

Electromagnetic Freezing – A novel approach to the improvement of previously frozen beef

by

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Abstract

The objective of this study was to determine the effect of electromagnetic field (EM) assisted freezing on the thawing yield, shelf-life, and tenderness of previously frozen beef striploins. Striploins were collected from both sides of 12 USDA Choice carcasses and halved into 2 equal parts (n=48), weighed, and temperature probes were inserted to measure internal temperature. Halves were randomly assigned to 0, 2, 4, or 8 kV of EM and frozen under the designated treatments in a walk-in freezer (-20°C) for 24 hours. Frozen cores were taken for X-ray computed tomography (XRCT) for ice crystal size analysis. Following the freezing treatment, striploin halves were thawed for 72 hours at 2°C, weighed, and fabricated into two 2.54 cm steaks and five 1.27 cm steaks from each portion. Steaks were then subjected to 10-day simulated display. Instrumental and descriptive color measurements were taken daily. Representative steaks from each portion were analyzed for muscle fiber spacing through histological analysis, Warner-Bratzler Shear Force (WBSF), purge and cook loss, fat content, moisture content, protein content, lipid oxidation, antioxidant capacity, protein degradation, pH, and free calcium analysis on days 0, 4, 7, and 10. Swabs and purge were also collected for aerobic plate count (APC) from both before and after freezing treatments. Samples from the higher treatments required a longer time to freeze than those from lower voltage and control ($P<0.05$), but total thawing yield was only higher in the samples from the 8 kV treatment ($P<0.01$). Degraded desmin and troponin-T percentages were lower in the higher voltage treatments compared to those from the lower voltage treatment and control ($P<0.01$). However, all samples with EM treatments reported a higher free calcium concentration compared to the those from the control ($P<0.01$). During the display period, 4kV samples showed least discoloration, while exhibiting highest a^* and b^* and hydrophilic antioxidant

capacity than those from the other treatments ($P < 0.01$). No differences were observed in APC, cook loss, WBSF, muscle fiber spacing, ice crystal size, lipid oxidation, or pH ($P > 0.05$). EM assisted freezing demonstrated potential for improving thawing yield and shelf-life, and these findings provided a foundation to better determine the feasibility of large-scale integration of EM in the industry.

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Dedication

I would like to dedicate this work to my parents. You have instilled in me a love of knowledge, integrity, and hope that are the backbone of not only this work but who I am as a person. I strive to be even half as impactful in someone else as you have been to me.

Chapter 1 - Review of Literature

Introduction

Finding new and inventive ways to increase the physical and visual shelf-life of previously frozen meat products has become increasingly more important due to the volatility of the supply chain, and as consumers gain more disposable income and families spend less time in the kitchen, the demand for easily accessible frozen foods increases (Zhang et al., 2023b). In order to encourage consumer repeat purchasing habits, an increasing amount of research has been dedicated to identifying interventions that will reduce quality degradation during the freezing process (de Araújo et al., 2022). Through this effort, many novel approaches such as wavelength-assisted freezing (Alizadeh et al., 2007; Dalvi-Isfahan et al., 2016; Jiang et al., 2023; Fei et al., 2024) have shown potential success in reducing ice crystal damage. However, mechanisms and methodologies vary significantly among each of these new approaches. It is important to determine which intervention is most effective and has the strongest potential to be upscaled to industry levels. Thus, the purpose of this review is to understand the process of freezing and compare these novel approaches as a potential industry option for improving the quality of previously frozen meat products.

Freezing Process

Supercooling

While freezing will always ultimately result in ice crystal formation, a heavily studied topic on long term preservation of food products is supercooling (Stonehouse and Evans, 2015). Supercooling is an intermediate state between the liquid and solid phase where the product is cooled to a temperature below freezing while the water molecules remain in a liquid state (Mok

et al., 2017). If energy fluctuations prevent the transition from liquid to solid, water molecules migrating into the crystalline structure of frozen water will remain in their arrangement but will not solidify until an external force provides the necessary energy (Zheng et al., 2024).

Supercooling does not always occur to a noticeable degree when a food product is chilled, and its pre-freezing state can influence the likelihood of this phenomenon happening on a larger scale (Lochan Poudyal et al., 2023). More importantly, supercooling is known to be extremely unstable. For instance, Fukuma et al. (2012) reported slight changes in temperature or minor vibrations to supercooled products were sufficient to trigger nucleation in supercooled products. Despite this challenge, extensive research has focused on modifying the refrigeration environment to harness the benefits of freezing temperatures while minimizing ice crystal damage (Stonehouse and Evans, 2015).

While freezing under some conventional methods can result in supercooling, novel methods have been introduced to establish an ideal environment for this phenomenon, which includes interventions such as electrostatic or ultrasound fields (Lochan Poudyal et al., 2023). Lochan Poudyal et al. (2023) reported increased supercooling in beef *Longissimus dorsi* frozen at -4°C with approximately 8 mT of static magnetic field compared to samples frozen at the same rate without the treatment. You et al. (2020) reported that both pulsed electric field and oscillating magnetic field treatments of 5 V and 95 V respectively extended the supercooling phase of top round beef samples throughout the duration of the study. Importantly, Kang et al. (2021) observed that the intensity of oscillating magnetic field was impactful on the ability of fresh-cut mango slices to maintain a supercooled status. This study reported that 50 mT successfully inhibited the formation of ice crystals and thus extended supercooling at traditional freezing temperatures while 100 mT resulted in the transition from liquid to solid, disrupting the

supercooling environment (Kang et al., 2021). It is important to note that due to the high potential for variation in studies of this nature, supercooling can increase or decrease under various conditions and treatments, offering a wide range of solutions for different food types and industrial applications.

Ice Crystal Formation

Ice crystal formation has gained attention throughout the years as a main target to improve the frozen product quality. The process of ice crystal formation can be divided into three major steps: 1. cooling the liquid portion of the product to the ice crystallization zone (pre-freeze chill), 2. removing the exchange of heat produced during crystallization (crystallization), and 3. ensuring post freezing chill until the product reaches the desired storage temperature (post-freeze chill) (Mok et al., 2015). During the crystallization step, nucleation begins and is the most critical step for altering ice crystal size in frozen products (Qian et al., 2022). Nucleation is the first step in the formation of ice crystals and occurs when a sufficient number of water molecules form hydrogen bonds with one another and create a hexagonal structure, establishing a nucleus (Dalvi-Isfahan et al., 2016). This formation occurs via two means, primary or secondary nucleation. Primary nucleation can occur in both homogeneous and heterogeneous environments. Homogeneous nucleation, which is less common, occurs when ice crystals form spontaneously in liquid water without any surface disruption or impurities. (Maeda, 2021). On the other hand, heterogeneous nucleation occurs when water molecules cluster around an external factor, such as the structural boundaries containing them or a sufficiently small particle that facilitates ice crystal formation. (Maeda, 2021). This heterogenous nucleation is the primary means of crystal production in meat products. After primary nucleation occurs, secondary nucleation expands on

the previously existing nuclei allowing for previously small crystals to grow and potentially disrupting the surrounding microstructure (Tan et al., 2021). The location and occurrence of nucleation play a crucial role in shaping the size and structure of ice crystals, which significantly impacts the quality of frozen products (Tan et al., 2021).

The rate of ice crystallization is determined by factors such as the temperature at which the product is frozen and how often that temperature fluctuates during the freezing period (Poudyal et al. 2023). As established, increasing the rate of cooling helps to maintain the quality of frozen products, but there is also a direct connection between cooling rate and the formation of ice crystals. The solid-liquid equilibrium of water is characterized by the conversion of molecules from ice to liquid water and back again, and during this time, ice crystals are able to increase in size as well as number (Jia et al. 2017). Kono et al. (2017) reported an exponential decrease in ice crystal size as freezing rate increased in salmon fillets, with diameters ranging from 450 to 20 μm at freezing rates increased from approximately 0.03 to 0.8 $^{\circ}\text{C}/\text{min}$. The degree of supercooling plays the critical role in how long food products are in this phase, and there is an inverse relationship between time in the solid-liquid equilibrium and the degree of supercooling (Sutariya and Sunkesula, 2020). A higher degree of supercooling leads to the early formation of more nuclei, leaving less water available for conversion and resulting in smaller ice crystals. Conversely, minimal supercooling allows more molecules to participate in secondary nucleation, producing larger ice crystals during solid-liquid equilibrium (Sutariya and Sunkesula, 2020).

Decreasing the cooling rate has generally been linked to increasing the time in which water in the meat product is held at this “maximum ice crystal formation zone” (Jiang et al. 2022). As cooling rates increase, the products’ water can form nuclei without attracting large

amounts of additional water molecules, resulting in smaller ice crystals (Jha et al. 2017). However, ice crystals are not always stable during the post-freezing phase, and migration of water molecules still occurs post freezing in a phase called recrystallization, which alters the number, size, and overall conformation of the ice crystal (Tan et al. 2021). Large ice crystals tend to have more jagged surfaces, resulting in a more unstable crystal with a higher surface to volume ratio (Dalvi-Isfahan et al., 2019). Simultaneously, smaller ice crystals contain a higher level of free energy (Dalvi-Isfahan et al., 2019). These combined features, small ice crystals tend to break up and reestablish themselves with large crystals, resulting in a rounder surface area (Dalvi-Isfahan et al., 2019).

Influences of Ice Crystal Size on Meat Quality

Research has generally established a negative relationship between ice crystal size and final product quality (Kim et al., 2018). It has been shown that ice crystal formation during freezing disrupts the structural integrity of meat products by promoting the growth of large ice crystals (Liu et al., 2022), leading to reduced color stability, increased purge loss, and a significant decrease in water-holding capacity post-thaw due to the conversion of immobilized to free water (Zhang et al., 2022), ultimately lowering consumer acceptance and repeat purchase rates (James et al., 2015).

