6 polyunsaturated fatty acids.
∑ n-3 PUFA = Sum of n-3 polyunsaturated fatty acids.
[5]∑ TFA = Total fatty acids.

Table 5.5. Pearson correlation between physico-chemical traits of LT muscle of young Holstein-Friesian bulls.

	WBSF[1]	WB-slope	WB-area	Cooking loss	Moisture	IMF[2]
pH (15°C)	-0.25	-0.37	-0.26	-0.45*	-0.39*	0.46*
pH (35°C)	-0.58***	-0.60***	-0.51**	-0.20	-0.24	0.57***
pHu[3]	0.20	0.06	0.20	-0.36*	0.32	-0.43*
L* 2h	-0.31	-0.20	-0.27	0.46*	-0.01	0.23
a* 2h	-0.28	-0.14	-0.31	0.23	-0.28	0.43*
b* 2h	-0.39*	-0.24	-0.38*	0.30	-0.25	0.46*
Croma 2h	-0.33	-0.18	-0.34	0.26	-0.27	0.45*
Hue angle 2h	-0.37*	-0.27	-0.28	0.31	-0.02	0.15
L* 24h	-0.02	0.05	0.03	0.57***	0.02	0.08
a* 24h	-0.39*	-0.22	-0.44*	0.23	-0.17	0.37*
b* 24h	-0.45*	-0.27	-0.48*	0.39*	-0.04	0.30*
Croma 24h	-0.42*	-0.24	-0.46*	0.29	-0.13	0.36*
Hue angle 24h	-0.02	-0.04	0.05	0.22	0.31	-0.23
WBSF		0.92***	0.96***	0.15	0.22	-0.33
WB-slope			0.83***	0.28	0.36*	-0.47*
WB-area				0.17	0.16	-0.24
Cooking loss					0.05	0.08

Significance: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

LT = *Longissimus thoracis* . [1]WBSF = Warner-Bratzler Shear Force; [2]IMF = Intramuscular fat; [3]pHu = ultimate pH.

Table 5.6. Pearson correlation between physico-chemical traits of ST muscle of young Holstein-Friesian bulls.

	WBSF[1]	WB-slope	WB-area	Cooking loss	Moisture	IMF[2]
pHu[3]	-0.24	-0.25	-0.12	-0.26	0.52**	-0.35*
L* 2h	0.01	0.03	0.01	0.12	-0.10	0.32
a* 2h	0.25	0.19	0.29	0.43*	-0.12	0.07
b* 2h	0.33	0.28	0.31	0.46*	-0.10	0.15
Croma 2h	0.29	0.23	0.31	0.46*	-0.12	0.11
Hue angle 2h	0.16	0.17	0.05	0.09	0.05	0.14
L* 24h	0.34	0.32	0.37*	0.34	0.01	-0.01
a* 24h	-0.10	-0.16	-0.01	0.09	-0.18	0.29
b* 24h	0.05	0.01	0.19	0.26	0.02	0.22
Croma 24h	-0.04	-0.10	0.07	0.17	-0.11	0.28
Hue angle 24h	0.27	0.31	0.31	0.25	0.38*	-0.26
WBSF		0.97***	0.92***	0.63***	0.11	-0.16
WB-slope			0.84***	0.53**	-0.02	-0.10
WB-area				0.68***	0.30	-0.26
Cooking loss					0.41*	-0.20

Significance: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.
ST = *Semitendinosus*. [1]WBSF = Warner-Bratzler Shear Force; [2]IMF = Intramuscular fat; [3]pHu = ultimate pH.

Table 5.7. Pearson correlation between physico-chemical traits of GM muscle of young Holstein-Friesian bulls.

	WBSF[1]	WB-slope	WB-area	Soluble collagen	Insoluble collagen	Total collagen	Collagen solubility
pHu[2]	0.48*	0.43*	0.50*	0.01	0.38	0.33	-0.40
L* 2h	-0.15	0.01	-0.15	0.02	0.15	0.14	-0.25
a* 2h	-0.41*	-0.31	-0.39*	0.06	-0.08	-0.06	0.17
b* 2h	-0.35	-0.21	-0.34	0.06	0.08	0.08	-0.03
Croma 2h	-0.40*	-0.28	-0.38*	0.06	-0.02	-0.01	0.10
Hue angle 2h	-0.03	0.13	-0.01	0.10	0.38	0.35	-0.38
L* 24h	0.04	0.02	0.10	0.38	-0.04	0.06	0.27
a* 24h	-0.47*	-0.42*	-0.46*	-0.19	-0.13	-0.16	-0.04
b* 24h	-0.43*	-0.30	-0.41*	-0.03	0.10	0.08	-0.20
Croma 24h	-0.47*	-0.39*	-0.46*	-0.14	-0.06	-0.09	-0.09
Hue angle 24h	0.17	0.29	0.17	0.35	0.45	0.48	-0.26
WBSF		0.94***	0.95***	-0.22	0.22	0.13	-0.45
WB-slope			0.88***	-0.19	0.36	0.26	-0.56*
WB-area				-0.32	0.24	0.12	-0.52*
Cooking loss				-0.44	-0.03	-0.14	-0.37

Significance: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.
GM = *Gluteus medius*. [1]WBSF = Warner-Bratzler Shear Force; [2]pHu = ultimate pH.

Table 5.8. Pearson correlation between fatty acid proportion (%) and IMF content, WBSF and cooking loss of three muscles of young Holstein-Friesian bulls.

	LT			ST			GM		
	IMF	WBSF	Cooking loss	IMF	WBSF	Cooking loss	IMF	WBSF	Cooking loss
C14:0	0.64*	-0.18	-0.11	0.23	-0.53*	-0.49	0.30	-0.38	0.16
C14:1	0.28	0.12	0.04	0.21	0.14	-0.28	0.43	0.22	0.09
C15:0	0.18	0.01	-0.19	-0.31	-0.42	-0.06	0.55*	0.35	-0.15
C15:1	-0.85***	0.17	0.23	-0.19	0.51*	0.41	-0.21	0.16	-0.41
C16:0	0.48	-0.01	0.10	0.06	-0.58*	-0.59*	0.48	-0.09	0.02
C16:1	0.34	0.10	-0.02	0.29	-0.23	-0.46	0.26	-0.21	0.15
C17:0	0.28	0.09	-0.16	-0.16	-0.48	-0.22	0.53*	0.28	0.08
C18:0	0.53*	-0.06	0.15	-0.10	-0.02	0.24	-0.28	0.25	-0.09
C18:1n9t	-0.03	0.01	-0.75*	-0.13	-0.28	-0.56	-0.09	0.68*	-0.10
C18:1n9c	0.62*	0.17	0.10	0.42	-0.34	-0.26	0.23	-0.27	0.49*
C18:1n7	-0.19	0.32	-0.12	-0.46	0.60*	0.55*	-0.56*	0.09	-0.38
C18:2n6c	-0.75***	0.13	0.03	-0.41	0.54*	0.57*	-0.36	0.18	-0.38
C18:3n3	-0.78***	0.24	0.38	-0.29	0.46	0.55*	-0.05	0.23	-0.07
C22:0	-0.84	0.83	0.89*	-0.25	0.42	0.21	-0.24	-0.10	-0.68*
C20:3n6	-0.83***	0.20	0.10	-0.25	0.37	0.59*	-0.26	-0.08	-0.33
C20:4n6	-0.85***	0.15	0.16	-0.25	0.45	0.45	-0.18	0.06	-0.46
C20:5n3	-0.84***	0.19	0.37	-0.29	0.34	0.48	-0.07	0.14	-0.19
C22:5n3	-0.81***	0.21	0.33	-0.19	0.36	0.31	-0.06	0.08	-0.34
∑ SFA[1]	0.56*	-0.04	0.08	0.36	-0.54*	-0.63*	0.28	-0.23	0.14
∑ UFA[2]	-0.56*	0.04	-0.08	-0.36	0.54*	0.63*	-0.28	0.23	-0.14
∑ MUFA[3]	-0.04	-0.08	-0.18	0.29	-0.40	-0.39	0.22	-0.09	0.40
∑ PUFA[4]	-0.81***	0.17	0.14	-0.36	0.51*	0.55*	-0.28	0.15	-0.39
∑ n-6 PUFA	-0.79***	0.13	0.06	-0.38	0.53*	0.56*	-0.32	0.15	-0.41
∑ n-3 PUFA	-0.80***	0.29	0.36	-0.24	0.36	0.39	-0.04	0.11	-0.21

Significance: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

[1]∑ SFA = Sum of saturated fatty acids.

[2]∑ UFA = Sum of unsaturated fatty acids.

[3]∑ MUFA = Sum of monounstaturated fatty acids.

[4]∑ PUFA = Sum of polyunsaturated fatty acids.

∑ n-6 PUFA = Sum of n-6 polyunsaturated fatty acids.

∑ n-3 PUFA = Sum of n-3 polyunsaturated fatty acids.

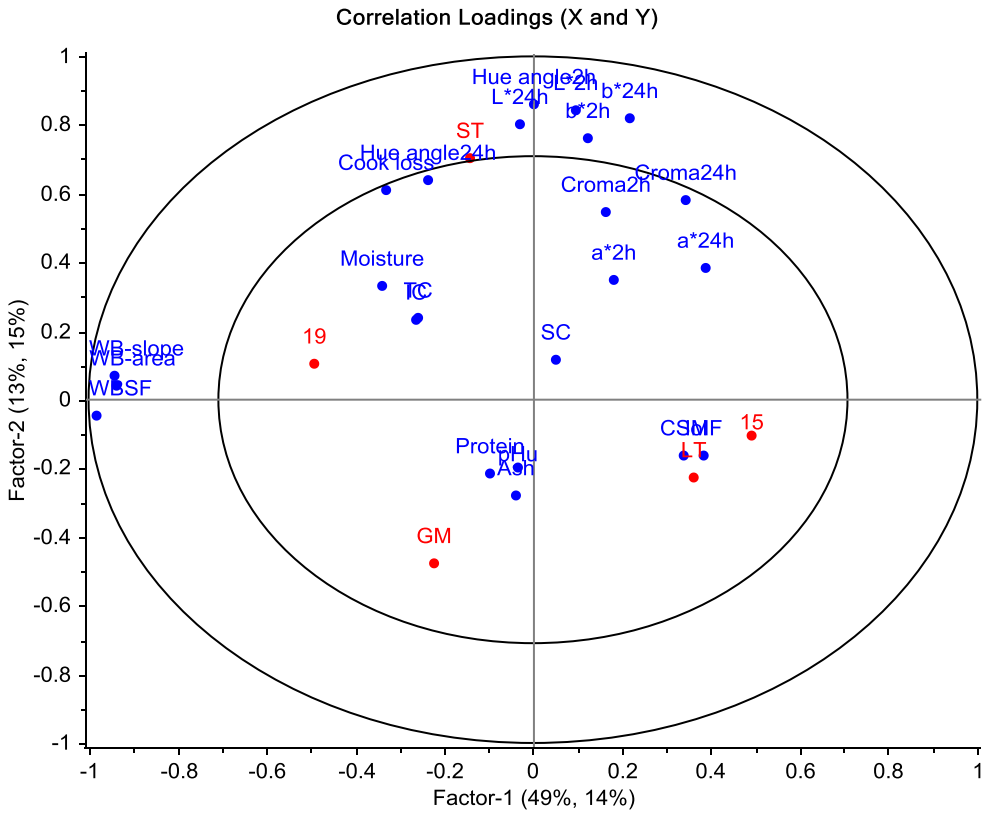
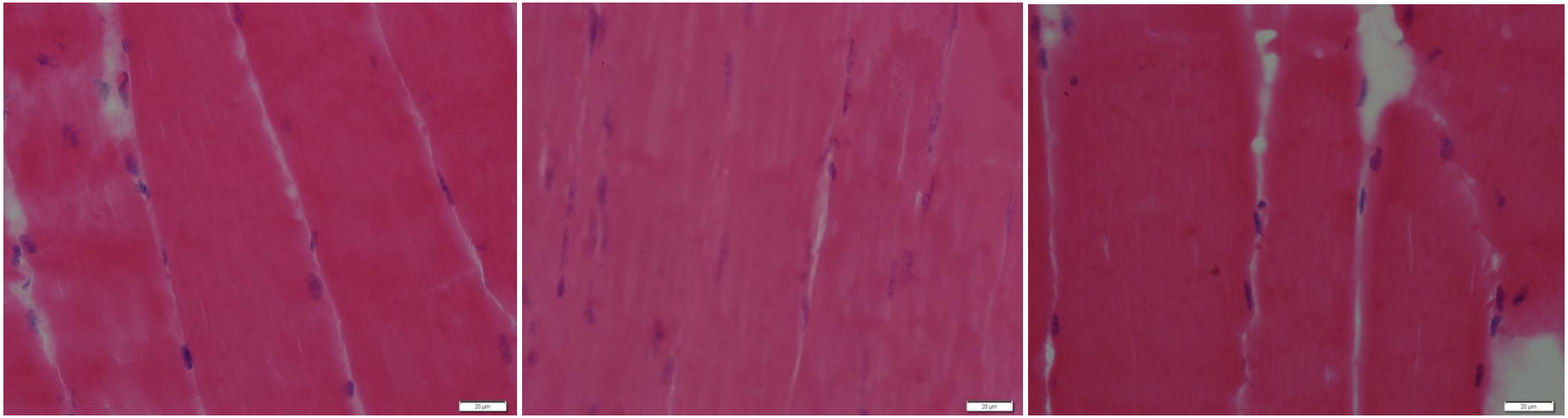


Figure 5.2. Partial least square regression (PLSR) correlation loadings plot of Factor 1 versus Factor 2. The model was derived from the quality traits in the X-matrix and muscle and age-production system in the Y-matrix. SC = Soluble collagen; IC = Soluble collagen; TC = Total collagen; CSol = Collagen solubility; WBSF = Warner-Bratzler Shear Force; IMF = Intramuscular fat. LT = *Longissimus thoracis*; ST = *Semitendinosus*; GM = *Gluteus medius*.



LT ST GM

Figure 5.3. *Longissimus thoracis* (LT), *Semitendinosus* (ST) and *Gluteus medius* (GM) muscles at 3 ageing days from Holstein-Friesian (HF) bulls at 19 months old (Light Microscopy - 60X magnification).

Chapter 6

Effect of castration and carcass suspension method on the quality and fatty acid profile of beef from male dairy cattle

6.1 Abstract

The use of bulls over steers in terms of beef manufacture offers some considerable production advantages, however, the eating quality of bull beef is an issue of marketing concern. This study assessed the physico-chemical characteristics of young Holstein-Friesian bull (n = 14) and steer (n = 15) beef. All bull carcasses and seven steer carcasses were suspended by the Achilles tendon suspension (AS) and eight steer carcasses suspended by pelvic suspension (PS). Post-mortem pH, ultimate pH (pHu), meat colour, proximate chemical composition, collagen characteristics and fatty acid (FA) composition were evaluated. Warner-Bratzler variables, including; WBSF, WB-slope, WB-area and cooking loss were measured after 3, 7, and 14 days ageing. Steer beef had higher redness, yellowness, and chroma values, while bull beef had higher pHu and darker muscle. WBSF, cooking loss at different ageing times and moisture content were higher for bull beef, whilst intramuscular fat (IMF) concentration was higher for steer beef. Bull beef had more insoluble and total collagen, while soluble collagen and collagen solubility were higher for steer beef. This suggests that for young dairy cattle, steer beef would likely have superior eating quality to bull beef. Ageing improved tenderness for both bull and steer beef. WBSF for aged bull beef indicated that it was acceptably tender after more than 7 days of ageing. Steer beef displayed the higher proportion of saturated (SFA) and monounstaturated fatty acid (MUFA), while bull beef had higher polyunsaturated fatty acid (PUFA) proportion, PUFA/SFA and n-6/n-3 PUFA ratios. The absolute contents of most FAs were higher for steers. In comparison to AS, PS increased redness and chroma after 24 h blooming; PS improved tenderness up to 7 days of ageing and accelerated the ageing process. WB-variables for different ageing times were positively correlated with cooking loss, moisture and insoluble collagen, and negatively correlated with IMF, soluble collagen content and collagen solubility. FA composition showed significant correlations with IMF content, WBSF and cooking loss.

Keywords: Castration, Dairy cattle, Fatty acids, Meat quality, Pelvic suspension, Tenderness

6.2 Introduction

After the ending of milk quotas in April 2015, there is significant potential for increased dairy output in Ireland. Undoubtedly, this will lead to a substantial increase in the number of male calves from the dairy herd. This is a potential new resource for the industry if they can be reared economically to produce meat of acceptable eating quality. Holstein-Friesian (HF) is the predominant Irish dairy breed owing to their high milk yield and reproductive efficiency.

Castration is performed to reduce animal aggressiveness and improve meat quality, while poor conformation is the marked disadvantage for raising these calves as steers. Leaving these males intact to develop as bulls may be a more viable option due to their improved growth rate, feed conversion efficiency and leaner carcasses compared with steers (Rodriguez et al., 2014). On average, bulls have 8.4% higher live weight gain, 9.5% heavier carcass weight and 20% greater lean meat yield than steers reared in the same way (Fallon et al., 2001). Moreover, lower carbon emissions from bull production systems will benefit sustainable farming and the environment (Dawson, 2010). Such an approach would provide a significant new source of income and opportunity for producers, increase alternative beef supplies and potentially open up new export markets for Irish beef. Although young bull beef production has increased in the last decade, it still only accounted for 19% of overall Irish male cattle slaughtered in 2015, down from 22% in 2014, 25% in 2013 and 30% in 2012 (DAFM, 2015). Any further growth in bull beef production is constrained by the reluctance of processors to purchase bulls arising from concerns about the acceptability of bull beef.

Meat from steers is often preferred by consumers over meat from bulls, due to greater marbling and improved sensory traits resulting from gender differences in the different muscle and fat depositions in the carcass (Mach et al., 2009; Zhang et al., 2010). It has been generally accepted that beef tenderness can be enhanced by post-mortem ageing (Rodriguez et al., 2014). However, little is known about how the ageing process affects beef from bulls and steers from the dairy herd. Moreover, castration effects on beef quality have been researched mainly on beef breeds (Peachey et al., 2002; Destefanis et al., 2003), and Holstein calves fed a high-concentrate diet (Mach et al., 2009; Marti et al., 2013). Little research has been

reported on the effect of castration on the quality characteristics of Holstein-Friesian calves fed a grass-based diet.

Fat and fatty acid (FA) play an important role in human health. In order to reduce the risk of cardiovascular diseases, guidelines for decreasing total FA intake, and replacing SFA with PUFA, especially those of the n-3 series, has been recommended by the World Health Organization (WHO, 2003). FA composition also plays an active role in the colour and palatability of beef, including; flavour, tenderness and juiciness (Wood et al., 2008). As it is susceptible to oxidation, PUFA is prone to colour deterioration and undesirable flavour development, thus reducing product shelf-life (Scollan et al., 2006). Variation in IMF content between animals strongly influences FA profile (Brugiapaglia et al., 2014). It is important to select beef production options which maximise both eating quality and healthiness. Several factors can modify FA composition and fat deposits, including; species, breed, diet, age, sex and anatomical location (Nürnberg et al., 1998). Of these, sex is important and is ascribed to hormonal differences which further affect fatness and associated changes in the triacylglycerol/phospholipid ratio (Eichhorn et al., 1985; Zhang et al., 2010).

Pelvic suspension (PS), also termed aitch-bone hanging or tenderstretch, has been implemented by the global meat industry to improve palatability, particularly tenderness, and to reduce the variation in tenderness of certain muscles (Sørheim & Hildrum, 2002). Even though PS requires more storage chilling space, extra labour and results in slight alterations in muscle shape, it is used for tenderness specifications by some retailers in the UK and within Northern Ireland. Moreover, a majority of beef carcasses exported from Ireland are aitch-bone hung (Ahnström et al. 2012a). The effect of PS on meat quality has been previously researched on and cattle of different genders, including; bulls, heifers, cows, primarily from beef breeds (Ahnström et al. 2012a; Kamatara et al., 2014), however, little information is available which pertains to the impact of aitch-bone hanging effects on beef quality parameters from dairy breeds.

The objectives of this study were to evaluate the effects of castration and carcass suspension method on the quality traits and FA profile of beef derived from young male dairy breed-type cattle under an Irish pasture-based system. The

interrelationships between physico-chemical traits and between physico-chemical properties and FA composition were also investigated.

6.3 Materials and methods

6.3.1 Animals

A total of 29 weaned, spring-born, male HF calves (10 to 12 weeks of age) were sourced and transported to Teagasc, Johnstown Castle Research Centre in 2013. They were turned out to pasture, predominantly perennial ryegrass (*Lolium perenne*), and supplemented with 1.5 kg concentrates during the first grazing season for 6 months (May-Nov). Concentrates consisted of 80% *Hordeum vulgare* (ground barley), 14% *Glycine max (L.) Merr* (soya bean meal), 4% *black treacle* (molasses) and 2% minerals. Fifteen calves were selected at random to be castrated at an age of approximately 6 months using Burdizzo clamps. Animals were then housed together and were offered grass silage supplemented with 2 kg concentrates during the winter period for 4 months (Nov-Mar 2014). They were all turned out again and fed pasture only for the second grazing season (bulls: Mar-June and steers: Mar-Sep). Bulls and steers were then finished on pasture plus 5 kg concentrates over a 100- or 60-day period, respectively, and subsequently slaughtered. Bulls were slaughtered at 19-months of age and steers were slaughtered 50 days later than bulls, in order to reduce differences in mean carcass weights. Mean carcass weights for bulls and steers were 292 kg and 279 kg, respectively.

6.3.2 Slaughter and sampling

At a commercial abattoir, cattle were stunned by captive-bolt, exsanguinated within 30 sec, centrally-split into two sides and weighed. Conformation and fat scores were graded automatically by certified video image analysis systems already installed at the abattoirs and were evaluated based on EUROP system criteria. Bull carcasses were conventionally suspended by the Achilles tendon (AS). Eight steer carcasses with higher conformation score (between 4 and 6) were selected and suspended by the obturator foramen of the pelvic bone (PS), while the remaining seven steers of a lower conformational score (2 or 3) were hung by AS in the chilling room at 4 °C. Although suspension method was confounded by conformation score owing to

market requirements, European conformation score has previously been shown to have no relationship with beef eating quality (Bonny et al., 2016).

The pH and temperature of the *longissimus thoracis* (LT) muscle at the 10th rib on the left side of each carcass were measured hourly for up to 6 h. The LT was excised from the cube roll (ribs 6 to10) from the left-hand side of each carcass at 48 h post mortem. After holding for 72 h at 4 °C, the ultimate pH (pHu) of the LT samples was measured, and then the muscle was cut into individual slices (25 mm in thickness). The fresh cut surface of the first slice from the 10th rib end was used for colour measurement, and the rest of the slices were vacuum-packed. Steaks for chemical composition, collagen, FA determinations, WBSF and cooking loss at day 3 were stored at -20 °C immediately, samples for WBSF and cooking loss were also aged for 7 and 14 days at 4 °C and then frozen at -20 °C for further analysis.

6.3.3 Post-mortem pH, temperature and ultimate pH

A portable pH meter model 420A (Orion, Germany) and an Amagruss pH electrode EC-2010-11 (Refex sensors Ltd., Westport, Co. Mayo, Ireland) were calibrated using two standard buffer solutions (pH 4.0 and pH 7.0). The temperature probe and pH electrode were inserted approximately 50 mm into the LT muscle immediately anterior to the 10th rib by making a scalpel incision. The electrode was rinsed thoroughly with distilled water between measurements. The post-mortem pH decline rate was calculated by the following equation:

$$\text{pH decline rate (\%)} = (pH_{5h} - pH_{1h})/4 \times 100$$

6.3.4 Meat colour

The method was described in Chapter 2.

6.3.5 Warner-Bratzler shear force and cooking loss

The method was described in Chapter 2.

6.3.6 Proximate chemical composition

The method was described in Chapter 2.

6.3.7 Collagen content and solubility

The method was described in Chapter 3.

6.3.8 Fatty acid analysis

The method was described in Chapter 5.

6.3.9 Imaging

The method was described in Chapter 5.

6.3.10 Statistical analysis

The data from steers were analysed using the GLM procedure of one-way ANOVA with type Ⅲ sums of squares (SAS, 2002) with suspension method as the factor. The Tukey-Kramer test was used to compare mean values with a significance level of $P < 0.05$. For the parameters which were significantly affected by suspension method ($P < 0.05$) and for all WB-variables, the gender comparison was conducted by the ANOVA test on bulls and Achilles tendon-suspended steers. All other variables were subjected to ANOVA test on bulls and all steers (both suspension treatments) including gender as the fixed effect.

Pearson's correlations coefficients were calculated using the CORR procedure of SAS (2002). Meat quality traits were also analysed by Partial Least Squares Regression (PLSR) to achieve an overview of their correlations and to visualize and determine the degree of the contribution of castration and suspension method to the variation in quality traits. The corresponding PLSR correlation loading plot and standardized regression coefficients were performed using Unscrambler Software, Version 10.3 (CAMO ASA, Oslo, Norway).

6.4 Results and discussion

6.4.1 Sex effect

6.4.1.1 Post-mortem pH-temp decline and ultimate pH

According to the Meat Standards Australia (MSA) post-mortem pH/temperature Model, cold shortening occurs when the muscle pH is greater than 6.0 while the temperature is below 15 ºC as ATP is still available for muscle contraction, thus

increasing meat toughness. Conversely, heat toughening can occur if the pH is below 6.0 while the temperature is above 35 ºC, as the combination of low pH and high temperature causes early decrease of proteolytic activity in muscle, thereby rendering the product incapable of ageing (Thompson, 2002). In this study, four bull carcasses were within the 'cold shortening window', however, no bull carcass was determined to be within the 'heat toughening window' (Figure 6.1a). The more rapid temperature fall may be a result of the relative lighter carcass of these four bulls compared with the other bulls used within this study (mean carcass weight of four bulls 268 kg versus mean carcass weight of rest bulls 301 kg). Only one steer carcass was determined to be within the 'cold shortening window' and one steer carcass within the 'heat toughening window' (Figure 6.1b), and these were the lightest (242 kg) and heaviest (311 kg) steer carcasses, respectively. Although the mean carcass weight was similar for bulls and steers, fat scores were lower for bulls (5.0) than steers (8.0), indicating that the subcutaneous fat covering on bull carcasses was insufficient to prevent an excessively rapid decrease in temperature, thereby increasing the risk of cold shortening.

The pH values at 35 ºC and at 2 and 3 h post-mortem were higher for bulls than steers ($P < 0.05$; Table 6.1). The pH at 15 ºC and at 1, 4, and 5 h post-mortem tended to be higher for bulls than steers ($P < 0.10$; Table 6.1). Likewise, bulls had a higher ultimate pH (pHu) than steers ($P < 0.01$; Table 6.1). This was probably due to the faster chilling rate resulting from insufficient subcutaneous fat cover in bull carcasses. Another reason which could be more important for this is likely due to the excitable temperament and the physical contests between bulls, consequently causing ante-mortem glycogen depletion, thus leading to insufficient lactic acid generation post slaughter, thereby limiting pH fall (Monin, 1990). The mean pHu of both bulls and steers were within the normal range $5.4 \leq \text{pHu} \leq 5.7$ (Tarrant, 1989), four bulls could be classified as DFD (dark, firm, dry, pHu > 5.9) meat, while no DFD meat was determined in steers.

6.4.1.2 Meat colour

After 24 h blooming, HF bull beef was darker than steer beef ($P < 0.05$; Table 6.1), in agreement with Zhang et al. (2010) for Qinchuan cattle and Marti et al. (2013) for Holstein calves. Since the myoglobin content has been shown to be similar between

both sexes by Destefanis et al. (2003), this probably results from reduced myofiber disruption, as indicated by the higher pHu and lower marbling (Muir et al., 1998b). Redness, yellowness and chroma were all higher in steer beef after both 2 and 24 h blooming ($P < 0.001$), thereby indicating a redder, deeper and more yellow colour in steers, in line with Marti et al. (2013). In contrast, hue angle was higher in bull beef after 24 h blooming, thereby indicating a less red hue ($P < 0.01$).

Redness has been shown to correlate with pHu, and redness decreases with an increase in pHu (Guignot et al., 1993). Thus, it is reasonable that bull beef was less red in colour, given the higher pHu. The higher yellowness is likely due to the higher IMF content of steer beef which correlates to b* value (Waritthitham et al., 2010a). The higher beef value for each colour parameter after 24 h rather than after 2 h blooming for both bulls and steers suggests that blooming of the LT muscle for young dairy cattle is incomplete after 2 h of exposure (Table 6.1).

6.4.1.3 Warner-Bratzler shear force

Castration improved meat tenderness, with HF steer beef being more tender on all ageing days ($P < 0.001$). This is in agreement with many studies which have shown that steers produce more tender and more palatable meat than bulls (Morgan et al., 1993a; Purchas et al., 2002; Rodriguez et al., 2014). The more tender beef from steers can be partly explained by the lower insoluble, total collagen content and lower cooking loss, higher collagen solubility and higher IMF concentration determined in this study, compared with bull beef (Table 6.2). Moreover, the higher risk of post-mortem cold shortening occurring in bull beef over steer beef could also contribute to meat tenderness variation. Although most studies state that bulls contain a higher percentage of red fibres than steers, which has a positive relationship with tenderness, androgens (testosterone in intact bulls) appear to increase the fibre diameters of all muscle fibres (Seideman et al., 1986), which has a markedly negative effect on LT tenderness in cattle (Chriki et al., 2013).

WBSF of LT muscle of HF bulls and steers both decreased during ageing ($P < 0.01$; Figure 6.2). Morgan et al. (1993a) stated that for the *longissimus* muscle of young beef breed cattle, 24 h post-mortem m-calpain activity was similar ($P > 0.05$) and μ-calpain activity tended to be higher ($P < 0.08$) in bulls rather than in steers, whereas calpastatin activity (endogenous calpain inhibitor) was 81.0% greater in bull muscle

compared to steers. They concluded that the higher calpastatin activity in bulls most likely decreased the amount of myofibrillar protein proteolysis by μ-calpain. In contrast, within the present study, WBSF decline during ageing was more marked for bull beef. The high association between the activity of calpastatin and the rate of post-mortem proteolysis has been found in beef from *Bos indicus* breeds of cattle (Morgan et al., 1993a). However, calpain and calpastatin systems varied largely between breeds and species (Monsón et al. 2005), thus, the balance of enzyme and inhibitor activity, and their interaction with post-mortem proteolysis of muscles from dairy (*Bos taurus)* breed cattle, needs to be investigated further. Moreover, it should be noted that calpain systems are responsible for beef tenderness changes in the early post-mortem period (24 h), while the effect of cathepsin could be expected thereafter (Sentandreu et al., 2002). Accordingly, differences in cathepsin activity between bull and steer beef would probably explain tenderization rates observed upon ageing between 3 and 14 days.

The lower post-mortem pH at each hour, combined with higher steer carcass temperature probably led to early exhaustion of proteolytic enzyme activity and reduced the potential for ageing (Dransfield, 1993). In addition, the different extent of ageing between 1 and 14 days could be associated with the different average diameter of type I fibres (Zamora et al., 1996), though this was not evaluated in the present study.

Based on the beef tenderness classification of Shackelford et al. (1991), HF bull beef after 3 days ageing is defined as 'tough' (WBSF > 45.08 N), and after 7 days ageing would be classified as 'intermediate tender' (WBSF between 38.22 and 45.08 N), whereas by 14 days it would be classified as 'tender' (31.36 < WBSF < 38.22 N). Steer beef was surprisingly tender even after only 3 days of ageing as it was within the 'very tender' category (WBSF < 31.36 N) (Figure 6.2). Although it is recommended that ageing can improve the organoleptic qualities of meat, the results suggest that HF steer beef needs less ageing than bull beef to achieve an acceptable degree of tenderness.

6.4.1.4 Cooking loss

Several reports have demonstrated that castration significantly reduced cooking loss of the *longissimus* muscle (Dikeman et al., 1986; Zhang et al., 2010). This is

consistent with our finding that the cooking loss was higher for beef from HF bulls than steers at all ageing times ($P < 0.01$; Table 6.2). This was probably due to the higher IMF content as fatter muscles sustain lower cooking loss than leaner muscles (Pordomingo et al., 2012). Furthermore, in the present study, cooking loss at different ageing times was positively correlated to collagen content and particularly, to thermally stable collagen ($P < 0.05$; Table 6.6-6.8). Hence, the lack of testosterone promotion of collagen synthesis could cause lower cooking loss from castrated beef (Gerrard et al., 1987). In addition, bulls contain larger muscle fibre diameters than steers due to the effect of androgens (Seideman et al., 1986) and cooking loss increases with increasing cross-sectional area of muscle fibres (Waritthitham et al., 2010b). Cooking loss from beef did not change during ageing for both bulls and steers (Table 6.2), in line with that for beef breeds (Morgan et al., 1993a).

6.4.1.5 Proximate chemical composition

Castration increased IMF ($P < 0.001$) and decreased water content of LT muscle ($P < 0.001$; Table 6.2), and which is in agreement with other findings for dairy (Mach et al., 2009; Marti et al., 2013) and beef breeds (Mandell et al., 1997). Total protein content was similar for both bulls and steers ($P > 0.05$), which is not consistent with Padre et al. (2006) who reported that bulls had higher protein content than steers. IMF metabolism is subjected to hormonal regulation. Changes in fat and muscle tissue metabolism have been attributed to different endocrine environments (Goodpaster & Kelley, 1998). Firstly, the lack of testicular hormones, particularly testosterone in castrates, leads to decreased growth rate and a reduction in muscular development capacity, thus more fat deposition and rapid fattening occur in steers (Eichhorn et al., 1985). Secondly, the lower fat deposition and increased muscle gain in bulls could be related to increased protein muscle accretion arising from the stimulating effect of testosterone on the reduction of muscle protein degradation (Morgan et al., 1993b). Finally, androgens in some manner, control mitochondria to assist in depositing fat in intact bulls (Seideman et al., 1986).

IMF levels between 3.0% and 7.3% in beef have been generally considered acceptable in terms of visual quality and health concerns (Miller, 2002). IMF also plays an important role in beef palatability with regard to improvement of flavour, tenderness and juiciness (Costa et al., 2012). Thus, the steer beef in this study had a

satisfactory IMF level, whereas for bull beef, it was much lower than the acceptable level. A higher IMF content in the LT of young bulls (2.6%) and steers (4.1%) was reported for Holstein cattle fed a high-concentrate diet (Mach et al., 2009). The lower IMF content of HF cattle in the present study may partly result from the lower energy diet, with the feeding effect being more marked for bulls. Compared with the present study, a higher IMF content for bulls (1.7%) and a slightly lower IMF content for steers (3.4%) have been determined in *longissimus* muscle for Nelore × Angus cattle fed a pasture diet at 20 months of age (Padre et al., 2006).

6.4.1.6 Collagen content and solubility

HF bulls had higher insoluble ($P < 0.001$) and total collagen ($P < 0.05$) contents than steers, whereas soluble collagen ($P < 0.001$) and collagen solubility ($P < 0.001$) were higher in steers than bulls (Table 6.2), which indicated that collagen in HF bull beef had more thermally stable intermolecular cross-links. The reduced total collagen content in castrates, as observed in this study, is in accordance with other reports (Boccard et al., 1979; Destefanis et al., 2003). The reduced testosterone in steers is believed to decrease stimulating collagen synthesis (Gerrard et al., 1987) and these authors also found that intramuscular collagen solubility was higher in Charolais × Angus castrates than in intact males. The reduced collagen turnover, particularly the decreased degradation rate of newly synthesized collagen in intact males, may lead to more enzymatic cross-linking, thereby resulting in a more thermally stable collagen complex (Gerrard et al., 1987).

