The effect of freezing and *post-mortem* ageing on beef quality

By

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DECLARATION

I declare that this thesis for the degree M. Agric - Animal Production Management at the University of Pretoria has not been submitted by me for a degree at any other University

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DEDICATION

To my loving mother Flora, and my siblings Nurse, Jeanette, Patrick, Ishmael, my son Gabriel and my late sister Queen and father Rooijan Mosimanyana.

ACKNOWLEDGEMENTS

Abstract

The aim of this study was to evaluate the effect of freezing methods and *post-mortem* ageing days for frozen beef *m. longissimus lumborum* (LL) on colour, moisture characteristics; characteristics and sensory characteristics. There were 3 freezing treatments: fresh, never frozen (FR); quick-frozen (QF), and slow-frozen (SF). All fresh and frozen beef *m. longissimus lumborum* samples were aged for 3 or 14-days and frozen beef samples were frozen before ageing.

Materials and methods

Three replications of 7 beef samples (steaks) per treatment (total of 3-days, *n*=126) were used. Fresh beef samples (FR) were kept in a cold room at 3 ˚C until the next morning (Day 3). Slow-frozen samples (SF) were packed in a single layer on 3 shelves of a house hold freezer (AEG Skandiluxe) to reach a core temperature of -20 °C. Quick-frozen samples (QF) were placed in a single layer on 3 shelves of an IcematicT15-2P blast freezer to a reach to core temperature of -30°C in 3 hours. All samples for the two freezing treatments were stored after the freezing processing at -20°C freezer room and were then thawed in vacuum packages for 18 hours at 3 °C in a dark chiller before analyses.

Results

Moisture characteristics

The freezing methods and ageing days had a significant effect (*P<0.001*) on moisture characteristics but showed no effect on cooking losses. Drip loss and more pressed out water were higher for samples aged for 3-days compared to 14-days ageing *post-mortem*. The effect of ageing days on moisture characterises was different for fresh compared to frozen beef samples (interaction: *P<0.001*). Drip loss of frozen steaks during 3-days ageing *post-mortem* was significantly higher *(P<0.001)* than those aged for 14-days. Drip loss between fresh samples aged for 3 and 14-days nor between frozen samples aged for 3 or 14-days.

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Colour characteristics

Most colour characteristics were significantly affected by freezing methods and *post-mortem* ageing days. Fresh beef samples had significantly higher ($P<0.001$) values for redness (a*) and chroma, lower values ($P<0.05$) for hue (closer to typical red colour) and were brighter (higher L*) than samples of both freezing methods. Higher (*P<0.001*) OxyMb and lower MetMb were recorded for fresh compared to frozen samples. Beef samples aged for 3-days had lower ($P<0.05$) b* (less yellow) and hue angle values than those aged for 14-days. DeoxyMb and OxyMb were higher and MetMb lower ($P<0.05$) in 3-days samples compared to 14-days samples.

Sensory characteristics

Freezing method had a significant effect (*P<0.001*) on WBSF, tenderness related sensory attributes and meat flavour. Fresh samples were tougher than frozen samples according to WBSF and sensory scores for first bite, overall tenderness, and residual connective tissue. In addition, quick-frozen samples were also significantly more tender (*P<0.001*) than slow-frozen samples according to all three tenderness related sensory parameters.

Conclusion

Overall, freezing methods and *post-mortem* ageing days influenced moisture and colour characteristics of beef. Quick freezing method improves tenderness measured as WBSF or sensory tenderness, but neither freezing method nor *post-mortem* ageing period (day 3 and day 14) had a meaningful effect on cooking losses.

LIST OF ABBREVIATIONS

AMSA	American Meat Science Association
DeoxyMb	Deoxymyoglobin
FAO	Food and Agriculture Organisation
FR	Fresh
L	Loin
LSD	Lowest Standard Deviation
Mb	Myoglobin
MetMb	Metmyoglobin
MetMbRA	MetMb reducing activity
MLA	Meat and Livestock Australia
OxyMb	Oxymyoglobin
pHu	Ultimate pH
NADH	Nicotinamide adenine dinucleotide hydride
NPPC	National Pork Producers Council
QF	Quick freezing
SE	Sensory evaluation
SF	Slow freezing
WHC	Water holding capacity
WBSF	Warner Bratzler Shear Force

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CHAPTER 1

Literature review

1.1 Introduction

In South Africa, most red meat is purchased in a fresh state by consumers. In rural areas where consumers normally have limited access to refrigerating or freezing facilities, meat will be consumed on the day of purchase or at least the following day. More affluent consumers will have facilities for storing fresh meat in refrigerators to be consumed within a few days of purchase. Other consumers may consider purchasing meat in bulk, and store the portioned meat for use over prolonged periods. Judged by the relative limited availability of frozen meat in retail stores and butcheries, it can be assumed that the quality of frozen meat on retail shelves is not trusted.

It is well known that freezing extend the shelf life of meat stored in bulk over extended periods (Obuz & Dikeman, 2003). However, freezing may influence quality characteristics like thaw loss, water holding capacity (WHC), colour stability, cooking losses, sensory characteristics (aroma, first bite, juiciness, tenderness, residue, flavour) as well as Warner Bratzler Shear Force (WBSF). The extent of quality loss of meat depends on many factors, including freezing and thawing rates, storage temperature and fluctuation, duration of storage, and retail display (Gambuteanu, Patraşcu & Alexe, 2014; Akhtar, Khan & Faiz, 2013; Li & Sun, 2002; Srinivasan, Xiong & Blanchard, 1997).

The rate of freezing (quick and slow freezing) and its relation to formation of ice crystals are critical for minimizing the damage to meat tissue and that of drip loss during thawing (Akhtar *et al.*, 2013). Generally speaking, the faster the rate of freezing, the smaller the ice crystals; and the slower the rate of chilling, the larger the ice crystals formed (Stuby, Lamken & Dolezal, 1993). The ice crystals formed within and between the muscle fibres during freezing, physically damage the ultra-structure of the meat (Akhtar *et al.*, 2013), leading to defects. The current study will focus more on the effect of freezing and *post-mortem* ageing on the quality of beef. The study included *post-mortem* ageing as additional factor (3 and 14-days) because meat presented for sale may be aged for various periods.

1.2. Muscle structure

Understanding certain fundamental aspects regarding muscle structure, will clarify the nature and definition of meat quality and the way in which it develops (Norström, 2011). Muscle contains approximately 75% water, 18% protein, 5% fats/lipids, carbohydrates 1%, and 1 % vitamins, sugars, minerals and glycogen (Norström, 2011) (Figure. 1.1).
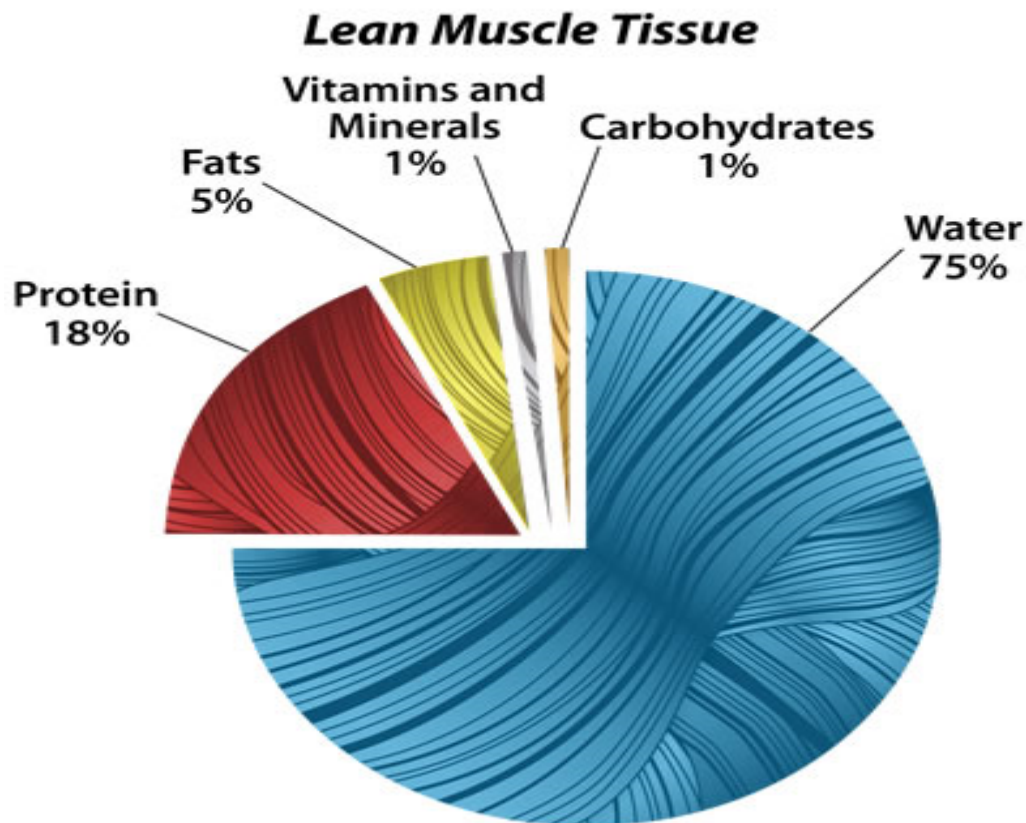
Figure 1.1 Lean muscle tissue content (Goldwyn, 1980)
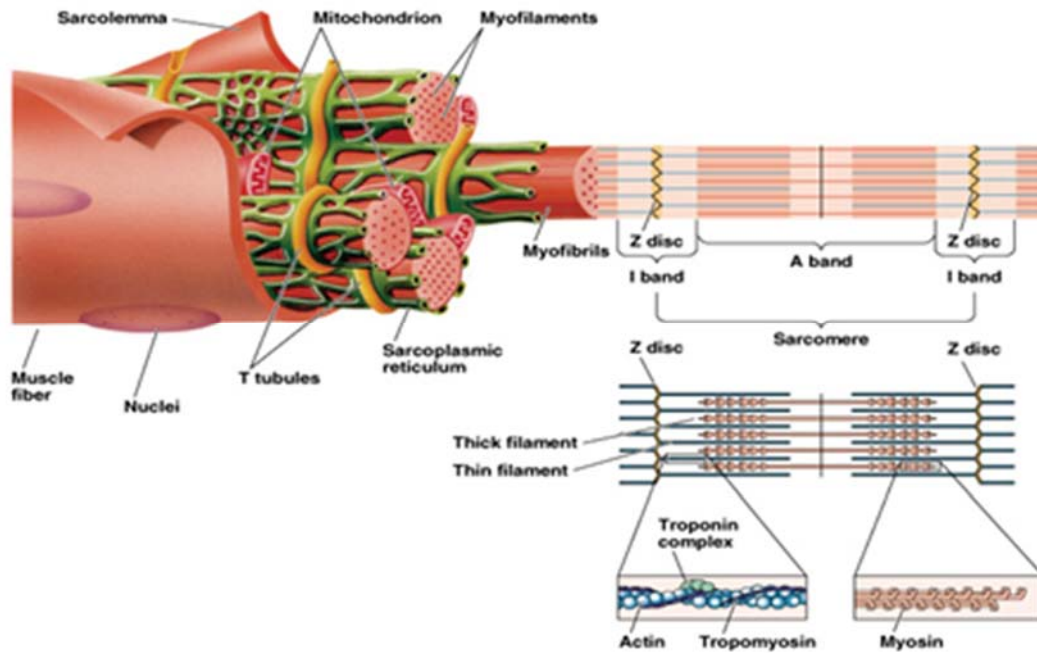
Figure: 1.2 The fibrous microstructure of meat (Gunenc, 2007)

In its simplest form, the structure of meat can be considered as being a collection of parallel fibres (Palka & Daun, 1999), myofibrillar structure, where each fibre is a single cell associated with connective tissue (Huff-Lonergan & Lonergan, 2005). The muscle fibres are bound together in bundles whereby each fibre in itself consists of bundles of myofibrils and surrounded by the plasma membrane called the sarcolemma (Huff-Lonergan & Lonergan, 2005). Overlying the sarcolemma of each muscle fiber is the endomysium, which is a thin layer of connective tissue (Bailey & Light, 1989). However, there are regular striations in muscle seen only when viewed under a microscope, which are caused by specialised contractile organelles found in the muscle cell within the myofibril. These striations are from dense protein A-bands and less-dense I-bands (Huff-Lonergan & Lonergan, 2005). A-bands are made up of thick filaments while I-bands are made up of overlapping thin filaments (Norström, 2011). Bisecting the I-bands is a dark line known as Z-disc or Z-line (Huxley & Hanson, 1954) and the area between two Z-discs is called a sarcomere, which can be considered to be the primary structural and functional unit directly responsible for muscle contraction (Lawrie & Ledward, 2006). Figure 1.2. illustrates that sarcomeres are composed of two filamentary proteins called actin and myosin, which slide over one another when the sarcomere contracts (Filgueras, Gatellier, Aubry, Thomas, Bauchart, Durand,

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Zambiazi & Sante-Lhoutellier, 2010). Myosin consists of the tail and the head. The tail forms the backbone of the thick filament and the head extends from the thick filament and interacts with actin in the thin filament (Huff-Lonergan & Lonergan, 2008; Moss, Diffee & Greaser, 1995) (Figure 1.3).
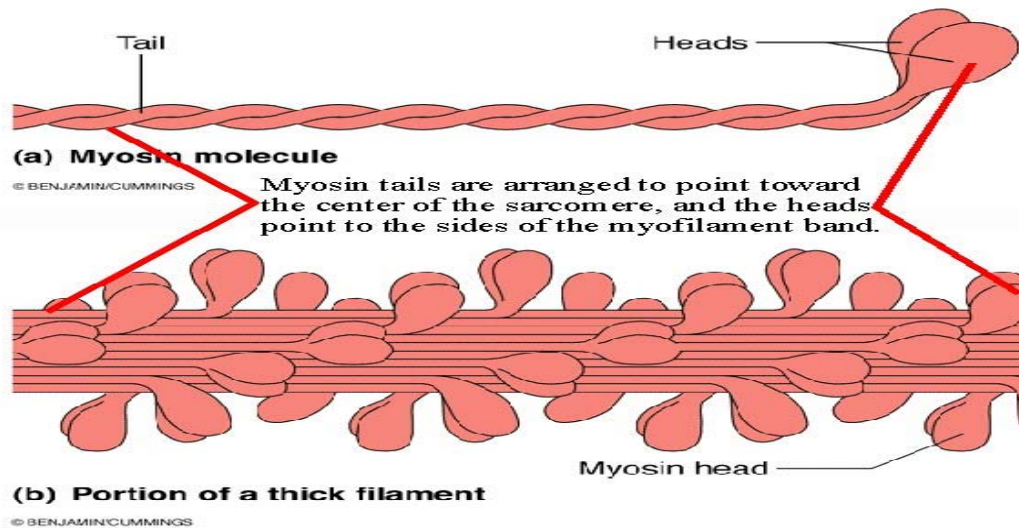
Figure 1.3 Diagram of myosin's heads & tail (Pearson education, 2006)

1.3 Freezing

Meat industries commonly utilise freezing to preserve the quality of meat for an extended period of time (Campañone, Roche, Salvadori & Mascheroni, 2006; Obuz & Dikeman, 2003). The process minimises microbial and chemical activities to a certain degree. Despite the advantages of destroying or inhibiting the growth of microorganisms (e.g. trichina) which may cause food spoilage (Obuz & Dikeman, 2003) freezing can cause undesirable alterations in the texture (fat could become granular and could crumble) and organoleptic properties. Most manufactures believe that any type of freezing reduces meat quality (Boles & Swan, 1996) although the degree of damage on frozen meat quality depends mainly on the rate of freezing, the size and the shape of the ice crystals formed (Ballin & Lametsch, 2008). Variants in the shape of ice crystals are perceived to cause tissue damage and reduce meat quality (Lagerstedt, Enfält, Johansson & Lundström, 2008; Brewer & Harbers, 1991). For example, slow conventional freezing leads to irregularly shaped, and relatively large ice crystals (Zhu, Ramaswamy & Simpson, 2004) which increase the structural damage to the meat (Devine, Bell, Lovatt, Chrystall & Jeremiah, 1996). The formation

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of larger, mostly intracellular ice crystals, probably causes most of the tissue damage (Yu, Ma, Zheng, Liu & Sun, 2011; Ahmad, Yaghmaee & Durance, 2010; Streit, Corrieu & Beal, 2010; Delgado, Zheng & Sun, 2009; Ming, Rahim, Wan & Ariff, 2009), resulting in a loss of elasticity and ability to hold water (Bhatnagar, Bogner & Pikal, 2007). Moreover, larger ice crystals damages and concentrates the solutes in the meat, leading to alterations in the biochemical reactions that arise at the cellular level and impact on the physical quality parameters of meat (Leygonie, Britz & Hoffman, 2012). As water in meat freezes, the concentration of the remaining solutes (proteins, carbohydrates, lipids, vitamins and minerals) increases, thereby disrupting the homeostasis of complex meat system (Lawrie, 1998). Smaller ice crystals are formed when rapid freezing is applied (Stuby, Lamkey & Dolezal, 1993) and this results in a uniform extracellular spacing immediately after freezing (Sigurgisladottir, Ingvarsdottir, Torrissen, Cardinal & Hafsteinsson, 2000). The formation of smaller ice crystals distributed evenly both inside and outside the cells, leads to better preserved quality of the product due to less tissue damage (Sun & Zheng, 2006).

Quality deterioration and possible risks are usually increased by a fluctuating time-temperature environment such as freeze/thaw cycles during storage (Fu & Labuza, 1992). Deterioration in quality due to freezing can also occur through lipid oxidation and protein degradation (Zhang, Farouk, Young, Wieliczko & Podmore, 2005).

There are contradictive reports in literature regarding the effect of freezing on organoleptic and technical quality of meat (Lagerstedt *et al.*, 2008; Zhang *et al.*, 2005; Farouk, Wieliczko. & Merts, 2003; Ngapo, Berge, Culioli, Dransfield, De Smet. & Claeys*,* 2002). Ngapo, Babare, Raynolds & Mawson (1999) found several physico-chemical changes in meat caused by freezing that could lead to the worsening of organoleptic quality. Gambuteanu, Borda & Alexe (2013) suggested that it is necessary to take into account all changes encountered by meat until thawing when considering the effects of freezing on meat quality. Therefore, freezing rates, freezing meat after a certain degree of ageing, as well as the storing conditions (temperatures and fluctuations) while freezing must be taken into consideration.