Rodezno et al. (2024) found that ice crystal size and the distribution were more uniform and ubiquitous under cryogenic freezing (-123.3°C) compared to air-blast freezing (-20°C) when freezing oyster meat. Ice crystal cross sectional area was found to be approximately 40 μm^2 when frozen at -20°C compared to 9.4 μm^2 at -123.3°C (Rodezno et al., 2024). Similarly, Qian et al. (2022) reported a smaller range of ice crystal size when freezing beef *Longissimus dorsi* at -80°C

compared to -40°C or -20°C. It was clear that rapid freezing reduces the size and variability of ice crystals as Ren et al. (2021) showed that beef *Longissimus lumborum* lost 0.5 to 4% more water when frozen at the -18°C compared to the lower -35°C. In chicken breast and legs frozen at -7, -12, and -18°C, the -7°C samples were found to have the highest amount of protein and lipid oxidation (Soyer et al., 2010). On the other hand, Hergenreder et al. (2013) reported that beef *Longissimus thoracis*, *Longissimus lumborum*, and *Gluteus medius* frozen at -28°C with high air velocity took approximately one to two days longer to reach 40% discoloration compared to samples frozen with low air velocity. The disruption in color stability could potentially be due to mechanical damage of muscle fiber due to the crystals and release of oxidative enzymes. As oxidation increases, color stability decreases (Gorelik and Kanner, 2001). However, it should be noted that the freezing environment is not the only factor affecting the downstream quality of a previously frozen meat product. Interestingly, Kobayashi et al. (2015) found that ice crystal formation caused greater damage with increased aging time, suggesting that products should be frozen as fresh as possible. Conversely, studies such as Setyabrata and Kim (2019) found when *Longissimus lumborum* and *semitendinosus* were frozen prior to aging, it resulted in greater muscle fiber spacing in samples compared to those frozen after aging, suggesting meat should be frozen after aging. Additionally, increasing the number of freeze/thaw cycles a product is subjected to decreases the overall quality. Rahman et al. (2014) found that beef forelimb subjected to three different freeze/thaw cycles with storage of 40 days for the first cycle, 20 days for the second cycle, and 15 days for the third cycle found that samples from the third cycle experienced higher amounts of cook loss compared to those from the first cycle, each being attributed to the repeated outgrowth of ice crystals during the freezing process.

Koohmaraie (1990) found that calpastatin, an endogenous inhibitor known to reduce the activity of calpains, can be reduced up to 50% following freezing. However, in that same study, calpain activity remained the same even with calpastatin activity reduced, implying that proteolytic activity does not respond in a universal manner (Koohmaraie, 1990). Ren et al. (2021) reported lower shear force values for cubed beef *Longissimus* samples frozen at -18°C compared to -35°C, attributing this difference to the larger ice crystals formed during slower freezing which more effectively damaged the muscle structure. Contrastingly, Hergenreder et al. (2013) reported that blast frozen *Longissimus thoracis* subprimals at -28 °C had no differences shear force values than the sample muscle frozen under minimal air movement and reported no differences in consumer tenderness. It was concluded that the size of the subprimal better buffered the freezing process, helping to alleviate some of the quality issues generally seen in steaks frozen under similar conditions (Hergenreder et al., 2013). However, Rahman et al. (2014) reported a decrease in consumer tenderness scores from the first freeze/thaw cycle to the third freeze/thaw cycle for beef forelimb. This decrease in tenderness could be a result of higher amounts of total drip loss, ultimately producing a tougher steak. Ultimate tenderness of a steak cannot be directly linked to one facet, and considering the proteolytic, mechanical, and drip loss effects of freezing can help establish a more complete understanding of how to improve the final acceptability of meat products.

Current Industrial Use of Freezing

With an increase in global population, the need for inventive ways to produce and store quality foods becomes more necessary (Yu et al., 2024). One of the most common methods of preservation in the food industry is freezing (Dwivedi et al., 2017). Relating to the meat industry,

the use of freezing is essential for the food supply, providing producers with the ability to maintain a consistent inventory throughout the year (Carrillo-Lopez et al., 2021). Freezing provides the consumer with one of the most similar eating experiences to fresh products in comparison to other further processing interventions (Jiang et al., 2023). Due to freezing, outgrowth of microbes is limited and proteolytic enzymes are reduced to minimal activity, establishing a longer shelf life before it reaches the consumer (Jha et al., 2017b). Furthermore, nutritional and structural integrity is maintained more efficiently than other means of long-term preservation such as drying or fermentation (Dalvi-Isfahan et al., 2016; Jha et al., 2017b). Transportation requirements and the ability to create a backstock of products makes this intervention necessary for the industry at large (Jiang et al., 2023). Currently, the food industry has implemented various methods such as blast chilling, plate freezing, and fluidized-bed freezing to quickly and effectively freeze a variety products, securing many of these positive features for a variety of products (Liu et al., 2020). It should be noted that many of these features of freezing can be lost if proper techniques are not established to maintain quality throughout both the freezing and thawing periods (Qian et al., 2019).

Novel Freezing Methods

Due to the extreme variation that can occur during the freezing process, studies have been conducted to find external forces that can increase or decrease the overall quality of frozen products. Novel methods such as ultrasound, electrostatic, static magnetic, and electromagnetic – including radio and microwaves, and AC and DC fields – have all been applied to various food products (Tan et al., 2019).

Ultrasound Assisted Freezing

The predominant theory regarding the function of ultrasound assisted freezing concludes that due to the high velocity of the water molecules, cavitation bubbles are created (James et al., 2015). These bubbles disturb the formation of ice crystals and create an abundance of primary nucleation points that result in smaller crystals (James et al., 2015). It has also been hypothesized that the ultrasound waves could break the already formed crystals allowing for the establishment of smaller, isolated secondary ice crystals (Jiang et al., 2023). Ultrasound interventions have been studied in numerous applications, both in fresh and frozen products. Reyes-Villagrana et al. (2020) reported higher tenderness in fresh rabbit meat treated with 12 W/cm² for 15 minutes compared to the control. Similarly, Peña-González et al. (2017) reported lower shear force values in beef *Longissimus dorsi* treated with 40 kHz of ultrasound waves for 1 hour after 0, 7, and 14 days. Gómez-Salazar et al. (2018) also found that the tenderness of rabbit meat increased when treated with 2.25 W/cm² of ultrasound, but a* values decreased while L* values increased due to the treatment. Li et al. (2024) observed higher L* and a* values in braised beef frozen under immersion freezing at -20°C with 200, 400, and 600 W of ultrasound treatment compared to samples only frozen under immersion. This study also reported lower cooking loss in the 200, 400, and 600 W treatments compared to the control and the highest moisture content in the 400 W treatment (Li et al., 2024). In a similar experimental design, Li et al. (2022) observed porcine muscles frozen under 150, 300, or 450 W of ultrasound under -20°C immersion freezing had no differences in thawing loss compared to the control but did have lower cooking loss in the 300 and 450 W treatment groups. Furthermore, L* values were also lower in each treatment group regardless of intensity (Li et al., 2022). Research has also incorporated the use of ultrasound assistance in thawing of previously frozen meat. Guo et al. (2021) found that vitamin and

mineral content of yak meat increased with the use of ultrasound thawing at 0, 200, 400, or 600 W treatments and that thawing time decreased with increasing strength. However, Sun et al. (2023) found that 600 W did increase purge loss and decrease a^* value, but treatments resulted in the lower shear force in duck meat. While the success of ultrasound freezing is promising, the use of this method in commercial settings has not yet been proven, even while potential integration methods have been provided (Zheng and Sun, 2005).

Electrostatic Field Assisted Freezing

Electrostatic freezing is a novel addition to the freezing process as it appears to orient the dipole nature of water, thus altering the nucleation and potentially prevents excessive clustering of the molecules (Dalvi-Isfahan et al., 2016). Similarly to other interventions, electrostatic freezing is theorized to increase the temperature in which spontaneous nucleation occurs (Jha et al., 2017b). This would also decrease cellular destruction and improve overall quality post treatment and thawing (Jha et al., 2017b). It is believed that this method could be scaled to industry standards at a more affordable price. Significant variation has been reported on the success of electrostatic freezing from studies showing no changes to significant increases in quality parameters (Dalvi-Isfahan et al., 2016; Qian et al., 2019). These inconsistencies are not unexpected, however, as variations in wattage, ambient temperature, humidity, and protein type make the current body of research difficult to compare.

Liu et al. (2022) found that the speed of freezing increased as the intensity of the electrostatic field increased. Crystals were more evenly distributed and were holistically smaller at the higher voltage treatments than the control (Liu et al., 2022). Similar results were found when electrostatic field ranging from 0 V/m to 6.0×10^6 V/m were used (Orlowska et al., 2009).

It was determined that as the field increased in intensity, the rate of ice nucleation increased. Wang et al. (2024a) froze grass carp under electrostatic treatments of 0, 3, 6, and 9 kV, and the samples froze under the 6 kV treatment resulted in the largest reduction of purge loss compared to those from the control. However, Xu et al. (2024) and Liu et al. (2022) did not find any difference in purge loss from pork loin and shrimp, respectively, frozen under electrostatic below 5 kV. Zhang et al. (2023a) also reported that using 3kV electrostatic while freezing yellow croaker resulted in brighter surface color with shinier muscle tissues throughout the 18-day display period. On the other hand, Jia et al. (2017) found that 10 kV electrostatic assisted freezing on pork tenderloin resulted in a lower L*, a* and b* values immediately following thawing, implying high intensity electrostatic assisted freezing may decrease shelf-stability of meat. While conflicting results do exist among the current body of electrostatic research, it is clear that potential for the improvement of previously frozen products exists.

Magnetic Fields

Static magnetic fields have been utilized in the freezing and supercooling of a variety of food products including meat tissues, and appear to generate smaller and rounder ice crystals through the uniform organization of water molecules in alignment with the waves (Jha et al., 2017a). Long et al. (2024) reported the fastest freezing rate of fried rice under static magnetic fields of 0-10 mT strengths to be the 2 mT treatment, followed by 5 mT, with 6 mT requiring the longest time to freeze. While no pattern emerges between treatment intensity and time to freeze, nucleation time was also the shortest in the 2 mT treatment, aligning with commonly understood mechanisms of freezing (Long et al., 2024). Similarly, Yang et al. (2024) found no consistent relationship between channel catfish frozen under increasing magnetic field strengths from 0 to 3

mT and transition time. However, this study did report a decrease in troponin-T, and myosin light chain in the 1, 2, and 3 mT treatments compared to the control, hypothesizing that magnetic fields could cause myofibrillar destruction (Yang et al., 2024). Zhou et al. (2022) observed no differences in hardness of bread dough frozen under 2 mT magnetic field at -20°C after 3 and 7 freeze/thaw cycles. However, this study did report a decrease in hardness in the magnetic field assisted samples when the samples were subjected to 10 freeze thaw cycles (Zhou et al., 2022).