Total collagen content in beef within the present study was similar to that observed for *longissumus thoracis et lumborum* (LTL) muscle for Piedmontese cattle at around 18 months of age with 3.97 mg/g for bulls and 2.76 mg/g for steers (Destefanis et al., 2003). As shown by Christensen et al. (2011), LT from Holstein bulls aged between 13-16 months of age had 21.7% collagen solubility, while the lower collagen solubility of bulls in this study was probably due to the older animals used in this study, as collagen solubility decreases with animal age (Bailey, 1985).

6.4.1.7 Fatty acid composition

6.4.1.7.1 Fatty acid proportion

Oleic acid (C18:1n9c) was the most abundant FA in beef for both bulls and steers, comprising of 28.5% and 39.9% of the total lipid fraction for HF bulls and steers,

respectively (Table 6.3). Palmitic acid (C16:0) and stearic acid (C18:0) were the second and third most abundant beef FAs, respectively. The observation that oleic, palmitic and stearic acid percentages in beef accounted for 68.9% of total FAs in bulls, and 82.4% of total FAs in steers is consistent with Eichhorn et al. (1985) for young beef breeds. Likewise, previous studies reported similar proportions of these three FAs, accounting for 73.3% of the total FAs for young HF bull beef (Lengyel et al., 2003) and 79.7% of the total FAs for young HF steer beef (Moreno et al., 2008). The lower proportion of the sum of the above three FAs in bull beef was due to the higher relative proportion of C18:2n6c. For PUFA, C18:2n6c, C18:3n3 and C20:4n6 tend to predominate in beef from both HF bulls and steers.

In cattle, an association between sex and FA composition exists. Our data showed that steer beef had higher proportions of C14:0 ($P < 0.01$), C14:1 ($P < 0.01$), C16:0 ($P < 0.001$), C16:1 ($P < 0.001$), C18:1n9c ($P < 0.001$), total SFA ($P < 0.05$) and total MUFA ($P < 0.001$) than bull beef (Table 6.3). Bull beef contained higher proportions of C15:1 ($P < 0.001$), C18:0 ($P < 0.01$), C18:2n6c ($P < 0.001$), C18:3n3 ($P < 0.001$), C20:3n6 ($P < 0.001$), C20:4n6 ($P < 0.001$), C20:5n3, EPA ($P < 0.001$), C22:5n3, DPA ($P < 0.001$), total UFA ($P < 0.05$), total PUFA ($P < 0.001$), total n-6 PUFA ($P < 0.001$) and total n-3 PUFA ($P < 0.001$) (Table 6.3). The results are in agreement with Eichhorn et al. (1985) who observed that the *longissimus* muscle of young beef breed castrates had higher proportions of C14:0, C15:0, C16:0, C16:1, C18:1n9c, total SFA and lower proportions of C18:2n6c, C18:3n3, C20:4n6, total UFA and PUFA than intact males. However, C15:0 did not differ between bull and steer beef in the present study ($P > 0.05$). Padre et al. (2006) found that *longissimus* muscle of Nelore × Aberdeen Angus bulls contained higher proportions of C15:1, C18:0, C18:2n6, C18:3n3, C20:5n3, total n-6 and n-3 PUFA and lower C16:0, C18:1 and total MUFA than steers, which is in line with what was determined in this present study. However, the higher 18:1n7 value for steer beef and the lack of difference in C22:5n3 content reported in their study is not consistent with our data. Likewise, Monteiro et al. (2006) reported that muscle from Mertolenga steers had higher C18:1n9c and lower C18:2n6c percentages compared with muscle from intact males. In addition, four FAs (C12:0, C20:0, C20:1n9 and C20:4n3) were detected in HF steer beef, but were not present in bull beef in the present study (Table 6.3).

The higher PUFA, lower SFA and lower MUFA percentages in bulls were due to their lower IMF content. Lower IMF content is related to fewer and smaller adipocytes, containing fewer triglycerides, hence it is accompanied by an increase in phospholipids as a percentage of total lipids (Marmer et al., 1984). Generally, percentages of total SFA and PUFA are negatively related because PUFA are preferentially deposited in phospholipids (which are membrane components in muscle tissue), whereas SFA and MUFA are mainly located in neutral lipids which are present primarily as triacylglycerols and contain less than 4.0% PUFA components (Eichhorn et al., 1985; Wood et al., 2008). Eichhorn et al. (1985) also pointed out that bulls have a lower triacylglycerol: phospholipid ratio and consequently, a higher relative proportion of PUFA, because bulls have leaner muscle tissue and consequently a lower marbling score compared with steers.

Although cellular and molecular mechanisms underlying the direct role of sex hormones in the FA profile of cattle are still not fully understood, it was suggested that the manipulation of sex hormonal status of cattle could affect lipid metabolism in both muscle tissue and adipose tissue, as gender differences are known to be related to hormonal changes and their possible influence on enzymatic systems (Malau-Aduli et al., 1998). Testosterone has been found to have an inhibitory effect on lipogenic enzyme activities in adipose tissue (Prior et al., 1983). The higher percentage of C18:1n9c in steer beef, accompanied by a lower C18:0 proportion probably results from the elevation of Δ^9 desaturase activity in steers. C18:0 can be converted to its respective n-9 monounsaturated counterpart by Δ^9 desaturase enzyme, which is encoded by the stearoyl-CoA desaturase (SCD) gene (Smith et al., 2009). In agreement with others, the possible elevated activity of Δ^9 desaturase exists in fatter animals (Brugiapaglia et al., 2014), like that of steers in the present study. Additionally, the higher proportions of long chain (C20-22) PUFAs observed in bulls indicate that testosterone could increase the activity or expression of Δ^5 and Δ^6 desaturase and elongase enzymes, which can accelerate the active conversion from C18:2n6 and C18:3n3 to form long chain PUFA in muscle (Wood et al., 2008). According to Monteiro et al. (2006), the higher proportion of linoleic acid (C18:2n6c) in bulls probably reflects a depression in ruminal biohydrogenation of dietary PUFA from pasture. Factors contributing to the differences in ruminal biohydrogenation pattern between genders could involve any factor affecting the

rumen microbial ecosystem, such as; feeding behaviour, changes in intake and passage rates, saliva secretion, rumen motility or volume (Monteiro et al., 2006), all of which need to be further investigated.

Thereby, two factors may explain the influence of gender on FA profile of HF beef. Firstly, differences in the degree of marbling and fattening induced by testosterone may affect the fat:lean ratio in meat and thus, the triacylglycerol: phospholipid ratio (Eichhorn et al., 1985). Secondly, gene expression, or varied activity of enzymes associated with FA desaturation, synthesis or chain elongation could be affected by hormonal changes (Monteiro et al., 2006).

6.4.1.7.2 Nutritional value

Castration had an effect on the ratios commonly used to evaluate the nutritional effect of fat composition on human health. A minimum value of 0.4 for PUFA/SFA and a maximum value of 4.0 for n-6/n-3 PUFA of dietary fat intake have been recommended for human diets, as these play important roles with regard to blood cholesterol elevation and in reducing the risk of coronary heart disease (Department of Health, 1994). HF bulls contained higher values of these two ratios in comparison to steers ($P < 0.05$; Table 6.4), in line with Monteiro et al. (2006) for the Mertolenga breed. The mean n-6/n-3 PUFA ratio for both bull and steer beef in the current study was within the recommended range. The PUFA/SFA ratio of bulls was also within the recommended value, whilst steers had less than the recommended value. Compared with the current study, a lower n-6/n-3 (1.69) and PUFA/SFA ratio (0.11) have been reported for Nelore × Aberdeen Angus bulls retained at pasture, whereas the n-6/n-3 (2.05) and PUFA/SFA ratios (0.11) for steers were similar to our results (Padre et al., 2006). Weglarz (2010) observed a higher n-6/n-3 (4.53) and a lower PUFA/SFA ratio (0.10) in Polish HF young bulls, than those in the present study. Likewise, slightly higher n-6/n-3 (2.10) and lower PUFA/SFA ratios (0.08) for young HF steers in New Zealand have been reported (Moreno et al., 2008). The lower n-6/n-3 PUFA and higher PUFA/SFA ratios of young HF bulls in the current study indicated a more healthy nutritional profile compared with young HF cattle employed in the studies described above.

Absolute values for C18:2n6c ($P < 0.05$), PUFA/SFA ($P < 0.001$) and n-6/n-3 ratios ($P < 0.05$) were higher in bulls, while absolute values for most FAs, including; C14:0

($P < 0.01$), C14:1 ($P < 0.01$), C15:0 ($P < 0.05$), C16:0 ($P < 0.001$), C16:1 ($P < 0.001$), C17:0 ($P < 0.01$), C18:0 ($P < 0.01$), C18:1n9c ($P < 0.001$), C18:1n7 ($P < 0.001$), C20:3n6 ($P < 0.001$), C20:5n3 ($P < 0.05$), C22:5n3 ($P < 0.001$), total SFA, UFA, MUFA, TFA ($P < 0.001$) were higher in steer beef (Table 6.4). As the total lipid content of muscle was also termed IMF or marbling fat (Wood et al., 2008), the results reflected the IMF trend between bulls and steers. In line with the higher total muscle lipid content of steers, the content of individual FAs was also higher, especially for those with a greater proportion of the neutral lipid fraction, such as SFA and MUFA (Pavan & Duckett, 2013). However, phospholipids are commonly considered to constitute a small and constant content, regardless of gender condition (Lengyel et al., 2003). This could explain why the total SFA and MUFA absolute concentrations were higher in steers compared to bulls, while the total PUFA absolute concentration was similar between bulls and steers.

In general, SFA, especially myristic (C14:0) and palmitic (C16:0) acids, were higher in steers which has a detrimental implication for health due to their serum cholesterol raising risk. However, the higher C18:0 concentration in steers has a neutral effect on the total plasma cholesterol concentration, with no hypercholesterolemic effect like that for other SFA (Daley et al., 2010). It should be noted that even though there are nutritionally beneficial FA in HF bull beef, particularly the ideal PUFA/SFA and n-6/n-3 ratios, a daily intake of desirable FAs, such as MUFA (mainly oleic acid, C18:1n9c), would have a beneficial effect of lowering blood cholesterol levels and could in fact be greater when consuming beef from steers due to its greater total fat content (Smith et al., 2009; Daley et al., 2010). Thus, the challenge is to maximize the intake of desirable FAs while reducing the intake of total fat and non-desirable FAs (Pavan & Duckett, 2013).

The dietary guidelines recommend the consumption of n-3 PUFA as 1.6 g/day for men and 1.1 g/day for women (Institute of Medicine of the National Academies, 2002). If we consider a common beef serving to be 100 g, steaks from HF bulls and steers would provide 40 and 47 mg n-3 PUFA, respectively (Table 6.4). For n-6 PUFA, the consumption would be 99 and 91 mg from HF bulls and steers, respectively compared with the recommended intake of 17 and 12 g for men and women, respectively (Institute of Medicine of the National Academies, 2002). Furthermore, the absolute amounts of specific PUFA intake are paid more attention

now, and it is recommended by the dietary guidelines to increase the consumption of long chain (LC)-PUFA, mainly the n-3 series. The sum of EPA and DPA were determined to be 21.2 and 28.2 mg/100g for HF bulls and steers, respectively; the sum of C20:3n6 and C20:4n6 were 24.5 and 28.4 mg/100g for HF bulls and steers, respectively (Table 6.4). Thus, the total n-3 or n-6 LC-PUFA for young HF cattle only correspond to approximately 10.0% of the minimum recommended daily intake for humans (250-2000 mg/day) (Elmadfa & Kornsteiner, 2009). Consequently, beef from this study would make a relatively small contribution to the recommended consumption of PUFA.

6.4.2 Carcass suspension effect

6.4.2.1 Post-mortem pH and meat colour

There was no significant difference in beef pHu between the two suspension methods utilised in this study ($P > 0.05$; Table 6.5), which in agreement with Hou et al. (2014). Colour parameters after 2 h blooming were also similar between AS and PS ($P > 0.05$), while PS increased redness and chroma values after 24 h blooming ($P < 0.05$). It is reasonable to conclude that for young HF cattle in this study, full blooming was achieved after 24 h rather than 2 h. However, most authors reported no effect of PS on beef colour (Bayraktaroglu & Kahraman, 2011; Hou et al., 2014), while Ahnström et al. (2012b) reported that meat from PS heifers had higher yellowness ($P < 0.05$) and tended to have higher redness ($P < 0.10$) than that of AS. Kamatara et al. (2014) explained that sarcomeres were stretched in muscles suspended by PS, thereby leading to less overlap between thick and thin filaments, thus interfilament space is expanded, which enables more room for water and less cooking loss. It is hypothesised that the larger interfilament space may also provide more room for myoglobin remaining after chilling for 24 h, which may induce more saturated and intense red colour. In addition, the rate of decline in pH post-mortem was considered to be another reason for colour differences (Bayraktaroglu & Kahraman, 2011), even though the pH decline rate did not differ between the two suspension methods in the present study (Table 6.5, $P > 0.05$).

6.4.2.2 Warner-Bratzler shear force

WBSF was lower in LT samples from PS in comparison to AS within 7 days post-mortem. The difference in WBSF between AS and PS was 3.91 N on the 7th post-

mortem day (Figure 6.3; $P < 0.05$). WBSF at 3 days of PS was also lower than that of AS by 2.36 N, but this difference was not significant ($P > 0.05$). This result indicated that the improvement of meat tenderness by PS can also be applied to dairy steers, which agrees with the general finding that PS can increase tenderness or decrease shear forces in several muscles from bulls (Ahnström et al., 2006; Kamatara et al., 2014), heifers and cows (Ahnström et al., 2012a; Ahnström et al., 2012b). This is due to the increased tension on the hind limb and loin as produced using PS and which increases sarcomere length and reduces muscle fibre diameter. The stretching of the myofibrillar proteins further restricts myofibril shortening during rigor and improves tenderness (Sørheim & Hildrum, 2002).

PS reduced WBSF of the LT muscle for HF steers up to 7 days of ageing compared with AS, while the reduction of WBSF between the two suspension methods was eliminated at 14 days of ageing (Figure 6.3). This result supports previous findings that the effect of suspension method on WBSF of LD from cows was reduced after ageing for 7 days and even disappeared after 21 days (Enfält et al., 2004). A similar trend was also found for other muscles; WBSF reduction in *biceps femoris* (BF) for HF cattle by PS only occurred after 2 days of ageing, and after 2 days post-mortem, WBSF difference between two suspension methods disappeared altogether (Bayraktaroglu & Kahraman, 2011). The results from Bayraktaroglu & Kahraman (2011) pertaining to HF beef may suggest that the rate and extent of the influence PS depends on muscle type. It seems reasonable that the stretching occurs at the beginning of rigor before ageing, when stretching plays the predominant role, particularly as observed for HF steers used in this study which demonstrated a slow rate of proteolysis. With the increasing extent of ageing, bonds between myosin and actin are broken down by cathepsins and the actomyosin complex loosens (Lonergan et al., 2010). At this later ageing period, the myofibril structure has already been disintegrated by proteolysis, which does not require external force, such as stretching, to improve tenderness any further. Hence in the later period of ageing, the effect of PS on tenderness disappears.

In the AS group, WBSF decreased from 7 days of ageing, while in the PS group, WBSF decreased from 3 days of ageing (Figure 6.3, $P < 0.05$), which indicated that PS can accelerate ageing in HF steers. Additionally, WBSF for PS beef at 3 days was lower than that of AS beef after 7 days, suggesting that PS can improve tenderness

markedly during the earlier ageing period and this method can improve tenderness of steer beef rapidly in comparison with AS. This trend is similar to a previous study which showed that WBSF decreased significantly up to 21 ageing days in the AS group of LD from Chinese young cattle, whereas in the PS group, WBSF decreased significantly only after 1 and 7 days of ageing and then remained stable (Hou et al., 2014). PS has been applied in the Meat Standards Australia (MSA) model for beef palatability, allowing the recommended ageing time to be reduced for some cuts from PS carcasses (MLA, 2007). However, proteolysis is a natural complex process involving the combined action of multiple proteases, and the degradation of several target substrate proteins which are located in different sites of muscle fibres (Lonergan et al., 2010), thus how PS affects the rate of proteolysis still needs to be further investigated.

6.4.2.3 Cooking loss and other traits

Cooking loss values for LT from HF steers at different ageing times were not affected by suspension method ($P > 0.05$; Figure 6.4). This result is in line with other studies in BF muscle from HF bulls (Bayraktaroglu & Kahraman, 2011), in five different muscles [*semimembranosus* (SM), LD, *gluteus medius* (GM), *psoas major* (PM), *adductor* (AD) from Swedish red cattle (bulls and heifers) (Ahnström et al., 2012a) and in the LD muscle from Chinese fattened cattle (Hou et al., 2014). It is suggested that cooking loss is related to pHu (Honikel, 1999), and as there was no difference in pHu in the present study, cooking loss was similar. Conversely, a decreased percentage cooking loss by PS was reported for Ankole bulls (Kamatara et al., 2014). Other quality traits, including; FA composition were not affected by hanging method in the present study ($P > 0.05$), thus the related comparison results were not listed. This observation is in accordance with the previous finding that total collagen and collagen solubility did not differ between AS and PS methods (Kamatara et al., 2014).

6.4.3 Correlations

6.4.3.1 Correlations between physico-chemical variables

The correlations were analysed using all samples from bulls and steers (both suspension methods). The pHu value was positively correlated with WB-variables, cooking loss, moisture content and negatively correlated with IMF content ($P < 0.05$;

Table 6.6-6.8). Although pH at 15 ℃ and 35 ℃ were not correlated with any WB-variables ($P > 0.05$), pH (15 ℃) had a positive correlation with cooking loss (d 3 and 14; $P < 0.05$); pH (35 ℃) had positive correlations with insoluble and total collagen content ($P < 0.01$). The pHu value was negatively correlated with a*, b*, chroma after both 2 and 24 h blooming ($P < 0.01$; $r^2 = -(0.49\text{-}0.71)$), but positively correlated with hue angle after 24 h blooming ($P < 0.001$; $r^2 = 0.61$). Purchas et al. (2002) also reported a negative correlation between pHu and colour parameters including L*, a* and b*. The positive correlation of L* after 24 h blooming with IMF ($P < 0.05$) agreed with Muir et al. (1998b) that more marbling concentration increases lightness (higher reflectance values). Colour a*, b* and chroma after 24 h blooming were positively associated with IMF ($P < 0.001$) and collagen solubility ($P < 0.05$), while negatively associated with moisture ($P < 0.001$), insoluble and total collagen content ($P < 0.05$), WB-variables and cooking loss at different ageing times ($P < 0.001$), indicating that fatter and more tender meat with better WHC had higher colour intensity. This could be due to better WHC, expressed here as lower cooking loss, as this can increase the amount of myoglobin remaining after chilling for 24 h, which enhances redness. Similarly, the higher level of IMF has been reported to be correlated with the b* value of beef (Waritthitham et al., 2010a).

WB-variables and cooking loss at different ageing times (3 vs 7 vs 14 days) had similar correlations with other quality parameters as shown in Table 6.6-6.8. For example, WB-variables at the three ageing times were positively correlated with cooking loss at the corresponding ageing time, and with moisture ($P < 0.001$) and insoluble collagen content ($P < 0.05$) and negatively correlated with IMF ($P < 0.001$), soluble collagen content and collagen solubility ($P < 0.05$). This is in line with others who found that IMF can improve meat tenderness (Costa et al., 2012; Monteiro et al., 2013) because IMF dilutes muscle fibrous protein contributing to the decrease of muscle resistance under shearing (Wood et al., 1999). The contribution of cooking loss to beef toughness is in agreement with Monteiro et al. (2013). The results are consistent with several published reports that collagen solubility instead of total collagen content was the critical factor contributing to meat tenderness (Bailey, 1989; Schönfeldt & Strydom, 2011). As the mature multivalent transverse cross-links lead to a dramatic increase in the tension generated upon heating (Bailey, 1985). Samples with higher cooking loss at three ageing times had higher moisture content

($P < 0.001$), which was in accordance with Chambaz et al. (2003). Cooking loss was negatively correlated with IMF content ($P < 0.001$), which was partially attributed to the increased marbling amount and the melting of fat by heat protecting against moisture loss of steaks during cooking. The positive correlations for cooking loss at three ageing times with insoluble, total collagen content and the negative correlation with collagen solubility all concur with those findings reported by Jeremiah et al. (2003c) and who showed that beef muscles containing higher amounts of insoluble and total hydroxyproline sustain greater cooking loss.

6.4.3.2 Correlations between fatty acid composition and other quality variables

The correlations were analysed using all samples from bulls and steers (both suspension methods) (Table 6.9). Most PUFA proportions were negatively correlated with IMF content including C18:2n6t, C18:2n6c, C18:3n3, C20:3n6, C20:4n6, C20:4n3, C20:5n3, C22:5n3, total UFA, PUFA, n-6 PUFA and n-3 PUFA ($P < 0.05$). In contrast, the major saturated and monounsaturated FAs C14:0 ($P < 0.01$), C16:0 ($P < 0.001$), C16:1 ($P < 0.001$), C18:1n9c ($P < 0.01$), total SFA ($P < 0.01$), total MUFA ($P < 0.01$) were positively correlated with IMF content. These correlations are in accordance with the finding by Brugiapaglia et al. (2014) for Piedmontese, Limousin and Friesian breeds. This result also confirms the finding that SFA and MUFA increased markedly as total lipid increased, while the phospholipid fraction PUFA decreased with increasing fatness (Marmer et al., 1984; Eichhorn et al., 1985). This result also indicates that SFA and MUFA could improve the eating quality of beef from male dairy cattle, but PUFA showed the opposite trend, owing to the positive relationship between IMF and tenderness, juiciness and flavour. However, some minor MUFAs had a negative correlation with IMF content, including C15:1 ($P < 0.001$), C18:1n9t ($P < 0.01$), and C18:1 n7 ($P < 0.05$).

Most FAs were correlated with IMF content and with WBSF and cooking loss, but in all cases, correlation with IMF was the opposite to that for the latter two variables at all ageing times (Table 6.9). This arises from the strong positive correlation between WBSF and cooking loss and the negative correlations of each with IMF. Hence, most SFA and MUFA were negatively correlated with WBSF and cooking loss at all ageing times ($P < 0.05$) while PUFA were positively correlated with both ($P < 0.05$). Similarly, Duckett et al. (1993) pointed out that PUFA of beef was negatively

correlated with tenderness (r = -0.49) ratings from taste panel evaluation. However, it should be noted that in the present study steaks with higher C18:0, C15:1 and C18:1n7 percentage had higher WBSF and cooking loss ($P < 0.05$). It is hypothesized that the higher percentages of SFA, MUFA and lower percentages of PUFA, C18:0 and C15:1 in HF steer beef in comparison to bull beef might contribute to their more tender texture and improved WHC.

6.4.3.3 Correlations analysed by PLSR

Partial least squares regression (PLSR) model reflected the correlations between physico-chemical quality parameters and identified the factors sex or suspension method that contributed positively and negatively to the variables (and their levels of influence). Factors 1 and 2 totally explained 89% of the variance of the X matrix and 51% of the variance of Y matrix (Figure 6.5). WBSF at different ageing times, pHu and moisture were positively correlated with cooking loss at different ageing times, insoluble and total collagen, hue angle and pH at 15 °C and 35 °C, as they were located in the same quadrant of the plot. However, they had negative correlations with lightness, IMF, soluble collagen and collagen solubility, redness, yellowness and chroma, as these two clusters are located in opposite quadrants of the plot. The outer ellipse and inner ellipse indicate 100.0% and 50.0% of the explained variance, respectively. A few variables were placed between these ellipses, indicating they were well explained by the PLSR model.

The columns in Figure 6.6 represent the standardized coefficients, located on the positive and negative portions of the Y axis. The size of the columns represents the extent of influence, thus larger columns indicate a greater influence or higher regression coefficients. Moreover, the jack-knife uncertainty test was applied to inspect the significant variables by calculating estimated regression coefficients. When the vertical line that represents the estimated uncertainty limits of 95.0% confidence interval crosses the X axis, the corresponding attribute does not have significant influence on the factor (solid column). Bulls and steers (both AS and PS) showed the opposite trend of regression coefficients for all variables (Figure 6.6 a, b & c), indicating a marked discrimination of quality traits between the sexes. Although of similar size of regression coefficients were, the traits for L* after 24 h blooming, insoluble collagen and collagen solubility were significant for PS steers (*P*

< 0.05) but not for the AS group (Figure 6.6 b & c). This probably was due to small sample size in the present study.

6.5 Conclusions

Beef of acceptable eating quality can be produced from male Holstein-Friesian calves from the dairy herd whether they are raised as young bulls or castrated and raised as steers. In the case of young bulls, the beef requires a longer ageing time of up to 14 days to reach an acceptable level of tenderness, while steer beef was surprisingly tender after only 3 day of ageing. Steer beef has superior palatability and needs less ageing than bull beef. Castration increased IMF content and decreased cooking loss, insoluble and total collagen content which would be expected to have a positive effect on eating quality. The very low IMF content of the young dairy bulls could be of concern in this respect. Steer beef was paler but was more red, more yellow hence had a more intense colour than bull beef.

Castration greatly affected fatty acid composition. Holstein-Friesian bulls had a nutritionally enhanced fatty acid profile compared with steers. Although bull beef had higher PUFA content and PUFA/SFA ratio which improves nutritional value, it produced less tender beef with lower WHC and less marbling. Conversely, a higher IMF content resulted in higher SFA and MUFA proportions more tender beef and higher WHC. Pelvic suspension accelerated tenderization compared with Achilles tendon suspension and improved tenderness up to 7 days ageing and colour intensity of dairy steers. Cooking loss, ultimate pH, IMF, moisture and collagen characteristics greatly contributed to the variation in WB-variables at different ageing times reflected by both Pearson correlations and the PLSR model.

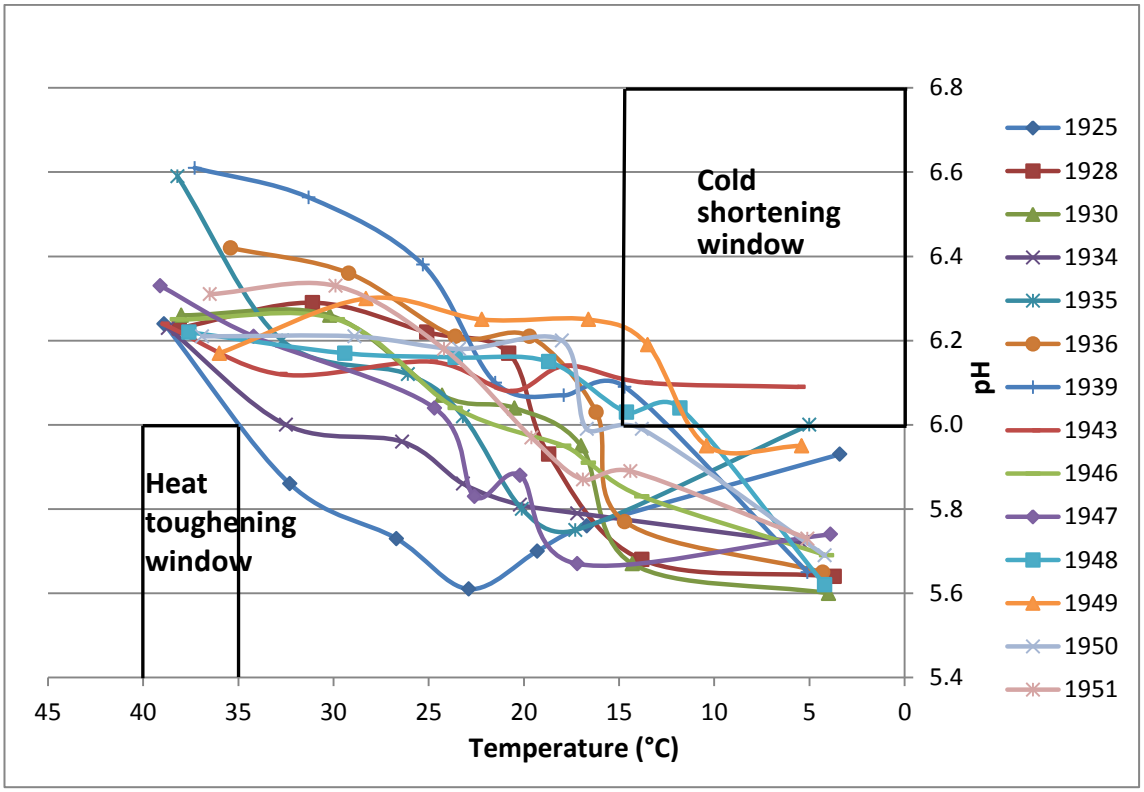
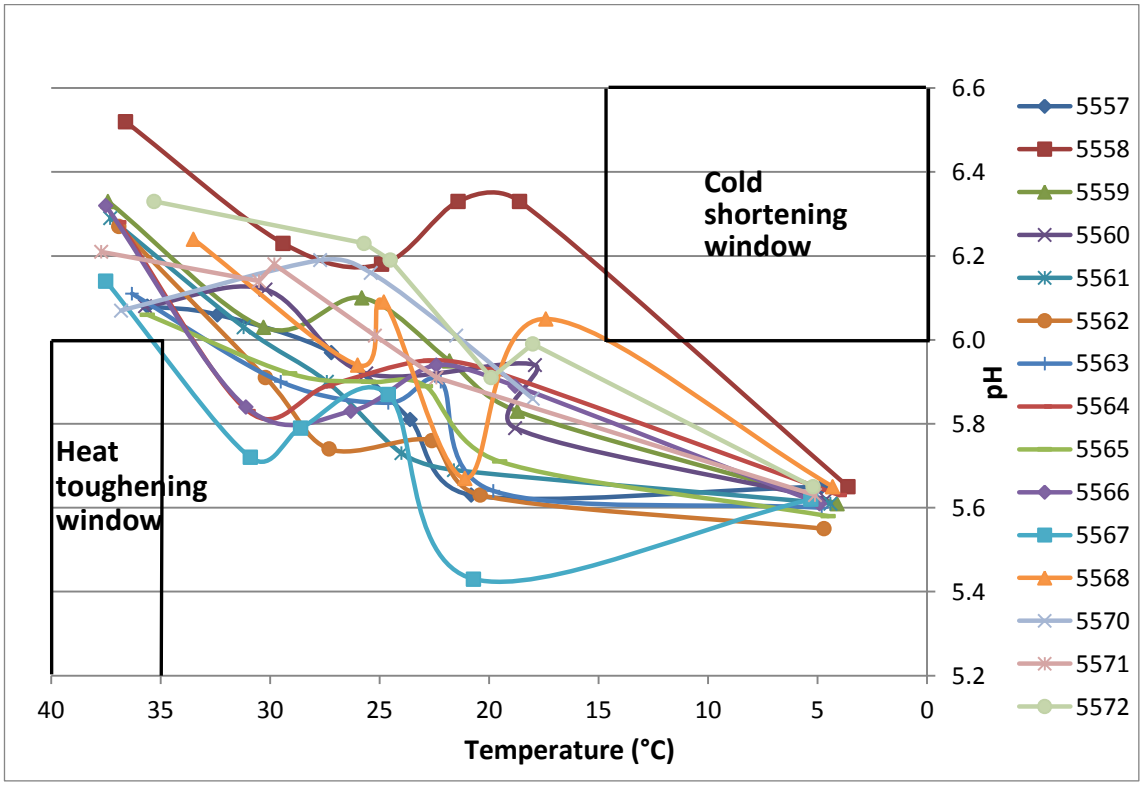


Figure 6.1. a). Post-mortem pH-temperature decline of Holstein-Friesian (HF) bulls.



b). Post-mortem pH-temperature decline of Holstein-Friesian (HF) steers.

Table 6.1. pH and colour after 2 and 24 hour blooming of LT muscles of young male Holstein-Friesian cattle.

Item	Bulls		Steers		*P*-value
	LSM	SEM	LSM	SEM	
pH (15°C)	5.88	0.05	5.76	0.05	0.091
pH (35°C)	6.25[a]	0.04	6.14[b]	0.03	0.049
pH1h	6.31	0.04	6.22	0.03	0.097
pH2h	6.22[a]	0.04	6.01[b]	0.04	0.001
pH3h	6.12[a]	0.04	5.98[b]	0.04	0.021
pH4h	6.03	0.04	5.91	0.04	0.060
pH5h	5.95	0.05	5.82	0.05	0.067
pHu[1]	5.67[a]	0.01	5.62[b]	0.01	0.002
pH decline rate (%)	8.95	1.22	10.0	1.18	0.528
L* 2h	41.8	0.35	42.2	0.34	0.381
a* 2h	11.7[b]	0.34	15.8[a]	0.33	<.001
b* 2h	8.33[b]	0.25	11.3[a]	0.24	<.001
Chroma 2h	14.4[b]	0.41	19.4[a]	0.39	<.001
Hue angle 2h	35.4	0.35	35.7	0.34	0.470
L* 24h	42.7[b]	0.54	44.6[a]	0.52	0.016
a* 24h	13.8[b]	0.57	18.4[a]	0.80	<.001
b* 24h	11.2[b]	0.32	14.2[a]	0.32	<.001
Chroma 24h	17.8[b]	0.61	22.9[a]	0.86	<.001
Hue angle 24h	39.3[a]	0.67	36.0[b]	0.65	0.002

LSM = least square means; SEM = standard error of LSM.
[a, b] Means within a row with different superscripts significantly differ ($P < 0.05$).
[1] pHu = ultimate pH.

