effects of different freezing/thaw-ageing regimens on physicochemical, biochemical characteristics and meat quality attributes of lamb loins

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Abstract

New Zealand red meat is in demand around the world; consumers recognise the safe, healthy, clean and green values of New Zealand's pasture-raised beef lamb and venison. Meat is transported chilled in containers to overseas markets, and recently container ships have started to travel slower to reduce fuel consumption. This has resulted in the meat spending longer in the containers, meaning that key quality attributes of lamb cannot be guaranteed anymore. Finding an alternative method to provide the overseas consumers with chilled meat of the required quality is a top priority for the industry. Currently during container transport, meat is ageing for 6/8 weeks but with the newly introduced longer transport time to overseas markets it is too long for the meat in terms of food quality. Frozen meat is traditionally aged first before freezing. Previous observations show that 2 weeks ageing for lamb is enough to give equivalent quality attributes to the meat as ageing for 8 weeks. In the current study meat is frozen first and then thawed-aged and it was therefore hypothesised that by increasing the thawing-ageing temperature the ageing process could be accelerated.

This thesis focused on determining the effects of different freezing/thawing-ageing regimens on meat quality and physical and biochemical characteristics of lamb loins. To study the effect of the different regimens, 10 treatments were set up and 90 loins in total have been used. The loins were randomly assigned to three different freezing conditions named Slow Freezing (SF), Fast Freezing (FF) and Very Fast Freezing (VFF) followed by three different thaw/ageing regimens: -1.5°C, 1°C and 3.5°C. A control group was included as treatment number 10, the Control group represents a non-frozen treatment with only 2 weeks of ageing.

The effects on the quality of the loins were evaluated through the use of quality measurements such as pH, colour, water holding capacity, tenderness and lipid oxidation. Other analyses used to give an indication of the mechanisms for any quality differences were histology and metabolomics. The data suggest that FF and VFF resulted in a greater water-holding capacity and colour stability than SF. Ageing at 3.5°C showed a general trend for accelerated lipid oxidation compared with ageing at -1.5°C and 1°C. SF seems to contribute to damage along the fibre whereas FF and VFF show obvious holes within the fibres. These different types of damage may lead to lesser or varying meat quality.

Overall, this work found that FF and VFF showed benefits over SF in several aspects of meat quality, but the differences were small. The results also suggest that FF or VFF could be used to avoid some of the common quality defects associated with freezing first and then thaw-ageing. This may potentially provide an opportunity for the New Zealand meat industry to utilise frozen storage/shipping and so saving money while still meeting the consumer demands for high quality fresh meat.

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1.1 From farm to plate

In order to understand the different factors affecting meat quality, it is important to have an overview of processes involved in the slaughter and handling of the carcass. Strict guidelines around such aspects as hygiene and animal welfare regulate the slaughter and process handling of the carcass. Although slaughter processes vary between abattoirs, the methods and treatments are similar.

1.2 The slaughter and carcass handling of lamb

Stunning is the first step of the slaughter process in most New Zealand meat processing plants. This can be achieved by several methods, but the most common is by passing an electric current through the brain of the animal. This causes an epileptic shock by stimulating all the parts of the brain simultaneously, rendering the animal unconscious and unable to feel pain or discomfort. An incision is then made in the neck with a knife, resulting in the animal's exsanguination and death. Following death, various electrical currents are applied to ensure that post-mortem reflex movement do not impede the slaughter operation, the pelt, head, legs and intestines are removed, leaving the carcass as the final product of the animal slaughter process (Committee, 2010).

After a period of approximately 24 hours, rigor mortis occurs, with the muscles becoming stiff, rigid and contracted (James & James, 2002). After the carcass has been held for the required 24 hours it is boned into commercial cuts. This method is known as cold boning because the carcass has reached the chiller temperature and rigor mortis is complete before boning(Strydom, Frylinck & Smith, 2005). The cuts are then packed using appropriate

oxygen barrier materials, such as vacuum bags. Within 30 minutes of excision from the carcass the cuts are back under refrigeration.

1.3 The metabolic conversion of muscle to meat

1.3.1 Structure of the muscle

The carcass is the product of the animal slaughter process. Carcass meat consists of muscle, fat, and connective tissue. The most important component of the carcass is the muscle. The animal body has more than 600 muscles of various shapes, sizes and actions. Specific characteristics of each muscle are dictated by the specialised function that particular muscle performs. Each muscle is covered with a connective tissue sheet, which continues into the interior of the muscle (Aberle, Forrest, Gerrard, Mills, Hedrick & Judge, 2001).

Muscles from mammals have a similar structure. The entire muscle is surrounded by a connective tissue sheet termed the epimysium. Within the muscle, smaller units named fasciculi consist of muscle fibres. These muscle fibres are formed from syncytium, multinucleate cells formed by the fusion of the plasma membranes of individual cells(Lawrie, 1979) (Figure 1). Therefore, they contain all organelles normally found in cells such as nuclei, mitochondria and the extensive sarcoplasmic reticulum. The mitochondria contain the enzymes involved in aerobic metabolism, while the sarcoplasmic reticulum acts as a store for calcium ions, which are used during muscle contraction. Each individual myofibre within the perimysium is surrounded by yet another sheet of connective tissue called the endomysium. Myofibres are mostly made up of myofibrils, which are 1-2 μm in diameter and are composed of around 10,000 serially repeating sarcomeres and, because of the ordered structure, they can contract under specific conditions. Sarcomeres are made up

of thick filaments and thin filaments. The thick filaments consisting mainly of the protein myosin and myosin binding proteins and thin filaments are composed mainly of the proteins actin and nebulin, as well as other regulatory proteins (Martini, 2001).

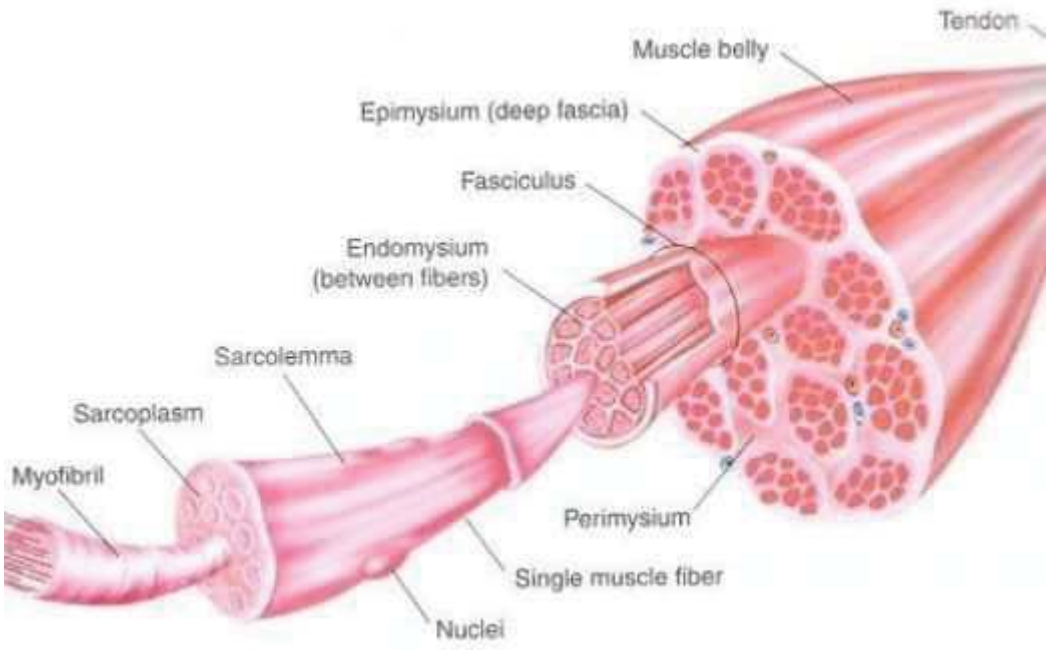


Figure 1: Internal view of muscle split into various component parts (Baechle *et al.*, 2008)

Sarcomeres have three distinct regions: the A-band (anisotropic band), where the thick (myosin) and thin (actin) filaments overlap; the I-band (isotropic band), where only actin filaments are present; and the H zone (Hele-Scheibe zone), which contains myosin but not actin filaments. At the boundary of each sarcomere is the Z-disc, which allows the entire filament network to be anchored. In the centre of the H-zone is the M-line, this line binds to the myosin filaments and ensures that they are in the correct orientation for contraction, as actin and myosin only pull past one another in one directions (Martini, 2001; Warriss, 2000a).

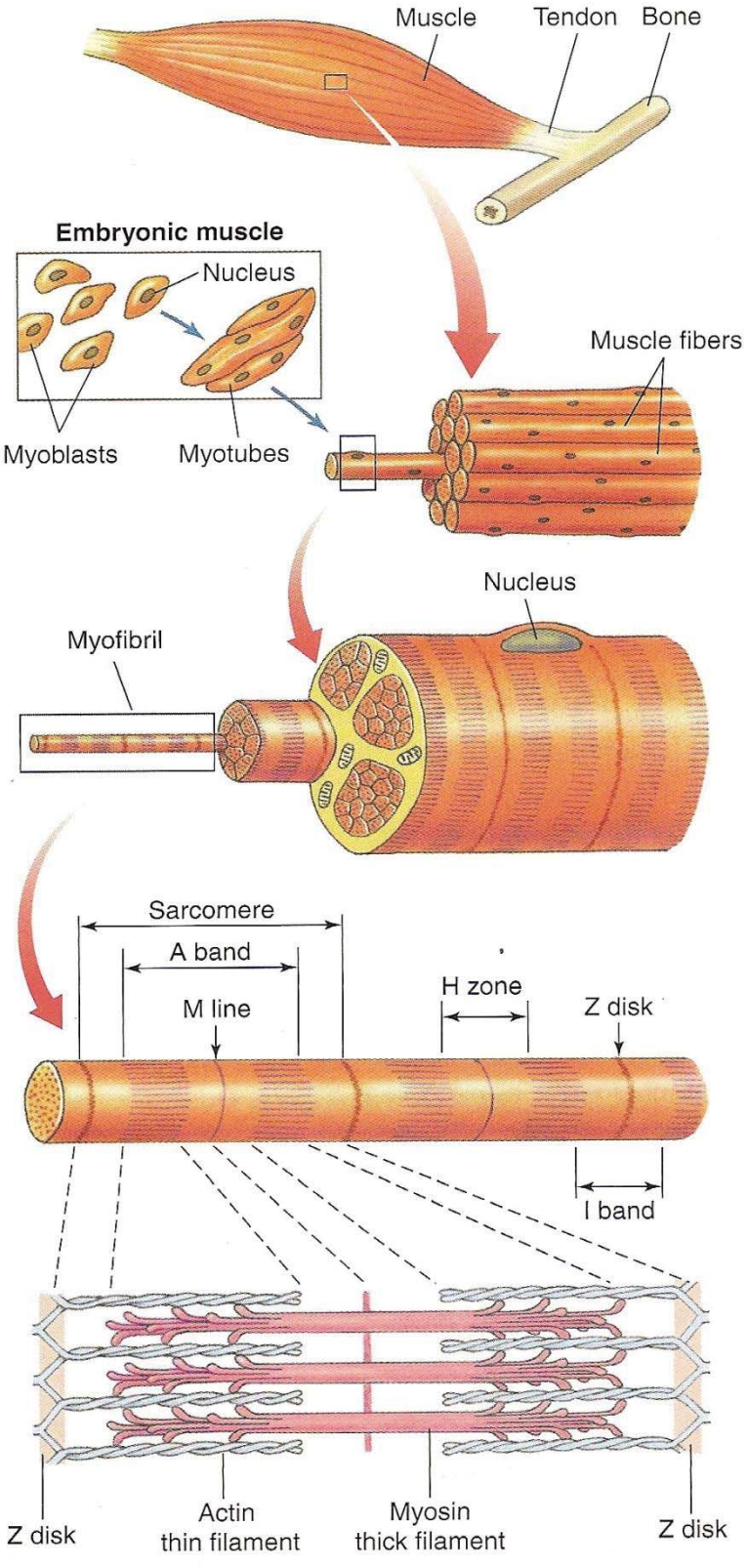


Figure 2: Diagram of a skeletal muscle and its constituents from top to bottom; skeletal muscle, a bundle of muscle fibers, a muscle fiber showing the myofibrils, a myofilament (Hendrick *et al.*, 1994)

Energy is stored by muscles as a polysaccharide called glycogen. In a live animal, glycogen reacts with oxygen to produce carbon dioxide, water and energy in the form of a compound called ATP (adenosine triphosphate). When a muscle has to work, ATP supplies energy directly to the myofibrillar proteins for the contractions of the myofibrils.

During muscle contraction the thick and thin filaments (Figure 2) slide over each other and become linked by cross bridges formed by actin and myosin proteins called actomyosin (Huxley & Hanson). This ATP-powered process involves the myosin head forming a reversible complex with actin, thus allowing the two filaments to slide past each other. The Z-disks pull closer together and thereby decrease the sarcomere length. During contraction the A-band stays the same length whereas the I-band and the H-band decrease in length (Huxley & Hanson 1957).

1.3.2 From muscle to meat

There are two main energy sources available to the muscle; aerobic and anaerobic metabolism. Aerobic metabolism, which occurs in the mitochondria, requires oxygen and is driven by adenosine triphosphate (ATP), a compound that stores chemical energy from nutrients. Anaerobic metabolism is known as anaerobic glycolysis and refers to the breakdown of glycogen to glucose, and does not require oxygen. One of the by-products of this process is lactic acid. The aerobic metabolism produces the largest amounts of energy with the greatest efficiency. On the whole, the production pathway is very complex but only a brief outline is needed for the scope of this thesis. Glycolysis is the major pathway of glucose metabolism and occurs in the cytosol of all cells. It can occur aerobically or anaerobically depending on whether oxygen is available. This is clinically significant because

oxidation of glucose under aerobic conditions results in 32 mole of ATP per mole of glucose. However, under anaerobic conditions, only 2 mole of ATP can be produced for each mole of glucose used (Lawrie, 1979). Therefore aerobic metabolism is more efficient than anaerobic.

During intensive exercise the body cannot deliver oxygen to the muscles fast enough to initiate the chemical reactions required for the aerobic process, so the body relies on anaerobic processes for the first couple of minutes.

After death, a series of events occur that can produce major structural or biochemical changes. One of the most serious consequences of circulatory collapse is that the oxygen supply to the muscle is disrupted (Aberle *et al.*, 2001). As the oxygen supply becomes depleted, the mitochondrial electron transport chain stops functioning. Energy metabolism then shifts to the anaerobic glycolytic pathway, such as after intensive exercise.

A major consequence of anaerobic metabolism is the accumulation of lactic acid. Since the circulatory system is no longer available to remove metabolites, lactic acid builds up in the muscle tissue resulting in lowering of pH. In response to the falling pH and depletion of metabolites, ATP resynthesis becomes inhibited and eventually no ATP is left.

With optimum pre-slaughter glycogen levels and no significant immediate stress, the pH falls in a predictable manner to a value of ~5.5, depending on muscle, temperature and animal species. In a typical chilling environment this takes about 16-24 hours for sheep. When the pH is falling but is still above 6.0, the muscle is ‘alive’ and can respond to changes in temperature or other stimuli. If the temperature control is not well maintained the muscle can shorten, which results in a tough meat. After approximately 24 hours the meat is through rigor mortis (Hendrick *et al.*, 1994). In this state, actomyosin bridges form and

sarcomeres shorten creating a state of muscle contraction. During a contraction, muscle fibres slide across each other, pulling the Z-lines together causing rapidly cycling cross bridges to form between actin and myosin, generating force. After death, almost 100 percent of these possible cross bridges form, as opposed to only 20 percent of cross bridges used during normal muscle contraction (Aberle *et al.*, 2001).

1.3.3 Changes in muscle structure during ageing

Post mortem storage, also known as ageing, is necessary for producing tender meat (Davey & Gilbert, 1976a). Tenderness is achieved by proteolytic enzymes that break down the muscle structures to produce meat that will yield easily during chewing (Ouali, 1992; Sentandreu, Coulis & Ouali, 2002). This mainly occurs in the Z-disk, although other myofibrillar structures are also affected (James *et al.*, 2002). The Z-disk keeps the structure of meat intact by keeping the thin filaments and, indirectly the thick filaments, intact. Ageing causes the degradation of the Z-disk and its associated proteins and therefore leads to the fragmentation of the myofibrils, resulting in increased meat tenderness.

The process of ageing is still not completely understood but the extent and speed of the ageing process depends on several factors, including temperature, pre-slaughter conditions chilling rate and condition of the animal (Savell, McKeith & Smith, 1981). Depending on all these different factors it can take one week for lamb, and up to several weeks for beef to reach maximum tenderness (Speck, Clarke & Morris, 1997). The amount and location of connective tissue and collagen have also been suggested to influence overall meat tenderness, but their role during ageing is believed to be minimal (Devine & Werner Klinth, 2004). Unfortunately, the muscle enzymes usually associated with meat ageing and

tenderisation, such as calpains and cathepsins, do not break connective tissue, and cuts of meat which contain a high proportion of connective tissue do not benefit from ageing to the same extent as cuts with a lower proportion. For example, rump is improved by ageing more than chuck (Spooncer, Smith & Powell).

Traditionally, beef is aged by hanging the carcasses in a cool room (<4°C) for several days. The muscles tenderise but also lose weight, and can spoil if hung too long. The modern practice is to age cuts in vacuum packs. Although there is some fluid loss, the shelf life for vacuum packed meat is much longer than the traditional ageing.

1.4 Freezing and Thawing / storage

1.4.1 Freezing

Freezing is a phase transition in which a liquid turns into a solid when its temperature is lowered below its freezing point. Freezing is a useful technique for preserving food. It preserves the flavour and nutritional properties of food for extended periods, and also minimises microbial and enzymatic activity within the food product itself (Alizadeh, Chapleau, de Lamballerie & Le-Bail, 2007). Ideally, from a microbiological perspective, meat should be frozen as soon as possible after slaughter and boning. However, such a procedure usually severely compromises product tenderness, during the first few hours post-mortem the components of muscle undergo an extensive series of biochemical changes and the ultimate structure of meat is determined partly by the rate and extent of events in this phase. Furthermore cold shortening may occur, meaning the temperature drops too quickly in relation to the pH fall (Davey & Gilbert, 1976b) (Marsh & Leet, 1966). Therefore this approach is only used when meat is to be used for processed foods (i.e. emulsion type

products such as sausages or hamburgers) as the meat will be unsuitable for consumption as whole tissue products (Jeremiah, 1996).

Depending on the muscle, meat contains around 70% water. Muscle fibres hold 85-95% of the total water (extracellular), with the remaining 5-15% water being held outside the fibre walls (James *et al.*, 2002). The freezing point of meat is between -1°C and -7°C, where 80% of the water within the meat will be frozen (Bevilacqua & Zaritzky, 1980).

The physical and chemical properties of meat may be influenced by a wide range of factors, including the temperature of the freezing medium, type and movement of the freezing medium, packaging materials used, and the composition of meat products to be frozen.

Air is a poor heat transfer medium and therefore freezing in air is usually a slow process, even when high air speed is applied. Rapid heat transfer is required for fast freezing which can be achieved by alternative systems such as spray application where the carcass is sprayed with cold water to diffuse heat faster or contact freezing methods that are based on heat transfer by contact between products and metal surfaces.

A slow freezing rate could be associated with the formation of large ice crystals in extracellular spaces, or chemical damage by concentration of solutes such as salts and sugars (Lawrie, 1979). During slow freezing, the temperature of meat remains near the initial freezing point (between -1°C and -7°C) for an extended time. Extracellular water freezes more readily than intracellular water because it has a lower solute concentration. With the freezing of the water in the extracellular space, the concentration of salts in the extracellular space increases to such an extent that an osmotic gradient is set up between the extracellular and intracellular spaces that in turn results in the migration of water from

the intracellular to the extracellular spaces. Consequently the extracellular ice crystals grow further and larger meaning the crystal growth breaks through the cell walls into the intracellular spaces. This causes an osmotic gradient between the inside and outside of the fibre cells. In order to equalise this osmotic pressure, water diffuses out from the cells into the extracellular spaces. This water freezes by 'sticking' to crystals already formed causing them to grow further (Lawrie, 1979; Petrović, Grujić & Petrović, 1993).

Conversely, when meat is frozen very quickly, internal temperatures fall rapidly below the initial freezing point. Numerous small ice crystals are formed both intra and extracellularly at approximately the same speed. Because of the rapid freeze rate there is little opportunity for the ice crystals to grow in size, and there is not enough time for the water to leave the fibres and the water subsequently freezes inside them (Grujić, Petrović, Pikula & Amidžić, 1993). Thus, a fast freezing rate results in the formation of many small ice crystals and very little translocation of water.