Conditions of frozen storage affect beef quality (Farouk *et al.*, 2003; Shanks, Wulf & Maddock, 2002). Several studies indicated the progressive deterioration of frozen meat quality over time in storage (Shanks *et al.*, 2002; Ngapo *et al.*, 1999; Wheeler, Miller, Savell & Cross, 1990). Furthermore, if the temperature in a freezer fluctuates, the length of time that products can be stored is significantly decreased (Akhtar,

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Khan& Faiz, 2013). The actual storage temperature also affects the quality of frozen foods in a significant way. Any elevation in temperature higher than the designed storage temperature is likely to decrease the value of frozen foods (Singh & Heldman, 2001). Other authors Bertram, Andersen & Andersen (2007), Farouk *et al.* (2003) found no effect of storage temperature on most sensory properties in frozen beef.

1.3.1 Freezing methods

Food is generally frozen using different methods. Common methods include: 1) Air blast freezing, where cold air is circulated over the product at high velocity, removing heat from the product and releasing it into an air/refrigerant heat exchanger before being circulated. 2) Contact freezing, where packaged or unpackaged food is placed in contact with a cold metal surface and cooled by any of the refrigerants such as ammonia or cold brine. 3) Cryogenic freezing, an especially rapid freezing method, where unpacked or thinly packed food is placed in a medium that is cooled by a cryogenic fluid such as liquid nitrogen with a boiling point of -196°C, or by liquid or solid carbon dioxide which sublimes at a temperature of -78°C (Smith, 2011; Hung & Kim, 1996).

1.3.1.1 Air blast freezing

Air blast freezing is normally used for freezing products between -35°C and -45°C (Dempsey & Bansal, 2010). The time needed to freeze a product varies between 12 and 48 hours, depending on its type and dimensions.

1.3.1.2 Contact freezing

The product can be in indirect or direct contact with the freezing medium. For direct contact, the product being frozen is fully surrounded by the freezing medium, the refrigerant, maximizing the heat transfer efficiency. For indirect contact, the product is exposed to the freezing medium while in contact with the belt or plate, which is in contact with the freezing medium (Mallett, 1993). It commonly requires flat surface or an almost a flat surface to be in contact with the refrigerant plates for a solid or

packaged product. In this way contact can be established between these plates and 1 or 2 surfaces of the food or food container (Potter, 1997)

1.3.1.3 Cryogenic freezing

With cryogenic freezing, the food maybe placed in direct contact with the liquid refrigerants: the food releases heat to provide the inactive heat of vaporisation of the refrigerant which then experiences a stage of change (Smith, 2011). Cryogenic freezing with liquid nitrogen is done by firstly passing liquid nitrogen vapour at about -50°C over the food and then freezing it by directly spraying the refrigerant onto the food (Smith, 2011).

1.4 The effects of freezing on muscle structure

The extent of muscle tissue damage caused by freezing depends on the rate of freezing (quick freezing and slow freezing) (Grujic, Petrovic, Pikula & Amidzic, 1993). For many years, it has been suggested that foods which are 'quick-frozen' yield optimum quality (Hanenian & Mittal, 2004). With this method of freezing, the ice crystals do not have time to grow in size before all the liquid is frozen. Subsequently structural damage to meat is limited, destroying fewer pathogens (Lund, 2000). In this case nucleation occurs inside the cells, causing intracellular ice. The faster food is frozen, the more nuclei will form, leading to a large number of small crystals. It also depends on the amount of solutes (e.g. soluble salts, proteins and carbohydrate) which affect the temperature at which the ice crystals are formed (Gill, 2002). If the freezing rate is slightly reduced, only one or a few large intracellular crystals will form (Pham, 2008).

A slow-freezing rate is considered to be below 1cm/hour compared to quick freezing which is above 5cm/hour (FAO, 1972). Most of the microorganisms move into the unfrozen fraction of water in the food during slow freezing (Gill, 2002). This type of freezing rate causes more of the meat fiber to rupture due to formation of large ice crystals, leading to excessive water loss in the form of drip when thawed (Farkas, 1997). Unlike quick freezing, fiber dehydration occurs in slow freezing, which could be

caused by the sarcoplasmic fluid migration towards the extracellular area where the extracellular ice is exclusively formed. The formation of large ice crystals damages the texture of the meat, increases oxidation rates during storage and after thawing and accelerates the activity of enzymes (Doughikollaee, 2012). At slow freezing rates, solutes become concentrated in unfrozen water as the extracellular ice forms. This increases loss of water from the bacterial cells and exposes them to osmotic pressure over a long period of time (Farkas, 1997). The changes that occur in the extracellular pH and in ionic strength are as a result of osmotic pressure. The pressure subsequently interferes with metabolic processes, denatures other proteins, and inactivates enzymes (Whyte, Hudson &Tuner, 2005). The damage which occurs in the membrane and its transport system could also be irreparable and the bacteria can become more sensitive to oxidative pressure (Farkas, 1997)

Slow freezing extensively characterizes dislocation of water and consequently the cells have a shrunken appearance in the frozen state (Fennema, 1996). This freezing rate gives higher cooking losses than that of a fast rate and consequently increases the risk of lower juiciness (Wheeler, Savell, Cross, Lunt & Smith, 1990). Deatherage & Hamm, (1960) reported a small amount of water holding capacity from beef cuts that were frozen slowly at -15°C.Furthermore, when the meat is frozen slowly, the colour turns to be dark and glassy, "translucent" (James & James, 2002), while that of quick-frozen meat is pale and opaque. The reason is that, the large crystals formed by slow freezing do not scatter as much light as small crystals formed by quick freezing (James & James, 2002).

1.5 The effects of freezing on meat quality

1.5.1. The effects of freezing on moisture characteristics

Drip loss is a very important quality aspect in the meat industry. It is caused by irreversible damage during the freezing, storage (recrystallization) and thawing processes (Pham & Mawson, 1997). This aspect is economically detrimental due to loss yield (weight) and to loss of soluble nutrients. It also causes the meat to have an unattractive appearance.

Freezing rate has an influence on drip loss (Ngapo *et al.*, 1999). Slow freezing causes more meat fibres to rupture due to formation of large ice crystals, leading to excessive water loss in the form of drip when thawed (Li & Sun, 2002). Unlike quick freezing, fibre dehydration occurs in slow freezing, caused by the sarcoplasmic fluid migration towards the extracellular areas where the extracellular ice is exclusively formed. This type of freezing rate gives higher cooking losses than fast freezing, resulting in the risk of lower juiciness (Wheeler, Savell, Cross, Lunt & Smith, 1990). In quick freezing, the Ice crystals do not have time to grow in size before all the liquid is frozen and because of that, less damage to the structure of the meat occurs (Lund, 2000). In this case nucleation occurs inside the cells, causing the formation of intracellular ice crystals. The faster food is frozen, the more nuclei will form, leading to large number of small crystals. The efficiency of this process also depends on the amount of solutes (e.g. soluble salts, proteins and carbohydrates) which affect the temperature at which the ice crystals are formed (Gill, 2002). Ramsbottom & Koonz (1939) indicated that the rate of freezing is not the main factor contributing to drip. They demonstrated that the amount of reabsorbed moisture after thawing will be higher if the size of the cut is large in relation to the cut surface area. Likewise, if the volume of meat is small compared with the area of the cut surface, the rate of freezing is significant in lowering the amount of drip loss, i.e. a high freezing rate will reduce drip loss. Ngapo *et al.* (1999) reported that the drip loss in quick-frozen pork (for 12-120 minutes) was the same as for refrigerated meat. Yet, in the case of slowly-frozen meat (for 240-900 minutes), the drip loss was significantly higher than for that of refrigerated meat. Their results also showed that slow freezing had a higher drip loss than fast freezing, but the difference disappeared after 4 weeks of frozen storage.

According to Huff-Lonergan & Lonergan (2005) the water holding capacity of meat is dependent upon the storage before freezing and the time of ageing, the temperature of frozen storage, the duration and the conditions of frozen storage, as well as the rate of temperature decline. During frozen storage, the fluctuation of temperature is unpreventable particularly in small freezing units. This problem is more serious during transportation of meat, in display cabinets, in home freezers, as well as during storage (Xia; Kong; Liu & Liu, 2009).

The cooking loss of frozen meats mainly depend on the processing of meat before freezing, specifically rigor-onset temperature (Farouk & Swan, 1998), and on the cooking method, specifically the cooking temperature (Perez-Chabela & Mateo-

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Oyague, 2006). Genot (2000) showed that cooking loss is higher when freezing rates are slow. Ferrier & Hopkins (1997) indicated that the effect of the freezing rate on cooking loss was not significant. Even though a lot of fluid losses make meat less attractive, this does not significantly influence its eating quality after cooking, except in the case of very large fluid losses which could affect juiciness and tenderness (Genot, 2000).

1.5.2 The effects of freezing on colour

Frozen meat (beef) colour normally appears darker, less red, and more yellow as compared to fresh meat especially after long periods in frozen storage (Farouk & Swan, 1998; Moore & Young, 1991). The less attractive darker colour could be caused by exudate loss during thawing. In certain cases, this could lead to the difficulties regarding the product's acceptance in the market place.

Bye (1993) compared the colour of fresh and frozen patties and found a significant difference in Hunter L, a, b values, as well as in hue and chroma angles. The frozen patties were less red and darker in colour.

Farouk *et al.* (2003) found lighter colour in slow-frozen beef than in fast-frozen beef. The reason was the difference in thaw drip which may have resulted in higher light reflection and light colour in the samples which were slowly-frozen and thawed.

1.5.3 The effects of freezing on flavour and aroma

The compounds of flavour may originate from lipid and peptide components in the meat (Spanier, Vercellotti & James, 1992). All these parameters are affected by freezing, frozen storage and subsequent thawing (Leygonie *et al.*, 2012). During longterm frozen storage, some important alterations in flavour caused by lipid (fat) oxidation can occur, and these changes results in rancid or stale flavoured meat (Aidani, Aghamohammadi, Akbarian, Morshedi, Hadidi, Ghasemkhani & Akbarian, 2014).

Jeremiah, Gibson & Tong (1993) found no effect on flavour as a result of freezing and thawing, of beef while Kemp, Johnson, Stewart, Ely & Fox (1976) found no effects on pork. However, Wheeler *et al.* (1990) found that freezing decreases the flavour intensity in beef. Some of the contrasting evidences were due to differences in freezing

rates and temperatures, as well as variations in the periods of freezer storage (Stephens, 2001).

1.5.4 The effects of freezing on juiciness

Contrasting results regarding the effects of freezing on juiciness are found in the literature. According to Moreno, Perez, Oliete, Carballo, Franco & Moserrat (2007) meat that was chilled or frozen and held for the same length of time did not affect juiciness. In contrast, Kandeepan & Biswas (2005) found that chilling and freezing improved juiciness during longer storage duration. According to Lawrie (1998), beef that was stored for 20 weeks at -10°C was found to be significantly less juicy than the same beef that was stored for a few days at 0°C.

1.5.5 The effects of freezing on tenderness

Several authors have shown general agreement that the meat tenderness improves with freezing and thawing when measured with peak shear force (Lagerstedt *et al.*, 2008; Farouk *et al.*, 2003; Shanks *et al.*, 2002; Wheeler *et al.*, 1990). However, poor freezing practices can cause severe toughening and meat of poor eating quality (James, 2002). This toughness is caused by the undesirable muscle fibre contraction that can occur during 'cold shortening' when pre-rigor meat is frozen (Aidani *et al.*, 2014).

According to Akhtar *et al.* (2013) there are mechanisms involved in the meat tenderisation during freezing which are: loss of structural integrity caused by the formation of ice crystals and muscle fibre break-down by the enzymatic activity during thawing. Most importantly, the rate of freezing plays an important role in meat ageing (Dransfield, 1994). Unlike slow freezing, the small intracellular ice crystals caused by quick freezing increase the rate of ageing by 3 times the rate of chilled meat (Dransfield, 1994). These occurrences are probably the results of protease-enzyme release, which lead to an increased ageing rate (Vieira, Diaz, Martinez & Garcia-Cacha, 2009). Also, Crouse & Koohmaraie (1990) found lower shear force from samples aged after freezing than samples aged for 6 days before freezing. Their results are in line with the hypothesis which states that freezing meat before ageing could improve the *post-mortem* proteolysis (Gambuteanu *et al.*, 2013). Ageing after

freezing is probably enhanced by the loss of Ca-dependent inhibitors of protease (Whipple & Koohmaraie, 1992). Langestedt *et al.* (2008) obtained contradictory results from sensory evaluation tenderness where shear force values of frozen meat were significantly less tender than chilled meat. Paul & Child (1937) indicated that freezing did not have an influence on meat tenderness.

1.6 *Post-mortem* ageing

Post-mortem ageing is a process that occurs naturally in muscle tissue improving the palatability attributes of meat, especially the cuts from the loins and ribs (Teye & Okutu, 2009), whether vacuum packed or on the carcass. Ageing of meat is a multifactorial process, depending on many *ante-mortem* and *post-mortem* factors, such as the type of muscle fibre, animal age, gender, amount and solubility of collagen, sarcomere length, ionic strength, degradation of myofibrillar protein (Goll, Boehm, Geesink & Thompson, 1997; Koohmaraie, 1994) and animal species (Valin & Ouali, 1992).

The major protease activity in *post-mortem* proteolysis of myofibribrilar protein is widely considered to be represented by calpain (Huff-Lonergan *et al.*, 2010; Koohmaraie & Geesink, 2006). However, calpastatin which is a specific endogenous inhibitor of calpains, receives much attention regarding tenderisation of meat *post-mortem* (Huang, Huang, Zhang, Chuo, Zhang & zhou, 2014). It was demonstrated by several studies that the amount of calpastatin gradually decreases during *post-mortem* ageing (Boehm, Kendall, Thompson & Goll, 1998) and this causes a significant difference in beef tenderness (~40%) (Shackelford, Koohmaraie, Cundiff, Gregory, Rohrer & Savell, 1994).

1.6.1 The effects of *post-mortem* ageing on muscle structure

During the *post-mortem* ageing period, muscle structures become separated due to deterioration of the cytoskeleton and myofibrillar proteins. These alterations are known as the tenderisation process of meat or ageing (Koohmaraie, 1996).

According to James & James (2002), the presence of proteolytic enzymes in the muscle causes tenderisation by catalysing the breakdown of some muscle proteins. This weakens the muscle fiber and allows meat to be pulled apart easily. The two

enzymes thought to be responsible are: calpains, which are active at neutral pH immediately after slaughter, and cathepsins that are active at acid pH after rigor (Offer, Knight, Jeacocke, Almond, Cousins, Elsey, Parsons, Sharp, Starr & Purslow, 1989). Tenderisation begins when calpains are to be activated, normally at about pH 6.3 in beef and rapidly increases as more calpains are activated (James & James, 2002). Calpain become activated and causes a further tenderisation at about 16 hour's *post-mortem*. When activated, both of these enzymes are changeable and become continuously less effective with storage (Prates, 2002).

Calpains do not degrade actin and myosin (Koohmaraie, 1994) but degrade all proteins constituting costameres, which are titin, nebulin and troponin-T as well as vinculin, desmin and dystrophin (Beohm *et al.*, 1998). According to Spooncer, Smith & Powel (1979) meat is at its toughest when it is set in rigor and gradually becomes more tender after rigor, as enzymes break down and weaken the myofibrillar proteins. This is thought to improve tenderness of meat during ageing (Koohmaraie, 1994). Titin and nebulin are two large muscle proteins, which are anchored at the ends of N-and C-terminalsin the Z-disk (Taylor, Geesink, Thompson, Koohmaraie & Goll, 1995) towards the center of the sarcomere (Wright, Huang & Wang. 1993) (Figure 1.4). These two myofibrillar proteins are reported to form the N2 lines and are believed to be degraded completely in 3-days of *post-mortem* ageing (Taylor *et al.,* 1995).