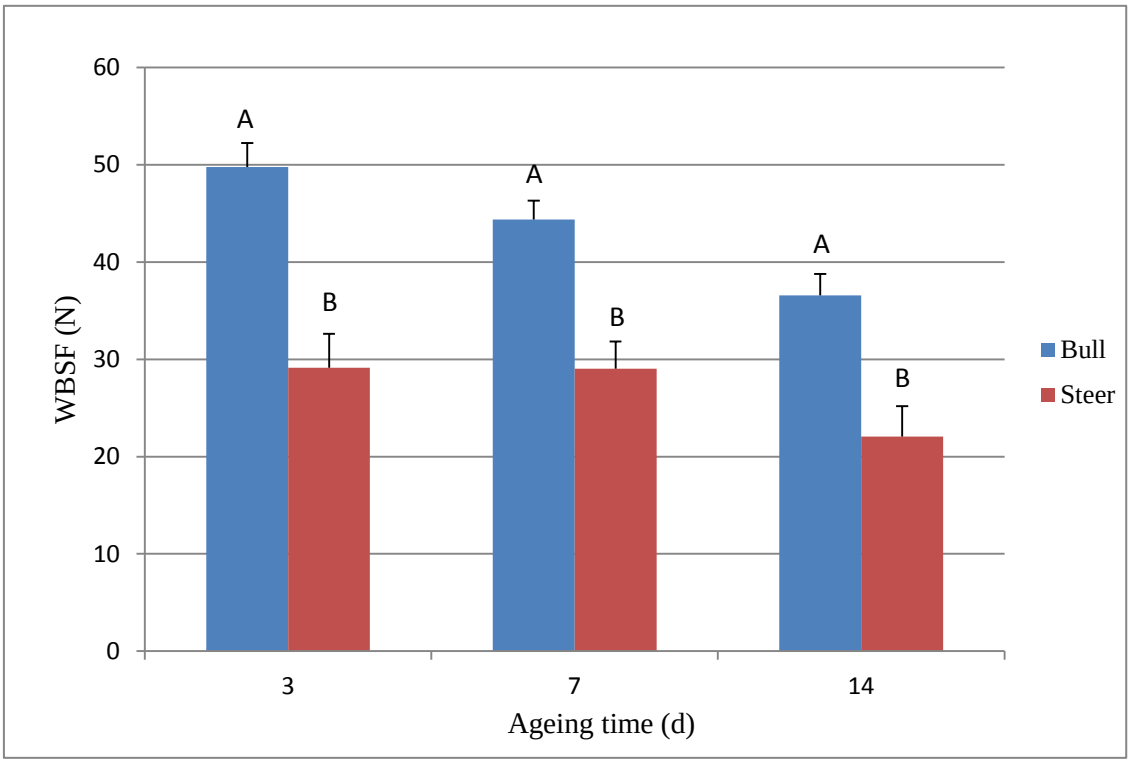


Figure 6.2. WBSF of LT muscles of Holstein-Friesian bulls and steers at different ageing times.

Table 6.2. Chemical composition, collagen characteristics and cooking loss at different ageing times of LT muscles of young male Holstein-Friesian cattle.

Item	Bulls		Steers		*P*-value
	LSM	SEM	LSM	SEM	
Moisture (%)	75.0[a]	0.26	72.1[b]	0.25	<.001
IMF[1] (%)	0.5[b]	0.36	3.8[a]	0.35	<.001
Protein (%)	23.2	0.11	23.0	0.10	0.390
Soluble collagen (mg/g)	0.49[b]	0.05	0.74[a]	0.04	0.001
Insoluble collagen (mg/g)	3.04[a]	0.19	2.16[b]	0.13	0.001
Total collagen (mg/g)	3.53[a]	0.19	2.90[b]	0.13	0.011
Collagen solubility (%)	14.6[b]	2.00	25.8[a]	1.36	<.001
Cooking loss (%, d3)	31.7[a]	0.33	28.8[b]	0.32	<.001
Cooking loss (%, d7)	30.9[a]	0.38	29.1[b]	0.37	0.003
Cooking loss (%, d14)	32.8[a]	0.33	29.9[b]	0.32	<.001

LSM = least square means; SEM = standard error of LSM.

[a, b] Means within a row with different superscripts significantly differ ($P < 0.05$).

[1]IMF = Intramuscular fat.

Table 6.3. Fatty acid proportion (g/100 g of total fatty acids) of LT muscles of young male Holstein-Friesian cattle.

Item	Bulls		Steers		*P*-value
	LSM	SEM	LSM	SEM	
C12:0	-	-	0.06	0.01	-
C14:0	1.80[b]	0.27	2.89[a]	0.16	0.004
C14:1	0.45[b]	0.07	0.67[a]	0.03	0.008
C15:0	0.33	0.02	0.32	0.02	0.799
C15:1	0.36[a]	0.03	0.10[b]	0.02	<.001
C16:0	23.0[b]	0.73	27.9[a]	0.50	<.001
C16:1	2.15[b]	0.25	4.33[a]	0.17	<.001
C17:0	0.91	0.10	0.80	0.07	0.367
C18:0	17.4[a]	0.66	14.6[b]	0.45	0.002
C18:1n9t	1.30	0.23	0.87	0.15	0.133
C18:1n9c	28.5[b]	1.27	39.9[a]	0.87	<.001
C18:1n7	1.88	0.09	1.69	0.06	0.108
C18:2n6t	0.19	0.03	0.14	0.01	0.161
C18:2n6c	12.9[a]	1.17	2.85[b]	0.80	<.001
C18:3n3	3.50[a]	0.37	1.04[b]	0.28	<.001
C20:0	-	-	0.08	0.06	-
C20:1n9	-	-	0.10	0.09	-
C21:0	0.31	0.08	0.34	0.04	0.741
C22:0	0.12	0.03	0.09	0.01	0.383
C20:3n6	0.62[a]	0.05	0.30[b]	0.03	<.001
C20:4n6	3.46[a]	0.27	1.08[b]	0.18	<.001
C20:4n3	-	-	0.15	0.01	-
C20:5n3	1.52[a]	0.13	0.53[b]	0.09	<.001
C22:5n3	1.87[a]	0.15	0.83[b]	0.10	<.001
∑ SFA[1]	42.4[b]	1.28	46.0[a]	0.88	0.032
∑ UFA[2]	57.6[a]	1.28	54.0[b]	0.88	0.032
∑ MUFA[3]	34.1[b]	1.53	47.5[a]	1.05	<.001
∑ PUFA[4]	23.5[a]	2.01	6.52[b]	1.37	<.001
∑ n-6 PUFA	17.0[a]	1.46	4.31[b]	1.00	<.001
∑ n-3 PUFA	6.54[a]	0.62	2.21[b]	0.43	<.001

LSM = least square means; SEM = standard error of LSM.

[a, b] Means within a row with different superscripts significantly differ ($P < 0.05$).

[1]∑ SFA = Sum of saturated fatty acids.

[2]∑ UFA = Sum of unsaturated fatty acids.

[3]∑ MUFA = Sum of monounstaturated fatty acids.

[4]∑ PUFA = Sum of polyunsaturated fatty acids.

∑ n-6 PUFA = Sum of n-6 polyunsaturated fatty acids.

∑ n-3 PUFA = Sum of n-3 polyunsaturated fatty acids.

Table 6.4. Fatty acid concentration (mg/100g meat) of LT muscles of young male Holstein-Friesian cattle.

Item	Bulls		Steers		*P*-value
	LSM	SEM	LSM	SEM	
C14:0	13.2[b]	17.0	72.4[a]	9.81	0.009
C14:1	4.50[b]	3.16	15.4[a]	1.41	0.006
C15:0	2.69[b]	1.46	7.61[a]	0.92	0.010
C15:1	2.23	0.11	2.08	0.07	0.255
C16:0	163.8[b]	89.4	652.8[a]	61.1	<.001
C16:1	16.9[b]	11.8	99.7[a]	8.09	<.001
C17:0	6.01[b]	2.89	18.9[a]	1.98	0.001
C18:0	120.8[b]	57.5	346.5[a]	39.3	0.004
C18:1n9t	9.78	4.43	18.1	2.90	0.135
C18:1n9c	207.3[b]	115.5	925.3[a]	78.9	<.001
C18:1n7	12.2[b]	4.11	38.3[a]	2.81	<.001
C18:2n6t	2.27	0.69	3.16	0.19	0.233
C18:2n6c	74.8[a]	4.85	60.6[b]	3.32	0.025
C18:3n3	20.7	2.40	23.6	1.83	0.343
C21:0	3.81	3.18	8.41	1.42	0.216
C22:0	1.52	0.52	1.73	0.20	0.714
C20:3n6	3.81[b]	0.31	6.28[a]	0.21	<.001
C20:4n6	20.7	1.32	22.1	0.90	0.392
C20:5n3	9.19[b]	0.51	10.9[a]	0.35	0.013
C22:5n3	12.0[b]	0.69	17.3[a]	0.47	<.001
∑ SFA[1]	298.4[b]	162.0	1084.7[a]	110.7	<.001
∑ UFA[2]	386.9[b]	138.1	1236.1[a]	94.4	<.001
∑ MUFA[3]	247.8[b]	132.8	1097.8[a]	90.7	<.001
∑ PUFA[4]	139.1	9.63	138.3	6.58	0.941
∑ n-6 PUFA	99.3	5.99	90.9	4.09	0.257
∑ n-3 PUFA	39.8	4.34	47.2	2.97	0.175
∑ TFA[5]	692.1[b]	299.1	2331.8[a]	204.3	<.001
MUFA/SFA	0.80[b]	0.05	1.04[a]	0.03	<.001
PUFA/SFA	0.58[a]	0.06	0.14[b]	0.04	<.001
n-6/n-3 PUFA	2.89[a]	0.26	2.02[b]	0.18	0.011
SFA/UFA	0.75[b]	0.04	0.86[a]	0.03	0.041

LSM = least square means; SEM = standard error of LSM.
[a, b] Means within a row with different superscripts significantly differ ($P < 0.05$).

[1]∑ SFA = Sum of saturated fatty acids.

[2]∑ UFA = Sum of unsaturated fatty acids.

[3]∑ MUFA = Sum of monounstaturated fatty acids.

[4]∑ PUFA = Sum of polyunsaturated fatty acids.

∑ n-6 PUFA = Sum of n-6 polyunsaturated fatty acids.
∑ n-3 PUFA = Sum of n-3 polyunsaturated fatty acids.
[5]∑ TFA = Total fatty acids.

Table 6.5. pHu and colour after 2 and 24 hour blooming of LT muscles of Holstein-Friesian steers suspended by AS and PS.

Item	AS		PS		*P*-value
	LSM	SEM	LSM	SEM	
pHu[1]	5.62	0.01	5.61	0.01	0.395
pH decline rate (%)	11.6	1.34	8.2	1.44	0.113
L* 2h	42.4	0.60	42.1	0.56	0.735
a* 2h	15.1	0.45	16.4	0.42	0.065
b* 2h	11.0	0.36	11.6	0.34	0.230
Chroma 2h	18.7	0.55	20.1	0.51	0.090
Hue angle 2h	36.0	0.52	35.5	0.49	0.464
L* 24h	44.6	0.63	44.7	0.59	0.891
a* 24h	18.4[b]	0.55	20.5[a]	0.51	0.013
b* 24h	13.6	0.47	14.8	0.44	0.094
Chroma 24h	22.9[b]	0.66	25.3[a]	0.62	0.018
Hue angle 24h	36.5	0.67	35.7	0.62	0.364

LSM = least square means; SEM = standard error of LSM.

[a, b] Means within a row with different superscripts significantly differ ($P < 0.05$).

[1]pHu = ultimate pH.

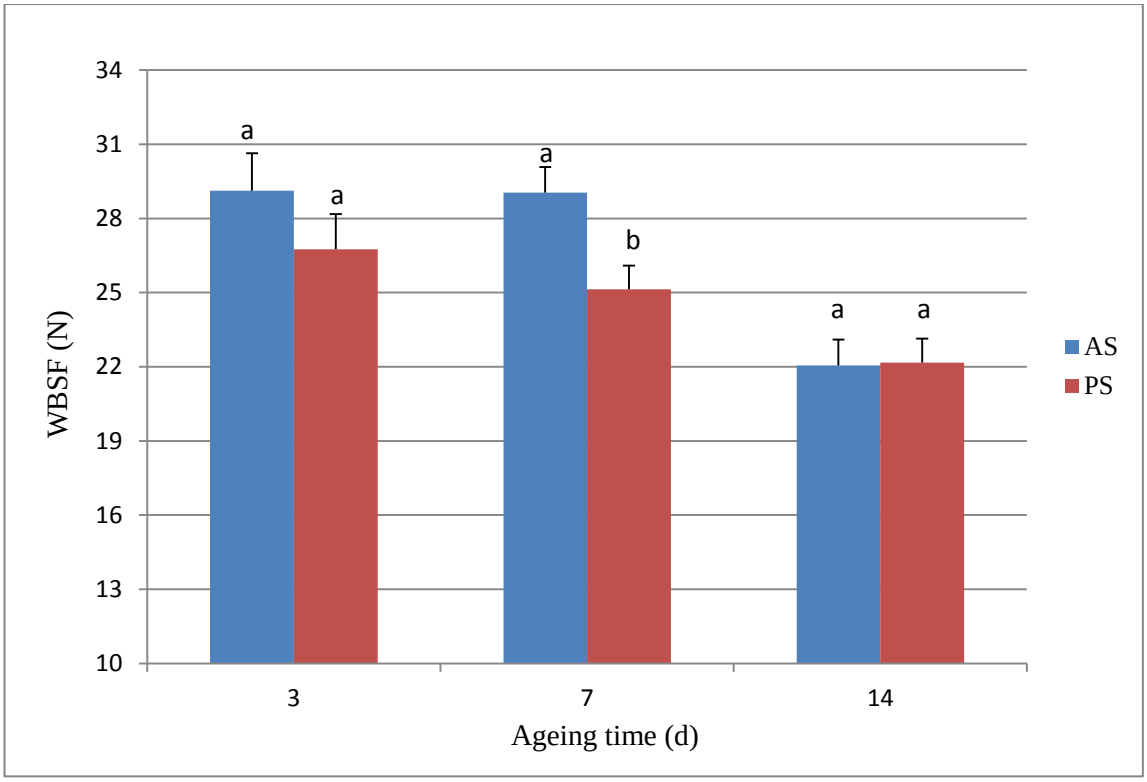


Figure 6.3. WBSF of LT muscles of Holstein-Friesian steers suspended by AS and PS at different ageing times.

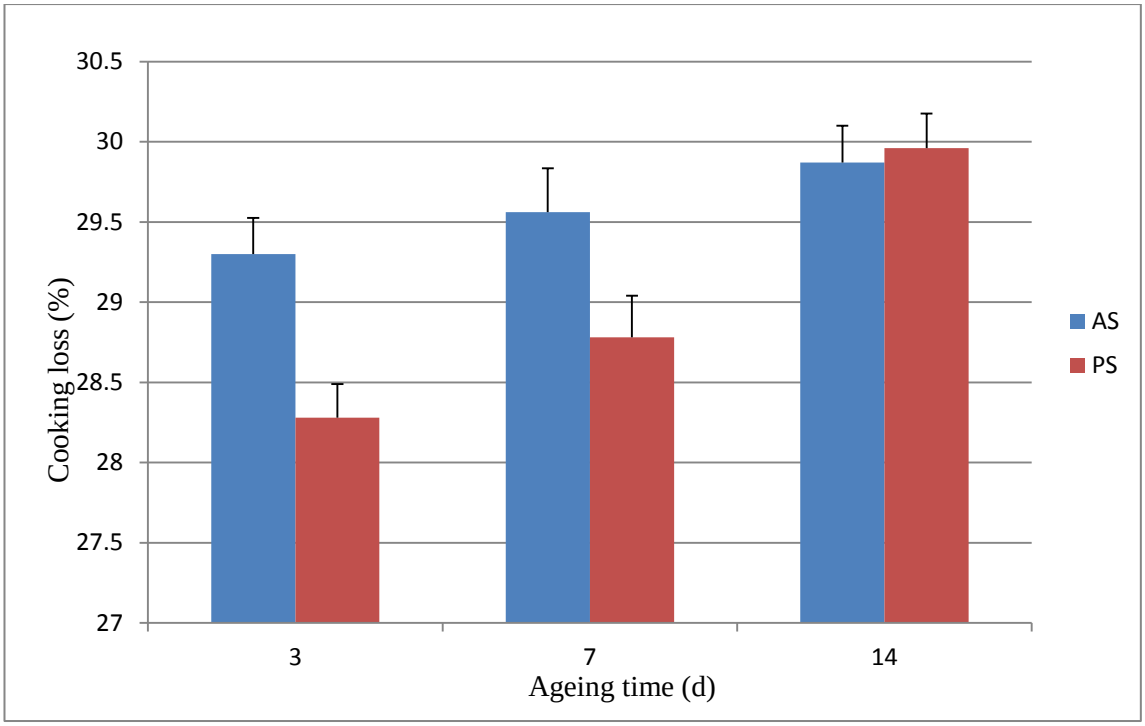


Figure 6.4. Cooking loss of LT muscles of Holstein-Friesian steers suspended by AS and PS at different ageing times.

Table 6.6. Pearson correlation coefficients between pH, colour, moisture, IMF, collagen characteristics, WB-variables and cooking loss on day 3 of LT muscles of young male Holstein-Friesian cattle.

	WBSF[1] (d3)	WB-slope (d3)	WB-area (d3)	Cooking loss (d3)	Moisture	IMF[2]	Soluble collagen	Insoluble collagen	Total collagen	Collagen solubility
pHu[3]	0.69***	0.61***	0.72***	0.57***	0.55**	-0.46*	-0.50*	0.28	0.15	-0.45*
pH (15°C)	0.25	0.22	0.30	0.56**	0.24	-0.16	-0.28	0.25	0.19	-0.27
pH (35°C)	0.12	0.16	0.06	0.35	0.23	-0.15	-0.29	0.60**	0.59**	-0.47*
L* 2h	-0.14	-0.09	-0.10	-0.22	-0.09	0.16	-0.08	-0.03	-0.06	-0.04
a* 2h	-0.78***	-0.74***	-0.71***	-0.67***	-0.70***	0.63***	0.57*	-0.32	-0.18	0.46*
b* 2h	-0.77***	-0.72***	-0.70***	-0.66***	-0.72***	0.68***	0.52*	-0.36	-0.24	0.45*
Croma 2h	-0.79***	-0.74***	-0.71***	-0.68***	-0.71***	0.65***	0.56*	-0.34	-0.20	0.46*
Hue angle 2h	-0.07	-0.06	-0.07	-0.04	-0.16	0.25	-0.01	-0.18	-0.21	0.06
L* 24h	-0.38*	-0.38*	-0.29	-0.35	-0.41*	0.39*	0.16	-0.19	-0.16	0.17
a* 24h	-0.78***	-0.73***	-0.69***	-0.75***	-0.75***	0.66***	0.46*	-0.53*	-0.45*	0.54*
b* 24h	-0.74***	-0.69***	-0.66***	-0.70***	-0.74***	0.68***	0.27	-0.60**	-0.60**	0.48*
Croma 24h	-0.78***	-0.73***	-0.70***	-0.75***	-0.76***	0.68***	0.41	-0.57*	-0.51*	0.54*
Hue angle24h	0.51**	0.48*	0.47*	0.51**	0.48*	-0.39*	-0.49*	0.23	0.10	-0.39
WBSF (d3)		0.98***	0.92***	0.72***	0.82***	-0.76***	-0.50*	0.44*	0.33	-0.51*
WB-slope (d3)			0.89***	0.71***	0.82***	-0.78***	-0.50*	0.46*	0.36	-0.52*
WB-area (d3)				0.77***	0.77***	-0.72***	-0.43*	0.38	0.29	-0.39
Cooking loss (d3)					0.77***	-0.70***	-0.39	0.53*	0.48*	-0.48*

Significance: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.
[1]WBSF = Warner-Bratzler Shear Force; [2]IMF = Intramuscular fat; [3]pHu = ultimate pH.

Table 6.7. Pearson correlation coefficients between pH, colour, moisture, IMF, collagen characteristics, WB-variables and cooking loss on day 7 of LT muscles of young male Holstein-Friesian cattle.

	WBSF[1] (d7)	WB-slope (d7)	WB-area (d7)	Cooking loss (d7)	Moisture	IMF[2]	Soluble collagen	Insoluble collagen	Total collagen	Collagen solubility
pHu[3]	0.72***	0.55**	0.68***	0.17	0.55**	-0.46*	-0.50*	0.28	0.15	-0.45*
pH (15°C)	0.32	0.31	0.34	0.34	0.24	-0.16	-0.28	0.25	0.19	-0.27
pH (35°C)	0.14	0.11	0.19	0.29	0.23	-0.15	-0.29	0.60**	0.59**	-0.47*
L* 2h	-0.09	-0.08	0.11	-0.17	-0.09	0.16	-0.08	-0.03	-0.06	-0.04
a* 2h	-0.83***	-0.74***	-0.71***	-0.30	-0.70***	0.63***	0.57*	-0.32	-0.18	0.46*
b* 2h	-0.79***	-0.70***	-0.64***	-0.29	-0.72***	0.68***	0.52*	-0.36	-0.24	0.45*
Croma 2h	-0.82***	-0.74***	-0.69***	-0.30	-0.71***	0.65***	0.56*	-0.34	-0.20	0.46*
Hue angle 2h	0.06	0.05	0.16	0.04	-0.16	0.25	-0.01	-0.18	-0.21	0.06
L* 24h	-0.52**	-0.46*	-0.39*	-0.27	-0.41*	0.39*	0.16	-0.19	-0.16	0.17
a* 24h	-0.84***	-0.80***	-0.75***	-0.53**	-0.75***	0.66***	0.46*	-0.53*	-0.45*	0.54*
b* 24h	-0.78***	-0.75***	-0.66***	-0.54**	-0.74***	0.68***	0.27	-0.60**	-0.60**	0.48*
Croma 24h	-0.84***	-0.80***	-0.73***	-0.54**	-0.76***	0.68***	0.41	-0.57*	-0.51*	0.54*
Hue angle24h	0.59***	0.54***	0.59**	0.31	0.48*	-0.39*	-0.49*	0.23	0.10	-0.39
WBSF (d7)		0.95***	0.87***	0.43*	0.83***	-0.76***	-0.43*	0.37	0.28	-0.44*
WB-slope (d7)			0.80***	0.48*	0.83***	-0.78***	-0.38	0.34	0.27	-0.38
WB-area (d7)				0.48*	0.72***	-0.66***	-0.32	0.40	0.35	-0.36
Cooking loss (d7)					0.57***	-0.57***	-0.18	0.65***	0.68***	-0.42*

Significance: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

[1]WBSF = Warner-Bratzler Shear Force; [2]IMF = Intramuscular fat; [3]pHu = ultimate pH.

Table 6.8. Pearson correlation coefficients between pH, colour, moisture, IMF, collagen characteristics, WB-variables and cooking loss on day 14 of LT muscles of young male Holstein-Friesian cattle.

	WBSF[1] (d14)	WB-slope (d14)	WB-area (d14)	Cooking loss (d14)	Moisture	IMF[2]	Soluble collagen	Insoluble collagen	Total collagen	Collagen solubility
pHu[3]	0.58***	0.49*	0.55**	0.35	0.55**	-0.46*	-0.50*	0.28	0.15	-0.45*
pH (15°C)	0.20	0.17	0.23	0.47*	0.24	-0.16	-0.28	0.25	0.19	-0.27
pH (35°C)	0.08	0.11	0.09	0.32	0.23	-0.15	-0.29	0.60**	0.59**	-0.47*
L* 2h	-0.02	0.02	-0.04	0.05	-0.09	0.16	-0.08	-0.03	-0.06	-0.04
a* 2h	-0.70***	-0.67***	-0.57***	-0.52**	-0.70***	0.63***	0.57*	-0.32	-0.18	0.46*
b* 2h	-0.69***	-0.66***	-0.59***	-0.47*	-0.72***	0.68***	0.52*	-0.36	-0.24	0.45*
Croma 2h	-0.70***	-0.67***	-0.58***	-0.51**	-0.71***	0.65***	0.56**	-0.34	-0.20	0.46*
Hue angle 2h	-0.07	-0.05	-0.15	0.11	-0.16	0.25	-0.01	-0.18	-0.21	0.06
L* 24h	-0.37*	-0.37*	-0.27	-0.23	-0.41*	0.39*	0.16	-0.19	-0.16	0.17
a* 24h	-0.65***	-0.67***	-0.53**	-0.67***	-0.75***	0.66***	0.46*	-0.53*	-0.45*	0.54*
b* 24h	-0.65***	-0.66***	-0.60***	-0.56**	-0.74***	0.68***	0.27	-0.60**	-0.60**	0.48*
Croma 24h	-0.67***	-0.68***	-0.56**	-0.65***	-0.76***	0.68***	0.41	-0.57*	-0.51*	0.54*
Hue angle24h	0.36	0.40*	0.19	0.57**8	0.48*	-0.39*	-0.49*	0.23	0.10	-0.39
WBSF (d14)		0.96***	0.88***	0.56**	0.71***	-0.64***	-0.48*	0.34	0.24	-0.48*
WB-slope (d14)			0.80***	0.59***	0.73***	-0.67***	-0.48*	0.42*	0.33	-0.51*
WB-area (d14)				0.41*	0.51**	-0.45*	-0.51*	0.41	0.30	-0.54*
Cooking loss (d14)					0.74***	-0.69***	-0.52*	0.64***	0.56*	-0.65***

Significance: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

[1]WBSF = Warner-Bratzler Shear Force; [2]IMF = Intramuscular fat; [3]pHu = ultimate pH.

Table 6.9. Pearson correlation coefficients between fatty acid proportion (%) and IMF content, WBSF and cooking loss at three ageing times of LT muscles of young male Holstein-Friesian cattle.

	IMF	WBSF-d3	Cooking loss-d3	WBSF-d7	Cooking loss-d7	WBSF-d14	Cooking loss-d14
C12:0	0.73	0.01	-0.70	0.65	-0.47	-0.07	-0.53
C14:0	0.76**	-0.80***	-0.68**	-0.82***	-0.62**	-0.41	-0.65*
C14:1	0.40	-0.14	-0.36	-0.17	-0.28	-0.42	-0.43
C15:0	0.22	-0.17	0.001	-0.28	-0.22	0.17	-0.13
C15:1	-0.75***	0.87***	0.70***	0.91***	0.49*	0.75***	0.79***
C16:0	0.70***	-0.85***	-0.55**	-0.86***	-0.36	-0.71***	-0.72***
C16:1	0.69***	-0.78***	-0.52**	-0.80***	-0.38	-0.74***	-0.71***
C17:0	-0.11	-0.07	0.20	-0.05	0.07	0.27	0.16
C18:0	-0.36	0.42*	0.33	0.42*	0.28	0.55*	0.45*
C18:1n9t	-0.59**	0.27	0.37	0.22	0.26	0.32	0.26
C18:1n9c	0.59**	-0.80***	-0.63**	-0.83***	-0.38	-0.76***	-0.71***
C18:1n7	-0.48*	0.51*	0.30	0.55*	0.19	0.34	0.45*
C18:2n6t	-0.56*	0.26	0.15	-0.03	0.04	0.16	0.34
C18:2n6c	-0.69***	0.87***	0.60**	0.90***	0.39	0.76***	0.74***
C20:0	0.38	-0.33	-0.44	-0.10	-0.41	-0.46	-0.27
C20:1n9	-0.43	0.07	0.43	-0.09	0.61	-0.45	0.55
C18:3n3	-0.66**	0.88***	0.56*	0.89***	0.35	0.76***	0.71***
C21:0	-0.04	-0.07	-0.14	-0.11	-0.16	-0.01	-0.08
C22:0	-0.49	0.45	0.36	0.20	0.05	-0.06	-0.10
C20:3n6	-0.72***	0.77***	0.66**	0.86***	0.48*	0.66***	0.75***
C20:4n6	-0.73***	0.84***	0.66***	0.89***	0.49*	0.72***	0.76***
C20:4n3	-0.77*	0.48	0.45	0.52	0.46	-0.18	0.21
C20:5n3	-0.68***	0.83***	0.62**	0.88***	0.47*	0.75***	0.74***
C22:5n3	-0.72***	0.84***	0.66***	0.90***	0.57*	0.66***	0.73***
∑ SFA[1]	0.63**	-0.69***	-0.40	-0.69***	-0.33	-0.42*	-0.51*
∑ UFA[2]	-0.63**	0.69***	0.40	0.69***	0.33	0.42*	0.51*
∑ MUFA[3]	0.58**	-0.78***	-0.60**	-0.82***	-0.38	-0.74***	-0.70***
∑ PUFA[4]	-0.69***	0.88***	0.62**	0.91***	0.43*	0.74***	0.74***
∑ n-6 PUFA	-0.70***	0.86***	0.62**	0.90***	0.41*	0.75***	0.75***
∑ n-3 PUFA	-0.64**	0.88***	0.61**	0.90***	0.45*	0.70***	0.71***

Significance: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

[1]∑ SFA = Sum of saturated fatty acids.

[2]∑ UFA = Sum of unsaturated fatty acids.

[3]∑ MUFA = Sum of monounstaturated fatty acids.

[4]∑ PUFA = Sum of polyunsaturated fatty aci

∑ n-6 PUFA = Sum of n-6 polyunsaturated fatty acids.

∑ n-3 PUFA = Sum of n-3 polyunsaturated fatty acids.

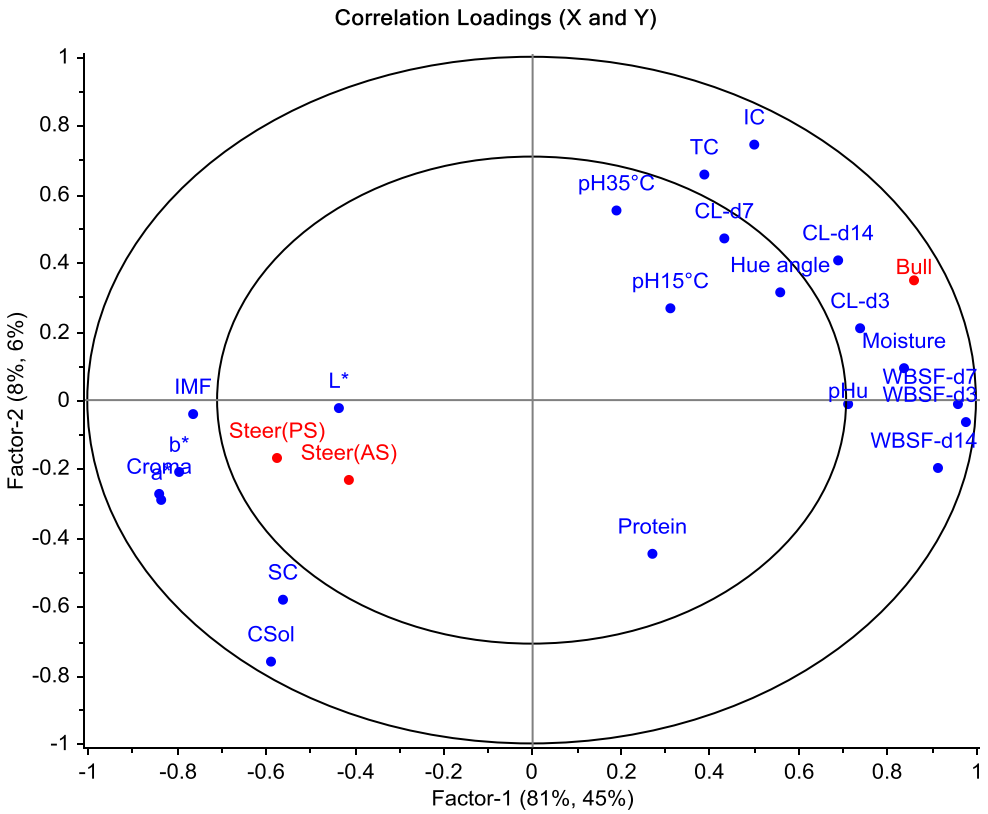


Figure 6.5. Partial least square regression (PLSR) correlation loadings plot of Factor 1 versus Factor 2. The model was derived from the quality traits in the X-matrix and sex and suspension method in the Y-matrix. CL = Cooking loss; SC = Soluble collagen; IC = Soluble collagen; TC = Total collagen; CSol = Collagen solubility; WBSF = Warner-Bratzler Shear Force; IMF = Intramuscular fat.

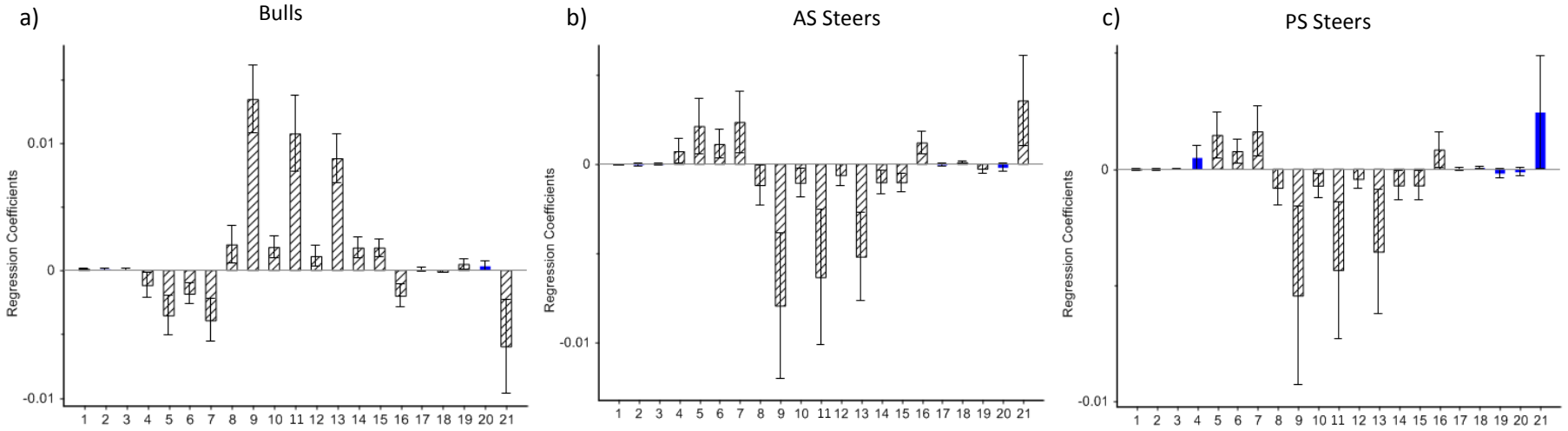
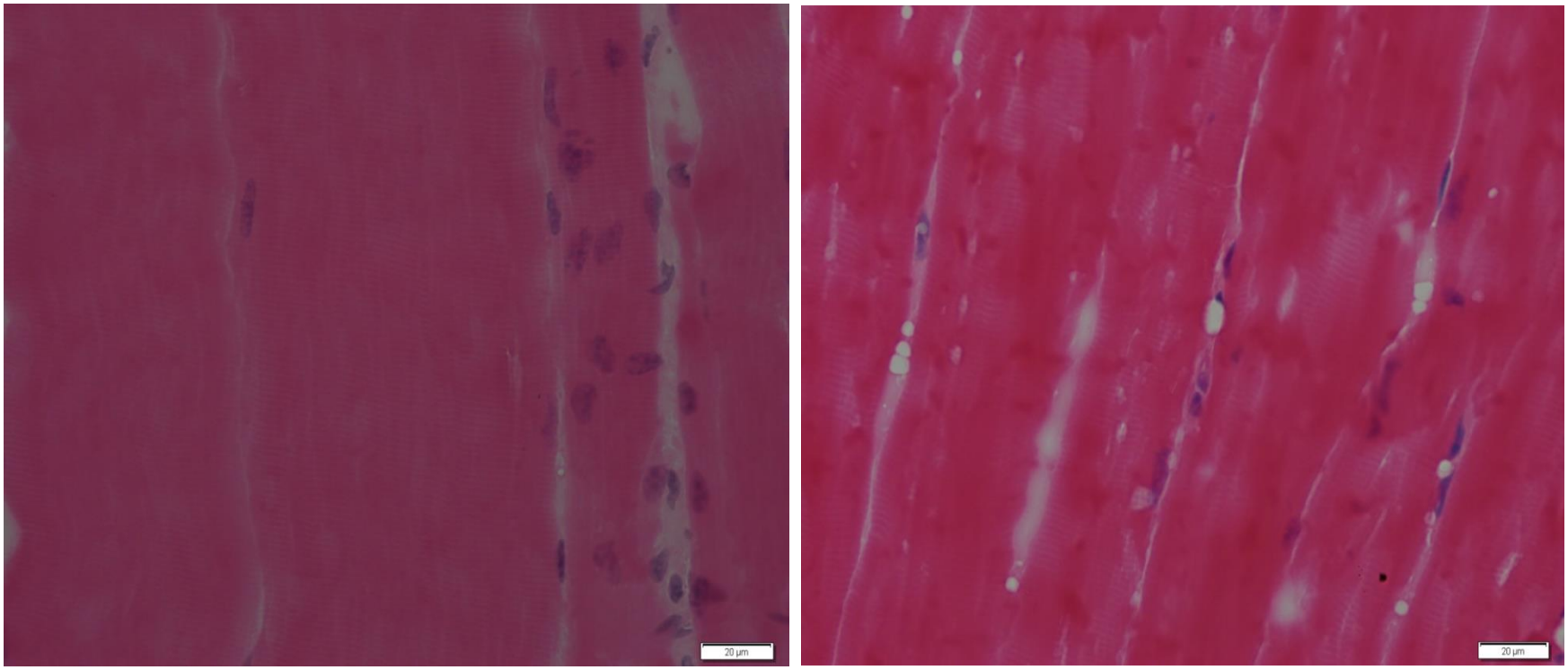


Figure 6.6. a-c). Standardized regression coefficients and significance indications from PLSR prediction models for sex and suspension method from the X-matrix – quality traits. Columns with shading are significant ($P < 0.05$). Quality traits of 1-21 are: 1. pHu, 2. pH (15°C), 3. pH (35°C), 4. L* 24 h, 5. a* 24 h, 6. b* 24 h, 7. Croma 24 h, 8. Hue angle 24 h, 9. WBSF-d3, 10. Cooking loss-d3, 11. WBSF-d7, 12. Cooking loss-d7, 13. WBSF-d14, 14. Cooking loss-d14, 15. Moisture, 16. IMF, 17. Protein, 18. Soluble collagen, 19. Insoluble collagen, 20. Total collagen, 21. Collagen solubility.



