Effects of freezing on physicochemical properties of Wagyu and crossbred beef

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Abstract

This study investigates and compares the effects of different freezing rates (slow and fast freezing) on the meat quality parameters of Wagyu and crossbred beef. To study the effects on the meat quality of Wagyu and crossbred beef, different treatments were set up, and 60 beef rump samples were used in total. The samples were randomly assigned to three different regimens named FR (fresh, never frozen), FF (fast frozen), and SF (slow frozen), followed by thawing of the FF and SF samples at 4 °C for 24 hours. A compositional analysis was performed to compare the moisture and fat levels of both breed meat samples. The impact of moisture and fat levels on the freezing rate was then analyzed by mathematically modeling the freezing kinetics of Wagyu and crossbred beef. This analysis aimed to highlight the primary differences in thermal behavior between the two breeds. The effects of different regimens on meat quality were also evaluated using various meat quality measurements such as pH, color, tenderness, and water holding capacity. Both the uncooked and cooked meat samples of Wagyu and crossbred beef were used to compare various meat quality attributes. Other analyses used to indicate quality differences between raw meat samples were light and transmission electron microscopy (TEM).

The experiment data shows that Wagyu beef generally outperforms crossbred beef in terms of tenderness (warner- bratzler shear force, WBSF), lower thaw, and cook losses. In addition, Wagyu beef consistently exhibited superior color characteristics, with higher values for lightness, redness, yellowness, chroma, and hue angle

compared to crossbred beef. The freezing rate and breed significantly affected the moisture and fat content ($p < 0.0001$). Wagyu beef exhibited higher fat and low moisture content compared to crossbred beef. The freezing kinetics indicated that crossbred beef cools faster compared to Wagyu beef. During the pre-cooling phase of fast freezing, despite the rate constant ($k = 1$) being the same in both breeds, the cooling process for crossbred beef was faster, with a larger pre-exponential factor of 10.017 against 9.523. Additionally, during the subcooling phase of slow freezing, crossbred beef cools slightly faster than Wagyu beef, as evident by the larger value of the rate constant (0.69 versus 0.646) and the pre-exponential factor (17.245 versus 16.15).

Regarding the meat quality analysis, Wagyu beef had a higher pH compared to crossbred beef for both uncooked and cooked samples. Freezing rate (slow or fast freezing) did not significantly affect pH ($p > 0.05$), but there was a statistically significant ($p < 0.05$) interaction between freezing rate and breed for both uncooked and cooked samples. Wagyu beef exhibited lower warner-bratzler shear force (WBSF) values for cooked samples, indicating greater tenderness compared to crossbred beef. Both freezing rate and breed type significantly affected warner-bratzler shear force (WBSF) ($p < 0.05$), with slow-frozen samples being the most tender. Crossbred beef (both fast and slow frozen samples) had a higher thaw loss ($p < 0.05$) than its Wagyu counterpart. Slow-frozen samples of both breeds exhibited greater thaw loss than fast-frozen samples ($p < 0.05$). Fast-frozen samples (both breeds) showed the least cook loss, while slow-frozen crossbred samples had the highest cook loss, indicating the freezing rate significantly affected cook loss ($p < 0.05$). Breed type did not significantly affect cook loss ($p > 0.05$), but there was a statistically significant interaction between freezing rate and breed ($p < 0.05$). Light microscopy (LM) and transmission electron microscopy (TEM) images of uncooked samples revealed that Wagyu samples, both slow and fast frozen, maintained their structure more effectively than crossbred samples under each regimen, as evidenced by their lower WBSF, thaw loss, and cook loss values.

The difference between uncooked and cooked meat samples related to moisture retention, tenderness, and structural changes was also observed. Uncooked samples, particularly Wagyu, had higher fat and lower moisture, with thaw loss more pronounced in slow-frozen crossbred beef. Cooking reduced moisture further, with fast-frozen samples showing less cook loss. In terms of tenderness, uncooked Wagyu beef was naturally more tender than crossbred beef, but this difference became more pronounced after cooking, with slow-frozen samples of both breeds being more tender due to the structural breakdown of muscle fibers during freezing and cooking. Color differences, with Wagyu showing superior lightness and redness, persisted after cooking, maintaining better visual quality.

Significant advantages were identified for the meat processing industry by the results of the Wagyu and crossbred beef quality experiments. Overall, these findings suggest that Wagyu beef, with its superior fat content and tenderness, offers distinct quality advantages over crossbred beef. The findings also suggest that fast freezing (FF) should be preferred method for both Wagyu and crossbred beef to minimize moisture loss during thawing and cooking. If tenderness is the primary concern, slow freezing (SF) method may be considered, particularly in case of Wagyu beef. However, the increase in thaw and cook loss must be taken into account.

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List of abbreviations

a*: Redness

AFFCO: Allied farmers farmers' cooperative organization

AMSA: American meat science association

ANOVA: Analysis of variance

AOAC: Association of official analytical chemists

b*: Yellowness

BM: Breast muscle

CB: Crossbred beef

CF: Cryogenic freezing

DeoxyMb: Deoxy myoglobin

DFD: Dark, firm, and dry

EPR: Enhanced preservation rate

FAO: Food and agriculture organization

FF: Fast freezing / fast frozen

FR: Fresh

IMF: Intramuscular fat

L*: Brightness

LM: Light microscopy

LM: Leg muscle

MetMb: Metmyoglobin

MYD: Myofibril width

MFD: Muscle fiber diameter

MPI: Ministry for primary industries (New Zealand)

N/A: Not applicable

NCT: Natural convection thawing

OxyMb: Oxymyoglobin

PSE: Pale, soft, and exudative

QC: Qinchuan

RF: Rapid freezing

RO: Reverse osmosis

RT: Running water thawing

SEM: Standard error of the mean

SF: Slow freezing / slow frozen

SL: Sarcomere length

TA.XT: Texture analyzer from stable microsystems

TEM: Transmission electron microscopy

WHC: Water holding capacity

WBSF: Warner-bratzler shear force

WU: Wagyu

1. Introduction

The quality of meat is a pivotal factor influencing consumer preferences and market value. High-quality meat is characterized by its favorable attributes such as taste, tenderness, juiciness, and appearance, all of which contribute to overall consumer satisfaction. Among the various types of beef available, Wagyu and crossbred beef hold prominent positions due to their distinctive qualities.

Wagyu beef, originating from Japan, is highly prized for its exceptional marbling, which results in a rich flavor, superior tenderness, and a buttery texture. The unique genetics of Wagyu cattle lead to intramuscular fat deposition that is unparalleled by other breeds. This high degree of marbling enhances the sensory attributes of the meat, making it a luxury item in the global market (Matsuishi et al., 2001; Motoyama et al., 2016).

In contrast, crossbred beef represents a combination of traits from different cattle breeds, aiming to balance quality and production efficiency. Crossbreeding strategies are often employed to improve specific characteristics such as growth rate, feed efficiency, and overall meat quality. While crossbred beef may not achieve the same marbling as Wagyu, it can offer a cost-effective alternative with satisfactory quality attributes, including good tenderness and flavor (NZ Beef & Lamb, 2021).

The storage conditions of meat, particularly the methods of freezing, play a crucial role in maintaining or altering its quality attributes. Fresh storage, where meat is kept refrigerated without freezing, is generally preferred for short-term preservation to maintain optimal quality. However, freezing is a widely used method to extend the shelf life of meat, allowing for longer-term storage and distribution (Leygonie et al., 2012).

Freezing methods can be broadly divided into two categories: fast freezing and slow freezing. Fast freezing involves rapidly lowering the temperature of the meat, typically through methods such as blast freezing or cryogenic freezing. This process aims to minimize the formation of large ice crystals that can damage muscle fibers and degrade meat quality. On the other hand, slow freezing occurs at a more gradual pace, often leading to larger ice crystals and potential negative impacts on meat texture and overall quality (Leygonie et al., 2012).

Given the significant impact of freezing methods on meat quality, it is essential to understand how these storage conditions affect meat composition especially, moisture and fat content, and various other quality parameters, including pH, color, tenderness, water holding capacity (WHC), and structural integrity. pH is a critical factor influencing meat's biochemical properties and overall stability. Color is a primary quality attribute affecting consumer perception and acceptability. Tenderness is a key sensory attribute determining the meat's palatability. WHC affects the juiciness and yield of the meat, while structural integrity, examined through microscopy and transmission electron microscopy (TEM), provides insights into the microscopic changes occurring in the meat tissues during storage (Kim et al., 2015).

This study aims to investigate and compare the effects of different freezing rates (fast frozen, and slow frozen) on these meat quality parameters in New Zealand raised Wagyu and crossbred beef. By integrating quality parameter analysis with microstructural examination using advanced microscopy techniques, this research seeks to provide a comprehensive understanding of how storage conditions influence meat quality in these two types of beef, offering valuable insights for the meat industry to optimize storage practices and enhance meat quality preservation.

Furthermore, extensive research has been done on Japan's Wagyu beef, highlighting its superior meat quality attributes (Matsuishi et al., 2001; Motoyama et al., 2016). On the other hand, there has been very little investigation into the quality characteristics of Wagyu beef produced in New Zealand. Given that crossbred beef is already well-established and popular in New Zealand, the objective of this study is to address the knowledge gap by comparing the quality of New Zealand Wagyu with crossbred beef. New Zealand is renowned for its pasture-based livestock farming as compared to grain-based in other countries, which significantly influences the fatty acid profile and sensory characteristics of beef (Pethick et al., 2004). By identifying potential quality advantages of New Zealand Wagyu, the results of this research can provide the meat industry with significant benefits, thereby assisting producers in the development of marketing strategies and product differentiation.

2. Literature review

2.1 Meat muscle structure

The nature and definition of meat quality will become clear when we have a solid understanding of the basic concepts pertaining to muscle structure (Norstrom, 2011). According to Norstrom (2011), the composition of muscle tissue is 75 % water, 17–18 % protein, 5 % fats, 1 % carbs, and 1 % glycogen, vitamins, and minerals. Meat structure can be summed up as a network of parallel fibers, or myofibrillar structure (Palka & Daun, 1999). Each fiber consists of a single cell attached to connective tissue (Huff-Lonergan & Lonergan, 2005). Bundles of muscle fibers are interconnected, with each fiber being made up of bundles of myofibrils and encircled by the sarcolemma, a type of plasma membrane (Huff-Lonergan & Lonergan, 2005). Each muscle fiber has an endomysium, which is a narrow layer of connective tissue covering the sarcolemma (Bailey & Light, 1989). Nevertheless, the regular striations in muscle that are only visible under a microscope are the result of specialized contractile organelles that are located within the muscle cell's myofibril. According to Huff-Lonergan & Lonergan (2005), these striations originate from less dense l-bands and dense protein A-bands. According to Norström (2011), I-bands are composed of overlapping thin filaments, whereas A-bands are composed of thick filaments. The distance between two Z-discs is referred to as a sarcomere, and the l-bands are divided by a dark line known as the Z-disc or Z-line (Huxley & Hanson, 1954). Sarcomeres are thought to be the main structural and functional units that are directly in charge of muscle contraction (Lawrie & Ledward, 2006). Actin and myosin, two filamentary proteins that slide over one another when a sarcomere contracts, are what make up sarcomeres, as Figure 1 shows (Filgueras, Gatellier, Aubry, Thomas, Bauchart, Durand, Zambiazi, & Sante-Lhoutellier, 2010).

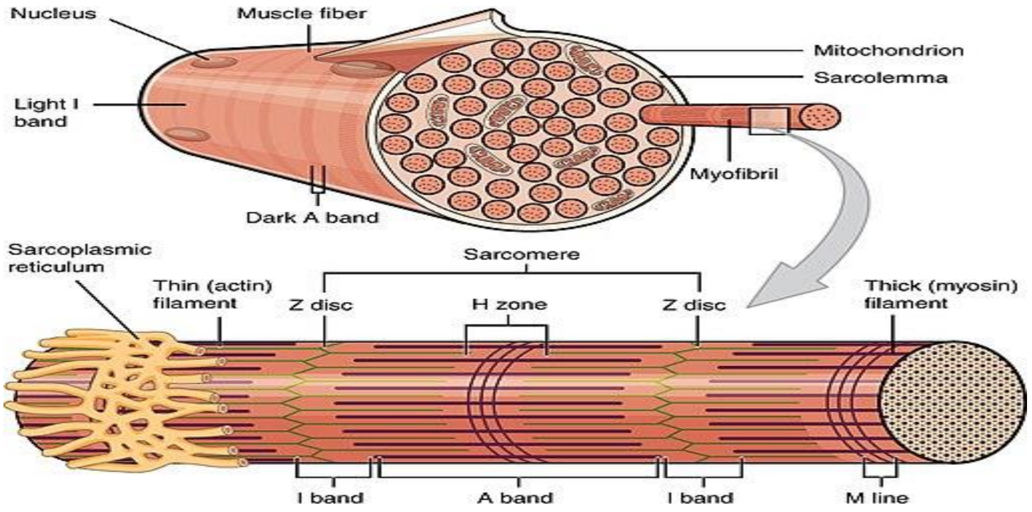


Figure 1: The detailed structure of a muscle, exhibiting myofibrils and myofilaments (Openstax anatomy and Physiology, 2016)

The head and tail make up myosin (Figure 2). The tail upholds the thick filament, while the thin filament is generated by the head extending from the thick filament and interacting with actin (Moss, Diffee, & Greaser, 1995).

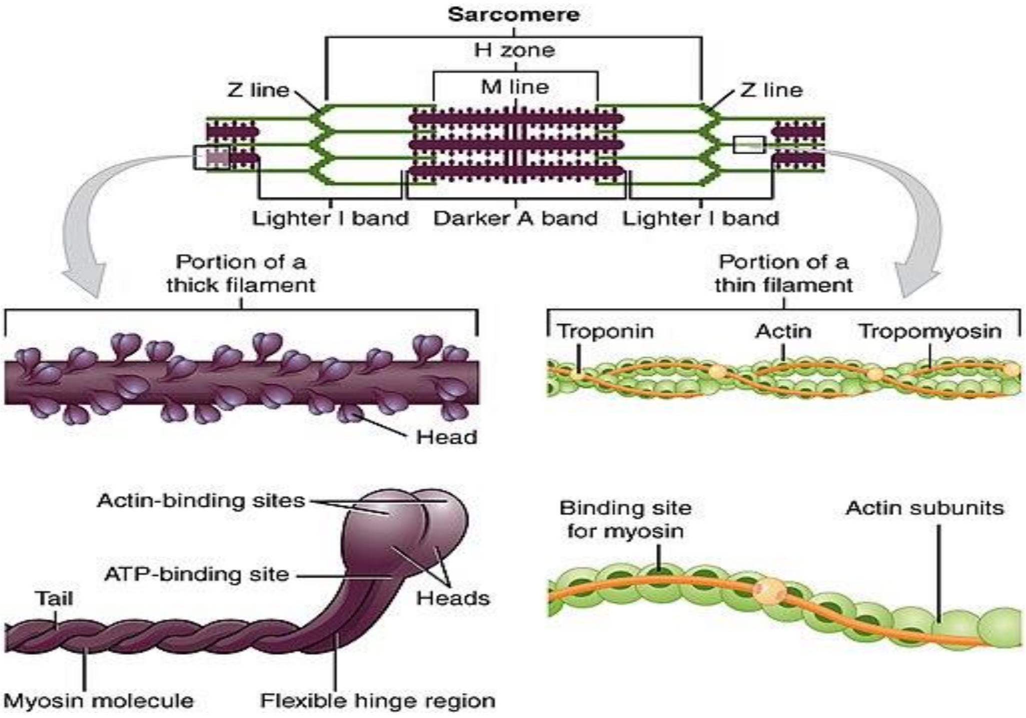


Figure 2: Myosin's head and tail diagram (Openstax anatomy and physiology, 2016)

2.2 Freezing and thawing

The meat industry often employs freezing to preserve the freshness of meat for an extended period of time. To a certain extent, this process reduces microbiological and chemical reactions. Unfavorable changes to the texture (fat may become grainy and disintegrate) and organoleptic qualities can result from freezing, regardless of the benefits of eliminating or preventing the growth of microorganisms (Obuz & Dikeman, 2003). The majority of manufacturers hold the belief that any form of freezing diminishes the quality of meat (Boles & Swan, 1996), regardless of the fact that the quality of frozen meat is predominantly influenced by the rate of freezing and the shape and size of the ice crystals that develop (Ballin & Lametsch, 2008). Different ice crystal shapes are thought to damage tissue and lower the quality of meat (Brewer & Harbers, 1991). Slow conventional freezing, for instance, results in comparatively large and irregularly shaped ice crystals, which increase damage to the meat’s structure (Devine, Bell, Lovatt, Chrystall, & Jeremiah, 1996). Most of the tissue damage is likely caused by larger, mostly intracellular ice crystal formation (Zheng & Sun, 2009; Ming, Rahim, Wan & Ariff, 2009). This results in a decrease in the tissue's ability to retain water and elasticity (Bhatnagar, Bogner, & Pikal, 2007).

Furthermore, biochemical reactions that occur at the cellular level are altered, and the physical quality parameters of the meat are influenced by the concentration and damage of the solutes by larger ice crystals (Leygonie, Britz, & Hoffman, 2012). As soon as the water in the meat freezes, the percentage of the remaining solutes, comprising proteins, carbohydrates, lipids, vitamins, and minerals, increases, disrupting the homeostasis of the intricate meat system (Lawrie, 1998). According to a study conducted by Stuby, Lamkey, and Dolezal (1993), rapid freezing results in the formation of smaller ice crystals, which in turn leads to a consistent spacing between cells. As a result of less tissue damage, the product's quality is better preserved when smaller ice crystals form and are evenly distributed both inside and outside of the cells (Sun & Zheng, 2006). The risk of quality deterioration is typically elevated in a time-temperature environment that varies, such as cycles of freezing and thawing during storage (Fu & Labuza, 1992). Lipid oxidation and protein degradation are additional processes that can lead to quality degradation because of freezing (Zhang, Farouk, Young, Wieliczko, & Podmore, 2005). In the literature, there are conflicting reports regarding the effect of freezing on the organic and technological quality of meat (Zhang et al., 2005; Farouk, Wieliczko, & Merts, 2003).

According to Ngapo, Babare, Raynolds, and Mawson (1999), freezing causes a number of physio-chemical alterations in meat that may deteriorate organic quality. Gambuteanu, Borda, and Alexe (2013) suggested that it is important to analyze all modifications that meat undergoes prior to thawing when examining the impact of freezing on meat quality. Thus, it is necessary to consider freezing rates, freezing meat after a specific age, and storage conditions (temperature fluctuations) during the freezing process. Beef quality is impacted by frozen storage conditions (Shanks, Wulf & Maddock, 2002). Research has shown that frozen meat quality gradually deteriorates while it is stored (Ngapo et al., 1999; Wheeler, Miller, Savell & Cross, 1990). In addition, fluctuations in refrigeration temperatures significantly reduce the duration of product storage (Akhtar, Khan & Faiz, 2013).

The quality of frozen foods is also significantly impacted by the actual storage temperature. The frozen food value is expected to drop with any temperature increase above the intended storage temperature (Singh & Heldman, 2001). Several sensory attributes of frozen beef were not affected by storage temperature, as indicated by Farouk et al. (2003). In addition, thawing has received far less attention in the literature than freezing or chilling. Nonetheless, thawing frozen materials is crucial for food processing. In order to minimize microbial growth, chemical deterioration, and excessive water loss, thawing time should be minimized (Taher & Farid, 2001). Meat's water content is mostly affected by freezing and thawing. Freezing happens faster than thawing. The temperature increases rapidly during the thawing process, reaching the freezing point, and then continues to rise. Large ice crystals may have the chance to recrystallize—form into new, even larger ice crystals—if the thawing process proceeds too slowly (James et al., 2002).

The literature lists a number of thawing conditions, the most popular being thawing under refrigeration. Other methods include immersion in water, convection heating, microwave radiation, infrared radiation, radio frequency heating, applying pressure, and cooking to thaw (Eastridge & Bowker, 2011). For research reasons, the American Beef Science Association recommends thawing beef in the refrigerator at a temperature between 2 and 5 °C till the meat's internal temperature approaches that specific range. Thawing is typically described as a broad timeframe process, such as overnight or 18 to 24 hours. It is accepted that the process of thawing can be influenced by factors such as the number of items being thawed, the dimension and type of refrigerator door openings, the surrounding temperature, and the circulation of air. According to Eastridge et al. (2011), beef steaks can be quickly thawed in a water bath in accordance with food safety regulations without compromising the quality of the meat.

2.2.1 Food freezing process and freezing diagrams

Food freezing has several steps, and it is a very complicated process. The temperature-time diagrams are used to describe the freezing diagrams during food freezing. When freezing water is done, it occurs in three different stages. The first phase is called the sensible or pre-cooling phase, in which the water cools down to its freezing point. After the water has reached its freezing point, supercooling occurs, which lowers the temperature of the water below its freezing point, but icing does not take place (Miyawaki et al., 1989). The process is followed by a second phase, which is called the phase-changing phase, during which ice crystal formation takes place and ice crystals co-exist with water. During this phase, the temperature of the water remains almost constant. The next phase is called the subcooling phase, where the temperature starts to drop according to the external environment (Pham, 2014). Freezing food is quite similar to freezing water, as most food materials consist of 60–80% water content, but food freezing becomes more complicated due to the presence of different compositions in the food. The food materials consist of various water solutes, such as minerals, salts and fat, which also depress the freezing point of food (Heldman, 1974b). When freezing of pure water is done, there is a sharp transition between the three phases of freezing, whereas in the case of food freezing, the transition between phases of freezing is found to be more gradual and complicated due to the presence of different compositions.

One of the important parameters during food freezing is the freezing time, which is usually considered the time taken by the slowest cooling point of food (usually geometric center or thickest part) to attain the final preselected temperature (Heldman & Lund, 2006; Pham, 2014).

2.2.2 Ice crystals formation and food quality

The creation of ice crystals during freezing can have significant implications for the texture and quality of foods, including meat. Several factors contribute to the impact of ice crystal development on food quality. Ice crystal formation within the food matrix can lead to physical damage to cell structures, including cell membranes and organelles. This damage disrupts the integrity of the food's cellular structure and can result in textural changes such as softening, loss of firmness, and increased water loss upon thawing. Research by Sun et al. (2022) demonstrated that ice crystal formation during freezing caused structural damage to muscle fibers in fish fillets, resulting in reduced firmness and increased drip loss upon thawing. Water Redistribution: Ice crystal formation can disrupt the distribution of water within the food matrix, leading to the migration of water from areas of higher concentration to areas of lower concentration.

Research by Jia et al. (2022) found that ice crystal formation during freezing induced protein denaturation in frozen egg whites, resulting in changes in gelation properties and texture. This water redistribution can result in changes in texture, such as the formation of ice crystals, pockets of free water, or dehydration, depending on the freezing conditions and food composition. In a study by Zhu et al. (2020), ice crystal formation during freezing led to changes in water distribution and texture in frozen fruits, affecting their overall quality and sensory attributes. Ice crystal formation can induce changes in protein structure, including denaturation and aggregation, which can impact the texture, juiciness, and mouthfeel of frozen foods. Protein denaturation can occur due to mechanical stress from ice crystal growth or temperature fluctuations during freezing and thawing (Jia et al., 2022).