Extremely low temperatures, which can be achieved by using liquid nitrogen (-195°C) or dry ice (-98°C), are called cryogenic. Freezing meat at temperatures below -70°C results in an adverse effect on its quality. A great number of small ice crystals form within the fibres, significantly disturbing the ultrastructure. Petrović *et al.* (1993) reported that meat frozen at cryogenic temperatures loses some water during freezing and thawing, but a lot more during later heat treatment, resulting in cooked meat that is drier than meat frozen at higher temperatures.

1.4.2 Thawing

Thawing has received much less attention in the literature than either chilling or freezing. However, in food processing the thawing of frozen materials is important. Thawing time should be minimised to reduce microbial growth, chemical deterioration and excessive loss of water (Taher & Farid, 2001). Freezing and thawing mainly affect the water fraction of meat. Thawing occurs more slowly than freezing. During thawing, temperatures rise rapidly to the freezing point (meat starts to freeze at -1.8°C depending on pH) and then continues rising throughout the thawing time. If thawing happens too slowly it can potentially create an opportunity for big ice crystals to reform into new and even bigger ice crystals (recrystallisation) (James *et al.*, 2002).

A variety of thawing conditions are cited in the literature, the most common of which is thawing under refrigeration. Other methods include submersion in water, microwaves, heat convection, infrared, radio frequency, pressure, and thawing by cooking (Eastridge & Bowker, 2011). For research, the American Meat Science Association recommends thawing in a refrigerator at 2 to 5°C until the internal temperature reaches 2 to 5°C. Thawing time is usually described as an imprecise amount of time, such as overnight or 18 to 24h. It is recognised that factors such as the size and type of refrigerator, door openings, product load, ambient temperature and air circulation may affect thawing. Eastridge *et al.* (2011) reported that beef steaks can be rapidly thawed in a water bath according to food safety guidelines without deleterious effects on meat quality.

1.4.3 Frozen storage

The final temperature to which meat is frozen and subsequently stored determines the amount of unfrozen water that remains available for chemical reactions to proceed.

Petrović *et al.* (1993) showed that biochemical reactions could still take place in meat frozen and stored at temperatures higher than -18°C, since sufficient unfrozen water remained available at these temperatures for such reactions to occur. For freezing temperatures above -5°C, oxidation occurs rapidly in meat exposed to oxygen and rancidity is noticeable after a few weeks of storage. At freezing temperatures of -18°C the reaction takes place more slowly. The colder the storage temperature the slower the oxidation takes place (Spooncer *et al.*). The optimum temperature for the frozen storage of meat has been reported to be -40°C, as only a very small percentage of water is unfrozen at this point (Estévez, 2011).

The freezing of the water fraction causes an increase in the solute concentration in both intracellular and extracellular compartments, which is thought to be the reason for the increased chemical reactions during frozen storage at more mild temperatures (Hamm, 1979). The chemical reactions that can occur during storage are reported by Hansen, Juncher, Henckel, Karlsson, Bertelsen and Skibsted (2004). The authors reported accelerated lipid oxidation in frozen-thawed meat that was subjected to a refrigerated shelf-life study. The concentration of the secondary products of lipid oxidation is generally measured using thiobarbituric acid reactive substances (TBARS) method. These secondary products cause rancid, fatty, pungent and other off-flavours in the meat. This can lead to radical secondary lipid oxidation upon thawing, leading to adverse changes in colour, odour, flavour and healthfulness (Grujić *et al.*, 1993). Most of these reactions could be eliminated by lowering the storage temperature to -80°C but such temperatures are not possible in real-life situations as it is very expensive to run storage at this temperature.

1.5 Meat quality

With regards to meat production, the term "quality" is difficult to objectively define as some sort of number or other graded value due to numerous factors affecting the meat. Functional quality refers to the attributes in meat that affects its appearance and palatability. Functional quality will be addressed in this section as consumers usually refer to these types of parameters when discussing meat quality. The three dominant attributes by which consumers judge meat quality are appearance, texture and flavour (Faustman & Cassens, 1990). The conformance of these attributes to consumers' expectations is therefore important as it affects meat marketability.

1.5.1 pH

If the animal experiences high stress or significant exercise before slaughter, it will deplete the glycogen stores which are found within the muscle and used as an alternate anaerobic energy source when turned into glucose within the muscle. This depletion results in the ultimate pH being greater than 6.0, creating dark, firm and dry (DFD) meat (Young, Reid & Scales, 1993). Stress and exercise use up the animal's glycogen reserves and therefore post-mortem lactic acid production is diminished. It is the lactic acid produced within the muscle after slaughter that lowers the muscle pH. Despite the unattractive look and inferior cooking characteristics, the meat is critical in terms of the development of micro flora. The issues associated with meat having a high ultimate pH are applicable to beef, venison and lamb. Low ultimate pH meat can be a problem in pork, where a high rate of post-mortem glycolysis results in pale, soft and exudative (PSE) meat, due to stress prior to slaughter (James *et al.*, 2002).

The pH of meat that has been frozen and thawed tends to be lower than it was prior to freezing (Leygonie, Britz & Hoffman, 2012b). As pH is a measure of the amount of free hydrogen ions (H^+) in a solution, it is possible that the exudate production during freezing could cause denaturation of buffer proteins, the release of hydrogen ions and a subsequent decrease in pH. Alternatively, the loss of fluid from the meat during thawing may cause an increase in the concentration of the solutes, which can result in a decrease in the pH. This phenomenon has been confirmed by Leygonie *et al.* (2012b) , who reported a decrease in pH following the freeze/thaw processing of fresh ostrich meat, and suggested that the slight decrease in pH was most likely due to the loss of minerals and small protein compounds called exudates and thereby changing the ionic balance in the meat, which resulted in a decreased pH.

1.5.2 Meat colour

Of the various quality attributes of fresh meat, colour is the most important factor at the moment of purchase (Mancini *et al.*, 2005). Consumers can only judge the quality of meat on the colour at the point of sale, with a bright cherry-red colour associated with fresh meat (Suman & Joseph, 2013). During retail display, meat undergoes discolouration. Discoloured meat will be either discounted or the whole product will be discarded (Kim & Hunt, In Press). As a result, nearly 15% of retail beef is discounted in price due to surface discolouration, resulting in an annual loss of $1 billion in the US (Smith, Belk, Sofos, Tatum, & Williams, 2000).

Myoglobin (MB) is the protein largely responsible for meat colour. In the living cell it has two functions; it serves as an oxygen-storage and oxygen-delivery molecule (Livingston,

1983). The capacity of MB to bind oxygen depends on the presence of a haem. A haem is a non-polypeptide prosthetic group consisting of protoporphyrin and a central iron atom. Of the six bonds available, four connect the iron atom to the haem ring, the 5th attaches to the proximal histidine-93, and the 6th site is available to reversibly bind ligands, including oxygen, carbon monoxide and nitric oxide (AMSA, 2011). The nature of the group attached to the iron, and the state (covalent or ionic) of the iron determines meat colour. Since MB contains haem iron, it is rather susceptible to oxidation. MB oxygenation, also known as blooming, depends on time, temperature, pH and competition for oxygen by mitochondria. More specifically, the competition for oxygen between MB and mitochondria determines oxygen penetration beneath the meat's surface, which significantly affects the intensity of surface colour(Kropf, 2008).

In uncut meat which is not exposed to the oxygen in air, MB exists in its reduced ferrous state (Fe^{2+}) with no bound oxygen. Meat in this stage is purple-red in colour. This form of MB is known as deoxymyoglobin (DMB) (AMSA, 2011). This purplish-red or purplish-pink colour is also typical of vacuum packed meat due to the presence of oxygen. However, by opening the vacuum bag or by cutting the meat, DMB reacts with oxygen and forms a pigment called oxymyoglobin (OMB), leading to a bright red colour consumers associate with fresh meat (Mancini *et al.*, 2005). At low oxygen concentrations, OMB tends to undergo oxidation (where the Fe^{2+} in the haem is oxidised to Fe^{3+}) to form the undesirable brown pigment metmyoglobin (MMB). This change is prevented if no oxygen is present. The change from OMB to MMB occurs quite readily, but the reverse is more difficult (O'Keeffe & Hood, 1982).

Different packaging conditions can influence the appearance of the meat as they can potentially delay the development of MMBs characteristic brown colour.

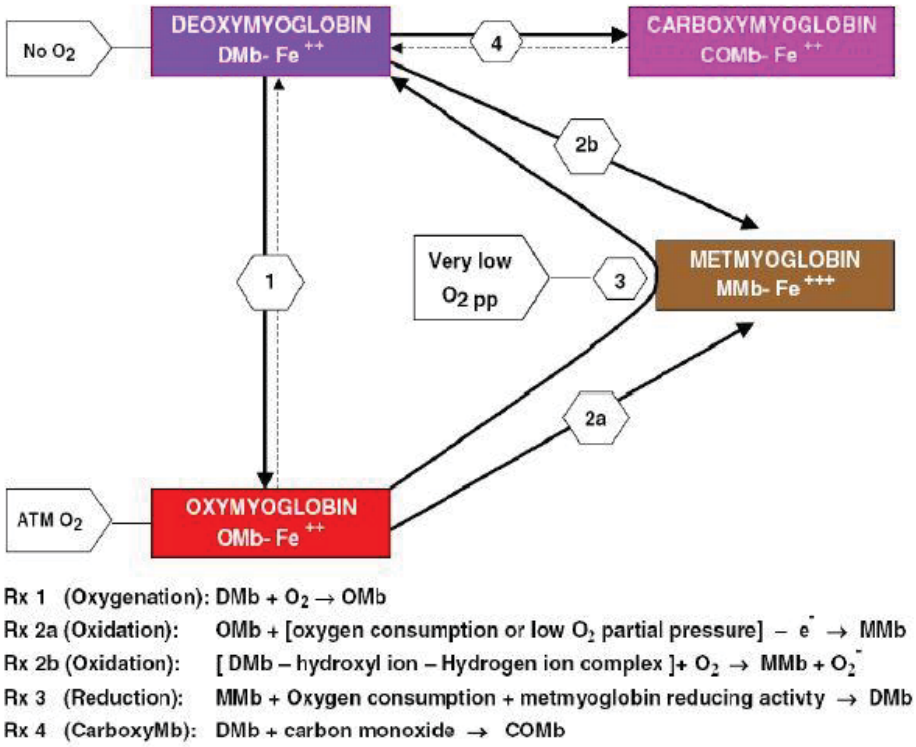


Figure 3: The different chemical states of myoglobin, the colour of the meat is regulated by the oxidative state of iron in the haem group (Mancini *et al.*, 2005)

Packaging which maintains low oxygen levels, like a vacuum pack or Modified Atmosphere Packaging (MAP), results in the formation of the purple-red colour DMB. However, when the meat is opened or repacked in a high-oxygen MAP, oxygen binds to the haem ring and forms OMB. When muscle is stored in high oxygen conditions (80% oxygen and 20% carbon dioxide), oxygen penetrates deeper into the meat surface and thus creates a much thicker layer of OMB and consequently a more stable red colour (King & Whyte, 2006).

When low oxygen is present in meat, a thin third layer of MMB forms between the OMB and DMB layers (Figure 4). This intermediate layer of MMB becomes thicker and moves toward the meat surface with time, while the OMB layer becomes thinner. A thinner layer of OMB and a more pronounced layer of MMB dulls the meat's red appearance and eventually the OMB layer is completely replaced by MMB, resulting in total discolouration of the meat surface (MIA, 2012/13).

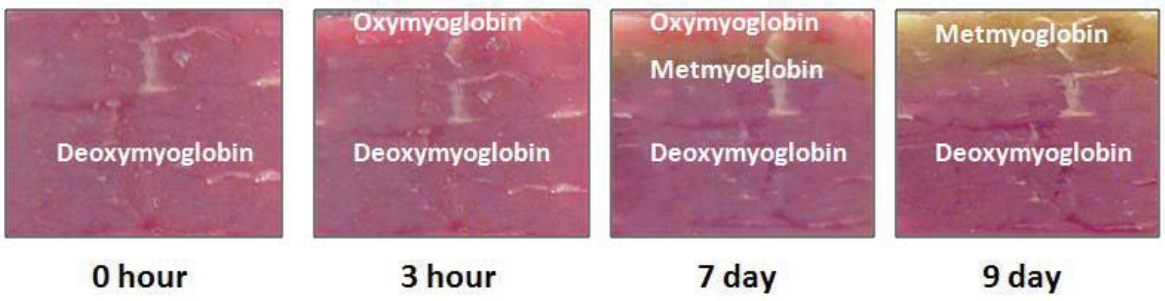


Figure 4: Illustration of DMB, OMB and MMB formation in the surface layer of a cut of meat by increasing oxygen exposure (Photo courtesy of Dr. D.H. Kropf, Kansas State University).

The existence of an enzyme system capable of reducing MMB back to MB was proposed by Livingston and Brown (1981) and was termed the metmyoglobin reducing activity (MRA). The theory is that in fresh muscle the enzyme is very active and the MMB formed is quickly reduced to DMB and oxygenated back to OMB, thereby retaining the bloomed colour. However, as the meat ages or is frozen, the activity of the MRA is decreased, and MMB begins to accumulate rapidly on the surface of the meat (Ben Abdallah, Marchello & Ahmad, 1999).

Meat colour is also affected by its pHu (Figure 5). Meat with a pHu of less than 5.8 has the ideal bright cherry red appearance, while the dark colour of high pH meat (DFD or Dark,

Firm, Dry) is often considered unattractive. DFD meat has a higher water holding capacity and tighter structure than meat with a normal pH, and it has been suggested that this may decrease the rate of oxygen diffusion into the muscle and consequently inhibit the formation of OMB, resulting in the darker colour (Young & West, 2001).

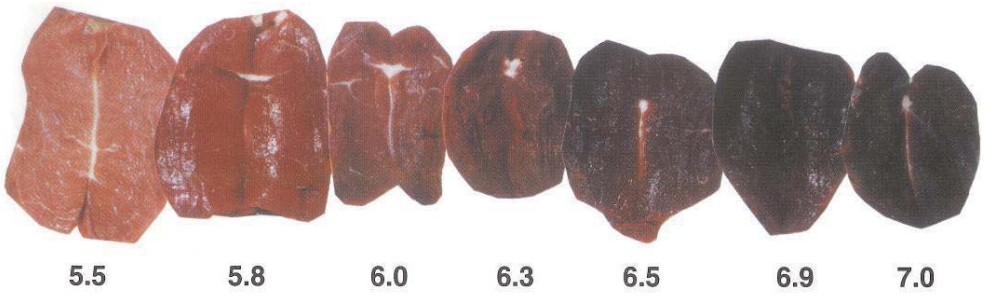


Figure 5: The colour of meat at various pH levels (MIRINZ, 1999)

Different muscles have been shown to have different colour profiles and stabilities. Kim, Keeton, Smith, Berghman and Savell (2009) reported that the Longsissimus dorsi muscle had the highest level of MRA (and subsequently the most stable colour), followed by the Semimembranosus muscle and finally the Psoas major muscle. Mancini, Suman, Konda and Ramanathan (2009) reported that every muscle with a different colour profile would respond differently to packaging under modified atmosphere (MAP) and therefore have different discolouration profiles.

Meat colour can be measured either visually or instrumentally. Although human judgement better reflects the total impression of the whole meat surface being evaluated, the consistency and repeatability of visual evaluation can be influenced by personal preference, external lighting and visual deficiencies within the eye. On the other hand, instrumental

measurements are much more consistent. The most common method for measuring meat colour instrumentally is by using the colour scale CIE L* (lightness), a* (redness) and b* (yellowness) with a 10° observer angle, which is more representative of what the consumer sees than some other methods.

1.5.3 Meat tenderness

Although the purchase decision of raw meat is primarily affected by colour, the likeability of meat is markedly affected by the tenderness of the cooked product. Tenderness is also linked to other meat attributes such as juiciness, texture and fat content. Only when the tenderness of cooked meat is acceptable can the evaluation of flavour and juiciness be independently made (Dumont, 1981). Meat tenderness is not simple to define and is more than just a measure of biting effort. The perception of tenderness involves ease of fragmentation, mealiness, texture and the adhesion of muscle fibres during mastication (Hendrick & Hartl, 1993). The tenderness of meat is not always consistent following cooking, this is due to many intrinsic properties of meat that determine tenderness, including pHu, cold shortening and connective tissue content (Purchas & Werner Klinth, 2004). The abundance of connective tissue surrounding the muscle fibres, bundles and the entire muscle is an important source of variation in the tenderness of meat (Purchas, 2004). The proteins, collagen and elastin are of particular interest. Collagen content in meat increases with the age of the animal, and results in a chewy texture to meat, while elastin has elastic properties and is extremely insoluble, which may contribute to meat toughness (Purchas *et al.*, 2004).

There is a general agreement in the literature that the tenderness of meat increases with freezing and thawing when measured by peak force (tenderness) (Lagerstedt, Enfält, Johansson & Lundström, 2008). The formation of large, extracellular ice crystals disrupt the

structure, largely breaking myofibrils apart and resulting in tenderisation. The tenderising effect of freezing seems to be negated when the meat was sufficiently aged prior to freezing (Vieira, Diaz, Martínez & García-Cachán, 2009). However, contradictory results have been found from sensory evaluation of tenderness, where lower peak forces were reported in freeze/thaw samples compared to chilled meat. The sensory results from the trained panel rated the freeze/thawed meat less tender than the chilled meat. This result was attributed to the loss of fluid during thawing that resulted in less water available to hydrate the muscle fibres, thus increasing the perception of toughness (Liu, Xiong & Chen, 2010).

1.5.4 Water holding capacity

Water-holding capacity (WHC) is defined as the ability of meat to retain moisture (Hamm, 1986). This will influence meat quality parameters, and therefore is important to the industry and consumers alike. Poor WHC results in high water loss and can represent a significant loss of weight from the carcass or cut muscle. In the muscle, the actin and myosin filaments bond together, squeezing out water from the filament lattice into the sarcoplasmic space. When a muscle is cut, the fluid will drain from the surface under gravity.

It is commonly accepted by researchers that WHC is correlated to juiciness, although many publications have reported that the actual results of measuring WHC and its effect on juiciness are conflicting (Winger & Hagyard, 1994). However, WHC is still important to the industry from an economic perspective, as it represents not only loss of product weight, but also loss of a significant amount of protein (Savage, Warriss & Jolley, 1990).

There are a number of different types of WHC of relevance to meat. The development of fluid loss in a retail pack in the supermarket (referred to as drip loss) produces an

unattractive product and can lead to a reduction in sales. The use of dry pads in the retail packages or trays, which have a moisture absorbing layer, is common in order to reduce the amount of fluid visible to the consumer (Kapper *et al.*, 2014). The fluid created and exuded into a package by a cut of meat from the time it is packed to a given time point, is called purge loss. This can, for example, be from the time of vacuum-packaging after boning until a vacuum-package is reopened prior to shelf life display. Cook loss is the amount of fluid that comes off the meat during cooking. During heating, different meat proteins will denature at various temperatures (37-75°C). Due to denaturation, structural changes in the muscle fibres, such as shrinkage and aggregation, fibres lose the ability to hold water and this results in cooking loss (Honikel, 1998; Hughes, Oiseth, Purslow & Warner, 2014). The correlation between the WHC of the raw muscle and the percentage of cook loss can be quite high, and is dependent on the cooking temperature.

There is a general agreement in the literature that freezing, storage and thawing all contribute to a decrease in the WHC of meat and therefore to an increase in drip loss, purge loss and cooking loss (Añón & Calvelo, 1980; Ngapo, Babare, Reynolds & Mawson, 1999a; Vieira *et al.*, 2009). This is true for meat intended for domestic consumption which has been stored for a maximum of 2-3 weeks. The effects of storage beyond this period have not been studied to any large extent, as it is only relevant for countries such as New Zealand that export chilled meat to markets in Europe and Asia, where the travel time is 8 weeks or longer. However, research on the long term storage of lamb meat indicates that WHC improves with extended storage time. The hypothesis behind the improvement after long term cold storage is that the channels through which water is lost are disrupted as a result of muscle/meat structural breakdown, formatting a “sponge effect” that traps the water

within the meat. The effect is enhanced by the increase in the viscosity of the drip due to higher amounts of soluble proteins in the drip with ageing and the cold gelation of those proteins at -1.5°C (Farouk, Mustafa, Wu & Krsinic, 2012).

Ngapo *et al.* (1999a) investigated the influence of freezing rate on drip loss. Fast frozen pork had the same amount of drip loss as refrigerated meat, while drip loss from slowly frozen pork meat was significantly higher. The author also reported that the protein concentration of the drip obtained after different treatments (frozen and thawed vs frozen, stored and thawed) had no observed differences.

Gonzalez-Sanguinetti, AÑOn and Calvelo (1985) concluded that a decrease in thawing time (time elapsed from -5°C to -1°C) to below 50 minutes resulted in a decrease in drip. This was attributed to the melting of the ice in the extracellular spaces causing an increase in water activity, resulting in the net flow of water into the intracellular spaces and its subsequent re-absorption by the dehydrated fibres. Haugland (2002) also proposed that increased rate (or decrease in time) of thawing caused less exudate to form. Ambrosiadis, Theodorakakos, Georgakis and Lekas (1994) reported that rapid thawing of meat by submergence in water decreased the drip loss. On the other hand, it was found in the latter study that microwave thawing (35 min to reach 0°C) increased the drip loss to within the same range as ambient air thawing (5-7h), but this drip loss was still less marked than in the case of refrigerated thawing (28h), which resulted in the highest drip loss.