HF bulls HF steers

Figure 6.7. LT muscles at 3 ageing days from Holstein-Friesian (HF) bulls vs steers (Light Microscopy - 60X magnification).

Chapter 7

Assessment of physico-chemical traits related to eating quality of young dairy bull beef at different ageing times using Raman spectroscopy and chemometrics

7.1 Abstract

Raman spectroscopy and chemometrics were investigated for the prediction of eating quality related physico-chemical traits of Holstein-Friesian bull beef. Raman spectra were collected on the 3rd, 7th and 14th days post-mortem. A frequency range of 1300-2800 cm^{-1} was used for partial least squares (PLS) modelling. PLS regression (PLSR) models for the prediction of WBSF and cooking loss achieved an R^2CV of 0.75 with RMSECV of 6.82 N and an R^2CV of 0.77 with RMSECV of 0.97%w/w respectively. For the prediction of intramuscular fat, moisture and crude protein content, R^2CV values were 0.85, 0.91 and 0.70 with RMSECV of 0.52%w/w, 0.39%w/w and 0.38%w/w respectively. An R^2CV of 0.79 was achieved for the prediction of both total collagen and hydroxyproline content, while for collagen solubility the R^2CV was 0.88. All samples (100%) from 15- and 19-month old bulls were correctly classified using PLS discriminant analysis (PLS-DA), while 86.7% of samples from different muscles (*longissimus thoracis*, *semitendinosus* and *gluteus medius*) were correctly classified. In general, PLSR models using Raman spectra on the 3rd day post-mortem had better prediction performance than those on the 7th and 14th days. Raman spectroscopy and chemometrics have potential to assess several beef physical and chemical quality traits.

Keywords: Beef, Chemometrics, Eating quality, Physico-chemical traits, Post-mortem ageing, Raman spectroscopy

7.2 Introduction

Meat quality is a complex concept that involves intrinsic cues (i.e. safety, shelf-life, nutritional value, eating quality) and extrinsic cues (i.e. brand, quality label, origin, convenience of the product). Of these, eating quality is a critical parameter to determine consumer preferences, including sensory quality (i.e. tenderness, juiciness and flavour) and physico-chemical traits: technological quality (i.e. Warner-Bratzler shear force (WBSF) and cooking loss; compositional quality (i.e. intramuscular fat (IMF), collagen and moisture content) (Prieto et al., 2009; Troy & Kerry, 2010).

The amount and solubility of intramuscular connective tissue (IMCT) and post-mortem proteolysis of myofibrillar proteins influence beef tenderness predominately. Collagen, as a major component of IMCT, is believed to contribute to the "background" toughness of beef after prolonged ageing. It has been generally accepted that higher levels of total collagen and particularly lower collagen solubility are associated with reduced beef tenderness (Jeremiah et al., 2003a). IMF produces marbling effects in beef, which is positively linked to beef tenderness, juiciness and flavour (Scollan et al., 2006). A higher level of moisture in beef can lead to higher cooking loss and lower tenderness (Chambaz et al., 2003). An increased cooking loss has a negative effect on beef tenderness (Silva et al., 1999).

Ageing is the most influential primary processing factor involving complex changes in muscle metabolism in the post slaughter period. Post-mortem proteolysis is a pronounced action during ageing, which greatly contributes to meat tenderization (Muchenje et al., 2009). Moreover, water mobility and biomechanical changes of IMCT in meat during ageing are also associated with changes in quality parameters, including juiciness and tenderness (Pearce et al., 2011; Nishimura, 2015).

Previous studies have demonstrated the potential of Raman spectroscopy (RS) combined with chemometric approaches to measure WBSF and cooking loss of aged meat. A regression coefficient determination of cross validation (R^2CV) of 0.75 for the prediction of shear force (SF) in roasted beef silversides was reported by Beattie et al. (2004) while R^2CVs of 0.33-0.79 were obtained for the prediction of intact fresh bovine *gluteus medius* (GM) muscles SF at the 14^{th} day post-mortem using a portable Raman system (Bauer et al., 2016). R^2CVs of 0.79-0.86 for SF and 0.79-0.83 for cooking loss were obtained for intact frozen/thawed sheep meat after ageing

for 5 days using a prototype handheld Raman system (Schmidt et al., 2013). In contrast, a very low R^2CV of 0.06 was obtained using a handheld Raman device for SF prediction on intact fresh lamb muscle at the 1st day post-mortem by Fowler et al. (2014). However, none of these studies have reported on homogenised meat samples, and previous studies only focused on one type of muscle and one specific ageing time; the prediction ability for different muscle types and different ageing times has not been investigated to date.

A rapid and applicable method for compositional quality assessment would be highly appreciated by the meat industry. Near infrared spectroscopy (NIRS) has been employed to predict chemical composition of beef with R^2CVs of 0.16-0.82 for predictions of crude protein (Alomar et al., 2003; Ripoll et al., 2008), R^2CVs of 0.76-0.99 for IMF (Rødbotten et al., 2000; De Marchi et al., 2007), R^2CVs of 0.09-0.91 for moisture (Cozzolino et al., 2002; De Marchi et al., 2007) and R^2CVs of 0.18-0.44 were reported for collagen prediction (Alomar et al., 2003; De Marchi et al., 2007). Hyperspectral imaging has been reported to be effective for the prediction of hydroxyproline content in chicken meat (R^2CV-0.87) (Xiong et al., 2015). Compared with NIRS, RS has been claimed to provide more detailed information on chemical structures and physical forms for the identification of substances by their characteristic spectral patterns – 'fingerprinting' and for quantitative detection of the amount of a substance in a sample (Smith & Dent, 2005). However, recent studies using RS on lamb were unable to predict collagen and for IMF obtained an R^2CV of 0.02 (Fowler et al., 2015). The authors of this study are not aware of any previous research investigating the use of RS to determine chemical composition of beef particularly for collagen characteristics. Moreover, the previous studies were mainly focused on sole physical or chemical trait of meat, while the prediction performance of RS on a wide range of beef physical and chemical traits has not been explored.

Beef quality can also be largely affected by on-farm production factors, such as animal breed, slaughter age, sex, feeding regime, muscle location etc. (Frylinck et al., 2013). The clear discrimination of beef according to production factors could not just solely be used to identify meat origin, but also as a marker to select meat cuts based on expected quality properties. In addition to the conventional analytical methods for muscle identification such as DNA, immunological and chromatographic techniques, RS has been shown to be a potential tool for rapid assessment of food adulteration

and discrimination between species and muscle groups within species (Herrero, 2008; Damez & Clerjon, 2008).

The objectives of this study are to use RS and chemometrics to (1) develop models for the prediction of key physico-chemical traits of young bull beef; (2) select the most representative wavelengths for these predictions; (3) compare prediction performance during beef ageing; (4) discriminate beef samples from three muscle types or from two slaughter ages.

7.3 Materials and methods

7.3.1 Source of materials

For the prediction models, Holstein-Friesian (HF) bulls (n = 49) were slaughtered in a commercial abattoir. The *longissimus thoracis* (LT) muscle samples were removed from the carcasses of 35 bulls at 48 h post-mortem at 4 °C. LT and *semitendinosus* (ST) muscles were removed from the carcasses of the remaining 14 bulls. At 72 h post-mortem, muscle samples (n = 63) were cut into individual slices (~ 25 mm thick) and vacuum-packed using five-layer (PA/tie/PE/tie/PE) coextruded nanocomposite films (Versatile Packaging Ltd., Ireland) and a VG 400 ILPRA sealing machine (Vigevano, Italy). Samples for chemical analysis and Raman spectra measurement on the 3rd day post-mortem were immediately stored at -20 °C; while samples for Raman spectra measurements at the 7th and 14th days post-mortem were aged for 7 and 14 days at 4 °C respectively and then stored at -20 °C.

For the discrimination models, 30 bulls were slaughtered at 15-months (n = 15) and 19-months (n = 15) of age respectively. LT, ST and GM muscles were collected from 10 bulls (15-months of age), and were used for muscle type discrimination. For age discrimination, 26 muscles (LT & ST) were collected from 15-month old bulls, and 29 muscles (LT & ST) were collected from 19-month old bulls. Two individual slices were cut from each muscle and vacuum-packed after ageing for 7 and 14 days at 4 °C respectively, and then frozen at -20 °C prior to Raman spectra measurements. In addition, LT muscles from Holstein-Friesian (HF) (n = 56) and Jersey × Holstein-Friesian (JEX) (n = 33) young bulls were used for discrimination analysis on breed.

7.3.2 Warner-Bratzler shear force and cooking loss

The method was described in Chapter 2 and beef steaks used in this measurement had been aged for 3 days.

7.3.3 IMF, moisture and protein

The method was described in Chapter 2 and beef steaks used in this measurement had been aged for 3 days.

7.3.4 Collagen content and solubility

The method was described in Chapter 3 and beef steaks used in this measurement had been aged for 3 days.

7.3.5 Sample preparation and Raman measurements

Raman spectroscopic data were collected from scanning aged beef samples on the 3rd, 7th and 14th days post-mortem. Before measurements, frozen steaks were removed from −20 °C storage and allowed to thaw at 4°C for ∼16 h. Each sample was homogenized using a Robot Coupe R301 ultra (Vincennes, France) for 1 min. Approximately 10 g of homogenized beef sample was wrapped in PVC clingfilm to form a ball. Raman spectra were collected on a DXR SmartRaman spectrometer (ThermoFisher Scientific UK Ltd., Loughborough, UK) equipped with a diode laser operating at 780 nm to minimize sample fluorescence issues and a charge coupled device (CCD) detector operating at −50 °C. A smooth side of the wrapped sample was then placed over the aperture (50 μm slit) of the universal platform sampling (UPS) accessory. All spectra of each sample were accumulated for 5 min (i.e. 15 s exposure time × 20 exposures) using a 150 mW laser power. Samples were scanned in random order at ambient temperature (∼20 °C). Raman intensity counts per second (cps) were recorded over the wavelength range 250−3380 cm^{-1} at 2 cm^{-1} intervals. Cosmic spikes were removed automatically by the supplied software. Instrument control, spectral acquisition, and file conversion were performed using the supplied OMNIC software v 9.2.98 (Thermo Fisher Scientific Inc., USA). Each sample was scanned twice, once each at two different scan sites on the sample ball; the mean of these replicate spectra was used in subsequent chemometric operations.

7.3.6 Spectral data processing

Raw Raman spectra were exported from OMNIC software as JCAMP.DX files and imported into Matlab 2014a (The Mathworks, Natick, MA, USA), the mean spectrum of each sample was calculated and also imported into The Unscrambler v.10.3 (Camo, Trondheim, Norway) for different data-pretreatments and chemometric operations.

Baseline correction of Raman data was carried out using the Savitzky-Golay (S.G.) derivation. First derivatives were calculated using a fifth-degree polynomial and 7 smoothing points; second derivatives were calculated using a fifth-degree polynomial and 9 smoothing points. Multiplicative effects of the spectroscopic data were removed using unit vector normalization. In order to find the optimal data pre-processing method for partial least squares model development, other baseline correction methods such as polyfit using a fifth-degree polynomial were also employed.

7.3.7 Chemometric analysis

For the prediction of beef quality traits, partial least squares regression (PLSR) models were developed using pre-processed Raman spectroscopic data collected on the 3^{rd} , 7^{th} or 14^{th} days post-mortem using selected frequency ranges (i.e. 250-3380 cm^{-1}, 900-1800 cm^{-1}, 1300-2800 cm^{-1}) respectively combined with the reference values of the physico-chemical traits measured on the 3^{rd} day. Full cross-validation PLSR models were developed using 63 samples to predict WBSF, cooking loss, IMF, moisture and protein content and 36 samples for the prediction of total collagen (TC), hydroxyproline (HYP) and collagen solubility (CSol). It was assumed that prediction performance of these PLSR models developed using Raman spectral data collected after a longer post-mortem duration would have lower accuracy as the reference data was obtained from the 3^{rd} post mortem day. Evaluation of PLSR model prediction performance was carried out using the statistics parameters such as root mean square error of calibration (RMSEC) and cross-validation (RMSECV) and the coefficient of determination on calibration (R^2C) and cross-validation (R^2CV). For a satisfactory prediction performance, the value of R^2 is expected to be close to 1 while values of RMSE and bias are expected to be close to 0.

Partial least squares discriminant analysis (PLS-DA) models were developed using pre-processed Raman spectroscopic data of 55 and 30 beef samples (aged for both 7 and 14 days) in the frequency range of 1300-2800 cm^{-1} for the classification of slaughter age (15- and 19-month old) and muscles (LT, ST and GM), respectively. For the PLS-DA models developed for the detection of bull age, dummy y values of 1 and 2 were assigned to samples (1 for beef from 15-month old bulls, 2 for beef from 19-month old bulls); the threshold selected for classification was set empirically at 1.5. Likewise, for muscle determination, the dummy y values of 1, 2 and 3 were given to samples of LT, ST and GM muscles respectively with the empirical thresholds of 1.5 for the classification of LT and ST and 2.5 for the classification of ST and GM. To evaluate the performance of PLS-DA models, confusion matrices were developed for the classification of slaughter age and muscle based on the explanatory matrices (Table 7.1a & b). The correct classification (CC) used to evaluate PLS-DA models was expressed as percentage:

$$CC = \frac{TP+TN}{TP+FP+FN+TN} \quad (1)$$

In the current study, all PLS models were calibrated using the nonlinear iterative partial least squares (NIPALS) algorithm. Improvements in performance of PLS models were attempted using a reduced number of Raman spectral variables. The Martens' uncertainty test was applied to select spectral variables based on the variability of their regression coefficients during cross-validation of model development (Martens & Martens, 2000). Other informative variable selection algorithms such as variable importance on projection (VIP) (Chong & Jun, 2005) and significance multivariate correlation (sMC) (Tran et al., 2014) were also explored.

7.4 Results and discussion

7.4.1 Meat quality results

WBSF of samples ranged from 25.1-86.6 N (Table 7.2), with 4 samples below 31.36 N, which was categorized into a 'very tender' group; 4 and 9 samples ranged from 31.36-38.22 N and 38.22-45.08 N, belonging to 'tender' and 'intermediate tender' categories, respectively. The WBSF of the other 46 samples was above 45.08 N, considered as a 'tough' group (Shackelford et al., 1991). In the current study, cooking

loss ranged between 26.6%w/w and 36.7%w/w, which was in accordance with previous work (Jeremiah et al., 2003c).

IMF content in the current study ranged from 0.05-5.81%w/w, and most samples were within the range of 0.76%-6.00%w/w reported for beef in previous studies (Jeremiah et al., 2003c; Muchenje et al., 2008). Generally, 3-7%w/w of IMF is considered as an ideal range to ensure palatability while not being detrimental to human health (Miller, 2002). Moisture, the most abundant component in beef, varied from 71.6-77.5%w/w with a standard deviation of 1.27%w/w. Total protein content varied from 21.2 to 24.3%w/w with a low standard deviation of 0.69%w/w and a mean value of 22.7%w/w, which is in agreement with the reported range for beef chemical composition values (Muchenje et al., 2009). The mean value of total collagen (3.27 mg/g) or collagen solubility (18.0%) was in agreement with studies by other authors (Archile-Contreras et al., 2010; Christensen et al., 2011). The wide range of collagen values used in this study was due to the different muscles selected as inter muscle comparisons is considered as an important reason for samples obtaining large variation in collagen content, which consequently leads to the strong relationship between collagen characteristics and cooked meat tenderness (Dransfield, 1977).

7.4.2 Raman spectra

Raw Raman spectra (250-3380 cm^{-1}) of all bull beef samples (n = 189, the sum of samples of all ageing times) including the background fluorescence are shown in Figure 7.1a. All sample spectra have very similar spectral profiles. Averaged spectra derived from the raw spectra (n = 63) of each ageing time are shown in Figure 7.1b. The spectra of the 3^{rd} and 7^{th} days almost overlap each other, while the spectrum of the 14^{th} day is separated from them in the frequency range of 250-2800 cm^{-1}. In Figure 7.1c, fluorescence effects and multiplicative effects have been removed; detailed Raman signals are shown for the mean spectrum of all bull beef samples in the frequency range of 500-2800 cm^{-1}. The Raman spectral signal around 670 cm^{-1} is related to methionine and disulphide S-S stretching vibration (Beattie et al., 2004). Signals of myoglobin in meat are shown at 714, 755, 855, 1125, 1340 and 1540 cm^{-1}; signals of tyrosine (aromatic amino acid side chains) are shown at 825 and 855 cm^{-1} (Bauer et al., 2016). The spectral regions of 890-1060 and 1645-1685 cm^{-1} assigned

to the amide I bands involve C-C stretching vibrations, C=O stretching and N-H in-plane bending of peptide bonds; the region of 1200-1350 cm^{-1} assigned to amide III bands mainly involve C-N stretching and N-H in-plane bending vibrations of the peptide groups (Herrero et al., 2008). Peaks at 1270 and 1336 cm^{-1} have been related to the secondary and tertiary structures of proteins, i.e., amide III bands. A peak at 1650 cm^{-1} is probably due to α-helical structures, while those at 1270 and 1300 cm^{-1} have been attributed to α globular- and α fibrous-helix formations of amide III (Beattie et al., 2004). Phenylalanine is a strong scatter at 1003 cm^{-1}, tryptophan shows specific peaks at 1353 and 1550 cm^{-1}, which are another two typical signals attributed to the aromatic amino acid side chains. Peaks at 1439-1447 cm^{-1} have been attributed to a CH_2 scissoring vibration in proteins (Beattie et al., 2004). Raman spectral peaks at frequencies of 1744, 1653, 1439, 1300, 1270, 1125, and 920 cm^{-1} have also been assigned to the C=O, C=C stretching bonds, CH_2 scissoring or twisting bonds, and C−H in-plane deformation bonds of aliphatic chains in lipids (Li-Chan, 1996). The peak shown at 2327 cm^{-1} is most likely related to C≡N stretching bonds of aliphatic nitriles (Socrates, 2001).

7.4.3 Prediction of physico-chemical traits based on PLS regression models

Performance of PLS models developed on pre-treated Raman data using Savitzky Golay (S.G.) derivation or polyfit with 5^{th} baseline correction methods were examined; models developed in selected ranges (i.e. 250-3380 cm^{-1}, 900-1800 cm^{-1} and 1300-2800 cm^{-1}) were also explored. Results showed that PLS models developed using S.G. derivation pre-treated Raman spectra over 1300-2800 cm^{-1} performed best for both prediction and discrimination purposes; especially for the prediction of TC, HYP and CSol. In the current study, the Martens' uncertainty test was demonstrated to be the most effective method to select the informative Raman spectral variables for enhancing the PLS model performance. Therefore, all models were developed using retained Raman spectral variables in the Raman shifts of 1300-2800 cm^{-1} after the Martens' uncertainty test, and results derived from 900-1800 cm^{-1}, 250-3380 cm^{-1} and from other informative spectral variable selection algorithms (i.e. VIP and sMC) are not discussed in this paper.

7.4.3.1 Prediction of WBSF and cooking loss

Summary statistic results of the PLSR models developed for the predictions of WBSF and cooking loss are shown in Table 7.3. Generally, one or two latent variables were required to attain RMSECV values of 6.82-9.98 N for WBSF and 0.97-1.51%w/w for cooking loss. The models developed using Raman spectra which were collected on the 3rd day post-mortem had a higher prediction performance than those collected on the 7th and 14th days. For the prediction of WBSF, the best prediction results achieved an R^2C of 0.88, R^2CV of 0.75, RMSEC of 4.7 N and RMSECV of 6.82 N (Figure 7.2a). For the prediction of cooking loss, an R^2C of 0.83 with RMSEC of 0.82%w/w and R^2CV of 0.77 with RMSECV of 0.97%w/w were obtained (Figure 7.2b). The results show the changes in beef tenderness during ageing.

It is well established that ageing can be used to reduce WBSF values during post-mortem storage due to the proteolysis of myofibrillar proteins (Muchenje et al., 2009). Cooking loss also changed considerably during ageing. It also has been reported that cooking loss generally increases with ageing in beef GM and LL muscles (Colle et al., 2015). Most of the water loss during cooking is from the juice expelled by heating-induced shrinkage which occurs in the myofibrillar matrix due to protein denaturation (Hughes et al., 2014). During ageing, myofibrillar strain is reduced by proteolysis contributing to an inflow of extra-myofibrillar water to the intra-myofibrillar space. The swelling of the intra-myofibrillar space appears to increase water storage before heating (Pearce et al., 2011). Also protein denaturation (myofibrillar shrinkage) during heating can be accelerated by the destabilisation of the structure of myosin and actin after ageing. Therefore, the weakened protein structure in aged meat is unable to retain or trap as much water during cooking (Hughes et al., 2014). Accordingly, changes in the WBSF and cooking loss in beef at the 7th and 14th days post-mortem would not be expected to be reflected in the Raman spectral information of the 3rd day, hence reducing the potential of spectra on the 7th and 14th days to predict WBSF and cooking loss measured on the 3rd day.

7.4.3.2 Prediction of IMF, moisture and protein

A performance summary of PLSR models for predicting IMF, moisture and protein in each independent sample group is shown in Table 7.4. R^2C values (0.74−0.92) of

the calibration were generally higher than their R^2CV values (0.63−0.85) of the leave-one-out cross-validation for IMF prediction. R^2C values of 0.73-0.97 with RMSEC of 0.22-0.65%w/w and R^2CV values of 0.61-0.91 with RMSECV of 0.39-0.82%w/w were obtained for moisture prediction. For the protein content prediction, the obtained R^2C and R^2CV values are in the ranges of 0.75-0.82 and 0.61-0.70 with RMSEC and RMSECV values in the ranges of 0.28-0.32%w/w and 0.37-0.43%w/w respectively. The best performing models were developed using the Raman spectra pre-treated by Savitzky Golay 2nd derivative using 2nd polynomial with 9 smoothing points. Most informative spectral variables were enhanced using the 2nd derivative. Therefore, a reduced number of spectral variables were retained for developing PLSR prediction models after the Martens' uncertainty tests (Table 7.4). Models developed using Raman spectra collected on the 3rd day post-mortem had a better prediction for IMF and moisture than those collected on the 7th and 14th days, while there was no significant difference for protein prediction. Calibration and cross-validation results of the best performing PLSR models are shown in Figure 7.2 c, d & e. This phenomenon reflects the qualitative or quantitative changes of moisture and IMF during ageing.

Changes of IMF during ageing are mainly due to lipid oxidation, which is responsible for quality deterioration of meat during storage. Lipid peroxidation occurs mostly in phospholipid fraction, and particularly high degree of polyunsaturated fatty acids are more prone to be oxidation, which leads to off-flavour formation (rancidity), colour changes, drip loss, etc. (Wood et al., 2003). Although low temperature and vacuum packaging can prevent the rapid development of lipid oxidation, the process may continue due to a small amount of residual air in the package or the oxygen transmission through the packaging film. There is no change in IMF content during ageing while any chemical structure change may influence the prediction accuracy.

Moisture loss increases with ageing (Colle et al., 2015). Proteolysis of cytoskeletal proteins during ageing will affect water distribution. The larger water channels are mainly due to the extra-myofibrillar water in meat, which is related to integrin degradation as integrin attaches the cytoskeleton to the extracellular matrix. Moreover, the water channels could also be due to water resulting from the

degradation of water-binding proteins during the process of ageing (Pearce et al., 2011).

Changes of protein structure occur during the ageing process. A series of endogenous enzymatic systems have been demonstrated to contribute to softening of the myofibrillar structure and improved tenderness; the degraded proteins mainly include myosin, actin and cytoskeletal proteins (Longergan et al., 2010). Although the total amount of amino acid within muscle doesn't change during proteolysis, the location of individual amino acid may change or be split off to form free amino acids (Beattie et al., 2008). However, the results in the current study showed no significant difference in prediction performance of protein between the 3rd and 14th days probably due to the limitation of sample size.

7.4.3.3 Prediction of total collagen, hydroxyproline and collagen solubility

Results of PLSR models for the prediction of TC, HYP and CSol are summarised in Table 7.5. Models developed using the Raman spectra of the 3rd day post-mortem show a more satisfactory performance for the prediction of TC and HYP while for CSol, the best prediction performance was obtained on the 14th day. In particular, the models developed using S.G. 1st derivative and unit vector normalisation pre-treated Raman data achieved an R^2C of 0.97 and R^2CV of 0.79 with RMSEC of 0.19%w/w and RMSECV of 0.56%w/w for the prediction of TC (Figure 7.2f); with RMSEC of 0.03%w/w and RMSECV of 0.07%w/w for the prediction of HYP (Figure 7.2g); and an R^2C of 0.95, R^2CV of 0.88, RMSEC of 1.30%w/w, RMSECV of 2.15%w/w for the prediction of CSol (Figure 7.2h). The slightly lower prediction ability of PLSR models for TC and HYP based on Raman spectra of the 7th and 14th days post-mortem compared to the 3rd day can be explained by the ultrastructural and quantitative changes of collagen during ageing.

From the ultrastructure point of view, proteoglycan is degraded, thus the linkage between collagen fibrils is weakened. The disintegrated total collagen networks appear to decrease the mechanical strength of IMCT and lead to an improvement of tenderness in uncooked meat (Nishimura, 2015). Furthermore, the perimysium and epimysium also undergo damage as a result of proteolytic attack. Within the collagen fibrils of muscle, collagenases or Zn^{2+} metalloproteinases are able to break down matrix components and cleave the triple collagen helix (Woessner, 1991). After helix

cutting, the single α-helix can be hydrolysed by cathepsins, thus peptides and free-amino-acids can be released in lysosomes (Feidt et al., 1996). For the quantity aspect, total free hydroxyproline in LD of bovine increased from 3%w/w at the 3rd day post-mortem to 11%w/w at the 14th day (Feidt et al., 1996).

Dutson & Lawrie (1974) reported that soluble collagen increased from 1 hour to 14 days post-mortem expressed as more hydroxyproline after hydrolysis due to the size of collagen fragments decreasing during storage. Similarly, a weak but significantly increased solubility of collagen fractions was observed in bovine muscles after ageing for 14 days (Stanton & Light, 1987). In the current study, the solubility (CSol) prediction showed an increased trend during ageing.

7.4.4 Regression coefficients of the prediction equations

The relevant regression coefficient plots of the best performing models are shown in Figure 7.3. Regression coefficient plots show multiple maxima and minima of intensities, which consistently happened in the 1300-2800 cm^{-1} range for the prediction of WBSF, cooking loss, moisture and IMF (Figure 7.3 a, b, c & e). Figure 7.3 f & g show regression coefficient intensities at similar Raman frequencies for the prediction of total collagen and hydroxyproline, which shows the strong correlation between them. Theoretically, collagen is the only molecule in muscle which contains hydroxyproline, a type of pro-collagen which accounts for 14% of collagen. (Bailey & Light, 1989). For the prediction of protein content, intensity changes specifically occurred around 1300-1339 cm^{-1} which is likely related to *cis* form secondary amides (amide III group); 1609-1655 cm^{-1} correlates with arginine, phenylalanine, tryptophan in the region of amide I group (Beattie et al., 2004); 2252-2256, 2459-2728 cm^{-1} may relate to the carbonyl group of proteins (Smith & Dent, 2005). For collagen solubility prediction, regression coefficient intensity shifts happened at 1444-1490 cm^{-1} which have been assigned to C=O stretching, C=C stretching, CH_2 scissoring and C-H bonds of aliphatic chains of lipids (Smith & Dent, 2005); 1525-1557 cm^{-1} have been assigned to C=N stretching bonds or tryptophan (amide II); 1857-1860 cm^{-1} have been assigned to C-H and S-H stretching bonds and 2430-2746 cm^{-1} may be assigned to P-H stretching bonds of phosphines or S-H stretching bonds of mercaptans, aliphatic thiols and thiophenols (Socrates, 2001). Other possibly related function groups of chemical compounds and Raman shifts are listed in

Appendix II. However, compared with the fingerprint range (900-1800 cm^{-1}) of Raman shifts, the related chemical bonds in the range of 1300-2800 cm^{-1} are not widely reported for meat studies.

7.4.5 Discrimination between production systems

7.4.5.1 Slaughter age discrimination

For the discrimination of beef from 15- or 19-month old bulls, 100% correct sample identification was achieved by PLS-DA models developed using Raman spectral data collected at the 7th day post-mortem and pre-treated by S.G. 1st derivative using 5th polynomial with 7 smoothing points with normalization on unit vector. After spectral variable selection, 282 of the spectral variables (n = 1557) were retained for the model development. A 94.5% correct sample identification was achieved by PLS-DA models developed using Raman spectral data collected at the 14th day post-mortem using 161 retained spectral variables (Table 7.6). It has been reported that 19-month old bulls produced tougher beef than that from 15-month old bulls (Renand et al., 2001). Collagen characteristics were greatly influenced by slaughter age, with older animals having a higher proportion of heat-stable cross-links, which contributed to the lower collagen solubility (Bailey, 1985). The best performing PLS-DA model for the determination of beef samples from two age groups is shown in Figure 7.4c; these two sample groups can be completely defined by the arbitrary cut-off line at 1.5 on the y-axis. The score plot of samples (Figure 7.4a) shows that components (PC1&2) explain 23% of the x-variance and 99% of the y-variance. Figure 7.4b shows the relevant regression coefficients of spectral variables in the 1300-2800 cm^{-1} range for the best performing PLS-DA models developed.

7.4.5.2 Muscle discrimination

Beef samples from three different muscles (LT, ST and GM) were discriminated with 70.0-86.7% correct classification using PLS-DA models (Table 7.7). Results showed PLS-DA models developed using 53 to 72 similar spectral variables after data pre-treatments by S.G. 1st derivative using 5th polynomial with 7 smooth points (with normalisation on unit vectors) or S.G. 2nd derivative using 2nd polynomial with 9 smooth points. The best performing model was developed using 72 retained spectral variables collected at the 7th day post-mortem with 86.7% correct classification. In Figure 7.4f, 4 samples were misclassified, including two samples of the LT group

which are above the arbitrary cut-off line at 1.5 on the y-axis, one of the ST group was above the line at 2.5 on the y-axis and one of the GM group was under the line at 2.5. The relevant regression coefficient plot is shown in Figure 7.4e. The score plot show that samples of these three muscle groups can be explained using components (PC1&2) with 8% of x-variance and 100% of y-variance explained (Figure 7.4d). In this score plot, the cluster of GM was located in the middle between the other two muscle clusters; this corresponds to the difference in eating quality of three muscle types. It has been noted that ST had higher WBSF than LT while WBSF of GM took an intermediate position between ST and LT (Belew et al., 2003). In contrast, the IMF content was higher in LT than ST, and GM also took an intermediate position. ST and GM had higher total collagen content than LT, and collagen solubility was greater in LT and GM than ST (Jeremiah et al., 2003c).

Generally, PLS-DA modelling based on Raman spectra of the 7th day post-mortem showed similar discrimination results to those based on the spectra of the 14th day.

LT muscles (n = 89) aged for 21 days from two breed types HF and JEX were discriminated with 80.3% correct classification using PLS-DA models. The score plot showed that samples of two breed groups can be explained using components (PC1&2) with 14% of x-variance and 77% of y-variance (Figure 7.5). The two clusters can be clearly separated with JEX beef were located in the positive part of PC1 while most HF beef were in the negative part of PC1.

7.5 Conclusions

This study demonstrated the potential of Raman spectroscopy to assess eating quality related physico-chemical characteristics in young dairy bull beef aged for different times. PLSR models based on the 1300-2800 cm^{-1} wavelength range yielded the best results for both prediction and discrimination purposes. The prediction ability of PLSR models developed using spectra of the 7th or 14th day post-mortem was lower (except for collagen solubility and protein prediction) than those at the 3rd day, reflecting the ultrastructural changes in beef with ageing. Improved prediction performance can be achieved using multiple muscle types. PLS-DA modelling showed that Raman spectroscopy has potential to discriminate beef characteristics such as age and muscle type and results were not greatly influenced by post-mortem ageing. Future work should investigate the application of Raman spectroscopy for

on-line assessment of meat eating quality related physico-chemical traits. Furthermore, the Raman frequency range of 1300-2800 cm^{-1} still merits further investigation for meat sensory analysis.

Table 7.1. a. Confusion matrix for the classification of samples at the age of 15-month (defining as and 19-month defining as true or false – positive and true or false – negative).

		Measured bull age	
		15-month	19-month
Predicted bull age	15-month	True positive (TP)	False positive (FP)
	19-month	False negative (FN)	True negative (TN)

b. Confusion matrix for the classification of samples from beef cuts of LT, ST and GM muscles (defining as true or false – positive and true or false negative).

			Measured muscle type		
			LT	ST	GM
PLS-DA modelling for LT determination	Predicted muscle type	LT	True positive (TP)	False negative (FN)	False negative (FN)
		ST	False positive (FP)	True negative (TN)	True negative (TN)
		GM	False positive (FP)	True negative (TN)	True negative (TN)
PLS-DA modelling for ST determination	Predicted muscle type	LT	False negative (FN)	False positive (FP)	False negative (FN)
		ST	True negative (TN)	True positive (TP)	True negative (TN)
		GM	True negative (TN)	False positive (FP)	True negative (TN)
PLS-DA modelling for GM determination	Predicted muscle type	LT	True negative (TN)	True negative (TN)	False positive (FP)
		ST	True negative (TN)	True negative (TN)	False positive (FP)
		GM	False negative (FN)	False negative (FN)	True positive (TP)

Table 7.2. Reference values of meat quality traits of young dairy bull beef on the 3rd day post-mortem.