Electromagnetic Freezing

General Electromagnetic Fields

Electromagnetic (EM) waves are formed through changing electromagnetic fields and encompassing a wide variety of frequencies, hertz, and wavelengths. When applied during the freezing process of food products, these wavelengths interact with the dipole water molecules and result in torque-assisted rotation or oscillation (Hanyu et al., 1992). This theory further went on to explain that this rotation decreases the opportunity for secondary nucleation during freezing because the rotation results in instability in the water molecule (Hanyu et al., 1992). More recently, Xanthakis et al. (2014) hypothesized that not only does the torque assisted rotation result in instability but creates small areas of localized heat, which thaw ice crystals that then rapidly refreeze. This helps contribute to smaller and more uniform ice crystals that are generally reported with the use of EM (Xanthakis et al., 2014).

EM waves, specifically microwaves, have gained attention in the food industry primarily because of their ability to improve food safety (Tang, 2015; Zhang et al., 2024). It is generally believed that EM waves are able to cause non-thermal damage to microbes, either through pore creation in the cellular membrane or denaturation of proteins, ultimately resulting in cellular

death but this topic is still heavily debated (Banik et al., 2003; Shamis et al., 2011; Bhatt et al., 2020). Regardless of its functionality, Kizhekkedath et al. (2017) has reported that studies on EM radiation in both processed and fresh meat products have shown decreases in spoilage and pathogenic bacteria.

Otero et al. (2017) tested 0%, 50%, or 100% power of an oscillating EM generating freezer on crab sticks at -25 °C, and they found no differences in color stability, water holding capacity, or shear force between treated and control groups. Conversely, using an EM resonance freezer, Choi et al. (2015) reported consistently smaller ice crystal size in beef loin and round steaks frozen to -55 °C under EM compared to the air blast frozen samples. Additionally, this study reported lower thawing loss in loin and round steaks throughout 8 months of storage compared to those frozen under blasted air (Choi et al., 2015). Under a AC electric field strength of 150 V/cm at a frequency of 50 Hz, Wu et al. (2023a) observed beef *Longissimus dorsi* samples were less effected by the mechanical damage of freezing and a reduction of protein oxidation was observed compared to the control. Wu et al. (2023b) similarly concluded that beef *Longissimus lumborum* frozen under the same AC electric field resulted in a faster freezing time and less purge loss compared to samples frozen without the intervention. Combined with the histological assessment, it was determined that the intervention resulted in smaller ice crystal formation and less damage to the myofibrillar structure during freezing (Wu et al., 2023b). Frozen at -18°C, Lin et al. (2025) reported that samples under an AC electric field of 2.2 kV with a frequency of 50 Hz had a smaller ratio of ice crystal area to total cell area than those frozen under traditional conditions and purge loss was lower.

Radiofrequency Assisted Freezing

Radio waves are a subtype of EM waves and are characterized by wavelengths greater than 100 millimeters (Mattson et al., 2013). Much research has been done relating to the use of radio waves in food preservation and cooking (Laycock et al., 2003; Uemura et al., 2010; Wang et al., 2012). However, Anese et al. (2012) showed that a treatment of 5 pulses of radiofrequency waves at 10 seconds each in freezing pork loin under flowing liquid nitrogen resulted in approximately 2% lower drip loss without alterations to the post thaw color compared to the control. In a similarly structured study, Manzocco et al. (2022) observed a decrease in drip loss and smaller ice crystals in pork loin treated with 2 kV of pulsed and continuous radiofrequency frozen under continuous liquid nitrogen at -80°C compared to samples without the treatment. Hafezparast-Moadab et al. (2018) observed that rainbow trout frozen at -30°C under pulse patterns of 10 seconds on/20 seconds off, 20 seconds on/20 seconds off, or 30 seconds on/20 seconds off. They found no differences in the freezing rate and found no variation in ice crystal size based on the pattern application. These same authors did find, however, a decrease in purge loss and ice crystal formation when electrode spacing was increased from 2 to 4 cm (Hafezparast-Moadab et al., 2018). Although radio waves show potential for improving previously frozen meat products, converting this intervention for industry application has seen limited success.

Microwave Fields

Microwaves are a subtype of EM waves that are characterized by wavelengths of 100 millimeters to 1 millimeter (Mattson et al., 2013). Xanthakis et al. (2014) used 40, 50, and 60% microwave power during the freezing of pork tenderloins, and they found that microwave power

shortened freezing time compared to the control. In contrast, Vardanjani et al. (2024) froze button mushrooms under 0%, 10%, 20%, or 30% microwave power, and they concluded that any microwave intervention resulted in a longer time to freeze, attributing the localized heating effect caused by the Ohmic effect (Vardanjani et al., 2024). This effect is caused by the friction resulting from running wavelengths through a food product and has been traditionally studied for its effects in food processing and sterilization (Varghese et al., 2014; Pereira et al., 2024). Xanthakis et al. (2014) also found that there was a decrease in overall cellular damage with any of the microwave treatments applied. Similarly, Atani et al. (2022) found that freezing lamb at 40, 50 or 60% microwave power had rounder and smaller ice crystals, leading to the potentially lower amounts of cellular disruption and thus higher quality once thawed. This same study found the samples with higher microwave power (50 and 60%) had a higher color stability once thawed, implying a decrease in myoglobin and hemoglobin damage and a decrease in purge loss. Similarly, button mushrooms treated with any microwave power level resulted in less drip loss but a higher browning index, concluding that longer period to freeze increase the likelihood for color instability (Vardanjani et al., 2024). Although microwave assisted freezing has shown promising effects in improving food quality, variability in the food product and the effectiveness of microwave-assisted freezing methods leads to significant inconsistencies in results.

Conclusion

Ice crystal nucleation is the starting point for reducing the overall damage of previously frozen products. Methods like EM assisted freezing target the crucial phase transition period when water molecules are converting back and forth between solid and liquid phases. Increasing the rate of nucleation, thereby generating more nuclei while reducing their overall size, is the key

to minimizing damage. As more research focuses on these interventions, targeting more specific treatments and limiting external variables will allow for a greater understanding and thus focus meaningful consideration into the upscaling for industry. Resultingly, this study aims to determine the effects of electromagnetic freezing on the industry applicable shelf-life and biochemical processes of beef striploin.

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Chapter 2 - Electromagnetic Freezing – A novel approach to the improvement of previously frozen beef

Freezing beef allows for more effective management of backstock in the meat industry (Carrillo-Lopez et al., 2021; Jiang et al., 2023), and this practice has become increasingly important for packers and further processors in the recent years due to supply and demand volatility (Yu et al., 2024). During the freezing process, ice crystal nuclei are initially formed, and secondary nucleation occurs as surrounding water molecules migrate to the primary nucleation site, leading to the growth of larger crystals (Jiang et al., 2023). The critical ice crystallization zone is traditionally comprised of temperatures between -1 to -2°C (Farouk et al., 2013), and the prolonged recruitment of water molecules and extended time in this solid-liquid equilibrium phase will result in the formation of larger ice crystals compared to those from rapid freezing (Jia et al., 2017; Tan et al., 2021). These larger ice crystals can damage muscle tissues by tearing and disrupting their structural and cellular integrity resulting in lower meat quality such as shorter post-thaw shelf-life, greater purge loss, and lower consumer acceptability (Qian et al., 2019). Therefore, it is generally accepted that an inverse relationship exists between the ice crystal size and meat quality attributes (Dalvi-Isfahan et al., 2016; Qian et al., 2019).

To better combat the negative effects of slow freezing, several different freezing aids such as ultrasound (Reyes-Villagrana et al., 2020), electrostatic (Dalvi-Isfahan et al., 2016), microwave (Tan et al., 2019), and electromagnetic (EM) fields (Otero et al., 2017) have been investigated. These aids have become of interest due to their potential improvement in the distribution, size, and structural nature of ice crystals formation in meat (James et al., 2015). Out of those mentioned, EM has shown promising results in a variety of food products and thus has

become of interest due to its potential for large scale industry application (Otero et al., 2017). EM, as the name implies, is a combination of electric and magnetic fields and serves as an overarching name for a variety of fields including radiofrequency and microwave (Chrispin and Nazarian, 2017). The theory underlying the functionality of EM freezing was first introduced by Hanyu et al. (1992), who hypothesized that torque-assisted rotation produced by EM wavelengths could reduce opportunities for secondary nucleation. More recently, Xanthakis et al. (2014) proposed that this torque-assisted rotation not only reduces secondary nucleation but also generates sufficient heat to briefly thaw ice crystals before they rapidly refreeze, resulting in smaller, more uniformly distributed ice crystals.

Xanthakis et al. (2014) reported that applying EM during the freezing of button mushrooms at 10%, 20%, or 30% microwave power resulted in smaller ice crystals in the treatment groups compared to those from the control, as evidenced by reduced cellular damage. The same authors also reported that it took longer to freeze for the EM samples in comparison to the control. Muscle tissues appear to behave similarly. Atani et al. (2022) found that smaller and rounder ice crystals were produced in lamb meat with EM treatments of 40, 50, or 60% microwave power compared to the control. It was inferred that less structural damage would have occurred due to the formation of those smaller and less prominent ice crystals (Atani et al., 2022). Although studies have reported EM assisted freezing as a promising intervention for cellular damage control in various foods, very few studies can be found using this technology on meat products. Furthermore, with variations in experimental designs from the existing studies, it can be challenging to determine an encompassing understanding of the effects EM has on frozen beef products. Thus, the objectives of this study were to characterize the effect of EM assisted

freezing on 1) freezing speed, yield (purge loss), microbial growth and beef shelf-life for future applications to the beef industry and 2) the biochemical characteristics of beef loin subprimals.

Materials and Methods

Sample Collection and Preparation

Longissimus lumborum (LL) were collected from both sides of 12 USDA Choice carcasses from a commercial beef processing facility in Arkansas City, Kansas on two separate days. On each day of collection, LL from both sides of six carcasses at 48 h postmortem were selected, vacuum packaged and transported to the Kansas State University meat lab. The LLs were swabbed (3M Sponge Stick with 10 mL neutralizing buffer; 3M Microbiology, St. Paul, MN) from an approximate 10 X 10 cm² area at the center of the ventral side and purge was collected for aerobic plate count (APC). These measurements were conducted prior to the treatments to establish a baseline for microbial load. The LLs were then halved, weighed, and the four sections of each pair were assigned to the four EM freezing treatments (0 kV, 2 kV, 4 kV, or 8 kV) in a manner that established an equal representation of each section in the four treatments to avoid location effect. A temperature probe attached to a temperature logger (THS-291-401, ThermoWorks Inc., American Fork, UT) was inserted into the geometric center of the halves to monitor and record the internal temperature throughout the freezing process.