Ice crystal formation is a critical aspect of the freezing process and has profound effects on the texture and quality of foods, including meat. Understanding the mechanisms underlying ice crystal formation and its impact on food quality is essential for optimizing freezing techniques, minimizing textural changes, and preserving the sensory attributes of frozen products. By controlling freezing conditions and implementing appropriate handling practices, food manufacturers and consumers can ensure the production of high-quality frozen foods with desirable texture, appearance, and eating experiences (Kaale et al., 2014).

2.2.3 Frozen storage

Freezing is the preferred long-standing preservation strategy for meat because it minimizes autolytic reactions and prevents microbiological deterioration. However, the quality of meat is irreparably compromised during slow freezing and subsequent storage in the freezer. The finest ice crystals are formed, and the solute concentration in the defrosted matrix is maximized by this process. The natural protein structure can be altered when it is frozen due to the greater number of ions in the defrosted phase, which have a strong ionic strength and may contend with existing electrostatic bonds. Based on research conducted by Setyabrata and Kim in 2019, the color, nutritional value, and flavor of frozen meat can all take a hit as lipid oxidation shortens its shelf life. The rate of lipid oxidation in frozen meats can be accelerated by using insufficient freezing and thawing procedures, which lead to a higher salt content and a low water activity. Furthermore, it is known that the autoxidation of heme pigments begins at these lower water activity levels. Thus, by rapidly freezing and thawing, the pace of lipid oxidation can be reduced, and the microstructure of the food matrix can be minimized due to the low preservation temperature and the creation of tiny ice crystals. Unfortunately, the fast-freezing method isn't practical for bigger meals because of the low heat transfer that occurs with them (da Silva Bernardo et al., 2020).

The temperature of the freezer plays a crucial role in preserving meat quality. Freezing meat at lower temperatures, typically around -18 °C or lower, helps maintain its texture, flavor, and nutritional value. Lower temperatures inhibit microbial growth and enzymatic activity, reducing the risk of spoilage. The rate of freezing meat can influence the formation of ice crystals, which, in turn, affects meat quality. Rapid freezing, such as in blast freezers, promotes the creation of smaller ice crystals, minimizing harm to cell structures and preserving moisture content. Slow freezing, on the other hand, can lead to the formation of larger ice crystals, causing cell rupture and moisture loss. Proper packaging is essential for minimizing exposure to air and preventing freezer burn, which can adversely affect meat quality. Vacuum-sealed packaging or airtight containers help preserve the value of frozen meat by preventing oxidation and dehydration (Rahman et al., 2015).

Prolonged frozen storage can cause changes in meat consistency, resulting in a loss of tenderness and juiciness. This is often attributed to ice crystal formation, which can disrupt muscle fibers and cell structures. Additionally, prolonged frozen storage may lead to protein denaturation, affecting the meat's texture and mouthfeel. Beef steaks stored for longer durations (over six months) exhibited decreased tenderness compared to steaks stored for shorter periods. Frozen storage can affect the flavor of meat due to lipid oxidation and protein degradation over time. Lipid oxidation can lead to off-flavors and rancidity, while protein degradation can result in the growth of undesirable odors and flavors (Motoyama et al., 2016).

According to a study by Lu et al. (2022), prolonged frozen storage of pork resulted in increased lipid oxidation and changes in flavor attributes, including increased bitterness and decreased sweetness. Color changes are common in frozen meat, with prolonged storage often resulting in discoloration. Oxidative processes and enzymatic reactions can lead to changes in meat color, including darkening and loss of vibrancy. In a study by Kim et al. (2015), frozen storage of beef led to changes in color attributes, including decreased redness and increased yellowness, particularly after extended storage durations. Frozen storage can impact the nutritional quality of meat, including the preservation of vitamins, minerals, and other nutrients. While freezing helps preserve the nutritional content of meat, prolonged storage can lead to nutrient degradation over time. Frozen storage of fish fillets resulted in a gradual decrease in vitamin C content over six months, highlighting the importance of monitoring nutrient retention during frozen storage (Lu, Ma & Sun, 2022).

2.2.4 Effects of moisture and fat content on the meat freezing rate

The freezing rate of meat has a significant impact on its structure, texture, and overall quality. Two critical factors that influence the freezing rate are the moisture and fat content of the meat (Kaale et al., 2011; James & James, 2013). The moisture content is a major influencing factor in the beef freezing process. The high-water content of beef produces more ice crystals during the freezing process. The faster the freezing rate, the more ice crystals are created inside the smaller muscle fibers. Slow freezing, on the other hand, produces bigger ice crystals that can penetrate through cell membranes and cause drip loss when thawed, reducing meat texture and juiciness (Leygonie et al., 2012). Kaale et al. (2011) demonstrated that the initial moisture level of meat influences its thermal conductivity. Higher moisture content often improves thermal conductivity, increasing the rate of heat transfer during freezing. However, it also indicates that more energy must be extracted up to the level of the latent heat of fusion. In this aspect, while the initial rate of freezing may be high due to increased conductivity, the freezing process is actually slowed down since more energy is required to freeze off the surplus water content.

Another physical component that has a significant impact on the freezing rate of beef is fat content. Beef with a larger percentage of fat freezes slower than lean beef because fat has a lower thermal conductivity than water. Fat functions as insulation during freezing, slowing the rate of heat loss from the meat into the surrounding environment (James and James, 2013). Furthermore, because fat has a lower specific heat capacity than water, cooling and freezing require less energy. Thus, while excessive marbling of beef in intramuscular fat content may accelerate the first drop in temperature, the total rate of freezing is tempered to some extent by the fat's insulating capabilities. In this freezing dynamic, fat and moisture content interact to create a complex scheme, resulting in zones with varying compositions freezing at extremely varied rates.

In terms of freezing rate, the combined impacts of moisture and fat content are critical to understanding how the freezing process affects meat quality. In general, high moisture content accelerates the initial rate of freezing due to superior thermal conductivity; nevertheless, it requires a lot of energy, which can extend the freezing period. On the other hand, a large fat content slows freezing due to its low thermal conductivity and specific heat capacity.

2.2.5 Impact of freezing and thawing on meat quality

Both freezing and thawing can affect the quality of meat in various ways, including texture, juiciness, flavor, and color. Several factors influence the extent of these effects. During freezing, large ice crystals can rupture muscle fibers and cell membranes, leading to structural damage and moisture loss. Quick freezing minimizes the creation of large ice crystals, helping preserve meat quality. Drip loss occurs as meat thaws because it releases moisture. When this happens too quickly or the meat isn't wrapped properly, it might impact the meat's juiciness and tenderness. Denaturation, a result of structural changes brought about by freezing and thawing, can alter the meat's texture and flavor. Protein denaturation can be reduced with careful handling and the right way to thaw. Thawing meat in an oxygen-rich environment can cause lipid oxidation, which in turn can give unpleasant flavors and aromas to the meat. Careful handling and proper thawing methods can help minimize protein denaturation. Exposure to oxygen during thawing can promote lipid oxidation, leading to off-flavors and odors in the meat. Packaging meat properly and minimizing exposure to air can help reduce the risk of oxidative rancidity.

Freezing and thawing are essential processes in the preservation and preparation of meat products. While freezing helps inhibit microbial growth and prolong shelf life, proper thawing is critical to preserving meat quality and ensuring food safety. Understanding the basics of freezing and thawing, as well as their effects on meat quality, can help producers, processors, and consumers make informed decisions about handling and storing meat products. By employing appropriate freezing and thawing methods and practices, it is possible to maintain the quality and integrity of meat throughout the preservation and preparation process (Bowker & Zhuang, 2017).

2.2.6 Effects of freezing on muscle structure

Freezing induces structural changes in muscle tissue, affecting its texture, juiciness, and overall quality. When meat is frozen, ice crystals form within the muscle fibers and extracellular spaces, causing mechanical impairment to cell membranes and disrupting the structure of muscles. During freezing, ice crystal formation can rupture cell membranes and cause protein denaturation, leading to the release of moisture upon thawing. This loss of moisture contributes to drip loss, resulting in a drier and less juicy texture in the thawed meat. Moreover, freezing can also affect the integrity of muscle fibers, leading to textural changes such as increased toughness or a loss of tenderness. The extent of these changes depends on factors like freezing rate, storage temperature, and the presence of cryoprotectants. Thus, freezing induces structural changes in muscle tissue, primarily through ice crystal formation, which disrupts cell membranes, causes protein denaturation, and alters the texture and juiciness of the meat upon thawing (Bowker & Zhuang, 2017).

The freezing rate, whether it is fast or slow, directly impacts the extent of muscle tissue damage (Grujic, Petrovic, Pikula & Amidzic, 1993). It has been long held that fast-frozen foods yield the highest level of quality (Hanenian & Mittal, 2004). Quick freezing is defined as occurring at a rate greater than 5 cm/hour, while slow freezing occurs at a rate less than 1 cm/hour (FAO, 1972). During slow freezing, the majority of microorganisms enter the portion of food's water that isn't frozen (Gill, 2002). When large ice crystals form at this freezing rate, more meat fiber ruptures, resulting in excessive water loss as drips when the meat thaws (Farkas, 1997). In contrast to rapid freezing, slow freezing results in fiber dehydration. This phenomenon results from the movement of sarcoplasmic fluids into the extracellular region, where ice forms only in the extracellular space. When big ice crystals form, the meat's texture is harmed, oxidation rates rise both during and after thawing, and enzyme activity is accelerated (Doughikollaee, 2012).

According to Fennema (1996), slow freezing is characterized by extensive dislocation of water, which causes the cells to appear shrunken when frozen. Lower juiciness is a potential consequence of this freezing rate since it results in greater cooking losses than a fast rate (Wheeler, Savell, Cross, Lunt & Smith, 1990). Deatherage & Hamm (1960) found that beef pieces that were gradually frozen at a temperature of -15 °C exhibited a minimal water-holding capacity. Furthermore, meat that has been frozen gradually develops a black and glossy appearance (James & James, 2002). whereas meat that has been frozen quickly is opaque and pale. According to James and James (2002), this is due to the fact that small crystals formed by rapid freezing scatter light more than large crystals formed by slow freezing.

2.2.7 Effects of freezing on moisture characteristics

In the meat business, drip loss is a crucial component of quality. It is a consequence of permanent damage that occurs throughout the processes of freezing, thawing, and preservation (Pham & Mawson, 1997). The economic impact of this aspect stems from the loss of soluble nutrients and yield (weight). Additionally, it gives the meat an ugly appearance. The impact of freezing rate on drip loss is known (Ngapo et al., 1999). Because big ice crystals form during slow freezing, more meat fibers break when the meat thaws, resulting in excessive water loss as drips (Li & Sun, 2002). Slow freezing, as opposed to quick freezing, results in fiber dehydration because extracellular ice forms exclusively in the extracellular areas where sarcoplasmic fluid migrates. As per Wheeler, Savell, Cross, Lunt, and Smith (1990), this freezing rate leads to greater cooking losses than rapid freezing, which increases the likelihood of lower juiciness. According to Lund (2000), the meat's structure is less affected by rapid freezing since the ice crystals lack the opportunity to expand before the entire liquid is frozen. In this instance, intracellular ice crystals are formed as a result of nucleation taking place inside the cells. Food will form more nuclei and produce a greater number of small crystals the faster it freezes. The quantity of solutes, including proteins, carbohydrates, and soluble compounds, which alter the temperature at which ice crystals form, also affects how efficient this process is (Gill, 2002).

It has been suggested by Ramsbottom & Koonz (1939) that drip is not primarily caused by the rate of freezing. They proved that if a cut is large in comparison to its surface area, there will be more moisture reabsorbed after it thaws. Similarly, when the area of the sliced surface exceeds the volume of meat, a rapid freezing rate will lead to less drip loss. This is due to the fact that drip loss is reduced with a high freezing rate. According to Ngapo et al. (1999), pork that was rapidly frozen had exactly the same drip loss as meat kept inside a refrigerator. However, the drip loss was much greater for meat that had been slowly frozen (for 240–900 minutes) than it was for meat that had been refrigerated. Additionally, their findings indicated that drip loss was higher with slow freezing than with fast freezing; but four weeks of freezer storage caused this difference to disappear.

Huff-Lonergan & Lonergan (2005) state that the temperature at which meat is frozen, the length of time it is frozen, the conditions under which it is frozen, and the rate at which the temperature drops all affect how much meat can hold water. Temperature fluctuations occur naturally during frozen storage, especially in small freezer units. The severity of this issue increases when meat is transported, stored, and kept in display cabinets and home freezers (Xia; Kong; Liu & Liu, 2009). The key elements that influence the cooking loss of frozen meats are the rigor-onset temperature during meat processing and the cooking temperature during the cooking technique. Genot (2000) demonstrated that slow freezing rates result in greater cooking loss. According to Ferrier & Hopkins (1997), there was no discernible correlation between cooking loss and freezing rate. Though high fluid losses detract from the appearance of meat, they have no discernible effect on the meat's eating quality once it has been cooked, with the exception of extremely high fluid losses, which may impact the meat's tenderness and juiciness (Genot, 2000).

2.2.8 Effects of freezing on meat color

Freezing alters meat color due to the formation of ice crystals, which disrupt the structure and cause oxidation, leading to discoloration. Factors influencing color stability include storage time, temperature fluctuations, packaging methods, and oxygen exposure. Extended storage times and fluctuating temperatures accelerate color deterioration, while vacuum or modified atmosphere packaging and antioxidants can help preserve color (Suman & joseph, 2013). When beef is frozen for extended periods of time, its color typically appears darker, less red, and more yellow than when it is fresh (Farouk & Swan, 1998; Moore & Young, 1991). The loss of exudate during thawing may be the reason for the less appealing, darker color. Occasionally, this may lead to challenges with the product's market acceptability. Bye (1993) conducted a comparison between the color of fresh and frozen patties and found a notable difference in L*, a*, b*, hue, and chroma angle values. The frozen patties had a darker color and were less red. Slow-frozen beef had a lighter color than fast-frozen beef. Farouk et al. (2003) attributed this phenomenon to the fluctuation in thaw drip, which potentially resulted in increased light reflection and a brighter hue in the samples subjected to slow freezing and thawing.

2.2.9 Effects of freezing on meat tenderness

Freezing processes impact meat tenderness by disrupting muscle structure, causing protein denaturation, and increasing the release of water upon thawing. Ice crystal formation during freezing can break cell membranes and affect the integrity of muscle fibers, leading to textural changes. Thawing further exacerbates tenderness changes as ice crystals melt and cause drip loss, resulting in a drier texture. The mechanisms behind tenderness changes involve proteolysis, where enzymes break down muscle proteins, and structural damage to muscle fibers, influencing the overall tenderness of the meat (Bowker & Zhuang, 2017). There is broad consensus among authors (Leygonie et al., 2012; Vieira et al., 2009) that meat tenderness improves with freezing and thawing. However, inadequate freezing procedures can result in extremely tough meat and low-quality meat for human consumption (James, 2002). This toughness results from the unwanted contraction of muscle fibers that can happen when freezing pre-rigor meat, a process known as "cold shortening" (Huff-Lonergan & Lonergan, 2005).

Akhtar et al. (2013) discovered two processes that contribute to the tenderization of meat during freezing: the enzymatic activity-induced breakdown of muscle fibers during thawing and the loss of structural integrity caused by the formation of ice crystals. Most significantly, meat aging is influenced by the rate of freezing (Dransfield, 1994). Rapid freezing, in contrast to slow freezing, results in small intracellular ice, which triples the rate of meat aging relative to chilled meat (Dransfield, 1994). Protease-enzyme release, which accelerates the aging process, is most likely the cause of these events (Koohmaraie, 1992). In addition, Crouse & Koohmaraie (1990) found that samples that were aged after freezing exhibited lower shear force compared to samples that were aged six days before freezing. According to the theory put forth by Gambuteanu et al. (2013), freezing meat before it ages could enhance post-mortem proteolysis. Their findings support this theory. The loss of Ca-dependent protease inhibitors is likely what accelerates aging after freezing (Whipple & Koohmaraie, 1992). Langestedt et al. (2008) conducted a sensory examination of meat tenderness and discovered conflicting outcomes: the shear force values of frozen meat were notably lower compared to those of chilled meat. According to Farouk et al. (1992), freezing had no effect on the tenderness of the meat.

2.3 Meat quality analysis

Meat quality is a multifaceted concept encompassing various attributes that collectively determine its desirability and suitability for consumption. Several criteria are employed to assess meat quality, spanning sensory, nutritional, safety, and technological aspects. Sensory attributes such as color, aroma, flavor, juiciness, tenderness, and overall palatability are primary indicators of meat quality. These attributes are often assessed through sensory evaluation by trained panels or consumers, employing methods like descriptive analysis or hedonic scales. Nutritional quality, including protein content, fat composition, vitamin and mineral content, and amino acid profile, plays a crucial role in determining the nutritional value of meat (Hoffman et al., 1996). When considering meat production, defining the term "quality" becomes challenging due to the numerous aspects that influence the meat. Functional quality pertains to the characteristics of meat that impact its visual appeal and taste. This section will focus on the functional attributes that meat buyers typically consider when evaluating meat quality. The texture, appearance, and flavor of the meat are the most important attributes through which consumers judge meat quality (Troy and Kerry, 2010).

2.3.1 pH

The pH of the meat depends on the glycogen stores in the animal body. When an animal experiences extreme stress or engages in vigorous exercise before being slaughtered, the glycogen reserves inside its muscle tissue are depleted. The depletion of glycogen reserves inside animals' results in a pH greater than 6.0. Thus, it leads to dry and dark meat (Scanga et al., 1998). The postmortem lactic acid production gets diminished as the glycogen reserves get used up during exercise or stress. The lactic acid production within muscles after slaughter is responsible for lowering the muscle pH. This also leads to inferior cooking characteristics and unattractive looks.

Lamb, venison, and beef are susceptible to quality issues when their pH levels are elevated, which leads to dark, firm, and dried (DFD) meat. In contrast, porcine meat is prone to low pH issues as a result of the rapid rate of postmortem glycolysis, resulting in pale, soft, and exudative (PSE) meat (James et al., 2002). Furthermore, the pH of frozen and thawed flesh is typically lower than that of the meat prior to freezing (Leygonie, Britz, & Hoffman, 2012).

The pH is a quantitative measure of the quantity of unbound hydrogen ions (H+) in a solution. Freezing causes the denaturation of buffer proteins, resulting in the release of hydrogen ions and a subsequent fall in pH. In addition, the fluid loss that occurs after thawing leads to a rise in the concentration of solutes, resulting in a further decrease in pH. Leygonie et al. (2012) provided evidence of a pH reduction following the freezing and thawing of ostrich flesh. They proposed that the pH decline was caused by the depletion of minerals and protein molecules, known as exudates. The pH of the meat is reduced as a consequence of the resultant ionic imbalance.

2.3.2 Color

Color is one of the most important attributes of fresh meat quality considered by consumers at the time of purchase (Carpenter et al.,2001). When purchasing meat, consumers judge the quality of the meat on the basis of color and consider bright cherry red meat to be fresh meat (Suman & Joseph, 2013). When the meat is put on retail display, it usually undergoes discoloration with time. The meat that gets discolored is usually put on discounted offers, or the whole meat product is discarded if not sold out (Mancini and Hunt, 2005). Due to surface discoloration, about 15% of the beef meat gets a discounted price. As a result, nearly 15% of retail beef is discounted in price, which results in a US$1 billion annual loss in the United States (Smith et al.,2000).

The protein primarily responsible for meat color is myoglobin (Mb). It serves two crucial functions in living cells: oxygen storage and oxygen delivery (Mancini & Hunt, 2005). The ability of Mb to bind with oxygen depends on the haem group, which has an iron atom at its center. The haem group is a non-polypeptide prosthetic group with six coordination bonds. Four of these bonds connect to the nitrogen atoms in the porphyrin ring of the heme; the fifth bond attaches to the proximal histidine (His-93), and the sixth bond can bind oxygen, nitric oxide, or carbon monoxide (Faustman & Cassens, 1990).According to Faustman and Cassens (1990), the color of the meat is determined by the oxidation state of the iron in the haem group. Due to the presence of iron in the haem group, Mb has the potential to undergo oxidation. Several variables, such as temperature, pH, and duration, have an impact on the oxidation process in Mb, which ultimately defines the external appearance of meat.

When meat is not exposed to air, myoglobin does not have any oxygen attached to it, and it is referred to as deoxymyoglobin, or DeoxyMb. During this stage, meat exhibits a purplish-red hue (Faustman and Cassens, 1990). The vacuum-packed meat has a similar hue of purplish-pink due to the absence of oxygen. When the vacuum-sealed meat is opened, DeoxyMb reacts with oxygen from the air to create oxymyoglobin, known as OxyMb, which causes the surface to appear brilliant red. This gives the meat a fresh and appealing look to the consumer. Nevertheless, when there is a short supply of oxygen, OxyMb undergoes additional oxidation, resulting in the formation of a less desirable brown pigment called metmyoglobin, or MetMb.

color of the meat is influenced differently by the varied packaging conditions. pH is a crucial factor in determining the color of meat. When the pH of the meat is less than 5.8, it has a vivid cherry-red color. However, when the pH is high, the color of the meat becomes dark and brown. The dark-colored meat is unattractive to consumers and has high WHC. The meat has a tighter structure compared to normal pH meat with less oxygen diffusion, which in turn limits the synthesis of OxyMb and leads to the formation of a deeper hue (Abril et al., 2001; Mancini & Hunt, 2005).

The color of meat is usually measured visually or instrumentally. The visual method is subjective and dependent on individual color preference and on variables caused by external lighting conditions. Instrumental measurement offers an accurate and more consistent reading. Instrumental measurement of meat color is done using the CIE L* (lightness), a* (redness), and b* (yellowness) color space with a 10° observer angle. This method provides a more objective representation of what consumers observe in meat color (Hunt et al., 1991; AMSA, 2012).

2.3.3 Meat tenderness

The consumer's choice to purchase raw meat is influenced by both its color and the tenderness of the cooked meat. Meat tenderness is associated with various meat quality parameters, including texture, juiciness, and fat content. The meat quality evaluation regarding flavor and juiciness can be done independently only if the meat has reached acceptable tenderness after cooking (Miller et al., 2001). Meat tenderness is not just a measure of biting force. According to Warner et al. (1998), meat tenderness is the ease of fragmentation, mealiness, and separation of muscle fibers during chewing. Moreover, post-cooking, the meat tenderness is not always uniform. This is the result of a variety of factors, including the pH of the meat, the amount of connective tissue, and the process of cold shortening during refrigeration (Koohmaraie, 1996; Miller et al., 2001). There is a lot of connective tissue around the muscle fibers, which results in the meat tenderness variation (Purchas, 2004). There are other factors that affect meat tenderness, including the presence of collagen and elastin. The presence of collagen results in a chewy texture, and its content increases with the animal’s age. On the other hand, elastin gives elastic properties, is quite insoluble, and increases meat toughness (Purchas et al., 2004). Meat freezing and thawing also affect its tenderness. The tenderness increases with freezing and thawing (Lagerstedt, Enfält, Johansson & Lundström, 2008).

In the process of meat freezing, the formation of extracellular ice crystals may disturb the meat structure and break the myofibrils apart, thus increasing tenderness. If the meat is aged adequately before freezing, this tenderizing effect of freezing becomes ineffective (Leygonie, Britz, & Hoffman, 2012). However, during sensory evaluation regarding meat tenderness, the results are quite contradictory. Peak forces were frequently observed to be lower in the freeze/thaw samples than in chilled meat. This can be a result of fluid loss during the thawing process. This loss of fluids lowers the water content available to hydrate the muscle fibers, thus toughening the meat (James & James, 2002).