	N[3]	Mean	Min	Max	SD[4]
Physical traits					
WBSF[1] (N)	63	53.0	25.1	86.6	13.4
Cooking loss (%w/w)	63	32.7	26.7	36.7	2.03
Chemical traits					
IMF[2] (%w/w)	63	1.69	0.05	5.81	1.31
Protein (%w/w)	63	22.7	21.2	24.3	0.69
Moisture (%w/w)	63	74.7	71.6	77.5	1.27
Total collagen (mg/g)	36	3.27	1.51	6.02	1.19
Total hydroxyproline (mg/g)	36	0.44	0.20	0.81	0.16
Collagen solubility (%)	36	18.0	6.95	30.6	5.73

[1]WBSF = Warner-Bratzler shear force; [2]IMF = intramuscular fat;[3] n = numbers of samples; [4]SD = standard deviation.

Table 7.3. Summary of PLSR[1] model performances (Raman shift 1300-2800 cm^{-1}) for WBSF and cooking loss prediction in bull beef.

	Sample	Raman data pre-treatment with the Martens' uncertainty test	Numbers of spectral variables retained	# PLS loadings[6]	R^2C[7]	RMSEC[8]	R^2CV[9]	RMSECV[10]
WBSF[2]	**D 3[11] (n[14]=63)**	**S.G.[3] 1st der.[4] using 5th polynomial with 7 smooth points + nor.u.v.[5]**	**130**	**2**	**0.88**	**4.70**	**0.75**	**6.82**
		S.G. 2nd der. using 2th polynomial with 9 smooth points	60	2	0.70	7.22	0.45	9.98
	D 7[12] (n=63)	S.G. 1st der. using 5th polynomial with 7 smooth points + nor.u.v.	107	2	0.84	5.12	0.70	7.22
		S.G. 2nd der. using 2th polynomial with 9 smooth points	87	2	0.86	4.86	0.73	6.98
	D 14[13] (n=63)	S.G. 1st der. using 5th polynomial with 7 smooth points + nor.u.v.	56	1	0.76	6.29	0.70	7.26
		S.G. 2nd der. using 2th polynomial with 9 smooth points	62	1	0.72	6.91	0.63	7.97
Cooking loss	**D 3 (n=63)**	**S.G. 1st der. using 5th polynomial with 7 smooth points + nor.u.v.**	**97**	**1**	**0.83**	**0.82**	**0.77**	**0.97**
		S.G. 2nd der. using 2th polynomial with 9 smooth points	73	2	0.71	1.08	0.54	1.39
	D 7 (n=63)	S.G. 1st der. using 5th polynomial with 7 smooth points + nor.u.v.	78	2	0.76	0.98	0.55	1.36
		S.G. 2nd der. using 2th polynomial with 9 smooth points	56	2	0.61	1.24	0.45	1.51
	D 14 (n=63)	S.G. 1st der. using 5th polynomial with 7 smooth points + nor.u.v.	60	2	0.81	0.85	0.62	1.21
		S.G. 2nd der. using 2th polynomial with 9 smooth points	60	1	0.58	1.26	0.48	1.41

[1]PLSR = partial least squares regression models; [2]WBSF = Warner-Bratzler shear force; [3]S.G. = Savitzky Golay; [4]der. = derivatives; [5]nor.u.v. = normalisation on unit vectors; [6]# PLS loadings = number of PLS loadings; [7]R^2C = coefficient determination of calibration; [8]RMSEC = root mean square error of calibration; [9]R^2CV = correlation coefficient of determination in cross-validation; [10]RMSECV = root mean square error of cross-validation; [11]D3 = the 3rd day post-mortem; [12]D7 = the 7th day post-mortem; [13]D14 = the 14th day post-mortem; [14]n = numbers of samples.

Table 7.4. Summary of PLSR[1] model performances (Raman shift 1300-2800 cm^{-1}) for IMF, moisture and protein prediction in bull beef.

	Sample	Raman data pre-treatment with the Martens' uncertainty test	Numbers of spectral variables retained	# PLS loadings[6]	R^2C[7]	RMSEC[8]	R^2CV[9]	RMSECV[10]
IMF[2]	**D3[11] (n[14]=63)**	S.G.[3] 1st der.[4] using 5th polynomial with 7 smooth points + nor.u.v.[5]	176	2	0.91	0.38	0.78	0.62
		S.G. 2nd der. using 2nd polynomial with 9 smooth points	**110**	**2**	**0.92**	**0.38**	**0.85**	**0.52**
	D7[12] (n=63)	S.G. 1st der. using 5th polynomial with 7 smooth points + nor.u.v.	195	2	0.91	0.39	0.73	0.68
		S.G. 2nd der. using 2nd polynomial with 9 smooth points	105	3	0.92	0.36	0.81	0.57
	D14[13] (n=63)	S.G. 1st der. using 5th polynomial with 7 smooth points + nor.u.v.	117	1	0.74	0.67	0.67	0.76
		S.G. 2nd der. using 2nd polynomial with 9 smooth points	86	2	0.76	0.64	0.63	0.80
Moisture	**D3 (n=63)**	S.G. 1st der. using 5th polynomial with 7 smooth points + nor.u.v.	132	2	0.94	0.30	0.86	0.47
		S.G. 2nd der. using 2nd polynomial with 9 smooth points	**109**	**3**	**0.97**	**0.22**	**0.91**	**0.39**
	D7 (n=63)	S.G. 1st der. using 5th polynomial with 7 smooth points + nor.u.v.	164	2	0.90	0.40	0.72	0.69
		S.G. 2nd der. using 2nd polynomial with 9 smooth points	132	2	0.75	0.65	0.61	0.82
	D14 (n=63)	S.G. 1st der. using 5th polynomial with 7 smooth points + nor.u.v.	128	1	0.73	0.64	0.67	0.72
		S.G. 2nd der. using 2nd polynomial with 9 smooth points	105	2	0.79	0.57	0.65	0.75
Protein	**D3 (n=63)**	S.G. 1st der. using 5th polynomial with 7 smooth points + nor.u.v.	159	2	0.82	0.29	0.62	0.43
		S.G. 2nd der. using 2nd polynomial with 9 smooth points	**33**	**3**	**0.82**	**0.29**	**0.70**	**0.38**
	D7 (n=63)	S.G. 1st der. using 5th polynomial with 7 smooth points + nor.u.v.	128	2	0.81	0.28	0.66	0.38
		S.G. 2nd der. using 2nd polynomial with 9 smooth points	63	2	0.78	0.30	0.61	0.40
	D14 (n=63)	S.G. 1st der. using 5th polynomial with 7 smooth points + nor.u.v.	111	1	0.75	0.32	0.68	0.37
		S.G. 2nd der. using 2nd polynomial with 9 smooth points	76	2	0.78	0.32	0.69	0.37

[1]PLSR = partial least squares regression models; [2]IMF = intramuscular fat; [3]S.G. = Savitzky Golay; [4]der. = derivatives; [5]nor.u.v. = normalisation on unit vectors; [6] # PLS loadings = number of PLS loadings; [7]R^2C = coefficient determination of calibration; [8]RMSEC = root mean square error of calibration; [9]R^2CV = correlation coefficient of determination in cross-validation; [10]RMSECV = root mean square error of cross-validation; [11]D3 = the 3rd day post-mortem; [12]D7 = the 7th day post-mortem; [13]D14 = the 14th day post-mortem; [14]n = numbers of samples.

Table 7.5. Summary of PLSR model performances (Raman shift 1300-2800 cm^{-1}) for TC, HYP and CSol prediction in bull beef.

	Sample	Raman data pre-treatment with the Martens' uncertainty test	Numbers of spectral variables retained	# PLS loadings	R^2C	RMSEC	R^2CV	RMSECV
TC	**D3**	**S.G. 1st der. using 5th polynomial with 7 smooth points + nor.u.v.**	**131**	**3**	**0.97**	**0.19**	**0.79**	**0.56**
	(n=36)	S.G. 2nd der. using 2th polynomial with 9 smooth points	21	1	0.82	0.49	0.78	0.56
	D7	S.G. 1st der. using 5th polynomial with 7 smooth points + nor.u.v.	121	1	0.74	0.59	0.67	0.69
	(n=36)	S.G. 2nd der. using 2th polynomial with 9 smooth points	106	2	0.80	0.51	0.59	0.77
	D14	S.G. 1st der. using 5th polynomial with 7 smooth points + nor.u.v.	53	1	0.66	0.67	0.58	0.76
	(n=36)	S.G. 2nd der. using 2th polynomial with 9 smooth points	49	1	0.62	0.72	0.52	0.82
HYP	**D3**	**S.G. 1st der. using 5th polynomial with 7 smooth points + nor.u.v.**	**112**	**3**	**0.97**	**0.03**	**0.79**	**0.07**
	(n=36)	S.G. 2nd der. using 2th polynomial with 9 smooth points	21	1	0.82	0.07	0.78	0.08
	D7	S.G. 1st der. using 5th polynomial with 7 smooth points + nor.u.v.	121	1	0.74	0.08	0.67	0.09
	(n=36)	S.G. 2nd der. using 2th polynomial with 9 smooth points	106	2	0.80	0.07	0.59	0.10
	D14	S.G. 1st der. using 5th polynomial with 7 smooth points + nor.u.v.	81	2	0.80	0.07	0.62	0.10
	(n=36)	S.G. 2nd der. using 2th polynomial with 9 smooth points	59	2	0.70	0.09	0.54	0.11
CSol	D3	S.G. 1st der. using 5th polynomial with 7 smooth points + nor.u.v.	68	2	0.88	1.96	0.66	3.38
	(n=36)	S.G. 2nd der. using 2th polynomial with 9 smooth points	58	1	0.73	2.94	0.65	3.45
	D7	S.G. 1st der. using 5th polynomial with 7 smooth points + nor.u.v.	61	2	0.87	2.20	0.72	3.34
	(n=36)	S.G. 2nd der. using 2th polynomial with 9 smooth points	58	1	0.80	2.75	0.73	3.29
	D14	S.G. 1st der. using 5th polynomial with 7 smooth points + nor.u.v.	74	1	0.92	1.72	0.79	2.84
	(n=36)	**S.G. 2nd der. using 2th polynomial with 9 smooth points**	**102**	**2**	**0.95**	**1.30**	**0.88**	**2.15**

[1]PLSR = partial least squares regression models; [2]TC = total collagen; [3]HYP = hydroxyproline; [4]CSol = collagen solubility; [5]S.G. = Savitzky Golay; [6]der. = derivatives; [7]nor.u.v. = normalisation on unit vectors; [8]# PLS loadings = number of PLS loadings; [9]R^2C = coefficient determination of calibration; [10]RMSEC = root mean square error of calibration; [11]R^2CV = correlation coefficient of determination in cross-validation; [12]RMSECV = root mean square error of cross-validation; [13]D3 = the 3rd day post-mortem; [14]D7 = the 7th day post-mortem; [15]D14 = the 14th day post-mortem; [16]n = numbers of samples.

Table 7.6. Summary of PLS-DA performances (Raman shift 1300-2800 cm^{-1}) for the detection of samples from 15- and 19-month old bulls.

				classification results of cross-validation				
Sample	Data type	Numbers of spectral variables retained	No. of PLS loadings	TP (True positive)	TN (True negative)	FP (False positive)	FN (False negative)	% correct classification (CC)
D7[1]	S.G. 1st der. using 5th polynomial	1557	2	26	29	0	0	100
(n^3=55)	with 7 smooth points + nor.u.v.	282	1	26	29	0	0	100
D14[2]	S.G. 1st der. using 5th polynomial	1557	1	19	22	7	7	74.5
(n=55)	with 7 smooth points + nor.u.v.	161	1	25	27	1	2	94.5

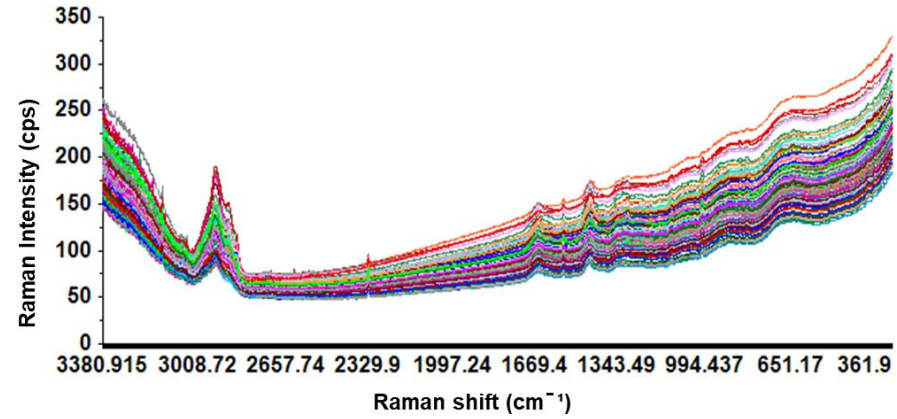
[1]D7 = the 7th day post-mortem; [2]D14 = the 14th day post-mortem; [3]n = numbers of samples.

Table 7.7. Summary of PLS-DA performances (Raman shift 1300-2800 cm^{-1}) for the detection of bull beef samples of LT, ST and GM muscles.

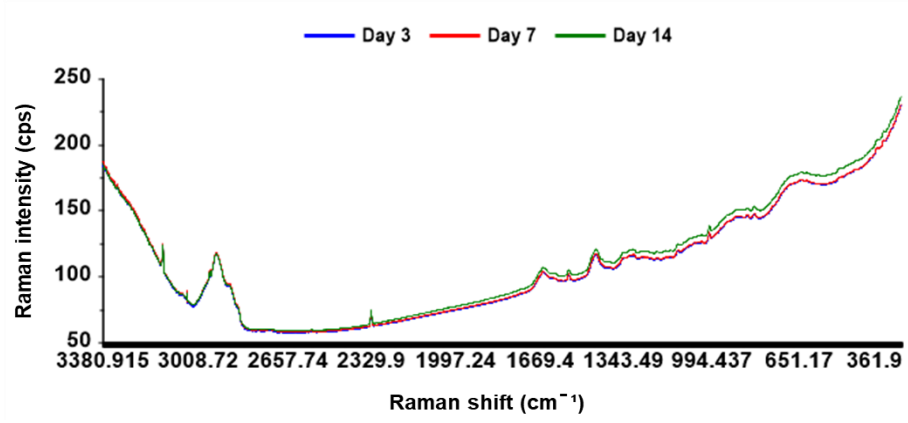
Sample	Data type	Numbers of spectral variables retained (1300-2800 cm)	No. of PLS loadings	predicted muscle types	classification results of cross-validation TP (True positive)	TN (True negative)	FP (False positive)	FN (False negative)	% correct classification (CC)
D7[1] (n[3]=30)	S.G. 1st der. using 5th polynomial with 7 smooth points + nor.u.v.	70	1	1-LT	7	18	3	2	
				2-ST	10	15	0	5	
				3-GM	8	17	2	5	83.3
	S.G. 2nd der. using 2th polynomial with 9 smooth points	72	1	1-LT	8	18	2	2	
				2-ST	9	17	1	3	
				3-GM	9	17	1	3	86.7
D14[2] (n=30)	S.G. 1st der. using 5th polynomial with 7 smooth points + nor.u.v.	55	1	1-LT	7	14	3	6	
				2-ST	9	12	1	7	
				3-GM	5	16	5	4	70.0
	S.G. 2nd der. using 2th polynomial with 9 smooth points	53	2	1-LT	9	16	1	4	
				2-ST	8	17	2	3	
				3-GM	8	17	2	3	83.3

[1]D7 = the 7th day post-mortem; [2]D14 = the 14th day post-mortem; [3]n = numbers of samples.

a)



b)



c)

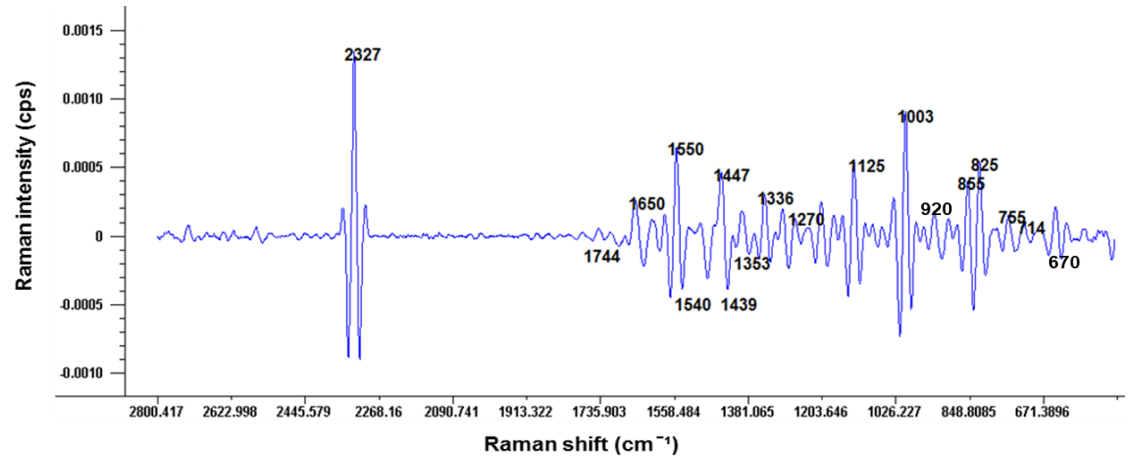


Figure 7.1. a) Raw Raman spectra (250-3380 cm^{-1}) of all bull beef samples (n = 189); b) Averaged raw Raman spectra (250-3380 cm^{-1}) of bull beef samples (n = 63) from the 3rd, 7th and 14th day post-mortem, respectively; c) Averaged Raman spectra (500-2800 cm^{-1}) of all bull beef samples (n = 189) pre-treated by the Savitzky Golay second derivative using a fifth-degree of polynomial and 7 smoothing points and normalization.

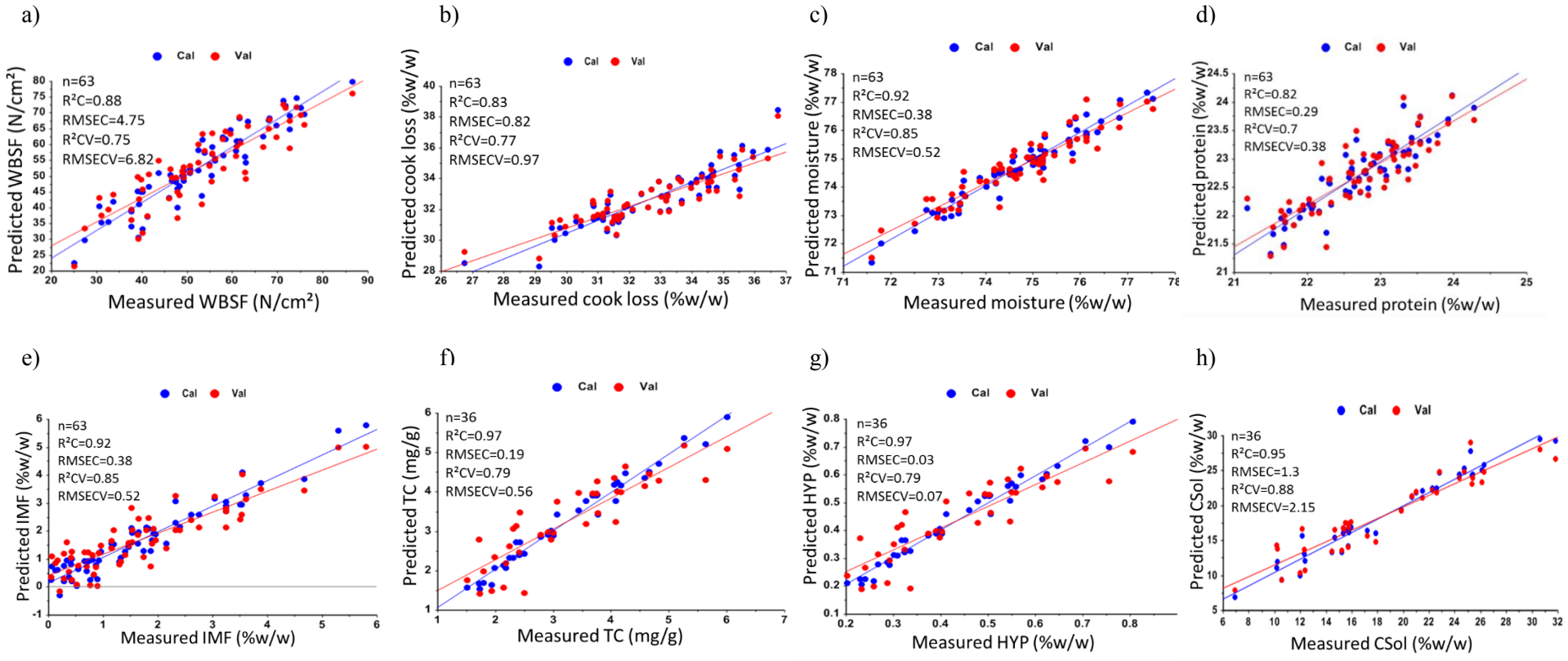


Figure 7.2. PLS linear regression plots of measured reference (x-axis) versus predicted (y-axis) values of a) Warner-Bratzler shear force (WBSF) (N/cm^2); b) cooking loss (%w/w); c) moisture (%w/w); d) protein (%w/w); e) IMF (%w/w); f) total collagen (TC) (mg/g); g) hydroxyproline (HYP) (%w/w); h) collagen solubility (CSol) (%w/w).

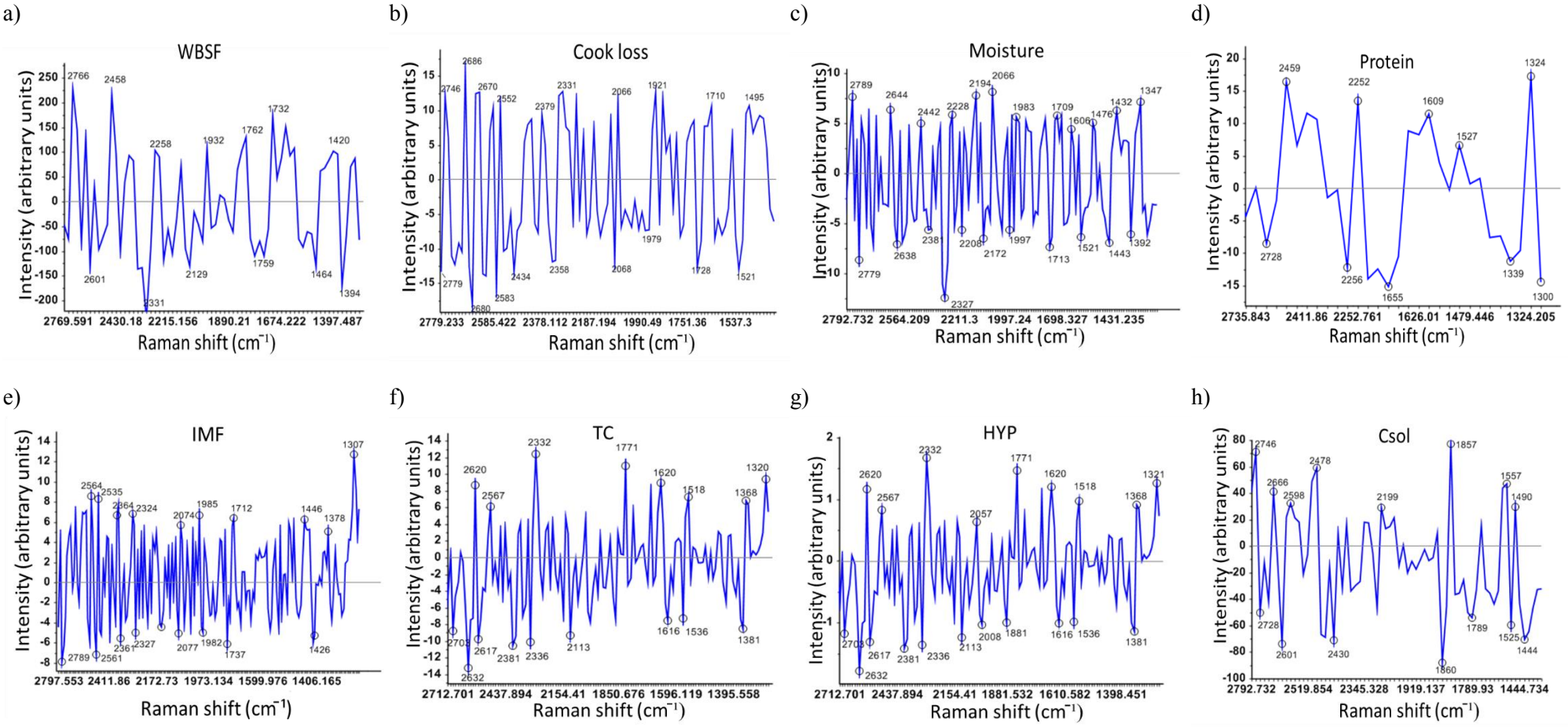


Figure 7.3. Regression coefficients plots of: a) Warner-Bratzler shear force (WBSF); b) cooking loss; c) moisture; d) protein; e) IMF; f) total collagen (TC); g) hydroxyproline (HYP); h) collagen solubility (CSol).

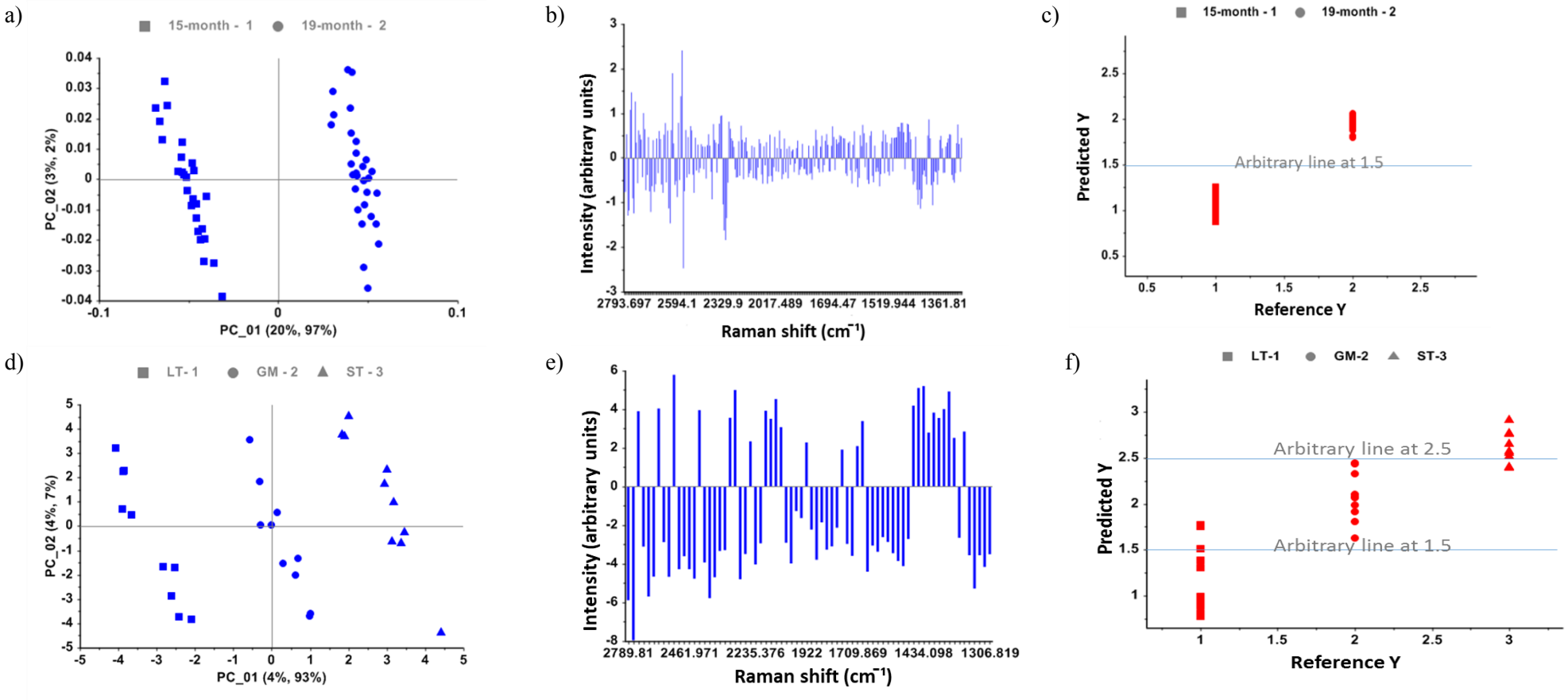


Figure 7.4. Score plots of a) sample groups of 15-month and 19-month old, d) sample groups of LT, GM and ST muscles; regression coefficient plots of b) sample groups of 15-month and 19-month old, e) sample groups of LT, GM and ST muscles; example of PLS-DA results of c) predicted Y values of sample groups of 15-month and 19-month old, f) predicted Y values of sample groups of LT, GM and ST muscles.

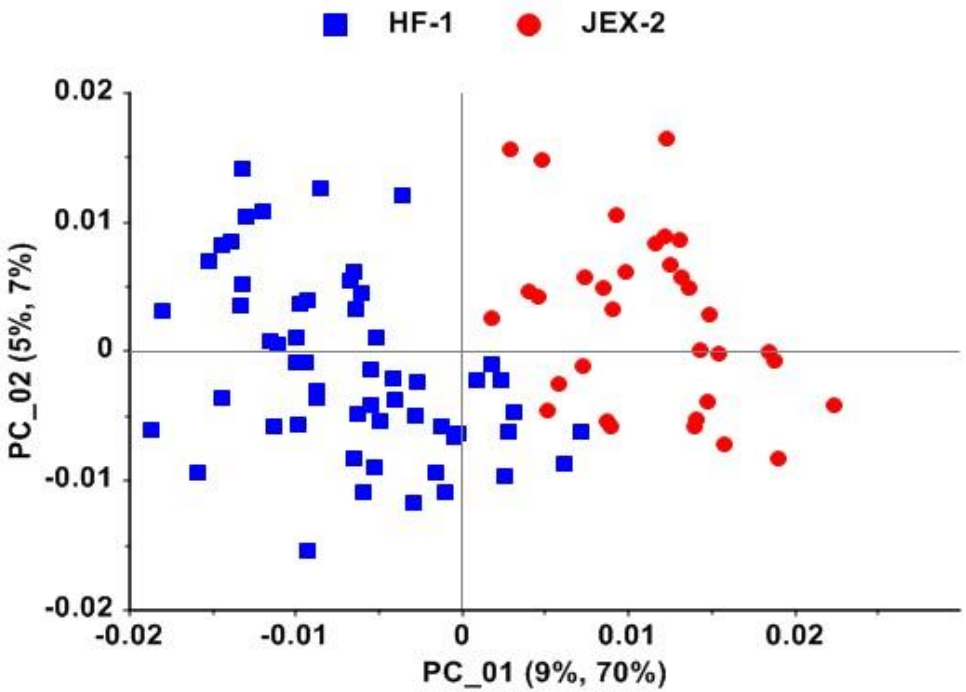


Figure 7.5. Score plot of sample groups from two breeds using PLS-DA model. HF- Holstein-Friesian (n = 56); JEX-Jersey × Holstein-Friesian (n = 33).

Chapter 8

Assessment of beef quality traits of young male dairy cattle using near infrared spectroscopy

8.1 Abstract

This study aims to evaluate the potential of near infrared spectroscopy (NIRS) to predict physico-chemical quality traits and to identify different production factors of young male dairy cattle. Samples were collected based on different amounts of concentrate feeding, slaughter age (15 vs 19 months old), muscle type (LT: *longissimus thoracis* vs *ST: Semitendinosus* vs GM: *gluteus medius*) and gender (bull vs steer). Full near infrared spectroscopy (NIR, 400-2500 nm), visible and near infrared reflectance (Vis-NIR, 400-1900 nm), and near infrared transmittance (NIT, 850-1100 nm) spectroscopy were measured on the 3rd post-mortem day. Calculation equations were developed from reference data (n = 88 to 221) of ultimate pH (pHu), colour parameters (L*, a*, b*, chroma, hue angle) after blooming for 2 and 24 hour using both MiniScan and UltraScan, WB-variables and cooking loss after ageing for 3, 7 and 14 days, proximate chemical composition, soluble and total collagen content. The best results for prediction were obtained using Vis-NIR spectroscopy. Predictions using partial least squares regression (PLSR) model were good (R^2CV = 0.60-0.76 and RMSECV of 1.23-2.75) for colour parameters after 24 hour blooming; moderate (R^2CV = 0.30-0.60 and RMSECV of 0.25 mg/g-10.8 N) for pHu, some colour parameters after 2 hour blooming, WB-variables and cooking loss at 3 different ageing times, intramuscular fat, moisture and soluble collagen content; unsatisfactory for protein (R^2CV = 0.11) and total collagen content (R^2CV = 0.15). Sensory attributes were not able to be predicted. Beef cuts from different production systems were classified into different clusters by principle component analysis (PCA) using the full wavelength 400-2500 nm. The results showed that NIRS offered potential for the development of rapid and non-destructive methods for the assessment of quality traits and identification of samples from young male dairy cattle.

Keywords: Dairy bull beef, near infrared spectroscopy, physico-chemical quality traits, partial least squares regression (PLSR), principle component analysis (PCA)

8.2 Introduction

Eating satisfaction is considered to be the main criteria for purchasing fresh meat in addition to other factors including nutritional value, safety and convenience (Hocquette et al., 2014). At the point of sale, colour is the most important attribute of meat quality as it is the first quality clue seen by the consumer who uses discoloration as an indicator of freshness and wholesomeness. An attractive bright cherry-red colour of beef normally would meet consumer preference (Hood & Mead, 1993). Although beef colour was sufficient to influence consumers' likelihood to purchase, it is not correlated to sensory attributes. It is likely that after a decision to purchase beef is made in the market, eating satisfaction at home will depend only on the eating quality properties, i.e. tenderness, juiciness and flavour (Carpenter et al., 2001), from which tenderness is the most important factor. Cooking loss, which can be related to water holding capacity (WHC), is also important for beef eating quality because of its role in moulding muscle structure, for example it can affect juiciness of beef on mastication (Hughes et al., 2014). Chemical composition and collagen characteristics are background properties that mostly influence beef acceptability. Intramuscular fat (IMF) (or marbling) is positively correlated with juiciness, flavour and tenderness and in many countries marbling is used for carcass grading purpose (Li et al., 2006). It has been well established that intramuscular connective tissue characteristics is one of the two main factors contributing to beef tenderness along with the myofibrillar component. According to Young & Braggins (1993), collagen content has been reported to be more closely related to sensory panel tenderness, while collagen solubility was more closely associated with shear force value.

Although several conventional methods were widely used to assess beef quality, including instrumental analysis, chemical procedure, and sensory evaluation, they are often time-consuming, expensive and destructive, consequently not suitable for large scale application (Liu et al., 2003). In contrast to conventional methods, near infrared spectroscopy (NIRS) is one of the most promising techniques for the determination of meat quality parameters because it is fast, sensitive, non-destructive with simple sample preparation and allows low cost predictions of numerous traits (Cozzolino & Murray, 2002; Alomar et al., 2003).