1.5.5 Meat flavour

Flavour is a combination of two sensory responses; taste and smell (Pegg, Shahidi & Werner Klinth, 2004). The flavour of meat arises from the interaction and degradation of a host of compounds during cooking, and is ultimately determined by the chemical composition of the raw meat (Oddy, Harper, Greenwood & McDonagh, 2001). Raw meat is a heterogeneous matrix including proteins, fat, vitamins, sugars and nucleotides, which are the flavour precursors for the cooked meat.

Part of the flavour profile for cooked meat can be attributed to compounds generated by the Maillard reaction (Miller & Werner Klinth, 2004). The Maillard reaction begins when a carbonyl group from a reducing sugar reacts with an amino group (NH_2) of an amino acid or peptide (Warriss, 2000b). This reaction is accelerated by heat. After this initial reaction, the Amadori compounds (part of the Maillard reaction) degrade to compounds such as furfurals, furanones and dicarbonyls. These further react with compounds such as amino acids, aldehydes and ammonia to generate a host of aroma compounds. Thiamine and sulphur-containing amino acids have been identified as major contributors to meat flavour compounds because Maillard reaction products contain sulphur. Such compounds have very low odour thresholds (the minimal concentration of a substance that can be detected by a human nose) (Warriss, 2000b).

Another important contributor to cooked meat flavour is the oxidative degradation of fats in meat during cooking. Phospholipids and triacylglycerols are particular contributors to meat aroma. Flavour derived from fat is due to a plethora of carbonyl compounds (aldehydes and ketones) generated from the oxidation of unsaturated fatty acids and the volatilisation of fat soluble compounds during cooking (Pegg *et al.*, 2004). However, these fat oxidation products are also responsible for unpleasant aromas when generated in excess (Oddy *et al.*,

2001). This can occur when meat is stored, chilled or frozen. The pHu also contributes to the variation of flavour in meat. For beef, the lower concentrations of sugars in high pH meat reduces the extent of the Maillard reaction and leads to poorer flavour (Warriss, 2000b).

1.5.6 Meat structure

Most of the severe fibre deterioration during freezing and thawing is due to the formation of intra and extracellular ice crystals. (Hansen, Appelgren Trinderup, Hviid, Darré & Skibsted, 2003) state that when ice crystals form between fibres they generate pressure, which pushes the fibres apart. In contrast, the ice crystals that form within the fibres generate pressure in the opposite direction.

A significant number of the studies focused on meat freezing and thawing has been to try to better understand the effect of different freezing rates on meat structure by using electronic microscopes (TEM or SEM). Rahelić, Puač and Gawwad (1985) analysed histological changes in beef which had been frozen at different temperatures, and noted a link between the degree of meat degradation and the position of ice crystals. Less severe deterioration was noticed when only intracellular ice crystals were formed, when the pressure is generated in one direction only. Moreover, the structure of the frozen meat was reported by Hansen *et al.* (2003) according to the size of the holes which become visible when using an electronic microscope. The size of the holes in frozen meat can indicate the size of the ice crystals, while for fresh meat they correspond to the space occupied by the extracellular fluid. It was noticed that after thawing, the structure of the meat samples almost fully recovered from the damage observed in the frozen samples (Ngapo, Babare, Reynolds & Mawson, 1999b).

1.6 New Zealand meat industry and processing

New Zealand red meat is in demand around the world; consumers recognise the safe, healthy, clean and green values of New Zealand's pasture-raised beef, lamb and venison. This contrasts with the USA and Europe, where many animals are finished on a grain-based diet for varying periods of time and some are housed indoors. New Zealand is the world's largest lamb exporter, accounting for 40% of global exports by volume in 2008 (MIA, 2009).

The New Zealand export meat industry owes its existence to the development of refrigerated shipping. The first shipment of frozen meat from New Zealand to England was in 1882. In the early days, almost all meat products exported from New Zealand was frozen, the largest percentage of sheep meat as carcasses and beef as quarters. Nowadays, only about 3% of lamb is exported as carcasses, and instead a huge variety of cuts, both boneless and bone-in, are prepared for export. Chilled exports did not really start till the development of vacuum packaging and Controlled Atmosphere Packaging. These and other techniques make it possible for meat to be transported by sea to international markets and arrive in a fresh condition, with several weeks of shelf life remaining. Vacuum packs protect the meat from oxidation and fluid loss during storage. The oxygen-free environment inhibits aerobic bacterial growth and provides better yields by preventing weight loss from evaporation.

Supplying overseas markets relies on production, processing and transport within New Zealand, the transport phase from New Zealand to the market and finally the distribution, secondary packaging and sale of the product in the final marketplace.

Recently, prices for the diesel used by container ships have increased, resulting in the ships travelling more slowly to reduce fuel consumption in order to manage the travel costs. A journey in a container can now take 12-14 weeks when originally it took 6-8 weeks, meaning that key quality attributes for chilled (lamb) meat cannot be guaranteed anymore. The industry now has to think about alternative sea freight options in order to guarantee the quality and food safety of the meat.

One option is frozen transport of the meat. Freezing is among the most widely practised and one of the most effective methods of food preservation (James *et al.*, 2002). However, freezing/thawing of meat has been generally considered to result in inferior meat quality attributes such as colour, flavour and textur inconsistencies as well as with fluid loss when compared to unfrozen meat. This has meant that markets prefer to receive meat chilled, and consumers associate chilled meat with higher quality than frozen product. However, it has been found that fast freezing of previously aged meat minimised the amount of water loss due to the freezing/thawing processes (Kim, Frandsen & Rosenvold, 2011). A recent study by Balan, Kim, Choe, Kemp, Salerno and Stuart (2013) reported that fast freezing then thawing/ageing improved meat quality attributes in comparison to slow freezing. This has led to the hypothesis that fast freezing of early post mortem muscle (24h) then thawing/ageing could produce a high quality chilled product similar to that obtained through the traditional chilled aging process. If this is correct, then it could support the development of a novel way of producing chilled meat with the quality attributes required by consumers, but with superior shelf-life due to the ability to transport frozen. This would have significant benefits for the meat industry. This research project will further explore the possibilities of the freezing/thawing/ageing approach using a range of freezing speeds and ageing temperatures.

Therefore the objectives in this project were:

1. To determine the effects of different freezing/thawing-ageing regimens on meat quality and physical and biochemical characteristics of lamb loins

2. To establish an optimal freezing/thawing/ageing combination process to maximise the effect and efficiency of the freezing/thawing/ageing treatment on meat quality

2. Material and methods

2.1 Raw material & processing

Sixty whole lamb racks were chilled following best practice procedures at AFFCO (former Auckland Farmers Freezing Company) Rangiuru abattoir to between 2-4°C and then transported in chilly bins to AgResearch, Ruakura campus. At 24 h *post mortem*, both loins (M. longissimus dorsi) from 45 of the initial 60 lamb racks were excised (90 loins in total) for the trial. An extra 18 loins were also collected for temperature data recording only. The loins with ultimate pH (pH_{24h}) of 5.8 or below were selected at the beginning of processing by inserting a calibrated Hanna HI99163 with a FC232D combined pH/temperature probe (HANNA instruments, RI, USA) at two points along each loin. The first 90 loins with an acceptable pH were selected for the trial and then labelled and vacuum packed.

The loins were randomly assigned to one of three different freezing conditions:

- Slow freezing (SF)
- Fast Freezing (FF)
- Very Fast Freezing (VFF)

After the freezing period, the loins within each freezing condition group were randomly assigned to one of three different thaw/ageing regimens:

- -1.5°C for 2 weeks
- 1°C for 1 week
- 3.5°C for 1 week.

This provided a total of nine different treatments (table 1). In addition to the freezing thaw/ageing, further 9 loins were allocated to a control group and were kept chilled (never frozen) while being aged for 2 weeks at -1.5°C. At the end of each of the respective regimen treatment times the loins were sent for analysis, explained in the following sections.

Table 1: Schedule of freezing methods and thawing/ageing regimens

Groups (n=9)	Freezing	Thawing ageing
Slow Freezing -1.5°C (SF -1.5°)	Slow Freezing -18°C	-1.5° for 2 weeks
Slow Freezing 1°C (SF 1°C)	Slow Freezing -18°C	1°C for 1 week
Slow Freezing 3.5°C (SF 3.5°)	Slow Freezing -18°C	3.5°C for 1 week
Fast Freezing-1.5°C (FF -1.5°C)	Fast Freezing -18°C	-1.5° for 2 weeks
Fast Freezing 1°C (FF 1°C)	Fast Freezing -18°C	1°C for 1 week
Fast Freezing 3.5°C (FF 3.5°)	Fast Freezing -18°C	3.5°C for 1 week
Very Fast Freezing -1.5°C (VFF -1.5°C)	Very Fast Freezing -40°C	-1.5° for 2 weeks
Very Fast Freezing 1°C (VFF 1°C)	Very Fast Freezing -40°C	1°C for 1 week
Very Fast Freezing 3.5°C (VFF 3.5°C)	Very Fast Freezing -40°C	3.5°C for 1 week
Control group -1.5°C	Non frozen	-1.5° for 2 weeks

2.1.1 Freezing processes

Samples assigned to Slow Freezing (SF -18°C) were placed in an air freezer. This is a standard freezer chamber constructed of Poly Panel (metal-clad polystyrene) sheets, with a ceiling-hung evaporator fan assembly and insulated concrete floor.

For the Fast Freezing (FF -18°C) regimen, a 250L insulated immersion tank fitted with a stainless steel tube cooling coil was used. This cooling coil was connected via a stainless steel centrifugal circulating pump to an in-house designed refrigeration unit consisting of a Kirby Polar Pack condenser fitted with a titanium plate heat exchanger and PID controller (Proportional-integral-derivative, a widely used industrial controller). An aqueous solution of 30% calcium chloride and a corrosion inhibitor (Hood Chemicals WM8699) was pumped through both the plate and the tube heat exchangers, and also used as the immersion fluid in the tank. To keep the immersion fluid in the tank circulating, a water pump (Aqua One Maxi 105, 2200L/hr) was used.

For the Very Fast Freezing (VFF -40°C) regimen, a 250 litre immersion tank was placed in a custom environmental chamber (designed for MIRINZ by APZ) to be operated and controlled between +20°C and -40°C with +/- 0.5°C accuracy. The environmental chamber is constructed of Poly Panel (metal-clad polystyrene sheets) and fitted with a special low thermal mass floor. Ambient air flow in the chamber is controlled by fans mounted on the environmental chamber's cooling refrigeration evaporator. The immersion tank was placed in the chamber at least six days before the trial started, to ensure the immersion fluid temperature had equilibrated to -40°C. To keep the immersion fluid in the tank circulating, a water pump (Aqua One Maxi 105, 2200L/hr) was used.

The core temperature of the loin samples during all freezing processes was monitored to confirm the freezing rate and final temperature using a Grant 1000 Series Squirrel Data Logger fitted with T-type thermocouples (a temperature-measuring device) that had been calibrated.

After 24h all the loins (irrespective of freezing regimen) were put in boxes and stored at -18°C in an air freezer for 4 weeks. Subsequent thawing and ageing was carried out in a Skope VF1300 2-door vertical freezer with individual chambers adjusted to the required temperatures (-1.5°C, 1°C and 3.5°C).

At the end of each regimen, i.e., after the freezing and thaw/ageing period, the loins were removed from their vacuum packs and divided into six segments, starting from the cranial orientated end of the loin, as follows:

1 - 6 cm from the front of the loins was allocated to shear-force/cook loss measurements

2 - 1 cm for histological analysis

3 - 2 cm for colour display measurement Day 1 and T-bars

4 - 2 cm for colour display measurement Day 4/7 T-bars

5 - 2 cm for drip loss

6 - 1 cm for biochemical analysis

The methodology for each of the procedures mentioned above is described later in this chapter.

2.2 Meat quality attributes

2.2.1 pH

It has been shown that meat with a high pHu (24 h post mortem) is more likely to have a higher freezing temperature, which could confound the freezing regimens (Farouk, Kemp, Cartwright & North, 2013). Given this condition, loins with a pHu lower than or equal to 5.8 were selected for this study.

The pH was measured at two random positions along the loin by inserting a calibrated pH probe (Hanna 99163 pH meter with a FC232D combined pH/temperature probe, Hanna Instruments, Rhode Island, USA) directly into the meat along the uncut loin. A two-point calibration of the pH meter electrode was performed with standardised buffers (pH 4.0 and 7.0) prior to each measurement day. Two sets of readings were taken for each sample: one at 24 h *post mortem* (before the treatments) and the other after the assigned freezing thawing/ageing regimens.

2.2.2 Purge and drip loss

The loins were weighed (initial weight), packaged under vacuum and then frozen according to the three freezing regimes. After the thaw-ageing period, the loins were removed from the vacuum bags, dried on paper towels and reweighed (final weight). The purge loss was calculated as weight lost expressed as a percentage of the original sample weight.

The purge liquid (the liquid that was expelled from the loin during the freezing and thaw/ageing treatments) from each vacuum bag was collected in 1.7 ml tubes, frozen in liquid nitrogen and stored at -80°C for later analysis (section 2.6).

The drip loss was measured after the assigned freezing and thaw/ageing period following the procedure of Honikel (1998). Approximately 45 grams of loin with all skin and connective tissue removed was dried with paper towels, weighed, placed in a plastic 'onion' net and then suspended by a hook within a closed container without contact to any side of the container. After 48 h at 3±1°C, the sample was removed, blotted dry and then

reweighed. The drip loss was calculated as weight lost expressed as a percentage of the original sub-sample weight.

2.2.3 Cook loss and shear force

The samples for shear force measurements were cooked to a core temperature of 75°C in a water bath set at 99°C. After cooking, the samples were immediately placed in an ice-water slurry to cool down to below 10°C. The temperature was monitored using a Digi-Sense scanning temperature logger (Eutech Instruments Pte Ltd., Singapore) positioned in the centre of each sample. The weight of the samples was recorded before and after cooking, with samples blotted dry before weighing and the loss of weight due to cooking calculated as weight lost expressed as a percentage of the original sample weight.

Shear force was measured using a MIRINZ Tenderometer (MIRINZ Inc., Hamilton, New Zealand). Once cooled on ice, 10 mm × 10 mm cross section samples (n=10 from each sample) were cut from the cooked meat samples and measured with the meat fibres perpendicular to the cutting tooth of the Tenderometer. The results were expressed as shear force (kgF) (Chrystall & Devine, 1991; MacFarlane & Marer, 1966).

2.2.4 Colour

The cuts for colour measurements were placed in Cryovac® food grade trays (Cryovac TQD-0900; 22.5 cm × 17.3 cm × 4.1 cm; CRYOVAC, Sealed Air Corporation, South Carolina, USA) with the cross sectional side up and then sealed with oxygen barrier film using Cryovac® LID 1050 (CRYOVAC, Sealed Air Corporation, South Carolina, USA) into a high-oxygen modified

atmosphere (HiOx MAP). This was comprised of 80% O_2/20% CO_2 (Certified Standard within ± 5%, BOC GASES; Hamilton, New Zealand) and was accomplished using a ROSS® Inpack™ Junior A10 Packaging Machine (Ross industries packaging division, Midland, USA) by applying vacuum, then flushing the package with the gas mixture and sealing.

The packaged cuts were displayed for 7 days at 3±1°C under continuous fluorescent natural white light (2800 lx, CRI=82, Colour temperature=4000 K; Osram, Auckland, New Zealand). The gas composition of the HiOx-MAP trays was monitored by using a headspace oxygen/carbon dioxide analyser (PBI Dansensor, Glen Rock, NJ, USA), confirming that a high-oxygen level (>70% O_2) was maintained during the display period. The gas mixture composition inside each pack was also checked before opening with a PBI Dansensor, CheckPoint handheld gas analyser (Ringsted, Denmark) by piercing the top layer and reading the oxygen and carbon dioxide levels.

Instrumental surface colour of the loins was measured on day 1, 4 and 7 at three random places on each sample with a Minolta Colour Meter (Illuminant D65, 1 cm diameter aperture, 10° standard observer; CR-300; Konica Minolta Photo Imaging Inc., Tokyo, Japan) using the CIE*LAB* colour space. The surface meat colour was scanned with the colour meter while covered with the same film sealing the HiOx-MAP tray. Calibration was performed by using a standard white tile prior to the colour measurement. L^*, a^*, and b^* values were used to calculate chroma $[(a^{*2}+b^{*2})^{1/2}]$ and hue angle $[(b^*/a^*)^{\tan-1}]$ (AMSA, 2011).

After measuring the colour of the samples on day 1, the cuts were removed from the package, cut finely and immediately frozen in liquid nitrogen. After measuring the colour of the samples on day 4, however, the cuts were immediately repacked under HiOx-MAP and left for another 3 days on display. On day 7, the last day of measuring the colour, the cuts

were cut finely and immediately frozen in liquid nitrogen. The frozen samples collected on day 1 and day 7 were stored at -80°C until used for lipid oxidation analysis (Section 2.3).

2.3 Lipid oxidation

Measurement of Thiobarbituric Acid Reactive substances (TBARS) was performed on the samples frozen on days 1 and 7 from the colour assessment (Section 2.2). Lipid oxidation was measured using the 2-thiobarbituric acid method described by Buege and Aust (1978) and Kim et al. (2009) with a few modifications. The finely cut frozen samples were ground to a fine powder using Freezer/Mill 6970 EFM USA, (3 minutes run time 9 cycles per second), then 5.0 g of each sample was homogenised in 15 ml distilled water and centrifuged (Eppendorf 5810R, Hamburg, Germany) at 2000 rpm for 10 min at 4 °C. An aliquot of the homogenate (2 ml) was transferred to test tubes and 4 ml of trichloroacetic acid and thiobarbituric acid (TCA/TBA) and 100uL butylated hydroxyl anisole (BHA) was added. The samples were incubated for 15 min in a water bath at 80°C and then cooled for 10 min in ice cold water. After centrifuging the combined sample once more at 2000 rpm for 10 min at 25°C, the supernatant was filtered through a Whatman filter paper #4. The absorbance of the supernatant was read at 531nm using a 96-well plate. (Spectrophotometer Multiskan GO Thermo scientific Finland, skanit 3.2 Research Edition).

TBARS level was calculated using known concentrations of malonaldehyde (MDA) as a standard curve. The protein oxidation was calculated based on the free thiol contents as µM thiol/mg protein.

2.4 Data analysis

All the methods described in sections 2.2-2.3 have been statistically analysed as follows:

The left and right side loin samples of 45 animals were randomly assigned to one of three different freezing conditions (Control, SF, FF and VFF) and to the thawing/ageing temperatures (-1.5°C, 1°C and 3.5°C).

All statistical analysis was done using Genstat 16th edition. Colour data was analysed using repeated measures ANOVA. Animal was included as the blocking variable, and the regimens (Control -1.5°C, SF -1.5°, SF1°C, SF3.5°C and FF-1.5°, FF1°C, FF3.5° and VFF -1.5°, VFF1°C, VFF3.5°C) as the treatment variable. Least Significant Differences (LSD) at 5% level of significance was used to compare means. To explore the main effects of freezing (SF,FF,VFF) and ageing (-1.5°C, 1°C and 3.5°C) temperatures, ANOVAs blocked by animal with a factorial + added control treatment structure were also fitted. Temporal trends in the colour data were investigated using a repeated measures ANOVA, block by animal, and with a uniform correlation of time.

TBARS was analysed using mixed effects models (REML). The Control, treatments (SF, FF and VFF), ageing temperature (-1.5°C, 1°C and 3.5°C) and day (1, 7) were included as the fixed effects, while the side (left or right loin) was included as the random effect. Wald test was used to determine statistical significance of fixed effects. Means were compared using the approximate LSD at the 5% level, based on the maximum standard error of a difference.

The other data (pH, Colour, Drip loss, Purge Loss, Cook loss and Shear force) was analysed using ANOVA. Animal was included as the blocking variables, and the regimens (Control -1.5°C, SF -1.5°, SF1°C, SF3.5°C and FF-1.5°, FF1°C, FF3.5° and VFF -1.5°, VFF1°C, VFF3.5°C) as

the treatment variable. LSD at 5% level of significance was used to compare means. The factorial + added control ANOVA was also fitted to analyse the main effects of freezing (SF, FF, VFF) and ageing (-1.5°C, 1°C and 3.5°C) temperatures.

2.5 Histology

After each freezing/thawing ageing regimen, 4 out of 9 loins were randomly selected for histological analysis. The loins were cut in transversal and longitudinal samples (1 x 1 mm), fixed in 10% buffered formalin solution (see Appendix) for 48 hours then rinsed in 70% ethanol and stored in 70% ethanol at 4°C until use.