According to the findings of Huff-Lonergan, Parrish & Robson (1995), the complete degradation of titin occurs in 14-days *post-mortem* and that of nebulin in 7-days. Hergenreder (2011) reported that meat was more tender after a degradation of titin in 7-days *post-mortem* and of nebulin in 3-days *post-mortem*. The degradation of the proteins results in greater fragmentation of myofibrils, with the most fragmentation at or near the Z-line (Huff & Parrish, 1993; Koohmaraie, 1994; Taylor *et al.*, 1995). According to Goll, Taylor, Christiansen &Thompson (1992) in the first 7 to 10-days, 90% or more of the tenderisation that occurs during *post-mortem* storage is due to calpains.
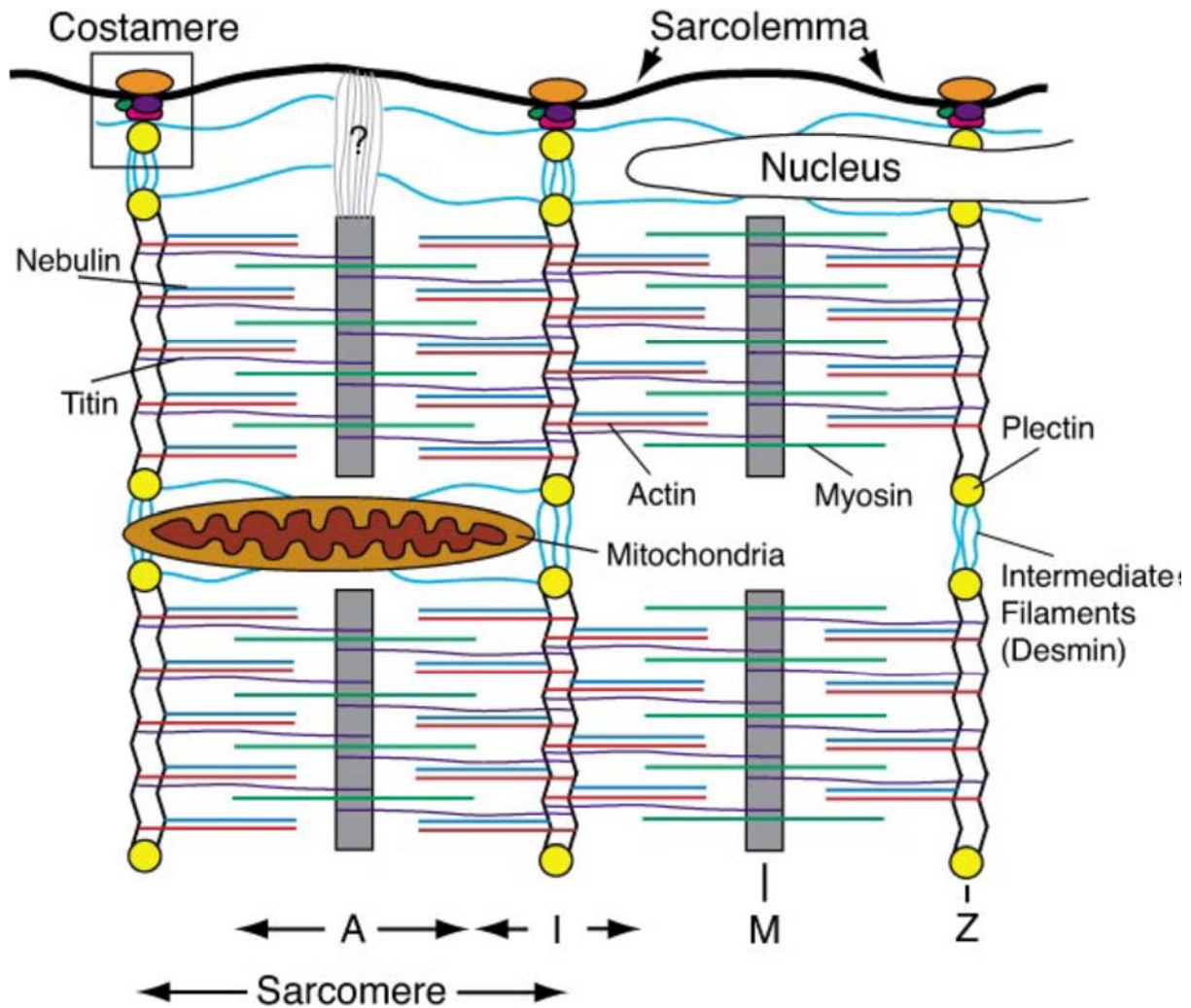
Figure 1.4 Schematic overview showing the structure and protein composition of costameres in striated muscle relative to Z-disks and the myofibrillar lattice (Carlsson & Thornell, 2001)

1.6.2 The effects of *post-mortem* ageing on meat quality

1.6.2.1 The effects of *post-mortem* ageing on moisture characteristics

Drip loss is generally considered to be the result of intracellular water from myosin denaturation which is lost from muscle fibres *post-mortem*, expelled by a pH and calcium-induced contraction of myofibrils *post-mortem* during early rigor development (Bertram, Aaslyng & Andersen, 2004; Brewer, 2004; Honikel, 1997; Offer *et al*., 1989). It is primarily affected by the extent of sarcomere shortening. There are two main reasons for this. Firstly, as the muscle goes into rigor, the development of rigor bonds

decreases the amount of spaces available for water to reside in the myofibril (Huff-Lonergan, Baas, Malek, Dekkers, Prusa & Rothschild 2002). Honikel, Kim, Hamm & Roncales (1986) showed that drip loss increases with a decrease in sarcomere length in the muscle cells. Secondly, as the muscle reaches total stiffness, pH of the tissue which is near the isoelectric point of many major proteins, especially myosin, affects the amount of water that is absorbed into protein structures in the myofibril (Huff-Lonergan *et al.*, 2002). The combination of these factors could significantly increase drip loss.

According to Li, Wang, Mao, Zhang, Niu, Liang, Zhu & Luo (2014) and MLA (2014), drip loss increases with ageing time. Similar results were found by Moron & Zamorano (2003), Lonergan, Huff-Lonergan, Wiegand & Kriese- Anderson (2001), Lesiak, Olson, Lesiak & Ahn (1996), Karlsson, Enfalt, Essh-Gustavssont, Lundstrom, Rydhmer & Stern (1993). These findings contradict Sobczak, Lachowicz, Kamieniecki, Wójcik, Ga¬jowiecki, Żochowska, Żych, Kotowicz, Sablik & Rzewucka (2005). They found no changes in drip loss during the *post-mortem* ageing time.

Drip loss and water holding capacity are closely interconnected (Zhang, Lonergan, Gardner & Huff-Lonergan, 2006). Maggiolino, Tateo & Centoducati (2014), De Palo, Maggiolino, Centoducati & Tateo (2013), De Palo, Abdelhadi, Babiker, Hocquette, Picard, Durand & Faye (2013) showed that during *post-mortem* storage, the drip loss increased with storage time and water holding capacity decreased.

Some of the factors which have been demonstrated to have a direct influence on entrapping water are as follows: the pH; ionic strength; oxidation, which affects the functionality of myofibrillar protein; myofibrils and muscle cells. It is clear that the same factors (pH decline, ionic strength, and oxidation) also affect proteolysis of key cytoskeletal proteins in *post-mortem* muscle (Huff-Lonergan & Lonergan, 2005)

Cooking loss is dependent on ultimate pH, sarcomere length, and the conditions of cooking (Trout & Schmidt, 1983). The myofibrillar proteins alter structurally with *post-mortem* ageing resulting in significantly lower cooking loss for aged steaks rather than non-aged steaks (Bouton, Harris & Shorthose, 1972). This statement was supported by Kadim, Mahgoub, Al-Ajmi, Al-Maqbaly, Al-Saqri & Ritichie (2004), Kannan, Kouakou & Gelaye (2001) who reported lower cooking losses on loin cuts during *post-mortem* ageing. However, these findings contrast with the findings of Hanzelkova, Simeonovova, Hampel, Dufek & Subrt (2011) who found increased cooking losses as

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a result of *post-mortem* ageing. Similarly, Stanišić, Petričević, Živković, Petrović, Ostojić-Andrić, Aleksić & Stajić (2012) found increased cooking loss with storage duration in both *m longissimus dorsi* and *m. gluteus medius* muscles.

1.6.2.3 The effects of *post-mortem* ageing on colour characteristics

Post-mortem ageing has advantageous effects on the initial display colour of beef, whereby a lighter and more intense redness in colour was recorded for aged steaks compared to fresh steaks (Mancini, 2012; Boakye & Mittal, 1996).

Myoglobin content is one of the three factors that affect meat colour (Seideman & Durland, 1984; MacDougall, 1982). According to Mancini (2012), it is likely that increased *post-mortem* ageing time enhances initial colour intensity by higher initial OxyMb but limited colour stability due to limited mitochondria-mediated MetMb-reducing activity. Some enzymes can convert MetMb back to Mb and hence to OxyMb, (MLA, 2006). However, the formation of brown MetMb can be prevented by enzymes in fresh meat, but as the meat ages, these systems become less active and discolouration becomes faster. When the temperature in aged meat is low; the formation of MetMb becomes slow because low temperatures slow the biochemical reaction and preserve the activity of enzymes (MLA, 2006).

1.6.2.4 The effects of *post-mortem* ageing on flavour and aroma

During *post-mortem* ageing, proteolytic and lipolytic enzymes cause changes in the structures of compounds like peptides, free amino acids, and fatty acids, which result in flavour alteration (Phillip, 2011). The production of these compounds can be influenced by the interaction effect of *post-mortem* ageing and the cooking through enzyme redistribution and activity (Kerth, Miller & Ramsey, 1995). The enzymes μ- and m-calpain which are primarily known for textural changes, also influence flavour by producing flavour related peptides during *post-mortem* ageing (Kerth *et al*., 1995). These two enzymes may also relate to an increase in rancid, sour and salty flavours (Toldrá & Flores, 2000). The amount of carbonyl derived from lipid oxidation also increases as a result of *post-mortem* ageing, some of which contributes to the increase of off-flavours (Phillip, 2011).

Post-mortem ageing increases beef flavour-intensity but eventually degradation sets in and rancidity increases (Touraille & Girarad, 1985). Studies have shown that *post-mortem* ageing up to 14-days increases fatty flavour and positive flavours such as beefy and brothy. However, beyond 14-days of ageing, negative flavours, such as painty, cardboard, bitter, and sour also increases (Gorraiz, Beriain, Chasco & Insausti, 2002; Spanier, Flores, McMillin & Bidner, 1997). According to Stetzer, Tucker, McKeith & Brewer (2006a, b) positive flavour compounds (Pentanal and 3-hydroxy-2-butanone) decrease with ageing and negative compounds (nonanal, butanoic acid and 1-Octene-3-ol) increase.

The environment (availability of oxygen, temperature, humidity and *post-mortem* ageing time) under which beef is aged also influences the ultimate flavour of the meat (Kerth *et al.*, 1995).

1.6.2.5 The effects of *post-mortem* ageing on juiciness.

Juiciness is dependent on the amount of moisture retained in a cooked meat product and the amount of intra-muscular fat. *The post-mortem* ageing of meat can increase water retention (Trout & Schmidt, 1983) and thus improve juiciness (Hoffman, Vermaak & Muller, 2012). According to MLA (2010), Teye & Okutu (2009) and Bianchi, Bentancur, Garibotto, Feed, Franco & Sañudo (2006) beef steaks become juicier with increasing time of ageing. Other studies Kim & Lee (2003), Goll, Carlin, Anderson, Kline & Walter (1965) found no significant differences in juiciness over extended *post-mortem* ageing.

1.7 Meat quality

The quality of meat is described by those traits the consumer perceives as desirable and these include both visual and sensory characteristics, meat safety, health aspects and more intangible traits such as environmental impact of meat production and welfare status of the production system (Becker, 2000). Other properties and perceptions used to describe meat quality include carcass composition and conformation and technological aspects (Popova, Marinova, Vasileva, Gorinov & Lidji, 2009). For instance, illustrating complexity of the "quality" problem, health of animals

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and ethical practices of farmers could be as important as technological and sensory characteristics of meat (Sanudo, Nute, Campo, Maria, Baker, Sierra, Enser & Wood, 1998). The consumers generally combine some of these factors to give an overall assessment of the quality of meat.

The first critical point of judgement of the quality of meat occurs when the consumers buy the product. Thus, they are part of the definition characterizing meat quality (Joo, Kim, Hwang & Ryu, 2013). From the consumer's point of view, uniformity in colour, amount of fat and purge are highly indicative of quality in their decision-making process to purchase fresh meat (Glitsch, 2000). The subsequent eating quality will also be as important as physical appearance, as well as the price and the wholesomeness of the product (Boleman, Boleman, Miller, Taylor, Cross, Wheeler, Koohmaraie, Shackelford, Miller, West, Johnson & Savel, 1997). Tenderness, juiciness and flavour, are the most important sensory attributes (Gunenc, 2007) determined by consumer satisfaction once the meat is cooked (Glitsch, 2000). The variability in eating quality, mainly in tenderness, is the main reason for consumer complaints and this is the primary cause of failure to repurchase (Bindon & Jones, 2001; Tarrant, 1998). Since muscle is naturally highly-arranged the complex in structure, this is not surprising. The present study is focussing on the factors that can affect the eating quality and the appearance of meat, such as freezing and *post-mortem* ageing.

1.7.1 Moisture characteristics

1.7.1.1 Water holding capacity

Water holding capacity is also one of the important quality aspects of meat (Norström, 2011) and it contributes to the sensation of juiciness and tenderness (McKeith, Brewer & Buggen, 1994). It can be explained as the ability of meat to retain its water even though external forces of action (like gravity, heating, centrifugation, pressing) are applied to it (Huff-lonergan, 2010). Water in the muscle is classified as *bound*, *immobilized* or *free (*Huff-Lonergan & Lonergan, 2005; Offer & Knight, 1988).
Bound water: Water does not easily move to other compartments. It is found near proteins of non-aqueous constituents (Huff-Lonergan & Lonergan, 2005). Bound water is also unaffected by freezing and is driven off by conventional heating (Fennema,

1985). Less than a tenth of the total water in the muscle is made up of bound water with the total concentration of 200 mg/g of protein, and very small changes take place in *post-rigor* muscle (Offer & Knight, 1988). Therefore, depending on the measurement system used, approximately 0.5 gm of water per gram is estimated to be tightly bound to proteins (Lonergan & Lonergan, 2005).

Immobilized /entrapped water: the water molecules may be held either by the steric and/or attraction to bound water (Lonergan & Lonergan, 2005). Immobilised water is held within the structure of the muscle but not bound to protein (Lonergan & Lonergan, 2005). This type of water can easily be converted to ice during freezing but does not flow freely in early *post-mortem*, it can also be removed by drying (Lonergan & Lonergan, 2005).

Free water: Unimpeded water flow from the tissue, is termed free water. This fraction of water is mainly held in the meat by the weak surface forces (Lonergan & Lonergan, 2005; NPPC, 2000). Free water is not readily seen in pre-rigor meat but can develop as conditions develop that allow bound water to move from the structures where it has originally been lodged. (Fennema, 1985)

Water serves as a lubricant and as a medium for transport metabolites in muscle fibre (Puolanne & Halonen, 2010). A significant amount of water in muscle is held within the muscle structure and in muscle cells (Norström, 2011), particularly within and between myofibrils and between myofibrils and the cell membrane (sarcolemma). However, most water is present in the myofibrils, in the spaces between the thin filaments of actin or tropomyosin and thick filaments of myosin (Lawrie, 1998). Water holding capacity can be influenced by factors such as a decline in pH (Huff-Lonergan & Lonergan, 2005), as well as by temperature during storage. According to Norström (2011), high temperatures during storage can contribute to more purge loss. The size of the sample also affects WHC, since a thin slice renders an excessive amount of purge loss as compared to a larger cut (Huff-Lonergan, 2009).

1.7.1.2 Drip loss

Drip loss in general (purge from fresh meat or thawing loss) is described as the fluid, mainly consisting of water and water-soluble proteins, which can be expelled from a piece of meat without any force other than gravity (Fisher, 2007). It depends on the conditions under which it is measured; therefore, a general definition does not exist.

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Development of drip loss mostly develops during the conversion of muscle to meat. Therefore, it depends on the shortening of sarcomeres regulated by the interaction of muscle temperature and rigor development (Fischer, 2007). Nomenclature which designates drip includes purge loss, press loss and thaw loss (James & James, 2002). Myoglobin, glycolytic enzymes and some of the sarcoplasmic proteins, amino acid and vitamins are found in purge (Huff-Lonergan & Lonergan, 2005).

The amount of drip loss in meat is influenced by factors such as: diet, sex, age, pre-slaughter stress, slaughter method, storage temperature and time as well as meat properties such as: intramuscular fat content and pH (Lawrie, 1991).

Down (2013) stated that losses of 1-2% of evaporation can be as a result of boning and cutting. Further long-term storage can lead to far greater losses of up to 12% of drip. Drip loss on this scale represents a large reduction in the yield of meat leading to financial losses as well as affecting the appearance, nutritional value and palatability of the meat to the consumer (Down, 2013). A significant amount of protein contained in the aqueous solution can be lost from *post-mortem* muscle, (112 mg per millilitre of fluid) (Huff-Lonergan, Zhang & Lonergan, 2010).

1.7.1.3 Cooking loss

Cooking loss is defined as the total loss that occurs during cooking of meat and is the combined losses of drip and evaporation (Obuz & Dikeman, 2003). The greater part of the volatile loss is from evaporation of water released from tissue by denaturation and coagulation of proteins, and by water soluble materials such as salts, sarcoplasmic proteins and non-protein nitrogenous compounds (Kendall, 1973). Losses such as the heat-composed fat and protein products, aromatic compound and the small amount of fat (fat droplets) that have splashed out of the pan may be included in the volatile portion (Kendall, 1973). Drip cooking losses consists of melted fat oozing out of meat by heating, but water and non-volatile water-soluble materials (salts and sarcoplasmic proteins) may also be included (Paul, 1972). Myosin denatures at 40 to 53°C (Bendall & Restall, 1983), causing a transversal contraction in the myofibrils and a slow loss of water from the myofibrils. Collagen of the basement membrane contracts at 60°C, resulting in rapid moisture loss from the myofibrils at these temperatures (Bendall & Restall, 1983). Collagen of the perimysium and endomysium networks shrinks at temperatures above 64°C (Sims & Bailey, 1981) and therefore

causes much pressure on the aqueous solution, leading to a quick volume loss in cooked meat. Endpoint cooking temperature significantly affects cooking losses and subsequently loss of juiciness and tenderness. According to Aalhus *et al.* (2009), Obuz & Dikeman (2003) beef cooked to a rare endpoint (63°C) tended to be more tender and juicy than meat cooked to a well-done endpoint of 71°C.

1.7.2 Meat colour

Figure 1.5 demonstrates the relationships between different states of myoglobin (Mb) in fresh meat:
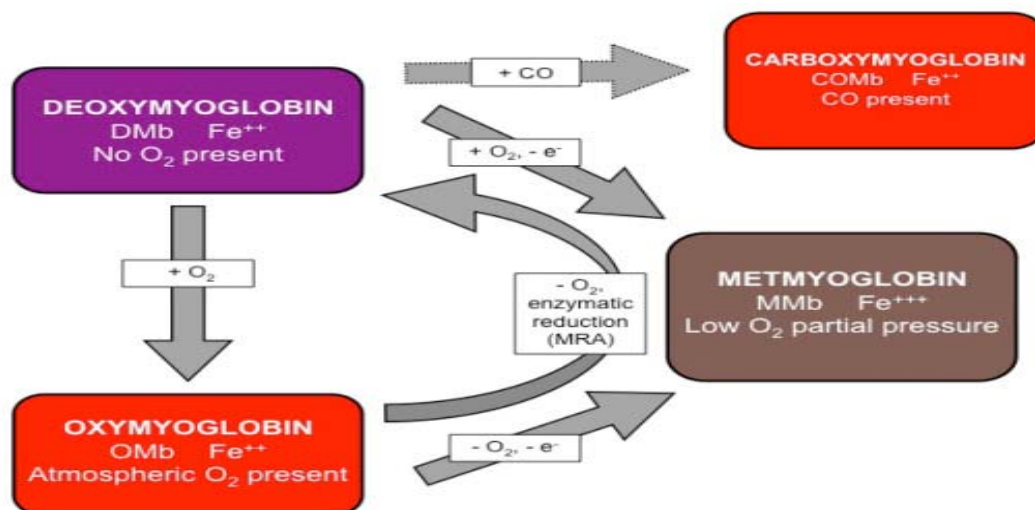
Figure 1.5 Forms of Mb (Mancini & Hunt, 2005)

Visual appearance is the most important quality characteristic of meat evaluated by the consumer at its point of sale, (Tateo, De Palo, Maggiolino & Centoducati, 2013; James & James, 2002). Meat colour is used by the consumer as an indicator of freshness and it strongly influences the purchase decision (Olivera, Bambicha, Laporte, Cárdenas & Mestorino, 2013). Consumers discriminate against meat that is not a bright cherry red (Young, Priolo, Simmons & West, 1999). Therefore, high colour stability is essential for the meat industry, since the colour gives the consumer an indication of the quality at point of purchase (Troy & Kerry, 2010). Colour of meat is determined by the relative quantity of the different forms of Mb, which are deoxymioglobin (DeoxyMb), oxymyoglobin (OxyMb) and metmyoglobin (MetMb). DeoxyMb is the reduced form of Mb which gives a purple colour when oxygen is absent

(Norström, 2011). OxyMb is the oxygenated form of Mb responsible for the bright red colour, while MetMb, is the oxidised form responsible for browning (Bekhit, Geesink, Morton & Bickerstaffe, 2001). Table 1.1 and Figure 1.5. Oxygenation of Mb begins when the meat is exposed to oxygen (O_2). MetMb formation depends on numerous factors such as temperature, partial pressure of oxygen, pH and meat's reducing activity. In some cases, growth of micro-organisms as well as genetic variation in animals also have an influence (Bekhit, Cassidy, Hurst & Farouk, 2007; Mancini & Hunt, 2005).