NIRS has shown promise as a rapid and effective tool for predicting beef chemical components (moisture, IMF, protein, ash, myoglobin) and some technological traits (pH, colour, tenderness, WHC). However, the results for technological traits are not completely satisfactory and in many cases it shows low potential. In addition, NIRS showed limited ability for estimating sensory attributes of beef (Prevolnik et al., 2004; Prieto et al., 2009). The above prediction potential was mainly focused on cattle of beef breed, and the potential of applying NIRS to dairy breed has not been explored yet. Moreover, wavelengths at which NIRS are more closely related to the properties of meat quality have recently been researched and analysed (De Marchi et al., 2013). However, the wavelength range which might improve the prediction ability is not available for this type of beef.

Chemical composition, technological and sensory traits of beef are highly affected by pre-slaughter factors including age, sex, breed, feeding, anatomical location, animal management (Thompson, 2002; Rhee et al., 2004; Frylinck et al., 2013). The possibility of measuring beef quality traits using NIRS might be an opportunity to identify meat origin and select muscles based on their quality characteristics. Discrimination between different types of meat using NIRS has already been used in some studies. Beef and kangaroo meat as well as broiler and local chicken can be distinguished using NIRS (Ding & Xu, 1999; Ding et al., 1999). Alomar et al. (2003) successfully used NIRS for muscle recognition (LT vs ST vs *supraspinosus)* and breed identification (Friesian vs Hereford) of steers. Moreover, another useful application of NIRS is the detection of beef product adulteration. For example, unadulterated beef hamburgers and those adulterated with mutton, pork, wheat flour or skim milk powder can be reliably identified using NIRS (Ding & Xu, 2000). Nevertheless, how the discrimination performance of NIRS on specific production systems of beef from male dairy cattle has not been studied yet.

Therefore, the objectives of this study are to 1) examine the accuracy of NIRS for the prediction of eating quality traits (technological, chemical compositional and sensory attributes) of beef from young male dairy cattle; 2) select the most representative wavelength for these predictions; 3) visualize any separation among beef samples from different production systems including three feeding regimes, three muscles, two slaughter ages or two sexes.

8.3 Materials and methods

8.3.1 Source of materials

Young male dairy cattle were reared and slaughtered over three consecutive years. Totally 32 Holstein-Friesian (HF) and 22 Jersey × Holstein-Friesian (JEX) bulls were assigned to three feeding regimes with different levels of concentrate during the second grazing season or finishing system (19-HC, 19-MC and 19-LC) and were slaughtered at 19 months of age (referring to Chapter 3). Another 45 HF bulls were slaughtered in 2013 at 15 (n = 27) and 19 months (n = 18). In 2014, two sets of cattle were slaughtered; 30 HF bulls were equally assigned to two production systems to finish at 15 or 19 months with a pasture mixed concentrate based feeding (referring to Chapter 5). Another set was 14 HF bulls slaughtered at 19 months while 15 HF steers were slaughtered at 21 months reared under pasture based feeding (referring to Chapter 6).

Cattle were slaughtered at a commercial abattoir; only for the first set of cattle slaughtered in 2014, the *longissimus thoracis* (LT), *semitendinosus* (ST) and *gluteus medius* (GM) muscles were excised from the left-hand side of each carcass at 48 h post-mortem. For the other cattle, only the LT muscle was removed and collected. After holding until 72 h post-mortem, the ultimate pH (pHu) of the muscles was measured, and they were cut into individual slices (~ 25 mm thick). The fresh cut surface of the first slice from the 10^{th} rib end of the LT and the anterior end of the ST and GM were used for colour measurement, and the rest of the slices were vacuum-packed. Samples for chemical composition including collagen determination and WB-variables and cooking loss on day 3 were frozen immediately after cutting. Samples for WB-variables and cooking loss on day 7 and 14 were aged at 4 ºC for 7 and 14 days respectively and frozen. Samples for sensory evaluation were aged for 14 days and frozen until required.

8.3.2 Meat colour (MiniScan)

Freshly cut samples were wrapped with an oxygen-permeable polyvinylchloride film (oxygen permeability of 580 $mL \cdot m^{-2} \cdot h^{-1}$) and left to bloom at 4 °C for 2 h and 24 h. CIE L* (lightness), a* (redness) and b* (yellowness) were analysed using a portable dual beam spectrometer Hunter Lab system (MiniScan XE, Hunter Lab., VA, USA).

Illumination was matched to daylight (D65, 10°) with an 8° viewing angle and a 25.4 mm port size. Standardisation was performed using a white tile and a black tile. Readings were taken as the average of 3 measurements and data was used to calculate the hue angle (arctan b* / a*) and saturation $(a^{*2} + b^{*2})^{1/2}$. Meat colour tested by Ultrascan was described in Chapter 2.

8.3.3 Warner-Bratzler shear force and cooking loss

The method was described in Chapter 2.

8.3.4 Chemical composition

The method was described in Chapter 2.

8.3.5 Collagen content and solubility

The method was described in Chapter 3.

8.3.6 Sample preparation and spectra measurements

The PVC clingfilm wrapped intact steak samples used for colour measurements were then used for NIR spectra measurements on the same day. Near infrared analyses were performed using a benchtop instrument (Model 6500 NIR Systems. Inc., Maryland, USA) in reflectance mode. Absorbance data were stored as log (1/R), R being the reflectance. Samples were canned in random order at ambient temperature (~ 20 ºC) over the wavelength range 400-2500 nm (2 nm intervals) with a 16-32 reference sample scan sequence. Each sample was scanned twice using a New Bulb Brick (see the photo below) with each surface of the steak scanned for each replicate. Between samples, the bulb brick probe surface was carefully cleaned using tepid water with detergent and dried. Spectral acquisition and file conversion were performed using WINISI software (version 1.04; Infrasoft International, Port Matilda, USA). The mean of duplicate scan results was used in subsequent chemometric operations.



8.3.7 Chemometric operations

Spectra were exported from WINISI software as JCAMP.DX format (Rutledge and McIntyre, 1992) and imported directly into the Unscrambler software v.10.3 (Camo, Trondheim, Norway) for different data-pretreatments and chemometric operations.

Raw spectra of all samples over the full scan range were plotted first and visually examined to detect unusual samples and outliers. To develop partial least squares regression (PLSR) models, spectral data were used both in un-treated (raw) and pre-treated forms. Pre-treatments used in this study included baseline correction, standard normal variate (SNV), multiplicative scatter correction (MSC), extended multiplicative scatter correction (EMSC) transformation and Savitzky-Golay algorithm (2^{nd} derivative with 2^{nd} polynomial and 21 smoothing points). These mathematical treatments were able to remove baseline shifts, slope ranges, scatter and other effects from spectral data which reduced irrelevant noise in the spectra (Zhao et al., 2013). Moreover, it is easier to interpret the chemical basis of signals by the 2^{nd} derivative as it enhanced the maintenance in band intensity and peak location and apparently improved band resolution relative to those in the raw spectra pattern (Naes et al., 2002).

PLSR quantitative models in the present study were developed using several spectral ranges: full near infrared spectroscopy (NIR, 400-2500 nm), visible and near infrared reflectance (Vis-NIR, 400-1900 nm), and near infrared transmittance (NIT, 850-1100 nm). Leave-one-out cross-validation was performed to evaluate the performance of PLSR models using statistical parameters of the correlation coefficient of determination in cross-validation (R^2CV) and root mean square error of cross-

validation (RMSECV), the coefficient of determination on calibration (R^2C) and root mean square error of calibration (RMSEC). For a satisfactory prediction performance, the value of R^2 is expected to be close to 1 while values of RMSE and bias are expected to be close to 0. Moreover, to assess the practical utility of the prediction models, the ratio performance deviation (RPD) was calculated by standard deviation (SD) of the reference data divided by RMSECV (Edney et al., 1994). High values of PRD are desirable; normally RPD higher than 2.5 is adequate for analytical purposes (Williams & Sobering, 1993). The best model for each trait was selected based on the highest coefficient of determination in cross-validation (R^2CV) and the lowest RMSECV (Hubert & Vanden Branden, 2003).

Principal component analysis (PCA) was conducted on spectra without modification (raw) to calculate scores and produce scatter plots of the different sample groups. The PCA scores plot can be used to interpret differences and similarities among samples and to visualize the original separation between sample clusters.

8.4 Results and discussion

8.4.1 Meat quality results

The coefficient of variation (CV) was highest for IMF content (79.4%), which was surprisingly high; besides, WB-variables at three ageing times, soluble collagen and total collagen content also had high variability (CV ≥ 30%); all the colour parameters and cooking loss at three ageing times showed intermediate variability (CV: 6.18-22.5%); whereas pHu, intramuscular moisture and protein content had lower variability (CV: 2.3-3.4%) (Table 8.1 & 8.2). This indicated exploitable variability existing to develop calibration models, which was mainly related to samples derived from different breed, sex, age and muscle type. The low variability of pH has been confirmed by several studies that investigated the use of NIRS in beef (Andrés et al., 2008; Prieto et al., 2008). The low variability of intramuscular moisture and protein content seems reasonable because of the real narrow range of these components in beef. Intramuscular moisture and protein content of most samples in the present study are within the normal range reported for beef with 73.9–77.9% for moisture and 20.0–22.9% for protein (Muchenje et al., 2009).

The CV values of pH and cooking loss at three ageing times are in accordance with De Marchi et al. (2013) who reported 3.0% for pH and 9.9% for cooking loss of young bull beef. CV values of a*, b* and WBSF in the present study are higher than 16.8%, 14.2% and 24.3%, respectively observed by De Marchi et al. (2013), while the CV of L* was lower than that from their study, which was 10.6%. The slight differences between studies could be due to the different production systems and analytical techniques used. The CV of total collagen content is similar to that observed by Prieto et al. (2006) (37.0%) who investigated the use of NIRS in oxen meat.

Although the CV values of colour parameters measured by MiniScan are close to those measured by UltraScan, the mean L* value from MiniScan is relatively lower than that from UltraScan, while mean a*, b*, chroma and hue angle are all higher in MiniScan compared with UltraScan (Table 8.1). As expected, WBSF decreased significantly during ageing with an average 4.9 N reduction from the 3rd to 7th post-mortem days and an average 2.9 N reduction from the 7th to 14th post-mortem days (Table 8.2). This confirmed the general finding that beef tenderness can be improved by post-mortem ageing (Muchenje et al., 2009). Cooking loss didn't change during ageing while the mean values of cooking loss are similar to those reported by Cuvelier et al. (2006) for Limousin (30.8%) and Aberdeen Angus (33.1%) bulls.

8.4.2 NIR spectra data

The raw spectrum［log(1/R)］corresponding to the complete sample set (221 beef samples from young male dairy cattle) and its pre-treated spectrum with standard normal variate (SNV) and 2nd derivative of different wavelengths are also presented in Figure 8.1. All spectra recorded in reflectance mode had the same shape for different samples. The spectral features are similar to those reported for young bulls (Ripoll et al., 2008; De Marchi et al., 2013). Seven spectral broad bands can be clearly identified: 432, 576, 760, 980, 1193, 1448 and 1900 nm (Figure 8.1a).

Bands at 415 nm and 435 nm were reported to be related to myoglobin (Cozzolino et al., 1996; Andrés et al., 2008). Previous studies also confirmed signals of meat pigments, mainly with myoglobin, from 425 nm to 550 nm (Lawrie, 1985; Brøndum et al., 2000). Bands at approximately 574 nm and 762 nm were associated with the oxidation of myoglobin or deoxymyoglobin (Cozzolino & Murray, 2004). Liu et al.

(2003) also confirmed that an absorption band produced by myoglobin oxidation was at 760 nm. As stated by several authors, the band at around 1200 nm was related to C-H bonds second overtone (Murray & Williams, 1987; Rødbotten et al., 2000; Leroy et al., 2003). Bands at 1450 nm are associated with O-H first overtone (water absorption) and generally these signals are stronger than those of other components, thus some signals can be hid by water signals, such as at 1485 nm for proteins (Murray, 1986). Signals of water absorption are also shown at regions around 980 nm (O-H second overtone) and 1900-1950 nm (O-H combination tone) (Murray, 1986; Leroy et al., 2003). In addition, signals at 1620-1780 nm and 2200-2400 nm correspond to C-H stretch first overtone and C-H combination bands respectively in the fat fraction (Prieto et al., 2006). Signals related to crude protein are from the absorption of the N-H bonds at 1460-1570 nm and 2000-2180 nm (Murray, 1986; Murray & Williams, 1987; Shenk et al., 1992).

From Figure 8.1 a & c, no remarkable absorption bands were found in the region 2000-2500, probably because of obscuring by the water signal (Prieto et al., 2006). The spectral pattern calculated by the 2^{nd} derivative showed negative absorption peaks, but an apparent band resolution enhancement occurred (Shenk et al., 1992). All peaks in the 2^{nd} derivative spectra (Figure 8.1 c & d) are located at the same wavelength with the background raw spectrum with a better definition in comparison to the raw spectra but inverted.

8.4.3 PLSR prediction models

8.4.3.1 PLSR prediction of pHu and colour

The best prediction ability for all quality traits was developed by Vis-NIR (400-1900 nm), and the results of PLSR models investigated using the NIR (400-2500 nm) and NIT (850-1100 nm) wavelength ranges were inferior to the 400-1900 nm range, thereby, results of those wavelength ranges are not listed in Table 8.1 & 8.2. Also, only the pre-treatment equations with the best predictability for each variable are shown.

Similar to our results (Table 8.1), a R^2CV of 0.47 was obtained for pH on adult steers and young cattle meat (Prieto et al., 2008), but a more accurate prediction was reported by Cozzolino & Murray (2002) and Andrés et al. (2008) for beef samples. The better results of the latter two studies are probably due to the wider range of

reference data than that of the present study. The R^2CV, RMSECV and RPD of pHu are close to the previous findings of 0.62, 0.10 and 1.50, respectively reported by De Marchi et al. (2013).

The wavelength ranges that showed the highest regression coefficients for the prediction of pHu were related to the absorption of myoglobin (476 nm) and C-H bonds (1120 nm) (Figure 8.2a). This is in accordance with the previous finding that pH value had a significant correlation with colour (L*, a*, b*) (Purchas et al., 2002). However, Andersen et al. (1999) found a high correlation between pH and absorbance at 1400 and 1900 nm (O-H bond), while the coefficients of those absorptions in the present study were lower.

The colour prediction results using MiniScan are similar to that by UltraScan (Table 8.1). The best prediction for L* and hue angle were obtained using MiniScan after 24 h blooming with R^2CV of 0.66 for both parameters; the best prediction for a*, b* and chroma values were obtained using UltraScan after 24 h blooming. Particularly, high R^2CV combined with sufficient RPD for a* and chroma (R^2CV of 0.76 and 0.73, PRD of 2.02 and 1.90, respectively) indicate a successful prediction ability of Vis-NIR spectroscopy for the colour of intact beef samples.

The prediction ability of L* and a* are in agreement with the findings from De Marchi et al. (2013) with R^2CV of 0.70 and 0.73, RMSECV of 1.97 and 1.37, respectively, while b* in the present study is relatively more accurate than their study (R^2CV of 0.60, RMSECV of 1.33). Leroy et al. (2003) obtained a more accurate prediction of L* (R^2CV of 0.83, RMSECV of 1.55) and b* (R^2CV of 0.75, RMSECV of 0.77), but lower accuracy for a* (R^2CV of 0.39, RMSECV of 1.15) compared with the present study. Andrés et al. (2008) obtained a higher accuracy of L* (R^2CV of 0.75, RMSECV of 1.36), but lower accuracy of a * (R^2CV of 0.29, RMSECV of 1.28) and b* (R^2CV of 0.46, RMSECV 0.99).

The best predictions for colour were obtained using Vis-NIR spectra indicating that the visible spectra region are related to the prediction of beef colour. The successful prediction of a* and chroma using Vis-NIR spectra region seems reasonable because a* is related to the detection of myoglobin concentration and to the different forms (oxymyoglobin, deoxymyoglobin and metmyoglobin) in the visible region (Mancini, 2005). The higher regression coefficient for the prediction of L*, b* and chroma

were similar and were located at absorbance at 440, 600, 1206, 1898 nm (Figure 8.2 b, d & e). The bands at 440 and 600 nm are related to myoglobin absorption (Cozzolino et al., 1996; Cozzolino & Murray, 2004) and bands at 1206 and 1898 nm are associated with C-H bonds second overtone (fat absorption) and O-H combination tone (water absorption), respectively (Murray & Williams, 1987; Leroy et al., 2003). Similarly, Prieto et al. (2008) reported that L* and b* values correspond to C-H second overtone and C-H combination bands (1230-1400 nm) and C-H first overtone (1600-1710 nm), which are related to the absorbance of long C-H chains of fatty acids. This is reasonable because of the high correlation between IMF and lightness in meat samples (Ruiz et al., 2003). In addition, it has been reported that b* is highly correlated with IMF content in beef of young cattle ($r = 0.87$, $P < 0.001$; Prieto et al., 2008). Similar to our results, Prieto et al. (2008) found the wavelength related to O-H bonds (1940 nm) also showed good correlation with L* due to the strongly negative relationship between IMF and water content in beef. Likewise, the highest regression coefficients for the prediction of a* (Figure 8.2c) corresponds to bonds at 586, 644, 764 nm, which were related to the oxidation of myoglobin or deoxymyoglobin (Cozzolino & Murray, 2004) and bonds at 1100 and 1224 nm, which were related to IMF (Leroy et al., 2003).

8.4.3.2 PLSR prediction of WBSF variables and cooking loss

Overall results for prediction of WB-variables at different ageing times with R^2CV ranged from 0.27 to 0.46 and RPD from 1.17 to 1.38 (Table 8.2). This is in accordance with De Marchi et al. (2013) (R^2CV of 0.34, RMSECV of 9.39 N) and Leroy et al. (2003) (R^2CV of 0.12-0.41, RMSECV of 7.68-11.19 N) for beef WBSF. However, they were worse than those reported by Ripoll et al. (2008) (R^2CV of 0.74, RMSECV of 10.37 N). On the other hand, De Marchi et al. (2007) didn't find any prediction of WBSF with R^2CV of 0.03 in Piemontese beef samples. Prieto et al. (2014) pointed out that the overall relative low accuracy of prediction of WBSF by NIRS could be due to some factor, such as the heterogeneity in muscle tissue, which could cause a high variability of WBSF reference values within the same muscle, which further increases the difficulty to predict this parameter.

Cooking loss prediction performance at different ageing times was also moderate with the R^2CV ranging from 0.30 to 0.45 and RMSECV of 1.92-2.33% w/w (Table 8.2). Similar to WB-variables, ageing didn't change the prediction accuracy of

cooking loss significantly. Prieto et al. (2009) concluded that NIRS has limited capacity to estimate WHC of beef. For example, De Marchi et al. (2007) reported an R^2CV of 0.10 and RMSECV of 1.18% w/w; De Marchi et al. (2013) observed an R^2CV of 0.38, RMSECV of 3.02% w/w and Andrés et al. (2008) didn't find any correlation with an R^2CV of 0.02 and an RPD 1.01. Leroy et al. (2003) reported an R^2CV of 0.25 and RMSECV of 2.31% w/w for prediction of cooking loss at day 2 using NIR spectra at day 2 and an R^2CV of 0.38 and RMSECV of 1.81% w/w for prediction of cooking loss at day 8 using NIR spectra at day 8. According to Prieto et al. (2009), even though NIR can't directly predict WHC of beef, it reflects some chemical components that are related to WHC including protein, IMF and moisture. The lack of wide variability in the reference data (CV < 10) could be the reason for the limited ability to predict of cooking loss in the present study.

The regression coefficient plots of WBSF, WB-slope and WB-area were the same, indicating WB-variables were highly and positively correlated with each other (Figure 8.3 a, c & d). The main regression coefficients for the prediction of WB-variables and cooking loss were related to the absorption of myoglobin (458-620 nm) and C-H bonds (1100-1390 nm). In accordance with the present study, Prieto et al. (2008) and Prieto et al. (2014) reported for the estimation of WBSF value that the highest regression coefficient is related to the absorption of C-H molecular bonds (1124-1400 nm) in the fat fraction or fatty acids. This reflected the contribution of IMF to beef tenderness. Moreover, the correlation between cooking loss and IMF could also be expected as melted fat surrounds connective tissue and generally acts as a barrier against water loss during cooking (Hornstein et al., 1960). Additionally, the regression coefficient at the wavelength where absorbance data related to O-H bonds (1866 nm) was also selected for the prediction of WBSF (Figure 8.3 a, c & d). Consistent with this result, the considerable prediction of WBSF by O-H bond in NIRS has been reported by authors (Barlcco et al., 2006; De Marchi et al., 2013), which indicated the relationship between WBSF and moisture content.

8.4.3.3 PLSR prediction of proximate chemical composition

Compared with our results, Ripoll et al. (2008) found a higher accuracy of prediction of fat (R^2CV of 0.76, RMSECV of 0.49% w/w) and moisture (R^2CV of 0.72, RMSECV of 0.37% w/w), whereas the prediction of protein is consistent with our result with R^2CV of 0.16 and RMSECV of 1.02% w/w. Some authors reported a

higher prediction accuracy with R^2CVs of 0.99, 0.91, 0.64 and RMSECV of 0.13, 0.35, 0.33% w/w for IMF, moisture and protein, respectively (De Marchi et al., 2007). Alomar et al. (2003) also observed a good prediction with R^2CVs of 0.82, 0.77, 0.82 and RMSECV of 0.44, 0.58, 0.48% w/w for IMF, moisture and protein, respectively. The lower prediction accuracy of chemical composition in the present study compared with other studies was probably due to using intact samples. According to Prieto et al. (2009), sample presentation plays an important role in the reliability of NIR prediction, particularly lack of homogeneity of meat samples reduces the accuracy of prediction of chemical parameters. For example, Cozzolino & Murray (2002) and Barlocco et al. (2006) obtained markedly better prediction accuracy to estimate chemical composition using minced meat samples for NIR measurement compared with intact samples. In intact samples, the muscle fibres or myofibrils themselves could act as optical fibres, which may conduct light along their length due to a series of internal reflections. Moreover, compared with homogenized muscle, intact muscle absorbs more energy thereby giving less reflectance (Prieto et al., 2009). In contrast, the structure of homogenized muscle is disrupted, thus the fibre arrangement of the muscle is randomized and the effects of scattering by fibres are averaged (Barlocco et al., 2006). In addition, intact samples have a very heterogeneous distribution of fat, thus a lower correlation is to be expected. Ground or minced meat is a relatively homogeneous mixture which means the 'true' composition probably can be detected by a single NIR scan (Rødbotten et al., 2000).

As expected for the prediction of IMF content, the wavelengths showed the highest regression coefficients were related to the absorption of C-H bonds (1208 and 1722 nm) (Figure 8.4a), in agreement with that reported by Prieto et al. (2014). It is obvious that the regression coefficients plot of moisture (Figure 8.4b) shows the opposite trend to that of IMF because of the negative relationship between IMF and moisture content in beef. It has been reported that strong absorption bands associated to water were observed at wavelengths related to O-H bonds (1440 and 1940 nm) (Murray & Williams, 1987; Shenk et al., 1992). The regression coefficient of moisture at 1900 nm was remarkable in this study while that at 1440 nm was not apparent. The highest regression coefficients for the prediction of protein content were located at wavelengths of 450-600 nm (Figure 8.4c), which related to heme

protein, the muscle pigments oxymoglobin and myoglobin (Cozzolino & Murray, 2002). The wavelength range of 1132-1154 nm and 1384-1410 nm (mainly C-H bonds) in this study also showed the apparent regression coefficient to predict protein content. This is in agreement with Prieto et al. (2006) who found crude protein fraction showed the same correlation pattern as that for fat, but inverted. In addition, others also found some other wavelengths correlate well with protein content, which related to N-H bonds (1510, 1980, 2055 and 2570 nm) (Murray & Williams, 1987; Shenk et al., 1992; Alomar et al., 2003).

8.4.3.4 PLSR prediction of collagen characteristics and sensory attributes

The prediction results for collagen content agree with those reported by others (Alomar et al., 2003; Prieto et al., 2006; De Marchi et al., 2007) (Table 8.2). It was concluded that the overall unsatisfactory NIRS prediction ability of collagen found in beef could be due to the fact that the NIR spectrum of the collagen fraction and the myofibrillar proteins are not much different, while the latter are present in muscle at a 10 times higher concentration (Downey & Hildrum, 2004). Soluble collagen had the same regression coefficients as total collagen (Figure 8.4 d & e). The regression found between collagen and optical density was at around 540, 640, 1088, 1362, 1516, and 1740 nm. Mitsumoto et al. (1991) found a correlation between them at around 955 and 1080 nm. In accordance with our results, Prieto et al. (2006) reported the highest regression coefficients with collagen content at wavelengths related to C-H (1132, 1388 nm) and S-H (1740 nm) bonds. They also stated that these wavelengths correlate more with fat fraction, which could explain why collagen content could not be predicted well.

There was no correlation found between NIRS and sensory attributes in the present study. Likewise, the overall ability of NIRS to predict sensory attributes of beef in previous studies was low or moderate (R^2CV = 0.003-0.5; RPD = 0.57-1.67) (Byrne et al., 1998; Rødbotten et al., 2000; Venel et al., 2001; Liu et al., 2003; Ripoll et al., 2008). Firstly, the limited prediction ability for sensory parameters could be due to the heterogeneity of meat samples especially the intact meat used in NIRS measurements in the present study. Ripoll et al. (2008) obtained a relatively higher NIRS prediction ability for sensory attributes using minced beef (R^2CV of 0.98 for tenderness, R^2CV of 0.59 for juiciness and R^2CV of 0.59 for overall appraisal). Secondly, the low precision of the reference method could also be the reason because

of the subjectivity of the assessors even though they are well trained (Warriss, 2004). Thirdly, in NIRS, the fundamental molecular vibration modes are measured with overtones, which are often overlapped with yield broad bands. Hence it can not provide high resolution spectroscopic fingerprints of different molecular functional groups, which further limits the accuracy of the biochemical profiling of meat (Wang et al., 2012). Thus the combination of the influence of the features of NIRS itself and the possible moderate precision of the reference method contributed to the low prediction ability of NIRS for sensory analysis.

8.4.4 PCA analysis

Mean NIR spectra differences were detected for very tender (WBSF-d14 < 31.36 N) or tough (WBSF > 45.08 N) samples between 1450-2400 nm (Figure 8.5a). Absorption was higher for tough beef than for tender beef and similar findings were reported by others (Park et al., 1998; Rødbotten et al., 2000; Leroy et al., 2003). Beef samples with low IMF content (< 3%) had overall higher absorbance than that those with higher IMF content (> 5%) (Figure 5 b), probably due to increased light scattering from the fat.

Figure 8.6 showed the mean spectra corresponding to each group from different production treatment and breed. These mean spectra had the same shape and the same main absorbance bands as each other and as those presented in Figure 8.1. At all wavelengths, samples from treatment 3 had the lowest absorption values, followed by treatment 4 and 5 (Figure 8.6a). Beef from treatment 3 had higher IMF content (referring to Table 3.3 in Chapter 3) which may result in increased light scattering of tissue resulting in reduced light absorbance (Rødbotten et al., 2000). Only small spectral differences between breeds were detected but HF beef had lower absorption between 1448 and 2446 nm than JEX beef (Figure 8.6b). This was probably due to the direct effect of lightness value, and the paler colour (higher L*) of HF beef (referring to Table 2.3 in Chapter 2) related to the increased light scattering.

The PCA score plot showed that the first two principle components (PC) explained 94% of the variation among samples and the first principle factor seems to differentiate between slaughter ages (Figure 8.7 a&b). In the right part of the score plot few 15-month old bulls are present, while they dominate on the left side. The

score plot of 3D scatter is illustrated in Figure 8.7b, which showed a similar trend with the 2D plot in Figure 8.7a. However, no significant differences could be observed between the mean spectra of these two age groups slaughtered in year 2013 (Figure 8.7c).

Even though PC1 explained the highest variation among muscles (Figure 8.8a), the major separation was observed in PC2 (Figure 8.8b). ST muscles were mainly located in left part of plot, LT was in the right part, and GM in the middle. The mean spectra also confirmed this trend that at all wavelengths the absorption of GM was in the middle position between LT and ST (Figure 8.8d). This probably reflected the differences in several chemical fractions between muscles. It was interesting to note that in the wavelength region of 400 to 1400 nm, LT had the highest absorption, followed by GM and ST, while within the later region the inverse pattern was detected. Several quality traits differ between these muscles including colour, fat content, cooking loss and tenderness, and the combination effect of these quality traits on absorbance values still needs to be investigated.

The first two PC explained 84% of the variation of two age groups slaughtered in year 2014 (Figure 8.9 a&b). The 19-month old bulls were located in the left and lower part of the score plot while 15-month old bulls were in the right and upper part. Absorbance values were greater for 15- than for 19-month old bulls (Figure 8.9c). This ability to differentiate is probably partly based on the indirect effect of different chemical fractions between the two groups. As the direct lightness value was similar between two age groups (referring to Table 5.1 in Chapter 5), and IMF content difference between two age groups was only for LT muscle (referring to Table 5.2 in Chapter 5), the difference of cooking loss may contribute to the variation in absorption. Water loss during cooking is mainly from the juice expelled by the myofibrillar lattice shrinkage caused by protein denaturation. More muscle fibre shrinkage also creates larger gaps between fibres which could allow for an increased light scattering (Hughes et al., 2014). Accordingly, the higher cooking loss of beef from 19-month old bulls (referring to Table 5.2 in Chapter 5) results in increased light scattering in tissue, which also contributes to reduced light absorbance (Rødbotten et al., 2000).

The first two PC explained 96% of the variation of the two sexes with bulls mainly located in the positive part of PC2 score plot, while steers were mainly in the negative part (Figure 8.10 a&b). Overall spectrum absorption was greater for bulls than steers especially during the region between 440 to 1300 nm (Figure 8.10c). This was probably due to the darker muscle (lower L*) of bulls compared to steers (referring to Table 6.1 in Chapter 6). The paler appearance of steer beef is related to increased light scattering in muscle, which results in decreased light absorbance (Rødbotten et al., 2000).

8.5 Conclusions

In the present study, beef quality traits from young male dairy cattle were better predicted when using Vis-NIR spectra (400-1900 nm) rather than full NIR spectra (400-2500 nm) or NIT spectra (850-1100 nm). Predictions from intact samples were quite good for colour parameters, especially for CIE a* and chroma values while only moderately high or unsatisfactory for other quality traits, probably due to collecting NIR spectra on intact samples. Prediction results of WBSF and cooking loss did not significantly change during ageing. On the other hand, NIR spectra could be used as a helpful tool for identifying or separating beef samples with different production characteristics such as age, muscle type, sex or with different quality categories (varied tenderness or IMF% level). The present findings suggest that Vis-NIR might be used for monitoring or screening purposes to assess some quality traits where rapid and non-invasive methods are required. However in the future study, homogenised samples could be tried for NIRS measurement on this type of beef which may help to reduce heterogeneity thus improving the prediction capacity.