2.3.4 Water holding capacity

The ability of the meat to hold moisture is defined as its water-holding capacity (WHC) (Hamm, 1986). The WHC is a very important factor for the food industry and consumers as it influences the meat quality. If the meat has poor WHC, it results in a high loss of water, due to which there is a loss of carcass weight or muscle cut. Researchers have found WHC directly relates to the juiciness of meat. The actual results regarding WHC measurement and the effects it creates on the juiciness of meat tend to be rather conflicting (Bertram et al., 2002). Economically, WHC is a very important characteristic since it shows the weight loss of the product of meat (Huff-Lonergan & Lonergan, 2005). There are several factors that relate to WHC in meat. One such attribute is drip loss, or fluid loss in a retail meat pack, which results in an unattractive appearance and reduced sales. A related term is purge loss, which refers to the fluid loss from a meat cut from the time it was packed until a given point. Supermarkets often use absorbent pads to minimize visible fluid for consumers (Kristensen & Purslow, 2001). The third terminology in this context is cook loss, which refers to the amount of fluid lost from the meat during cooking. When the meat is heated, the proteins denature at different temperatures ranging from 37-75 °C, resulting in their shrinkage, aggregation, and finally changes in the structure of the muscle fibers that reduce the water holding capacity of the meat (Huff-Lonergan & Lonergan, 2005; Tornberg, 2005).

The literature indicates that freezing, storage, and thawing decrease the water-holding capacity (WHC) of meat and increase drip loss, purge loss, and cook loss (Huff-Lonergan & Lonergan, 2005; Leygonie, Britz, & Hoffman, 2012; Xia et al., 2009). However, if meat is stored at cold temperatures for extended periods (8 weeks or more), the WHC improves. During prolonged cold storage, the disruption of water channels due to muscle structure breakdown creates a spongy effect that retains water within the meat (Kim, Lonergan, & Huff-Lonergan, 2010).

Ngapo et al. (1999) reported that the rate of freezing affects drip loss. Pork frozen at a fast rate exhibited the same amount of drip loss as chilled meat, whereas slowly frozen pork showed higher drip loss. Leygonie, Britz, and Hoffman (2012) found that reducing the thawing time (the time elapsed from -5 °C to -1 °C to less than 50 minutes decreased drip loss. This was attributed to the melting of ice in the extracellular spaces, with water from the melted ice flowing into intracellular spaces and being reabsorbed by dehydrated fibers. Liu, Xiong, and Chen (2010) also reported that an increased thawing rate reduces exudate formation, hence reducing drip loss. Farouk and Swan (1998) achieved a reduced drip loss using rapid thawing techniques such as immersion of meat in water.

2.3.5 Light and transmission electron microscopy (TEM)

Light microscopy and transmission electron microscopy (TEM) are two essential techniques used in examining meat structure, each offering unique advantages for visualizing different aspects of tissue morphology and organization. Light microscopy, also known as optical microscopy, utilizes visible light to magnify and resolve structures within a specimen. It is widely used in various scientific fields, including biology, medicine, and food science. In meat science, light microscopy is invaluable for studying the gross morphology and microstructure of meat tissues. Light microscopy allows the examination of muscle fibers, connective tissues, blood vessels, and fat cells in meat samples. The technique provides moderate magnification (up to a few hundred times) and enables real-time observation of samples without the need for complex preparation procedures.

Light microscopy is particularly useful for routine analysis, as it provides a rapid assessment of tissue characteristics such as muscle fiber size, fiber arrangement, and the distribution of intramuscular fat. For example, in a study by James and Yang (2011), light microscopy was used to assess the effects of different cooking methods on the microstructure of beef muscle tissue. The researchers observed changes in muscle fiber integrity and connective tissue structure following thermal processing, providing insights into the effect of cooking on meat tenderness and texture (James & Yang, 2011). The microstructural analysis of the samples using both light microscopy (LM) and scanning electron microscopy (SEM) showed good agreement. Both the raw and cooked, vinegar-marinated steak displayed muscle swelling, a reduction in the spacing between muscle fibers, and a reduced observed "crimp."

Transmission Electron Microscopy (TEM) is a high-resolution imaging technique that uses electron beams to visualize the ultrastructure of biological specimens at the nanometer scale. Unlike light microscopy, which relies on visible light, TEM utilizes electrons that are transmitted through thin sections of the sample. TEM offers significantly higher resolution and magnification compared to light microscopy, making it ideal for studying the fine structure of meat tissues at the cellular and subcellular levels. In meat science, TEM can be used to investigate the organization of muscle fibers, myofibrils, mitochondria, the sarcoplasmic reticulum, and other cellular components. For instance, in a study by Feng et al. (2020), TEM was employed to examine the ultrastructure of pork muscle fibers and the distribution of intracellular lipid droplets. The researchers observed differences in lipid accumulation patterns between lean and marbled muscle samples, providing insights into the association between meat quality and intramuscular fat content (Figure 3).

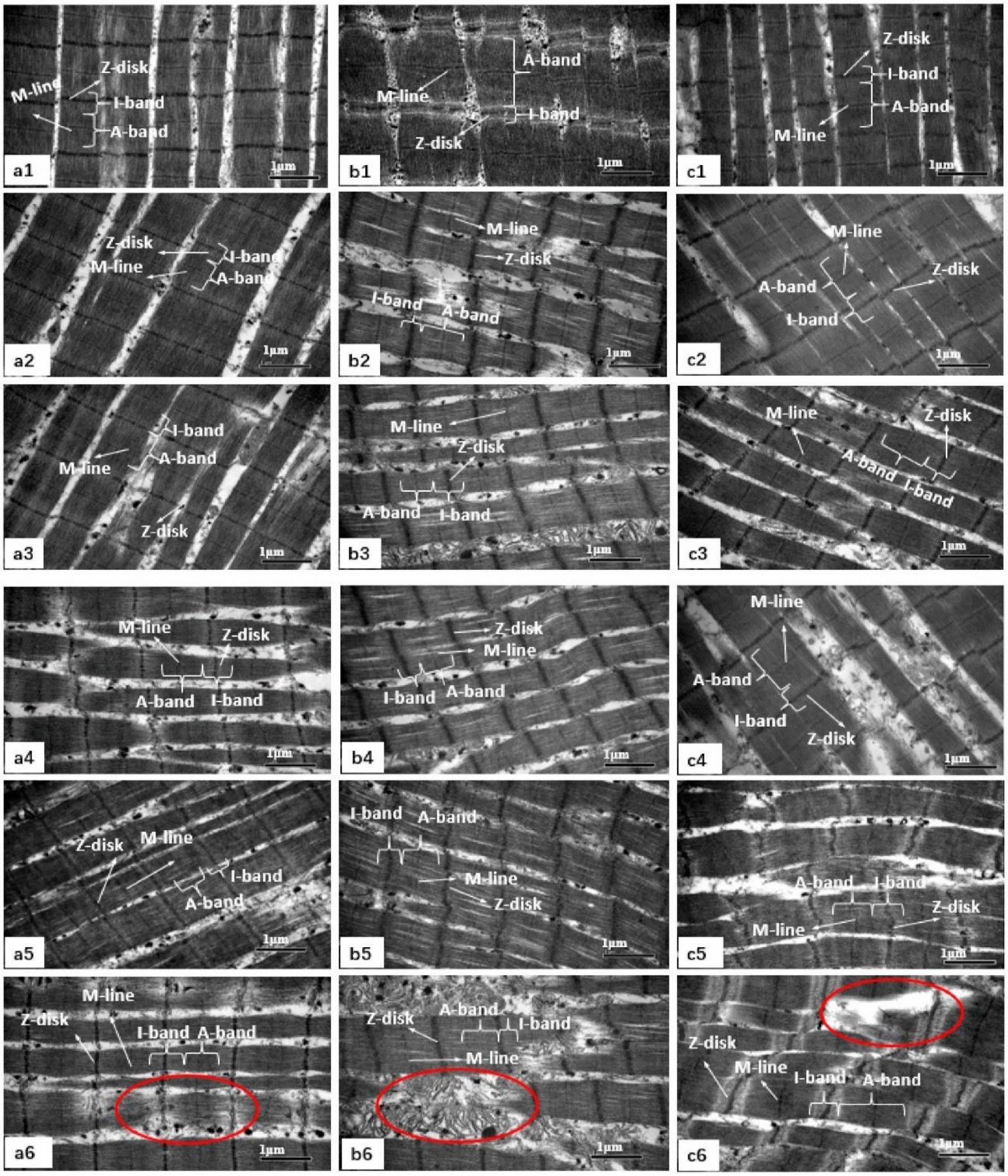


Figure 3: Beef muscle images at different stages of aging through transmission electron microscopy (Feng et. al., 2020) (a1 to a6): the semitendinosus (ST) microstructure at days 1, 3, 7, 9, 11, and 14. (b1 to b6): the rhomboideus (RH) microstructure at the 1st, 3rd, 7th, 9th, 11th, and 14th days. (c1 to c6): the infraspinatus (IN) microstructure at the 1st, 3rd, 7th, 9th, 11th, and 14th days. The red circles indicate the fracturing regions. (Source: Feng et. al., 2020)

Light microscopy and TEM are complementary techniques that offer different levels of resolution and visualization capabilities. While light microscopy provides a macroscopic view of tissue morphology and organization, TEM offers detailed imaging of cellular and subcellular structures. In meat research, integrating information obtained from both techniques can lead to a comprehensive understanding of tissue composition, architecture, and functional properties. For example, light microscopy may reveal overall muscle fiber arrangement and fat distribution, while TEM can provide insights into the ultrastructural changes associated with meat aging, processing, or disease conditions. Light microscopy and TEM are indispensable tools for examining meat structure and understanding the complex organization of meat tissues. By leveraging the unique capabilities of each technique, researchers can gain valuable insights into the composition, morphology, and functional properties of meat at different scales of observation (Feng et al., 2020).

2.4 New Zealand Wagyu and crossbred beef industry

New Zealand's beef sector is known for producing high-quality Wagyu and crossbred cattle, taking advantage of the country's favorable climate, rich agricultural tradition, and adherence to sustainable farming practices. Both forms of beef play important roles in the domestic and foreign markets, catering to distinct customer segments and tastes. Wagyu beef, which originated in Japan, is world-renowned for its excellent marbling, softness, and flavor. Wagyu cattle's unique genetic traits, combined with specialized feeding procedures, produce meat with high amounts of intramuscular fat, giving it a particular melt-in-the-mouth feel and rich flavor. Wagyu cattle in New Zealand are often bred in circumstances that are similar to their traditional Japanese upbringing. This includes stress-free environments and foods that are specifically tailored to increase marbling. Many New Zealand Wagyu producers attain the necessary meat quality using grass-fed systems supplemented by grain finishing. This strategy is consistent with New Zealand's overall emphasis on natural and sustainable farming practices. As a result, New Zealand Wagyu beef is marketed as a luxury product, appealing to high-end restaurants and discerning consumers both domestically and globally.

In contrast, crossbred beef in New Zealand combines established European and British breeds, such as Angus and Hereford, with other types to improve growth rate, meat yield, and resilience. The purpose of crossbreeding is to blend the best qualities from multiple breeds, thereby increasing overall productivity and meat quality. Crossbred cattle are mostly raised in extensive grass-fed systems, taking advantage of New Zealand's plentiful meadows. This grass-fed technique is advertised as providing leaner, healthier meat than grain-fed equivalents. Rotational grazing and other sustainable measures ensure that crossbred beef production is in line with New Zealand's environmental stewardship goals. Crossbred beef accounts for a large component of New Zealand's beef exports, providing to a diverse variety of markets, from commodity meat to luxury grass-fed segments.

Thus, the main objectives of this study are to assess and understand correlations between meat composition, structure, and meat quality parameters such as pH, color, and tenderness and drip and cook losses under varying freezing conditions and how they are influenced by breed type. The results of this study can help beef producers and processors choose the best freezing procedures to retain the quality of both Wagyu and crossbred cattle. By assuring greater meat quality preservation, the study can help meet customer expectations for both premium Wagyu beef and economically significant crossbred beef.

3. Material and methods

3.1 Introduction

To analyze the meat quality attributes of Wagyu and crossbred beef, the rump cuts were considered for testing. While the majority of studies on beef quality analysis focus on the longissimus dorsi muscle, also known as the loin or ribeye, the beef rump is largely unexplored. Beef rump is primarily sold in supermarkets in New Zealand due to its versatility, low cost, and widespread acceptance among consumers. In addition, the rump was significantly larger than other common cuts like tenderloin or ribeye, which enabled the extraction of multiple uniform samples. The size advantage facilitated the homogeneity of the samples in terms of muscle fiber and fat distribution, which was crucial for obtaining accurate and reproducible results during meat quality examination.

3.2 Raw material and processing

Three Wagyu whole rump cuts were brought from Greenlea farms (Hamilton, New Zealand) and three crossbred whole rump cuts from AFFCO (Fielding, Manawatu, New Zealand). Three distinct animals were selected and butchered following the New Zealand Code of Welfare: Commercial slaughter (MPI, 2018). Following slaughter, the carcasses were wet aged at 4 ± 1 °C overnight. The deboning and cutting process took place the day after the animals were slaughtered. The cuts were then vacuum-sealed and transported in a polystyrene box with cool packs to the Massey University

Manawatu campus. The received Wagyu beef rump cuts weighed 5-6 kg, while the crossbred beef rumps weighed 6.9- 7 kg. The beef cuts were then stored in a chiller at 2-4 °C after being received 24 hours postmortem, and testing was conducted 48 hours postmortem to reach a 2-day ageing period. Normally, 7-10 days of aging are done for beef, as it achieves the required tenderness after this period. The aging period of 2 days for beef was selected so that muscle structure degradation is kept to a to a minimum, as a longer aging period means more muscle degradation. The Z-disk keeps the structure of meat intact by keeping the thin filaments and, indirectly, the thick filaments intact. Aging causes the degradation of the Z-disk and its associated proteins, leading to the myofibril's fragmentation (James *et* al., 2002).

Each rump cut was removed from the vacuum bag, and any visible subcutaneous fat was uniformly removed. Each rump cut was then divided into ten rectangular samples with dimensions close to 15 x 10 x 4 cm (L x B x H) and weighing around 400 ± 20 g each. The number of samples used for testing was 60 (10 from each rump cut), consisting of 30 Wagyu rump samples and 30 crossbred rump samples. Six out of the ten samples from each rump were used for freezing, while the remaining four samples were used for testing the fresh meat. Out of the six samples allocated to freezing, three were used for fast freezing (FF), and the other three were for slow freezing (SF). Two out of the four samples allocated for testing fresh meat were used for cooking to examine the characteristics of cooked meat, while the other two samples were used for evaluating the composition (moisture and fat content) and characteristics of raw meat. Figure 4 shows the meat samples used in the proposed study.

Figure 4: Beef samples used for processing

The samples from both Wagyu and crossbred beef were randomly assigned to three different regimens:

Fresh, never frozen (FR).

Fast freezing (FF).

Slow freezing (SF).

The total number of treatments was 12 as mentioned in Table 1. The samples were promptly vacuum-sealed, weighed, and placed in the freezers. The temperature of the frozen samples was recorded using I buttons (Maxim integrated products, California, USA, Diameter-16mm, accuracy ± 0.5 °C). Before vacuum packing, the I buttons were placed in the geometric center of each sample by making a cut. The freezing of samples was done for 24 hours and then thawed in a refrigerator at 4 °C for 24 hours prior to testing their characteristics. Freezing processes are detailed in the next section.

Table 1: Freezing and thawing periods for Wagyu and crossbred beef.

Group	**Freezing**	**Thawing**
Wagyu fresh, raw	N/A	N/A
Wagyu fresh, cooked	N/A	N/A
Wagyu fast-frozen, raw	Fast freezing at -30 °C for 24 hours	2-4 °C for 24 hours
Wagyu fast- frozen, cooked	Fast freezing at -30 °C for 24 hours	2-4 °C for 24 hours
Wagyu slow-frozen, raw	Slow freezing at -18 °C for 24 hours	2-4 °C for 24 hours
Wagyu fast- frozen cooked	Slow freezing at -18 °C for 24 hours	2-4 °C for 24 hours
Crossbred fresh, raw	N/A	N/A
Crossbred fresh, cooked	N/A	N/A
Crossbred fast- frozen, raw	Fast freezing at -30 °C for 24 hours	2-4 °C for 24 hours
Crossbred fast- frozen, cooked	Fast freezing at -30 °C for 24 hours	2-4 °C for 24 hours
Crossbred slow- frozen, raw	Slow freezing at -18 °C for 24 hours	2-4 °C for 24 hours
Crossbred slow- frozen, cooked	Slow freezing at -18 °C for 24 hours	2-4 °C for 24 hours

3.3 Freezing processes

In this research, two freezing methods were used to evaluate the meat quality parameters.

1. Slow freezing (Set point -18 °C)
2. Fast freezing (Set point -30 °C)

3.3.1 Slow freezing

The slow freezing of the meat samples was carried out using a Fisher & Paykel chest freezer (Model RC376, Tamaki, New Zealand). The temperature was adjusted to -18 °C, and the air temperature inside the chest freezer was monitored by placing an I-button to document the supply air temperature. Preliminary tests were carried out by taking beef samples from the supermarket to observe the freezing time in the chest freezer. The meat samples with the same dimensions were found to achieve -18 °C at the meat sample's center within 9-10 hours.

3.3.2 Fast freezing

Correspondingly, the fast freezing of the meat samples was done by using the same chest freezer, and it was operated in fast freezing mode (-30 °C) this time. A small portable fan was also mounted inside the chest freezer to blow cold air above the meat samples to achieve fast freezing with an airflow of 3 m/s (Figure 5).

Figure 5: Portable fan installed inside chest freezer for fast freezing experiments

Pre-tests were conducted using meat samples from the supermarket by fixing an I button on the symmetrical center of the same-sized meat samples to calculate the freezing time, and the meat samples were found to be frozen to -30 °C in 3-4 hours by means of the proposed method. This is the standard freezing time followed by meat processing plants, where packed meat samples are frozen inside blast freezers before stowage. The inside air temperature was measured by using the I button to observe the supply air temperature.

After 24 hours of freezing, the samples were taken out of the chest freezer, and thawing of the meat samples was done in another chilling room operated at 4 °C for 24 hours. At the end of every freeze-thaw procedure, the meat samples were weighed again. The meat samples were removed from the vacuum packs, and the I buttons were used to document the temperature data. The meat samples were patted dry with a paper towel, and thaw loss was recorded. Each meat sample was then cut into two parts (6 cm in length) and used for testing as follows:

1. One portion was utilized to evaluate the pH, color, cutting force (WBSF), micro-structural analysis (microscopy and TEM), moisture and fat content of the raw meat sample.
2. Another portion was used for cooking and evaluating the cooking loss, pH, color, and cutting force (WBSF) of the cooked meat sample.

3.4 Sous vide cooking

The beef samples were prepared using the sous vide technique. Sous vide cooking has been extensively explored and documented for its ability to improve meat quality (Roldán et al., 2013). Sous vide, a common cooking technique in the culinary industry, involves cooking food in a water bath at a specific temperature while vacuum sealed. Six sub-samples measuring 5 x 4 x 2 cm and weighing 40 ± 2 g/slice each were used from each sample for cooking. The meat samples were vacuum packed and then submerged in a hot water bath at a temperature of 60 °C. The meat samples were cooked for a duration of 6 hours. Cooking red meat at 60 °C for over 4 hours is considered safe in terms of microbial safety (MPI 2015). According to Baldwin (2012), sous vide cooking of beef at 60 °C for 6 hours is effective in achieving desired meat quality attributes such as tenderness and juiciness. After completion of cooking times, the sous vide bags with meat samples were taken out of the hot water bath and immediately placed into ice-cold water for 15 minutes to halt the cooking process.

3.5 Freezing curve kinetics analysis

The kinetics of meat freezing are very important for understanding and optimizing the process of preservation. Temperature changes with time were analyzed during the fast-freezing and slow-freezing processes for Wagyu and crossbred beef samples. The temperature profile and identification of key phases in the process of freezing enabled us to explain the thermal behavior and efficiency of the freezing methods used.

Temperature measurements of Wagyu beef, CB beef, and the supply air were recorded at regular intervals over a period of 5 hours. These measurements were plotted to form a freezing curve which was then analyzed for distinct phases of the freezing process. This yielded cooling rates and durations for the different phases necessary to understand the kinetics involved. Curves at each freezing phase were also mathematically modeled. Mathematical modeling of freezing curves is the process of coming up with equations that describe how temperature changed with time during the freezing process for both Wagyu and crossbred beef. Data analysis revealed models that best explained thermal activity during the precooling, phase transition, and subcooling phases. This method was useful in understanding the kinetics of freezing, and it is commonly used to provide meaningful data for the optimization of freezing protocols to maintain the quality and texture of beef.

The first phase of freezing was the pre-cooling phase, during which there was a sharp downward temperature change when beef approaches its freezing point. This phase was modeled by an exponential decay-type function. The general form of the equation used was:

$$T(t)=T_i+(T_f-T_i)e^{-kt} \tag{1}$$

where:

- $T(t)$ represents the temperature at time t.
- T_i represents the initial temperature.

- T_f represents the final temperature at the end of the cooling phase.
- k represents the cooling rate constant.
- t represents the time.

The next important phase on the freezing curve was the phase shift phase, which occurred when the beef transitions from a liquid to a solid state. While the meat's temperature remains roughly constant, the water content shifts from liquid to solid. This occurred at a constant temperature, indicating that energy was being absorbed as latent heat of fusion. Following the phase transition phase, the final step involved a further temperature drop, known as the sub-cooling phase. The sub-cooling phase, like the cooling phase, was represented by an exponential decay function.

$$T(t)=T_f+(T_s-T_f)e^{-kt} \tag{2}$$

where:

- $T(t)$ represents the temperature at time t.
- $T_i$represents the initial temperature.
- T_f represents the final temperature at the end of the cooling phase.
- k represents the cooling rate constant.
- t represents the time.

3.6 Meat quality measurements

3.6.1 pH

pH is the measure of acidity or alkalinity in meat, and it is essential in deciding the quality of meat that we consume. An appropriate pH balance guarantees that the meat remains fresh, tender, and juicy. It also aids in keeping the meat fresh for an extended period of time. If the pH level of meat is either too high or too low, it can lead to spoilage, color change, and an unpleasant sour flavor. The pH of the uncooked and cooked beef sections was measured with a pH meter (Orion 3 Star, Thermo Electron Corporation, Waltham, MA, USA) equipped with an insertion glass electrode (Sensorex, Garden Grove, CA, USA). The probe was inserted into the center part of the meat for measurement. Before use, the pH meter was calibrated using buffers of pH 4 and 7 at room temperature (17–20 °C). After each measurement, the probe was rinsed using reverse osmosis (RO) water to obtain accurate readings. Six measurements were conducted at various locations on each sample and then combined to find an average.

3.6.2 Color

The color measurement of the beef samples was done in accordance with the suggested guiding principle of influential meat color measurements (Hunt et al., 2012). The samples were sliced to a thickness of 12–15 mm, then bloomed on a tray at 4 °C for 30 min. The cutting was done across the length of the muscle. After blooming, six distinct spots on the meat's sliced surface were chosen to serve as the six measurement points. The color values of lightness (L*), redness (a*), and yellowness (b*) were measured by a handheld Minolta Chromameter CR-400 (Aperture size of 3.18 cm, Konika Minolta sensing, Inc. Japan). The images were taken with a D65 illuminant at a 10° observer angle. Calibration of the device was also executed before every measurement using a standard white tile. Moreover, chroma values are given by C = [$a^*2 + b^*2$]1/2 along with hue angle HA = $\tan^{-1}$ [b^*/a^*] were calculated. Chroma is used to measure color intensity, and high values of chroma designate further concentrated red-colored meat (Chris, 2007).