The mechanisms and their effects of pH, temperature and partial oxygen pressure on meat quality is summarised in Table 1.2

Table 1.1 Characteristics of different states of Mb (Brewer *et al*., 2001)

Pigment	Type of bonding	State ofglobin	State of iron	State of heme	Colour
Deoxymyoglobin [DeoxyMb]	Ionic	Native	Fe++	Intact	Purple-red
Oxymyoglobin [OxyMb]	Covalent	Native	Fe++	Intact	Bright red
Metmyoglobin [MetMb]	Ionic	Native	Fe+++	Intact	Brown

Table 1.2 Factors affecting meat colour (Radetić *et al.*, 2007)

Temperature		Partial O₂ pressure		pH	
High	**Low**	**High**	**Low**	**High**	**Low**
Favours higher O_2 consumption by residual respiratory enzymes, as well as other processes that consume O_2	Facilitates increased penetration of O_2	Favours the OxyMb formation	Favours the MetMb formation	Accelerates the meat respiratory activity resulting in a thin layer of OxyMb on surface, below which Mb is present	Causes denaturation of globin followed by dissociation of O_2 from the heme
Promotes the dissociation of O_2 from OxyMb, with increasing tendency of autoxidation of created Mb	Increases solubility of O_2 in the fluid tissue			Muscle fibres swell with decreasing diffusion of O_2 and thus form OxyMb	Promotes Mb oxidation

MetMb-reducing activity/system (MetMbRA) is involved in conversion of MetMb back to the reduced and subsequently oxygenated red pigment OxyMb (Tikk, 2007). This process is determined by the efficiency of reducing enzyme systems, oxygen-scavenging enzymes and the NADH pool of muscle, which is limited in *post-mortem* muscle and continuously decreases as *post-mortem* time continues (Tikk, 2007). Therefore, the rate of discolouration of meat is believed to be dependent on both oxidative processes and enzymatic MetMb reducing activity (Faustman & Cassens, 1990). Usually, during storage and display, fresh and frozen meats undergo a gradual change in surface colour from red to brown. These changes occur as a result of thermal and photochemical changes to the brown MetMb (Andersen, Bertelson & Skibsted, 1990). During formation of MetMb, oxidation of $Fe^{2}+$ iron into $Fe^{3}+$ iron takes place, causing gradual discolouration (Brewer, 2008).

1.7.3 Sensory characteristics

1.7.3.1 Flavour and aroma

Meat consists of water, proteins, lipids, carbohydrates, minerals and vitamins. During flavour development, proteins, lipids and carbohydrates play a primary role because they consist of different compounds that develop into flavour precursors when heated (Mottram, 1998; Spainer & Miller, 1993). Each of these main components contributes to the overall identity of beef flavour. To date, thousands of volatile compounds have been identified in the constituents of cooked meat (Van Ba, Hwang, Jeong & Touseef, 2012) that contribute to the flavour of cooked meat (Oliveros, Ryu & Hwang, 2010; Rochat & Chaintreau, 2005; Machiels, Istasse & Van Ruth, 2004). Flavour of meat is usually influenced by species, age, and amount of fat, as well as the diet of the animal. For example, flavour characteristics of beef, pork and poultry are different. In general, this is due to different flavour precursors in the fat between species (Joo *et al.*, 2013). The species-specific flavour is mostly associated with the lipid portion of meat, because more than 650 fat-soluble volatiles are released in beef when heated (Shahidi, 1994; MacLeod, 1994). Most of the compounds contributing to the "meaty flavour" of meat are sulphur or carbonyl-containing compounds (Hogan, 2002). Gender affects meat flavour because it is highly related to testosterone and skatole, which are produced in intact males and females, respectively. For example, an unpleasant urine-like and sweaty odour in pork from intact males (boar taint), is related to the presence of androstenone (5α-androst-16-en-3-one) and skatole (3-methylindole) (Grindflek, Meuwissen, Aasmundstad, Hamland, Hansen, Nome, Kent, Torjesen & Lien, (2011). Androstenone is a metabolite of testosterone, and skatole is the main contributor to pastoralflavour (Teixeira, Batista, Delfa & Cadavez, 2005). According to Mottram (1991) off-flavours such as blood-like flavours found in meat, develop during cooking, due to the complex interaction of precursors obtained from lean as well as fat that produces volatile flavour compounds. Increasing serum-like bloody aromatic and metallic tastes are the results of higher levels of myoglobin in the meat of older animals (Joo *et al.*, 2013).

1.7.3.2 Juiciness

Juiciness is a critical trait in determining overall beef palatability (Montgomery & Leheska, 2012) and can be divided into two organoleptic components, namely: the impression of juiciness during first chews which is produced by rapid release of meat fluids, while sustained juiciness results from the stimulatory effect of fat on salivation (Lawrie, 1998). In general, juiciness and tenderness are closely related, that is, the more tender the meat the more readily juices are released during chewing.

Meat from a young and therefore leaner animal gives an initial impression of juicy meat but ultimately a dry sensation is experienced due to the absence of fat (Lawrie, 1998). The method, time and temperature of cooking affect the amount of moisture loss due to shrinkage during cooking. High temperatures melt fat and tend to destroy the structures retaining fat (Lawrie, 2006; Weir, 1960). Jedlicka (2009) stated that fast-cooked meat has a lower cooking loss and is juicier compared to slow-cooked meat at the same temperature. Therefore, the cooking procedure may be the most important factor influencing juiciness in cooked meat. High quality raw meat can be easily destroyed by poor cooking methods and techniques.

1.7.3.3 Tenderness

Tenderness is described as the ease of chewing, which includes initial ease of penetration, by the teeth (Lawrie, 1998). Meat then breaks into fragments and an amount of residue remains after chewing. Among sensory attributes, the combination of tenderness, juiciness and meat flavour determines palatability of meat. However, consumers regard tenderness as one of the important components affecting meat quality. They can discern between different tenderness categories namely: the content and state of connective tissue and the structure and state of myofibrils (Simela, 2005; Wheeler, Shackelford & Koohmaraie, 2004; Boleman, *et al.*, 1997) and they are willing to pay for improved tenderness (Boleman *et al.*, 1997). Inconsistency in beef tenderness is a major problem within the beef industry (Destefanis, Brugiapaglia, Barge & Dal Molin, 2008; Koohmaraie, 1994). According to Morton, Bickerstaffe, Kent, Dransfield & Keely (1999) and Monin (1998) the concept of meat tenderness is very complex, since it depends on the total amount of collagen and collagen solubility (connective tissue characteristics), muscle energy status which affects the extent of

the contraction of the muscle (Hope-Jones, Strydom, Frylinck & Webb, 2010), moisture losses and finally, the integrity of the myofibrillar component of muscle. Collagen molecules are bound to each other through intermolecular crosslinks that assist in supplying structure and strength. These crosslinks are reducible, but over a period of time are substituted by developed, thermally stable, and less soluble crosslinks which are the key factors in collagen-related toughness (Weston, Pas & Althen, 2002). The proportion of mature to reducible crosslinks increases with age, resulting in older animals that have less tender meat than younger animals (Weston *et al.*, 2002).

CHAPTER 2

2.1 Problem Statement

Household freezers have limited capacity for freezing large amounts of meat in a short time. Therefore, home freezing may potentially leave the customer with poor quality meat. Blast freezers can be used on a commercial scale by meat processors to accelerate the rate of freezing, and possibly improve the quality of frozen meat. A frozen product could then be presented to consumers with more confidence and without the need to freeze at home. However, since we do not know whether meat frozen in a domestic freezer will in fact lead to quality problems compared to meat frozen in a blast freezer, this study will compare the two freezing systems regarding various meat quality characteristics.

The objective of this study is to determine the physical and organoleptic changes of beef loin steaks using an air blast freezer (fast freezing method) with the ability to freeze beef steaks within 3 hours to -35°C and a domestic freezer (slow freezing method) with the ability to freeze beef steaks within 24 hours to -20°C. Since meat presented for sale may be aged for various periods, the study will also include the effect of *post-mortem* ageing as additional factor (3 and 14-ageing days). Fresh, unfrozen steaks were used as control treatment.

2.2 Research question: Do freezing method and the rate of freezing influence physical and sensory quality of beef *m. longissimus* steaks aged for 3 and 14-days?

2.3 Objectives and hypothesis

To determine the effect of freezing method, rate of freezing and *post-mortem* ageing on moisture characteristics (thaw loss, water holding capacity (WHC) and cooking loss) of *m. longissimus* steaks.

To determine the effect of freezing method and *post-mortem* ageing on colour characteristics (metmyoglobin (MetMb), deoxymyoglobin (DeoxyMb) and oxymyoglobin (OxyMb), CIE L*a*b*, chroma and hue) of fresh *m. longissimus* steaks.

To determine the effect of freezing method and *post-mortem* ageing on Warner Bratzler shear force (WBSF) of cooked *m. longissimus* steaks.

To determine the effect of freezing method and *post-mortem* ageing on acceptability of *m. longissimus* steaks using a trained sensory panel to evaluate aroma intensity,

first bite, impression of juiciness, muscle fibre tenderness, amount of connective tissues (residue) and overall beef flavour.

The following hypotheses were investigated:

Freezing method has no effect on moisture characteristics, colour measurements, shear force values (WBSF) and sensory characteristics of *m. longissimus* steaks.
Post-mortem ageing days has no effect on moisture characteristics, colour measurements, shear force values (WBSF) and sensory characteristics of *m. longissimus* steaks.

CHAPTER 3

Materials and methods

3.1 Meat

Seven pairs of loin primal cuts (*m. Longissimus dorsi, LD)* were collected from the same carcass during deboning at Chalmar Beef (Pty Ltd) commercial abattoir and deboning plant, on three different occassions (n= 42). The carcasses were selected from a group of grainfed steers, aged between 12 and 14-months and were slaughtered according to standard commercial procedures. These selected carcasses were uniform in weight (~250 kg) and in fatness (~5 mm subcutaneous fat thickness between 12th and 13th rib). Carcasses were electrically stimulated (ECS-1 Jarvis stimulator) (Output: Rectangular DC wave, 150V, maximum amplitude 17Hz, 5ms pulse width, RMS-voltage below 50V) (Jarvis Products Corporation RSA (Pty) Ltd) for 20s. Temperatures and pH of the loin muscle (posterior *m. longissimus dorsi)*, during the course of rigor mortis reflected ideal rigor conditions that Thompson (2002) described as pH>6 when muscle temperature was above 35°C and pH<6 when muscle temperature was below 10°C. In addition, all pH_u values (18 hours) were below 5.7.

Deboning and selection of cuts took place on the day following slaughter. The primal cuts were vacuum-packed and shipped to the meat laboratory of the Animal Production Institute of the ARC at Irene, Pretoria for further processing and sampling on the same day that the cuts were collected.

3.2 Processing and sampling

Table 3.1 describes the layout of the trial. One loin of each of the 21 pairs was processed to accommodate three treatments and various meat quality tests: The other of each pair was aged at 2°C in the dark for a further 12-days (14-days *post-mortem*) and processed according to the same procedure as the short-aged cut. All loins were weighed prior to further processing. Loin cuts were removed from the vacuum bags and processed and divided into 9 cm portions from the cranial (thoracic part) to the caudal (lumbar part) end, and each portion was subjected to a specific quality test.

Each 9-cm portion was subdivided into 3 x 3-cm steaks that were subjected to one of the three treatments, namely: Fresh; never frozen=FR; Slow-freezing=SF, and Quick-freezing=QF. The order of allocation of the steaks (positions 1, 2 or 3) to the 3 treatments was changed with each loin so that the possible effect of position within the 9-cm sub-samples were addressed in a satisfactory way (Figure 3.1 & 3.2). Loins were sampled in order and labelled as loin 1 to 21 as shown in Table 5. The various measurements, Warner Bratzler shear force; sensory evaluation, drip loss, colour and water holding capacity were measured on the same section of the loin each time.
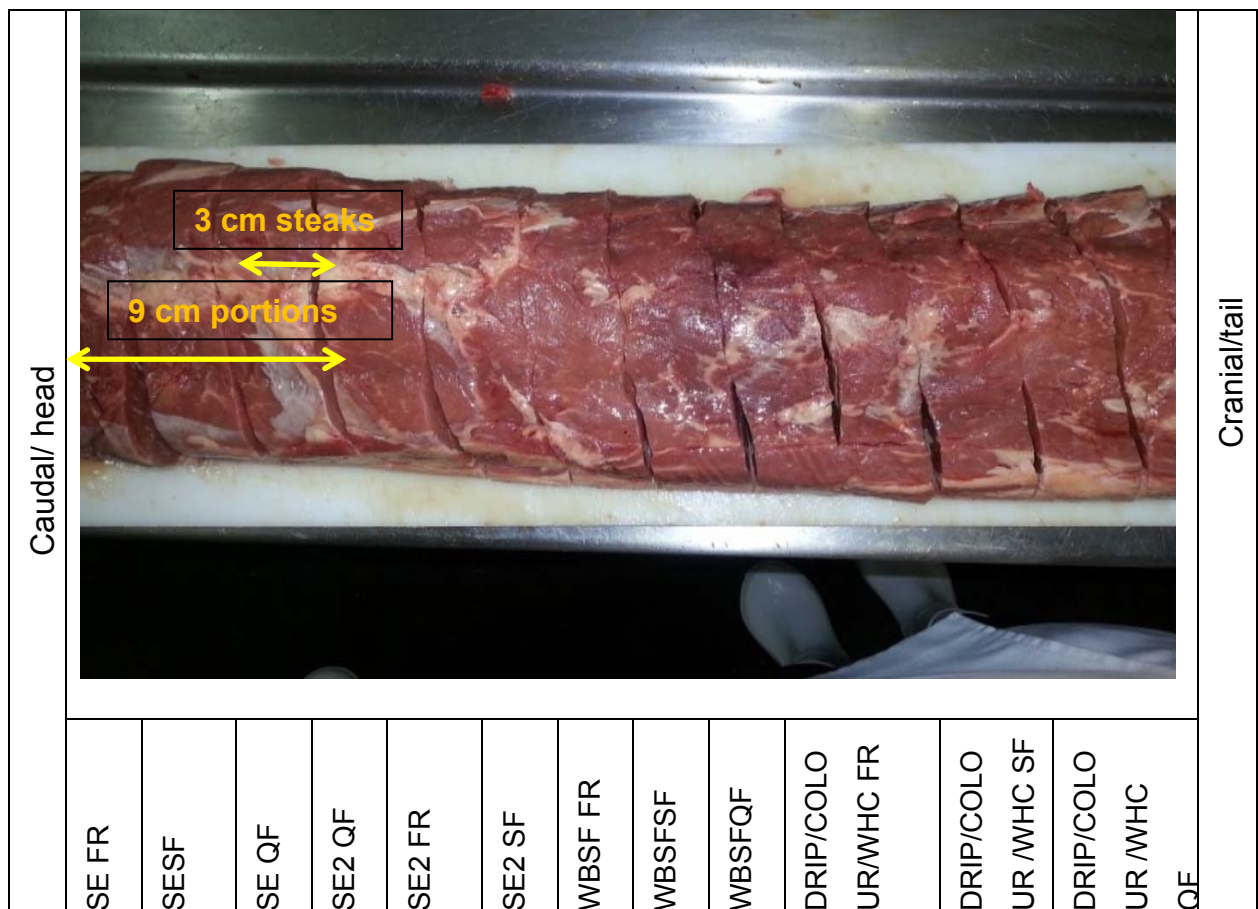
SE = Sensory evaluation, FR = Fresh, SF = Slow-freezing, QF = Quick-freezing, WBSF = Warner Bratzler Shear Force, WHC = Water holding capacity