The LL halves were frozen for 24 h in a walk-in freezer maintained at -20°C under continuous EM treatment throughout the freezing period. Specifically, two LL halves were placed on each stainless steel cart connected to a high voltage power supply equipped with a voltage controller (NDT-10KV, New Defrost Technology Inc., Taipei, Taiwan), with three carts assigned to each treatment daily over a four-day period (Figure. 1). The voltage applied to the

system was verified using a multimeter (177, Fluke Corp., Everett, WA) connected to a high voltage probe (80K-40, Fluke Corp.). Due to the limited size of the freezer, only two EM treatments could be applied on each day, involving a total of six carts. The first sample collection occurred on day 0 and the treatment schedule was as follows: On day 1, EM treatments of 0 and 2 kV were applied. On day 2, EM treatments of 4 and 8 kV were applied. The second sample collection occurred on day 2, and this same treatment sequence was inverted on days 3 and 4 for the samples from the second collection trip.

Following EM treatment, the LL halves were weighed. Three cores approximately 2.5 cm X 2.5 cm X 5.1 cm were taken from the center of the dorsal portion of each half LL under frozen conditions using a 2.5 cm core bit (Hole Dozer Bi-Metal Hole Saw, Milwaukee Tool, Brookfield, WI) attached to a cordless drill (DCD793B, Dewalt, Baltimore, MD). The coring process of the LL halves were staggered so only one LL half was out of the freezer at a time to ensure ice crystal integrity. Cores were immediately placed in a whirlpak bag and transferred to a -80°C freezer. The LL halves were weighed again to account for the weight of the cores taken. The cored LL halves were vacuum packaged and stored in the same -20°C freezer for another 8-10 days without EM treatment. After the designated freezing period, all LL halves were thawed in a walk-in cooler set at 2°C for 72 h. The temperature of the geometric center of all LL halves was at least 0°C before being transferred to the processing room and weighed. Purge was collected from the vacuum package, and swabs (3M Sponge Stick with 10 mL neutralizing buffer) were taken from an approximate 10 X 10 cm² from the ventral side of the LL halves at a different location than the initial swab. Overall thaw yield percentage was calculated as:

$$\left(1 - \frac{\text{Initial raw weight} - \text{Cores weight} - \text{Final thawed weight}}{\text{Initial raw weight}}\right) \times 100$$

The LL halves were then immediately fabricated into two 2.54-cm and five 1.27-cm steaks. One 2.54-cm steak was immediately cooked and used for Warner-Bratzler Shear Force (WBSF) and cook loss analysis. One 1.27-cm steak was immediately used for histological analysis to measure muscle fiber spacing. A second 1.27-cm steak was coarsely ground, packed in a petri-dish, and fat, moisture, and protein were determined using the FOSS FoodScan (FOSS Analytical, Hillerød, Denmark). The post-scan ground beef was frozen with liquid nitrogen, pulverized, and stored at -80°C for laboratory analysis as undisplayed samples.

The remaining 2.54-cm steak and the three 1.27-cm steaks from each portion were overwrapped with polyvinyl chloride (23,250 cc/m²/24 h at 23°C and 0% relative humidity; Sysco, Houston, TX) in Styrofoam trays (17S; Genpak, Charlotte, NC) lined with a tray absorbent pad (Dri-Loc AC25; Novipax, Oak Brook, IL). The overwrapped steaks were subjected to a 10-day simulated retail display under continuous fluorescent lighting (32 W Del-Warm White 3,000 K; Phillips Lighting Company, Somerset, NJ) with $2,143 \pm 113$ lx in a coffin style retail case (model DMF8; Tyler Refrigeration Corp., Niles, MI). Descriptive and objective color measurements were taken each day throughout the 10 display days on the second 2.54-cm steak cut from each subprimal (n=48). Panelists made up of at least six graduate students and faculty were trained on two separate days on discoloration identification before the study. Discoloration was measured on a continuous line scale with anchors at 0=0% discoloration, 50=50% discoloration, and 100=100% discoloration. The discoloration surveys (Qualtrics XM, Provo, UT) were accessed through a QR code, and the panelists submitted their responses through their personal cell phones.

Objective color was measured according to the American Meat Science Association (AMSA) Meat Color Measurement Guidelines as described by King et al. (2022). The average of

six measurements of L*, a* and b* were taken on the lean portion of each representative steak using a HunterLab MiniScan EZ spectrophotometer (Illuminant D65, 2.54-cm diameter aperture, 10° observer, Hunter Associates Laboratory, Reston, VA). The spectrophotometer was calibrated each day with standardized black and white tiles. On days 4, 7, and 10, one 1.27-cm steak from each portion was frozen with liquid nitrogen, powdered, and stored at -80°C until further analysis. The second 2.54-cm steak was cooked on day 10 and used for WBSF measurement and cook loss.

Microbial Analysis

Aerobic microbial analysis was performed as described by Corrette et al. (2024). The swabs and purge samples were diluted 1, 1/10, and 1/100 in sterilized phosphate-buffered saline. One mL of each dilution was plated in duplicate on 3M Aerobic Plate Count (APC) petrifilm plates (6406, 3M Microbiology). Plates were incubated at 37°C for 48 h in a Symphony incubator (89511-418, VWR, Radnor, PA). The APC was determined by counting the individually formed colonies according to 3M Petrifilm Interpretation Guide (3M Microbiology) following the incubation. The final count was transformed into log CFU for statistical analysis.

Warner-Bratzler Shear Force

WBSF was conducted as described by Corrette et al. (2024). Briefly, the 2.54-cm steaks from day 0 and 10 of each sample were cooked to 71°C using a Cuisinart Griddler Deluxe Clamshell (Cuisinart, Stamford, CT). Internal temperature was monitored by inserting a ThermoPen thermometer (MK4; Thermoworks Inc.) into the geometric center of each steak. Raw

and cooked weights were recorded for each steak, and the cook loss percentage was calculated as:

$$\left(\frac{\text{Initial raw weight} - \text{Cooked weight}}{\text{Initial raw weight}} \right) \times 100$$

Steaks were then cooled for 24 h at 4°C. Once cooled, six 1.27-cm-diameter cores were taken parallel to the muscle fiber for WBSF analysis. Cores were sheared perpendicular to the muscle fiber using a V-shaped blade (G-R Elec. Mfg., Manhattan, KS) attached to an Instron Universal Testing System (Model 5569; Instron Corporation, Norwood, MA) at 250 mm/min. The resulting shear force value for each steak was calculated from the mean of the six cores and reported in kilograms of force.

Hematoxylin and Eosin Stain for Muscle Fiber Spacing Analysis

Muscle fiber spacing was determined using hematoxylin and eosin staining as described by Corrette et al. (2024). Briefly, three 1 cm X 1 cm X 1 cm cubes per sample were prepared from one 1.27-cm steak parallel to the muscle fiber. Cores were submerged in optimal cutting temperature medium (OCT; Thermo Fisher, Hampton, NH) in 22 mm X 22 mm X 20 mm Eppredia Peel-A-Way disposable embedding molds (Thermo Fisher) and frozen in liquid nitrogen cooled isopentane. Cubes were then stored at -80°C until analysis.

Frozen cubes were sliced perpendicular to the muscle fiber using a microtome cryostat machine (Microm HM 550; Thermo Fisher). Samples were sliced at -20°C to a thickness of 25 µm and fixed to a charged microscope slide (Globe Scientific, Mahwah, NJ). The slice was allowed to dry at room temperature for 1 h. Samples were stained in a 1% hematoxylin solution for 5 min, rinsed in deionized water for 5 min, briefly dipped twice in a 50% eosin-y solution,

and immersed in 50% ethanol for 10 sec, 70% ethanol for 10 sec, 95% ethanol for 30 sec, and 100% ethanol for 30 sec. Finally, samples were dipped in xylene seven times before covering with a drop of mounting medium (Eupredia Shandon-Mount, Thermo Fisher) and a coverslip (Fisher Scientific, Pittsburg, PA). Slides were allowed to dry at room temperature for 1 h before imaging.

Slides were viewed under a 10X magnification on a Nikon Eclipse Ti2 microscope (Nikon Instruments, Toyko, Japan) and imaged using a Nikon DS-Fi3 camera (Nikon Instruments). ImageJ (v. 1.53, U.S. National Institutes of Health, Bethesda, MD) was used to determine distance between adjacent muscle fibers (Figure. 2). Approximately 30 measurements were taken per slide totaling 90 measurements per sample.

X-ray Computed Tomographic Analysis for Ice Crystal Size Determination

Frozen cores collected during sample preparation were shipped to the Agricultural Particulates Research Group at Purdue University in dry ice for X-ray computed tomographic (XRCT) analysis of ice crystal size. Frozen meat cores were scanned with the Skyscan 1272 desktop X-ray microscopy system (Bruker, Billerica, MA). Each sample was placed into a sample tube with sufficient dry ice to keep the core frozen during imaging. Each sample scanned for approximately 90 min under 40 kV and 100 μ A for the power settings. Camera imaging settings was 1640 X 2452-pixel resolution with an exposure time of 4.4 sec. Each pixel represented 9.2 μ m. Approximately 300 to 1,500 cross-sectional slices with a thickness of 11 μ m per slice were taken for each core depending on core length. Following imaging, samples were reconstructed using the NRecon reconstruction software (Bruker).

To characterize ice crystal size in each core, a Python code was written using the OpenCV image-analysis library (OpenCV Foundation, Palo Alto, CA) to count the number of ice crystals in a given image (Figure 3). Only every fifth slice in the stack of a core was analyzed yielding a separation of approximately 55 μm to avoid double counting the same crystals in consecutive images. As ice crystals absorb x-ray radiation more strongly than muscle tissue, each image was binarized such that all pixels above the 255 threshold are rendered white. Additionally, only contiguous white pixels between 19 μm and 100 μm were counted to reduce background noise and exclude intramuscular fat. Employing these criteria (intensity threshold and size), the code identifies and records the size, area, and location of each ice crystals in the image for each designated image in the stack. Typically, 3,000-10,000 ice crystals were counted per image, and approximately 800 slices were characterized per sample, resulting in > 1,000,000 ice crystals counted for each sample. The diameters of all ice crystals counted were averaged for each sample.

Lipid Oxidation

Lipid oxidation was determined through the thiobarbituric acid reactive substances assay (TBARS) as described by Dahmer et al. (2022). Briefly, approximately 0.2 g of powdered meat from each sample were combined with 1 mL of 20 mM trichloroacetic acid/15% thiobarbituric acid and 0.05 mL of 3% butylated hydroxytoluene in ethanol. Samples were homogenized (Bead Blaster 24; Benchmark Scientific, Sayreville, NJ) and centrifuged (5810 R; Eppendorf, Hamburg, Germany) for 5 min. Six hundred μL of the supernatant was transferred to glass tubes and incubated at 70°C for 30 min in a water bath (Precision 2872; Thermo Fisher) and submerged in a cold water bath for 5 min. Samples and a standard curve of 0 to 25 μM of

malonaldehyde (MDA) were plated on a 96-well plate in duplicate and read in a spectrophotometer (Eon; BioTek Instruments Inc., Winooski, VT) at 532 nm. Lipid oxidation was calculated based on the standard curve concentration and reported as milligrams of MDA/kg of muscle tissue.