3.6.3 Thaw loss (%)

The thawing loss of the frozen / thawed meat samples was measured using the thawing method as described by AMSA (1995). The thaw loss of the meat samples was calculated by weighing the vacuum-sealed beef samples before and after freezing/thawing. The meat samples were taken out of the vacuum-sealed bags once they had thawed. The meat samples were dried with a paper towel to eliminate purge, reweighed individually, and the weights were noted. The total no. of samples used for thaw loss measurement was 36 (6 samples per each regimen) and thaw loss value was reported as average of six samples for each regimen. Thawing drip loss is the percentage loss related to its initial sample weight and calculated using the formula:

(Thawing loss (%) = ((Initial weight – weight of sample after purging) / initial weight) × 100)

3.6.4 Cook loss (%)

Cooking loss is the decrease in beef weight while being cooked. Thawing, dripping, and evaporation are the primary factors contributing to cooking losses. Beef that exhibits high cooking loss usually looks unappealing and also reduces the tenderness and juiciness of the meat (Savell, Mueller, & Baird, 2005). The various elements contributing to cooking loss can differ based on the duration of aging (Smith, 1985). Furthermore, the ingredients used for cooking may be related. An escalation in cooking loss within the beef industry has significant financial consequences, as it leads to the reduction of crucial minerals and vitamins, ultimately affecting the nutritional value of beef (Lawrie & Ledward, 2006). The meat samples subjected to sous vide cooking were used for cooking loss measurements. Six sub-samples measuring 5 x 4 x 2 cm and weighing 40 ±2 g/slice each were used from each sample for cooking and cook loss values were reported as average of six sub samples. After being cooked, the samples were promptly placed in ice-cold water. The samples weight was measured before and after cooking, and they were dried with a paper towel before weighing. The percentage of weight lost during cooking was calculated based on the original sample weight. The cooking loss percentage indicated the percentage of weight lost in relation to the original sample weight (Baldwin, 2012).

(Cooking loss (%) = ((raw weight - cooked weight) / raw weight) × 100)

3.6.5 Meat tenderness

The primary textural attributes of meat include toughness (firmness or level of tenderness), cohesiveness, and juiciness. There are different techniques for assessing the texture of meat. The texture of meat samples, both raw and cooked, was measured using the TA.XT plus texture analyzer (Stable Microsystems, Godalming, UK). The uncooked and cooked samples were chilled overnight at 4 °C to obtain firm samples. Then a section of the samples (1 cm^2 shear area) was cut. The cutting was done in a fiber direction parallel to the muscle fiber. For each sample, six subsamples were obtained. Samples were sheared perpendicular to the muscle fibers using a V-notched cutting blade, with peak force measurements obtained using a 50 N load cell at a crosshead speed of 250 mm/min (Warner et al., 1998). The meat tenderness was reported to be the peak shear force in Newtons (N). The reported values were the average of six subsamples. This method for measuring meat tenderness is well-documented in the meat science literature, ensuring accurate and reliable results (Bouton & Harris, 1972; Shackelford et al., 1999).

3.7 Moisture and intramuscular fat content (%)

Beef samples were well prepared prior to analysis in order to come up with a precise and consistent analysis. Trimming was first done on the beef to eliminate connective tissues and visible fat, so only the muscle tissue was considered in this analysis. This was important for avoiding external fat that could give misleading results. The trimmed beef was then chopped finely to obtain standardized meat pieces, which was necessary for moisture and fat content analyses since it would permit even the drying or extraction of samples during the later analytical procedures.

The determination of the moisture content of the beef samples followed the air-oven method according to AOAC method 950.46 (AOAC, 2006). A 5 g chopped beef sample was transferred into a previously weighed moisture dish. After that, the sample was dried in the air oven at a temperature of 105 ± 1 °C for 3 hours. Critical among these preparation processes was the maintenance of the oven temperature and the drying time to achieve comprehensive removal of moisture without inducing thermal degradation of any kind on the samples. After drying, the sample was placed in a desiccator at room temperature to prevent moisture absorption from the atmosphere. The values were reported as average of three sub samples for each regimen. The dish's weight with the dried sample was again measured. Moisture content was determined by the difference in weight before and after drying, using the following equation:

(Moisture Content (%) = ((Initial Weight / Final Weight) / Initial Weight) / 100)

The content of intramuscular fat (IMF) was determined by the Soxtec extraction method, according to the AOAC method 991.36 (AOAC, 2000). A finely chopped beef sample (3 g) was weighed in a Soxtec extraction thimble. The sample was then put through the process of solvent extraction by treating it with petroleum ether as the extraction agent. The Soxtec system automated the process of heating, extraction, and recovery of the solvent. This involved boiling the sample thimble in the boiling solvent to extract and dissolve the fat; rinsing the thimble with the solvent to ensure ample extraction of fat; and recovering the solvent with the dissolved fat in a previously weighed flask. The solvent was then evaporated, and the extracted fat remained in the flask. The flask was then dried in an oven to get rid of residual solvent. Finally, the flask's weight with the extracted fat was weighed for the amount of fat. The values were reported as average of three sub samples for each regimen. The IMF content was calculated using the formula as follows:

(IMF Content (%) = (Weight of extracted fat / Initial Weight of sample) / 100)

3.8 Light and transmission electron microscopy (TEM)

The alterations in raw beef meat structure due to different freezing rates were investigated through a light microscope and TEM examination. The uncooked meat samples were sliced into 4 × 1.5 × 1.5 mm pieces with a carbon steel surgical knife. The samples were cut across the muscle fibers as well as in parallel to the muscle fibers. The samples cut across the muscle fibers were used for TEM to get a longitudinal view of the muscle structure, whereas the samples cut along the muscle fibers were used to get transverse images of the muscle fibers using light microscopy. Cut samples were immediately preserved in Modified Karnovsky’s Fixative, which consists of 3 % glutaraldehyde (v/v) and 2 % formaldehyde (w/v) in 0.1M phosphate buffer (pH 7.2). To ensure that the tissue structure was completely preserved, the fixation process lasted at least two hours. Following fixation, the samples were washed three times in 0.1M phosphate buffer (pH 7.2) for 10 minutes each to remove excess fixative. To further stabilize and contrast the samples, they were post-fixed for one hour with 1 % osmium tetroxide in 0.1M phosphate buffer. This was followed by three 10-minute washes in the same phosphate buffer to eliminate any residual osmium tetroxide. The dehydration process was carried out in a graded succession of acetone concentrations (25 %, 50 %, 75 %, 95 %, and 100 %), each lasting 10-15 minutes. To ensure thorough dehydration, the samples were immersed in two changes of 100 % acetone for an hour each. Following dehydration, the samples were infiltrated with a 50:50 combination of resin and acetone and swirled overnight to ensure complete resin penetration. The mixture was then replaced with fresh 100 % resin (Procure 812, Pro-SciTech, Australia) and mixed for another eight hours. To achieve solid embedding, the samples were placed in molds made of fresh resin and baked in a 60 °C oven for 48 hours.

For light microscopy, slices were cut to 1 micron thickness with an ultramicrotome (Leica EM UC7, Germany) and heat-fixed to glass slides. These sections were stained with 0.05 % toluidine blue for about 12 seconds to increase contrast before being inspected under a light microscope.

For transmission electron microscopy (TEM), the block was trimmed to the necessary size, and sections were cut at 70 nm with a diamond knife (Diatome, Austria). The ultrathin portions were stretched with chloroform and adhered to grids with a Quick

Coat G adhesive pen (Saiko, Japan). For four minutes, the grids were stained with saturated uranyl acetate in 50 % ethanol, then washed with 50 % ethanol and Milli-Q water. A four-minute staining stage with lead citrate (Venable and Coggeshall, 1965) was followed by a Milli-Q water wash. Finally, the stained grids were analyzed with FEI Tecnai G2 Spirit BioTWIN (Czech Republic) transmission electron microscopy.

3.8 Statistical analysis

A two-way ANOVA was used to evaluate meat quality parameters such as moisture and fat content, pH, color, thaw loss, and cooking loss. This study looked at how two independent variables, the kind of beef (Wagyu and crossbred) and the freezing method (fresh, fast-frozen, and slow-frozen), affected both uncooked and cooked meat samples. The two-way ANOVA was used to evaluate the main effects of each independent variable as well as any interaction effects between them. The one-way ANOVA was also used to evaluate the significance between the uncooked and cooked beef samples groups separately. Statistical significance was determined at $p < 0.05$. The data was evaluated using Microsoft Excel (version 2407). The results are shown as means ± standard deviations.

4. Results

This section shows the findings of this study that looked into the effects of various freezing rates (fresh, rapid frozen, and slow frozen) and breed types (Wagyu and crossbred) on meat quality qualities. The findings are divided into various categories, including meat composition (moisture and intramuscular fat content), freezing patterns, freezing curve kinetics, pH levels, warner-bratzler Shear Force (WBSF), thaw loss, cook loss, and color characteristics (lightness, redness, yellowness, chroma, and hue angle) for the meat samples. Statistical analyses were carried out to investigate the impact of freezing rate, breed type, and their interaction on these quality parameters. Other analyses used to indicate structural differences between uncooked meat samples were light microscopy and TEM. The findings are given in extensive tables and figures, providing a comprehensive explanation of how these factors influence meat quality and offering suggestions for optimizing meat preservation and preparation procedures to improve desirable features like tenderness, moisture retention, and color.

4.1 Moisture and intramuscular fat content (%)

Table 2: Effects of freezing rates and breed type on moisture and intramuscular fat (IMF) content for uncooked meat samples.

	Freezing rate / breed							Significance		
	Fresh (Wagyu)	*Fresh (crossbred)*	*Fast frozen thawed (Wagyu)*	*Fast frozen thawed (crossbred)*	*Slow frozen thawed (Wagyu)*	*Slow frozen thawed (crossbred)*	*SEM*	*Freezing rate*	*Breed*	*Freezing rate x breed*
Moisture (%)										
uncooked	62.83	67.20	61.10	65.07	60.17	63.07	0.35	<0.0001	<0.0001	<0.05
Intramuscular fat (%)										
uncooked	12.9	8.93	13.37	9.30	13.63	9.60	0.11	<0.0001	<0.0001	ns

'ns' (not significant), <0.05 (significant). Each parameter is presented with its standard error of the mean (SEM).

Analysis of moisture content reveals significant differences in freezing rate, breed, and interaction. Freezing rate had a significant effect on the moisture content of the meat ($p < 0.0001$). Fresh samples contain the highest percentage of moisture, followed by fast-frozen and slow-frozen thawed samples. This finding is supported by literature that indicates the existence of water loss through the formation of ice crystals and subsequent cell rupture during the freeze and thaw cycles. Breed also had a significant effect on the moisture content ($p < 0.0001$). Crossbred beef had more moisture content compared to Wagyu beef. This is in agreement with the standard characteristics of these breeds, where generally, a higher percentage of fat in Wagyu compared with other breeds is counterbalanced by a lower moisture content in Wagyu. Smith et al. (2019) have reported that Wagyu beef in general includes more fat than crossbred beef but less moisture. The interaction of freezing rate and breed was also significant ($p < 0.05$), which indicates the effect of freezing rate on moisture content varies between Wagyu and crossbred beef.

There were considerable differences in the content of IMF due to freezing rate and breed. No interaction was seen between the two factors. The freezing rate gives a p-value less than 0.0001, showing that it significantly affects the IMF content. Fast-frozen and slow-frozen samples had slightly higher IMF compared to fresh samples. This may be attributed to the concentration effect, whereby moisture loss during thawing raises the proportion of fat in the meat.

The breed also had a strong effect on the content of IMF ($p < 0.0001$). Wagyu beef had a significantly higher IMF content compared with crossbred beef, underpinning the genetic predisposition of Wagyu cattle for high marbling (Brown et al., 2020). There was no significant interaction, however, between freezing rate and breed for IMF content, which further means that the effect of freezing rate on IMF is independent of both Wagyu and crossbred beef.

4.2 Freezing

The freezing curves for Wagyu and crossbred beef samples during fast freezing and slow freezing are demonstrated in the Figure 6 and 7. During fast freezing, the cores of both meat samples reached their freezing temperature of -18 °C after 3.5 hours, whereas in slow freezing, both samples reached -18 °C after 12 hours.

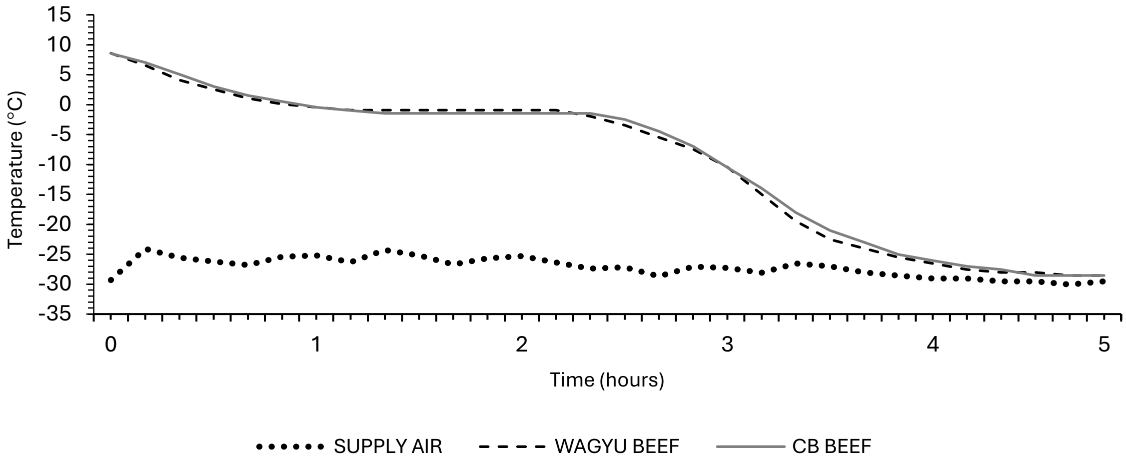


Figure 6: Changes in the core temperature of beef samples during fast freezing

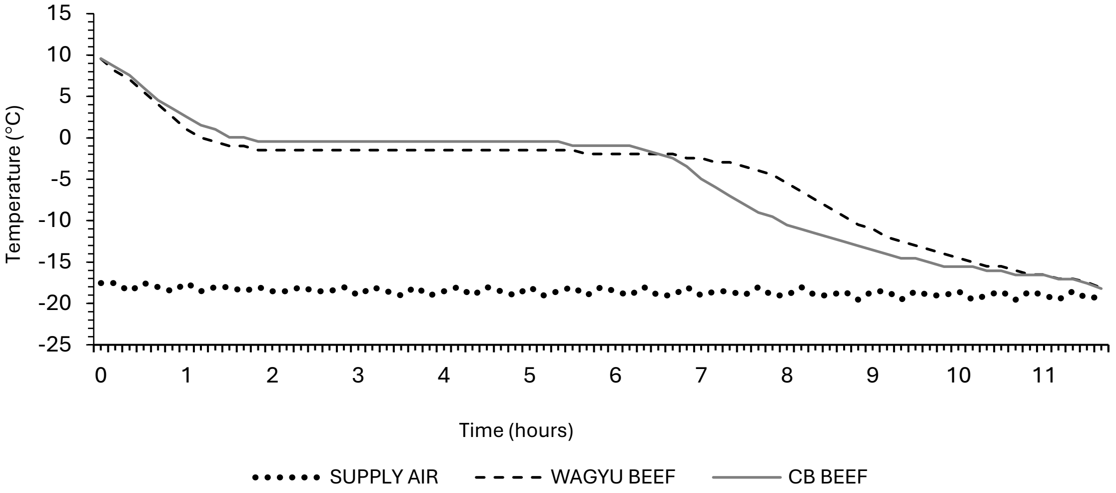


Figure 7: Changes in the core temperature of beef samples during slow freezing

4.3 Freezing curve kinetics analysis

Fast freezing

The temperature decline for the Wagyu and CB beef samples, as well as the constant temperature of the supply air, are displayed in the temperature curve in each phase of freezing. Throughout the freezing process, the supply air remained at a constant temperature of about -30 °C, which ensured that the meat samples' heat was effectively removed. During the initial precooling phase, the temperature of Wagyu and CB beef declined very fast, the readings dropped from around 5 °C to just below 0 °C in only 30 minutes (Figure 8). The cooling rate was computed to be 10 °C per hour for either type of beef within this period. This precipitous fall in temperature indicates that there was a high rate of heat transfer from the meat to the surrounding cold air; such a rate of heat transfer would reduce the formation of large ice crystals, thereby maintaining meat quality (Leygonie, Britz, & Hoffman, 2012).

For Wagyu beef, the cooling phase was modeled with an initial temperature of 8.588 °C and a final temperature of -0.935 °C. The cooling rate constant k was determined to be approximately 1, resulting in the equation (3):

$$T(t) = -0.935 + 9.523e^{-1t} \tag{3}$$

Similarly, for crossbred beef, the initial temperature was 8.548 °C and the final temperature was -1.469 °C, with a rate constant of 1, yielding the equation (4):

$$T(t) = -1.469 + 10.017e^{-1t} \tag{4}$$

The next crucial phase on the freezing curve is the phase change phase, during which meat transitions to solid form. This phase emerges in both Wagyu and CB beef between 1.25 and 2.25 hours, with a duration of about 1 hour (Figure 9). At this point, the temperature fluctuated from 0 °C to -5 °C, and the latent heat of fusion was absorbed with essentially little temperature change. When the foregoing change of phase happens, while the temperature of the beef remains relatively constant, the water in it transitions from liquid to solid. This is produced at a constant temperature, indicating that energy is absorbed as latent heat of fusion. This temperature is roughly -0.935 °C for Wagyu beef and -1.469 °C for crossbred beef. The duration of this phase is critical for producing little ice crystals that aid in the preservation of the meat's texture and structural integrity (Delgado & Sun, 2000; Leygonie, Britz, & Hoffman, 2012; Sun, 2016).

Following the phase change phase, the last chilling phase, commonly referred to as the subcooling phase, consisted of another temperature drop from -5 °C to -25 °C over the course of 2.5 hours (Figure 10). At this stage, both Wagyu and CB beef were cooled at 8 °C each hour. This phase describes the ongoing removal of sensible heat until the samples meet the temperature of the supply air, which ensures full freezing (Delgado & Sun, 2000; Sun, 2016).

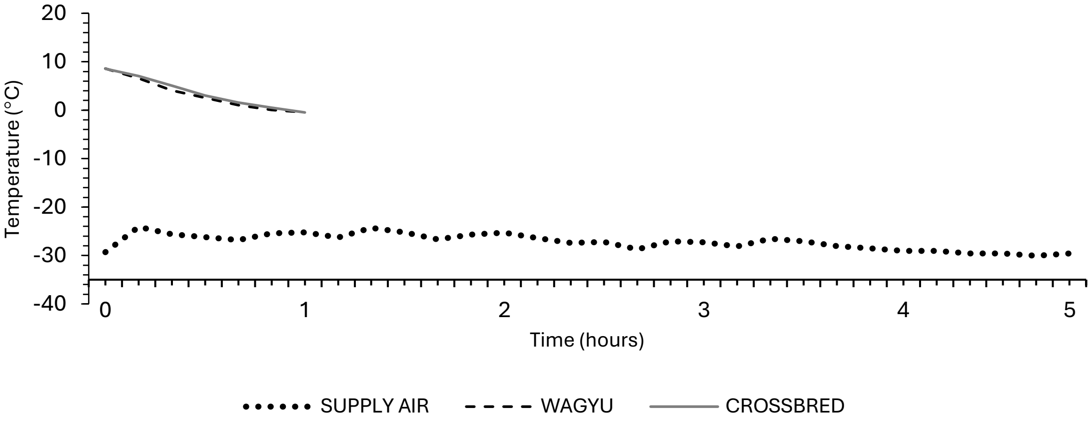


Figure 8: Precooling phase during fast freezing of Wagyu and crossbred beef

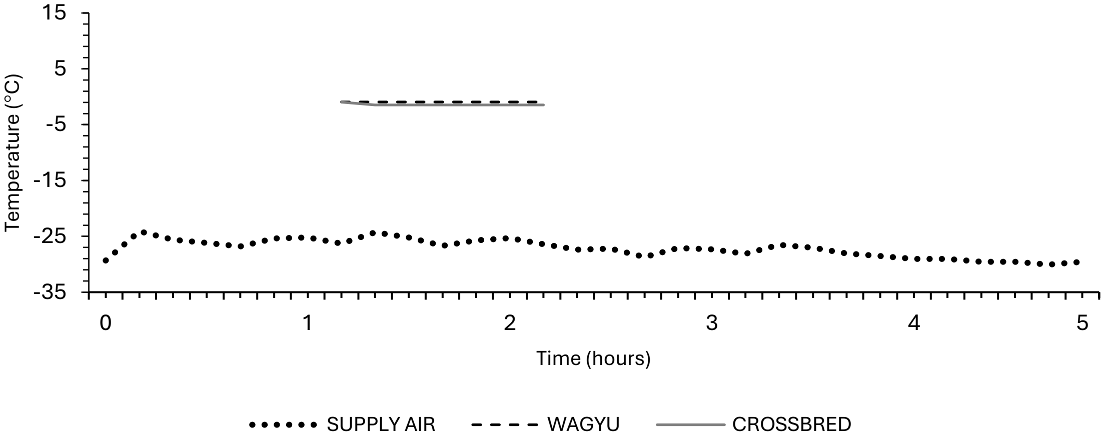


Figure 9: Phase change phase during fast freezing of Wagyu and crossbred beef

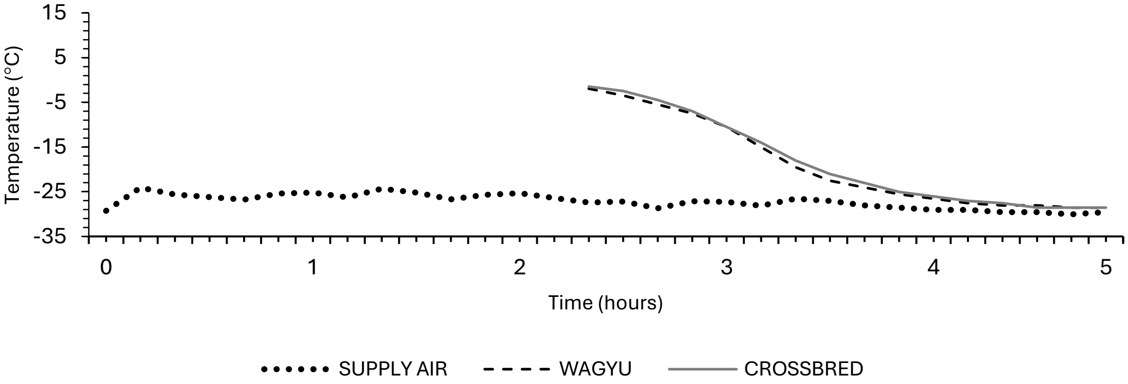


Figure 10: Subcooling phase during fast freezing of Wagyu and crossbred beef

For Wagyu beef, the sub-cooling phase was modeled with a final temperature of -0.935 °C and a storage temperature of -28.553 °C. The rate constant k was determined to be approximately 1, resulting in the equation (5):

$$T(t)=-28.553+27.618e^{-1(t-2)} \tag{5}$$

For crossbred beef, the final temperature was -1.469 °C and the storage temperature was -28.552 °C, with a rate constant of 1, yielding the equation (6):

$$T(t)=-28.552+27.083e^{-1(t-2)} \tag{6}$$

Slow freezing

Slow freezing was mathematically modeled in the same way as fast freezing, which was described above. In this scenario, the phase kinetics of Wagyu and crossbred beefs were studied to describe temperature variations over time. The slow freezing process, like fast freezing, is typically thought to occur in three major phases of interest: the precooling phase, the phase change phase, and the subcooling phase. To simulate each step, appropriate mathematical functions were used to produce rate equations that shed light on the beef's behavior during freezing. During the initial precooling process, both types of beef see a significant temperature reduction (Figure 11). Wagyu meat cools somewhat faster. This stage refers to the elimination of perceptible heat when the meat temperature approaches freezing. The large temperature difference between the meat and the supply air is a common cause of such fast-cooling rates. To represent this cooling phenomenon, an exponential decay function was used. This would be realistic, given the significant temperature difference between the beef and the cooling environment. The equation (7) describes the cooling phase rate for Wagyu beef:

$$T(t)=-1.469+11.018e^{-1t} \tag{7}$$

The equation demonstrates that the rate of temperature decrease for Wagyu beef is initially quick but gradually slows down as it approaches the freezing point. The rate constant, shown as $k = 1$, is directly proportional to the Wagyu beef's temperature behavior. On the other hand, the rate equation (8) describes the precooling phase for crossbred beef is:

$$T(t)=-0.463+10.035e^{-1t} \tag{8}$$

During this phase, meat from both beef breeds experiences a phase shift from liquid water to ice. The temperature remains practically constant because the removal of energy is used to modify the condition of the water within the meat rather than to lower its temperature. This phase is critical for ensuring that little ice crystals form, which aids in meat quality preservation. To keep things simple, this phase was approximated as a constant temperature. The temperature for Wagyu beef is roughly -1.469 °C, while crossbred beef is around -0.463 °C (Figure 12). The production of tiny ice crystals relies heavily on the constant temperature phase. These tiny crystals improve the texture and structure of meat (Leygonie, Britz, & Hoffman, 2012; Sun, 2016).