After approximately 2 months, the samples were taken from storage and processed using a Leica Peloris Rapid tissue processor, (Leica Biosystems Melbourne Pty Ltd, Victoria Australia) as follows:

Table 2: Processing schedule tissue processor

Step	Solvent	Time (minutes)	Temperature (°C)	Pressure
1	Formalin	60	45	Ambient
2	Ethanol	40	45	Ambient
3	Ethanol	40	45	Ambient
4	Ethanol	50	45	Ambient
5	Ethanol	50	45	Ambient
6	Ethanol	50	45	Ambient
7	Ethanol	60	45	Ambient

8	Xylene	50	45	Ambient
9	Xylene	60	45	Ambient
10	Xylene	60	45	Ambient
11	Paraffin wax	60	65	Vacuum
12	Paraffin wax	60	65	Vacuum
13	Paraffin wax	60	65	Vacuum

The processed samples were embedded in paraffin Wax ALP8330 and allowed to cool overnight before 7 µm sections were cut for histological staining.

2.5.1 Haematoxylin and Eosin staining:

In preparation for staining, sectioned samples were submerged in xylene (2 x 5 min) for dewaxing and rehydrated in consecutive baths of 100% ethanol (2 x 2 min), 95% ethanol (2 min), 70% ethanol (1 min) and 50% ethanol (1 min). Following rehydration, sections were immersed in Gill's Haematoxylin for 5 min to stain the nuclei. Excess dye was removed by rinsing under running tap water until the water flowed colourless followed by 2 min submersed in Scott's Tap Water. This was followed by another rinse under running tap water for a further 2 minutes.

Following nuclei staining, the slides were immersed in Eosin 1% solution for 2 minutes for cytoplasm staining, then rinsed under tap water. The slides were then dehydrated with graded strengths of alcohols as follows: 3 dips in 50% ethanol, 1 min in 70% ethanol, 1 min in 95% ethanol, 2 x 2 min in 100% ethanol and finally submersed in xylene (2 x 5 minutes).

Once the slides had dried, DPX mounting solution was applied before a cover slip was placed over the tissue sections. The slides provided a visual comparison of the effects of the regimens on the muscle tissue rather than quantitative data as statistical analyses were not performed on these samples.

2.6 Metabolomics

The purge collected as described in section 2.2.2 was used for metabolomic analysis. It is important to note that metabolomic analyses on purge loss was done with the purpose of looking for constituents in the purge, rather than finding a difference between the regimens. Five samples from each treatment were randomly selected from the total of 90 samples in this study, resulting in a total number of 50 samples. After defrosting on ice, the samples were centrifuged for 15 minutes at 13,500 relative centrifugal force (RCF) at 4 °C. 500 µL of the supernatant was transferred to 3 kDa cut-off centrifugal filters (NanoSep, Pall Science). The centrifugal filters were rinsed with 7 x 500 µL MilliQ water to remove glycerol residues from the filter prior to application of the drip supernatant. The samples were centrifuged for 2 hours at 13,500 RFC at 4°C to remove proteins. 200 µL of the filtered purge was transferred to standard 5 mm NMR tubes. 340 µL of 100 mM phosphate buffer (containing 10% D_2O and 90% H_2O, pH 7.0) and 60 µL internal standard solution (containing 5 mM 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS) as a quantitation standard and chemical shift reference and 100 mM imidazole as a pH indicator) was added. When the volume of the filtrate was less than 200 µL the total volume was adjusted to 600 µL by addition of MilliQ water. A quality control (QC) sample, consisting of a pool of 10 different samples was used as a reference spectrum.

2.6.1 NMR Spectroscopy

1-dimensional (1D) ^{1}H-NMR spectra were acquired on a Bruker 700 MHz Ultrashield NMR spectrometer at Massey University using presaturation to supress the large water peak. Spectra of the low-molecular weight fraction of the purge (<3kDa) were recorded using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence with the following parameters: number of scans: 256, spectral width: 8.33 kHz, number of points: 58k, recycle delay: 1.5 s, acquisition time: 3.5 s, echo delay: 78 ms, 90° pulse: 15.2 μs, and presatuaration field strength: 50 Hz.

2-dimensional NMR spectra (^{1}H,^{1}H-TOCSY (total correlation spectroscopy), ^{1}H,^{13}C-HSQC hetero nuclear single quantum coherence spectroscopy) were recorded on the quality control QC sample for metabolite identification.

2.6.2 Spectral processing

The MestreNova 8.1 software was used to process the data from the 1D ^{1}H-NMR spectra: Phasing and baseline correction was performed. The chemical shift reference DSS was binding to residual proteins in the samples resulting in peak broadening in samples with high protein content. This resulted in DSS being unsuitable as both a chemical shift reference and a quantitation standard. Therefore, the absolute concentrations of the purge metabolites could not be determined from the spectra and a different approach, 'binning', had to be used. Spectra were referenced to the alanine doublet at 1.466 ppm.

The spectral regions containing the water peak and peaks from DSS were deleted and the remaining regions of the spectra were divided into 0.04 ppm wide 'bins'. Bins containing resonances from pH sensitive compounds (e.g. carnosine) were merged.

The peaks in each bin were integrated to find the peak intensities and the sum of all peak intensities in each spectrum was set to 100 (normalisation) to account for the variation in the amounts of purge in the samples and also potential concentration differences between samples. The resulting intensity matrix (161 bins x 50 samples) was exported to Microsoft Excel where information about the different regimens was added.

2.6.3 Metabolite identification

Tentative identification of metabolites in the 1D NMR spectra of the QC sample was made using the Chenomx NMR Suite Professional software with an in-built 1D spectral database. These tentative identifications were then confirmed (or rejected) by comparing the 2D NMR spectra of the QC sample with 2D spectra of individual metabolites from the Human Metabolome Database (http://www.hmdb.ca/).

2.6.4 Metabolomic data analysis

The data was analysed using the web-based metabolomics software MetaboAnalyst 2.0 (http://www.metaboanalyst.ca/MetaboAnalyst/faces/Home.jsp) using both univariate and multivariate statistical methods. The intensity matrix was uploaded to MetaboAnalyst and the data was log-transformed and Pareto-scaled before analysis. Principal Component Analysis (PCA), an unsupervised multivariate data analysis method, was used as a first step to get an overview of the dataset. PCA is a multivariate projection method designed to extract and display the systematic variation in a large data matrix. In the current study PCA was used to represent all bins from each NMR spectrum as a point in a plot. Outputs of PCA are two plots: the scores plot which shows how similar or different spectra are from each

other and the loadings plot, which shows why the spectra of different treatment groups are different from each other. When a difference between treatment groups could be found using PCA, a supervised multivariate data analysis method called Partial Least Squares-Discriminant Analysis (PLS-DA) was used to further investigate the metabolites responsible for differences between groups. Information about class membership (e.g. FTA regime) was supplied and a model built trying to maximise the differences between the groups, for instance the metabolite differences between drip from the Control group and drip from the Very Fast Freezing group. Supervised methods are unfortunately prone to over-fitting, i.e. finding differences between groups when there is in fact no difference. Therefore, results need to be interpreted with caution and for this study PLS-DA was only used in a qualitative way.

Instead, t-tests were used to find the regions ('bins') of the NMR spectra that differed significantly between two treatment groups at a time ($P = 0.05$). False Discovery Rate (FDR) correction was used with the t-test since the large number of variables present in metabolomics data greatly increases the risk of Type I errors (false positives).

The regions in the NMR spectra which were found to differ significantly between two groups (t-test, FDR, $P < 0.05$) were examined in detail and the metabolites giving rise to peaks in these regions were identified.

3. Results

3.1 Freezing regimens

The different freezing regimens, SF, FF, and VFF substantially influenced the freezing rate of the loins (Figure 6). The SF regimen resulted in the meat taking approximately 12 h to reach an internal temperature of -18°C, compared to only 4 h with the FF.

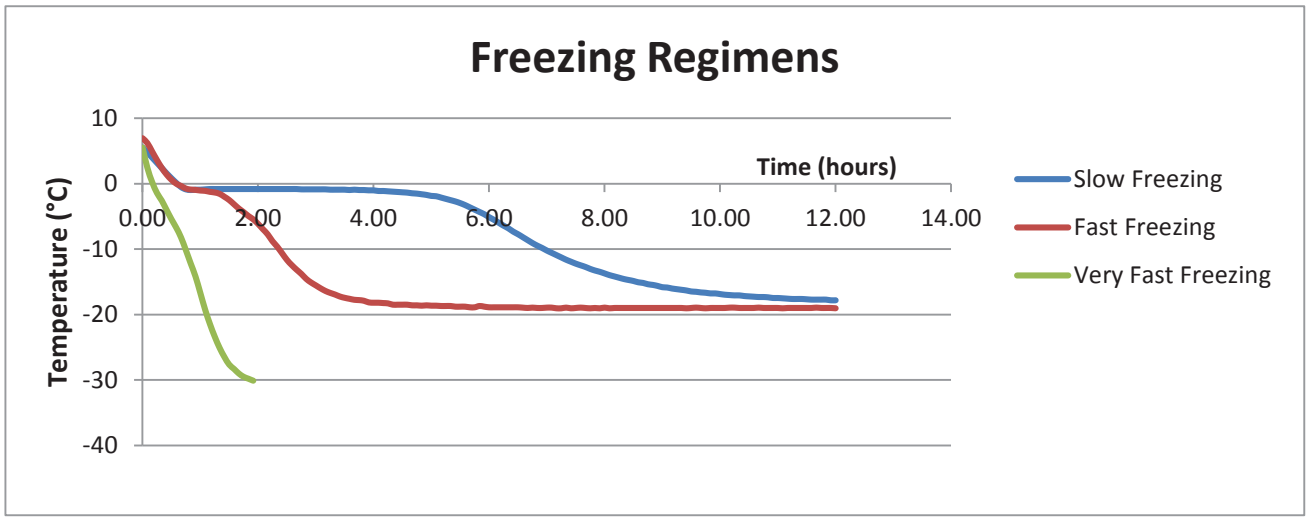


Figure 6: **Temperature decline rates of the lamb samples assigned to Slow Freezing, Fast Freezing or Very Fast Freezing regimens.**

The VFF regimens resulted in the samples reaching an internal temperature of -32°C in approximately 1.5 h, but the target of -40°C was not achieved due to the introduction of loins at ambient temperature increasing the immersion liquid temperature. Therefore, the target temperature for the VFF samples was adjusted to -32°C, and for the purpose of this thesis, VFF samples will be referred to as VFF -32°C.

The Control group was placed in a chiller and allowed to age at -1.5°C for 2 weeks. These samples were never frozen. After 4 weeks of storage at -18°C, the SF, FF and VFF samples

were assigned to 3 different thawing/ageing temperatures. This resulted in 9 regimens plus a control group.

3.2 Meat Quality Measurements

The three dominant attributes by which consumers judge meat quality are appearance, texture and flavour (Faustman *et al.*, 1990). Understanding the factors that influence consumers' perception of what meat quality is to them as an individual is critical for the meat processing plants, as the conformance of these attributes to consumers' expectations will affect the products marketability. In the next few sections, the effects of the different treatment regimens on parameters that have been shown to influence meat quality (pH, colour stability, lipid oxidation, tenderness and water holding capacity) will be addressed.

3.2.1 pH

A significant increase in pH was found only for the Control and VFF -1.5°C treatments ((P = 0.004) 0.04 units and 0.05 units respectively) over the total processing time.

Although there was a difference in final pH between the assigned treatments, the maximum differences were less than or equal to 0.1 pH units, which was not considered to be large enough to influence the meat quality attributes of the lamb loins. Overall, pH values of lamb loins from the different regimens varied from 5.62 to 5.73, which is considered to be within the normal range for lamb meat (Devine, Graafhuis, Muir & Chrystall, 1993).

3.2.2 Colour stability

The L* values (indication of lightness) showed no evidence of a treatment and display day interaction ($P = 0.632$) (Table 3). However, on day 1 of display, SF -1.5°C and FF -1.5°C were somewhat darker in colour than the other regimens. This trend continued on day 4 and day 7. On the other hand, the L* values for Control increased at day 4 and day 7, and were significantly higher in colour ($P < 0.001$) than that of the frozen/aged loins. Overall, the regimens had limited to no effect on the L* value.

The a* value (redness) of all freezing-ageing/thawing regimens were significantly ($P < 0.001$) lower than the control samples at all three display time points for all regimens (Table 3). The decrease was consistent for all regimens and therefore implies that any freezing/thawing treatment causes the a* value to decrease over the display period. Redness at day 1 and day 7 was highest for the Control, but there was no difference at the mid-point of display time (day 4). Interestingly, at day 7, SF 1°C and SF 3.5°C had a lower redness than FF and VFF within their thawing temperature group ($P < 0.001$).

b* is the scale from yellow to blue, where a positive number indicates yellow and a negative number indicates blue. The more the a* values decrease, the more prominent the b* (yellowness) appears, as the b* values decrease more slowly. Changes over the display period move towards browner (less desirable) colours after 7 days (Table 3). Evidence is found for a decrease in b* over display time for all regimens ($P < 0.001$) except SF -1.5°C, FF -1.5°C, and VFF -1.5°C; they all had a higher b* value at day 4 but did decrease again on day 7. There is also evidence of a treatment and time interaction ($P < 0.001$), where b* values for SF 1°C, FF 1°C, VFF 1°C, SF 3.5°C, FF 3.5°C and VFF 3.5°C are significantly lower after 7 days of retail display when compared to day 1 and 4. However, the Control, SF -1.5°C, FF -1.5°C, and VFF -1.5°C do not show this trend.

Table 3: Effect of different freezing thawing/ageing regimens (SF, FF and VFF and thawing/ageing temperatures -1.5°C, 1°C and 3.5°C) on L* value, a* value, b* value, Hue angle and Chroma value

		Colour Measurements				
Display time	Treatment	CIE L*	CIE a*	CIE b*	Hue angle (°)	Chroma
Day 1	**CO -1.5**	42.7	22.2	8.92	21.77	23.9
	FF -1.5	40	20.2	6.94	19.1	21.4
	FF 1	42.4	17.6	6.83	21.28	18.9
	FF 3.5	41.6	19	7.59	21.73	20.4
	SF -1.5	42.4	19.9	7.51	20.69	21.3
	SF 1	42	19.6	8.08	22.27	21.2
	SF 3.5	42.6	17.7	7.01	21.45	19.1
	VFF -1.5	40.8	19.4	6.09	17.58	20.4
	VFF 1	41.2	18.8	7.43	21.5	20.2
	VFF 3.5	41.6	18.5	7.35	21.61	19.9
	P value	0.002	<.001	<.001	<.001	<.001
Day 4	**CO -1.5**	44.3	17.6	6.93	21.5	18.9
	FF -1.5	41	17.3	8.15	25.3	19.1
	FF 1	42	15.7	6.25	21.7	16.8
	FF 3.5	41.7	16.7	6.85	22.2	18.1
	SF -1.5	41.8	17.3	8.53	26.3	19.3
	SF 1	42.3	16	6.51	22.2	17.2
	SF 3.5	42	15.5	6.19	21.8	16.7
	VFF -1.5	41	15.7	7.69	26.2	17.5
	VFF 1	41.5	16.1	6.49	21.9	17.4
	VFF 3.5	41.6	16.1	6.58	22.2	17.5
	P value	<.001	0.098	<.001	<.001	0.025
Day 7	**CO -1.5**	44.2	16.6	7	22.5	18
	FF -1.5	40.4	15	6.58	24	16.4
	FF 1	42	14.1	5.98	22.7	15.3
	FF 3.5	41.3	14	6.09	23.1	15.3
	SF -1.5	42.8	14.1	6.84	25.9	15.7
	SF 1	42.6	12.8	6.2	26.1	14.3
	SF 3.5	42.6	12	6.17	27.7	13.6
	VFF -1.5	41	13.8	5.84	23.6	15
	VFF 1	41.5	14.1	6.17	24	15.4
	VFF 3.5	41	13	6.23	25.8	14.4
	P value	<.001	<.001	0.2	<.001	<.001

Whereas L* a* and b* are individual wavelengths and components of the visible light spectrum, Chroma and Hue represent the total image and so better represent what the eye actually sees. Chroma, or saturation, is the value of the "colourfulness" (absolute colour intensity) (AMSA, 2011).

Chroma values for all the regimens decreased as display time progressed, with the decline throughout the display days being consistent for all regimens (Table 3). There was no interaction between display time and regimen as all values decreased relative to each other ($P = 0.107$). On days 1 and day 7, the Control had a higher colour intensity compared with all regimens ($P < 0.001$). On day 7, SF 3.5°C had the least intense colour.

Hue angle is an indication of discolouration and a higher value is associated with greater discolouration or change in colour from the original colour measurement i.e. the meat becomes browner (Table 3).

Between day 1 and day 4 hue angle for SF -1.5°C, FF -1.5° and VFF -1.5°C increased rapidly and then decreased again. This trend could be due to the fact that on days 4 and 7, a different slice of loin is used than on day 1. Day 7 values are the more relevant results, as this is critical to the consumer acceptability. SF -1.5°C, SF 1°C, SF 3.5°C and VFF 3.5°C had the most rapid accumulation of surface discolouration throughout the display period (Table 3). On day 7, FF -1.5°C, FF 1°C, FF 3.5°C, VFF -1.5°C and VFF 1°C had the lowest hue angle values, indicating the least discolouration and thus superior colour stability.

There is evidence of an interaction between regimens and display time ($P < 0.001$), with SF -1.5, SF 1, SF 3.5 and VFF 3.5 showing a faster browning of the samples. These results correlate with the previous results for b* value (yellowness).

It is of interest to note that the samples at day 4 of display, which show an increase for b* value and Hue angle is a different slice of the same loin compared to day 1. The consequences of this will be addressed in the discussion chapter.

Although significant differences were found for the colour measurements between the freezing-thawing/ageing regimens and the Control, they were still within the limits of what consumers would accept (Farouk, Bekhit, Dobbie & Waller, 2007). Figure 7 shows the difference between Control and SF 1°C after 7 days of retail display. These are an example of the two extremes found within the samples, and although both samples are still looking acceptable, SF 1°C is more discoloured than Control.

Figure 7: Effect of display time on sample Control (left) and SF 1 (right) after 7 days

3.2.3 Lipid Oxidation

Lipid oxidation, which results in the production of free radicals, is closely coupled with pigment oxidation (Muela, Sañudo, Campo, Medel & Beltrán, 2010). Although oxygen creates and maintains the desirable red colour of fresh meat, it also promotes the oxidation of lipids, creating a rancid flavour. TBARS values were measured to determine the level of lipid oxidation for the treatments.

Table 4: Effect of freezing thawing/ageing regimens and display time on the level of TBA (mg malondialdehyde/kg meat) SED = 0.012

Treatment	Day 1	Day 7	Difference
CO -1.5	0.32	0.38	0.06
SF -1.5	0.34	0.40	0.06
FF -1.5	0.33	0.34	0.01
VFF -1.5	0.29	0.33	0.04
SF 1	0.32	0.38	0.06
FF 1	0.30	0.32	0.02
VFF 1	0.30	0.35	0.06
SF 3.5	0.33	0.40	0.07
FF 3.5	0.29	0.35	0.06
VFF 3.5	0.27	0.38	0.11

No significant differences were found between the regimens overall (P = 0.094) (Table 4). However, strong evidence for an increase in TBARS values between day 1 and day 7 (P < 0.001) was found for all regimens. FF-1.5°C displayed the smallest increase (0.01 units) and VFF 3.5 the largest (0.11 units), with increases for the other regimens falling between these values. Since no evidence was found for any time treatment interaction overall (P = 0.052), it appears that the level of lipid oxidation was not affected by treatment, and all regimes displayed the natural increase usually observed at 7 days of retail display.

FF -1.5°C had the least amount of lipid oxidation of all samples, and showed no significant difference between day 1 and 7 of display, while FF 1°C also exhibited no significant difference between these days.

A trend was observed within the freezing groups, where FF had a consistently lower TBARS level on day 7 when compared with VFF and SF. Furthermore, FF -1.5°C also exhibited lower TBARS values between day 1 and day 7 when compared to thawing/ageing at 1°C and 3.5°C, although the difference between temperatures was not significant.

Even though there were differences in the TBARS levels between the regimens, it was only around a maximum of 0.4 mg MDA/kg meat, which is well below the level of 1 mg MDA/kg meat that is the threshold for detection by the consumer (Jayasingh, Cornforth, Brennand, Carpenter & Whittier, 2002).

3.2.4 Tenderness

Measuring the tenderness of the meat is important because the freezing regimens can influence protein degradation and so influence meat tenderness. Another reason that it is important is that, after colour, consumers mainly judge the experience of eating meat on tenderness.