Figure 3.1: Order of tests on the loin

SE

FR → SF → QF →

SF ← FR ← QF

SF → QF → FR

WBSF

FR → SF → QF →

SF ← FR ← QF

SF → QF → FR

DRIP, COLOUR & WHC

FR → SF → QF →

SF ← FR ← QF

SF → QF → FR
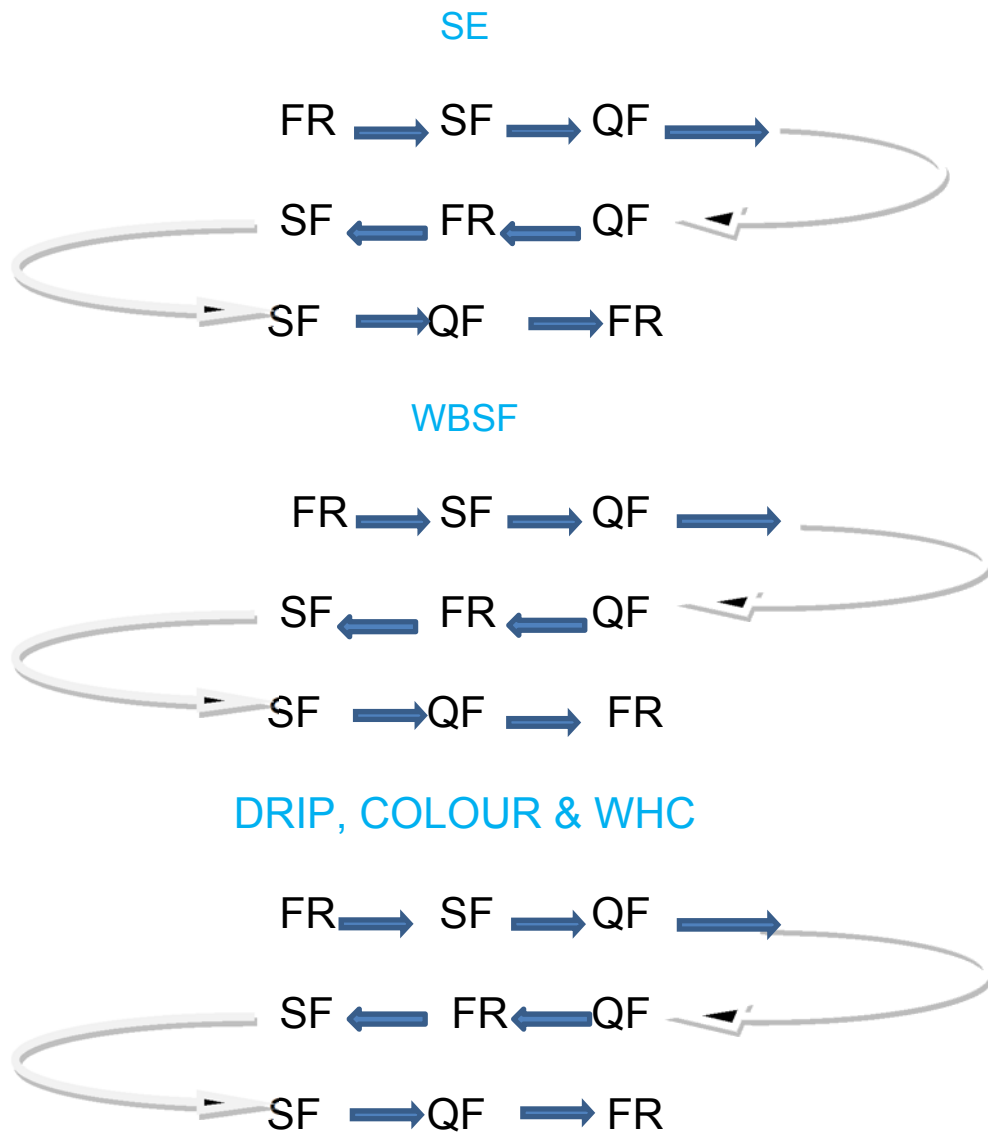
Applies for 2 x 21 loins for 3 and 14 ageing days

SE = Sensory evaluation, **FR** = Fresh, **SF** = Slow-freezing, **QF** = Quick-freezing, **WBSF** = Warner Bratzler Shear Force, **WHC** = Water holding capacity
Figure 3.2: Changing orders of treatments on the 9 cm portions of the loin cuts

The total number of steaks for the experiment n=126

Table 3.1 Analyses, replications, sampling dates, ageing days and treatments of 126 steaks

Analyses	Replications and sampling dates	Days aged	Treatments and number of steaks per treatment			
			FR	QF	SF	Total number. of steaks
Thaw drip loss, colour, WHC	Replication 1 (Day: 15) 7 x loins	**3**	7	7	7	21
		14	7	7	7	21
WBSF and temperature	Replication 2 (Day: 17) 7 x loins	**3**	7	7	7	21
		14	7	7	7	21
Sensory evaluation	Replication 3 (Day: 22) 7 x loins	**3**	7	7	7	21
		14	7	7	7	21
		Total= 126				

FR = Fresh, **QF** = Quick-freezing, **SF** = Slow-freezing, **WHC** = Water-holding capacity, **WBSF** = Warner Bratzler Shear Force

Table 3.2 Allocation order of treatments of the 21 loins processed on 3 different dates for the 3- and 14-day loin cuts

Date	Allocation order of treatments
October 15	**L1**=FR, QF, SF/**L2**=QF,SF,FR/**L3**=SF,F,QF/**L4**=FR,QF,SF/ **L5**=QF,SF,FR/**L6**=SF,FR,QF/**L7**=FR,QF,SF
October 17	**L8**=QF, SF,FR/**L9**=SF,FR,QF/**L10**=FR,QF,SF/**L11**=QF,SF,FR/ **L12**=SF,FR,QF/**L13**=QF,SF,FR/**L14**=SF,FR,QF
October 22	**L15**=FR,QF,SF/**L16**=QF,SF,FR/**L17**=SF,FR,QF/**L18**=QF, SF, FR/**L19**=SF,FR,QF/**L20**=FR,QF,SF/**L21**=QF,S,FR

L = loin, **FR** = Fresh, **QF** = Quick-freezing, **SF** = Slow-freezing

Each sample was labelled according to the purchasing and sampling dates, loin number, name of the treatment, name of the test and the number of ageing days (Figures 3.3 and 3.4)

Figure 3.3: Loin cutting

Figure 3.4: Loin labelling

Steaks were vacuum-packed and treatments were applied as follows:

3.3 Treatment procedures

Freezing samples included the slow-frozen loin samples (SF) quick-frozen loin samples(QF)compared to fresh loin samples (not frozen, FR). Fresh samples were kept in a cold room at 3 °C until the next morning (Day 3).

Slow-frozen samples (SF) were packed in a single layer on 3 shelves of a house hold freezer (AEG Skandiluxe) operating on setting 3 to reach a final temperature of -20 °C. The remaining freezer space was filled with unfrozen ice packs. The starting temperature before loading the samples was -20°C. The samples were frozen to a core temperature of -20°C in 24 hours.

Quick-frozen samples (QF) were placed in a single layer on 3 shelves of an IcematicT15-2P blast freezer. The samples were frozen to a core temperature of -30°C in 3 hours. The core temperatures of the steaks of both freezing treatments were continuously monitored using a YCT thermostat logger (YC-747UD model, Taiwan, type K thermo couple). All samples for the 2 frozen treatments were stored after the freezing processing a -20°C freezer room, and were then thawed in vacuum packages

for 18 hours at 3 °C in a dark chiller before analysis. All analyses for fresh samples were done on Day 3 *post-mortem* or a day after sub-sampling 14-day aged samples.

3.4 Freeze profiles

Figure 3.5 shows the typical freeze profile for slow and fast-frozen samples. Samples frozen in the conventional freezer took 18hours to reach a core temperature of -20°C, while the blast-frozen samples reached -20°C in less than 3 hours. QF samples were frozen to core temperatures of -30°C but both treatments were stored at -20°C until quality measurements were done.
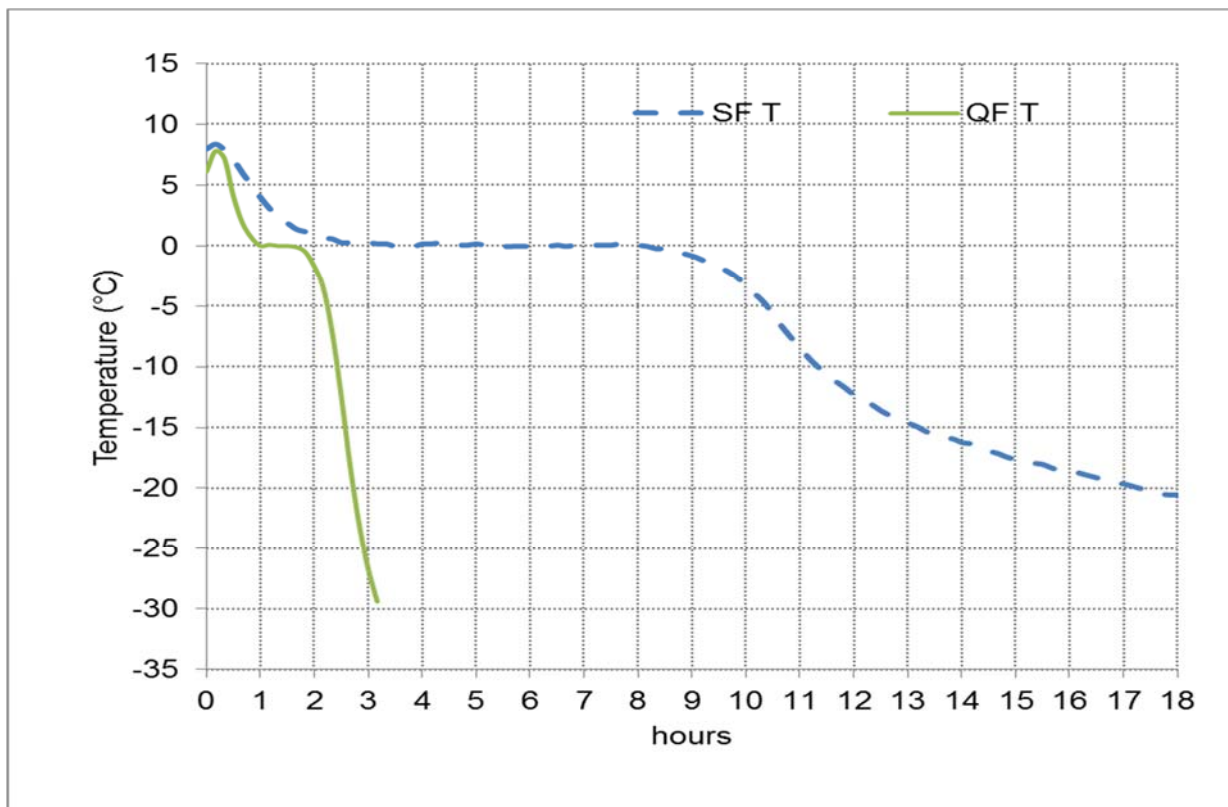
Figure 3.5: Temperature profile (T) of slow-frozen (SF) and quick-frozen (QF) samples (0h indicates commencement of the freezing process).

3.5 Analyses

The pH_u and temperatures of the samples were recorded on the day of collection and on each day of analysis to verify their consistency. Samples were analysed for sensory evaluation (SE), Warner Bratzler Shear Force (WBSF), drip loss (%) water holding

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capacity (%) and colour (MetMb, DeoxyMb and OxyMb, CIE L*a*b*, chroma and hue) as seen in Table 3.3.

Table 3.3 Analysis protocol

		ANALYSES				
Treatment	pH & Temperature	Thawing	Colour	WHC	Sensory	WBSF
Raw samples						
FR	x	x	x	x		
QF	x	x	x	x		
SL	x	x	x	x		
Cooked samples						
FR					x	x
QF					x	x
SL					x	x

3.5.1 Temperature measurement and pH

The pH values of samples were determined by using a pH-meter (Eutech Instrument, Cyber scan, portable pH meter) fitted with a specially designed meat electrode. The pH and temperature were measured at sampling on whole loins and again in the fresh and frozen thawed steaks, with a pH-calibration temperature of 4°C.Two-point calibration was carried out, and the pH of calibration buffers used was 7.0 and 4.0.

3.5.2 Thawing drip loss

Adjusted thawing method as described by AMSA (1995)

The thaw/drip losses of samples were measured by weighing the vacuum-packaged steaks and their labels using Radwag Wagi Elecktroniczne (Model, PS 750/C/2, Lasec/SA). Steaks and labels were then removed from the vacuum packaging bags, after which steaks were dried off with a paper towel to remove purge, individually

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weighed again, and weights recorded. Purge was emptied out of the vacuum-packaging bag and dried off with a paper towel, and the empty bag was weighed again. Thaw loss of fresh steaks was determined in the same way, and the accumulated overnight drip after sampling and packing was measured. The following formula was used for calculating thaw losses:

Thaw loss (%) = [(mass of bag + drip – mass of bag without drip) ÷ mass raw + thawing loss –mass of bag without drip] x 100

3.5.3 Water holding capacity (WHC)

Water holding capacity is described as the ability of meat to bind its own water or, under the influence of external pressure such as gravity, heating, and pressure, to bind additional water (Huff-Lonergan, 2010).

WHC was determined by calculating the ratio of meat area and liquid area after pressing a 400 to 600 mg fresh meat sample on a filter paper (Whatman 4) sandwiched between two Perspex plates, and pressed at a constant pressure of 300 psi for 60 seconds, according to the method described by Irie, Izumo & Mohri (1996). The areas were measured by means of video image analysis using a CC12 video camera (Olympus, Tokyo, Japan), as well as image processing and calculations using Analysis Life Science software package (Soft Imaging Systems Gmbh, Münster, Germany).

3.5.4 Colour measurement

Instrumental colour (CIE L*a*b*) and myoglobin fractions were measured on fresh, and thawed steaks using a portable spectrophotometer (CM-700/CM-600d, Model, Konica Minolta Sensing, Europe B.V) with a large-size colour LCD screen for setting, items and data display. The instrument also offers additional 8mm-measuring aperture for perfect adaptation to small sample sizes. The spectrophotometer was used with the software package Spectra Magic NX Pro (Konica-Minolta) and following configuration applied illuminant D65, geometry di 8° de 8°, observer angle 10°spectral component excluded (Krzywicki, 1979).

White calibration was performed 20 minutes before the colour reading. Each steak was removed from the vacuum bag, placed on a polystyrene tray and kept in a dark cold room at 3 °C to bloom for 60 minutes. Steaks were blotted with paper towel before

reading and three readings were taken at different locations on the surface of each steak. Reflectance was measured from 400 to 740 nm in increments of 10 nm. Colorimetric' parameters CIE L*, a*, b*, chroma and hue angle were measured. In the CIE Lab system L* denotes (lightness), +a* (redness), and +b* yellowness (AMSA, 1991). Chroma and hue were automatically calculated from a* and b*. Chroma measures colour intensity where the higher values indicate more intense red colour in meat (Chris, 2007). An increase in hue angle between 0° and 90° corresponds to a blending of yellowness or loss of redness, probably due to metmyoglobin formation in fresh meat (Chris, 2007). The proportions of different myoglobin redox forms metmyoglobin (MetMb), deoxymyoglobin (DeoxyMb) and oxymyoglobin (OxyMb) were calculated. Individual sample results were averaged and converted into reflectance attenuance (A= Log10 1/R), at isobestic point 572, 525, and 473 nm (analogous to calculation of absorbance from transmittance, as described by Shibata (1962) and Swatland (1983), calculated by the linear interpolation), and at 730 nm (Krzywicki, 1979).

3.6 Sensory analysis

3.6.1 Cooking procedure

Samples for fresh treatments were evaluated on different days than those of frozen samples (QF and SF) for practical reasons. Considering the dilemma of not being able to taste fresh and frozen samples on the same day, two additional loins of the same carcass were purchased to provide a standard sample for anchoring the sensory panel's scores on each day of tasting. The two loins were processed into 3 cm steaks, vacuum-packed and frozen until the day on which they were required. For frozen samples, 7 randomly sampled steaks from the QF and SF treatments and 1 anchor sample were thawed for 12hours in a 4°C cold room (Annexure 3). Eight non-stick frying pans + racks used for cooking samples were weighed a day before evaluation. On the day of evaluation, 3 steaks (1 for WBSF measurement and 2 for sensory evaluation) were put on the labelled (name of the treatment) cooking pan and weighed together with the rack and raw samples before cooking. Required cooking data was recorded on the cooking data form (Figure 3.6 & 3.7).

Figure 3.6: Weighing of raw samples
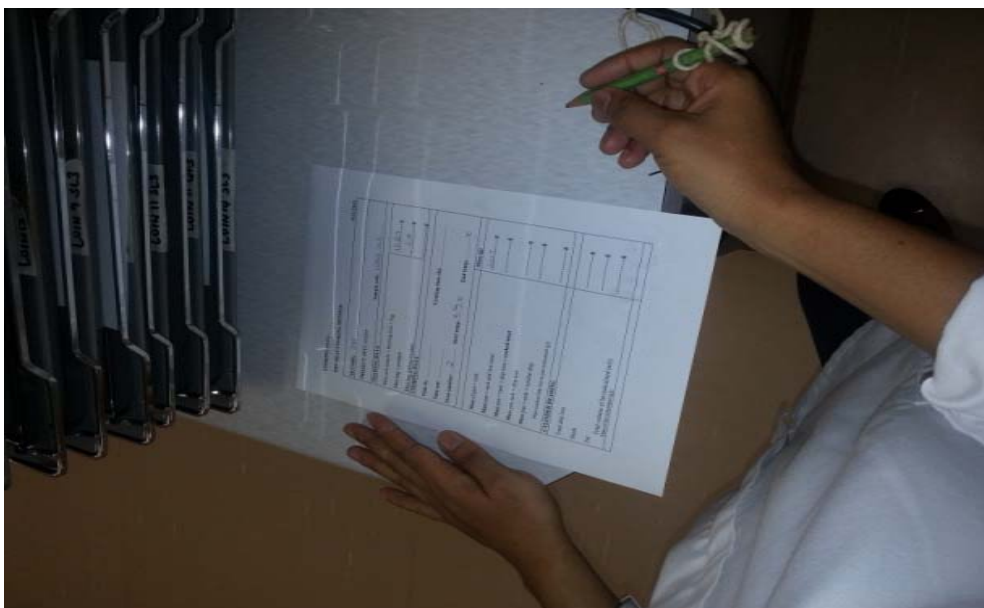
Figure 3.7: Recording of cooking data

Steaks were broiled in a preheated electric convention oven (Mielé, model H217, Mielé & Cie, Gütersloh, Germany) by direct radiant heat (> 200°C). The ovens were set on grill for 10 minutes prior to preparation. Samples were placed on an oven pan on a rack to allow meat juices to drain during cooking and placed in a preheated oven. The steaks were grilled for approximately 20 minutes until they reached a final internal core temperature of 70°C. The internal temperature of each steak was monitored by using

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an iron-constant thermocouple (T-type) (Hand-model Kane-Mane thermometer). The thermometer was placed in the approximate geometric centre of each steak. Steaks were removed from the oven after reaching a 69-70°C internal core-temperature. The cooked meat + pan +drip, were weighed. The following formulae were used as stated by AMSA (1995) to calculate cooking losses.