After the phase transition, the beef enters the subcooling phase, where it continues to cool until it reaches the appropriate storage temperature (Figure 13). The temperature of the beef drops during the sub-cooling process as it approaches the final storage temperature. This phase has a lower freezing rate than the first cooling phase. Crossbred beef cools slightly faster (2.77 °C/hour) than Wagyu beef (2.58 °C/hour). This phase is also represented by an exponential decay function, which describes the continued decline in temperature. Wagyu beef's sub-cooling phase rate equation (9) is as:

$$T(t) = -18.12 + 16.15e^{-0.646(t-6)} \tag{9}$$

This equation shows that after the initial phase change, the temperature of Wagyu beef drops from -1.469 °C to approximately -17.01 °C over a period of 6 hours. For crossbred beef, the sub-cooling phase rate equation (10) is:

$$T(t) = -18.21 + 17.245e^{-0.69(t-6)} \tag{10}$$

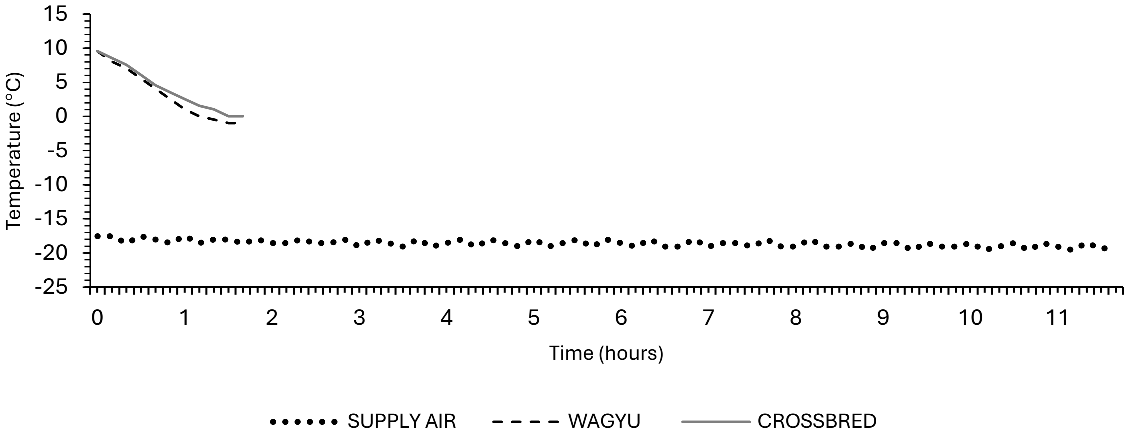


Figure 11: Precooling phase during slow freezing of Wagyu and crossbred beef

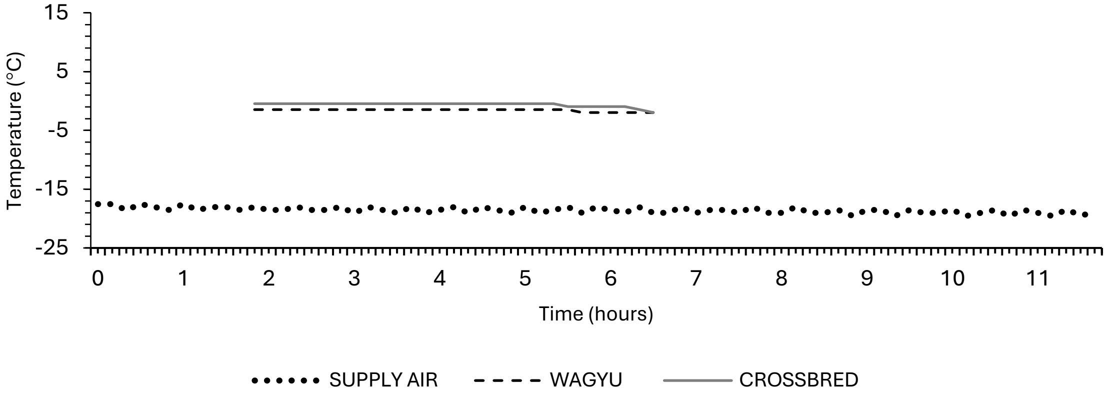


Figure 12: Phase changing phase during slow freezing of Wagyu and crossbred beef

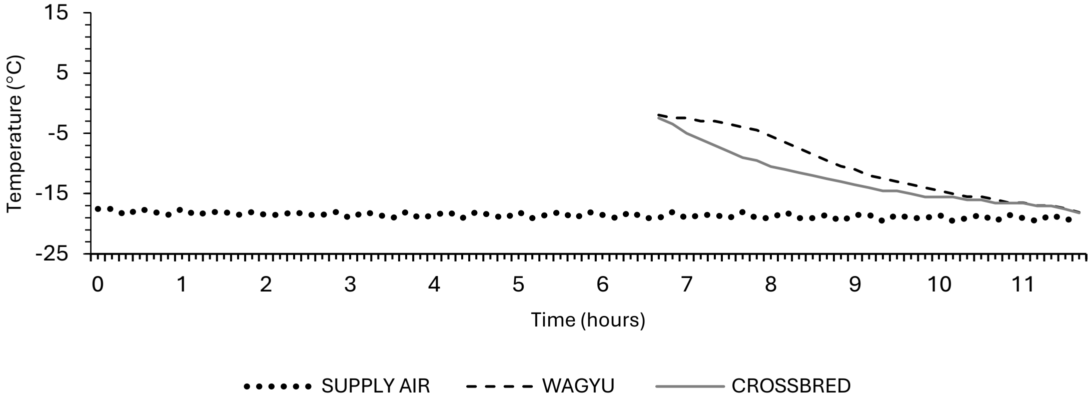


Figure 13: Subcooling phase during slow freezing of Wagyu and crossbred beef

The comprehensive mathematical modeling of Wagyu and crossbred beef freezing kinetics reveals important information about their thermal behavior during the freezing process. From the results of modeling the precooling phases of fast freezing, it is clearly indicated that the rate constants (k = 1) are the same for both beef breeds, but the exponential terms indicate that crossbred beef cools slightly faster due to the slightly bigger pre-exponential factor (10.017 vs. 9.523). The rate constants also show that (0.646 for Wagyu and 0.69 for crossbred) crossbred beef cools slightly more quickly, and the pre-exponential components also suggest a faster cooling rate for crossbred beef (17.245 vs. 16.15) during the subcooling phase of the slow freezing process. Therefore, in both fast and slow freezing methods, crossbred beef cools slightly faster than Wagyu beef. This attribute was due to variations in thermal conductivity and specific heat capacity caused by the variation in fat and moisture content in the meats of different breeds, which is also in good agreement to the moisture and fat content analysis (Delgado & Sun, 2000; Gotoh et al., 2018; Leygonie, Britz, & Hoffman, 2012).

4.4 Meat quality measurements

The three important factors by which consumers judge meat quality are appearance, color, and flavor (Faustmen et al., 1990). Assessing the quality of meat is a crucial aspect of the food industry, as it directly impacts consumer satisfaction, product value, and overall market acceptance. In the next sections, the effect of different regimens of fresh FR, FF, SF, and type of beef (Wagyu, crossbred) on various meat quality parameters (pH, color, WBSF, thaw loss, cook loss) for uncooked and cooked meat samples will be addressed. Table 3 below shows a two-way ANOVA for the pH, WBSF, thaw loss, and cook loss of the meat samples.

Table 3: Effects of freezing rates and breed type on pH, meat tenderness, thaw loss, and cooking loss for uncooked and cooked meat samples.

	Freezing rate / breed							Significance		
	Fresh (Wagyu)	*Fresh (crossbred)*	*Fast frozen (Wagyu)*	*Fast frozen (crossbred)*	*Slow frozen (Wagyu)*	*Slow Frozen (crossbred)*	*SEM*	*Freezing rate*	*Breed*	*Freezing rate x breed*
pH										
uncooked	5.54	5.49	5.58	5.49	5.59	5.45	0.0143	ns	<0.0001	<0.05
cooked	5.92	5.73	5.84	5.78	5.85	5.8	0.0128	ns	<0.0001	<0.05
WBSF										
cooked	37.6	44.11	34.97	35.98	28.22	33.92	0.496	<0.0001	<0.0001	<0.05
Thaw loss (%)										
uncooked	NA	NA	3.65	6.22	4.2	7.01	0.075	<0.05	<0.0001	ns
Cook loss (%)										
cooked	22.13	20.74	21.52	19.35	21.95	24.33	0.243	<0.0001	ns	<0.0001

WBSF (Warner-bratzler shear force), 'ns' (not significant), <0.05 (significant), NA (not applicable). Each parameter is presented with its standard error of the mean (SEM)

4.4.1 pH

Both uncooked and cooked Wagyu beef have a significantly ($p < 0.0001$) higher pH than their crossbred meat counterparts (Table 3). The statistical study indicates that freezing has no significant effect on the meat pH of both breeds. The interaction between freezing rate and breed is significant ($p < 0.05$), indicating that the effect of freezing rate on pH differs between the two breeds. Another important point to mention is that an increase in pH was observed upon cooking for both breeds (Figure 14). This might be due to the denaturation of muscle proteins during sous vide cooking at low temperatures for a long time. Denatured proteins lead to more amino group exposure, which is usually basic in nature, thereby increasing meat pH (Tornberg, 2005).

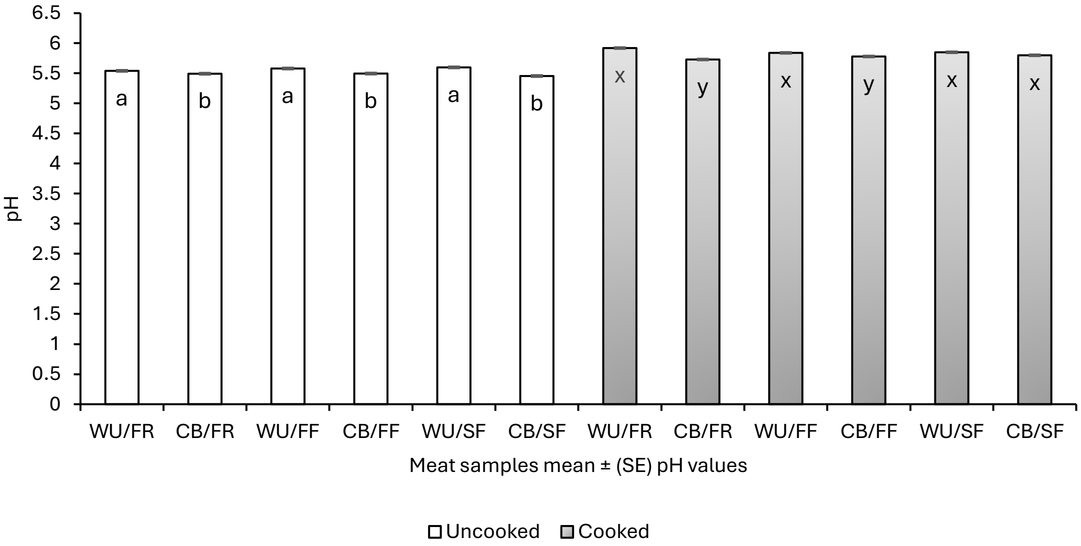


Figure 14: pH values of uncooked and cooked beef samples, (WU, Wagyu; CB, Crossbred; FR, Fresh; FF, Fast-frozen; SF, Slow-frozen)

*Means with same letter within cooked or uncooked samples did not differ significantly ($p < 0.05$)

4.4.2 Warner-bratzler shear force (WBSF)

WBSF, or warner-bratzler shear force, is a measurement of meat softness. This component was lower in Wagyu across all freezing settings (fast and slow frozen), indicating more delicate meat than crossbred. WBSF is a measure of meat softness; lower values indicate more tender meat. Freezing has a substantial influence on WBSF ($p < 0.0001$) for Wagyu as well as crossbred beef samples, indicating that meat tenderness increases after freezing (Table 3). Wagyu meat is tender than its crossbred meat counterpart ($p < 0.0001$), indicating a substantial effect of breed type. The interaction between freezing rate and breed is significant ($p < 0.05$), indicating that freezing rate has a different impact on tenderness between the two breeds. Wagyu samples have WBSF values of 37.6 (FR), 34.97 (FF), and 28.22 (SF). Crossbred samples have WBSF values of 44.11 (FR), 35.98 (FF), and 33.92 (SF). It can be noted that slow frozen samples (Wagyu and crossbred) have the lowest WBSF values (Figure 15). This can be attributed to the formation of large ice crystals during slow freezing that disrupt muscle structure, leading to more tender meat (Leygonie, Britz, & Hoffman, 2012).

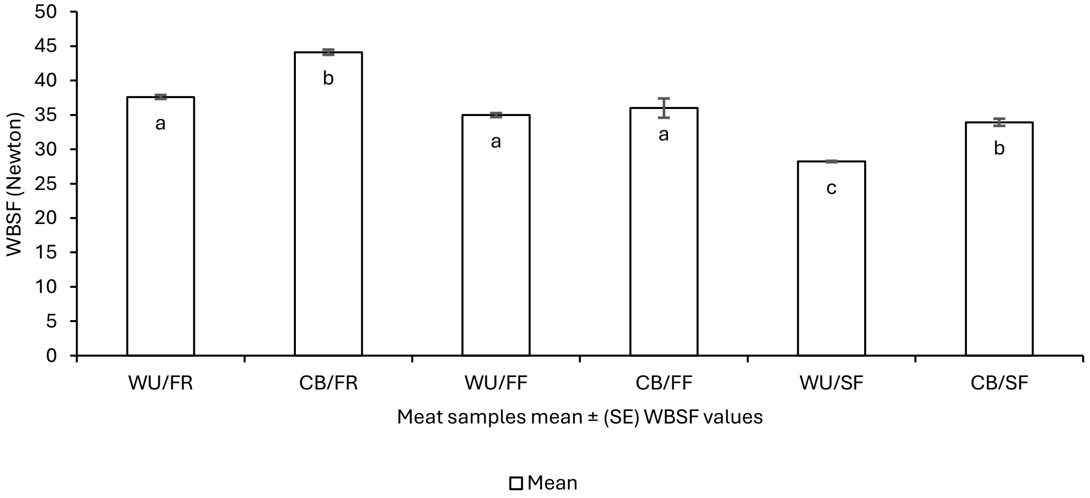


Figure 15: Warner-bratzler shear force (WBSF) values of cooked beef samples (WU, Wagyu; CB, Crossbred; FR, Fresh; FF, Fast-frozen; SF, Slow-frozen)

*Means with same letter did not differ significantly ($p < 0.05$)

4.4.3 Thaw loss (%)

Thaw loss, which is the loss of moisture during thawing, is only measured in frozen samples of uncooked beef. Thaw loss solely affects frozen samples of raw meat. The statistical analysis (Table 3) shows that freezing rate has a substantial effect on thaw loss ($p < 0.05$). Crossbred meat loses more moisture during thawing than Wagyu ($p < 0.0001$). The interaction between freezing rate and breed is not significant (ns), indicating that the impact of freezing rate on thaw loss is consistent across breeds. FF Wagyu's thaw loss is 3.65 %, while crossbred's is 6.22 %. The thaw loss for SF Wagyu is 4.2 %, while crossbred is 7.01 % (Figure 16). It can be noted that SF samples of Wagyu as well as crossbred meats have high thaw loss due to the formation of larger ice crystals that cause more extensive damage to the fibers, thereby higher thaw loss (Leygonie, Britz, & Hoffman, 2012).

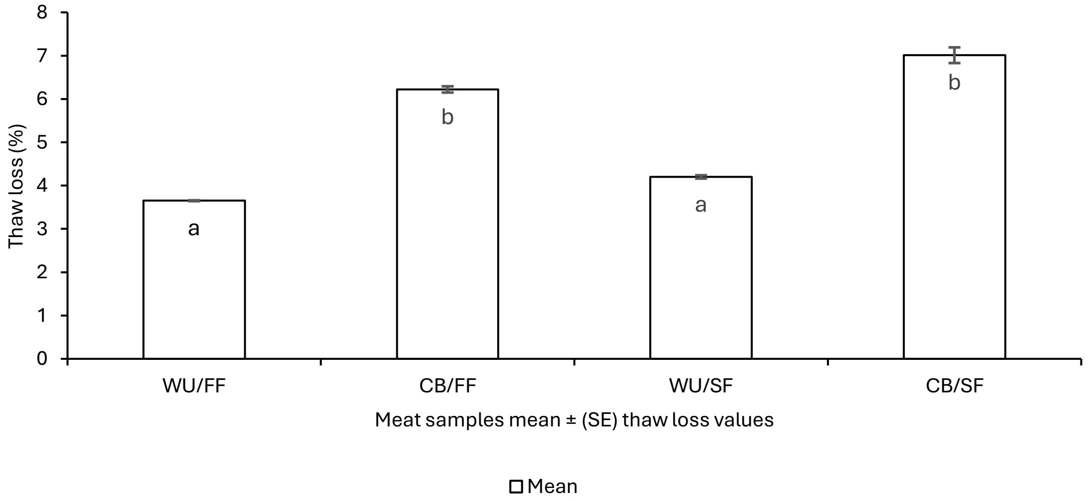


Figure 16: Thaw loss (%) of uncooked beef samples (WU, Wagyu; CB, Crossbred; FR, Fresh; FF, Fast-frozen; SF, Slow-frozen)

*Means with same letter did not differ significantly ($p < 0.05$)

4.4.4 Cook loss (%)

Cooking loss, which measures moisture loss during cooking, produced mixed findings in the cooked beef. The freezing rate significantly affects cooking loss (Table 3), with $p < 0.0001$, demonstrating that differing freezing rates influence the amount of liquid lost during cooking. The breed type has no significant effect on cooking loss ($p > 0.05$), implying that it does not have a major impact. The interaction between freezing rate and breed is extremely significant ($p < 0.0001$), demonstrating that the effect of freezing rate on cooking loss varies between the two breeds. The cooking loss for Wagyu samples is 22.13 % (FR), 21.52 % (FF), and 21.95 % (SF). The cooking loss in crossbred samples is 20.74 % (FR), 19.35 % (FF), and 24.33 % (SF). Figure 17 clearly shows that crossbred slow frozen samples had the highest cook loss. This clearly confirms the formation of large ice crystals that damage cell membranes and muscle fibers, releasing more water upon cooking (Leygonie, Britz, & Hoffman, 2012).

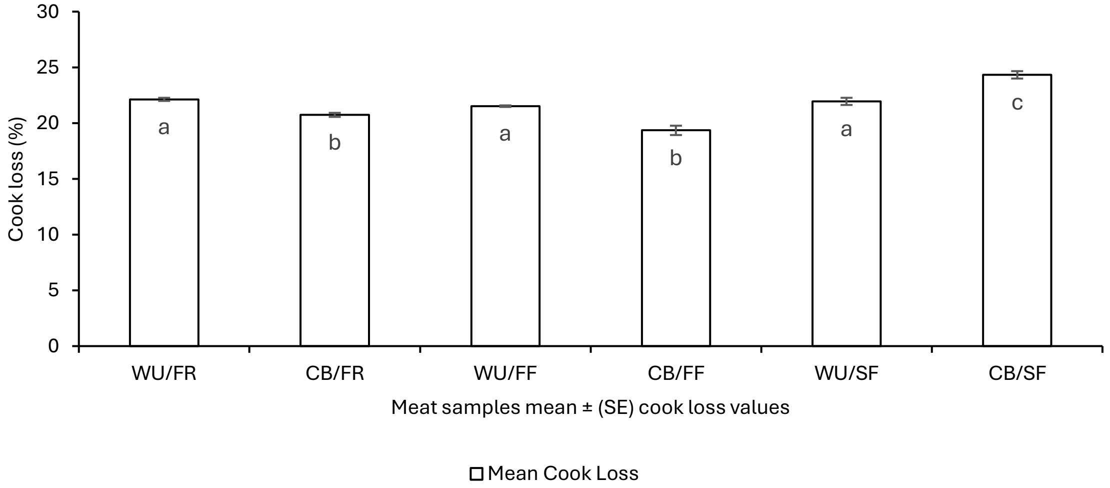


Figure 17: Cook loss (%) values of cooked beef samples (WU, Wagyu; CB, Crossbred; FR, Fresh; FF, Fast-frozen; SF, Slow-frozen)

*Means with same letter did not differ significantly ($p < 0.05$)

Thus, the breed type (Wagyu vs. crossbred) has a significant impact on all measured parameters, frequently resulting in more desirable features in Wagyu meat, such as lower thaw loss and lower WBSF, indicating more softness. The freezing rate has an impact on thaw loss and cook loss, with faster freezing generally preferable. The interaction between freezing rate and breed type is significant for pH in cooked samples, and cook loss, showing that the combined effects of these parameters may differ based on the specific parameter and meat state.

4.4.5 Color parameters

A statistical study (Table 4) shows that freezing rate and breed have a considerable effect on the color parameters, with breed displaying the most significant disparities across all parameters. A more detailed analysis of each parameter is provided in the following sections.