Oxygen radical absorbance capacity

Antioxidant capacity was determined through the oxygen radical absorbance capacity assay (ORAC) as described by Chun et al. (2023). Briefly, approximately 0.1 g of powdered sample from each half of days 0, 4, 7, and 10 of retail display were combined with 1 mL of hexane and homogenized (Bead Blaster 24; Benchmark Scientific, Sayreville, NJ). Samples were vortexed at a low speed for 1 h before centrifuged at 3,000 X g (5810 R; Eppendorf, Hamburg, Germany) for 10 min. The hexane layer was removed from the pellet and dried under a continuous flow of nitrogen. The sample was redissolved in 750 μ L of 7% randomly methylated cyclodextrin (RMCD) to obtain the lipophilic portion. The pellet was retained and combined with 1 mL of 20% ethanol and 80% ultrapure water. The sample was homogenized and centrifuged at 12,000 X g for 10 min before removing 750 μ L of the supernatant for the hydrophilic portion.

One hundred and fifty μ L of 0.1% fluorescein solution in phosphate buffer were first added into all experimental wells on a 96-well plate. Twenty-five μ L of standards and samples were plated in triplicate and incubated at 37°C for 30 min before adding 25 μ L of 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) into each occupied well. Samples were then read in a spectrophotometer (Synergy HTX Multi-Detection Microplate Reader; BioTek Instruments, Inc. Winooski, VT) under a fluorescence setting (excitation: 485 nm, 20 nm bandpass; emission: 528

nm, 20 nm bandpass). Measurements were taken every 60 sec for 2 h for a total of 360 measurements per sample. Lipophilic and hydrophilic antioxidant capacity was calculated using the net area under curve between the sample and the blank as described by Chun et al. (2023). Antioxidant capacity was then converted into Trolox equivalents (TE) based on a standard curve of 0 to 100 μ M of Trolox, and values were reported as TE/g of muscle tissue.

Proteolysis

Proteolysis of meat tissue were determined through the measurement of troponin-T and desmin degradation through a modified protocol as described by Corrette et al. (2024) and Hammond et al. (2022). Briefly, whole muscle protein was extracted from 0.2 g of pulverized meat sample with an extraction buffer containing 2% sodium dodecyl sulfate (SDS) and homogenized (Bead Blaster 24; Benchmark Scientific, Sayreville, NJ) for 30 sec. Samples were centrifuged at 4,000 X g (5810R; Eppendorf) for 5 min, and supernatant was collected and deemed as the protein stock. To determine protein concentration of the stock, a Pierce BCA Protein Assay Kit (Thermo Fisher) was utilized following manufacturer's instruction. Protein stocks were diluted and standardized to 2,000 μ g/mL.

To determine troponin-T and desmin degradation in a multiplex setting, gel electrophoresis and Western Blot were conducted. Sample was combined with 2X reducing Laemmli buffer (Thermo Fisher) at 1:1 ratio and heated at 95°C for 5 min. A total of 5 μ g of protein sample was loaded into each well of a 10% tris-glycine gel (XP00100BOX; Invitrogen, Carlsbad, CA) and ran with a Mini Gel Tank (Invitrogen) at 180 V for 50 min.

Once the gel electrophoresis was completed, protein was transferred to polyvinylidene fluoride (PVDF) membranes using a protein transfer system (iBlot2; Invitrogen) under the

setting of 20V for 1 min, 23V for 4 min, and 25V for 2 min. Once transferred, the membranes were blocked with 10 mL of OneBlock Western-FL Blocking Buffer (Genesee Scientific, San Diego, CA) for 1 h. Membranes were then incubated in anti-troponin-T mouse monoclonal primary antibody (JLT-12; Sigma Aldrich, Burlington, MA) and anti-desmin rabbit monoclonal primary antibody (RM234; Sigma Aldrich) in 1:2,000 dilution with blocking buffer for 1 h. Following primary antibody incubation, membranes were washed in 5 mL 1X Tris-buffered saline with 0.1% Tween-20 (TBS-T) three times for 5 min each. Membranes were then incubated in Alexa Fluor 488-anti-mouse secondary antibody (A32723; Invitrogen) and Alexa Fluor 647-anti-rabbit secondary antibody (A11001; Invitrogen) in 1:10,000 dilution with blocking buffer for 1 h. Membranes were then washed in 5 mL TBS-T 3 times for 5 min each with a final wash in 5 mL of TBS for 5 min. The membranes were imaged using the iBright Imaging System (FL 1500, Thermo Fisher) under the fluorescence setting: Alexa Fluor 488 (excitation peak: 488 nm and emission peak: 496 nm) and Alexa Fluor 647 (excitation peak: 650 nm and emission peak: 671 nm). The iBright Analysis Software was used for analysis (Thermo Fisher) in which troponin-T was analyzed under the 488 nm channel and desmin was analyzed under the 647 nm channel (Figure 4, Figure 5). Both troponin-T and desmin degradation were calculated by dividing the total amount of degraded products in a single lane by the sum of the intact and degraded bands in that same lane. Measurements were reported as a relative percentage of the degraded bands of troponin-T and desmin.

pH

The pH of samples was determined as described by Corrette et al. (2024) with modifications. Briefly, 3 g of powdered sample were weighed in duplicate into 50 mL conical

tubes and combined with 30 mL of ultrapure water. Samples were homogenized using an 850 homogenizer (15340169, Thermo Fisher) with a 10 mm x 115 mm stainless steel probe (15340178, Thermo Fisher) for 40 s at 10,000 rpm. An InLab Science Pro-ISM pH probe attached to a Seven Compact pH meter (Meter Toledo; Columbus, OH) was calibrated using 4.0, 7.0, and 10.0 pH standards before data collection. Sample pH were read by placing the probe into the tube under constant stirring using a stir bar.

Calcium concentration

The free calcium concentration of samples was determined as described by Welter et al. (2022). Briefly, 7 g of powdered sample was measured into each open-top thickwall polycarbonate ultracentrifuge tube (Beckman Coulter, Brea, CA) and centrifuged (Optima XE-90 Ultracentrifuge; Beckman Coulter) at 175,000 X g for 30 min. One mL of the supernatant was collected and 25 uL of that supernatant was used to determine protein concentration using a Pierce BCA Protein Assay Kit as previously described. The remaining supernatant was combined with 0.2 mL of 27.5% trichloroacetic acid and immediately vortexed. The sample was allowed to incubate at room temperature for 10 min, and samples were centrifuged (Eppendorf) at 10,000 X g for another 10 min. Three hundred uL of the supernatant was transferred into a glass tube and combined with 2.7 mL of ultrapure water. The sample was then filtered through a 13mm diameter Millex-LG 0.45 µm syringe filter (MilliporeSigma, Burlington, MA) into a new tube. Filtered samples were quantified using atomic absorption spectroscopy (Perkin-Elmer, AAnalyst 200, Norwalk, CT) under calibrated calcium concentration standards and measurements were taken at 422.7 nm. Measurements were converted to free calcium concentration using the calcium standards and was reported as nmol of calcium/ mg of protein.

Statistical Analysis

Data were analyzed under the PROC GLIMMIX procedure of SAS (v. 9.4, SAS Institute, Cary, NC). Freezing rate, yield, proximate analysis, muscle fiber spacing, and ice crystal diameter were analyzed as a randomized complete block design. The freezing day was designated as the block. The microbial analysis was analyzed as a one-way analysis of covariance where the fixed effect was represented by the EM treatment and the covariate was the initial microbial count. The Toeplitz covariance structure was selected because it had the smallest value for Akaike's information criterion. Cook loss, shear force, lipid oxidation, antioxidant capacities, pH, free calcium, and troponin-T and desmin degradation were analyzed as a split-plot where the whole plot is the EM treatment, and the split-plot is the display day. Instrumental and subjective color were analyzed as a split-plot with repeated measures design where the whole plot was represented by EM treatment and the repeated measure was the display day. The experimental unit was each cart ($n = 24$; 6 per treatment). Mean separation was conducted using least-squares mean procedures, and differences among means were detected at $P < 0.05$ level using the least significant difference.

Results

Effect of EM treatments on LL freezing characteristics and yield

Figure 6 illustrates the time it took to lower the subprimals' initial temperature ($\sim 3^{\circ}\text{C}$) to the four targeted end point temperatures for the four EM treatments. No treatment effect was observed from the initial temperature to 0°C among the treatments ($P > 0.05$). However, a treatment effect was found for the time it took to decrease from the initial temperature to -5°C , -10°C , and -18°C ($P < 0.01$). Samples from 0 kV and 2 kV treatments took a shorter amount of

time to reach -5°C compared to those from 4 kV treatments ($P<0.01$), while those from 8 kV treatment was not different from 2 or 4 kV in the time it took to reach -5°C ($P>0.05$).

Furthermore, the samples from 0 kV treatment took the shortest amount of time from the initial temperature to reach -10°C , followed by those from 2 kV, 8 kV, with the 4 kV samples took the longest time to reach -10°C ($P<0.01$). Finally, the 0 and 2 kV treatments took the shortest time from the initial temperature to reach -18°C , followed by those from 8 kV, with 4 kV samples once again took the longest amount of time to reach -18°C ($P<0.01$).

A treatment effect was found for overall yield of the LLs after thawing ($P<0.01$; Table 1). Samples from the 8 kV treatment were found to have greater overall yield percentage than those from the 0, 2, and 4 kV groups ($P<0.01$). However, no treatment effect was found for fat, moisture, or protein % nor the muscle fiber spacing and ice crystal size measurements ($P>0.05$; Table 1). Finally, no treatment effect was found for cook loss ($P>0.05$; Table 1).

Effect of EM treatments on LL tenderness and proteolysis

No interaction between treatment and display day nor a main effect of treatment was found for WBSF ($P>0.05$; Table 1). A display day effect was observed for WBSF in which steaks displayed for 10 days were more instrumentally tender than those not displayed ($P<0.01$; Table 2).