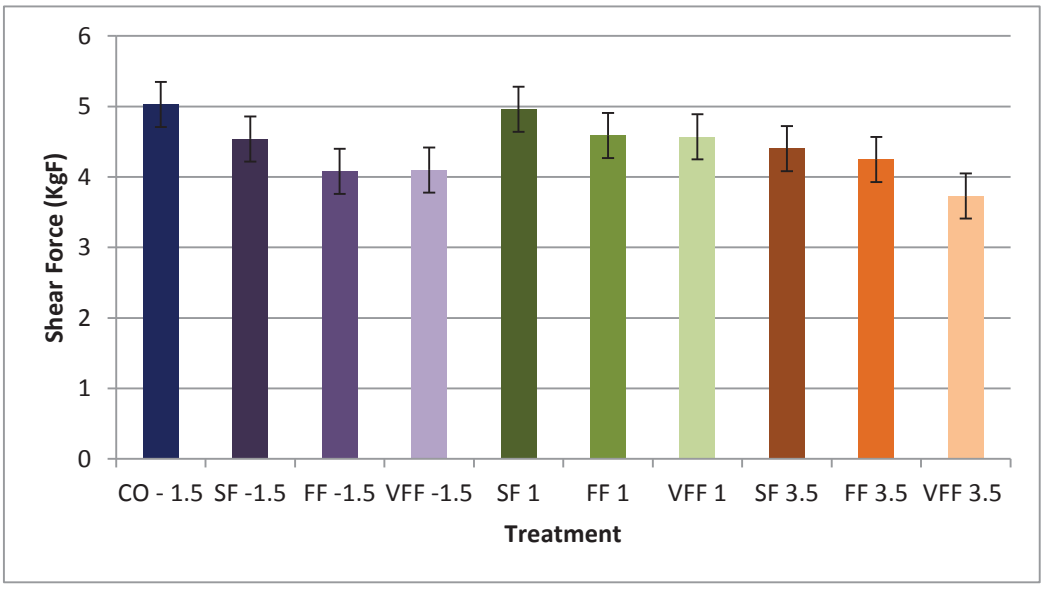


Figure 8: Effect of different freezing thawing/ageing regimens (SF, FF and VFF and thawing/ageing temperatures -1.5°C, 1°C and 3.5°C) on the shear force required to break/shear the loin fibres SED = 0.324

There are some significant ($P < 0.009$) differences between regimens (Figure 8), with the Control being less tender than FF -1.5°C, VFF -1.5°C, FF 3.5°C and VFF 3.5°C. However, all

values are below 5 kgF, and thus the consumer would consider all the samples to be very tender (Bickerstaffe, Bekhit, Robertson, Roberts & Geesink, 2001). This suggests that it is unlikely the consumer would be able to detect any significant differences between the samples.

3.2.5 Water holding capacity

Poor water holding capacity (WHC) results in high water loss from the carcass or cut up muscle. WHC was measured using three different methods: purge loss, drip loss and cook loss.

The different freeze, thaw/ageing regimens significantly affected the overall purge loss (Figure 9) of the loin samples ($P < 0.001$). Within the thawing/ageing at -1.5°C group, FF -1.5°C and the Control had similar amounts of purge loss, while the purge loss for VFF -1.5°C was higher than the Control sample but not statistically significant. However, SF -1.5°C is significantly higher ($P < 0.001$) than FF -1.5°C, VFF -1.5°C and the Control. Ageing at 1°C shows the same trend as -1.5, but SF 1°C lost more purge liquid ($P < 0.001$) than SF -1.5°C. An increase in purge loss for FF 1°C and VFF 1°C when compared to at -1.5°C was also detected; however, this difference is just a numerical trend and not significant. Interestingly, thawing/ageing at 3.5°C shows the same trend, with an increase in purge loss for SF 3.5°C when compared to FF 3.5°C and VFF 3.5°C. SF at all thawing/aging temperatures had higher purge losses (almost 2 times greater) compared to the other two freezing regimens.

The results show that the Control sample has a lower drip loss ($P < 0.001$) than SF -1.5°C, FF -1.5°C, VFF -1.5°C, FF 1°C VFF 1°C, FF 3.5°C and VFF 3.5°C (Figure 10) . Interestingly, SF 1°C and SF 3.5°C had drip loss volume comparable to the Control.

The total water loss in a sample is the combined values of purge loss and drip loss (Figure 11). The results show that the Control had the lowest total water loss ($P < 0.001$) in comparison to all regimens.

During heating, meat proteins will denature, and denaturation causes structural changes in the muscle fibres such as shrinkage or aggregation resulting in cook loss (Figure 12). Only one significant difference was found for cook loss; SF -1.5°C had a lower cook loss than Control ($P = 0.05$), however, there was a non-significant trend for the other -1.5°C regimens, (FF -1.5°C and VFF -1.5°C) to also have lower cook losses than the other regimens.

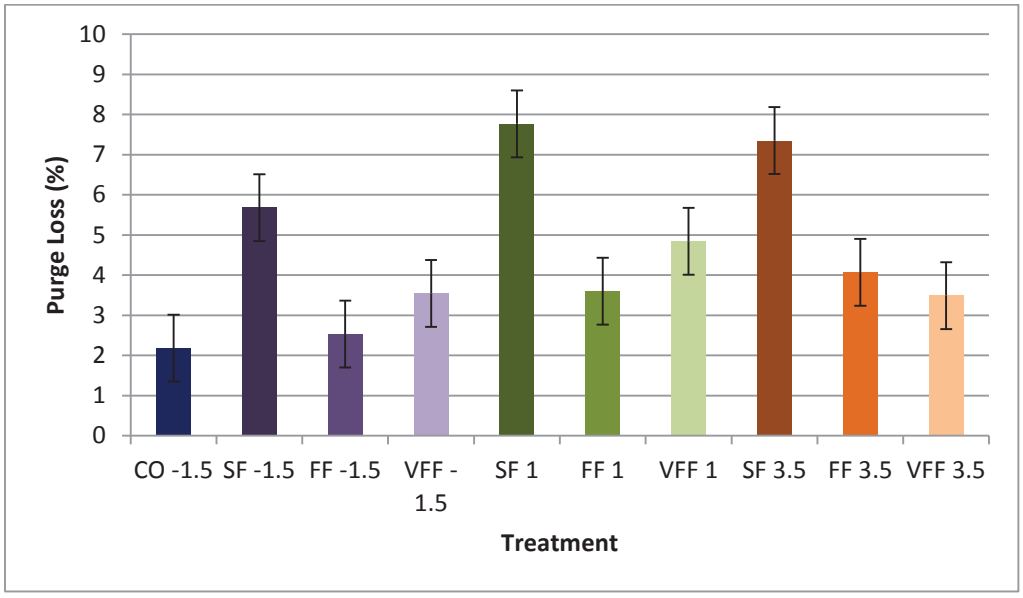


Figure 9: Effect of different freezing thawing/ageing regimens (SF, FF and VFF and thawing/ageing temperatures -1.5°C, 1°C and 3.5°C) on purge loss (%) ± SE 0.833

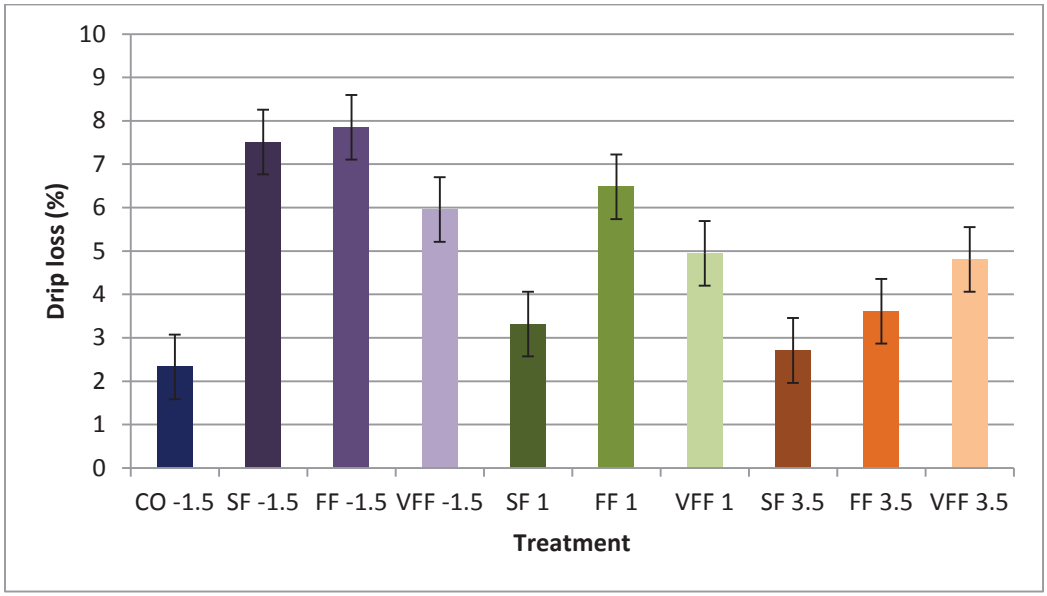


Figure 10: **Effect of different freezing thawing/ageing regimens (SF, FF and VFF and thawing/ageing temperatures -1.5°C, 1°C and 3.5°C) on drip loss (%) ± SE 0.754**

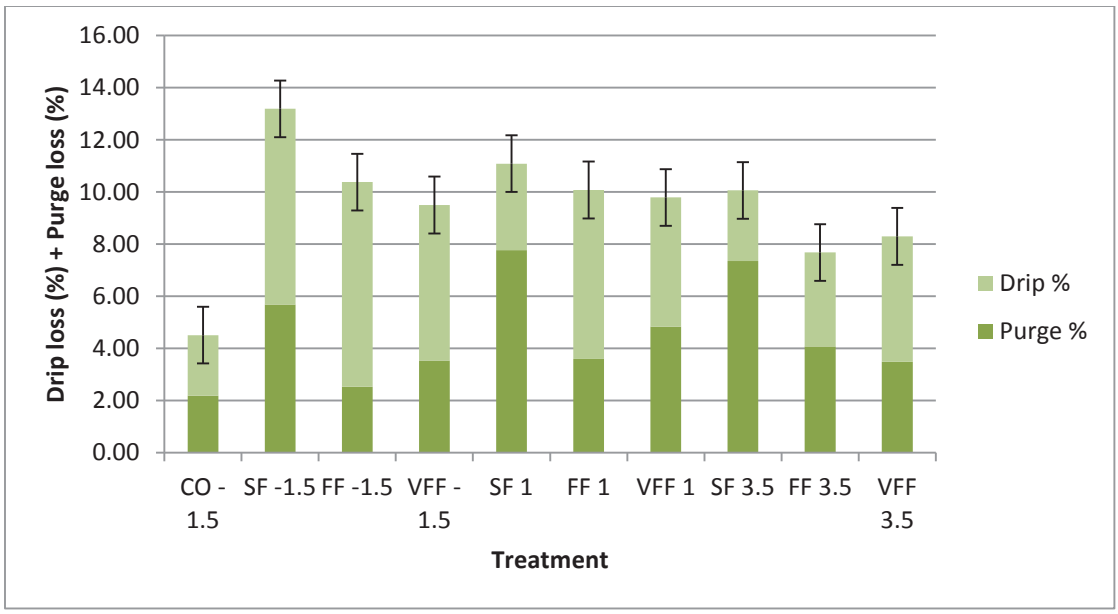


Figure 11: Effect of different freezing thawing/ageing regimens (SF, FF and VFF and thawing/ageing temperatures -1.5°C, 1°C and 3.5°C) on the water holding capacity – purge loss and drip loss SED = 1.091

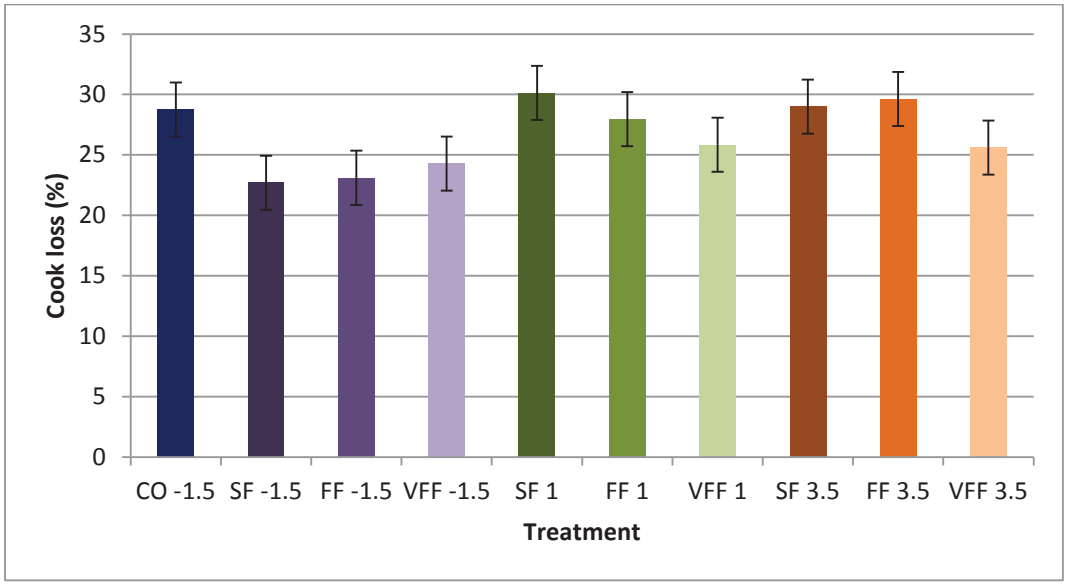


Figure 12: Effect of different freezing thawing/ageing regimens (SF, FF and VFF and thawing/ageing temperatures -1.5°C, 1°C and 3.5°C) on the cook loss SED =2.24

3.3 Histological analysis

As stated in the Material and Methods chapter, the loin samples from each regimen were cut transversally to the muscle fibre at the end of each assigned freezing thawing/ageing period. In order to determine if any obvious histological changes have occurred in the samples following each regimen, the tissues were processed for histological analysis and stained with Haematoxylin and Eosin (H&E staining) for determination of structural changes.

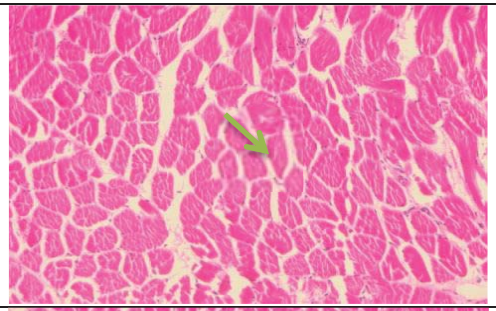
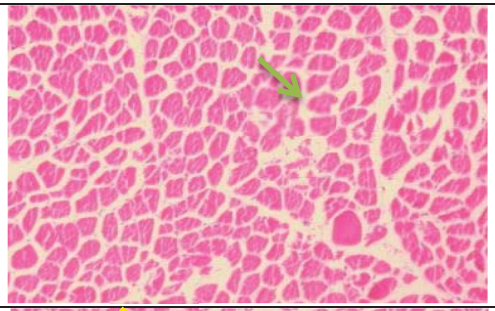
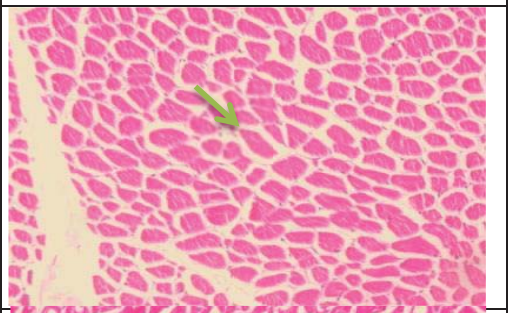
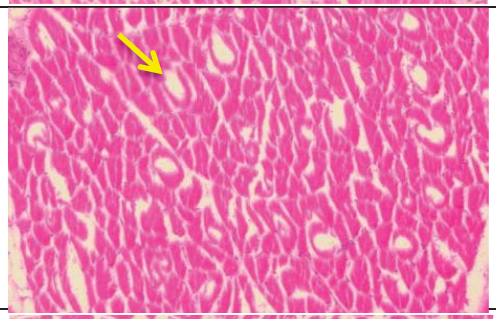
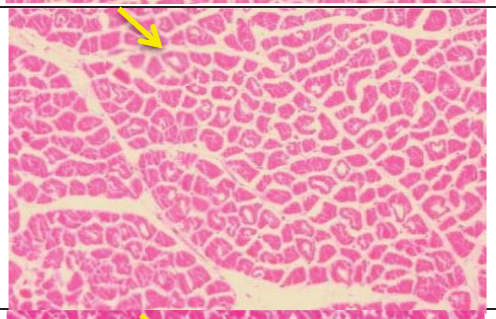
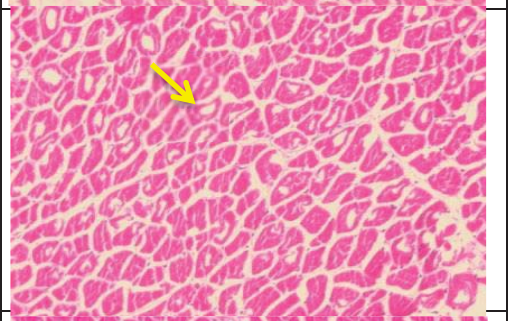
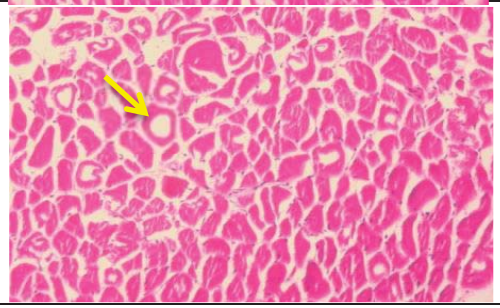
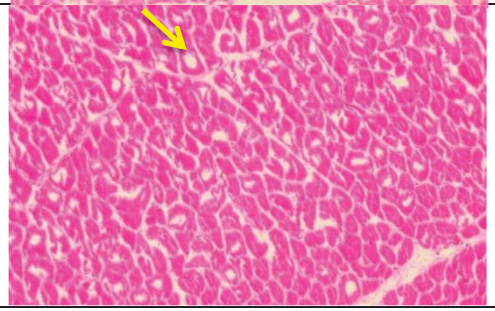
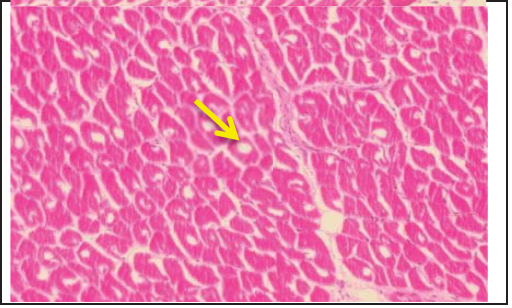
Haematoxylin colours the nuclei of cells blue, while Eosin staining, being an acidic dye, has an affinity for the cytoplasmic components of the cell, staining the cytoplasm pink/red.

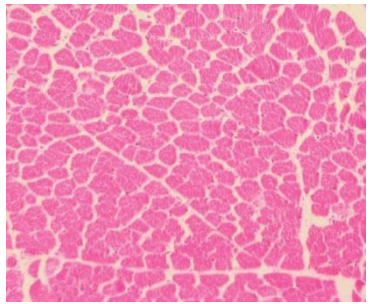
As seen in Table 5, H&E staining of samples from the different freezing regimens were compared to the control samples. As a non-frozen sample, the Control shouldn't show any freeze damage (cryodamage) to the tissue, and this is indeed what is observed. When compared to the control, SF samples show bigger gaps between the fibres (Indicated by green arrows) than their FF and VFF counterparts.

There are no observed differences between FF and VFF. Both regimes show obvious 'holes' within the fibres (Table 5, yellow arrows). This pattern of cryodamage is different from what is observed for SF and Control samples, and is a consequence of intracellular damage.

Overall, SF samples show intracellular damage, as a consequence of ice crystals being formed in-between the fibres, while both FF and VFF samples show extracellular damage to the tissue, as a consequence of ice crystals being formed within the fibre

Table 5: transverse section of the muscle at different freezing thawing/ageing regimens (SF, FF and VFF and thawing ageing temperatures -1.5°C, 1°C and 3.5°C)

Freezing regime	Thawing/ageing -1.5°C	Thawing/ageing 1°C	Thawing/ageing 3.5°C
Slow Freezing			
Fast Freezing			
Very Fast Freezing			



Control Sample – Non-frozen

3.3.1 Metabolomics results

There is currently no published data on the metabolite composition of purge from lamb meat. Finding a way of processing the purge samples is the priority of this work, rather than simply determining the composition of purge.

3.3.2 Metabolites in purge

The 1D NMR spectra confirmed that protein was still present in the majority of the samples and also that the degree of protein removal fluctuated between samples. The reason for this variation in protein removal is likely disruption of the membrane of the centrifugal filters during the washing procedure. As a result, both the volume and colour of the filtrate also varied between samples, reflecting the variation in protein content. Broad resonances originating from proteins were visible in the NMR spectra of the majority of the samples. This problem was circumvented by using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence, instead of the more commonly used noesypr1d pulse sequence. The CPMG pulse sequence attenuates signals from macromolecules, such as proteins, while keeping signals from small molecules, like metabolites, intact.