Table 4: Effects of freezing rates and breed type on color parameters for uncooked and cooked meat samples

	Freezing rate / breed							Significance		
	Fresh (Wagyu)	*Fresh (crossbred)*	*Fast frozen (Wagyu)*	*Fast frozen (crossbred)*	*Slow frozen (Wagyu)*	*Slow frozen (crossbred)*	SEM	*Freezing rate*	*Breed*	*Freezing Rate x breed*
Lightness (L*)										
uncooked	38.58	34.07	35.37	29.97	38.09	33.93	0.5	<0.05	<0.0001	ns
cooked	56.66	51.99	51.43	45.25	52.25	51.58	0.238	<0.0001	<0.0001	<0.0001
Redness (a*)										
uncooked	21.78	19.33	12.37	18.12	12.36	18.35	0.546	<0.0001	<0.0001	<0.0001
cooked	23.04	18.71	18.75	15.85	15.35	19.07	0.28	<0.0001	<0.0001	<0.0001
Yellowness (b*)										
uncooked	11.87	8.29	10.09	7.77	13.1	8.93	0.258	<0.05	<0.0001	ns
cooked	18.04	13.89	14.19	10.13	12.51	14.3	0.188	<0.0001	<0.0001	<0.0001
Chroma										
uncooked	24.86	20.93	15.96	19.66	18.06	20.38	0.573	<0.0001	<0.0001	<0.0001
cooked	29.26	23.3	23.51	18.69	19.79	23.84	0.285	<0.0001	<0.0001	<0.0001
Hue angle										
uncooked	28.53	23.19	38.98	23.06	46.65	25.73	0.475	<0.0001	<0.0001	<0.0001
cooked	38.06	36.6	37.1	32.6	39.17	36.85	0.385	<0.0001	<0.0001	<0.05

'ns' (not significant), <0.05 (significant). Each parameter is presented with its standard error of the mean (SEM)

4.4.5.1 Lightness (L*)

Statistical analysis indicates a significant effect (Table 4) of freezing rate on lightness ($p < 0.05$). Wagyu meat is lighter than crossbred meat, as indicated by the very significant influence of breed type ($p < 0.0001$). The interaction between freezing rate and breed is not statistically significant (ns), indicating that the effect of freezing rate on lightness is constant across breeds. Uncooked Wagyu samples show L* values of 38.58 (FR), 35.37 (FF), and 38.09 (SF). Crossbred samples have L* values of 33.93 (SF), 29.97 (FF), and 34.07 (FR). For cooked meat, Wagyu samples have L* values of 56.66 (FR), 51.43 (FF), and 52.25 (SF). Crossbred samples have a L* value of 51.99 (FR), 45.25 (FF), and 51.58 (SF). The freezing rate has a substantial effect on brightness ($p < 0.0001$). Cooked Wagyu meat is lighter than crossbred meat, indicating a substantial effect of breed type ($p < 0.0001$). The interaction between freezing rate and breed is very significant ($p < 0.0001$), suggesting that the influence of freezing rate on lightness differs between the two breeds. Figure 18 shows that the lightness values increased after sous vide cooking. This is due to protein denaturation, which increases the reflectivity of the meat surface, making it appear lighter (Baldwin, 2012).

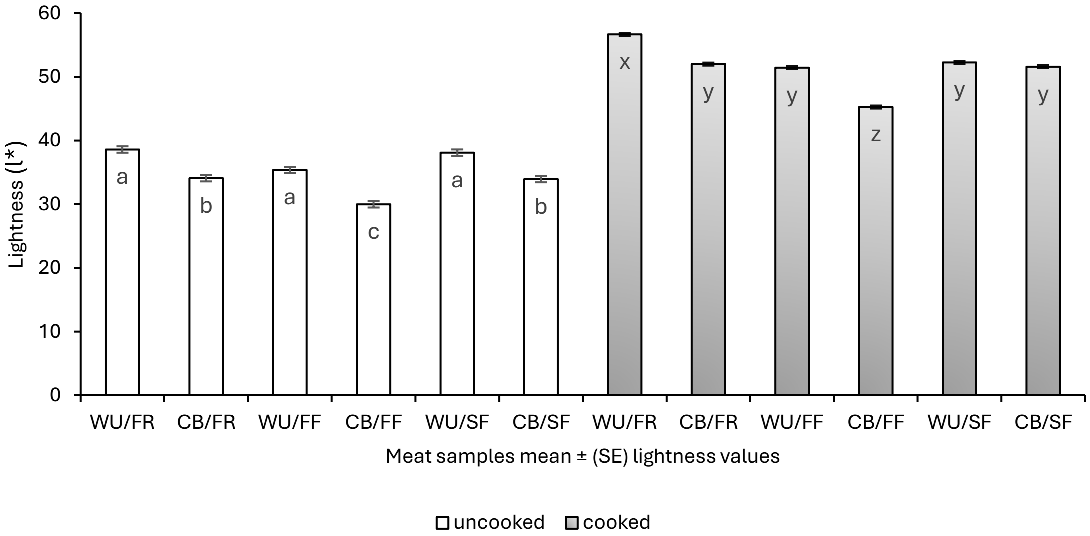


Figure 18: Lightness (L*) values of uncooked and cooked beef samples, (WU, Wagyu; CB, Crossbred; FR, Fresh; FF, Fast-frozen; SF, Slow-frozen)

*Means with same letter within cooked or uncooked samples did not differ significantly. ($p < 0.05$)

4.4.5.2 Redness (a*)

Redness (a*) is a measure of the intensity of the red color in meat. Wagyu samples with uncooked meat have a* values of 21.78 (FR), 12.37 (FF), and 12.36 (SF). Crossbred samples show a* values of 19.33 (FR), 18.12 (FF), and 18.35 (SF). The freezing rate significantly affects redness ($p < 0.0001$), according to the statistical study (Table 4). Wagyu beef is typically redder than crossbred meat, as evidenced by a highly significant impact ($p < 0.0001$). The interaction between freezing rate and breed is very significant ($p < 0.0001$), indicating that the impact of freezing rate on redness differs between breeds. Wagyu samples with cooked beef have a* values of 23.04 (FR), 18.75 (FF), and 15.35 (SF). Crossbred samples show a* values of 18.71 (FR), 15.85 (FF), and 19.07 (SF). The redness of cooked meat is significantly affected by freezing rate, breed type, and their interactions (p < 0.0001), indicating complex interactions between these parameters. Figure 19 clearly shows that each sample's redness changes slightly after cooking. The vacuum-sealed environment during sous vide cooking stabilizes myoglobin, the protein responsible for meat color, which maintains and enhances the red color of the meat by reducing oxidation (Roldán et al., 2013).

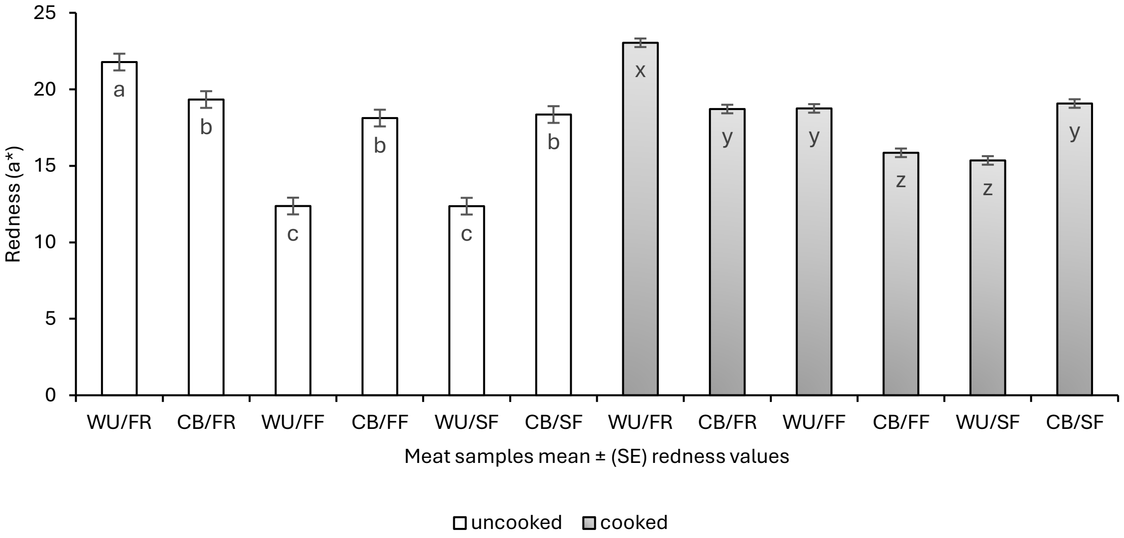


Figure 19: Redness (a*) values of uncooked and cooked beef samples, (WU, Wagyu; CB, Crossbred; FR, Fresh; FF, Fast-frozen; SF, Slow-frozen)

*Means with same letter within cooked or uncooked samples did not differ significantly (p < 0.05)

4.4.5.3 Yellowness (b*)

Yellowness (b*) refers to the intensity of the yellow hue in meat. A statistical study indicates a significant effect (Table 4) of freezing rate on yellowness (p < 0.05). Wagyu meat is often yellower than crossbred meat, as seen by the very significant influence of breed type (p < 0.0001). The interaction between freezing rate and breed is not statistically significant (ns), indicating that freezing rate has a consistent influence on yellowness across breeds. Wagyu uncooked beef samples have b* values of 11.87 (FR), 10.09 (FF), and 13.1 (SF). Crossbred samples exhibit b* values of 8.29 (FR), 7.77 (FF), and 8.93 (SF). For cooked beef, Wagyu samples have b* values of 18.04 (FR), 14.19 (FF), and 12.51 (SF). Crossbred samples' b* values are 13.89 (FR), 10.13 (FF), and 14.3 (SF). The yellowness of cooked meat is significantly influenced by freezing rate, breed type, and their interaction ($p < 0.0001$), indicating complex interactions between these factors. Figure 20 clearly shows that yellowness values increased for all samples after sous vide cooking. Moisture retention plays a crucial role during sous vide cooking because it prevents the loss of juices and lipids, thereby enhancing the yellowness of the meat samples (Tornberg, 2005).

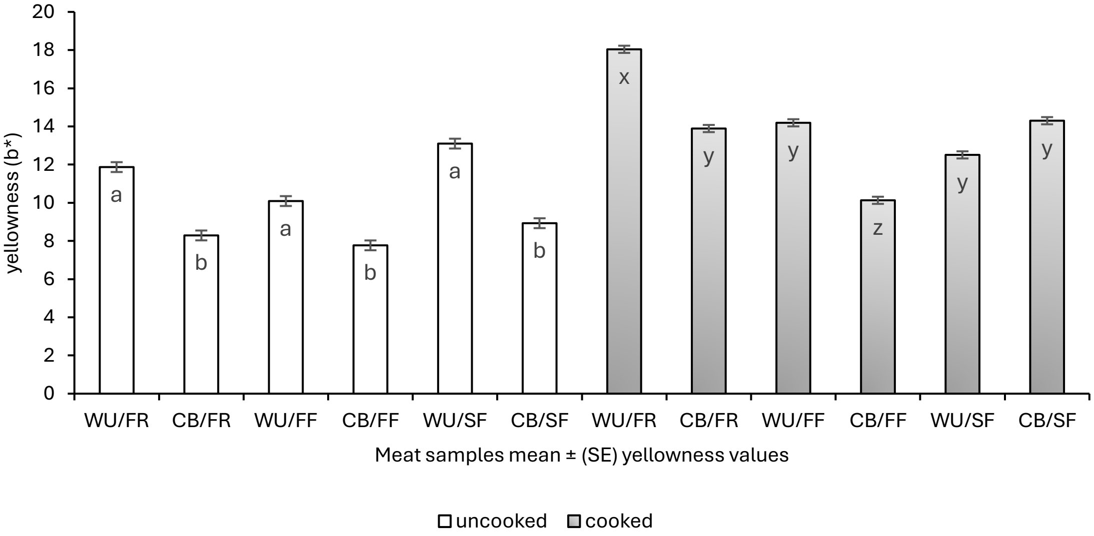


Figure 20: Yellowness (b*) values of uncooked and cooked beef samples, (WU, Wagyu; CB, Crossbred; FR, Fresh; FF, Fast-frozen; SF, Slow-frozen)

*Means with same letter within cooked or uncooked samples did not differ significantly ($p < 0.05$)

4.4.5.4 Chroma

The color's vividness or saturation is quantified by chroma. The statistical analysis demonstrates that the freezing rate, breed type, and their interaction have very significant effects on chroma (Table 4), with $p < 0.0001$, indicating that these parameters alter the color saturation of raw meat. Wagyu samples with uncooked meat have chroma values of 24.86 (FR), 15.96 (FF), and 18.06 (SF). Chroma levels in crossbred samples are 20.93 (FR), 19.66 (FF), and 20.38 (SF). Wagyu samples with cooked beef have chroma values of 29.26 (FR), 23.51 (FF), and 19.79 (SF). Chroma values for crossbred samples range from 23.3 (FR) to 18.69 (FF) and 23.84 (SF).

4.4.5.5 Hue angle

The hue angle measures the overall color tone, with higher values indicating a preference for yellow-green hues. The statistical research shows that freezing rate, breed type, and their interaction have highly significant effects (Table 4) on hue angle ($p < 0.0001$), indicating that these factors greatly modify the color tone of raw meat. Both freezing rate and breed type had substantial effects on hue angle ($p < 0.0001$), and the interaction between freezing rate and breed is significant ($p < 0.05$), suggesting that these parameters interact to alter the color tone of cooked meat. Figures 21 and 22 signify that even cooking achieved through sous vide cooking leads to less surface browning and preserves the natural colors of the meat, leading to more vivid color parameters (Baldwin, 2012).

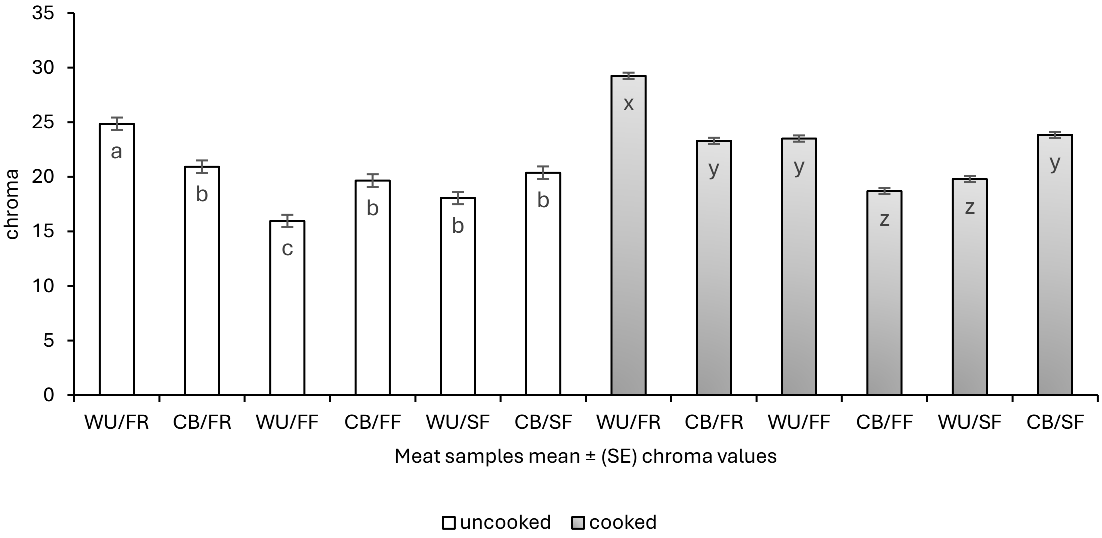


Figure 21: Chroma values of uncooked and cooked beef samples, (WU, Wagyu; CB, Crossbred; FR, Fresh; FF, Fast-frozen; SF, Slow-frozen)

*Means with same letter within cooked or uncooked samples did not differ significantly ($p < 0.05$)

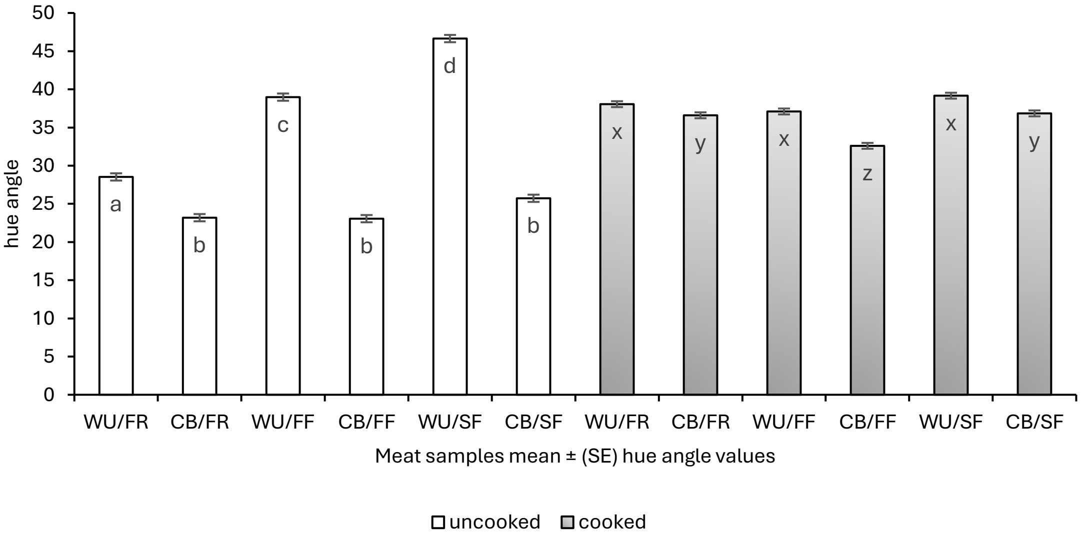


Figure 22: Hue angle values of uncooked and cooked beef samples, (WU, Wagyu; CB, Crossbred; FR, Fresh; FF, Fast-frozen; SF, Slow-frozen)

*Means with same letter within cooked or uncooked samples did not differ significantly ($p < 0.05$)

4.4.6 Muscle structure analysis

The Wagyu and crossbred rump meat samples after each freezing/thawing regimen were cut to view muscle fibers transversely under a light microscope. To check for structural changes transversely, the uncooked samples were cut along the muscle fibers. The Wagyu and crossbred beef light microscopy images show different colors due to different staining techniques. The microscopy images of Wagyu beef showed a dense network of fine muscle fibers (Figure 23). The tightly packed muscle fibers and minimal interstitial space indicate high muscle density, which is often associated with better beef texture. In contrast, in the microscopy images of crossbred beef, the muscle fibers appeared slightly looser than Wagyu. The fresh samples of both breeds did not show any tissue damage. There was very little difference between fresh and FF samples. There was minimal disruption of muscle fibers in FF samples. On the other hand, in SF samples, there was a high disruption of muscle fibers.

The microscopy images show the structural differences between Wagyu and crossbred beef under different freezing treatments. Wagyu beef always showed a higher density of fine muscle fibers, which means better meat quality. Wagyu beef is well-documented to exhibit a higher density of fine muscle fibers, which is a significant factor contributing to its superior meat quality (Gotoh et al., 2018). On the other side, crossbred beef, which tends to have larger, less densely packed muscle fibers, results in a different texture and potentially less tenderness (Nishimura, 2010). Freezing methods affect both types of beef's muscle structure. Fast freezing causes less structural damage than slow freezing and preserves the meat better.

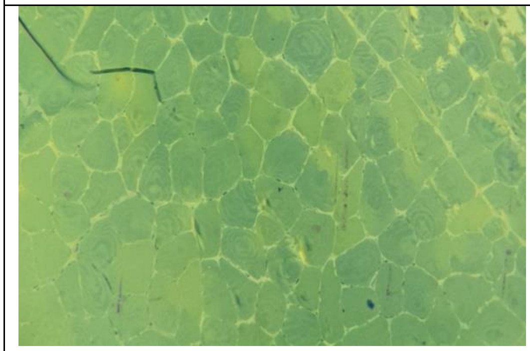
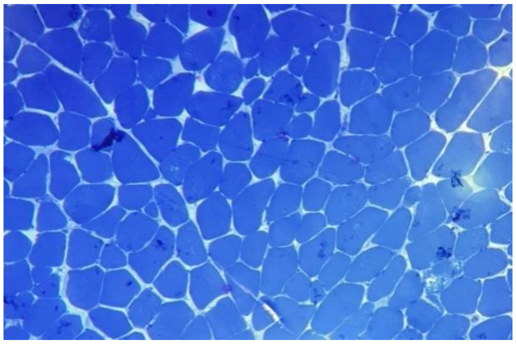
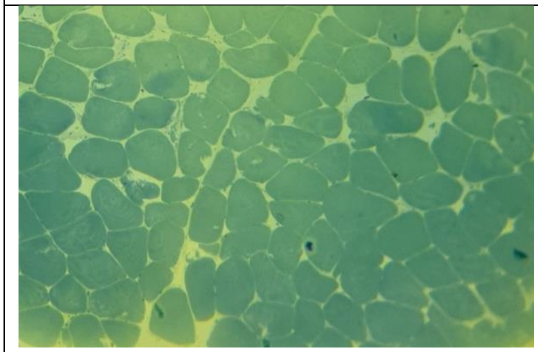
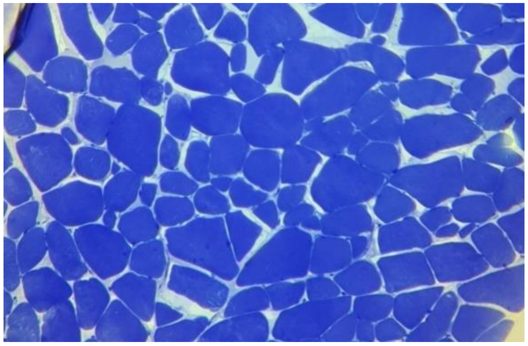
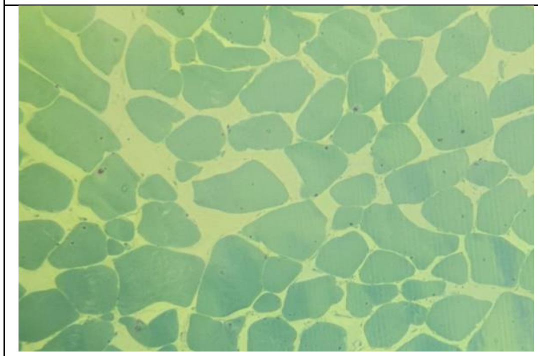
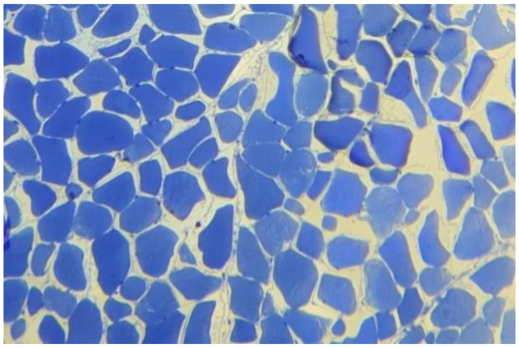
Wagyu	**Crossbred**
	
Fresh (FR)	*Fresh (FR)*
	
Fast frozen (FF)	*Fast Frozen (FF)*
	
Slow Frozen (SF)	*Slow frozen (SF)*

Figure 23: Light microscopy images of Wagyu and crossbred uncooked beef (FR/FF/SF).