In addition, no interactions were reported for the relative percentage of degraded troponin-T ($P>0.05$), but a treatment effect was observed ($P<0.01$; Table 1). The 8 kV samples had the lowest percentage of degraded troponin-T compared to those from 0, 2, and 4 kV ($P<0.01$). A representative image of the treatment effect is shown in Figure 4. A day effect was found for degraded troponin-T ($P<0.01$; Table 2). As expected, the relative percentage of

degraded troponin-T increased as display day increased ($P<0.01$; Table 1). No interactions were reported for the relative percentage of degraded desmin ($P>0.05$), but a treatment effect was also observed ($P<0.01$; Table 1). Similar to troponin-T degradation trend, a lower percentage of degraded desmin was shown in samples from 4 and 8 kV EM compared to those from 0 and 2 kV ($P<0.01$; Table 1). A representative image of the treatment effect is shown in Figure 5. A day effect was also found for degraded desmin ($P<0.01$). Again, degraded desmin percentages increased as display day increased ($P<0.01$; Table 2).

No interaction between display day and treatment was observed for free calcium concentrations ($P>0.05$). Both treatment and day effect were reported for free calcium ($P<0.01$; Table 1 and Table 2, respectively). For the treatment effect, 0 kV samples had a lower concentration of free calcium than those from 2, 4, and 8 kV samples ($P<0.01$). However, no difference in free calcium concentration was found among 2, 4, and 8 kV samples ($P>0.05$). Finally, free calcium concentration increased as display progressed ($P<0.01$).

Effect of EM Treatments on LL Shelf-Stability

An interaction between display day and EM treatment was observed for discoloration ($P<0.05$; Figure 3). All treatments remained the same until days 9 and 10, in which 4 kV samples had less discoloration than those from the other treatments on both day 9 and 10 ($P<0.05$). No interaction between display day and treatment was reported for L^* , a^* or b^* ($P<0.05$). Main effects for treatment and display were found for L^* , a^* , and b^* ($P<0.01$). For the treatment effect, L^* values for 0 and 2 kV groups were higher than those from 4 and 8 kV treatments ($P<0.01$; Table 1). On the other hand, the a^* and b^* values were highest for the 4 kV group, followed by 0 and 2 kV, while the 8 kV group had the lowest values ($P<0.01$; Table 1).

For the display effect, L^* increased until day 5 then remained constant through the remainder of display (Figure 8) while a^* (Figure 9) and b^* (Figure 10) values decreased throughout the 10-day display as expected ($P < 0.01$).

No interaction between display day and treatment or a treatment main effect was found for pH ($P > 0.05$; Table 1). However, a day effect was found for pH ($P < 0.01$, Table 2). Day 10 samples had the highest pH compared to those from the other display days ($P < 0.01$), and no differences were found among those from 0, 4, and 7 samples ($P > 0.05$).

No interactions were found for lipophilic, hydrophilic, or total antioxidant capacity ($P > 0.05$), but a treatment effect was found for all three antioxidant capacity measurements ($P < 0.01$; Table 1). For the lipophilic portion, samples from the 0 kV treatment had higher antioxidant capacity than those from the 2, 4, and 8 kV treatments ($P < 0.01$). For the hydrophilic portion, the 4 kV treatment samples had a higher antioxidant capacity than those from the 0, 2, and 8 kV treatments ($P < 0.01$). Finally, 4 kV treatment had higher total antioxidant capacity than those from 2 and 8 kV but was not different from the 0 kV treatment ($P < 0.01$). A display day effect was also observed for hydrophilic and total antioxidant capacity ($P < 0.01$; Table 2). Samples from days 0 and 4 for both hydrophilic and total antioxidant capacity reported similar values and were lower than those from day 7 and 10 samples ($P < 0.01$). No day effect was found for the lipophilic portion ($P > 0.05$; Table 2).

No interactions between treatment and display day or treatment effect were observed for lipid oxidation ($P > 0.05$; Table 1). However, a display day effect was found for lipid oxidation ($P < 0.01$). As expected, samples from days 0 and 4 reported the lowest amount of lipid oxidation, followed by samples from day 7, with samples from day 10 having the highest level of lipid

oxidation ($P < 0.01$; Table 2). Finally, no treatment effect was found for either APC count from swabs or purge ($P > 0.05$; Table 1).

Discussion

EM assisted freezing resulted in slower freezing rate but increased post-thaw yield

The results from this study showed that the samples from the higher voltage EM treatments resulted in longer time to reach our targeted temperature (-18°C) compared to those from the control. In agreement with our findings, Vardanjani et al. (2024) found that button mushrooms frozen with 10%, 20%, or 30% microwave applications took progressively longer to freeze as microwave power increased, with the 30% treatment requiring the longest freezing time. Similarly, Xanthakis et al. (2014) found that pork loins frozen under 40, 50, or 60% microwave power resulted in the same trend, with 60% treated samples requiring the longest time to freeze. Both studies recognized that the prolonged freezing time may be attributed to heat generation from the microwave application during freezing. Ohmic heating is a thermal processing technique in which an electrical current is passed directly through a food product, generating heat due to the electrical resistance of the material (Varghese et al., 2014). This resistance causes the oscillation of charged particles, thus resulting in friction that may produce heat uniformly throughout the product (Pereira et al., 2024). It is possible that the EM treatment could cause increased oscillation that produces localized friction and heat release, thus increasing the time to freeze. It should be noted that Jha et al. (2020) found no differences in the freezing rate of apples or potatoes when treated with continuous or pulsed EM field at 5, 15, or 20 W. They concluded that the lower power settings generated minimal friction, resulting in a negligible amount of heat.

Based on the established literature, rapid freezing is required to maintain product quality due to its ability to minimize ice crystal size, cellular damage and purge loss (Xie et al., 2021). However, as new interventions in freezing technology are developed, this commonly accepted belief has been challenged. In this study, the overall yield of LL after thawing was observed to be the highest in the 8 kV samples compared to those from the other treatments. Similarly, Anese et al. (2012) reported that applying a radiofrequency pulsed treatment at 2 or 7 kV to pork loin samples during freezing at -18 °C reduced drip loss by 2% compared to the control. On the other hand, Rodríguez et al. (2017) observed no differences in pork loin drip loss when using oscillating magnetic fields at 0, 50 or 100% during freezing at -30°C. Using the same oscillating magnetic field generator, Otero et al. (2017) reported the same no difference finding when freezing crab sticks with the same intensities of EM fields at -25 °C.

Although we found that 8 kV EM treatment increased the overall yield of LL post-thawing, no differences in muscle fiber spacing or ice crystal diameter were observed in this study. One theory behind all electrical energy based-assisted freezing is that it reduces the free energy required for primary nucleation during freezing, thereby significantly increasing the primary nucleation rate and decreasing the amount of free water available for secondary nucleation (Dalvi-Isfahan et al., 2016). This ultimately results in smaller ice crystal size due to minimized migration of water molecules. Increased abundance of ice crystals and reduced ice crystal size have also been associated with smaller spacing between the fibers (Ishiguro and Horimizu, 2008; Tippala et al., 2021). Furthermore, our results disagreed with Choi et al. (2015) and Hafezparast-Moadab et al. (2018), which both reported smaller ice crystal size when beef loin samples and rainbow trout were frozen under EM or pulses of radiofrequency waves, respectively. Given the large size of these LL subprimals in this study, there may be significant

resistance within each LL, leading to an uneven distribution of ice crystal sizes from the ventral to the dorsal end. It is possible that the reduction in ice crystal size is more pronounced on the ventral end of the LL as this is where the AC current contact is strongest. However, we only collected cores from the dorsal end, as performing an XRCT scan on the entire LL subprimal was not feasible. Finally, no differences in cook loss were found in this study in relation to EM treatments. It should be noted that the 8 kV treatment, which reported the highest overall yield following thawing, did not have a compensatory loss during cooking.

High voltage EM assisted freezing reduced proteolysis but had no effect on instrumental tenderness

In this study, no differences in WBSF were found among the treatments. Similarly, Hafezparast-Moadab et al. (2018) observed no differences in hardness nor ice crystal size among treatments when samples were frozen with pulsed radiofrequencies of 10, 20, or 30 sec. However, Anese et al. (2012) reported higher tenderness in pork loins frozen with 2 or 7 kV pulsed radiofrequency treatments at -18 °C under continuous liquid nitrogen flow compared to samples without the radiofrequency treatment. The same authors explained that the smaller intracellular ice crystals in pork loin samples froze with pulsed radiofrequency was able to retain more moisture by decreasing drip loss, thus improve tenderness. On the other hand, Dalvi-Isfahan et al. (2016) observed a direct relationship between tenderness and ice crystal size in lamb frozen under 4, 8 or 12 kV of electrostatic fields, and the authors attributed this finding to increased mechanical damage in muscle tissue caused by larger ice crystals. Perhaps, the reason why we didn't find any difference in WBSF was due to small differences in total yield between the treatments and the control, and no differences in ice crystal diameter in this study.

Although no significant differences in WBSF were observed, our results showed that proteolysis was reduced in samples treated with high-voltage EM fields. Similar to our findings, Suwandy et al. (2015) reported greater troponin-T and desmin degradation in beef LLs treated with 5 and 10 kV of pulse EM fields at 20 and 50 Hz than those from pulse EM field of 10 kV with 90 Hz. Both Suwandy et al. (2015) and Corrette et al. (2024) described higher voltage and frequency of EM can further contribute to physical damage of muscle tissue in addition to the damage from ice crystal formation. Tuell et al. (2022) showed that damage to the muscle structure may result in additional release of proteolytic enzymes and free calcium, resulting in higher levels of myofibrillar protein degradation due to increased activation of the calpain system. Interestingly, this study found that the 0 kV treatment had the lowest concentration of free calcium but with greater proteolytic activity compared to those from the high voltage EM treatments. Increased free calcium concentration is generally associated with an increase in calpain activity (Ji and Takahashi, 2006). However, this study observed the opposite. Calcium is often held by calcium-binding proteins such as calsequestrin and sarcocalumenin in sarcoplasmic reticulum but could be released due to sufficient amounts of protein denaturation (Conte et al., 2023). As previously noted, the friction generated by water molecule oscillation from EM may have produced heat, leading to the denaturation of calcium-binding proteins and calpains. This, in turn, could have reduced proteolytic activity despite the noted higher free calcium concentration.

Finally, after 10 days of display, shear force decreased in all samples compared to those at 0 days, aligning with the existing body of literature (Aroeira et al., 2016; Nair et al., 2019). As expected, desmin and troponin-T degradation also consistently increased throughout the 10 days of retail display. It is well established that as meat products age, proteolytic enzymes break down

meat tissue, resulting in a more tender product (Zhang et al., 2021; Tuell et al., 2022). Similarly, free calcium concentration increased throughout days of display in this study. As aging progresses, more calcium is released from calcium-binding proteins and continuously contributing to the activation of the calpain system and overall myofibrillar protein degradation (Ji and Takahashi, 2006).