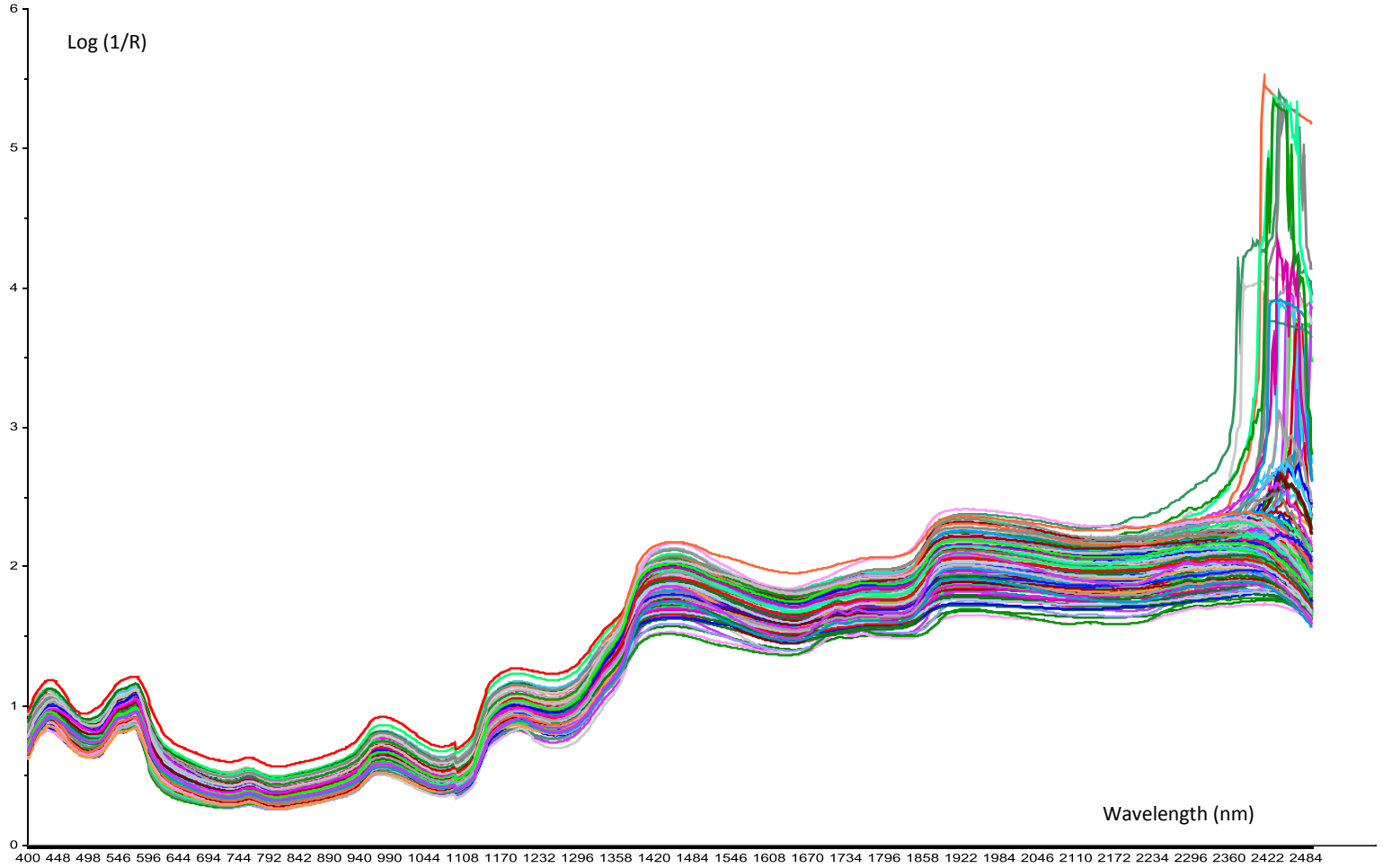
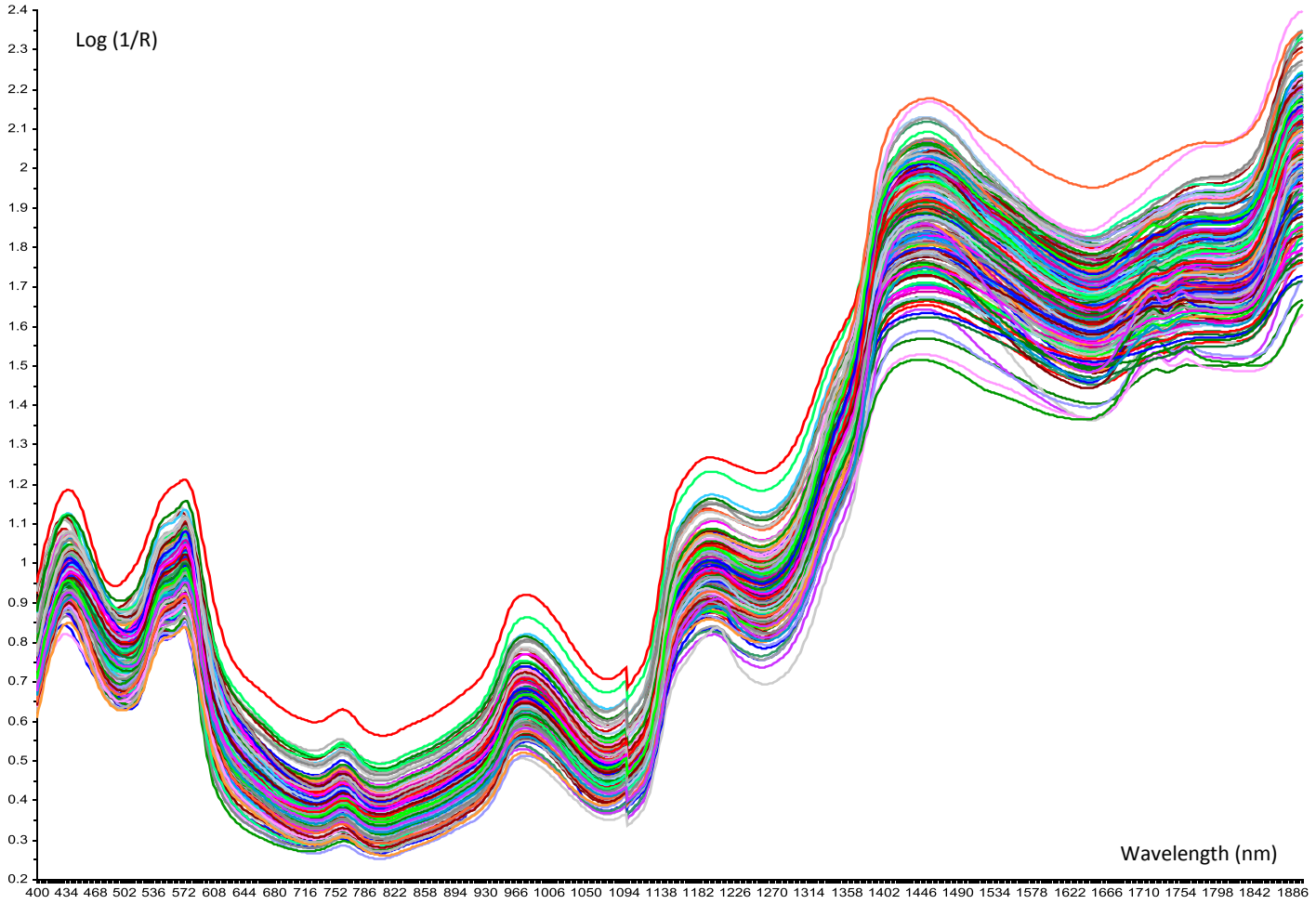
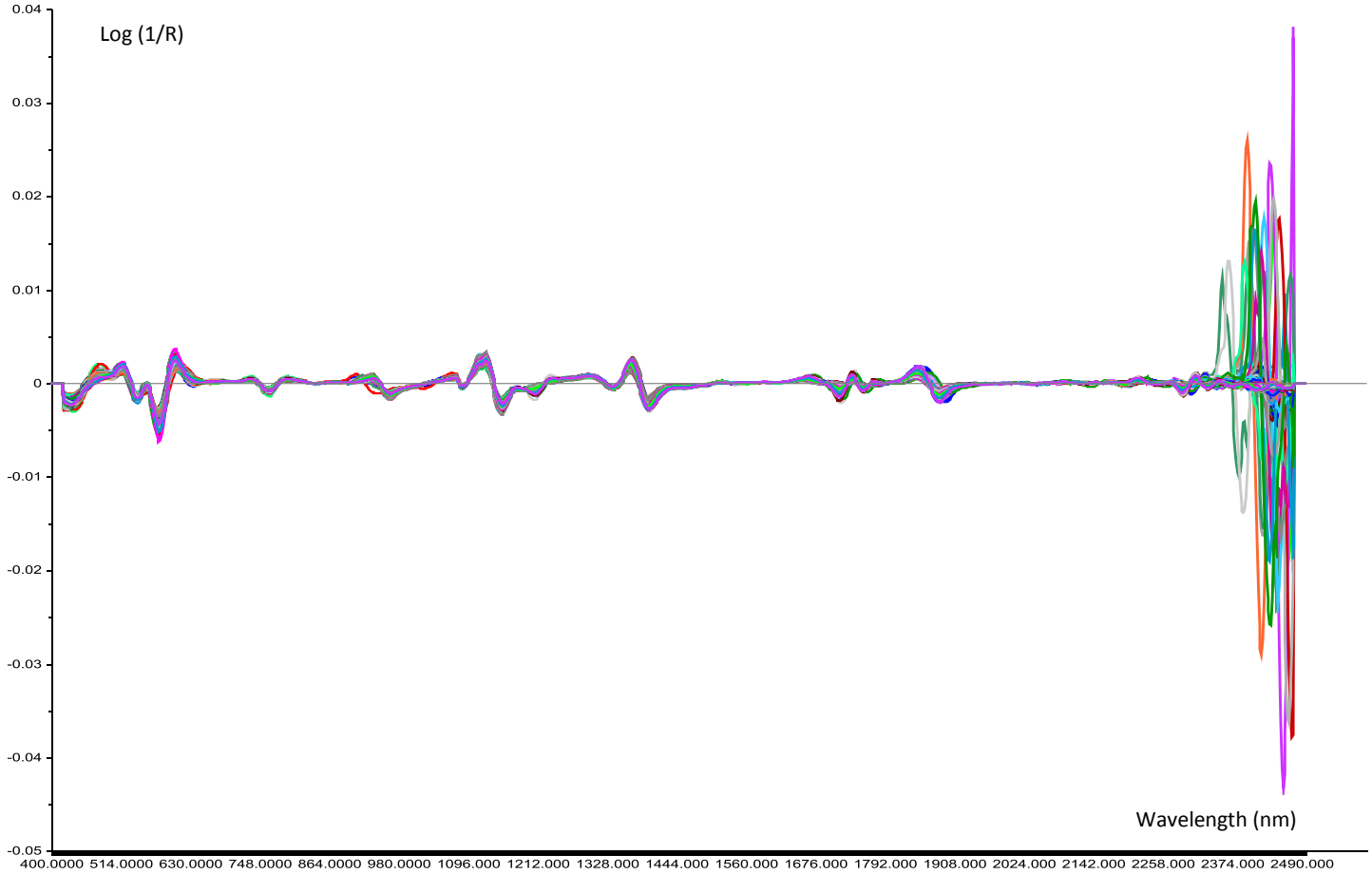


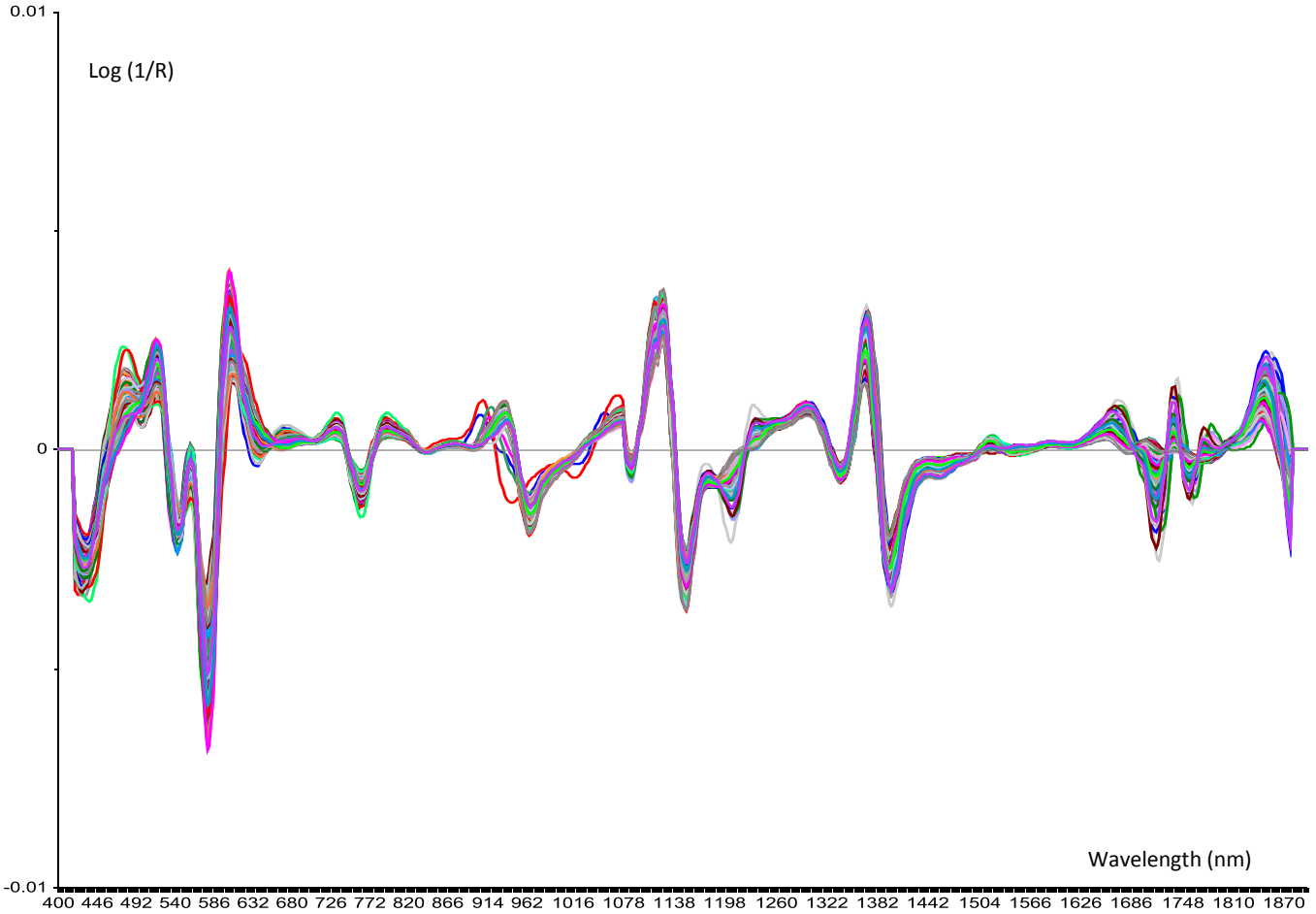
Figure 8.1. a) Raw near infrared spectrum of all bull beef samples (n = 221) over the wavelength range 400−2500 nm.



b) Raw near infrared spectrum of all bull beef samples (n = 221) over the wavelength range 400−1900 nm.



c) Averaged near infrared full spectrum (400-2500 nm) pre-treated by standard normal variate (SNV) and the Savitzky Goly second derivative (n = 221).



d) Averaged near infrared spectrum (400-1900 nm) pre-treated by standard normal variate (SNV) and the Savitzky Goly second derivative (n = 221).

Table 8.1. Description of laboratory reference values and statistics of prediction models for pHu and colour parameters developed using visible and near infrared reflectance spectroscopy (Vis-NIR, 400-1900 nm).

Spectral Data	n[1]	Range	Mean	SD[2]	CV[3]	Loading	R^2C[6]	RMSEC[7]	R^2CV[8]	RMSECV[9]	RPD[10]	Maths[4]
pHu[5]	210	5.24-6.59	5.68	0.19	3.35	3	0.54	0.13	**0.49**	0.14	1.36	SNV+2ndDer
MiniScan L* 2h	100	29.0-44.2	35.6	2.93	8.24	8	0.74	1.49	**0.63**	1.80	1.63	SNV+2ndDer
MiniScan a* 2h	100	13.3-24.3	17.5	2.48	14.2	6	0.73	1.28	**0.62**	1.54	1.61	SNV+2ndDer
MiniScan b* 2h	100	11.0-21.3	15.6	2.67	17.1	6	0.65	1.34	**0.57**	1.49	1.79	Log(1/R)
MiniScan Chroma 2h	100	17.5-31.0	23.5	3.29	14.0	6	0.70	1.79	**0.61**	2.08	1.58	SNV+2ndDer
MiniScan Hue angle2h	100	37.4-46.9	41.7	2.69	6.45	5	0.55	1.13	**0.42**	1.28	1.32	Log(1/R)
MiniScan L* 24h	88	27.8-45.9	37.3	3.38	9.05	9	0.76	1.66	**0.66**	1.99	1.70	Log(1/R)
MiniScan a* 24h	88	10.6-27.6	17.1	3.46	20.3	10	0.82	1.45	**0.68**	1.99	1.74	SNV+2ndDer
MiniScan b* 24h	88	8.90-24.4	15.8	3.38	21.4	9	0.74	1.71	**0.61**	2.08	1.63	Log(1/R)
MiniScan Chroma 24h	88	14.2-36.8	23.3	4.56	19.6	9	0.75	2.25	**0.64**	2.75	1.66	Log(1/R)
MiniScan Hue angle 24h	88	31.8-49.3	42.8	4.14	9.68	6	0.73	2.15	**0.66**	2.39	1.73	Log(1/R)
UltraScan L* 2h	196	36.7-58.4	43.3	3.08	7.11	3	0.50	2.16	**0.48**	2.22	1.39	Log(1/R)
UltraScan a* 2h	196	5.68-23.9	14.6	3.07	21.1	8	0.62	1.88	**0.52**	2.12	1.45	Log(1/R)
UltraScan b* 2h	196	3.96-20.2	11.3	2.55	22.5	10	0.71	1.38	**0.50**	1.81	1.41	SNV+2ndDer
UltraScan Chroma 2h	196	7.25-31.3	18.5	3.87	21.0	10	0.73	2.00	**0.53**	2.68	1.44	SNV+2ndDer
UltraScan Hue angle 2h	196	27.5-50.3	37.9	3.17	8.37	5	0.59	2.01	**0.54**	2.16	1.47	Log(1/R)
UltraScan L* 24h	196	35.0-55.3	44.7	2.76	6.18	3	0.48	1.98	**0.60**	2.05	1.35	Log(1/R)
UltraScan a* 24h	196	6.35-23.0	16.0	3.52	22.0	9	0.81	1.54	**0.76**	1.74	2.02	SNV+2ndDer
UltraScan b* 24h	196	6.27-18.5	12.8	2.22	17.4	5	0.73	1.15	**0.69**	1.23	1.80	Log(1/R)
UltraScan Chroma 24h	196	9.09-28.2	20.5	3.96	19.3	6	0.76	1.93	**0.73**	2.08	1.90	Log(1/R)
UltraScan Hue angle 24h	196	31.7-51.7	39.0	3.62	9.28	6	0.67	2.08	**0.60**	2.31	1.57	Log(1/R)

[1]n = number of samples; [2]SD = standard deviation; [3]CV = coefficient of variation; [4]Maths = mathematical treatments. [5]pHu = ultimate pH.
[6]R^2C = the coefficient of determination on calibration; [7]RMSEC = root mean square error of calibration; [8]R^2CV = the coefficient of determination in cross-validation; [9]RMSECV = root mean square error of cross-validation; [10]RPD = the ratio performance deviation.

Table 8.2. Description of laboratory reference values and statistics of prediction models for WB-variables, cooking loss at different ageing time, proximate chemical composition, soluble and total collagen developed using visible and near infrared reflectance spectroscopy (Vis-NIR, 400-1900 nm).

Spectral Data	n[1]	Range	Mean	SD[2]	CV[3]	Loading	R^2C[7]	RMSEC[8]	**R^2CV[9]**	RMSECV[10]	RPD[11]	Maths[4]
WBSF[5]-d3 (N)	130	17.9-86.6	46.7	15.2	32.5	7	0.55	10.1	**0.40**	11.9	1.28	SNV+2ndDer
WB-slope-d3 (Mpa)	130	0.28-1.71	1.02	0.32	31.4	7	0.53	0.21	**0.40**	0.25	1.28	SNV+2ndDer
WB-area-d3 (J)	130	0.14-0.60	0.33	0.10	30.3	6	0.48	0.07	**0.35**	0.08	1.25	SNV+2ndDer
Cooking loos-d3 (%)	130	26.7-36.7	32.2	2.28	7.08	6	0.45	1.68	**0.30**	1.92	1.19	SNV+2ndDer
WBSF-d7 (N)	166	19.2-75.4	41.9	12.6	30.1	5	0.41	9.66	**0.27**	10.8	1.17	SNV+2ndDer
WB-slope-d7 (Mpa)	166	0.24-1.51	0.91	0.27	29.7	6	0.45	0.20	**0.30**	0.23	1.17	SNV+2ndDer
WB-area-d7 (J)	166	0.10-0.54	0.29	0.09	31.0	4	0.45	0.07	**0.38**	0.07	1.29	SNV+2ndDer
Cooking loos-d7 (%)	166	22.4-37.0	30.7	2.86	9.33	7	0.57	1.86	**0.45**	2.12	1.35	SNV+2ndDer
WBSF-d14 (N)	166	18.8-79.0	39.0	12.9	33.2	5	0.42	9.83	**0.31**	10.8	1.20	SNV+2ndDer
WB-slope-d14 (Mpa)	166	0.33-1.44	0.82	0.26	31.7	8	0.52	0.18	**0.39**	0.21	1.24	SNV+2ndDer
WB-area-d14 (J)	166	0.12-0.64	0.30	0.11	36.7	7	0.55	0.07	**0.46**	0.08	1.38	SNV+2ndDer
Cooking loos-d14 (%)	166	23.7-39.4	31.6	2.98	9.45	6	0.49	2.13	**0.40**	2.33	1.28	SNV+2ndDer
IMF[6] (%)	221	0.05-9.77	2.33	1.85	79.4	7	0.54	1.25	**0.48**	1.33	1.39	SNV+2ndDer
Moisture (%)	221	68.4-78.6	74.2	1.67	2.25	7	0.53	1.15	**0.47**	1.22	1.37	SNV+2ndDer
Protein (%)	221	20.8-24.3	22.6	0.68	3.02	1	0.14	0.63	**0.11**	0.64	1.06	SNV+2ndDer
Soluble collagen (mg/g)	141	0.21-1.80	0.64	0.30	46.9	6	0.42	0.23	**0.32**	0.25	1.20	SNV+2ndDer
Total collagen (mg/g)	141	1.51-7.31	3.13	1.06	33.9	5	0.30	0.88	**0.15**	0.98	1.08	SNV+2ndDer

[1]n = number of samples; [2]SD = standard deviation; [3]CV = coefficient of variation; [4]Maths = mathematical treatments.
[5]WBSF = Warner-Bratzler shear force; [6]IMF = intramuscular fat.
[7]R^2C = the coefficient of determination on calibration; [8]RMSEC = root mean square error of calibration; [9]R^2CV = the coefficient of determination in cross-validation; [10]RMSECV = root mean square error of cross-validation; [11]RPD = the ratio performance deviation.

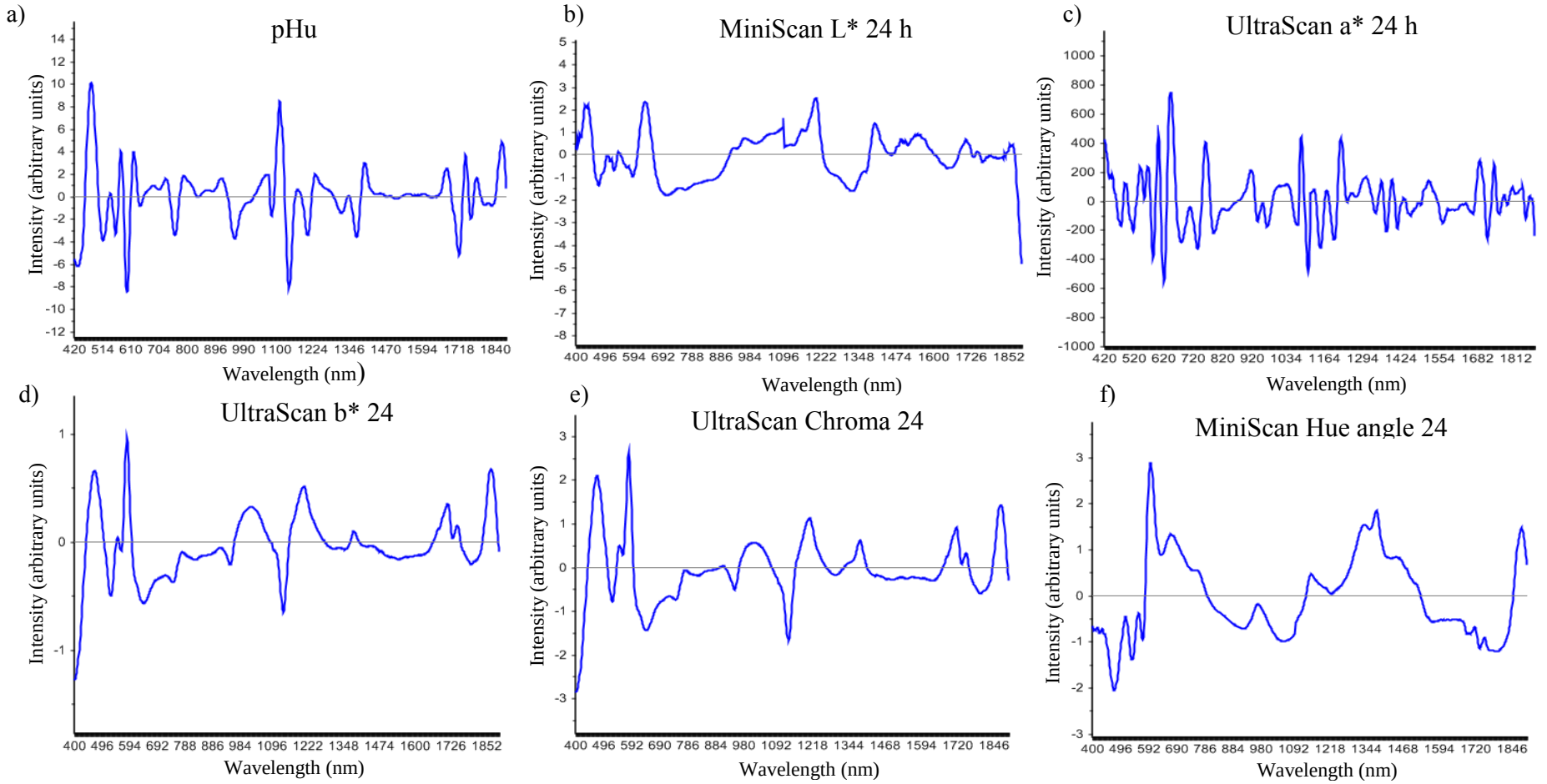


Figure 8.2. Regression coefficients plots of ultimate pH (pHu) and colour parameters after blooming for 24 hour. a) pHu; b) MiniScan L* 24 h c) UltraScan a* 24 h; d) UltraScan b* 24 h e) UltraScan Chroma 24 h f) MiniScan Hue angle 24 h.

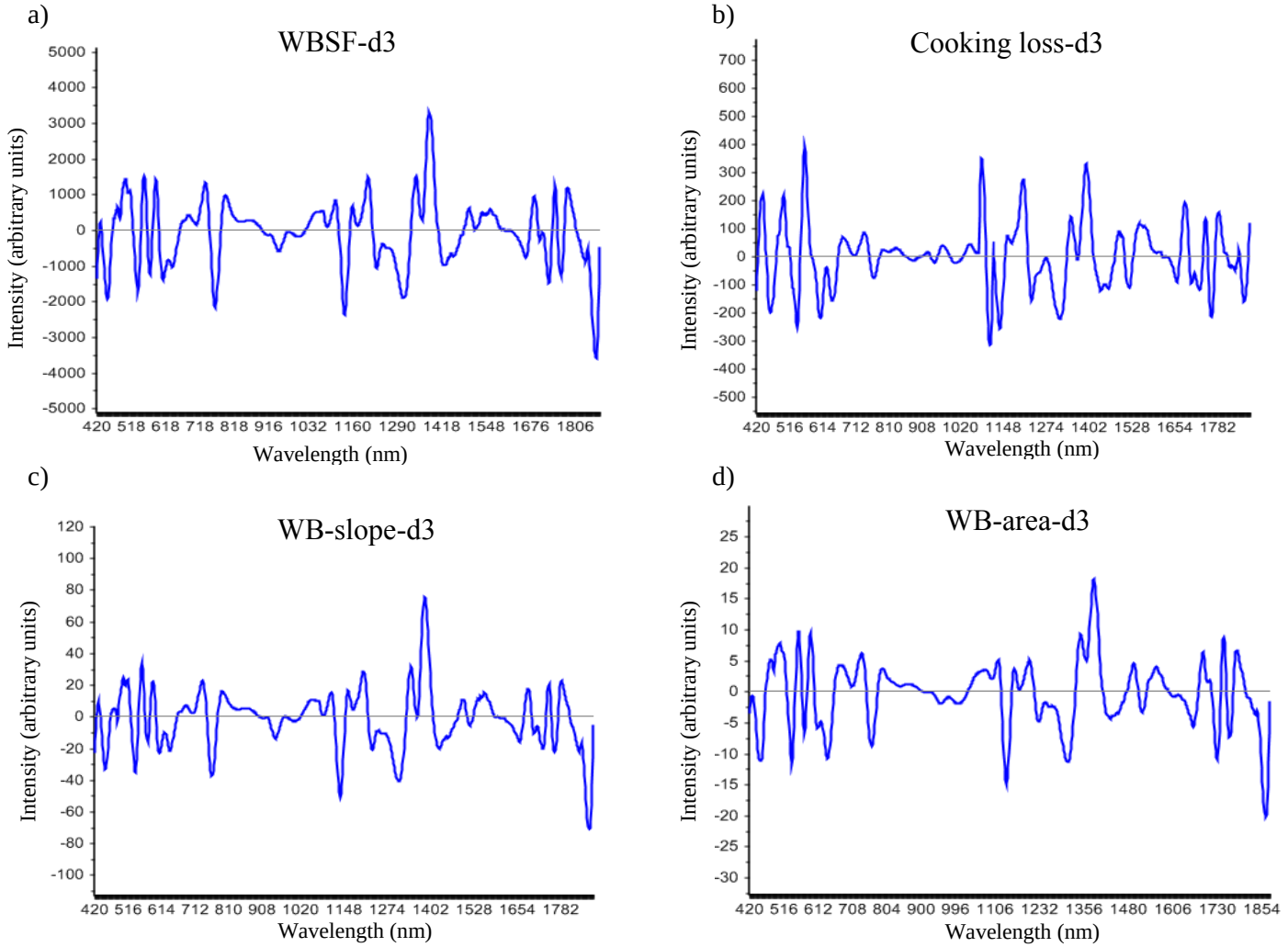


Figure 8.3. Regression coefficients plots of WB-variables (a) (c) (d) and cooking loss (b) on the 3rd post-mortem days.

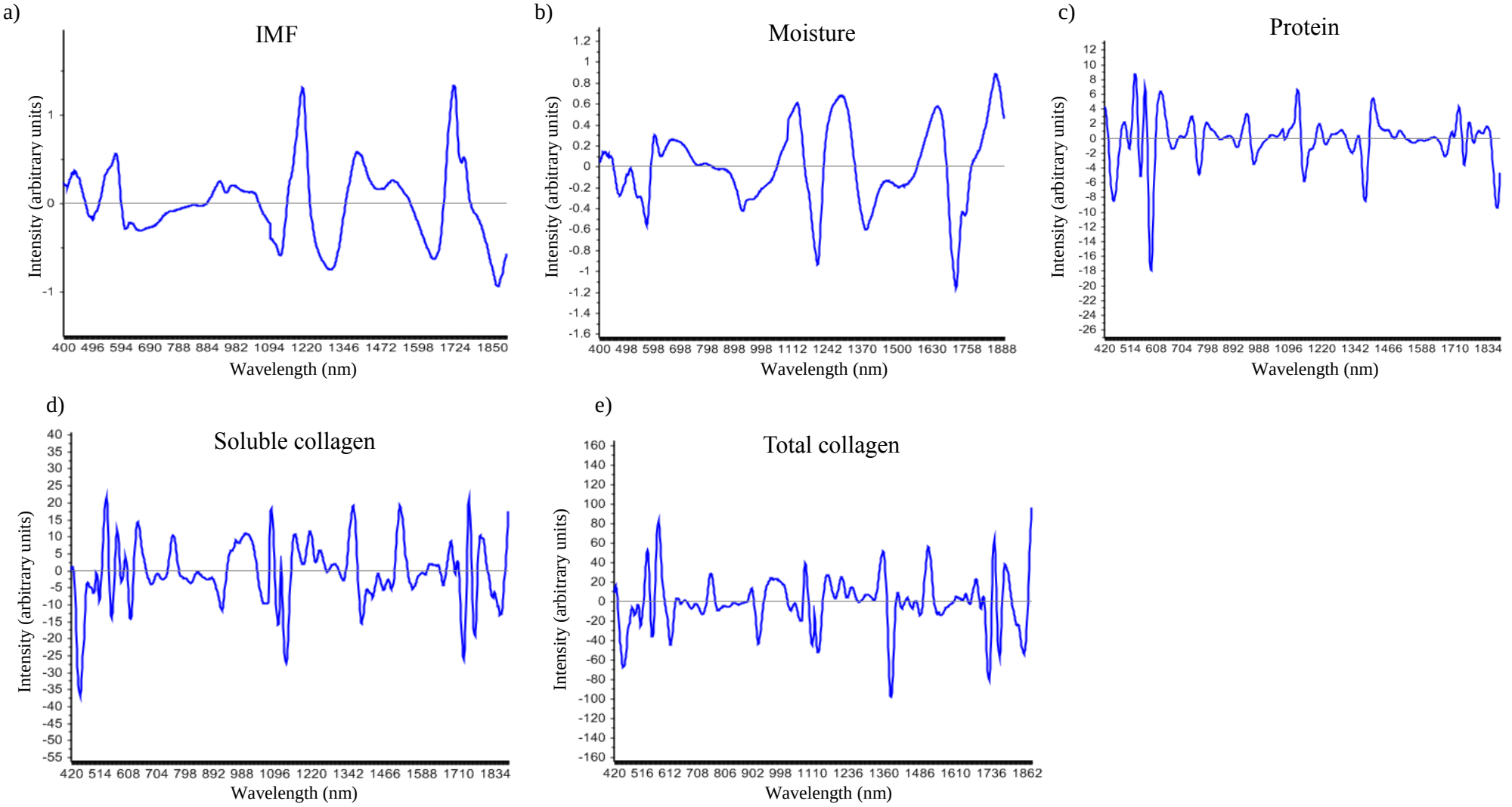


Figure 8.4. Regression coefficients plots of proximate chemical composition (a-c), soluble and total collagen (d & e).

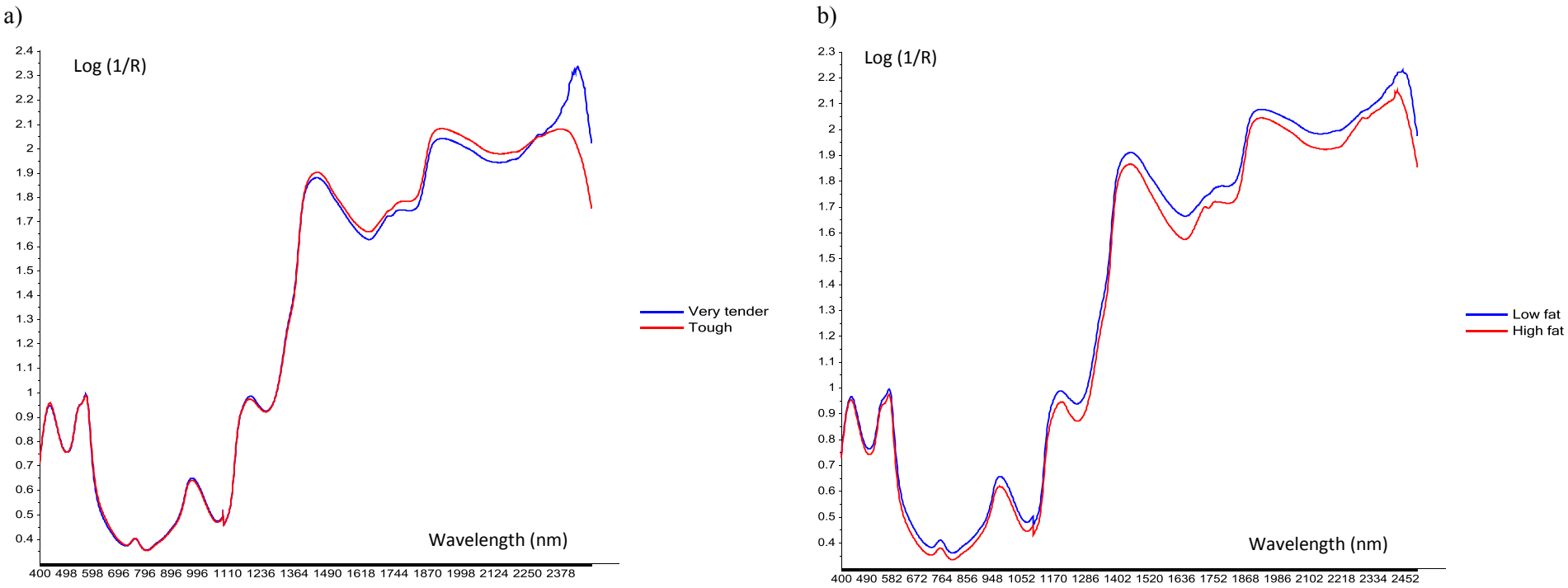


Figure 8.5. Average spectra of samples with high and low WBSF (day 14) (n = 166) (a) and IMF (n = 221) (b) obtained by the full wavelength range 400-2500 nm.