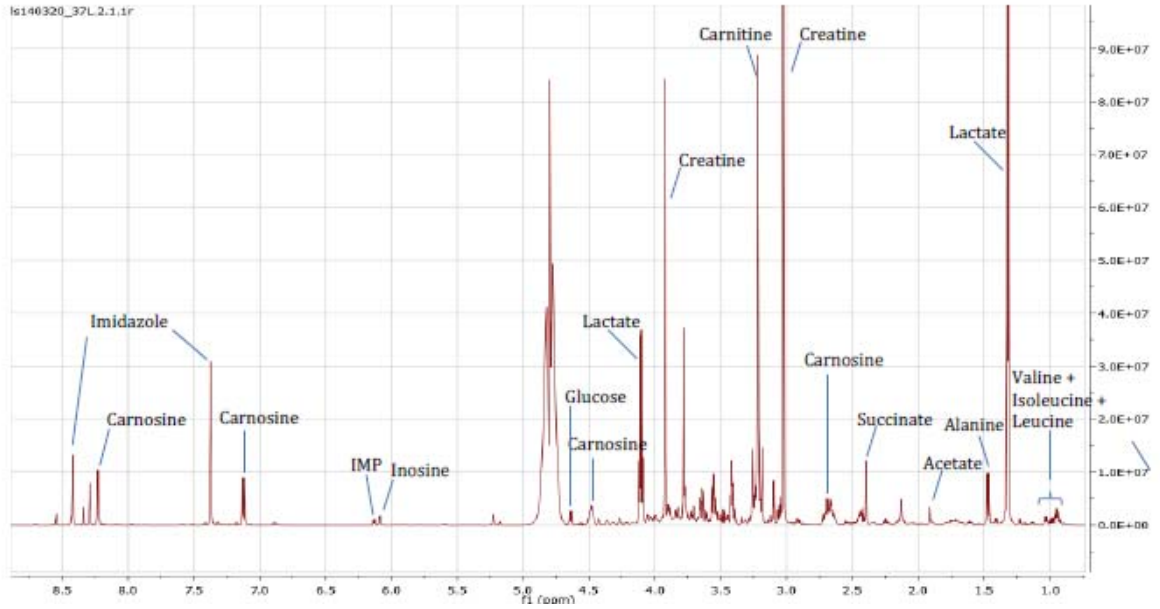


Figure 13: 1D ^{1}H-NMR spectrum of purge after regimens. Peaks arising from some common metabolites are indicated in the spectrum. Imidazole was added as a pH indicator

Each metabolite has a unique NMR spectrum arising from peaks from its H atoms (hydrogen atoms). The position, area and appearance of each peak are related to the chemical structure of the metabolite. A list of 32 metabolites (Table 6) identified in the purge is presented in order of decreasing concentration. The majority of the identified metabolites were amino acids, organic acids, carbohydrates and osmolytes.

The positions of some of the metabolite peaks (e.g. carnosine) varied greatly between the samples, due to small differences in pH. The pK_a of carnosine is close to 7, meaning that there will be peak fluctuations despite adding buffer (pH 7.0) to all samples.

Table 6: metabolites identified in purge in order of decreasing concentration

Metabolite	Metabolite type
Lactate	Anion of Lactic acid
Creatine	Organic acid
Carnosine	Dipeptide of β-alanine and histidine
Anserine	Methylated dipeptide of β-alanine and histidine

Carnitine	Quaternary ammonium compound made from synthesis of amino acids lysine and methionine
Glucose	Carbohydrate (monosaccharide)
Glycerol	Sugar alcohol compound (polyol)
Alanine	Amino acid
Taurine	Organic acid, osmolyte
Glutamine	Amino acid
Glycine	Amino acid, osmolyte
Succinate	Anion of succinic acid
Inosine	Nucleoside
Pyruvate	Anion of pyruvic acid
Leucine	Amino acid
Valine	Amino acid
Lysine	Amino acid
Acetate	Anion of acetic acid
Methionine	Amino acid
Proline	Amino acid
Betaine	Amino acid, osmolyte
Malate	Dicarboxylic acid, anion of malic acid
Isoleucine	Amino acid
Tyrosine	Amino acid
Phenylalanine	Amino acid
Nicotinurate	Organic acid
O-Acetylcarnitine	Acetylated form of L-Carnitine
Inosine Mono Phosphate	Nucleoside, acid
Formate	Anion pf formic acid
Histidine	Amino acid
Fumarate	Anion of fumaric acid
Tryptophan	Amino acid

3.3.3 Data analysis of the whole dataset

Principal Component Analysis (PCA) was used (Table 6) to get an overview of the entire data set. The different colours represent the freezing regimens (Control, SF, FF and VFF). Although no major differences between regimens were detected, the control group appears to be somewhat different from the other freezing regimens.

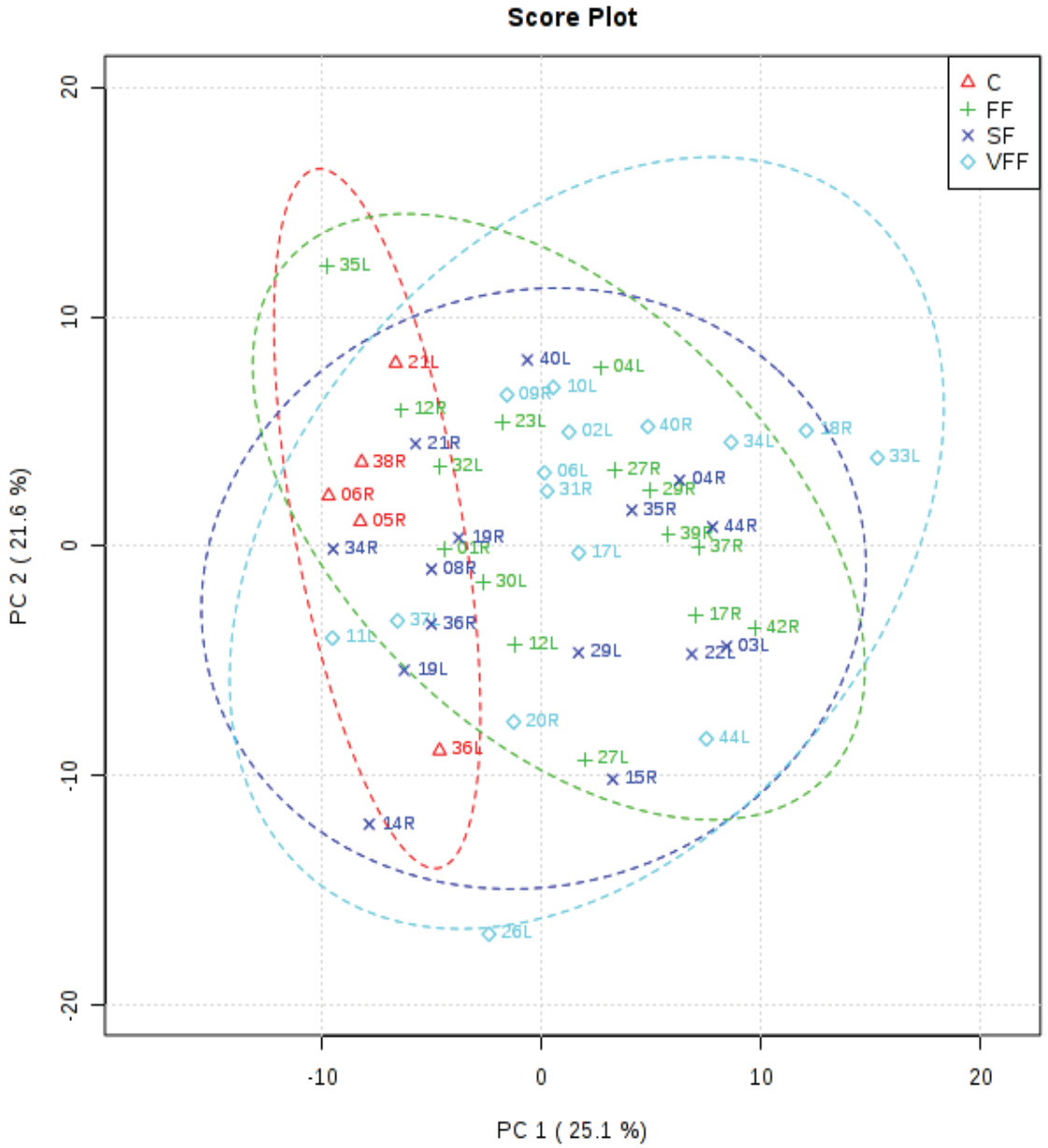


Figure 14: PCA scores plot of the whole dataset. Samples are coloured according the freezing regimens

3.3.4 Data analysis of thawing/ageing -1.5 °C subset

Pairwise comparisons were made of purge from controls and purge from each one of the three thawing/ageing regimes at -1.5°C (Figure 14). The Control was significantly different from the other regimens ($P < 0.05$). The largest differences was found when comparing the Control with VFF, where the peak areas in 40 of the bins were significantly different ($P =$

0.05) between groups; most of them were higher in the VFF group. The second largest differences were found in Control vs FF, where 31 bins were significant different (P = 0.05) and most of them higher in FF. Furthermore, 19 bins significantly differed between Control and SF and most of them were higher in SF. The corresponding metabolites from these pairwise comparisons are listed in appendix 2. No differences were observed between SF and FF, SF and VFF, or FF and VFF.

3.3.5 Effect of ageing temperature

Overall, the effects of ageing temperature on the metabolite profile were relatively small; the largest difference was between -1.5°C and 1°C.

3.3.6 Summary of metabolomics results

The metabolite profiles of purge from lamb meat frozen with SF, FF or VFF were different from that of the control. However, because of the sample preparation problems (variable protein removal), it was impossible to extract absolute quantitative information from the NMR spectra, which would have provided information about the amounts of each of the metabolites expelled in the purge. Instead, the peaks were normalised to the total area of all metabolite peaks in the NMR spectrum. The majority of metabolites lost in purge were amino acids.

4. Discussion

4.1 Introduction

Currently New Zealand prime cuts are mostly exported as chilled products to overseas markets. Due to environmental and economic issues the time it takes to ship meat has gone up from 6-8 weeks to 10-12 weeks. This increase in travel time can affect the meat quality attributes of the transported product. Freezing techniques could be used to compensate for this longer travel time, but it is well known that freezing can negatively influence some meat properties (Muela *et al.*, 2010).

Meat can be an attractive target for adulteration and there are many ways in which to achieve this. One very obvious kind is to illegally sell frozen and then thawed meat labelled as “fresh”. The attractiveness is obvious since fresh meat is more valuable than frozen meat. Legislative requirements are different in each of the countries New Zealand exports meat to. Nevertheless, none of the countries allows previously frozen meat to be sold as fresh. A few published investigations have shown that fraud or mislabelling is a problem in the case of thawed meat sold as fresh meat i.e. 43 meat samples collected in Switzerland were wrongly declared as fresh (Gremaud, G., Karlen, S., & Hulliger, K. 2002). Furthermore, an Internet search revealed that during recent years numerous cases have occurred where meat has been sold after frozen storage far exceeding the expiry date. Although there is an awareness of the overseas requirements this is not in the scope of the current thesis and will therefore not be included in the discussion and conclusion.

Recent studies have found that tenderness and drip loss differences between chilled and frozen meat can be reduced if the meat is aged sufficiently prior to freezing (Farouk,

Wiklund, Stuart & Dobbie, 2009b). Furthermore, the colour of frozen/thawed beef and venison meat can actually be improved by ageing the meat prior to freezing when compared to meat frozen at 48h post mortem (Farouk, Wiklund, Stuart & Dobbie, 2009a).

The usual order of processing frozen meat is to age prior to freezing. However, during the time of the current study a paper has been published by Grayson, King, Shackelford, Koohmaraie and Wheeler (2014), exploring the effect of just freezing and thawing, with no ageing, as well as freezing, thawing-aging on meat quality. However, the focus was mainly on the effect the processes had on tenderness, with very little focus on other meat quality parameters. They concluded that freezing-thawing/ageing may be a viable option to improve the consistency of meat tenderness and may be a viable option to improve the consistency of meat tenderness. This conclusion agrees with the current study although you can't necessarily translate it directly to this study without first repeating their experiment on ovine meat as it has been shown to sometimes display different properties in terms of tenderness (Bickerstaffe *et al.*, 2001).

Furthermore the study described here focused on the order of processing (freeze, thaw-ageing), as well as freezing speed and ageing temperatures, to find an ideal alternative for the current way of processing meat for export. It also is compatible with the strategy of stocking up on meat when the price is low due to seasonal price fluctuations and then being able to distribute it in times when meat may be more scarce, evening out the price throughout the year and being able to better guarantee the price of meat to both farmer and consumer.

4.1 Freezing rate and influence on muscle structure

Grujić *et al.* (1993) published that meat is best frozen around the eutectic point (-40°C to -50°C). Muscles under such conditions have the least damage because ice crystals will be formed exclusively inside fibres. Furthermore, at these temperatures all the water in the meat is frozen and thus not available for chemical reactions that could negatively influence the final quality attributes of the meat. Often papers don't provide details around the rate or final temperature value for freezing, but only use terminology such as "slow freezing" or "fast freezing" to refer to their freezing methods, making it challenging to draw conclusions from various published papers around this subject.

The present research did not achieve the desired temperature within the range of -40°C to -50°C for the VFF regime, only reached -32°C instead. Grujić *et al.* (1993) reported that freezing at -20°C had a considerably greater negative effect than freezing at -40°C, which gave a significantly smaller ice crystal diameter ($P < 0.05$). Although -32°C was significantly colder than the temperatures for the FF and SF regimes, it is possible that the ice crystal formation could still be detrimental to the muscle fibres.

The histology results confirmed that the damage in SF regimens was different than FF and VFF. The SF samples show intercellular damage while FF and VFF showed obvious 'holes' within the fibres, an indication of intracellular damage. There were no noticeable differences between the histological results from the FF and VFF regimens. This is in agreement with the results of Grujić *et al.* (1993) and Rahelić *et al.* (1985) who showed that large ice crystals were formed between fibres when frozen at relatively high temperatures. However, they were smaller and inside the fibres when frozen at a lower temperature. Grujić *et al.* (1993) sliced the samples while frozen before fixing for cryo-SEM (cryo-scanning

electron microscopy) analysis. However, ice crystal diameter was not measured in the current research, as the meat samples had been aged after the freezing period. Furthermore we did not have the capability to perform cryo-SEM analysis in our research facility.

4.2 Influence of freezing on pH

Measuring pH in meat is complicated because a muscle does not have a homogenous structure. The irregular structure means pH readings are unlikely to be consistent at different places along the meat cut (AMSA, 2011). To reduce the chance of an incorrect reading, the meat is measured at each end of the loin muscle and the results then averaged. To avoid the confounding effect of initial pH on the freezing rate of the samples as mentioned in the literature section, only loins with pH bellow 5.8 were used in the present study. However, a significant increase in pH over the total processing time was found only for the Control and VFF-1.5°C. Even though this increase is measureable and statistically significant, the differences were less than 0.1 points on the pH scale. Most importantly, the samples from all treatments fell well within the optimal lamb meat pH of 5.5 - 5.8 (Devine *et al.*, 1993). Therefore, the observed differences most likely would not have any noticeable influence on the meat quality attributes of the samples following the different freezing/thawing-aging regimens. Furthermore, these results also match with previous work by Balan *et al.* (2013), who found that although freezing/thawing-ageing treatments did influence pH of the loins after storage, the maximum difference between the treatments was less than 0.1. The researchers concluded that this range of pH change was unlikely to be a major influence on meat quality characteristics.

~~Farouk *et al.* (2013) found that meat pH possibly has an influence on the freezing rate of unprocessed beef. Their evidence suggests that high pH meat is more likely to have a higher freezing temperature and a more rapid rate of crystallisation than normal pH meat. Although the reasons for this rise in freezing point temperature are unclear, a reason may be that normal pH meats have a higher amount of dissolved solutes, considering that muscles with lower ultimate pH have a higher concentration of lactic acid and are more likely to contain residual glycogen than high pH muscles (Farouk *et al.*, 2013). However it is not easy to extend the results from the above mentioned study, which were obtained using a -80°C freezer, to what happens in industrial process method, where the meat is held in an environment of approximately -1.5°C for many weeks.~~

4.3 Freezing and the influence on colour

An increase in storage temperature has a marked effect in reducing colour stability (O'Keefe & Hood, 1982). On the other hand, the onset of discolouration in meat is delayed by low temperature storage due to slower enzymatic activity, leading to a decreased rate of myoglobin oxidation (Johnson, 1991). Maximum storage life is achieved when meat is held at -1.5°C (Rosenvold & Wiklund, 2011), which is the lowest temperature that can be maintained indefinitely without the meat freezing (Gill & Jones, 1992). Rosenvold *et al.* (2011) reported that even a short temperature abuse such as having the meat at 2°C for one week out of a total of 7 weeks at -1.5°C, had a large impact on retail colour display life. This could be due to a more rapid rate of MMB production at higher temperatures, resulting in a marked decrease in lightness, from day 5, in the retail display life of lamb loins.

In the present study, two different slices of meat had to be used for colour measurements for each regimen, due to limitations on the amount of readings that can be physically taken

from one slice of meat, and the minimum of three time points that was deemed necessary for an overview of discolouration during retail colour display. Although both slices were next to each other along the loin, the slices are likely not identical and could possibly show a differing discolouration pattern (AMSA, 2011). However, despite a slight discrepancy on the rate of discoloration for the regimens aged at -1.5°C on day 4, where the second meat sample was used, this was not significant and was consistent with results obtained using a single meat sample for all readings (Rosenvold *et al.*, 2011). Therefore, it is feasible to conclude that the use of a second sample for day 4 readings did not affect the results obtained.

Increased lightness of meat during ageing has been reported by several studies and agrees with the results presented here. The variation in lightness can be attributed to the different freezing regimens, since an increase in lightness can result from structural changes in the muscle protein during ageing (MacDougall, 1982). In addition, more free water within the aged meat might increase the light scattering coefficient, resulting in a lighter surface colour (Farouk, Mustafa, Wu, Stuart, Dobbie & Krsinic, 2010). Here, the Control was the lightest colour, which is in agreement with the work by (Moore & Young, 1991).

Previous studies using the same method and equipment have shown that a redder colour in meat is much preferred by the consumer (Farouk *et al.*, 2007; Wiklund, Stevenson-Barry, Duncan & Littlejohn, 2001). These studies indicate a* values of 12 as the cut-off point for the acceptability of venison and beef (Farouk *et al.*, 2007), and none of the lamb loin values obtained here dropped to this level. Furthermore, Farouk *et al.* (2009a) reported that a hue angle within the range of 19-25° is still acceptable to consumers. SF -1.5°C, SF 1°C SF 3.5°C and VFF 3.5°C had hue angles of 25.9°, 26.1°, 27.7° and 25.8° respectively, and are therefore

close to the limit of what consumers might find acceptable. However, the values for all other regimens were within the acceptable rage (below 25°) which is in agreement with the current study. Care must be taken before extrapolating the results from the present study to other muscles and/or other animal species when studying colour decline as a result of different chilling rates. Different muscles from the same animal, as well as different animal species, can display different physiological characteristcs that will influence pH decline and ultimaly meat colour (AMSA, 2011).

In New Zealand, lambs can be classified into two different age classes when sent to the abattoir for slaughter; new season lambs are 3 to 4 months old, and old season lambs are 10 to 11 months old. Kim, Stuart, Black and Rosenvold (2012) reported that new season lambs started with a greater L* value indicating lighter muscles than old season lambs. Old season lambs had a higher a* value indicating more redness than new season lambs. The present study used new season lambs, and because oxidative stability of meat can be adversely influenced by animal age (Kim, Bødker & Rosenvold, 2012) we can speculate that the oxidative damage to the meat observed in this study, regardless of the treatment, would be lower than that obtained from old season lambs subjects to the same conditions. Frozen storage is useful in matching supply with varying market demands, but experience shows that long-term frozen-stored meat may have quality problems such as inferior colour and rancid smell and taste (Xia, Kong, Liu & Liu, 2009).

In the current study, an increase in the level of lipid oxidation is found for all regimens over the retail colour display life, although none increased significantly. Furthermore, the TBARS values all remained under 1 mg MDA/kg meat, the threshold for consumer to detect off-flavours (Jayasingh *et al.*, 2002) and as the same methods were used (Buege & Aust, 1978)

in this study, direct comparisons be made allowing us to conclude that none of the increases in oxidation observed here are large enough that negative changes would be detected by consumers

It is possible that TBARS would have shown a difference between the treatments for display periods if conducted over a 10 days period, as at day 7 the samples were just starting to change colour, and lipid oxidation is closely related to discolouration.