3.6.2 Cooking loss

$$Cooking\ loss = \frac{\text{Total mass of drip loss}}{\text{Mass of raw meat}}\ x100$$

3.6.3 Drip loss

$$Drip\ loss = \frac{\text{Mass of bag + exudate} - \text{mass bag without exudate}}{\text{Mass of raw meat + drip loss + bag} - \text{mass of bag without exudate}}\ x100$$

3.6.4 Evaporation loss

Evaporation loss (%) = Total cooking loss – drip loss

3.7 Sensory panel training procedures

Ten experienced sensory panel members were used for sensory analysis at the Meat Industry Centre (ARC-Irene). The sensory analysis facility includes all the elements essential for a well-organised sensory plan (ASTM, 1989). During the two-day training sessions (two hours per day), the panel received representative beef loin samples of each of the different treatments (Fresh, not frozen, quick freezing and slow freezing) one at a time. The panel was trained in order to increase their sensitivity and ability to differentiate between specific loin samples and the sensory characteristics of each sample. A clear description of each characteristic was developed, based on ASTM in order to define the specific product characteristics to be analysed. The panel were given the opportunity to analyse the 3 treatments (FR, fresh not frozen, QF and SF) according to the descriptive sensory characteristics shown on table 3.4 (lexicon). The panel scored the samples on an 8-point hedonic scale for aroma intensity, first-bite, impression of juiciness, muscle fibre and overall tenderness, amount of connective

tissue (residue) as well as the overall beef-flavour intensity (see Annexure 2). Each sensory category attribute was expressed in words, e g. 8 indicated extremely tender and juicy meat, extreme flavour and aroma intensity and no residue, while 1 referred to extremely tough and dry meat, extremely bland flavour and aroma intensity and excessive residual connective tissue (Meilgaard, Civille & Carr, 1991)

3.7.1 Sensory evaluation

A ten-member, trained panel was used to evaluate the sensory characteristics of the cooked samples. The two steaks allocated for sensory analysis were removed from the grill and cut into 1cm² cubes using an electric knife. Cut samples were wrapped in an aluminium foil (25cm²), labelled using random numbers, put on a warm saucer and placed in the oven of 110°C for 5-8 minutes to reach a temperature of 60°C, and kept warm prior to being evaluated.

Two samples (1 per treatment) per session were evaluated and four sessions were hosted per day. In every first session, the panel members were evaluating a treatment sample together with the anchor sample. Sensory panels were conducted in a darkened room under soft red light in individual cubicles designed for objective sensory analysis. Panelists were instructed to cleanse their palate after each sample with the room-temperature distilled water and unsalted crackers. Results of ten members were averaged to obtain a single value for each sample (treatment repetition).

Table 3.4: Lexicon for the description of each sensory attribute used by sensory panel for the evaluation of the cooked steaks

ATTRIBUTES	DESCRIPTION
AROMA INTENSITY	Take a few short sniffs as soon as you remove the foil. This aroma is associated with cooked steaks and has an important influence on the flavour characteristics of cooked meat
FIRST BITE	The impression that you form on the first bite of the meat
IMPRESSION OF JUICINESS	The impression of juiciness that you form during chewing, or the experience of wetness produced by the rapid release of meat fluid during the first few chews
MUSCLE FIBRE & OVERAL TENDERNESS	Chew sample with a light chewing action. The impression of tenderness of the meat when chewing, and evaluating whether the meat breaks easily between the teeth, or has become tough or difficult to bite through
AMOUNT OF CONNECTIVE TISSUE (RESIDUE)	The chewiness of the meat
OVERALL <u>BEEF FLAVOUR</u> INTENSITY	The combination of taste while chewing and swallowing the meat

3.7.2 Shear force

The single steak to be used for WBSF was cooled down in an air-conditioned room for 2-3 hours, to reach an internal temperature of 16°C.Six cores were removed from each steak, parallel to the orientation of the muscular fibres. Each core of 12.7mm diameter was sheared once across its length. The mean shear force values were determined

by using Warner-Bratzler shear force, mounted on a Universal Instron apparatus (Model 4301, Intsron Ltd, Buckinghamshire, England, cross head speed = 200 mm/min). A mean value of the maximum force required to shear each set of cores in kg was used as shear force value (Humling *et al.,* 2008).

3.8 Statistical analysis

Data of WBSF, sensory analyses, drip loss/purge and colour attributes were subjected to analysis of variance for a split-plot design (GenStat® VSN International, Hemel, Hempstead, UK; Payne, Murray, Harding, Baird & Soutar, 2007) with the 2 ageing periods (3-days and 21-days) as whole plots and the 3 freezing treatments as sub-plots. Means for the interactions between whole plot and sub-plots were separated using Fisher's protected t-test least significant difference (LSD) at the 5% level (Snedecor & Cochran, 1980).

Annexure 1: ARC-API cooking loss form for the raw fresh and cooked steaks

Dry-heat cooking method	
SE CODE……………………. Date:………………….	
PROJECT **SAMPLE CODE:………………......**	
THAWING DATA	
Mass raw sample + thawing loss + bag	..........g
Mass bag + exudates	.........g
Mass bag without exudates	……..g
COOKING DATA	
Time in:......................Cooking time (h),................... **Time out:.........................** **Oven number:.................Start temp:°CEnd temp:.............°C**	
	Mass (g)
Mass of pan + rack	……..g
Mass pan + rack and raw meat	…….g
Mass pan + rack + drip loss + cooked meat	……..g
Mass pan rack + drip loss	. ……..g

Mass pan + rack + residue drip	
Mass residue drip loss in pan (calculated (g)	.........g
CYLINDER READING	
Total drip loss	...........g
Stock	...........g
Fat	...........g
Total volume of fat (calculated (ml)	
Mass of fat (calculated (g)	

Annexure 2: ARC-API trained panel sensory evaluation form

SENSORY ANALYSIS OF BEEF Session 1&2 Date:					
Name:..Panellistno:.........................					
Please evaluate the following samples of BEEF for the designated characteristics					
Characteristics	**Rating scale**	**Sample codes**			
AROMA INTENSITY Take a few short sniffs as soon as you remove the foil	1 = Extremely bland 2 = Very bland 3 = Fairly bland 4 = Slightly bland 5 = Slightly intense 6 = Fairly intense				

Bland = No flavour or taste factors perception	7 = Very intense 8 = Extremely intense				
Comments on aroma	**Please describe the aroma**				
FIRST BITE The impression that you form on the first bite	1 = Extremely bland 2 = Very bland 3 = Fairly bland 4 = Slightly bland 5 = Slightly intense 6 = Fairly intense 7 = Very intense 8 = Extremely intense				
IMPRESSIONOF JUICINESS The impression of juiciness that you form after chewing	1 = Extremely dry 2 = Very dry 3 =Fairly dry 4 = Slightly dry 5 = Slightly juicy 6 = Fairly juicy 7 = Very juicy 8 = Extremely juicy				
MUSCLE FIBRE & OVERALL TENDERNESS	1 = Extremely tough 2 = Very tough 3 = Fairly tough 4 = Slightly tough				

	5 = Slightly tender 6 = Fairly tender 7 = Very tender 8 = Extremely tender				
AMOUNT OF CONNECTIVE TISSUE (RESIDUE)	1=Extremely abundant 2 = Very abundant 3= Excessive amount 4 = Moderate 5 = Slight 6 = Traces 7 = practically none 8 = None				
OVERALL <u>BEEF FLAVOUR</u> INTENSITY	1 = Extremely bland 2 = Very bland 3 = Fairly bland 4 = Slightly bland 5 = Slightly intense 6 = Fairly intense 7 = Very intense 8 = Extremely intense				

Annexure 3: Sensory randomised cooking and serving order 3-digits coding form

Where **C = Control**, **FR** = Fresh, not aged (3-days), **FR1**4 = Fresh, aged for 14-days, **SF3** = Slow-Freezing, 3-days aged; **QF3** = Quick-Freezing, 3-days aged; **SF14** = Slow-Freezing, 14-days aged; **QF14** = Quick-Freezing, 14-days aged; **L =** Loin

		1	2	3	4	5	6	7	8
Day 1:									
Sample Group		C	FR	FR	FR	FR	FR	FR	FR
Sample Number		Control	L1	L2	L3	L4	L5	L6	L7
SE Code		**862**	**223**	**756**	**544**	**681**	**199**	**918**	**335**
Day 2:									
Sample Group		C	FR	FR	FR	FR	FR	FR	FR
Sample Number		Control	L8	L9	L10	L11	L12	L13	L14
SE Code		**653**	**749**	**522**	**475**	**894**	**116**	**381**	**968**
Day 3:									
Sample Group		C	FR	FR	FR	FR	FR	FR	FR
Sample Number		Control	L15	L16	L17	L18	L19	L20	L21
SE Code		**742**	**859**	**964**	**177**	**228**	**591**	**636**	**415**
Day 4:									
Sample Group		C	QF3	SF3	QF3	SF3	SF3	QF3	QF3
Sample Number		Control	L3	L6	L1	L2	L1	L7	L5
SE Code		**975**	**257**	**723**	**448**	**539**	**661**	**394**	**882**
Day 5:									
Sample Group		C	FR14	FR14	FR14	FR14	FR14	FR14	FR14
Sample Number		Control	L1	L2	L3	L4	L5	L6	L7
SE Code		**254**	**398**	**954**	**537**	**829**	**113**	**481**	**662**
Day 6:									
Sample Group		C	SF3	QF3	SF3	QF3	QF3	SF3	SF3
Sample Number		Control	L3	L4	L5	L6	L2	L7	L14

SE Code	776	489	834	967	172	333	218	641
Day 7:								
Sample Group	C	QF3	SF3	QF3	SF3	QF3	SF3	QF3
Sample Number	Control	L14	L13	L8	L12	L9	L10	L12
SE Code	755	421	878	593	638	355	214	167
Day 8:								
Sample Group	C	FR14	FR14	FR14	FR14	FR14	FR14	FR14
Sample Number	Control	L8	L9	L10	L11	L12	L13	L14
SE Code	982	349	973	752	395	524	881	469
Day 9:								
Sample Group	C	SF3	QF3	SF3	QF3	SF3	QF3	SF3
Sample Number	Control	L8	L13	L9	L10	L11	L11	L14
SE Code	847	295	452	784	363	926	519	630
Day 10:								
Sample Group	C	QF3	QF3	SF3	QF3	SF3	SF3	QF3
Sample Number	Control	L18	L21	L19	L15	L16	L17	L17
SE Code	769	476	352	291	526	848	633	184
Day 11:								
Sample Group	C	FR14	FR14	FR14	FR14	FR14	FR14	FR14
Sample Number	Control	L15	L16	L17	L18	L19	L20	L21
SE Code	151	513	326	895	879	762	248	437
Day 12:								
Sample Group	C	SF3	SF3	QF3	SF3	QF3	QF3	SF3
Sample Number	Control	L15	L20	L16	L18	L20	L19	L21
SE Code	194	538	485	747	919	888	272	658
Day 13:								
Sample Group	C	QF14	SF14	QF14	SF14	SF14	QF14	QF14
Sample Number	Control	L5	L3	L6	L7	L4	L2	L1
SE Code	351	797	289	145	463	874	626	512

Day 14:								
Sample Group	C	SF14	QF14	SF14	QF14	QF14	SF14	SF14
Sample Number	Control	L2	L4	L5	L7	L3	L6	L1
SE Code	**714**	**927**	**345**	**664**	**559**	**196**	**871**	**233**
Day 15:								
Sample Group	C	QF14	SF14	QF14	SF14	QF14	SF14	QF14
Sample Number	Control	L12	L11	L11	L8	L9	L14	L10
SE Code	**171**	**948**	**215**	**536**	**725**	**683**	**362**	**499**
Day 16:								
Sample Group	C	SF14	QF14	SF14	QF14	SF14	QF14	SF14
Sample Number	Control	L12	L13	L10	L8	L9	L14	L13
SE Code	**256**	**595**	**614**	**873**	**162**	**449**	**938**	**387**
Day 17:								
Sample Group	C	QF14	QF14	SF14	QF14	SF14	SF14	QF14
Sample Number	Control	L21	L20	L21	L19	L5	L18	L18
SE Code	**337**	**843**	**562**	**924**	**259**	**786**	**615**	**478**
Day 18:								
Sample Group	C	SF14	SF14	QF14	SF14	QF14	QF14	SF14
Sample Number	Control	L19	L20	L17	L16	L16	L15	L17
SE Code	**447**	**961**	**192**	**889**	**397**	**746**	**425**	**179**

CHAPTER 4

Results

4.1 Moisture characteristics

Freezing method had a significant effect ($P<0.001$) on thaw/drip loss and water-holding capacity (WHC) but not on any cooking losses (Table 4.1 and 4.4). The ageing period influenced drip/ thaw loss and WHC but showed no effect on cooking losses (Table 4.2 and 4.5). Thaw/drip loss was lower for fresh than for frozen samples, irrespective of freezing methods. Generally, higher levels of thaw loss and more pressed out water (lower WHC) were recorded for 3-day samples compared to 14-day samples. The number of ageing days had a different effect on fresh steaks than on frozen steaks regarding thaw/drip loss and WHC (interaction $P<0.001$). Frozen steaks that were aged for 3-days recorded significantly more ($P<0.001$) thaw loss than those that were aged for 14-days, but there was no difference in drip loss between fresh samples aged for 3 and for 14-days (Figure. 4.1).

Table 4.1: Effects of freezing method on moisture characteristics before cooking

	Freezing method				
Traits	FR	QF	SF	SEM	*P*-Value
Thaw/drip loss %	1.6[a]	4.3[b]	4.4[b]	0.1134	**<0.001**
WHC	0.4	0.4	0.4	0.0045	0.141

FR = Fresh, QF =Quick-freezing, SF =Slow-freezing, SEM = Standard error of mean, *P-Value* = Probability value, WHC = water-holding capacity
*Means in the same row with the same letter do not differ significantly. *(P<0.05)*

Table 4.2: Effects of *post-mortem* ageing on moisture characteristics before cooking

	Post-mortem ageing			
Traits	Day 3	Day 14	SEM	*P*-Value
Thaw/drip loss %	4.3	2.6	0.1273	**<.001**
WHC %	0.4	0.4	0.0058	**0.005**

FR = Fresh, QF =Quick-freezing, SF =Slow-freezing, SEM = Standard error of mean, *P-Value* = Probability value, WHC = water-holding capacity

Table 4.3: Effects of freezing method and *post-mortem* ageing on moisture characteristics before cooking

Traits	DAYS	Freezing method x *post-mortem* ageing			SEM	*P*-Value
		FR	QF	SF		
Thaw/drip loss %	3	1.7[a]	5.6[c]	5.8[c]	0.1826	**<0.001**
	14	1.6[a]	3.1[b]	3.1[b]		
WHC %	3	0.33[a]	0.35[a]	0.37[ab]	0.00774	**0.005**
	14	0.38[b]	0.37[ab]	0.37[b]		

FR = Fresh, **QF** =Quick-freezing, **SF** =Slow-freezing, **SEM** = Standard error of mean, ***P*-Value** = Probability value, **WHC** = water holding capacity
*Means of the same characteristic with the same letter do not differ significantly. *(P<0.05)*

Fresh samples recorded less (*P=0.005*) pressed out moisture (higher WHC) after 14-days' ageing compared to samples aged for 3-days (interaction: *P<0.001*) (Figure 4.2). Differences were found between frozen samples aged for 3 or 14-days (interaction: *P<0.001*).
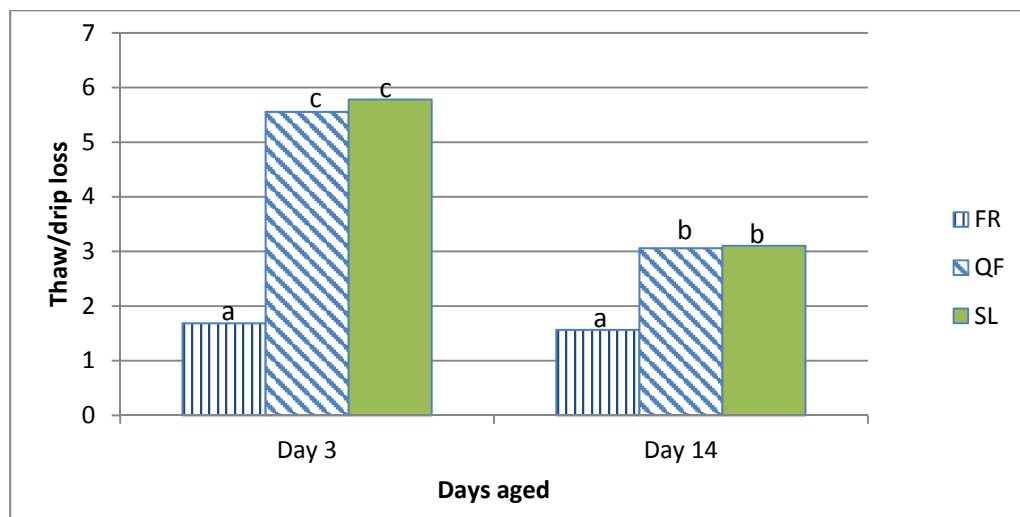
Figure 4.1: Interaction effects of freezing method and *post-mortem* ageing on thaw/drip loss

FR = Fresh, **QF** = Quick-freezing, **SF** = Slow-freezing
*Means in the same row with the same letter do not differ significantly. *(P<0.05)*
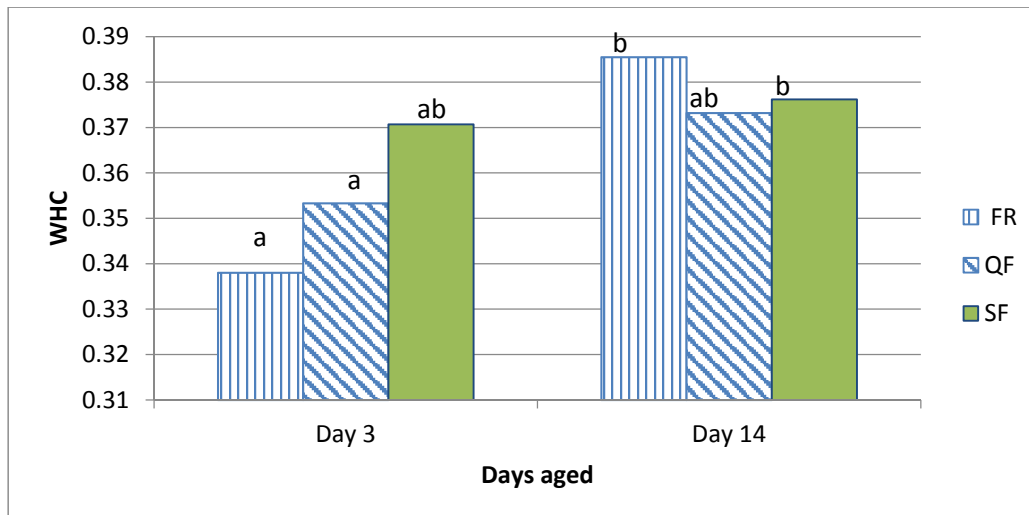
Figure 4.2: Interaction effects of freezing method and *post-mortem* ageing on WHC