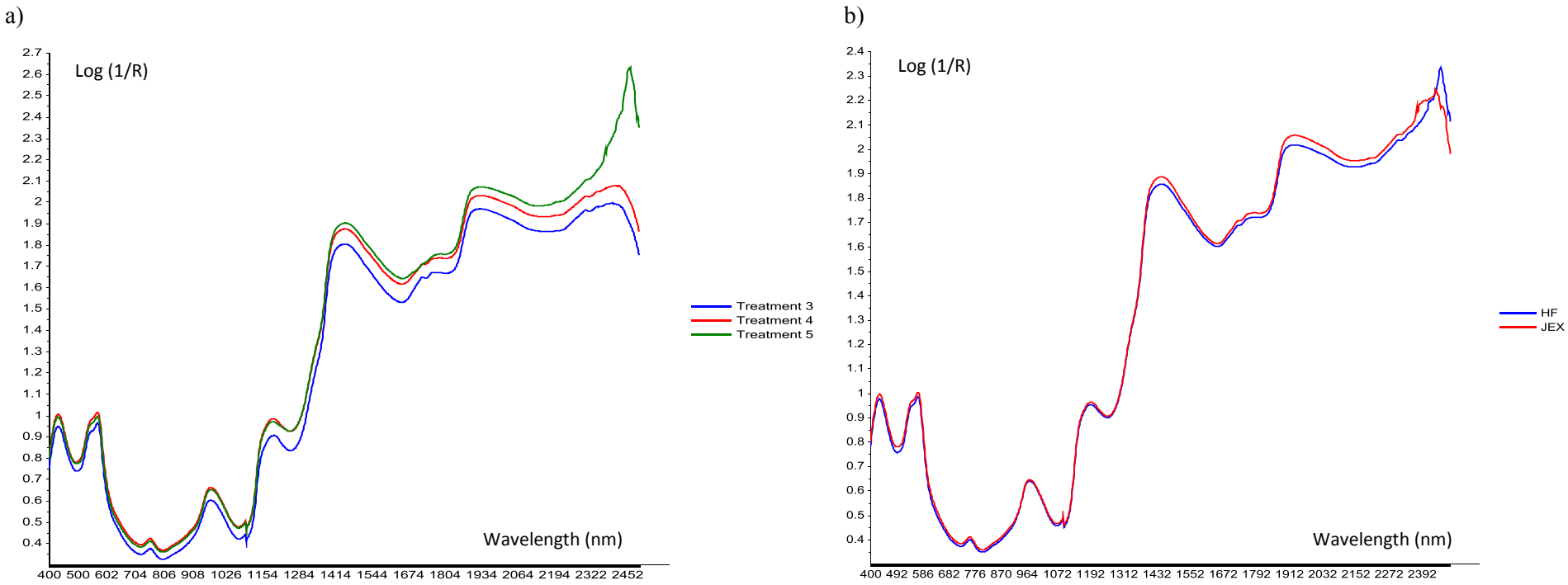


Figure 8.6. Average spectra of samples from three feeding treatments (a) and two breeds (b) slaughtered in 2012 (n = 54) obtained by the full wavelength range 400-2500 nm (Treatment 3–19-HC; Treatment 4–19-MC; Treatment 5–19-LC. HF–Holstein-Friesian; JEX–Jersey × Holstein-Friesian).

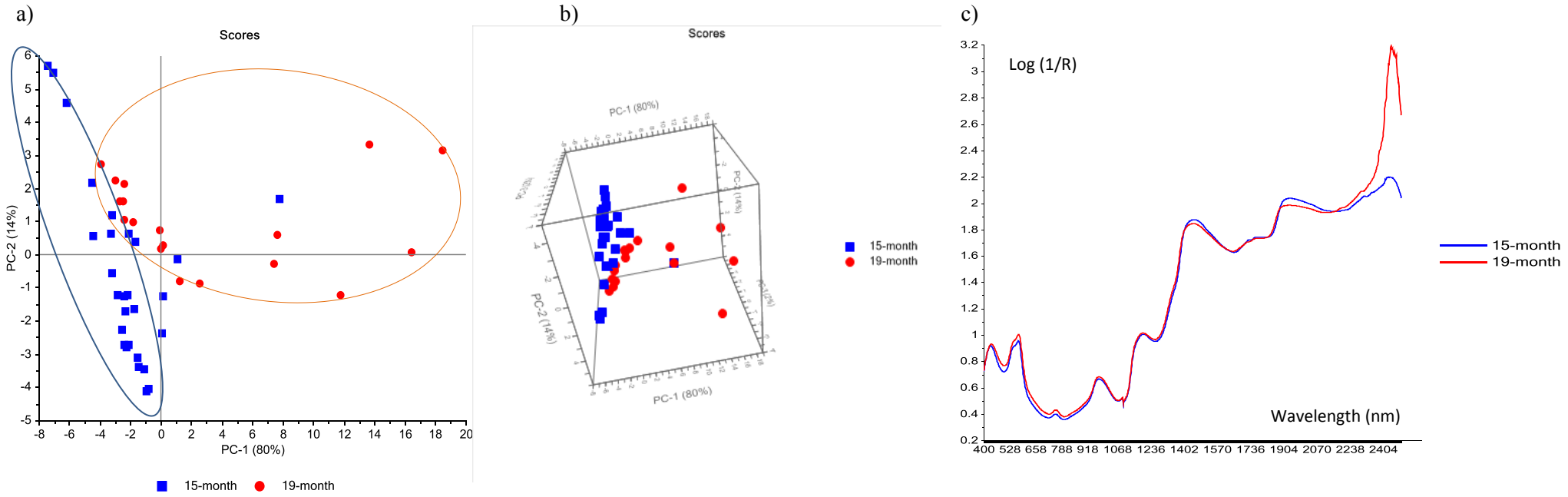
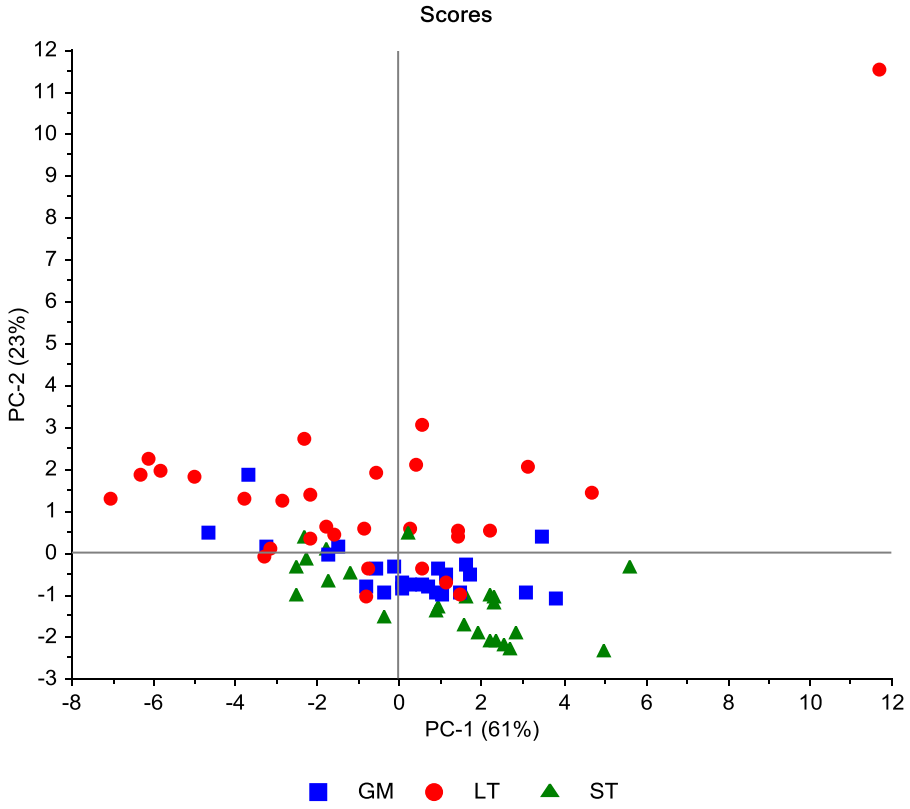
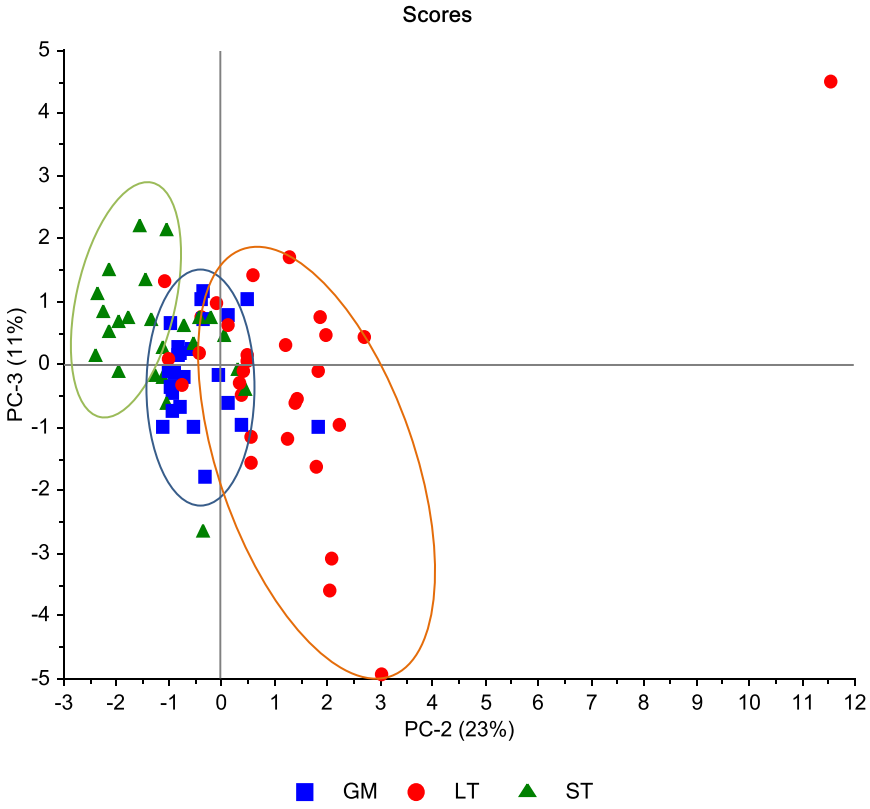


Figure 8.7. a) 2D scatter and b) 3D scatter PCA scores and c) average spectra of samples from two slaughter ages slaughtered in 2013 (n = 45) obtained by the full wavelength range 400-2500 nm.

a)



b)



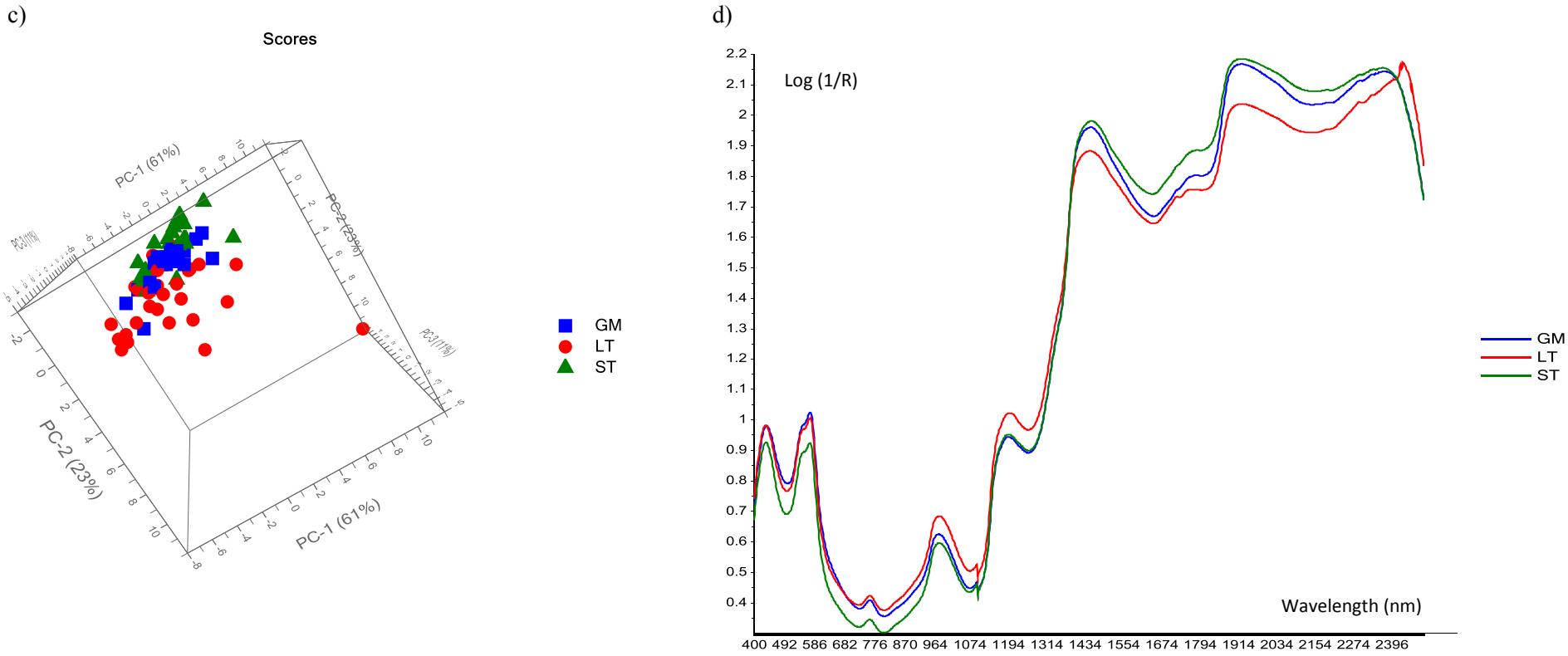


Figure 8.8. a), b) 2D scatter and c) 3D scatter PCA scores and d) average spectra of samples from three muscle types slaughtered in 2014 (n = 78) obtained by the full wavelength range 400-2500 nm.

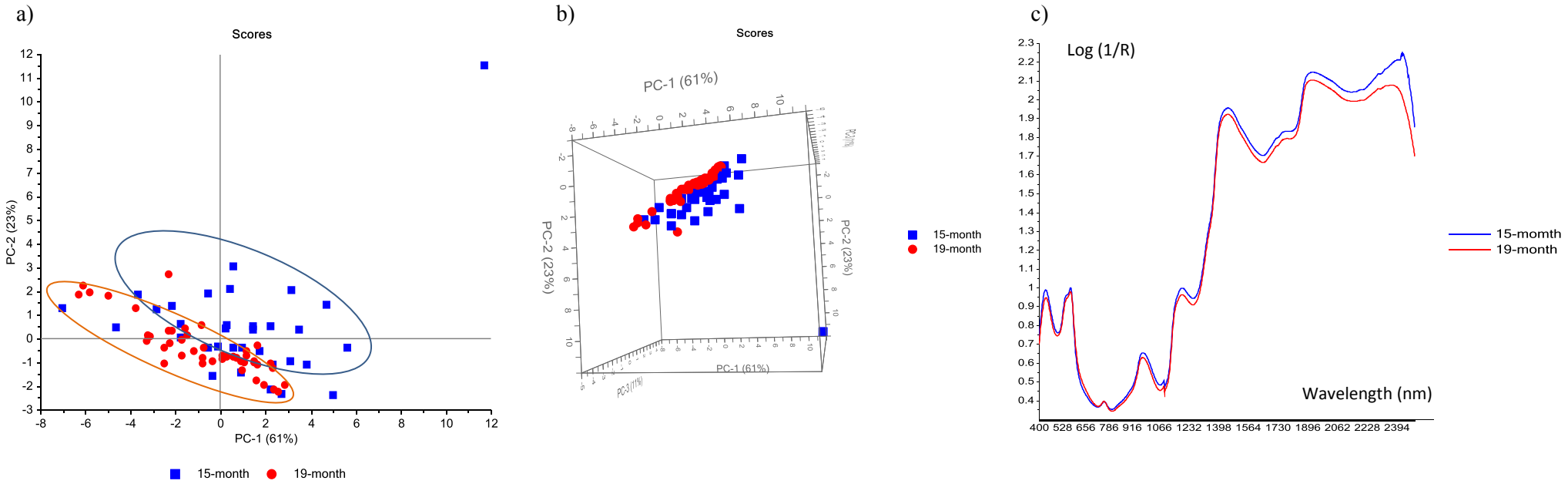


Figure 8.9. a) 2D scatter and b) 3D scatter PCA scores and c) average spectra of samples from two slaughter ages slaughtered in 2014 (n = 78) obtained by the full wavelength range 400-2500 nm.

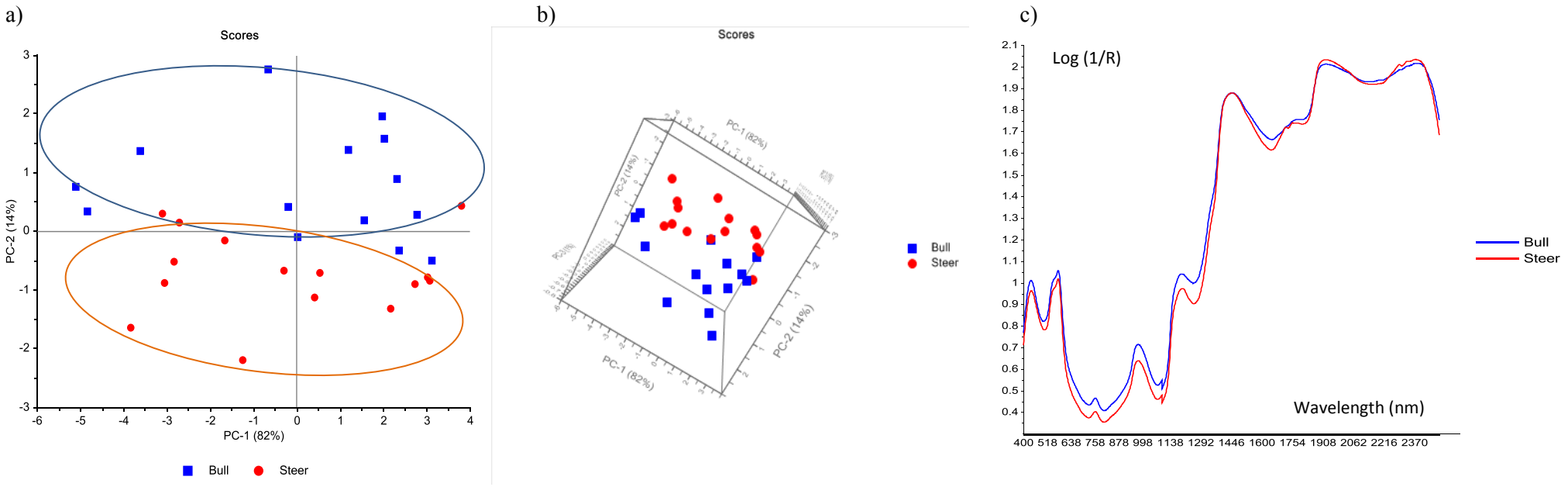


Figure 8.10. a) 2D scatter and b) 3D scatter PCA scores and c) average spectra of samples from two sexes slaughtered in 2014 (n = 29) obtained by the full wavelength range 400-2500 nm.

Chapter 9

Overall discussion and conclusion

At the consumer level, it has been shown that the strongest quality attributes for beef are tenderness, juiciness, taste (flavour), freshness, leanness, healthiness and nutritional values as intrinsic quality cues, as well as labels and brands as extrinsic quality cues (Verbeke et al., 2010). For the intrinsic quality cues interested in the present research, the following detailed categories for eating quality are evaluated in this thesis: technological quality traits including post-mortem pH-temperature window, pHu, colour, WB-variables and cooking loss; compositional quality traits including IMF, moisture, protein content and collagen characteristics; nutritional quality traits including FA composition; sensorial quality traits including 16 attributes.

Given the background, the general technological, compositional and sensorial quality traits of beef derived from two breeds and three slaughter ages (15, 19 and 22 months) were studied in Chapter 2. It showed good quality beef can be produced from young dairy bulls with different breed types expressed as most beef samples were acceptably tender after 21 days ageing. Breed type and age at slaughter affected some quality traits, but first season feeding system (pasture only vs pasture plus 2 kg concentrates) had no effect. With age at slaughter increasing from 15 to 22 months, beef became darker, moisture decreased and the meat was judged to hold a longer beef flavour length during sensory evaluation. JEX beef had lower cooking loss, was more greasy and more tender than HF beef.

Following Chapter 2, a larger scale study including technological, compositional and sensorial quality traits was established to better define the relative merits of the two breeds as well as production systems differing in dietary energy with slaughter at 15 and 19 months was undertaken in Chapter 3. This larger scale study confirmed the finding in Chapter 2 that improved beef quality of young dairy bulls by crossbreeding the Jersey breed with the HF due to the higher IMF content, sensory texture, flavour and juiciness scores and reduced instrumental WB-area value of JEX beef. It also agreed with the previous chapter that after 21 days ageing beef from young dairy bulls is generally acceptably tender. In agreement with the previous literature (Bailey, 1989; McCormick, 2009), insoluble and total collagen increased while collagen solubility decreased at the higher (19 month) slaughter age. It should be noted that the failure to find an effect on the darkness and flavour length between slaughter ages in Chapter 3 compared with Chapter 2 could be due to the shorter age

gap in the former (3 vs 6 months). The failure to find a significant difference in collagen characteristics between the two slaughter ages in previous chapter could be due to the method used. In Chapter 3, the novel UPLC-MS method was used for collagen determination, which could improve the accuracy over that of the traditional spectrophotometer method. The energy level of the diet had an obvious effect on quality properties and beef produced with a higher proportion of grass in the diet had inferior WHC of raw beef (higher thaw loss), but more intense red colour and increased moisture content and a higher score for astringent taste, but reduced IMF content, beef flavour intensity and shorter beef flavour length compared with the higher concentrate diet. However, similar to the first season effect in Chapter 2, different level of concentrate feeding in the first and second grazing seasons or silage mixed with concentrate had very limit effect on beef quality traits.

In order to investigate the nutritional quality of young dairy bull beef derived from different production systems and breed, the FA profile of subsamples from Chapter 3 was studied in Chapter 4. The relationship between FA composition and sensorial quality was also evaluated. Different dietary regimes markedly affected the FA composition of young dairy bull beef. Beef produced from a forage-based diet had relatively better nutritional characteristics than that from the concentrate-based diet expressed as containing less non-desirable SFAs and more total PUFAs or n-3 PUFA proportions. Again these significant findings were all from comparing finishing systems, while first and second grazing season feeding systems had very limited effect on the FA profile. Slaughter age also had no effect and breed only had slight effects on the FA profile. This chapter also showed that FA composition had a high correlation with beef palatability. However, the beneficial PUFAs, especially n-3 PUFA, were found to have negative correlations with IMF level, beef flavour intensity, tenderness and juiciness terms in sensory evaluation, thus an increase in PUFAs would have a detrimental effect on eating quality. Results also reflected the contribution of FA profile to the variation in sensory properties of beef between diets determined in Chapter 3.

The previous chapters only focused on the LT muscle, whereas beef quality of other muscles of dairy bulls was not explored yet. Moreover, the interaction between muscle type and dairy bull beef production system deserves to be investigated. Therefore, further research was undertaken in Chapter 5 to study technological,

compositional and nutritional quality traits of LT, ST and GM muscles from HF bulls finished at 15- and 19-months. Results in this chapter reflected the combination effect of age at slaughter and level of concentrate feeding on eating quality of young HF beef, which varied for individual muscles. In particular, the IMF content, total SFA and total FA concentrations were increased, but only in the LT muscle when bulls were finished at 15 months utilising a high energy diet. Tenderness and WHC of beef both decreased with increased slaughter age combined with the lower energy diet (19 months). Most physico-chemical quality traits varied between muscles. LT was consistently superior in tenderness, cooking loss and marbling. ST showed the highest cooking loss and lightest muscle, but had more intense colour. With the exception of GM and ST muscles from bulls produced from the of 19-month production system, HF bulls can produce acceptably tender beef after ageing for 14 days. In agreement with Chapter 3, the increased total collagen and insoluble collagen content but decreased collagen solubility at older age were also found in Chapter 5. Moreover, the changes observed in FA composition between age-production systems reflected mainly dietary influences rather than slaughter age, which is consistent with the finding in Chapter 4. Muscles varied in most individual and total FAs. The leaner ST muscle or meat derived from the 19-month production system had enhanced FA profiles, expressed as more desirable n-3 PUFA and PUFA/SFA ratio and less non-desirable SFAs and n-6/n-3 PUFA ratio.

In addition to the production factors described in the previous chapters, some post-mortem processing factors, i.e. hanging method, electrical stimulation, ageing etc., also play an important role in the eating quality of beef (Troy & Kerry, 2010). As the preliminary findings (Chapters 2, 3, 5) showed that young dairy bull beef is acceptably tender, post-mortem processing techniques other than ageing seem to be unnecessary to enhance the eating quality of young dairy bulls, thus the effect of post-mortem processing was not widely investigated in this thesis. However, how tenderness changes during ageing of beef from young male dairy cattle (both bulls and steers) is of interest and together with the effect of hanging method on HF steer beef quality (a market requirement) including technological, compositional and nutritional quality traits were studied in Chapter 6. In agreement with most findings by others (Morgan et al., 1993a; Purchas et al., 2002; Rodriguez et al., 2014) that castration can improve the palatability of beef, expressed as increased tenderness,

IMF content and decreased cooking loss, total collagen and insoluble collagen in the present study, which also applies to dairy breeds. FA composition was also greatly affected by castration. HF bulls had a nutritionally enhanced fatty acid profile compared with steers. Compared with Achilles tendon suspension, Pelvic suspension improved tenderness up to 7 days ageing and colour intensity of HF steers. Moreover, Pelvic suspension can accelerate ageing in comparison to Achilles tendon suspension.

As rapid and non-invasive techniques are required by the meat industry nowadays, optical spectroscopy methods including RS and NIRS were explored as alternative applications in this thesis to assess technological, compositional and sensorial quality traits of beef from young male dairy cattle in Chapters 7 and 8, respectively. Both RS and NIRS demonstrated considerable potential to assess a wide range of physical and chemical traits of young dairy beef. However RS showed the better predictive ability than NIRS. RS using a 1300-2800 cm^{-1} wavelength range was able to predict WBSF, cooking loss, IMF, moisture, protein, collagen content and solubility with high accuracy (R^2CV of 0.70-0.91 and RMSECV of 0.07 mg/g-6.82 N). On the other hand, visible and near infrared reflectance (Vis-NIR, 400-1900 nm) was only able to predict with moderately high ability WBSF, cooking loss, IMF, moisture and soluble collagen (R^2CV of 0.30-0.60 and RMSECV of 0.25 mg/g-10.8 N), and was unsatisfactory for protein and total collagen content. Furthermore, chemometrics of RS can monitor beef quality changes during ageing more sensitively than NIRS. Nevertheless, it should be noted that good prediction results were obtained using Vis-NIR spectroscopy to predict colour parameters (R^2CV of 0.60-0.70 and RMSECV of 1.23-2.75).

The most obvious difference in prediction performance between RS and NIRS in the present research is on sensorial quality traits. Compared with the non-correlation obtained for NIRS, RS-coupled with PLSR modelling is able to predict sensory attributes of young dairy bull beef. A moderate or high ability for prediction was obtained with R^2CV of 0.36-0.84 and RMSECV of 1.41-9.98 (referring to Appendix III). Particularly, RS yielded an excellent prediction performance for flavour terms including roast beef aroma (R^2CV of 0.76 and RMSECV of 5.01), roast beef flavour (R^2CV of 0.80 and RMSECV of 4.65) and residual roast beef flavour length (R^2CV of 0.84 and RMSECV of 4.21). The application of RS in the present research showed an overall better performance to predict physico-chemical quality traits and sensory

attributes of beef compared with other research on RS or other NIRS studies to date (Liu et al., 2003; Fowler et al., 2014; Fowler et al., 2015). The relatively lower prediction ability of NIRS compared with RS in the current thesis could be due to their own characteristics. In NIRS, the fundamental molecular vibration modes are measured with overtones, which are often overlapped to yield broad bands. Hence it can't provide high resolution spectroscopic fingerprints of different molecular functional groups, which further limits the accuracy of the biochemical profiling of meat (Wang et al., 2012). On the other hand, sample preparation could be another important reason for the limited predictive ability of optical spectroscopy methods (Prieto et al., 2009). In Chapter 7, minced beef samples were used for RS measurement rather than intact samples which were used for NIRS determination in Chapter 8, and homogenisation by definition gives a more representative sample with more homogeneity which thus could enhance the precision of assessment. In addition, RS and NIRS were both effective tools for the discrimination or separating dairy beef samples with different characteristics such as muscle and slaughter age, etc.

The knowledge gained in this thesis also provides valuable information on the detailed correlations among technological, compositional, sensorial and nutritional quality traits, which will help to understand the background principle of eating quality. Firstly, Chapters 2, 3, 5 & 6 agreed with each other for the correlations among technological and compositional parameters, which showed that IMF, cooking loss and soluble collagen content (or collagen solubility) were the main variables contributing to the variation of the WB-variables (cooked beef texture). Secondly, significant and consistent correlations between technological, compositional and sensorial parameters were observed in Chapters 2 & 3, which showed that higher WB-variables were associated with higher sensory score for chewiness, and lower scores for initial tenderness, ease of disintegration and juiciness. IMF was positively correlated with sensory tenderness terms, and negatively correlated with sensory toughness terms. Finally, Chapters 5 & 6 demonstrated consistent correlations between FA composition and some technological and compositional parameters, which indicated a negative relationship between nutritional value and eating quality. Dairy beef with a higher SFA proportion contained higher IMF content and lower cooking loss and was more tender. Furthermore, desirable PUFAs, especially n-3 PUFA, contributed to less

tender beef with lower WHC and less marbling. In addition, PLSR models conducted in Chapters 3, 5 & 6 showed the same trend as the Pearson correlations and confirmed the conclusions from the ANOVA analysis. Accordingly, the same correlations among quality parameters were found in beef of young male dairy cattle under different production conditions.

The pH-temperature profile in all chapters indicated that cattle produced from the specific production system with lighter carcass weight are more predisposed to cold shortening. Another agreement between chapters is that dairy beef achieved full blooming for colour after 24 h rather than 2 h. Colour parameters varied significantly between different production factors and the effects on colour could be important in terms of selecting carcasses for different markets. Although young male dairy cattle can produce good quality beef with good nutritional qualities, the relatively low amounts of IMF mean that this beef will make a relatively small contribution to the consumption of PUFAs as recommended in dietary guidelines (Chapters 5 & 6).

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Appendix I

Table. Main carcass traits of LT muscles of young dairy bulls.

	Treatment (T)										Breed (B)				*P*-value		
	15-AL		15-SC		19-HC		19-MC		19-LC		HF		JEX		T	B	T×B
	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM			
Slaughter weight (kg)	466^{b}	10.4	451^{b}	10.6	587^{a}	18.7	566^{a}	17.2	484^{b}	12.2	546^{a}	8.69	476^{b}	9.31	<0.001	<0.001	0.135
Hot carcass weight (kg)	237^{b}	6.14	228^{b}	6.29	305^{a}	11.1	291^{a}	10.2	249^{b}	7.22	283^{a}	5.15	241^{b}	5.52	<0.001	<0.001	0.115
Conformation score	4.69^{ab}	0.22	4.39^{ab}	0.23	5.21^{a}	0.40	5.13^{a}	0.37	3.81^{b}	0.26	5.13^{a}	0.19	4.17^{b}	0.20	0.011	0.001	0.904
Fatness class	5.39^{b}	0.24	5.08^{bc}	0.25	7.70^{a}	0.44	7.04^{a}	0.40	4.19^{c}	0.28	5.66	0.20	6.10	0.22	0.011	0.138	0.301

LSM = least square means; SEM = standard error of LSM. ^{a} ,^{b,} ^{c} Means within a row within a main effect with different superscripts significantly differ ($P < 0.05$).

15-AL–19-LC-Refer to the treatment design in Table 3.1.

Appendix II

Table. Raman shifts and structure correlations.*

Functional groups	Raman shifts (cm^{-1})	Assignments
Alkane (C-H)	2800-2750	-OCH_3 (m-s CH_3 str)
		CH_3(aliphatic) (m-s sym CH_3 str);OCH_3 (m-s, sh sym CH_3 str)
	1450-1320	Aldehydes (m-s CH in-plane rocking)
	1380-1355	CH_3 aliphatic (w-m sys CH_3 def)
Alkene (C=C)	2800-2750	vinyls,-CH=CH_2
	1425-1335	*cis* (sat) CH=CH (sat) (m-s CH def)
	1375-1340	thiophenes (s C=C in-plane vib)
	1350-1340	C=CH (hydrocarbons) (w CH in-plane def)
	1340-1260	*trans* (sat) CH=CH (sat) (s CH def)
Azo (–N=N–)	1465-1400	*trans*-aromatic azo compounds (s,p N=N str)
Alkyne (-C≡C-)	2310-2230	R-C≡C-R’
	2200-2100	alkyl alkynes (s C≡C str)
Nitrile (-C≡N)	2260-2200	aliphatic nitriles (s C≡N str)
Hydroxyl (O-H)	2800-2750	carboxylic acids (w,br OH str)
Ether (C-O-C)	~2780	−O-CH_2-O
	1475-1445	-OCH_3 (m-s CH_3 str)
	1470-1435	aliphatic nitriles (s C≡N str) (m-s sym CH_3 str)
Carbonyl (C=O)	1750-1725	α-Halo-ketones (m C=O str); Sat. aliphatic esters (m C=O str)
	1745-1715	Sat. aliphatic ketones (m C=O str)
	1740-1720	Sat. aliphatic aldehyde (w-m C=O str)
	1740-1700	Sat. aliphatic carboxylic acids (w-m C=O str)
Thiol (S-H)	1650-1590	Primary thioamides-Amide II band
	1480-1360	Primary thioamides-Amide III band
	1550-1500	Secondary thioamides-Amide III band
	2600-2500	aliphatic thiols and thiophenols (s, p S-H str); CH_2, SH (s, p S-H str)
Sulfur dioxide (O=S=O)	1380-1325	Sulphonamides (asym SO_2 str)
	1340-1320	Sulphonamides (sym SO_2 str)

Sat., saturated; sym, symmetric; asym, asymmetric; str, stretching; def, deformation; s, strong intensity; m, medium intensity; w, weak intensity.

*Adapted from Socrates, G. (2001).

Appendix III

Table. Summary of PLSR model performances (Raman shift 1300-2800 cm^{-1}) for the prediction of sensory attributes in bull beef (n = 72 and 21 days ageing). The results are all presented by S.G. 2nd der. using 2nd polynomial with 9 smoothing points, the data pre-treatment which showed the best results with the Martens' uncertainty test.

Attribute	# PLS loadings	R^2C	RMSEC	R^2CV	RMSECV	Bias
Roast Beef Aroma	2	0.85	3.92	0.76	5.01	-0.003
Initial Tenderness	2	0.54	8.84	0.45	9.83	0.00
Juiciness	2	0.69	5.41	0.47	7.1	0.00
Cohesiveness	2	0.72	5.15	0.61	6.12	-0.018
Ease of Disintegration	2	0.55	8.49	0.39	9.98	-0.061
Chewiness	2	0.68	7.79	0.55	9.33	0.031
Fattiness/Greasiness	2	0.67	2.68	0.48	3.38	0.032
Stringiness	3	0.78	2.27	0.52	3.37	-0.059
Astringent	2	0.63	2.66	0.52	3.04	0.03
Roast Beef Flavour	3	0.91	3.01	0.80	4.65	0.128
Metallic	2	0.55	2.47	0.36	3.01	-0.01
Stale/Rancid/Aged	2	0.61	1.17	0.45	1.41	-0.005
Res-Roast Beef Flavour Length	3	0.94	2.66	0.84	4.21	0.012
Res-Metallic	2	0.66	2.12	0.49	2.64	-0.003
Res-Fattiness/Greasiness	3	0.71	1.89	0.51	2.48	-0.005
Res-Dryness	2	0.67	2.54	0.48	3.25	-0.023

PLSR, partial least squares regression models; S.G., Savitzky Golay; der., derivatives; nor.u.v., normalisation on unit vectors;
PLS loadings, number of PLS loadings; R^2C, coefficient determination of calibration; RMSEC, root mean square error of calibration; R^2CV, correlation coefficient of determination in cross-validation; RMSECV, root mean square error of cross-validation. Res-' = Residual (after effects).