4.4 Tenderness

Tenderness is the most important factor influencing consumer satisfaction with the eating experience. There is a general agreement in the literature that the tenderness of meat increases with freezing and thawing (Leygonie, Britz & Hoffman, 2012a). It has also been found that the increase in tenderness is correlated to the length of frozen storage and the degree to which the meat was aged prior to freezing. This could be due to the breakdown of muscle fibres caused by enzyme activity and by ice crystal formation (Vieira *et al.*, 2009). The formation of large, extracellular ice crystals disrupts and breaks apart the large myofibrils, resulting in tenderisation, while the formation of small, intracellular ice crystals increases the rate of ageing, probably by the release of enzymes (Vieira *et al.*, 2009). However, alternative conclusions exist in the literature. For example, Shanks, Wulf and Maddock (2002) proposed that it is the muscle cell disruption due to intracellular ice formation during freezing that leads to the decreased peak force in frozen and thawed meat, although the study limited the freezing to only -16°C, which is not enough variation for evidence of multiple temperature effects.

A similar study by Grayson *et al.* (2014) on boneless striploin and boneless eye of round muscles of bovine animals shows an increase in tenderness when ageing occurred after a freezing/thawing period that could possibly be attributed to the proteolysis of a key structural myofibrillar protein. This protein, called μ-calpain, has been described previously to be the primary cause of meat tenderisation and is negatively regulated by calpastatin. Calpastatin, but not calpain, has been shown to be sensitive to frozen storage, decreasing over time.

Therefore freezing, thawing and then ageing might allow calpain to be more active and result in increased ageing and thus more tender results. A freezing, thawing, and subsequent ageing period could be used for meat from animals with higher calpastatin levels to improve the consistency of tenderness. Even though lamb and beef have similarities in terms of ageing processes, beef needs a much longer time to age than lamb and, therefore one could not just assume that lamb cuts would behave in the same way as beef. Regrettably in the current study Western Blot analysis was not performed due to time constraints. However, a previous study performed in our lab failed to show any differences between the treatments (Control, SF and FF) in the level of calpain activity (Balan *et al.*, 2013). Therefore, we expect that similar results would be seen in the present study.

The highest shear force value that was found for any regime in the current research was 5 KgF. According to a study on consumer perception on the tenderness of beef and lamb by Bickerstaffe *et al.* (2001), shear force values below or equal to 5 kgF for cooked beef and lamb meat were considered to be very tender by consumers. Therefore, in the present study, lamb loins from all treatments would still be considered very tender by consumers, regardless of the treatment. On the other hand, the current study only measured

tenderness by constant pressure, however, consumer satisfaction and their understanding of 'tenderness' is more than just the mechanical pressure needed to bite through a piece of meat, it is the whole eating experience. A sensory evaluation of tenderness by Lagerstedt *et al.* (2008) reported a lower peak force was required to bite through freeze/thaw samples compared to chilled-only meat. However, a trained sensory panel rated the freeze/thawed meat significantly less tender than the chilled meat. This conflicting result was ascribed to the loss of fluid during thawing that meant there was less water available to hydrate the muscle fibres. The subsequent higher density of the fibres seemed to increase the panel's perception of toughness. This means that even though mechanical measurements give a low acceptable shear force value for all regimes in this research, the actual eating experience could be different for the various freezing-thawing/ageing regimens in comparison to chilled meat.

4.5 Water holding capacity

The major difference between the various methods described in the literature is that some only use gravity to do the work, whereas in others the fluid is forced out of a piece of meat by mechanical means, such as the press or centrifugal methods (Trout, 1988). Purge loss is commonly measured for New Zealand meat during long-term chilled storage, as this reflects the conditions in the container during export from origin to its final market destination (Farouk *et al.*, 2012). Purge loss is considered an important meat quality attribute because it results in a decrease in meat weight, and subsequently a loss of potential money for the retailers (Payne, Durham, Scott & Devine, 1998).

In the current study, it is of interest to note that all SF regimens had a higher purge loss percentage than FF and VFF regimens. Moreover, the histology results confirm that the

damage in SF regimens was different than FF and VFF. As previously mentioned, the SF samples show intercellular damage while FF and VFF show obvious holes within the fibres, an indication of intracellular damage. These results are in agreement with Grujić *et al.* (1993) and Rahelić *et al.* (1985) who showed that faster freezing (as long as it was less than -70°C) provided small, intracellular ice crystals that minimised water loss from tissue damage.

Although most research in this area has been done on beef muscles, the observations are in agreement with our results obtained with lamb. Drip loss levels decreased over the thawing/ageing period while the purge increased. This was probably related to the fact that after water had already been released as purge, there was less water left to be released as drip loss. Cook loss is the last stage of measuring WHC, and simulates the experience the consumer will have while cooking the meat.

In the current study, cook loss was affected by the freezing-thawing/ageing regimens, however only the samples aged at -1.5°C showed significantly less cook loss than the Control and other treatments. Differences in cooking loss were most likely from damage done to fibres or cellular membranes during the aging period at the different temperatures. Damage to the fibres or cellular membranes could inhibit the ability to hold water, resulting in more cooking loss. Among treatments, cooking loss and purge loss could be affected by the temperature that the product is frozen, the rate of freezing, length of frozen storage and the rate of thawing (James *et al.*, 2002). Increases in fluid loss would be undesirable, and therefore it would be important to minimise the losses by optimising the processes. Pearson and Miller (1950) found no difference in the amount of drip loss or cook loss between different rates of freezing, however drip loss or cook loss tended to increase with increasing length of frozen storage time. Hiner, Madsen and Hankins (1945) determined that

intracellular ice crystal formation caused damage to the sarcolemma and myofibril, resulting in failing of the fibres to retain moisture. Hiner *et al.* (1945) also determined that freezing at -7.8°C resulted in higher drip loss compared to freezing at -40°C, and assigned the difference to where ice crystal formation occurred, concluding that ice crystals formed intracellularly could be reabsorbed in the fibre, leading to better fluid retention. These finding are in agreement with Grujić *et al.* (1993).

4.6 Metabolomics

Unfortunately, the results obtained here did not provide the insights originally hoped for in this research; however it is possible to use the results and techniques developed in this research to improve NMR methodology for the analysis of purge loss samples in the future. NMR has proven to be an extremely useful technique in the investigation and assessment of meat quality. A paper by Straadt, Aaslyng and Bertram (2011), that undertook a quality assessment of fresh pork and pork products from different breeds, was one of the few that discussed the constituents of drip loss in regards to NMR analysis in much the same way as the present study. They concluded that using drip/purge might be a more informative approach to assessing meat quality than the analysis of meat extracts.

The original intention of this research was to carry out quantitative metabolomics, as with this type of information it would have been possible to directly compare the metabolite concentrations in the purge between treatment groups. It would also have enabled correction for the total amount of purge, and the subsequent calculation of the amounts of each of the metabolites in the purge, allowing for a qualitative determination of nutrient loss for each of the different regimens. Unfortunately, since there were problems with the sample preparation, quantitative metabolomic data could not be obtained. The next option

was to normalise the peak area of an individual metabolite to the total area of all metabolite peaks in the NMR spectrum. This could show whether a certain metabolite increased in concentration in relation to the total concentration for a particular treatment. Regrettably, this approach did not enable an assessment of whether the overall levels of a particular metabolite are higher in one treatment than another. Therefore, no conclusions can be drawn on possible differences on metabolite/nutrient loss between the treatments.

5. Conclusion

This research investigated the effects of different freezing/thawing-ageing regimens on meat quality as well as on the physical and biochemical characteristics of lamb loins.

Freezing has had a "bad image", with consumers associating it with cheaper, low quality meat cuts because of inconsistent tenderness, more fluid loss, freezer burn and discolouration. However, the data from the current study suggest that FF and VFF regimens may reduce many of the negative changes caused by the standard freezing/thawing process high freezing temperatures (SF). The faster rates of freezing reduced the amount of water-loss, improving the product's appearance through reduced volumes of purge, as well as minimising the loss of soluble nutrients while maintaining equivalent tenderness, colour and shelf-life to the chilled only meat product.

Common quality defects associated with frozen/thawed meat such as fluid loss and a faster discolouration could be overcome by the application of FF and VFF. This can potentially provide an opportunity for the NZ meat industry by saving costs with a more economical alternative to shipping using chilled storage, increasing product life, while also supplying high quality meat products to high end markets throughout the year without compromising meat quality attributes. From these results it can be concluded that all VFF regimens were better than the SF but comparable to FF. The thawing rates did not seem to be able to overcome the differences caused by the freezing method.

6. Future directions

In summary, in the present study meat quality attributes of frozen meat have been found to be very similar to that of chilled meat. Furthermore, some of the freezing/thawing-ageing regimens described here have potential to become an alternative for the current export process. However still some variables should be investigated before introducing this to the industry.

The effect of freezing/thawing-ageing on different muscle types such as chuck and tenderloin could generate different results, as these muscles will differ in overall size, fibre type composition, as well as fat and collagen content. These variations can influence freezing rates, and therefore the ultimate effect of freezing/thawing-aging regimens on meat quality parameters could also be different. The same would be true for cuts from other meat animals, such as venison and beef. Therefore, further studies will be necessary to determine the required conditions to maintain high quality levels for individual cuts from different animal species, as well as to determine if a single regimen can be applied to different cuts from the same animal species.

The determination of the size and location of ice crystals formed in the meat during the freezing process and their relationship to water holding capacity should be the subject of further studies, especially considering that the size of ice crystals could be related to the freezing rate and its effect on meat quality characteristics such as WHC, thawing/ageing and protein degradation. Additional studies where cryo-SEM analysis is performed can help us further understand the influence of freezing rate on muscle integrity. Furthermore, the analysis of protein degradation in meat submitted to different freezing rates and different thawing-ageing temperatures can be revealing, as the freezing process can degrade

myofibrillar proteins when compared to chilled meat, and ultimately influence meat quality characteristics.

Most importantly, industry partners should be involved in this work to help with the scaling-up and optimisation required to take the process from laboratory to industrial scale. Especially challenging for future researchers and the industry will be fast thawing within the allowable safe temperature limits for pathogenic microbiological growth and development. To achieve this result a larger study should be conducted on the topic to validate the findings and to show the results hold for all categories of ovine stock.

References

Aberle, E. D., Forrest, J. C., Gerrard, D. E., Mills, E. W., Hedrick, H. B., & Judge, M. D. (2001). Growth and development of carcass tissues. *Principles of meat science,* 45-67.

Alizadeh, E., Chapleau, N., de Lamballerie, M., & Le-Bail, A. (2007). Effect of different freezing processes on the microstructure of Atlantic salmon (Salmo salar) fillets. *Innovative Food Science & Emerging Technologies, 8*(4), 493-499.

Ambrosiadis, I. N., Theodorakakos, S., Georgakis, & Lekas, S. (1994). Influence of thawing methods on the quality of frozen meat and drip loss. *Fleischwirtschaft, 74*284-286.

AMSA. (2011). Guidelines for the measurement of meat color. Champaign, Illinois, USA: American Meat Science Association.

Añón, M. C., & Calvelo, A. (1980). Freezing rate effects on the drip loss of frozen beef. *Meat Science, 4*(1), 1-14.

Baechle, T. R., & Earle, R. W. (2008). *Essentials of strength training and conditioning* Third ed.

Balan, P., Kim, B., Choe, J., Kemp, R., Salerno, M. S., & Stuart, A. (2013). Internal report - Effect of fast freezing then thaw-aging on meat quality attributes of lamb loins.

Ben Abdallah, M., Marchello, J. A., & Ahmad, H. A. (1999). Effect of Freezing and Microbial Growth on Myoglobin Derivatives of Beef. *Journal of Agricultural and Food Chemistry, 47*(10), 4093-4099.

Bevilacqua, A. E., & Zaritzky, N. E. (1980). Ice morphology in frozen beef. *International Journal of Food Science & Technology, 15*(6), 589-597.

Bickerstaffe, R., Bekhit, A. E. D., Robertson, L. J., Roberts, N., & Geesink, G. H. (2001). Impact of introducing specifications on the tenderness of retail meat. *Meat Science, 59*(3), 303-315.

Buege, J. A., & Aust, S. D. (1978). Microsomal lipid peroxidation. In *Methods in Enzymology*, (Vol. 52, pp. 302). New York, NY: Academic Press.

Chrystall, N. B., & Devine, C. E. (1991). Quality assurance for tenderness. *Meat Industries Research Institute of New Zealand Publication, No. 872.*

Committee, N. A. W. A. (2010). Animal Welfare (Commercial Slaughter) Code of Welfare 2010. In M. o. A. a. Forestry (Ed.). Wellington, New Zealand: The New Zealand Government.

Davey, C. L., & Gilbert, K. V. (1976a). The Temperature Coefficient of Beef Ageing. *Journal of Science of Food and Agriculture, 27*

Davey, C. L., & Gilbert, K. V. (1976b). The temperature coefficient of beef ageing. *Journal of the Science of Food and Agriculture, 27*(3), 244-250.

Devine, C. E., Graafhuis, A. E., Muir, P. D., & Chrystall, B. B. (1993). The effect of growth rate and ultimate pH on meat quality of lambs. *Meat Science, 35*(1), 63-77.

Devine, C. E., & Werner Klinth, J. (2004). CONVERSION OF MUSCLE TO MEAT | Ageing. In *Encyclopedia of Meat Sciences*, (pp. 330-338). Oxford: Elsevier.

Dumont, B. L. (1981). Beef quality, marketing and the consumer. In D. E. Hood, & P. V. Tarrant (Eds.), *The Problem of Dark-cutting in Beef*, (pp. 37-57). The Hague: Martinus Nijhoff.

Eastridge, J. S., & Bowker, B. C. (2011). Effect of Rapid Thawing on the Meat Quality Attributes of USDA select Beef Strip Loin Steaks. *Journal of Food Science, 76*(2), 156-162.

Estévez, M. (2011). Protein carbonyls in meat systems: A review. *Meat Science, 89*(3), 259-279.

Farouk, M., Mustafa, N. M., Wu, G., Stuart, A. D., Dobbie, P. M., & Krsinic, G. (2010). Meat ageing improvement in waterholding: a biophysical process?, *56th ICoMST*. Seoul, Korea.

Farouk, M., Wiklund, E., Stuart, A., & Dobbie, P. (2009a). Ageing prior to freezing improves the colour of frozen-thawed beef and venison. In *Proceedings 55th ICoMST* (pp. 786-790), 16-21 August 2009,Copenhagen, Denmark.

Farouk, M., Wiklund, E., Stuart, A., & Dobbie, P. (2009b). Ageing prior to freezing improves waterholding capacity in beef and venison. In *Proceedings 55th ICoMST* (pp. 781-785), 16-21 August 2009,Copenhagen, Denmark.

Farouk, M. M., Bekhit, A. E. D., Dobbie, P., & Waller, J. (2007). Towards benchmarking beef loin steak colour acceptability using Minolta and Hunter Colorimeters. In *Proceedings 53th ICoMST* (pp. 405-406),Beijing, China.

Farouk, M. M., Kemp, R. M., Cartwright, S., & North, M. (2013). The initial freezing point temperature of beef rises with the rise in pH: A short communication. *Meat Science, 94*(1), 121-124.

Farouk, M. M., Mustafa, N. M., Wu, G., & Krsinic, G. (2012). The "sponge effect" hypothesis: An alternative explanation of the improvement in the waterholding capacity of meat with ageing. *Meat Science, 90*(3), 670-677.

Faustman, C., & Cassens, R. G. (1990). The biochemical basis of discoloration in fresh meat: A review. *Journal of Muscle Foods, 1*(3), 217-243.

Gill, C. O., & Jones, S. D. M. (1992). Efficiency of a commercial process for the storage and distribution of vacuum packaged beef. *Journal of Food Protection, 55*880-887.

Gonzalez-Sanguinetti, S., AÑOn, M. C., & Calvelo, A. (1985). Effect of Thawing Rate on the Exudate Production of Frozen Beef. *Journal of Food Science, 50*(3), 697-700.

Grayson, A. L., King, D. A., Shackelford, S. D., Koohmaraie, M., & Wheeler, T. L. (2014). The Effects of Freezing and Thawing or Freezing, Thawing, and Aging Effects on Beef Tenderness. *Journal of Animal Science, 6*(92), 2735-2740.

Grujić, R., Petrović, L., Pikula, B., & Amidžić, L. (1993). Definition of the optimum freezing rate—1. Investigation of structure and ultrastructure of beef M. longissimus dorsi frozen at different freezing rates. *Meat Science, 33*(3), 301-318.

Hamm, R. (1986). 4 - Functional Properties of the Myofibrillar System and Their Measurements. In P. J. Bechtel (Ed.), *Muscle As Food*, (pp. 135-199). San Diego: Academic Press.

Hansen, E., Appelgren Trinderup, R., Hviid, M., Darré, M., & Skibsted, L. (2003). Thaw drip loss and protein characterization of drip from air-frozen, cryogen-frozen, and pressure-shift-frozen pork longissimus dorsi in relation to ice crystal size. *European Food Research and Technology, 218*(1), 2-6.

Hansen, E., Juncher, D., Henckel, P., Karlsson, A., Bertelsen, G., & Skibsted, L. H. (2004). Oxidative stability of chilled pork chops following long term freeze storage. *Meat Science, 68*(3), 479-484.

Haugland, A. (2002). Industrial thawing of fish — to improve quality, yield and capacity. Norwegian University of Sci. and Tech. Norway.

Hendrick, H. B., Aberle, E. B., Forrest, J. C., Judge, M. D., & Merkel, R. A. (1994). *Principles of Meat Science*. Third ed. Dubuque, Iowa: Kendall/Hunt Publishing Company.

Hendrick, J. P., & Hartl, F. U. (1993). Molecular chaperone functions of heat-shock proteins. *Annu Rev Biochem, 62*349-384.

Hiner, R. L., Madsen, L. L., & Hankins, O. G. (1945). HISTOLOGICAL CHARACTERISTICS, TENDERNESS, AND DRIP LOSSES OF BEEF IN RELATION TO TEMPERATURE OF FREEZING. *Journal of Food Science, 10*(4), 312-324.

Honikel, K. O. (1998). Reference Methods for the Assessment of Physical Characteristics of Meat. *Meat Science, 49*(4), 447-457.

Hughes, J. M., Oiseth, S. K., Purslow, P. P., & Warner, R. D. (2014). A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. *Meat Science, 98*(3), 520-532.

Huxley, H. E., & Hanson, J. (1957). Quantitative studies on the structure of cross-striated myofibrils: I. Investigations by interference microscopy. *Biochimica et Biophysica Acta, 23*(0), 229-249.

James, S. J., & James, C. (2002). Meat Refrigeration. In S. J. James, & C. James (Eds.): Woodhead Publishing.

Jayasingh, P., Cornforth, D. P., Brennand, C. P., Carpenter, C. E., & Whittier, D. R. (2002). Sensory evaluation of ground beef stored in high-oxygen modified atmosphere packaging. *Journal of Food Science, 67*(9), 3493-3496.

Jeremiah, L. E. (1996). *Freezing effects on food quality*. Agriculture and Agri-Food Canada Research Centre, Lacombe. Alberta, Canada.

Johnson, B. Y. (1991). Colour of chilled meat, *CSIRO Workshop - Production of Chilled Meat for Export*.

Kapper, C., Walukonis, C. J., Scheffler, T. L., Scheffler, J. M., Don, C., Morgan, M. T., Forrest, J. C., & Gerrard, D. E. (2014). Moisture absorption early postmortem predicts ultimate drip loss in fresh pork. *Meat Science, 96*(2, Part A), 971-976.

Kim, Y. H., Keeton, J. T., Smith, S. B., Berghman, L. R., & Savell, J. W. (2009). Role of lactate dehydrogenase in metmyoglobin reduction and color stability of different bovine muscles. *Meat Science, 83*(3), *376-382*.

Kim, Y. H., Keeton, J. T., Smith, S. B., Maxim, J. E., Yang, H. S., & Savell, J. W. (2009). Evaluation of antioxidant capacity and colour stability of calcium lactate enhancement on fresh beef under highly oxidizing condition. *Food Chemistry, 115*(1), 272-278.

Kim, Y. H. B., Bødker, S., & Rosenvold, K. (2012). Influence of lamb age and high-oxygen modified atmosphere packaging on protein polymerization of long-term aged lamb loins. *Food Chemistry, 135*(1), 122-126.

Kim, Y. H. B., Frandsen, M., & Rosenvold, K. (2011). Effect of ageing prior to freezing on colour stability of ovine longissimus muscle. *Meat Science, 88*(3), 332-337.

Kim, Y. H. B., & Hunt, M. C. (In Press). Advance technology to improve meat color. In S. T. Joo (Ed.), *Control of meat quality*. Kerala, India: Research Signpost.

Kim, Y. H. B., Stuart, A., Black, C., & Rosenvold, K. (2012). Effect of lamb age and retail packaging types on the quality of long-term chilled lamb loins. *Meat Science, 90*(4), 962-966.

King, N. J., & Whyte, R. (2006). Does it Look Cooked? A Review of Factors That Influence Cooked Meat Color. *Journal of Food Science, 71* (4), R31-R39.