WHC = Water holding capacity, **FR** = Fresh, **QF** = Quick-freezing, **SF** = Slow-freezing
*Means in the same row with the same letter do not differ significantly. *(P<0.05)*

Table 4.4 Effects of freezing method on moisture characteristics of cooked meat

	Freezing method				
Traits	FR	QF	SF	SEM	*P*-Value
Total cooking loss %	22.0	22.9	22.9	0.4130	0.218
Evaporation loss %	16.4	17.1	17.0	0.3550	0.333
Cooking drip loss %	5.7	5.9	6.0	0.3568	0.287

SEM =Standard error of mean, **P-Value** = Probability value

Table 4.5 Effects of *post-mortem* ageing on moisture characteristics of cooked meat

	Post-mortem ageing			
Traits	Day 3	Day 14	*SEM*	P-Value
Total cooking loss %	22.1	23.0	0.4020	0.125
Evaporation loss %	16.6	17.0	0.2980	0.390
Cooking drip loss %	5.6	6.0	0.3270	0.272

SEM =Standard error of mean, **P-Value** = Probability value

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Table 4.6 Interaction effects of freezing method and *post-mortem* ageing on moisture characteristics of cooked meat

Traits	Days	FR	QF	SL	SEM	*P*-Value
		Freezing method x *post-mortem* ageing				
Total cooking loss %	3	21.4	22.6	22.4	0.6240	0.912
	14	22.5	23.2	23.4		
Evaporation loss %	3	16.0	17.0	16.9	0.5070	0.822
	14	16.7	17.2	17.0		
Cooking drip loss %	3	5.5	5.6	5.5	0.3583	0.319
	14	5.8	6.0	6.3		

SEM = Standard error of mean, ***P*-Value** = Probability value

4.2 Colour characteristics

Most colour characteristics were significantly affected by the freezing method and by the number of *post-mortem* ageing days. Fresh samples recorded higher ($P<0.001$) values for redness (a*) and chroma, lower values ($P<0.05$) for hue (closer to typical red colour) and were brighter (higher L*) than any samples used for both freezing methods. Higher ($P<0.001$) OxyMb and lower MetMb were recorded for fresh vs frozen samples (Table 4.10). Samples aged for 3-days recorded lower ($P<0.05$) b* (less yellow) and lower hue-angle values than those aged for 14-days (Table 4.8). DeoxyMb and OxyMb were higher and MetMb lower ($P<0.05$) in 3-days samples compared to 14-days samples

Table 4.7: Effects of freezing method on instrumental colour characteristics

Traits	Freezing method				
	FR	QF	SF	SEM	*P*-Value
L *	37.2[c]	36.1[b]	35.7[a]	0.2110	**<0.001**
a *	15.3[b]	11.9[a]	12.2[a]	0.1530	**<0.001**
b *	13.6	13.2	13.1	0.3220	0.522
Chroma	20.7[b]	17.9[a]	18.0[a]	0.2760	**<0.001**
Hue angle	40.8[a]	47.7[b]	46.9[b]	0.7030	**<0.001**

FR = Fresh, **QF** = Quick freezing, **SF** = Slow freezing, **SEM** = Standard error of mean,
P-**Value,** Probability value, **L***= Lightness, + **a***= Red, + **b***= Yellow
*Means in the same row with the same letter do not differ significantly. (*P<0.05*)

Table 4.8: Effects of *post-mortem* ageing on instrumental colour characteristics

Traits	*Post-mortem* ageing		SEM	*P*-Value
	Day 3	Day 14		
L *	35.3	37.4	0.5670	**0.012**
a *	13.3	13.0	0.2447	0.328
b *	12.7	13.9	0.3520	**0.019**
Chroma	18.6	19.1	0.2820	0.180
Hue angle	43.3	47.0	0.9980	**0.011**

FR = Fresh, **QF** = Quick freezing, **SF** = Slow freezing, **SEM** = Standard error of mean,
P-**Value,** Probability value, **L***= Lightness, + **a***= Red, + **b***= Yellow

Table 4.9: Interaction effects of freezing method and *post-mortem* ageing on instrumental colour characteristics

Traits	Days	FR	QF	SF	SEM	*P*-Value
			Freezing method x *post-mortem* ageing			
L*	3	35.6[ab]	35.5[ab]	34.6[a]	0.618	**0.029**
	14	38.5[c]	36.7[b]	36.9[bc]		
a*	3	15.4	12.2	12.4	0.302	0.578
	14	15.2	11.6	12.0		
b*	3	12.9	12.7	12.5	0.572	0.844
	14	14.3	13.7	13.7		
Chroma	3	20.7	17.9	18.0	0.426	0.853
	14	21.0	18.0	18.3		
Hue angle	3	38.8	45.9	45.1	1.287	0.969
	14	42.8	49.5	48.7		

FR = Fresh, **QF** = Quick-freezing, **SF** = Slow-freezing, **SEM** = Standard error of mean, *P*-Value, Probability value, **L***= Lightness, + **a***= Red, + **b***= Yellow
*Means of the same characteristics with the same letter do not differ significantly. (*P<0.05*)

Table 4.10: Effects of freezing method on myoglobin redox reactions

Traits	FR	QF	SF	SEM	*P*-Value
	Freezing method				
MetMb	13.4[a]	34.3[c]	32.3[b]	0.8180	**<0.001**
DeoxyMb	22.2	20.8	22.2	0.7220	0.297
OxyMb	64.5[b]	44.9[a]	45.4[a]	0.8910	**<0.001**

FR= Fresh, **QF** = Quick-freezing, **SF** = Slow-freezing, **SEM** = Standard error of mean **MetMb** = metmyoglobin, **DeoxyMb** = deoxymyoglobin, **OxyMb** = oxymyoglobin
*Means in the same row with the same letter do not differ significantly. (*P<0.05*)

Table 4.11: Effects of *post-mortem* ageing on myoglobin redox reactions

	Post-mortem ageing			
Traits	**Day 3**	**Day 14**	**SEM**	*P*-Value
MetMb	23.4	29.9	0.9860	**<0.001**
DeoxyMb	23.4	20.1	0.6680	**0.001**
OxyMb	53.2	50.0	1.1180	0.047

FR= Fresh, **QF** = Quick-freezing, **SF** = Slow-freezing, **SEM** = Standard error of mean **MetMb** = metmyoglobin, **DeoxyMb** = deoxymyoglobin, **OxyMb** = oxymyoglobin

Table 4.12 Interaction effects of freezing method and *post-mortem* ageing on myoglobin redox reactions

	Freezing method x *post-mortem* ageing					
Traits	**Days**	**FR**	**QF**	**SF**	**SEM**	*P-Value*
MetMb	3	9.6	30.9	29.6	1.365	0.649
	14	17.1	37.6	35.0		
DeoxyMb	3	24.3	22.4	23.5	1.068	0.72
	14	20.1	19.3	21.0		
OxyMB	3	66.2	46.7	46.9	1.519	0.958
	14	62.8	43.1	44.0		

FR = Fresh, **QF** = Quick-freezing, **SF** = Slow-freezing, **SEM** = Standard error of mean
MetMb = metmyoglobin, **DeoxyMb** = deoxymyoglobin, **OxyMb** = oxymyoglobin

Ageing had no effect on colour characteristics irrespective of freezing method except for lightness (L*) where longer aged samples were generally brighter than shorter aged samples. The differences were more pronounced for fresh and slow-frozen samples than for quick-frozen samples (interaction: *P= 0.029*) (Figure 4.3).

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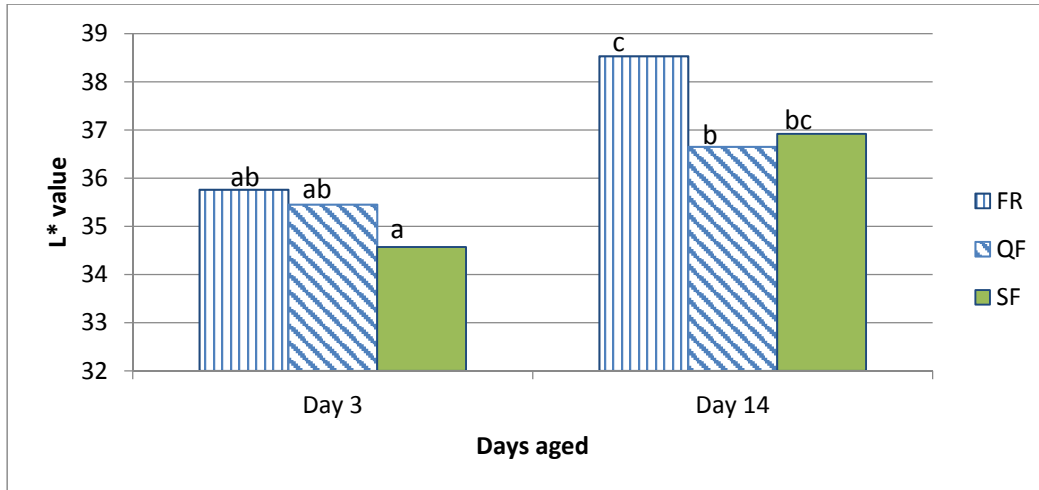
Figure 4.3: Interaction of freezing method and *post-mortem* ageing on L* values

WHC = Water holding capacity, **FR** = Fresh, **QF** = Quick-freezing, **SF** = Slow-freezing
*Means in the same row with the same letter do not differ significantly. (*P<0.05*)

4.3 Sensory characteristics and Warner Bratzler shear force (WBSF)

Freezing method had a significant effect (*P<0.001*) on WBSF, tenderness related sensory attributes and meat flavour (Table 4.13). Fresh samples were tougher than frozen samples according to WBSF and on sensory scores for first bite, overall tenderness, and residual connective tissue. In addition, quick-frozen samples were also significantly more tender (*P<0.001*) than slow-frozen samples according to all three tenderness related sensory attributes. Fresh samples scored lower *(P<0.05)* for flavour intensity than frozen samples did.

Samples aged for 14-days scored higher (*P<0.001*) for all tenderness-related sensory attributes as well as for aroma and flavour intensity (Table 4.14). WBSF-values confirmed that longer aged samples were more tender than those aged for 3-days (interaction: (*P<0.001*).

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Table 4.13: Effects of freezing method on sensory characteristics and Warner Bratzler shear force

| | Freezing method | | | | |
Traits	FR	SF	QF	SEM	*P*-Value
Aroma	5.4	5.4	5.4	0.0389	0.536
First Bite	5.0[a]	5.1[b]	5.3[c]	0.0623	**0.003**
Juicy %	5.0	5.0	5.0	0.0412	0.669
Tenderness	4.5[a]	5.0[b]	5.2[c]	0.0552	**<.001**
Residue %	4.5[a]	4.7[b]	4.9[c]	0.0561	**<.001**
Flavour	5.0	5.1	5.0	0.0370	**0.04**
WBSF	4.4[b]	3.5[a]	3.6[a]	0.0848	**<.001**

FR= Fresh, **SL** = Slow-freezing, **QF** = Quick -freezing, **WBSF** = Warner Bratzler Shear Force, **SEM** = Standard error of mean, ***P*-value** = Probability value
*Means in the same row with the same letter do not differ significantly. *(P<0.05*)

Table 4.14: Effects of *post-mortem* ageing on sensory characteristics and Warner Bratzler shear force

| | *Post-mortem* ageing | | | |
Traits	**Day 3**	**Day 14**	SEM	*P*-Value
Aroma	5.3	5.5	0.3810	**<.001**
First Bite	4.5	5.8	0.1233	**<.001**
Juicy %	5.0	5.0	0.0535	0.723
Tenderness	4.5	5.7	0.1258	**<.001**
Residue %	4.1	5.3	0.1197	**<.001**
Flavour	4.9	5.2	0.0432	**<.001**
WBSF	4.6	3.1	0.1416	**<.001**

FR= Fresh, **SL** = Slow-freezing, **QF** = Quick -freezing, **WBSF** = Warner Bratzler Shear Force, **SEM** = Standard error of mean, ***P*-value** = Probability value

Table 4.15 Interaction effects of freezing method and *post-mortem* ageing on sensory characteristics and Warner Bratzler shear force

Traits	Days	FR	SF	QF	SEM	*P*-Value
	Freezing method x *post-mortem* ageing					
Aroma	3	5.4	5.3	5.2	0.0589	0.391
	14	5.5	5.5	5.5		
First bite	3	4.3	4.6	4.6	0.1428	0.059
	14	5.7	5.6	6.0		
Juicy %	3	5.0	1.0	1.0	0.0716	0.723
	14	5.0	5.0	5.0		
Tenderness	3	4.3	4.5	4.6	0.1410	0.116
	14	5.6	5.6	5.9		
Residue %	3	3.9	4.2	4.3	0.1360	0.356
	14	5.2	5.2	5.5		
Flavour	3	4.7	5.0	4.9	0.0615	0.285
	14	5.1	5.2	5.2		
WBSF	3	5.2	4.2	4.5	0.1722	0.646
	14	3.6	2.7	2.8		

FR= Fresh, **SL** = Slow-freezing, **QF** = Quick-freezing, **WBSF** = Warner Bratzler Shear Force, **SEM** = Standard error of mean, *P*-value = Probability value

CHAPTER 5

Discussion

5.1 Treatment, ageing period, and interaction effects on moisture characteristics

The significant increase in thawing loss as a result of freezing demonstrated in the present study (Table 4.1, 4.3 and Figure 4.1) corresponds with the work of Muella *et al.*, (2010); Lagerstedt *et al.*, (2008) and Wheeler *et al.*, (1990). According to Benjakul, Vesessanguan, Thongkaew & Tanaka (2003), Bevilaqua, Zaritzky & Calvelo (1979), Rahelic & Puac (1980) and Wagner & Anon (1985), the disruption of the muscle-fibre structure by ice crystals and by protein denaturation leads to higher exudate although the latter is disputed by Ngapo *et al.* (1999). In the present study, both the slow-frozen and quick-frozen treatments recorded three times as much thawing loss after 3-days of ageing before freezing, and twice as much thawing loss when samples were aged 14-days before freezing, compared to the drip loss of the unfrozen samples of the respective ageing times *(P<0.001*, Figure 4.1). However, for both ageing regimes, freezing did not affect the amount of thaw drip which is in contrast to other studies that reported decreased thawing losses with increased freezing rates (Ngapo *et al.*, 1999; Sacks, Casey, Boshof & van Zyl, 1993; Li, Heaton & Marion, 1969; Deatherage & Hamm, 1960; Ramsbottom & Koonz, 1939).

Earlier and more recent studies by Grujić *et al.* (1993), Petrović *et al.* (1993) and Hiner *et al.* (1945), illustrated that ice-crystal formation during freezing, both intracellular and intercellular, damaged the sarcolemma and myofibrils, causing fibres to lose its water-retaining ability. They further illustrated that slow freezing caused higher drip loss due to larger crystals forming mainly outside muscle cells (intercellular), making the extracellular liquid more concentrated. Cells would lose water by osmosis which led to extensive dehydration and shrinking of the cells. Upon thawing, extracellular moisture was poorly re-absorbed. With quick-freezing methods, smaller intracellular crystals formed and water from these crystals were more readily re-absorbed upon thawing. Wagner & Anon (1985) reported that freezing also had a denaturing effect on myofibrillar protein, probably by the partial unfolding of the myosin head with accompanying exposure of hydrophobic groups. This effect was more pronounced

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with low freezing rates and with a consequently higher rate of water migration out of the myofibrillar space.

Anon & Calvelo (1980) defined freezing method and the amount of exudate in relation to time the sample takes to pass through a temperature range, from -1°C to -7°C; the so-called characteristic freezing time. This particular range was chosen as it defines the beginning of freezing of meat to the point where 80% of the meat is frozen. Anon & Calvelo (1980) recorded an increasing amount of exudate that peaked when the characteristic freezing time (t_{cf}) was between 16.5 and 19.5 minutes, and then decreased towards a constant value of up to a t_{cf} of 200 minutes. They also brought the earlier findings of Hiner *et al.* (1945) and others into context with t_{cf}. For t_{cf}'s shorter than 17 minutes, their studies mostly indicated the formation of small intracellular crystals that grew larger as the t_{cf} increased up to 17 minutes.

At t_{cf}'s longer than 30 minutes, the existence of large, mainly extracellular crystals were recorded. Since the formation of extracellular ice is different from that of intracellular ice, and since partial dehydration causes migration of sarcoplasmic liquid towards extracellular space, thawing losses will be affected accordingly. James, Nair & Bailey (1984) adjusted the t_{cf} range and reported no effect of freezing method for a t_{cf} between 2 and 70 minutes. Ngapo *et al.* (1999) recorded no differences in drip loss between unfrozen and frozen samples with t_{cf}'s between 12 – 120 minutes, while drip loss values for t_{cf} >900 minutes were significantly higher. Ngapo *et al.* (1999) noted that the size and nature of the sample would obviously affect the freezing gradient and the response to freezing, meaning that these times could not be declared absolute. Despite a large difference in t_{cf} between QF and SF samples of respectively 20 and 100 minutes in the present study, the lack of difference in drip loss (Table 4.1 and Figure 4.1) corresponds with the results of Ngapo *et al.* (1999).

Anon & Calvelo (1980) showed that unfrozen meat aged for six days had proportionally less exudate than unfrozen meat aged for 2-days, as a result of the greater absorption capacity of the former. They suggested that water-holding capacity of aged fresh meat will also impact reabsorption of moisture during thawing of frozen meat. Huff-Lonergan & Lonergan (2005) ascribed the reduction in exudate in aged meat to proteolysis of the intermediate filament proteins in early *post-mortem,* that reduce the amount of lateral shrinkage of the myofibrillar lattice and consequent minimizing of water flow from within the cell to drip channels. Increased water in drip channels when freezing commences, will invariably contribute to formation of ice crystals in the extracellular

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space, leading to potentially higher thawing loss. Farouk, Mustafa, Wu & Krsinic (2012) proposed a "sponge effect" hypothesis stating that structural changes both physically and chemically due to proteolyses disrupt the moisture loss channel and physically entrap free water, creating a sponge effect. Anon & Calvelo (1980) stated that that ageing time had virtually no influence on the difference in exudate amounts between frozen and unfrozen samples is, which disagrees with the present results.