Transmission electron microscopy (TEM) offers a detailed view of the ultrastructural characteristics of muscle tissues, providing critical insights into the effects of different freezing regimes on meat quality. This section presents an analysis of longitudinal TEM images of Wagyu and crossbred beef muscle fibers under fresh, fast frozen-thawed, and slow frozen-thawed conditions, highlighting the structural differences and their implications for meat quality attributes such as tenderness, juiciness, and texture (Figure 24). The TEM images of longitudinal sections of fresh Wagyu beef reveal a highly organized sarcomere structure with uniform Z-lines and well-aligned myofibrils. The sarcomeres appeared shorter, and the muscle fibers were smaller in comparison to the crossbred beef, contributing to the dense muscle fiber arrangement. Smaller muscle fibers are associated with increased tenderness (Calkins, & Sullivan, 2007). In contrast, the fresh crossbred beef TEM images show more variability in sarcomere length and organization.

The TEM images of FF Wagyu and crossbred beef demonstrate minimal disruption to the sarcomere structure as compared to SF samples. Fast freezing leads to the formation of small ice crystals, which cause limited damage to the myofibrils and Z-lines. The integrity of the muscle fibers is largely preserved. The TEM images of SF Wagyu and crossbred beef show significant structural damage due to the formation of larger ice crystals during the prolonged freezing process. These ice crystals disrupt the sarcomere structure, leading to noticeable ruptures in the myofibrils and misalignment of the Z-lines. The muscle fiber structures of the SF-TH samples were uneven with sarcomere dislocation and Z-line disintegration. The A-bands, I-bands, and Z-lines in Figure 24 are obvious. The overall integrity of the muscle fibers is compromised, leading to significant textural degradation.

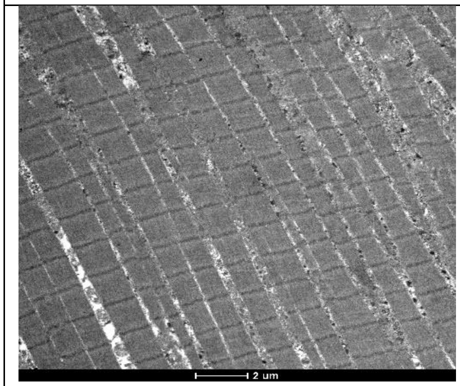
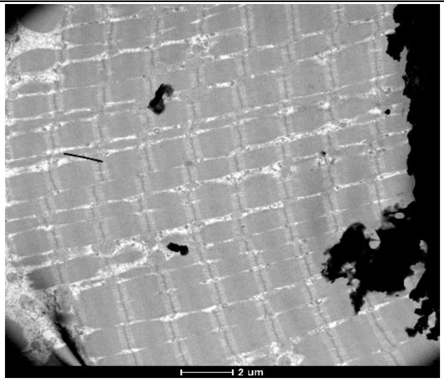
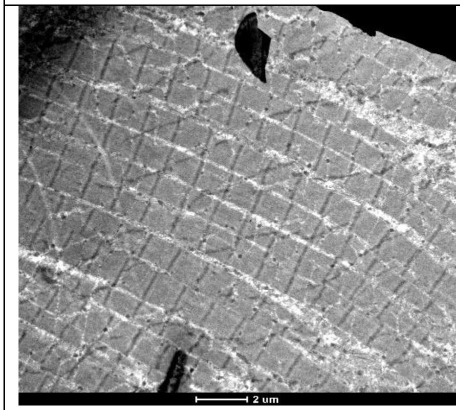
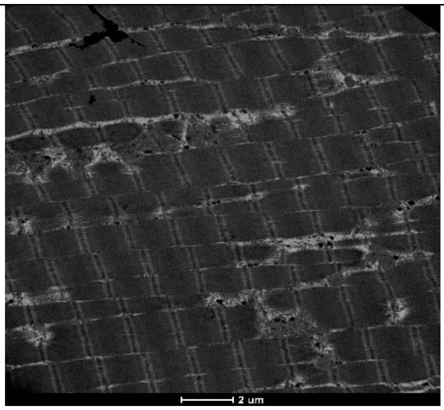
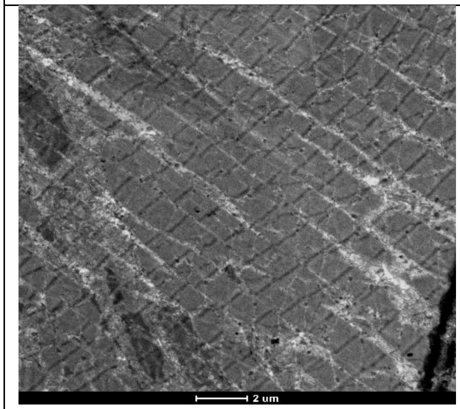
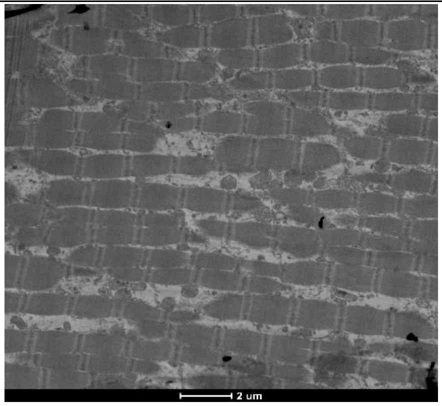
Wagyu	**Crossbred**
	
Fresh (FR)	*Fresh (FR)*
	
Fast frozen (FF)	*Fast Frozen (FF)*
	
Slow Frozen (SF)	*Slow frozen (SF)*

Figure 24: TEM images of Wagyu and crossbred uncooked beef (FR/FF/SF)

5. Discussion

This study explored the impact of different freezing rates and breed types on the quality attributes of meat, including moisture and fat composition, warner-bratzler shear force (WBSF), pH levels, cook loss, color parameters, and thaw loss. The freezing curve kinetic analysis was conducted to get insight into the thermal behavior of different beef breeds, specifically in terms of their moisture and fat composition. The insights aid in optimizing strategies for meat preservation as well as the preparation to inculcate desirable attributes like moisture retention, tenderness, and color in the meat. Earlier investigations have also been carried out in this domain to analyze the impact of breed types and the rate of freezing meat on its quality attributes to offer a solid foundation on the influence of these factors on the pH levels, tenderness, water holding capacity, and color of the meat (Dang et al., 2021; Santos et al., 2021).

Studies carried out by scientists like Dang et al. (2021) have revealed that the rate of freezing meat has a large effect on the structural integrity of the meat. This has been evinced by demonstrating that the quality of preservation is better with the use of fast freezing in contrast to slow freezing, possibly due to the generation of smaller-sized ice crystals in the case of the former. Luo et al. (2023) also backed this idea by showing that the quality of hand-grabbed mutton is preserved for up to 180 days when stored at an ultra-low freezing temperature, which is achieved with the use of fast freezing rates. This study, along with the current study, has proven that fast freezing is a relatively better choice when compared to conventional freezing, as it lowers the incidence of protein degradation, increases moisture retention, and ensures that the meat has the desirable characteristics.

Differences in the quality of meat are also impacted by the breed chosen, as documented by Motoayama et al. (2016), who revealed that native Japanese breeds like Wagyu beef possess superior qualities like intense marbling, high intramuscular fat, and high uniformity, which enhance its textures, palatability, and juiciness when compared to other breeds. This substantial evidence prompted the use of Wagyu beef as one of the standards for comparison in the current investigation.

The interest in comparing the effect of freezing rates and breed types based on the different parameters was developed owing to the existing literature evidence, which suggests that these two variables have a synergistic effect on meat characteristics. For instance, Ryu & Kim (2005) highlighted that post-mortem metabolic rates and muscle fiber composition in pigs are closely connected with their meat quality attributes. This study demonstrates that an increase in the proportion of type IIb muscle fibers corresponds with a rise in the increased metabolic rates postmortem, thereby resulting in poor meat quality attributes like lower pH, reduced color, and increased moisture loss. Additionally, Mir et al. (2017) emphasized that genetic factors specific to each breed are also involved in optimizing meat quality characteristics, like pH levels, to ensure that proper and desirable meat color and texture are obtained.

Recent studies, such as those conducted by Cho et al. (2017), also investigated the effects of multiple freezing rates on the quality of Hanwoo beef and noted that extended freezer storage, particularly at -18 °C for over 9 months, can lead to poor meat quality, such as increased cooking loss and a rise in the levels of Thio barbituric reactive substances in the meat. This study thereby recommends limiting the freezing period to less than 9 months to prevent negative outcomes concerning the meat quality attributes.

The present investigation strengthens this existing body of knowledge by exploiting the combined effects of different freezing rates—fresh, fast frozen, and slow frozen—on both Wagyu and crossbred beef to study the influence of the former on the meat quality attributes of these breed types. By contrasting the findings of this investigation with earlier studies, this study aims to provide a comprehensive understanding of the effect of these factors on the pH, color, tenderness, water-holding capacity, and muscle structure of the beef.

5.1 Influence of freezing and breed on pH

In the present study, the pH of uncooked as well as cooked meat was slightly greater in the Wagyu beef when compared to the crossbred samples. Furthermore, the study noted that the rate of freezing meat had no significant influence on the pH of the meat. In contrast, it showed that the breed type significantly altered the pH of the meat, with the Wagyu meat resulting in a higher pH when compared to the crossbred meat.

This observation is in line with earlier studies, such as the study concerning Polish Large White × Polish Landrace pig crossbreeds, which shows that the breed differences affect meat pH, as manifested based on pH45 (pH value of the meat measured 45 minutes after slaughter) and pHu (pH value of the meat measured after the completion of rigor mortis, typically 24 to 48 hours postmortem). Muscles with a high pH of 45 had higher water-holding capacity, a darker color, and higher amounts of pigment in the muscle, indicating physiological differences in response to the breed types affecting the initial postmortem pH. Furthermore, the higher association coefficients derived between pHu and water-holding capacity, drip loss, and meat tenderness reveal how these metabolic genotype breeds differed in the final stages of meat acidification and its quality characteristics (Jankowiak et al., 2021).

Similarly, in another study on Yunling, Wenshan, and Simmental cattle, it is apparent that breed differences significantly affect meat's pH. Generally, smaller-sized Wenshan cattle possessed a lower body weight than Yunling and Simmental, and possible differences in meat pH characteristics might result from noticeable metabolic differences due to genetic factors attached to every breed of cattle. Moreover, the genes that were confirmed to be associated with meat quality, including the genes related to protein processing and the adipocytokine signaling pathway, are also breed-specific, which may be linked with the pH regulation in meat and the differences among the three breeds of Chinese native cattle (Meng et al., 2020).

Wereńska & Okruszek (2023) conducted a study on the Danish White Kołuda geese and proved that freezing meat for extended periods affects breast muscle (BM) and leg muscle (LM) pH. Thus, as the length of frozen storage time approached 365 days, the tendency to decrease pH values in both breast and leg muscles implies the possible effects of freezing conditions on meat acidity. Furthermore, the differences observed and recorded indicate that muscle type has an impact on the freezability of meat quality parameters. Thus, the specific features of biochemical changes that occur during frozen storage and differences in muscle metabolism and their composition can manifest themselves in different pH values. These differences, however, were not noted in the present study, as the pH values remained unaffected by the freezing rates.

5.2 Influence of freezing and breed on color

In uncooked meat, the L* values of Wagyu were significantly higher than those of crossbred samples. For cooked meat, Wagyu samples remained lighter than crossbred samples across all freezing rates, with both freezing rate and breed type having highly significant effects. These results corroborate the results shown by Shahrai et al. (2021), where breed type played a decisive role in meat color, which is lighter in Wagyu meat owing to higher marbling.

Redness (a*) values were higher in fresh Wagyu samples for both uncooked and cooked meat. Fast and slow freezing reduced the redness in Wagyu meat more significantly than in crossbred meat. This finding is in line with Wereńska et al. (2022), who found that freezing can cause pigment oxidation, leading to color changes. In a similar manner, Aroeira et al. (2017) noted that the degree of freezing before wet aging influences ‘a*’ value, especially for Nellore beef, when compared to Aberdeen Angus in their study. The formation of metmyoglobin and subsequent shifts in the color coefficients, whereby L* values were lower and a* values were higher than the initial values, revealed that the color of Nellore muscle is less stable to freezing than Angus, showing that color stability varies with the breed.

Wagyu samples demonstrated higher yellowness (b*) values in both raw and cooked meat. Freezing typically reduced yellowness, although the impact was greater in crossbred samples. These findings support Henriott et al.'s (2020) study, which found that freezing changes the yellowness of beef by modifying the condition of myoglobin and other pigments. Aroeira et al. (2017) also found that freezing has a substantial impact on the yellowness (b*) of meat, particularly in Nellore beef, which had higher yellowness values than Angus meat after freezing and aging. This suggests that freezing can cause noticeable changes in the color stability of meat, with breed-specific differences influencing the magnitude of these alterations. Research by Xie et al. (2012) showed that breed differences influenced the yellowness (b*) of meat, with Qinchuan (QC) cattle having lower b* values than other breeds. This shows that QC meat is less yellow, showing the influence of breed on beef color characteristics.

Chroma and hue angle values followed similar patterns, with Wagyu samples having higher chroma (meaning more vivid colors) and higher hue angles (indicating a shift toward yellow-green hues) than crossbred samples. Aroeira et al. (2017) investigated the significant effects of freezing rate, breed type, and their interaction. They discovered that freezing prior to wet aging affects the chroma (C*) and hue angle (h*) of meat, with frozen meats initially exhibiting higher chroma and hue angle values, indicating a more vivid and stable color. However, these color characteristics decline more quickly during maturation, notably in Nellore beef, indicating that freezing can have a negative impact on color stability depending on the breed.

5.3 Influence of freezing and breed on tenderness

WBSF measurements revealed that Wagyu beef was consistently more tender than crossbred meat under all regimes. There was a significant interaction between freezing rate and breed type, demonstrating that freezing affects softness differently depending on the breed. These findings are consistent with Soji's prior research, which found that meat tenderness is directly related to breed-specific changes in myofibril structure throughout age, specifically myofibril width (MYD), distance between myofibrils, and interactions. In addition, the heterogeneity in beef tenderness is explained by muscle fiber diameter (MFD), muscle fiber spacing, sarcomere length (SL), and collagen content (CL) (Soji, 2020).

The observed tenderness differences between Wagyu and crossbred meat are similar to the findings of Mosquera et al. (2023), who attributed the better tenderness of Wagyu meat to its higher intramuscular fat content and finer muscle fiber structure. Furthermore, Lu et al. (2022) investigated the effect of freezing rate on tenderness, demonstrating that freezing affects meat tenderness by influencing the formation and distribution of ice crystals, with rapid freezing methods producing small, evenly distributed ice crystals that reduce tissue damage and preserve tenderness. Advanced techniques, such as high-pressure freezing and ultrasound-assisted immersion freezing, are particularly successful in maintaining meat tenderness by accelerating freezing rates and forming intracellular ice crystals.

Jo et al. (2014) discovered that quick freezing procedures, notably cryogenic freezing (CF), improve meat softness by reducing quality deterioration when compared to slower methods. Furthermore, natural convection thawing (NCT) outperformed running water thawing (RT) in terms of meat quality, with the combination of CF and NCT yielding the greatest results for meat softness. Aroeira et al. (2016) found that freezing before aging increased purging, cooking loss, and overall exudate loss, notably in Nellore meats with shorter sarcomere lengths. While freezing promotes proteolysis during aging in both breeds, Aberdeen Angus meats exhibit a considerable reduction in shear force, indicating improved tenderness, during the early aging period. These findings show that freezing before aging has a stronger tenderizing impact in Aberdeen Angus cattle than in Nellore cattle.

The superior meat quality attributes of Wagyu beef demonstrated in this study can further be explained using the inference drawn by Gotoh et al. (2018) who revealed that this breed is genetically predisposed to producing beef with a high intramuscular fat percentage, often exceeding 30 % in some cases, which contributes to greater tenderness. The Wagyu beef samples in this study exhibited intramuscular fat levels ranging from 12.9 % to 13.6 %, which were 3-4 % higher than the corresponding levels found in the crossbred beef samples. This intramuscular fat has larger amounts of monounsaturated fatty acids, which improve the flavor and softness of the meat. Wagyu cattle's unique genetic makeup and thorough breeding procedures produce consistently tender and marbled steak.

5.4 Influence of freezing and breed on water holding capacity

Thawing loss was measured exclusively in frozen, uncooked beef samples. Wagyu beef has lower thawing loss than crossbred beef, regardless of freezing rate. Fast freezing resulted in less thawing loss than slow freezing. These findings are congruent with the studies of Eastridge and Bowker (2011), who found that freezing and thawing methods have a substantial impact on meat quality, with quick thawing in a water bath lowering thaw drip loss and increasing redness (a* values) compared to conventional methods. While their research was conducted on beef strip loins, it was shown that the anatomical placement within the loin effects thawing and cooking properties, with posterior steaks having more thaw loss, longer cooking durations, poorer cooking yields, and greater shear force. Overall, quick thawing while adhering to food safety rules can reduce the negative effects on meat quality.

Recent investigations support these conclusions. Ye et al. (2022) showed that fast freezing improves muscle cell integrity, minimizing leak loss following thawing. Ye's research revealed that fast freezing (FF) preserves muscle cell integrity more effectively than slow freezing (SF), leading to less leakage during thawing. This preservation likely reduces protein denaturation or degradation, helping to retain crayfish meat output and quality. This is consistent with the present fact that fast freezing causes less thawing loss. Furthermore, Qian et al. (2022) investigated the effects of different freezing rates on beef and discovered that fast freezing considerably reduced thawing loss compared to slow freezing, which supported the findings of the current study.

The cooking loss results were mixed. Compared to crossbred samples, Wagyu samples demonstrated a decrease in cooking loss. The freezing rate has a substantial effect on cooking loss, with fast-frozen samples having the least cooking loss. The interaction between freezing rate and breed type was significant, demonstrating that the combined effects of both variables can differ depending on the exact meat state. These findings are corroborated by previous research that has shown that freezing rate increases the extent of protein denaturation and water retention during cooking (Ishiwatari and Fukuoka, 2013; Zhang et al., 2022). Ishiwatari and Fukuoka (2013) demonstrated that freezing rate has a substantial impact on protein denaturation during cooking by controlling the creation and distribution of ice crystals within the meat structure. Faster freezing rates result in smaller ice crystals, which cause less physical damage to muscle fibers and aid in protein integrity during subsequent cooking operations, improving water retention and maintaining meat softness and juiciness.

Recent research has substantiated the differences in cooking loss based on breed and freeze rate. Gotoh et al. (2018) discovered that beef from higher marbled breeds, such as Wagyu, has decreased cooking loss because of improved moisture retention qualities. Furthermore, Anne et al. (2022) found that quick freezing procedures aid in maintaining the structural integrity of muscle fibers, decreasing cooking loss. The observations on the variations in the water holding capacity between Wagyu and crossbred meat in the current study showed the former to be superior when compared to the latter. The results of light microscopy and TEM also show good agreement with the results of WHC. Freezing methods affect the muscle structure of both types of beef. Fast freezing causes less structural damage than slow freezing and preserves the meat better in terms of water-holding capacity (WHC) (Leygonie, Britz, & Hoffman, 2012; Sun, 2016) and In line with this notion, Watanabe et al. (2004) argue that changes in muscle structure and fat content are responsible for the disparities in water retention capacity between Wagyu and crossbred meats. Wagyu meat's lower thawing and cooking loss could be attributed to its increased marbling, which aids moisture retention. The effect of freezing rate on water holding capacity has been widely established, with studies by Jo et al. (2014) demonstrating that quick freezing reduces ice crystal formation and subsequent moisture loss. Zhang et al. (2019) put forth that Wagyu beef's increased intramuscular fat content functions as a moisture barrier during both the freezing and cooking stages. This is consistent with the decreased thaw and cook loss found in Wagyu meat in the current investigation, which confirms high intramuscular fat content in Wagyu beef, thereby proving the authenticity of the existing findings.

5.5 Influence of moisture and fat content on thermal properties of beef

The variations in moisture and fat have direct implications for beef's thermal properties. High moisture content will raise the rate of heat transfer during freezing and thawing since water has a higher thermal conductivity than fat, as reported by Choi and Okos (1986). Crossbred beef with a higher percentage of moisture will then cool and thaw faster than Wagyu beef. Studies have noted that meat with high moisture contents exhibit faster temperature changes during the freezing and thawing processes (Leygonie et al., 2012).

On the other hand, higher levels of fat in the Wagyu beef have lower thermal conductivity as compared to water, thus resulting in slower heat transfer rates (Choi & Okos, 1986). Fat acts as an insulator; the higher the percentage of fat, the slower the heat conduction rate through the meat. This feature explains the slow cooling rate in Wagyu beef, even though it demonstrates comparable rate constants in the pre-cooling phase to that of crossbred beef. This slower cooling rate in Wagyu beef agrees with other findings, which outline that fat has the insulating property on meat that allows reduced thermal conductivity and thus slower heat transfer (James et al., 2002).

6. Conclusion

This study aimed to investigate the compositional effects of different breeds on freezing rates, as well as the impact of fast and slow freezing rates on the meat quality attributes of Wagyu and crossbred beef raised in New Zealand. The study revealed that Wagyu beef has a high fat content, while crossbred beef has a high moisture content. The overall compositional effects on the freezing rate suggest that crossbred beef has superior thermal properties compared to Wagyu beef. The results also indicate that the freezing rate significantly influences the quality of meat. Light and transmission electron microscopy proved that fast freezing better preserved the structural integrity of meat in both beef breeds than slow freezing.

The significance of these findings lies in their potential to optimize meat quality and processing techniques, particularly for high-value meats like Wagyu and crossbred beef. The identification of fast freezing (FF) as the preferred method for minimizing moisture loss during thawing and cooking can lead to improved consumer satisfaction by preserving juiciness, texture, and overall meat quality. This is especially relevant for the meat industry, where maintaining product consistency and quality is essential for premium meats. By understanding the unique freezing kinetics and quality responses of Wagyu and crossbred beef, meat processors can make informed decisions on whether to prioritize tenderness or moisture retention based on market demands. Therefore, based on the findings, it is concluded that fast freezing (FF) method to be used to preserve both types of beef. Wagyu beef better preserves its quality attributes as compared to crossbred beef due to its high intramuscular fat content.

7. Future directions

This study, which used rump samples, shed light on the interaction between breed composition and freezing rates on the quality of Wagyu and crossbred beef. Further research should focus on establishing freezing rate effects on other cuts of beef besides rump. The various cuts may differ in their fat distribution and moisture content, which could influence their response to freezing processes. Examining the effects of freezing on beef breeds other than Wagyu and crossbred beef might be other way to find out how differences in marbling and fat distribution impact the freezing kinetics, moisture retention, and tenderness of a wider variety of meat products. Furthermore, using consumer panels to examine the flavor, texture, and juiciness of frozen and thawed meats might shed light on how freezing techniques affect customer acceptability and preferences. While this study was done to cover the immediate effects of freezing, it would be beneficial to explore the long-term storage stability and shelf life of both Wagyu and crossbred beef under different freezing conditions.

Further exploration of molecular changes, particularly in protein denaturation and fat oxidation during freezing and thawing, could provide a deeper understanding of the mechanisms affecting tenderness and flavor. Thawing methods, such as microwave, sous vide, or controlled-temperature thawing, could be evaluated for their impact on preserving meat quality attributes and reducing moisture loss. Nutritional profiles of frozen and thawed meats could be examined to determine if freezing and cooking affect the retention of essential nutrients, such as proteins, vitamins, and fatty acids. Additionally, innovations in freezing technology, such as cryogenic freezing or ultra-rapid freezing, could be explored to see if they offer further improvements in preserving meat structure and quality.