Kropf, D. H. (2008). Color Quality in Meat. In *Color Quality of Fresh and Processed Foods*, (Vol. 983, pp. 267-292): American Chemical Society.

Lagerstedt, Å., Enfält, L., Johansson, L., & Lundström, K. (2008). Effect of freezing on sensory quality, shear force and water loss in beef M. longissimus dorsi. *Meat Science, 80*(2), 457-461.

Lawrie, R. A. (1979). *Meat Science*. 3th edition ed.

Leygonie, C., Britz, T. J., & Hoffman, L. C. (2012a). Impact of freezing and thawing on the quality of meat: Review. *Meat Science, 91*(2), 93-98.

Leygonie, C., Britz, T. J., & Hoffman, L. C. (2012b). Meat quality comparison between fresh and frozen/thawed ostrich M. iliofibularis. *Meat Science, 91*(3), 364-368.

Liu, Z., Xiong, Y. L., & Chen, J. (2010). Protein Oxidation Enhances Hydration but Suppresses Water-Holding Capacity in Porcine Longissimus Muscle. *Journal of Agricultural and Food Chemistry, 58*(19), 10697-10704.

Livingston, D. J., & Brown, W. D. (1981). The chemistry of myoglobin and its reactions. *Food Tech., 35*238-252.

Livingston, D. J., Lamar, G.N. and BROWN, W.D. (1983). Myoglobin diffusion in bovine heart muscle. *Science 220,* 71.

MacDougall, D. B. (1982). Changes in the colour and opacity of meat. *Food Chemistry, 9*(1–2), 75-88.

MacFarlane, P. G., & Marer, J. M. (1966). An apparatus for determining the tenderness of meat. *Food Technologly, 838*134-135.

Mancini, R. A., & Hunt, M. C. (2005). Current research in meat color. *Meat Science, 71*(1), 100-121.

Mancini, R. A., Suman, S. P., Konda, M. K. R., & Ramanathan, R. (2009). Effect of carbon monoxide packaging and lactate enhancement on the color stability of beef steaks stored at 1 °C for 9 days. *Meat Science, 81*(1), 71-76.

Marsh, B. B., & Leet, N. G. (1966). Studies in Meat Tenderness. III. The Effects of Cold Shortening on Tenderness. *Journal of Food Science, 31*(3), 450-459.

Martini, F. H. (2001). *Fundamentals of Anatomy and Physiology*. New Jersey: Prentice Hall International Inc. .

MIA. (2009). Annual Report. Wellington: Meat Industry Association of New Zealand (Inc.).
MIA, M. I. A.-. (2012/13). 2013 Annual Report
Miller, R. K., & Werner Klinth, J. (2004). CHEMICAL AND PHYSICAL CHARACTERISTICS OF MEAT | Palatability. In *Encyclopedia of Meat Sciences*, (pp. 256-266). Oxford: Elsevier.
Moore, V. J., & Young, O. A. (1991). The effects of electrical stimulation, thawing, ageing and packaging on the colour and display life of lamb chops. *Meat Science, 30*(2), 131-145.
Muela, E., Sañudo, C., Campo, M. M., Medel, I., & Beltrán, J. A. (2010). Effect of freezing method and frozen storage duration on instrumental quality of lamb throughout display. *Meat Science, 84*(4), 662-669.
Ngapo, T. M., Babare, I. H., Reynolds, J., & Mawson, R. F. (1999a). Freezing and thawing rate effects on drip loss from samples of pork. *Meat Science, 53*(3), 149-158.
Ngapo, T. M., Babare, I. H., Reynolds, J., & Mawson, R. F. (1999b). Freezing rate and frozen storage effects on the ultrastructure of samples of pork. *Meat Science, 53*(3), 159-168.
O'Keefe, M., & Hood, D. E. (1982). Biochemical factors influencing metmyoglobin formation on beef from muscles of differing colour stability. *Meat Science, 7*(3), 209-228.
O'Keeffe, M., & Hood, D. E. (1982). Biochemical factors influencing metmyoglobin formation on beef from muscles of differing colour stability. *Meat Science, 7*209-228.
Oddy, V. H., Harper, G. S., Greenwood, P. L., & McDonagh, M. B. (2001). Nutritional and developmental effects on the intrinsic properties of muscles as they relate to the eating quality of beef. *Australian Journal of Experimental Agriculture, 41*(7), 921-942.
Ouali, A. (1992). Proteolytic and physicochemical mechanisms involved in meat texture development. *Biochimie, 74*251-265.
Payne, S. R., Durham, C. J., Scott, S. M., & Devine, C. E. (1998). The effect of non-vaccuum packaging systems on drip loss from chilled beef. *Meat Science, 49*(3), 277-287.
Pearson, A. M., & Miller, J. I. (1950). The Influence of Rate of Freezing and Length of Freezer-Storage upon the Quality of Beef of Known Origin. *Journal of Animal Science, 9*(1), 13-19.
Pegg, R. B., Shahidi, F., & Werner Klinth, J. (2004). HEAT EFFECTS ON MEAT | Flavour Development. In *Encyclopedia of Meat Sciences*, (pp. 570-578). Oxford: Elsevier.
Petrović, L., Grujić, R., & Petrović, M. (1993). Definition of the optimal freezing rate—2. Investigation of the physico-chemical properties of beef M. longissimus dorsi frozen at different freezing rates. *Meat Science, 33*(3), 319-331.
Purchas, R. W. (2004). Tenderness of beef *m. longissimus lumborum* at normal and intermediate ultimate pH before and after a period of ageing. *New Zealand Journal of Agricultural Research, 47,* 299-305.
Purchas, R. W., & Werner Klinth, J. (2004). TENDERNESS MEASUREMENT. In *Encyclopedia of Meat Sciences*, (pp. 1370-1377). Oxford: Elsevier.
Rahelić, S., Puač, S., & Gawwad, A. H. (1985). Structure of beef Longissimus dorsi muscle frozen at various temperatures: Part 1—histological changes in muscle frozen at −10, −22, −33, −78, −115 and −196°C. *Meat Science, 14*(2), 63-72.
Rosenvold, K., & Wiklund, E. (2011). Retail colour display life of chilled lamb as affected by processing conditions and storage temperature. *Meat Science, 88*(3), 354-360.
Savage, A. W., Warriss, P. D., & Jolley, P. D. (1990). The amount and composition of the proteins in drip from stored pig meat. *Meat Science, 27*289-303.
Savell, J. W., McKeith, F. K., & Smith, G. C. (1981). Reducing Postmortem Aging Time of Beef with Electrical Stimulation. *Journal of Food Science, 46*(6), 1777-1781.
Sentandreu, M. A., Coulis, G., & Ouali, A. (2002). Role of muscle endopeptidases and their inhibitors in meat tenderness. *Trends in Food Science and Technology, 13*398-419.
Shanks, B. C., Wulf, D. M., & Maddock, R. J. (2002). Technical note: The effect of freezing on Warner-Bratzler shear force values of beef longissimus steaks across several postmortem aging periods. *Journal of Animal Science, 80*(8), 2122-2125.

Speck, P. A., Clarke, J. N., & Morris, C. A. (1997). Effects of strain and selection for growth on tenderness in sheep and beef meats *Proceeding of the Association for the Advancement of Animal Breeding and Genetics, 12*567-570.

Spooncer, W. F., Smith, D. R., & Powell, V. H. The effect of chilling & freezing on meat quality CSIRO Meat Research Laboratory

Straadt, I. K., Aaslyng, M. D., & Bertram, H. C. (2011). Assessment of meat quality by NMR - an investigation of pork products originating from different breeds. *Magnetic Resonance in Chemistry, 1*(49), S71-S78.

Strydom, P. E., Frylinck, L., & Smith, M. F. (2005). Should electrical stimulation be applied when cold shortening is not a risk? *Meat Science, 70*(4), 733-742.

Suman, S. P., & Joseph, P. (2013). Myoglobin Chemistry and Meat Color. *Annual Review of Food Science and Technology, 4*(1), 79-99.

Taher, B. J., & Farid, M. M. (2001). Cyclic microwave thawing of frozen meat: experimental and theoretical investigation. *Chemical Engineering and Processing: Process Intensification, 40*(4), 379-389.

Trout, G. R. (1988). Techniques for measuring water-binding capacity in muscle foods--A review of methodology. *Meat Science, 23*(4), 235-252.

Vieira, C., Diaz, M. T., Martínez, B., & García-Cachán, M. D. (2009). Effect of frozen storage conditions (temperature and length of storage) on microbiological and sensory quality of rustic crossbred beef at different states of ageing. *Meat Science, 83*(3), 398-404.

Warriss, P. D. (2000a). *Meat Science - An Introductory Text*. Oxon, United Kingdom: CABI Publishing.

Warriss, P. D. (2000b). *Meat Science: An Introductory Text*. Oxon: CABI Publishing.

Wiklund, E., Stevenson-Barry, J. M., Duncan, S. J., & Littlejohn, R. P. (2001). Electrical stimulation of red deer (Cervus elaphus) carcasses — effects on rate of pH-decline, meat tenderness, colour stability and water-holding capacity. *Meat Science, 59*(2), 211-220.

Winger, R. J., & Hagyard, C. J. (1994). Juiciness - Its importance and some contributing factors. *Quality Attributes and Their Measurement in Meat, Poultry and Fish Products,* 94-124.

Xia, X., Kong, B., Liu, Q., & Liu, J. (2009). Physicochemical change and protein oxidation in porcine longissimus dorsi as influenced by different freeze–thaw cycles. *Meat Science, 83*(2), 239-245.

Young, O. A., Reid, D. H., & Scales, G. H. (1993). Effect of breed and ultimate pH on the odour and flavour of sheep meat. *New Zealand Journal of Agricultural Research, 36*(3), 363-370.

Young, O. A., & West, J. (2001). Meat Colour. In Y. H. Hui, W.-K. Nip, R. W. Rogers, & O. A. Young (Eds.), *Meat Science and Applications*, (pp. 39-70). New York: Marcel Dekker, Inc.

Appendix 1

10% neutral buffered formalin

Sodium phosphate, monobasic 4.0 gm

Sodium phosphate, dibasic 6.5 gm

Formaldehyde, 38% 100.0 ml

Distilled water 900.0 ml

Histology solution:

Gill's Haematoxylin

Haematoxylin (CI 75290)	4.0 g
Sodium iodate	0.4 g
Aluminium sulphate	35.2 g
Dist. Water	710 ml
Ethylene glycol	250 ml
Glacial acetic acid	40 ml

Eosin 1% solution

Thermo scientific: Eosin-Y alcohol with alcohol 71211

Scott's Tap Water:

Sodium bicarbonate ($NaHCO_3$)	2.0 g
Magnesium Sulphate ($MgSO_4$)	20 g
Distilled water	1000 ml
Crystal of Thymol	1 (optional)

Appendix 2: Metabolites that differ significantly in abundance in purge from control and VFF -1.5°C

Metabolite	More abundant in
Lysine	VFF
Isoleucine	VFF
Valine	VFF
Tyrosine	VFF
Phenylalanine	VFF
Leucine	VFF
Methionine	VFF
Proline	VFF
Inosine	VFF
Carnitine	VFF
Alanine	VFF
IMP	VFF
Carnosine	C
Malate	VFF
Acetate	VFF
Pyruvate	VFF
Unknown	C
Lactate	C
Unknown	C

Metabolites that differ significantly in abundance in purge from control and FF -1.5°C

Metabolite	More abundant in
Valine	FF
Leucine	FF
Lysine	FF
Isoleucine	FF
Carnitine	FF
Proline	FF
Methionine	FF
IMP	C
Glucose	FF
Tyrosine	FF
Phenylalanine	FF
Carnosine	C

Malate	FF
Lactate	C
Unknown	C

Metabolites that differ significantly in abundance in purge from control and SF -1.5°C

Metabolite	**More abundant in**
Lysine	SF
Valine	SF
Tyrosine	SF
Phenylalanine	SF
Isoleucine	SF
Leucine	SF
Methionine	SF
Proline	SF
Unknown	C

1

2

Appendix 3: statistical values

	Change in pH
Treatment	
p-value	0.004
CO -1.5	0.044
FF -1.5	-0.005
FF 1	-0.011
FF 3.5	-0.001
SF -1.5	-0.012
SF 1	0.002
SF 3.5	-0.002
VFF -1.5	0.046
VFF 1	0.006
VFF 3.5	-0.006
SEM	0.0099
SED	0.0139
LSD(5%)	0.0279
effective df	62.3
Factorial + Added Control	
Ageing temp	0.229
Freezing treatment	0.030
Ageing temp.freezing treatment	0.082

Summary of the results from ANOVA blocked by animal (n=9). Evidence of a difference between treatments means were assessed within the individual animal stratum using an F-test (9, 36 degrees of freedom). The corresponding p-value, the estimated treatment means, the standard error of the means (SEM), the standard error of the differences (SED) and the effective degrees of freedom is presented in first half of the table. Individual treatment means were compared using post-hoc Fisher's Least Significant Difference (LSD(5%)) at a 5% significance level. The overall effect of Ageing Temperature (-1.5°C, 1°C and 3.5°C) the overall effect of Freezing Temperature (SF, FF and VFF), and their interaction assessed using Factorial + Added Control ANOVA blocked by Animal (n=9). The P-values from the F-tests (36 denominator degrees of freedom) in the individual animal stratum for Ageing Temp, Freezing treatment and their interaction (Ageing temp. Freezing treatment) are given in the second half of the table.

	Day 1 L* value	Day 4 L* value	Day 7 L* value	Day 1 a* value	Day 4 a* value	Day 7 a* value	Day 1 b * value	Day 4 b* value	Day 7 b* value
Treatment									
p-value	0.002	<.001	<.001	<.001	0.098	<.001	<.001	<.001	0.200
CO -1.5	42.7	44.3	44.2	22.2	17.6	16.6	8.92	6.93	7.00
FF -1.5	40.0	41.0	40.4	20.2	17.3	15.0	6.94	8.15	6.58
FF 1	42.4	42.0	42.0	17.6	15.7	14.1	6.83	6.25	5.98
FF 3.5	41.6	41.7	41.3	19.0	16.7	14.0	7.59	6.85	6.09
SF -1.5	42.4	41.8	42.8	19.9	17.3	14.1	7.51	8.53	6.84
SF 1	42.0	42.3	42.6	19.6	16.0	12.8	8.08	6.51	6.20
SF 3.5	42.6	42.0	42.6	17.7	15.5	12.0	7.01	6.19	6.17
VFF -1.5	40.8	41.0	41.0	19.4	15.7	13.8	6.09	7.69	5.84
VFF 1	41.2	41.5	41.5	18.8	16.1	14.1	7.43	6.49	6.17
VFF 3.5	41.6	41.6	41.0	18.5	16.1	13.0	7.35	6.58	6.23
SEM	0.41	0.43	0.46	0.40	0.46	0.42	0.239	0.256	0.243
SED	0.58	0.60	0.64	0.57	0.65	0.60	0.338	0.363	0.344
LSD(5%)	1.17	1.20	1.29	1.14	1.29	1.20	0.675	0.723	0.689
effective df	49.1	61.5	57.1	64.7	71.0	45.8	65.2	69.6	54.7
Factorial + Added Control									
Ageing temp.	0.067	0.344	0.377	0.001	0.057	0.002	0.115	<.001	0.129
Freezing treatment	0.005	0.185	<.001	0.998	0.197	<.001	0.287	0.571	0.559
Ageing temp.freezing treatment	0.015	0.466	0.466	0.041	0.335	0.426	0.029	0.325	0.603

Colour per day was analysed using ANOVA. Animal was included as the blocking variables, and the as the treatment variable. LSD at 5% level of significance was used to compare means

	Hue Angle Day 1	Hue Angle Day 4	Hue Angle Day7	Chroma Value Day 1	Chroma Value Day 4	Chroma Value Day 7
Treatment						
p-value	<.001	<.001	<.001	<.001	0.025	<.001
CO -1.5	21.77	21.5	22.5	23.9	18.9	18.0
FF -1.5	19.10	25.3	24.0	21.4	19.1	16.4
FF 1	21.28	21.7	22.7	18.9	16.8	15.3
FF 3.5	21.73	22.2	23.1	20.4	18.1	15.3
SF -1.5	20.69	26.3	25.9	21.3	19.3	15.7
SF 1	22.27	22.2	26.1	21.2	17.2	14.3
SF 3.5	21.45	21.8	27.7	19.1	16.7	13.6
VFF -1.5	17.58	26.2	23.6	20.4	17.5	15.0
VFF 1	21.50	21.9	24.0	20.2	17.4	15.4
VFF 3.5	21.61	22.2	25.8	19.9	17.5	14.4
SEM	0.419	0.50	0.70	0.44	0.50	0.46
SED	0.592	0.70	0.99	0.63	0.71	0.65
LSD(5%)	1.186	1.40	2.00	1.25	1.41	1.31
effective df	55.4	60.5	42.3	66.3	71.5	48.3
Factorial + Added Control						
Ageing temp.	<.001	<.001	0.118	0.011	0.004	0.005
Freezing treatment	0.039	0.563	<.001	0.973	0.240	0.011
Ageing temp.freezing treatment	0.065	0.703	0.159	0.035	0.298	0.498

Colour per day was analysed using ANOVA. Animal was included as the blocking variables, and the as the treatment variable. LSD at 5% level of significance was used to compare means

Repeated measure analysis of colour data

	L* value	a* value	b* value	Hue angle	Chroma Value
p-value					
Treatment	<.001	<.001	<.001	<.001	<.001
Time	0.254	<.001	<.001	<.001	<.001
Time.Treatment	0.632	0.127	<.001	<.001	0.107
SEM	0.450	0.449	0.257	0.732	0.484
SED	0.636	0.636	0.364	1.035	0.684
LSD(5%)	1.301	1.307	0.767	2.060	1.417

Summary of the results from repeated measures analysis of the colour data. The colour data was assessed using repeated measures ANOVA blocked by animal (n=9) assuming uniform correlation over time. The overall effect of Treatment was assessed within the individual animal stratum using an F-test (9, 36 degrees of freedom). The overall effect of Time and the interaction (Time.Treatment) were assessed using F-tests with 2, 160 and 18, 160 degrees of freedom, respectively.

	kgF tenderness
Treatment	
p-value	0.009
CO -1.5	5.03
FF -1.5	4.09
FF 1	4.60
FF 3.5	4.25
SF -1.5	4.55
SF 1	4.97
SF 3.5	4.41
VFF -1.5	4.10
VFF 1	4.58
VFF 3.5	3.74
SEM	0.229
SED	0.324
LSD(5%)	0.650
effective df	51.0
Interaction	
Ageing temp.	0.012
Freezing treatment	0.021
Ageing temp.freezing treatment	0.731

Tenderness was analysed using ANOVA. Animal was included as the blocking variables, and the as the treatment variable. LSD at 5% level of significance was used to compare means

	Purge loss	Drip %	Cook Loss %	Purge%+ Drip%
Treatment				
p-value	<.001	<.001	0.043	<.001
CO -1.5	2.18	2.33	28.8	4.51
FF -1.5	2.53	7.85	23.1	10.37
FF 1	3.60	6.48	28.0	9.94
FF 3.5	4.07	3.61	29.6	7.65
SF -1.5	5.68	7.51	22.7	13.19
SF 1	7.77	3.32	30.1	11.14
SF 3.5	7.35	2.71	29.0	10.01
VFF -1.5	3.54	5.96	24.3	9.51
VFF 1	4.84	4.95	25.8	9.79
VFF 3.5	3.49	4.81	25.6	8.29
SEM	0.589	0.53	1.58	0.771
SED	0.833	0.745	2.24	1.091
LSD(5%)	1.659	1.484	4.46	2.172
effective df	75.4	75.6	78.9	77.1
Interaction				
Ageing temp.	0.165	<.001	0.031	0.107
Freezing treatment	<.001	0.004	0.169	0.021
Ageing temp.freezing treatment	0.761	0.049	0.132	0.697

Water Holding capacity was analysed using ANOVA. Animal was included as the blocking variables, and the as the treatment variable. LSD at 5% level of significance was used to compare mean

TBARS	Day 1	Day 7
Treatment		
p-value	0.03	0.105
CO -1.5	0.32	0.38
FF -1.5	0.33	0.34
FF 1	0.30	0.32
FF 3.5	0.29	0.35
SF -1.5	0.34	0.40
SF 1	0.32	0.38
SF 3.5	0.33	0.40
VFF -1.5	0.29	0.33
VFF 1	0.30	0.35
VFF 3.5	0.27	0.38
Approx. SEM	0.019	0.023
Approx. SED	0.027	0.033
LSR(5%)	0.164	1.189
denominator df	24	31

The log transformed TBARS data was analysed using linear mixed effects models (REML). The table contains the p-value for the Wald test used to test for differences between the treatment means, back-transformed means, approximate SEMs, approximate SEDs and the denominator degrees of freedom. Means were compared using post-hoc Fisher's Least Significant Ratio (LSR(5%)) at a 5% significance level