Firstly, the present study did not record lower exudate in 14-day aged, unfrozen samples compared to 3 day aged samples, but in both cases the amount of exudate was low. Secondly, Figure 4.1 suggests that the effects of freezing on the amount of exudate was significant irrespective of ageing time, but the difference between the amount of exudate between frozen and unfrozen samples was smaller for longer aged samples (14-days) than for shorter aged samples (3-days). Therefore, ageing probably minimized the flow of water to drip channels (Huff-Lonergan & Lonergan, 2005) that showed the advantage in thawing loss for aged beef samples that were frozen afterwards. However, for some reason this was not clearly demonstrated in unfrozen beef samples. The amount of pressed out water (WHC) of aged meat was the same for frozen and unfrozen samples and tended to be higher than that of unfrozen samples aged for 3-days, as well as that of quick-frozen beef samples. This partially corresponds with the theory of Anon & Calvelo (1980), i.e. that the higher reabsorption of moisture in aged frozen and unfrozen meat is independent of ageing time.

In contrast to the results of the present study, Lagerstedt *et al.* (2008), Huff-Lonergan & Lonergan (2005) and Anon & Calvelo (1980), reported increased drip for unfrozen meat after 7-days of ageing as compared to samples aged for 2-days, but no difference was reported between 2 and 14-day aged samples. Freezing resulted in greater moisture loss compared to unfrozen samples in their study at 2 and 7days' ageing, but no significant difference in moisture loss was recorded at 14-days. This agrees with the saving effect of ageing on thawing loss.

Various studies reported increased cooking losses when meat was frozen and thawed (Shanks *et al.* 2002; Crouse & Koohmaraie, 1990; Hildrum *et al.*, 1999). However, various factors and conditions involved in the period of freezing seem to influence the effect on cooking losses. Wheeler *et al.* (1990a) reported higher cooking losses for frozen and thawed meat, irrespective of ageing time.

Smith, Carpenter & King (1969) noted that freezing temperature influenced the amount of cooking loss, since freezing at -34° increased cooking losses, but not at -23°C.

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Smith *et al.* (1968) found that freezing had no effect on cooking loss of aged lamb. Shanks *et al.* (2002) recorded higher cooking losses for frozen steaks compared to non-frozen steaks after up to 10-days of ageing before freezing. However, no differences after 10 to 35-days of ageing were noted.

Cooking losses for frozen steaks remained constant over the different ageing scenarios while those of unfrozen steaks increased as ageing times were increased. The authors speculated that the degradation of protein due to ageing caused higher cooking losses in aged meat and that this effect was outweighed by cellular damage as a result of freezing. In the present study no losses prior to cooking was recorded and hence the differences in cooking losses should be interpreted with caution.

The fact that the current study found no differences in cooking losses among ageing regimes, nor between unfrozen and frozen samples, may be the result of losses already recorded during chilling or freezing, which did not reveal any additional differences upon cooking as suggested by Shanks *et al.* (2002). In agreement, Vieira *et al.* (2009) also did not find differences in cooking losses between aged and unaged beef, whether frozen or unfrozen, while higher press (frozen and unfrozen) and thawing losses (no drip loss of unfrozen samples recorded) were reported for longer aged samples. They attributed differences in the response of parameters to the origins of liquid lost in each process.

Cooking losses originate from constitutive water, but also from fat that melts, so that the difference due to ageing time could be attenuated (King, Dikeman, Wheeler, Kastner & Koohmaraie, 2003). The losses due to thawing and pressing mainly come from constitutive water that is a result of the effects of ageing on muscle-structure integrity and hence its ability to hold water. Lagerstedt *et al.* (2008) recorded higher thaw losses, as well as cooking losses for frozen samples compared to unfrozen samples when both were aged for seven days.

5.2 The treatment, ageing days and interaction effect on colour characteristics

Colour is one of the most important quality traits considered by consumers when making purchasing choices (Mortensen *et al.*, 2006; Hugo & Roodt, 2007). The colour and colour shelf life of meat is determined by the relative amounts of the three major chemical forms of the colour pigment. Myoglobin and the balance between these myoglobin forms are influenced by various processes (Mancini & Hunt, 2002),

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including *post-mortem* ageing and freezing (Abdallah, Marchello & Ahmad, 1999; Farouk & Swan, 1998; Lanari & Zaritzky, 1991; Ledward, 1985; Offer *et al.*, 1989; Leygonie *et al.*, 2012; Renerre & Bonhomme, 1991). These authors have compared the degree of bloom as well as the ability of the meat to resist MetMb formation through oxidation, between frozen and unfrozen meat. They found a general deterioration in colour and colour stability as a result of freezing which agrees with the present results (Table 4.7 and 4.10).

The present study did not involve measurement of colour stability over time, but it was clear that all colour attributes, apart from b* (yellowness/greenness) were affected negatively by freezing, irrespective of the method of freezing. Anon & Cavelo (1980) reported that freezing could influence colour due to the leaching of Mb exudate of meat that was frozen and thawed. In addition, Calvelo (1981) also demonstrated that denaturation of the globin moiety of Mb takes place during freezing, storage and thawing, exposing Mb to autoxidation that contributes to a lack of colour stability. A third factor to take into consideration is the loss of MetMb-reducing activity (MRA) during processes like ageing and freezing.

Livingston & Brown (1981) proposed a theory that MRA, which is predominant in fresh meat, quickly reduces MetMb to DeoxyMb that is subsequently converted to OxyMb, thereby retaining the bright red colour during blooming. According to Abdallah *et al.* (1999) ageing and freezing reduce the activity of MRA, causing the accumulation of MetMb on the meat surface which was clearly demonstrated in the present study. MetMb increased as a result of freezing, as well as of ageing, lowering both OxyMb and DeoxyMb in aged beef samples. This change was associated with poor colour measured as lower chroma (less vivid) and higher hue angles (moving away from typical red colour) as a result of freezing. Interestingly, only L* (light reflection) and hue angles were affected by *post-mortem* ageing and subsequently a significant interaction between freezing method or status, and duration of ageing was recorded for L*.

Hue angle increased significantly (browning) due to ageing. While the effect of freezing on lightness, L, * was negative, the interaction between freezing and ageing (*P*=0.029) demonstrated a general increase in L* due to ageing. However, this was more pronounced for unfrozen, aged samples and relatively less pronounced for frozen

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samples that were aged, in particular the quick-frozen samples. The only explanation for this phenomenon is that the unfrozen sample recorded lower drip loss and that more surface water resulted in higher reflectance, in particular for aged samples. Kim, Liesse, Kemp & Balan (2015) also reported higher L* values as well as redness and chroma for aged, unfrozen loin steaks compared to aged/frozen/thawed samples, which is similar to the present results. Furthermore, Farouk, Wiklund, Stuart & Dobbie (2009), Kim, Frandsen & Rosenvold (2011) and Kim *et al.* (2015) reported that aged/frozen/thawed steaks presented better colour results than directly frozen steaks, while the current study did not show any effects of ageing/freezing/thawing combinations, except for the higher L* value of SF-aged samples. This was significantly higher for aged beef samples, while QF samples recorded a numerical difference of two units.

Kim *et al* (2015) speculated that aged/frozen meat might maintain higher Mb redox stability and maintain a lower oxygen consumption, due to lower oxygen-consuming mitochondrial enzyme activities compared to aged and unfrozen steaks or directly frozen ones. However, present study showed consistently higher MetMb for all aged samples (frozen and unfrozen). This study did not record any effect of freezing method or interaction between freezing method and *post-mortem* ageing prior to freezing on colour attributes, which agrees with the results of Kim *et al.* (2015). The latter suggested that freezing method *per se* would neither impact on colour, nor on colour stability of frozen/thawed steaks. They reasoned that the meat surface region is relatively less influenced by freezing method compared to the centre portion of meat, i.e. mostly intracellular small ice crystal formation takes place on the surface, while physical cell damage and chemical changes in proteins in deeper sections are different for different freezing methods. In agreement with the present results, Kim *et al.* (2015), Farouk *et al.* (2003) reported no effect of freezing method on browning of meat (hue angle), although high values for lightness (L*) were recorded for SF and thawed beef compared to QF and thawed beef, which is in contrast with the present results. They attributed the difference to higher amounts of thaw drip in SF samples, leading to greater light reflection, while freezing method did not affect the amount of thaw drip in the present results. This explains the lack of difference in lightness.

5.3 Treatment, ageing, and interaction effect on Warner Bratzler shear force and sensory characteristics

There are conflicting reports in the literature regarding the effects of freezing on tenderness and it appears that the nature of freezing protocol determines its outcomes. Factors such as ageing before or after freezing, temperature and method of freezing, storage temperature and duration of storage, all seem to contribute to variation in the effect of freezing/thawing on tenderness. There has always been consensus in the literature that tenderness is improved by *post-mortem* ageing (Dransfield, Etherington & Taylor, 1992; Koohmaraie, 1996), mainly through the action of proteolytic enzymes (Taylor, Geesink, Thompson, Koohmaraie & Goll, 1995). Earlier studies of Law, Yang, Mullins & Fielder (1967) and Smith, Spaeth, Carpenter, King & Hoke (1968) reported positive effects of freezing on beef and lamb, respectively, but other studies showed detrimental effects in beef (Pearson & Miller, 1950) and lamb (Smith *et al.*, 1968).

Interestingly, Smith *et al.* (1968) found positive results with freezing rib chops of lamb but the opposite with loin chops and leg roasts, and attributed the contrasting effects to differences in anatomical position of cuts, time in frozen storage, temperatures employed in freezing and differences in cooking temperatures of different cuts. This emphasises the effect of protocol details on the outcomes of freezing effects. In this regard, Smith, Carpenter & King (1969) reported no effects of freezing on beef stored frozen for 3 to 6 weeks, but freezing resulted in lower WBSF-values when beef was stored for 4 months. In the present study all samples were stored for less than 4 weeks. Shanks *et al.* (2002) reported consistently lower WBSF-values for frozen meat compared with fresh meat, irrespective of ageing time (1 to 35 days), which agree with the present results. Lagerstedt *et al.* (2008) reported similar results for beef loin aged for 2 and 7-days, but with prolonged ageing (14-days), freezing negated the positive effect of ageing, which also agrees with the results of Vieira *et al.* (2009). Wheeler *et al.* (1990a) found no difference in WBSF or in sensory tenderness when loin steaks were either tested fresh after 13-days' ageing or frozen and thawed after 14-days' ageing. Farouk *et al.* (2003) recorded positive effects of freezing on WBSF, irrespective of freezing method, which agrees with the present results regarding WBSF, but not of sensory tenderness. The studies of Hiner *et al.* (1945) and Petrovic

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et al. (1993) reported positive effects on shear force values and sensory scores for fast frozen/thawed beef compared to slow-frozen counterparts. Paul & Child (1937) and Hergenreder *et al.* (2013) could not find any effect of freezing rate on tenderness.

Kim *et al.* (2015) attribute the discrepancies among studies to differences in freezing methods and also actual freezing rates. Hiner *et al.* (1945) and Petrovic *et al.* (1993) applied freezing at -40°C, while the targeted freezing temperature in the studies of Hergenreder *et al.* (2013) and Paul & Child (1937) ranged between -18 and -28°C. Kim *et al.* (2015) suggested that high freezing rates produced smaller intracellular ice crystals, causing less protein denaturation and therefore higher solubility of myofibrilar protein as well as more tender meat compared to lower freezing rates.

Sensory tenderness measured as "first bite", "overall tenderness" and "residual connective tissue" followed the same pattern as WBSF in the present study. Despite significant effects of ageing and freezing on tenderness, numerical differences among freezing methods were low; between 0.1 and 0.3 units. In contrast to our findings, Lagersteadt *et al.* (2008) reported lower WBSF values for frozen, compared to unfrozen loin steaks; although a trained sensory panel scored frozen steaks lower than unfrozen steaks for tenderness.

The way in which the structural effects of freezing impact on the two measuring tools was given as a reason for this discrepancy. Higher drip loss due to freezing/thawing would have led to less water to hydrate muscle fibres and consequently more fibres per surface area would increase the toughness as perceived by the taste panellist. In contrast, structural damage would have contributed to loss of membrane strength, resulting in less force needed to shear through the fibres (Li *et al.* 2012). However, it can be reasoned that loss of membrane strength would also lead to higher tenderness scores. Higher drip loss associated with freezing/thawing may also lead to lower scores for juiciness that indirectly influenced the perception of tenderness negatively, as reported by Lagerstedt *et al.* (2008). However, in the present study, scores for juiciness were neither affected by freezing or ageing, despite the effect of freezing on drip loss.

The current study can conclude that the balance between the structural damage, the amount of water loss, and increased dryness, probably explains the differences among the different studies in this regard.

Vieira *et al.* (2009) and Leygonie *et al.* (2012) suggested that the mechanism involved in improved tenderness caused by freezing/thawing is a combination of increased enzymatic action during proteolysis, and loss of structural integrity by formation of ice crystals of different sizes.

The rate of freezing is one of the factors that seemingly determines which of the two mechanisms will play the dominant role. Formation of small, mainly intracellular ice crystals during fast freezing increases the rate of ageing through the release of protease enzymes, as proposed by Shanks *et al.* (2002) and Vieira *et al.* (2009) and supported by Dransfield, (1986). Likewise, the formation of large ice crystals, mainly in the extracellular space during slow freezing, disrupts the structural integrity and produces more tender meat (Leygonie *et al.*, 2012)

In this study, the freezing method had no effect on WBSF, but slow freezing recorded slightly, though notably lower scores for all three attributes of sensory tenderness, compared to fast freezing, irrespective of ageing. Although not verified by measurement, we would expect that small ice crystals dominated the frozen structure of fast frozen samples in the present study, while larger extracellular crystals formed in the slow-frozen samples. As both mechanisms will theoretically contribute to increased tenderness, it is difficult to determine the reasons for difference in tenderness.

The present study found no difference in drip loss between the two used freezing treatments. This was supported by the results of Ngapo *et al.* (1999) who recorded no effect of freezing method on water binding if the characteristic freezing time was less than 120 min. Apparently, some mechanism gave a slight advantage to faster frozen beef samples in the present study. Kim *et al.* (2015) disputed the theory by Shanks *et al.* (2002), Vieira *et al.* (2009) and Dransfield (1986) that freezing increases the rate of ageing by releasing higher levels of proteolytic enzymes. Kim *et al.* (2015) found no effect of freezing method on shear force tenderness, irrespective of ageing.

In addition, qualitative Western blot analyses of desmin showed no effect of freezing rate on the extent of desmin degradation. Huff-Lonergan, Mitsuhashi, Beekman, Parrish, Olson & Robson (1996) reported a high relationship between desmin degradation and tenderness development. Kim, Luc & Rosenveld (2013) and Kim *et al.* (2015) also reported that ageing followed by freezing may show better results for WBSF than ageing alone (4 weeks and 9 weeks, respectively), even when frozen steaks were aged for shorter durations (2 and 3 weeks, respectively). However, they

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also agreed that meat frozen shortly after rigor mortis (1-day *post-mortem*) did not reach the same level of tenderness as unfrozen meat aged for longer durations (3 to 4-weeks). Despite the lower WBSF of samples aged for 3-weeks, followed by freezing and thawing, compared to those aged for 4-weeks, no differences in desmin degradation was found (Kim *et al.* 2015). This further supports the argument that improved tenderness due to freezing was probably caused by loss of structural integrity induced by ice crystal formation.

It was further argued that ageing followed by freezing will facilitate the formation of intracellular ice crystals between degraded myofibrils. These induce greater muscle-fibre fragmentation and structural weakening during thawing and therefore more tender meat compared to meat that was aged only. Although our design was not comparable with this study in all terms, the present results showed that the improvement in tenderness due to freezing was of the same ratio for samples frozen directly and those aged for longer.

The difference in sensory tenderness between the two freezing methods in the present study should also be viewed in context as mean scores for tenderness indicated that both treatments were scored as "slightly tender" at 3-days' ageing and "fairly tender" at 14-days' ageing. Therefore, the differences between the two treatments are probably of no commercial significance. Even the differences in sensory scores for different attributes between fresh and frozen samples were small and agree with the work of Damen & Steenbekkers (2007) that most consumers cannot distinguish between fresh and frozen/thawed meat. The present study also found no differences for other sensory attributes among freezing methods, except for flavour that recorded 0.1higher score for slow-frozen samples compared to unfrozen and quick-frozen samples. This is probably of no significance.

CHAPTER 6

Conclusion

In summary

The quality of frozen foods is closely related to the size and distribution of ice crystals within muscle tissues of meat. Large ice crystals which occur within the muscle tissues due to slow freezing could result in mechanical damage, drip loss and thus reduction in product quality. The effect of freezing, freezing method and *post-mortem* ageing have been studied in detail and the interaction effects on meat colour, moisture and sensory characteristics are well presented in the present study.

In conclusion:

Both slow-frozen and quick-frozen treatments recorded three times as much thawing loss after 3-days of ageing before freezing, and twice as much thawing loss when samples were aged 14-days before freezing, compared to the drip loss of the unfrozen samples of the respective ageing times. However, the ageing period did not affect moisture losses. These results support previous results that freezing both in domestic (slow) or industrial (fast) freezers will result in loss of yield due to thaw drip when compared with unfrozen meat. The type of freezer used in the present study did not have an effect on the amount of thaw drip. It seems that no additional loss as a result of freezing will be incurred during cooking.

Although colour stability over time on display was not evaluated in the present study, but clearly showed that freezing method contribute to MetMb formation resulting in colour defects soon after thawing has been completed. Colour measured as chroma (vividness) or hue angle (true red) deteriorated irrespective of freezing method probably due to the fact that colour development takes place on or close to the surface of the meat. Hence colour will not affected much by the method of freezing, since freezing on the surface probably takes place at the same rate.

Aged samples showed more browning than fresh samples, but there was no interaction between freezing methods and *post-mortem* ageing of meat. Freezing method improved tenderness measured as Warner Bratzler shear force or sensory tenderness and the results suggest that QF may have additional benefits in this regard. However, the differences in sensory scores were very small, even between unfrozen and frozen beef samples, and it is uncertain if consumers will notice the differences.

In conclusion, frozen storage of case ready meat (steaks) by the processing and retail industry to extend shelf life of meat has its challenges as far as loss of moisture during thawing and colour deterioration are concerned, although tenderness may be improved. Further challenges include the control of duration and temperatures of storage as these may affect the quality of frozen meat (not tested in this study). In addition, consumers need to be informed about the benefits of buying and storing, and the convenience of having frozen meat ready for use. The present results also suggest that slower freezing under domestic conditions can, within certain limits (e.g. time for core temperature to decline from -1 to -7°C), produce steaks of equally comparable quality to those frozen under certain commercial conditions (blast-freezing).

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