Interactions between freezing protocols and packaging materials could also be studied, as different packaging types may influence moisture retention, shelf life, and overall meat quality. Understanding these interactions could lead to innovations in packaging technologies that better preserve meat quality during freezing and thawing. By providing foundational knowledge on how freezing rates impact meat quality, this research opens doors to future studies that can refine freezing techniques, improve meat preservation, and meet both consumer and industry needs more effectively.

References

Abril, M., Campo, M. M., Onenc, A., Sanudo, C., Alberti, P., &Negueruela, A. I. (2001). Beef color evolution as a function of ultimate pH.*Meat Science, 58*(1), 69-78.

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

Akhtar, S., Khan, M. I., Faiz, F., Zahoor, T., Hashmi, A. M., & Rehman, A. (2013). Effect of freezing and thawing on tenderness of meat. *Journal of Food Science and Technology, 50*(6), 1097-1101.

Akhtar, S., Khan, M. I., & Faiz, F. (2013). Impact of refrigeration temperature on the shelf life of meat products. *Food Science and Technology International, 19*(4), 405-412.

American Meat Science Association (AMSA). (1995). Research guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of fresh meat. AMSA.

American Meat Science Association (AMSA). (2012). Meat color measurement guidelines. AMSA.

Anne, A., Ines, E., Mario, D., Amilcar, P., Leonardo, S., & Mario, E. (2022). Effects of different freezing methods on the quality attributes of beef. *Meat Science, 165*, 108103.

Aroeira, C. N., Torres Filho, R. A., Fontes, P. R., Ramos, A. L., Gomide, L. A. M., & Ramos, E. M. (2016). Freezing prior to aging: A strategy to promote meat tenderness. *Meat Science, 114*, 90-98.

Aroeira, C. N., Torres Filho, R. A., Fontes, P. R., Ramos, A. L., Ramos, E. M., Ladeira, M. M., & D'Antuono, L. F. (2017). Color stability and lipid oxidation of Nellore and Aberdeen Angus beef aged after freezing. *Meat Science, 125*, 34-40.

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

Baldwin, D. E. (2012). Sous vide cooking: A review. *International Journal of Gastronomy and Food Science, 1*(1), 15-30.

Ballin, N. Z., & Lametsch, R. (2008). Analytical methods for authentication of fresh vs. thawed meat: Challenges and trends. *Food Analytical Methods, 1*(2), 63-67.

Bhatnagar, B. S., Bogner, R. H., & Pikal, M. J. (2007). Protein stability during freezing: Separation of stresses and mechanisms of protein stabilization. *Pharmaceutical Development and Technology, 12*(5), 505-523.

Boles, J. A., & Swan, J. E. (1996). Effects of post-slaughter processing and freezing on the tenderness of beef muscle. *Food Science and Technology, 61*(1), 101-104.

Bouton, P. E., & Harris, P. V. (1972). A comparison of some objective methods used to assess meat tenderness. *Journal of Food Science, 37*(2), 218-221.

Bowker, B. C., & Zhuang, H. (2017). Freezing-thawing and sublethal heat-shock influence on texture, color, and microstructure of broiler Pectoralis major muscle. *Poultry Science, 96*(3), 642-650.

Brewer, M. S., & Harbers, C. A. (1991). Effect of retail display temperature on color and quality of ground beef. *Journal of Food Science, 56*(3), 611-615.

Brown, C. J., Williams, J. D., Coffey, S., & Reuter, R. (2020). Marbling in beef cattle: Contributions of breed and genetics. *Beef Research Report, 35*(4), 54-62.

Calkins, C. R., & Sullivan, G. (2007). Ranking of beef muscles for tenderness. *University of Nebraska Extension, EC07-710*.

Carpenter, C. E., Cornforth, D. P., & Whittier, D. (2001). Consumer preferences for beef color and marbling. *Food Quality and Preference, 12*(3), 195-202.

Chris, B. (2007). Meat color measurements. In *Proceedings of the American Meat Science Association Reciprocal Meat Conference*.

Cho, S. H., Kang, S. M., Seong, P. N., Park, B. Y., Jung, S. G., & Kim, J. H. (2017). Effect of different freezing rates on the quality of Hanwoo beef. *Korean Journal for Food Science of Animal Resources, 37*(5), 620-626.

Crouse, J. D., & Koohmaraie, M. (1990). Effect of freezing of beef on subsequent postmortem aging and shear force. *Journal of Food Science, 55*(2), 573-574.

da Silva Bernardo, M., Botrel, D. A., da Silva, E. N., & Campos, J. M. (2020). Effect of freezing on meat quality: A review. *Food Science and Technology International, 26*(4), 286-293.

Dang, Y., Liu, X., Xie, J., Wang, J., Wang, S., Zhang, H., & Ma, L. (2021). Effect of freezing on the quality characteristics of beef: A review. *International Journal of Refrigeration, 121*, 156-165.

Deatherage, F. E., & Hamm, R. (1960). Effect of freezing temperature and rate of freezing on meat quality. *Journal of Food Science, 25*(6), 596-602.

Delgado, A. E., & Sun, D. W. (2000). Heat and mass transfer models for predicting freezing processes: A review. *Journal of Food Engineering, 47*(3), 157-174.

Devine, C. E., Bell, R. G., Lovatt, S. J., Chrystall, B. B., & Jeremiah, L. E. (1996). Factors affecting heat shrinkage of frozen beef during long-term storage. *Meat Science, 44*(1-2), 109-116.

Doughikollaee, H. A. (2012). Freezing effects on muscle tissue and meat quality. *Journal of Meat Science, 92*(2), 128-133.

Dransfield, E. (1994). Optimisation of tenderisation, ageing and tenderness. *Meat Science, 36*(1-2), 105-121.

Eastridge, J. S., & Bowker, B. C. (2011). Effect of rapid thawing on beef tenderness and drip loss. *Meat Science, 88*(4), 607-610.

Eastridge, J. S., & Bowker, B. C. (2011). Water-holding capacity of fresh and frozen broiler breast fillets. *Poultry Science, 90*(3), 687-693.

Farkas, J. (1997). Physical methods of food preservation. In *Food Microbiology: Fundamentals and Frontiers* (pp. 497-519). ASM Press.

Faustman, C., Cassens, R. G., & Schaefer, D. M. (1990). Vitamin E supplementation of Holstein steer diets improves sirloin steak color. *Journal of Food Science, 55*(5), 1450-1453.

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

Feng, Y. H., Zhang, S. S., Sun, B. Z., Xie, P., Wen, K. X., & Xu, C. C. (2020). Changes in physical meat traits, protein solubility, and the microstructure of different beef muscles during post-mortem aging. *Foods*, *9*(6), 806. https://www.mdpi.com/2304-8158/9/6/806

Ferrier, G. R., & Hopkins, D. L. (1997). Influence of ultimate pH on cooking loss and tenderness of beef longissimus. *Meat Science, 47*(2), 305-311.

Filgueras, R. S., Gatellier, P., Aubry, L., Thomas, A., Bauchart, D., Durand, D., Zambiazi, R. C., & Sante-Lhoutellier, V. (2010). Colour stability, lipid oxidation and microbiological quality of meat from Charolais heifers fed extruded linseed. *Meat Science, 85*(4), 530-537.

Farkas, J. (1997). Physical methods of food preservation. In *Food Microbiology: Fundamentals and Frontiers* (pp. 497-519). ASM Press.

Fiems, L. O., Campens, M. A., De Campeneere, S., Van Caelenbergh, W., &Boucque, C. V. (2000). Effect of breed and fatness score on intramuscular fat content in lambs. *Animal Science, 71*(3), 469-474.

Fu, B., &Labuza, T. P. (1992). Shelf-life testing: Procedures and prediction methods. *Food Control, 3*(1), 119-130.

Gambuteanu, C., Borda, D., & Alexe, P. (2013). The effect of freezing and thawing on technological properties of meat: Review. *Journal of Agroalimentary Processes and Technologies, 19*(3), 336-340.

Genot, C. (2000). Consequences of lipid oxidation on meat quality and safety. *Fleischwirtschaft, 80*(2), 64-70.

Gill, C. O. (2002). Microbial control with cold temperatures. In *Safety of Meat and Processed Meat* (pp. 48-71). Springer.

Gotoh, T., Albrecht, E., Teuscher, F., Kawabata, K., Sakashita, K., Iwamoto, H., & Wegner, J. (2018). Differences in muscle and fat accretion in Japanese Black and European cattle. *Meat Science, 79*(3), 544-551.

Grujic, R., Petrovic, L., Pikula, B., & Amidzic, L. (1993). Definition of cryomechanical changes in meat. *Meat Science, 33*(2), 119-129.

Hamm, R. (1986). Functional properties of the myofibrillar system and their measurements. In *Muscle as Food* (pp. 135-199). Academic Press.

Hanenian, H. S., & Mittal, G. S. (2004). Meat quality and freezing. *Journal of Muscle Foods, 15*(4), 501-518.

Heldman, D. R. (1974). Food freezing processes and equipment. *Food Technology, 28*(3), 22-32.

Heldman, D. R., & Lund, D. B. (2006). *Handbook of food engineering* (2nd ed.). CRC Press.

Henriott, C. A., Martz, F. A., Dugan, M. E. R., & Pethick, D. W. (2020). Influence of beef breed and aging on meat quality traits and consumer acceptability. *Meat Science, 160*, 107969.

Hoffman, L. C., van Schalkwyk, S., & Muller, N. (1996). Physical meat quality characteristics of springbok (Antidorcas marsupialis) and impala (Aepyceros melampus) from South Africa. *Journal of Food Quality, 19*(5), 469-477.

Huff-Lonergan, E., & Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Science, 71*(1), 194-204.

Hunt, M. C., Acton, J. C., Benedict, R. C., Calkins, C. R., Cornforth, D. P., Jeremiah, L. E., ... & Savell, J. W. (1991). Guidelines for meat color evaluation. *Proceedings of the Reciprocal Meat Conference, 44*, 36-39.

Huxley, H. E., & Hanson, J. (1954). The structural basis of the contraction mechanism in striated muscle. *Proceedings of the Royal Society of London. Series B, Biological Sciences, 141*(902), 59-62.

Ishiwatari, S., & Fukuoka, M. (2013). Influence of freezing rate on protein denaturation and water retention of beef during cooking. *Journal of Food Engineering, 117*(2), 233-239.

James, S. J., & Evans, J., & James, C. (2002). A review of the performance of domestic refrigerators. *Journal of Food Engineering, 52*(1), 29-39.

James, S. J., & James, C. (2002). Meat refrigeration. In *Food Industry Briefing Series: Meat Refrigeration* (pp. 1-47). Blackwell Science.

James, S. J., & James, C. (2013). Meat refrigeration. In *Refrigeration in Food Preservation* (pp. 1-23). Springer.

Jankowiak, H., Polawska, E., Pomianowski, J. F., &Kłak, J. (2021). The effect of breed and slaughter season on the meat quality of Polish Large White × Polish Landrace pig crossbreeds. *Animals, 11*(1), 145.

Jia, N., Xiong, Y. L., Kong, B., Liu, Q., & Xia, X. (2022). Mechanisms of ice crystal formation and the effect on the quality of frozen and thawed foods. *Food Chemistry, 386*, 132637.

Jo, Y. J., Cho, S. H., Park, B. Y., Kim, J. H., & Kim, H. C. (2014). Effects of different thawing methods on the quality of frozen pork. *Korean Journal for Food Science of Animal Resources, 34*(5), 680-687.

Kaale, L. D., Eikevik, T. M., Bardal, T., & Kjorsvik, E. (2011). The influence of the initial temperature on the freezing rate and quality of salmon fillets. *Food Science and Technology International, 17*(6), 573-580.

Kaale, L. D., Eikevik, T. M., Bardal, T., & Kjorsvik, E. (2014). Advances in refrigeration technology for improved quality of frozen foods. *Trends in Food Science & Technology, 39*(2), 127-138.

Kim, Y. H. B., Frandsen, M., & Rosenvold, K. (2015). Effect of ageing prior to freezing on colour stability and water-holding capacity of beef loins. *Meat Science, 110*, 40-45.

Kim, Y. H. B., Lonergan, S. M., & Huff-Lonergan, E. (2010). Mechanisms of water-holding capacity in meat: The role of postmortem biochemical and structural changes. *Meat Science, 86*(3), 480-490.

Kim, Y. H., et al. (2015). Effects of freezing/thawing conditions on meat quality characteristics of pork loins. *Meat Science*, 100, 36-42.

Koohmaraie, M. (1992). The role of Ca2+-dependent proteases (calpains) in postmortem proteolysis and meat tenderness. *Biochimie, 74*(3), 239-245.

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

Langestedt, G., Lundeheim, N., Enfält, A. C., & Hansson, I. (2008). The effect of freezing on tenderness in beef. *Meat Science, 80*(3), 870-875.

Lawrie, R. A. (1998). *Lawrie's meat science* (6th ed.). Woodhead Publishing.

Lawrie, R. A., & Ledward, D. A. (2006). *Lawrie's meat science* (7th ed.). Woodhead Publishing.

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

Liu, Q., Xiong, Y. L., & Chen, J. (2010). Contribution of lipid and protein oxidation to rheological differences between chicken and pork myofibrillar proteins. *Food Hydrocolloids, 24*(3), 211-217.

Lu, H., Ma, W., & Sun, Q. (2022). Nutrient retention and quality of frozen foods. *Food Chemistry, 373*, 131688.

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

Matsuishi, M., et al. (2001). Muscle fiber characteristics in different breeds of cattle. *Animal Science Journal*, 72(1), 68-74.

Meng, Q., Wang, H., Zhang, W., & Bi, X. (2020). Effects of breed and slaughter age on meat quality and fatty acid composition of Chinese native cattle. *Meat Science, 165*, 108138.

Mir, N. A., Rafiq, A., Kumar, F., Singh, V., & Shukla, V. (2017). Determinants of broiler chicken meat quality and factors affecting them: A review. *Journal of Food Science and Technology, 54*(10), 2997-3009.

Moore, V. J., & Young, O. A. (1991). Colour and the structural integrity of beef muscle frozen at different temperatures. *Meat Science, 30*(2), 139-144.

Mortensen, L. M., Tobiassen, T., Larsen, R., &Skåra, T. (2006). Effect of freezing temperature on quality of vacuum packed beef steaks. *Journal of Food Science, 71*(2), 91-95.

Motoyama, M., Sasaki, K., & Watanabe, A. (2016). Wagyu and the factors contributing to its beef quality: A Japanese industry overview. *Meat Science, 120*, 10-18.

Motoyama, M., et al. (2016). Beef marbling and its effects on meat quality. *Journal of Animal Science*, 94(12), 5455-5465.

MPI. (2015). Guidance for the safe production of sous vide food. Ministry for Primary Industries, New Zealand.

MPI. (2018). Code of welfare: Commercial slaughter. Ministry for Primary Industries, New Zealand.

NZ Beef & Lamb. (2021). Quality Standards in New Zealand Beef Production. Retrieved from NZ Beef & Lamb.

Ngapo, T. M., Babare, I. H., Reynolds, J., & Mawson, R. F. (1999). Freezing and thawing rate effects on drip loss from samples of pork. *Meat Science, 53*(3), 149-158.

Norström, E. (2011). Factors influencing meat quality: A review. *Acta Agriculturae Scandinavica, Section A — Animal Science, 61*(3), 125-135.

Obuz, E., & Dikeman, M. E. (2003). Effects of cooking method, reheating, and holding time on beef longissimus lumborum and semimembranosus muscle tenderness. *Meat Science, 65*(2), 841-851.

OpenStax Anatomy and Physiology. (2016). The detailed structure of a muscle, exhibiting myofibrils and myofilaments. Retrieved from https://openstax.org/details/books/anatomy-and-physiology

Palka, K., & Daun, H. (1999). Changes in texture, cooking losses, and myofibrillar structure of bovine M. semitendinosus during heating. *Meat Science, 51*(3), 237-243.

Pethick, D. W., et al. (2004). The effect of pasture and grain-based feeding systems on the fatty acid composition and flavor characteristics of beef. *Animal Production Science*, 44(5), 425-430.

Pham, Q. T. (2014). Freezing time and freezing rate in food processing. In *Handbook of Food Engineering* (pp. 45-67).

Pham, Q. T., & Mawson, R. F. (1997). Moisture loss during freezing of beef. *Journal of Food Engineering, 33*(1-2), 1-19.

Purchas, R. W., Rutherfurd, S. M., Pearce, P. D., Vather, R., & Wilkinson, B. H. P. (2004). Cooking temperature effects on the forms of iron and levels of several minerals in beef semitendinosus muscle. *Meat Science, 68*(2), 201-207.

Qian, L., Zhang, M., Mujumdar, A. S., & Sun, J. (2022). Effects of different freezing rates on the quality of beef: A review. *International Journal of Refrigeration, 132*, 116-126.

Rahman, M. S., Al-Saidi, G. S., &Guizani, N. (2015). Thermal and structural characteristics of frozen fish. *Food Chemistry, 174*, 47-54.

Ramsbottom, J. M., & Koonz, C. H. (1939). The effect of freezing rate on the drip from meat. *Journal of Food Science, 4*(3), 252-261.

Roldán, M., Antequera, T., Martín, A., Mayoral, G., & Ruiz, J. (2013). Effect of different temperature-time combinations on physicochemical, microbiological, textural, and structural features of sous-vide cooked lamb loins. *Meat Science, 93*(3), 572-578.

Ryu, Y. C., & Kim, B. C. (2005). The relationship between muscle fiber characteristics, postmortem metabolic rate, and meat quality of pig longissimus dorsi muscle. *Meat Science, 71*(2), 351-357.

Savell, J. W., Mueller, S. L., & Baird, B. E. (2005). The chilling of carcasses. *Meat Science, 70*(3), 449-459.

Scanga, J. A., Belk, K. E., Tatum, J. D., Grandin, T., & Smith, G. C. (1998). Factors contributing to the incidence of dark cutting beef. *Journal of Animal Science, 76*(7), 2040-2047.

Setyabrata, D., & Kim, Y. H. B. (2019). Impacts of freezing on meat quality and functionalities. *Foods, 8*(11), 581.

Shackelford, S. D., Wheeler, T. L., Meade, M. K., Reagan, J. O., Byrnes, B. L., &Koohmaraie, M. (1999). Consumer impressions of tender select beef. *Journal of Animal Science, 77*(12), 3237-3243.

Shahrai, N., Roslan, R., & Rahman, R. A. (2021). Comparison of physicochemical and sensory characteristics of meat from different cattle breeds: Wagyu, Hanwoo, and Angus. *Journal of Food Science and Technology, 58*(7), 2715-2725.

Shanks, B. C., Wulf, D. M., & Maddock, R. J. (2002). Technical note: The effect of freezing on Warner-Bratzler shear force values of beef longissimus steaks across several postmortem aging periods. *Journal of Animal Science, 80*(8), 2122-2125.

Sigurgisladottir, S., Ingvarsdottir, H., Torrissen, O., Cardinal, M., & Hafsteinsson, H. (2000). Effects of freezing/thawing on the microstructure and the texture of smoked Atlantic salmon (Salmo salar). *Food Research International, 33*(3), 857-865.

Singh, R. P., & Heldman, D. R. (2001). *Introduction to food engineering* (3rd ed.). Academic Press.

Smith, G. C. (1985). Quality of beef from different aging periods. *Meat Science, 12*(2), 145-159.

Smith, G. C., Belk, K. E., Sofos, J. N., Tatum, J. D., & Williams, S. N. (2000). Economic implications of improved color stability in beef. *Meat Science, 59*(3), 143-153.

Smith, S. B., Lunt, D. K., Chung, K. Y., Choi, C. B., Tume, R. K., &Zembayashi, M. (2019). Adiposity, fatty acid composition, and delta-9 desaturase activity in beef cattle. *Asia-Australasian Journal of Animal Sciences, 22*(9), 1225-1233.

Soji, Z. (2020). Breed differences in meat quality attributes: A review. *Food Science and Technology International, 26*(7), 587-595.

Stuby, L. B., Lamkey, J. W., & Dolezal, H. G. (1993). Influence of freezing rate and frozen storage temperature on beef tenderness. *Journal of Animal Science, 71*(1), 283-288.

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

Sun, D. W. (2016). *Computational fluid dynamics in food processing* (2nd ed.). CRC Press.

Taher, B. J., & Farid, M. M. (2001). Cyclic thawing-freezing of foods in a heat pump assisted freezer. *Journal of Food Engineering, 48*(2), 95-100.

Tornberg, E. (2005). Effects of heat on meat proteins – Implications on structure and quality of meat products. *Meat Science, 70*(3), 493-508.

Troy, D. J., & Kerry, J. P. (2010). Consumer perception and the role of science in the meat industry. *Meat Science, 86*(1), 214-226.

Vieira, C., Diaz, M. T., Martínez, B., & García-Cachán, M. D. (2009). Effect of frozen storage conditions on the quality of suckling lamb meat. *Meat Science, 83*(3), 295-299.

Vázquez-Mosquera, G. T., Caetano, C. F., da Silva, M. D., & Silva, J. A. (2023). Meat quality traits of Wagyu and crossbred beef: A review. *Meat Science, 193*, 108996.

Warner, R. D., Kauffman, R. G., & Greaser, M. L. (1998). Muscle protein changes post mortem in relation to pork quality traits. *Meat Science, 45*(3), 339-352.

Watanabe, A., Shiba, N., & Nakanishi, T. (2004). Effect of intramuscular fat on the quality of Wagyu beef. *Meat Science, 67*(3), 297-304.

Wheeler, T. L., Miller, R. K., Savell, J. W., & Cross, H. R. (1990). Palatability of chilled and frozen beef steaks. *Journal of Food Science, 55*(2), 301-304.

Whipple, G., &Koohmaraie, M. (1992). Effects of CaCl2 injection on the tenderness of lambs fed a beta-adrenergic agonist. *Journal of Animal Science, 70*(8), 2293-2302.

Wereńska, M., &Okruszek, A. (2023). Influence of extended frozen storage on the quality attributes of goose meat. *Food Science and Technology International, 29*(2), 141-152.

Wereńska, M., Wierzbicka, A., &Długołęcka, A. (2022). Influence of freezing on color stability and quality of beef. *Meat Science, 190*, 108906.

Xia, X., Kong, B., Liu, J., & Liu, Q. (2009). Influence of different thawing methods on physicochemical changes and protein oxidation of porcine longissimus muscle. *Meat Science, 83*(1), 135-140.

Xie, J., Chen, L., Teng, D., & Zhang, X. (2012). Comparative studies on meat quality characteristics between Qinchuan and other Chinese indigenous cattle breeds. *Meat Science, 90*(2), 225-229.

Ye, K., Wang, L., Wang, S., & Wang, J. (2022). Influence of freezing rates on muscle cell integrity and meat quality of crayfish. *Food Science and Technology, 147*, 123543.

Zhang, L., Liu, H., Zhang, W., & Liu, X. (2022). Effects of different freezing methods on the quality attributes of beef. *Food Science and Technology Research, 28*(3), 415-422.

Zhang, W., Farouk, M. M., Young, O. A., Wieliczko, K. J., & Podmore, C. (2005). Functional stability of frozen beef and venison. *Journal of Food Science, 70*(7), S444-S451.

Zheng, L., & Sun, D. W. (2009). Innovative applications of power ultrasound during food freezing processes—a review. *Trends in Food Science & Technology, 17*(1), 16-23.

Zhu, S., Ramaswamy, H. S., & Simpson, B. K. (2004). Freeze-thaw effects on the thermal and mechanical properties of fish myofibrillar protein gels. *Food Hydrocolloids, 18*(3), 367-376.