EM assisted freezing increased color stability and antioxidant capacity during retail display

Samples from the 4 kV treatment reported lower total discoloration on days 9 and 10 of display along with the highest a^* compared to those from the other treatments. Similarly, Wu et al. (2023b) reported a higher a^* values through 7 days of display in beef striploins frozen with 2.2 kV of AC field at -18°C compared to untreated samples. However, no improvements were reported for pork loin samples frozen under -30°C with oscillating magnetic fields of 0, 50, and 100% power in L^* , a^* , or b^* (Rodríguez et al., 2017). Li et al. (2020) also reported no differences in L^* , a^* , or b^* values in salmon frozen and thawed under a pulsed EM treatment of 1 kV/cm and a frequency of 50 Hz. Although research has shown inconsistent results in EM's ability to improve color stability in various food products, the greater color stability findings from the 4 kV samples from this study are further supported by samples from 4 kV treatment also had the greatest antioxidant capacity. Higher antioxidant capacity is well documented in preventing protein oxidation and thus delayed the conversion of oxymyoglobin to metmyoglobin in meat (Echegaray et al., 2021). Arteaga et al. (2022) observed blueberries frozen under magnetic field strengths of 36.8 mT or 44.7 mT with 30, 60, 90, or 120 Hz frequencies and reported samples frozen with the 44.7 mT treatment had higher antioxidant capacity compared to

the frozen control regardless of frequency intensity. Although we noted greater antioxidant capacity, our study showed no changes in the concentration of MDA regardless of EM treatment. Manzocco et al. (2022) determined that protein and lipid oxidation was not altered by the intervention of pulsed radiofrequency and thus resulting in no differences in instrumental color. As this enhancement in color stability from the 4 kV EM samples cannot be attributed to the EM's ability to reduce ice crystal size, it has to be explained through other mechanisms. Sadot et al. (2020) reported that heat induced mild denaturation in proteins may allow for the exposure of amino acids with antioxidant abilities. Perhaps, the slower freezing rate of the 4 kV EM treatment is an indication of heat produced during the freezing process, and this mild heat has resulted in an increase in antioxidant capacity in those samples, thus allowing the 4 kV samples to exhibit better color stability compared to the others.

Discoloration has also been linked to pH and the growth of spoilage microbes during refrigerated display (Li et al., 2015; Neethling et al., 2017). EM treatments are known to reduce microbial populations by inducing single- and double-strand breaks in microbial DNA, thereby limiting their reproduction (Albert et al., 2021). However, this study reported no differences in pH or aerobic bacterial growth in relation to EM treatment. Similarly, Corrette et al. (2024) reported no changes in bacterial load nor pH after thawing previously frozen beef under AC EM at 2.5, 5 or 10 kV. The authors attributed the lack of difference in microbial growth among the treatments to low thawing temperatures at 2°C. On the other hand, the freezing process itself is known to reduce the growth of certain bacteria (Speck and Ray, 1977; El-Kest and Marth, 1992; Dang et al., 2021). Although the EM treatments may be effective in inhibiting microbial growth, it is likely that freezing itself was sufficient to reduce the initial bacterial load and thus mask the treatments' effect. This study also reported an increase in pH on day 10 of display, which some

spoilage bacteria, such as the *Psuedomonas* subspecies, are known to produce alkaline byproducts and thus can cause an increase in pH throughout display (Marcelli et al., 2024).

Conclusion

While high voltage EM assisted freezing was not found to decrease ice crystal size, it has appeared to increase thawing yield in previously frozen beef LLs through an unknown mechanism. Secondly, the incorporation of EM during the freezing process has shown promising results for increasing the antioxidant capacity, which resulted in shelf-life improvement of previously frozen beef, with no change in instrumental tenderness. The small increase in post-thaw yield and shelf-life of the product has the potential to provide a significant economic incentive for packers and further processors to utilize EM assisted freezing in commercial scale. Further research on EM-assisted freezing is needed to explore its feasibility for large-scale integration into commercial freezer systems.

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Figure 2.1: Representative image of EM treatment set up with temperature probes

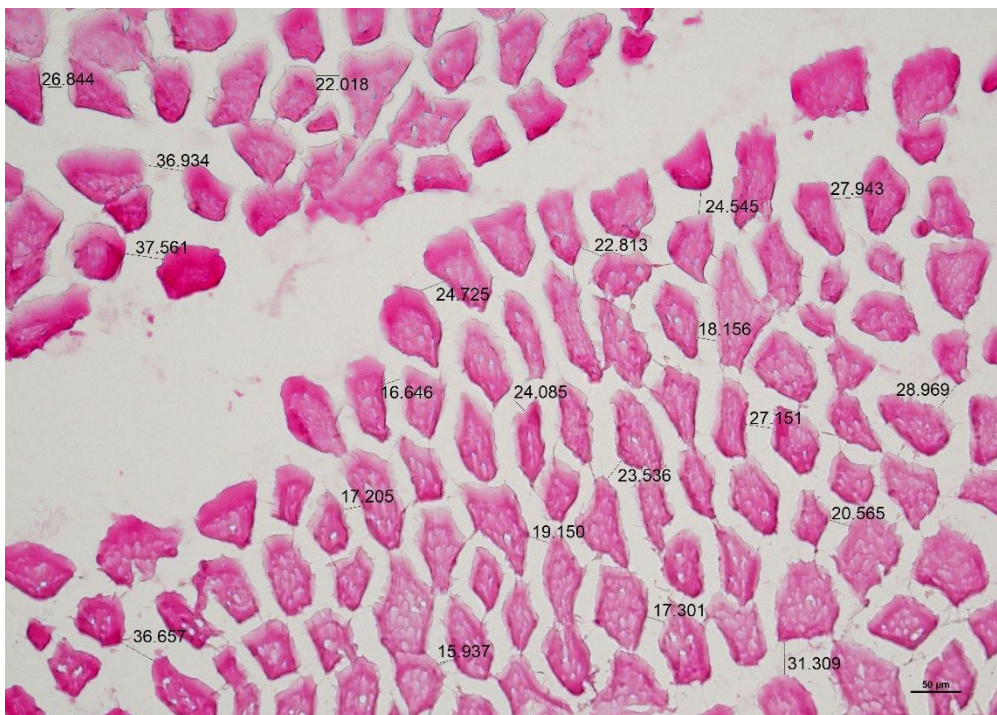


Figure 2.2: Representative measurements of muscle fiber spacing

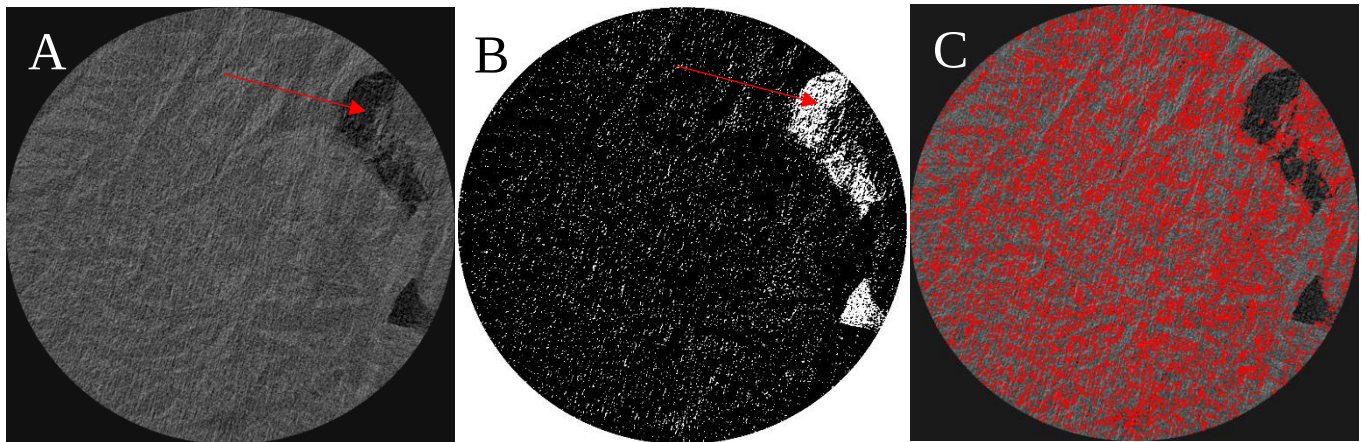


Figure 2.3: Representative *Longissimus Lumborum* core X-ray computed tomographic (XRCT) slice image and analysis for ice crystal size. A) Original XRCT slice image where light gray represents lean muscle tissue, and large portions of black area (denoted by the red arrow) are the intramuscular fat deposits in the muscle tissue. B) Binarized image of the same slice, where now black represents lean muscle tissue, and the white specks represent ice crystals. Large portions of white area (denoted by red arrow) are the intramuscular fat deposits and were excluded in ice crystal analysis. C) Overlaying of the original slice image on the binarized image to demonstrate ice crystal counting.

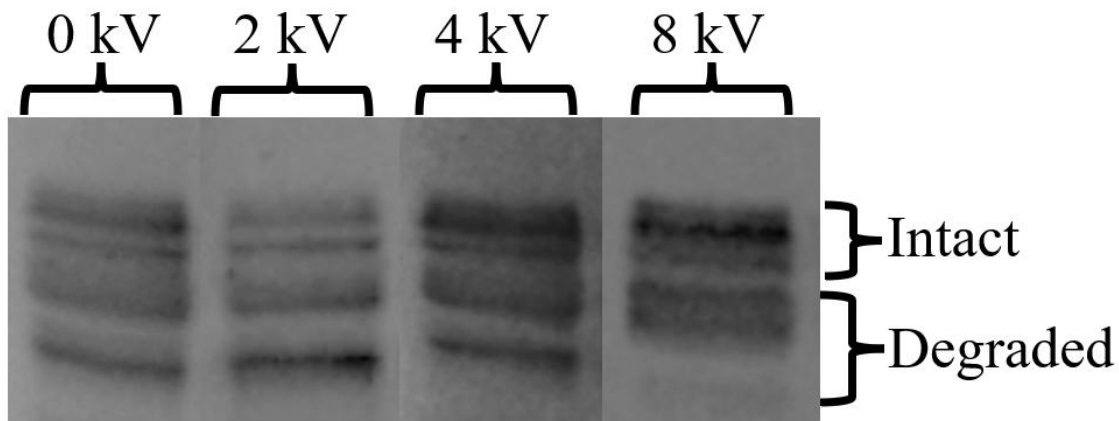


Figure 2.4: Representative measurements of troponin-T on day 0 of display through the four EM treatments

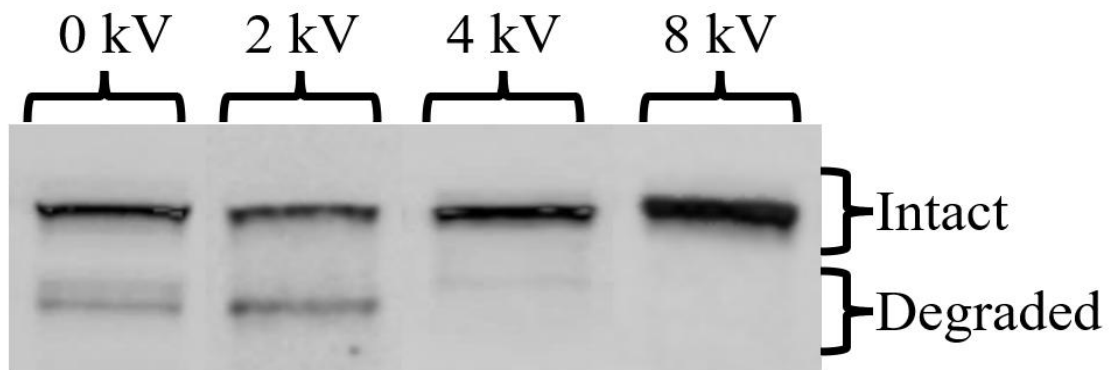


Figure 2.5: Representative measurements of desmin on day 0 of display through the four EM treatments

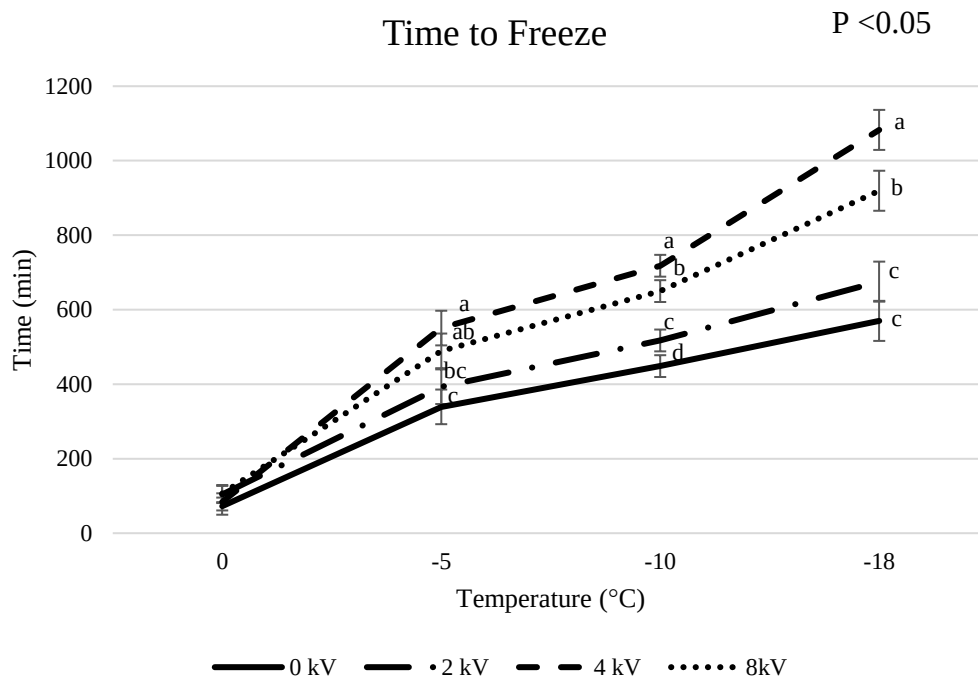


Figure 2.6: Main effect of EM treatment on freezing time of beef LLs

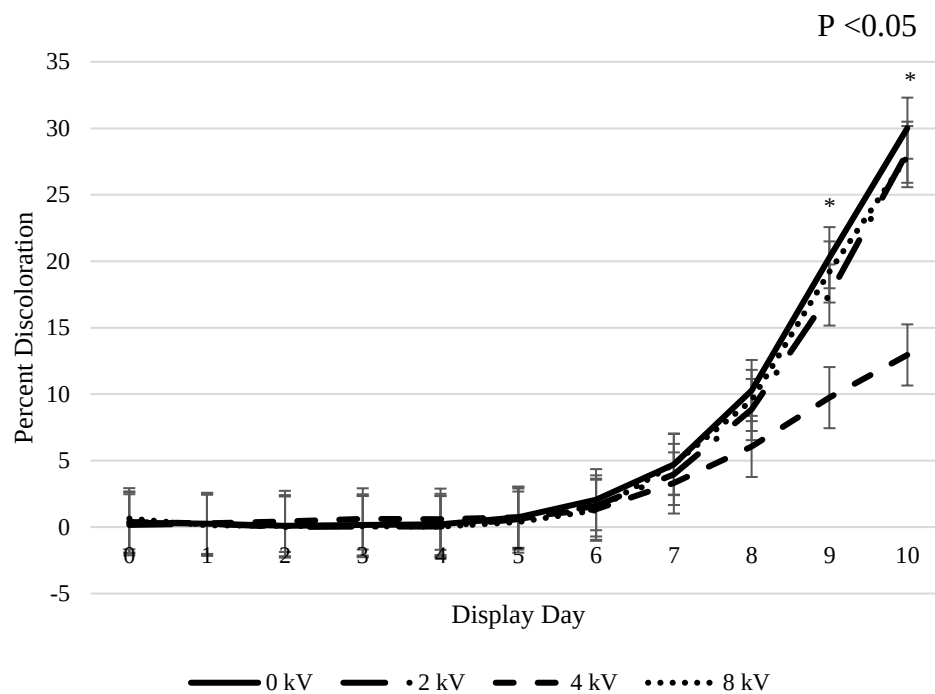


Figure 2.7: Main effect of display day on percent discoloration of beef LLs

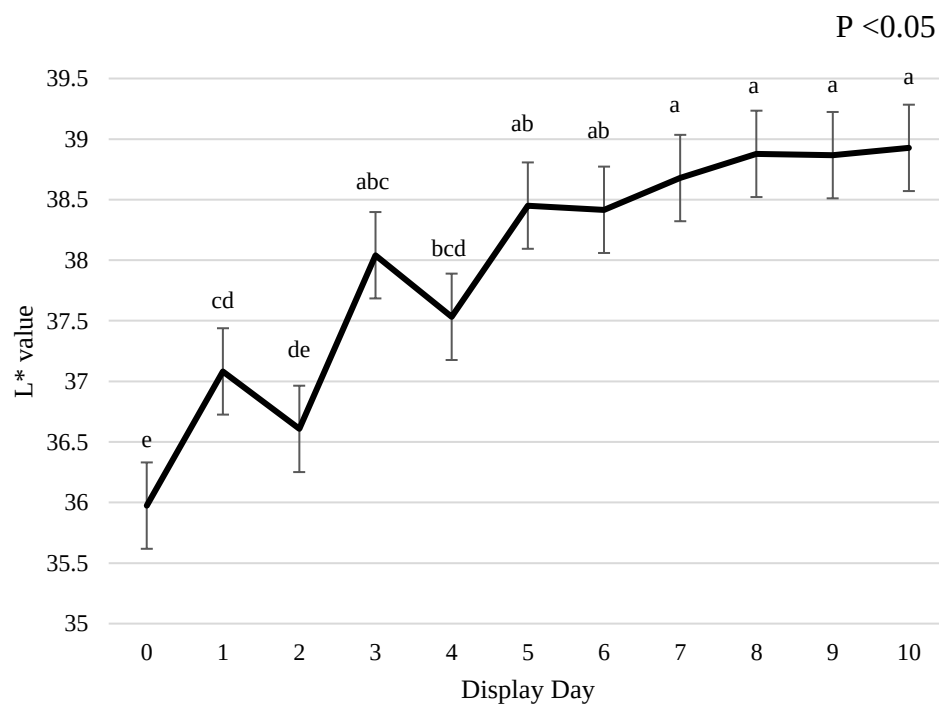


Figure 2.8: Main effect of display day on L* value of beef LLs

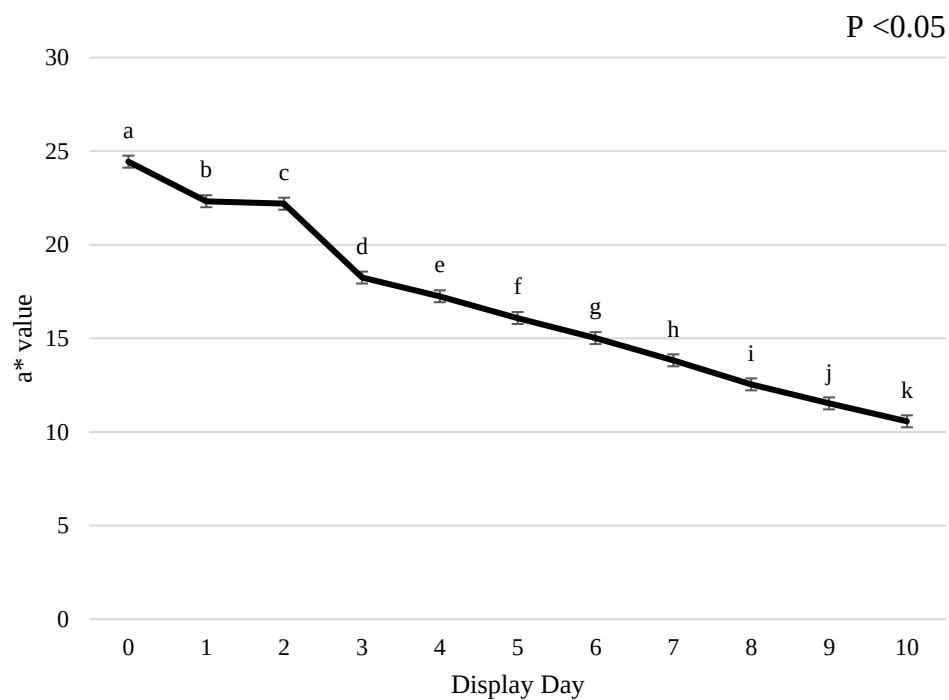


Figure 2.9: Main effect of display day on a* value of beef LLs

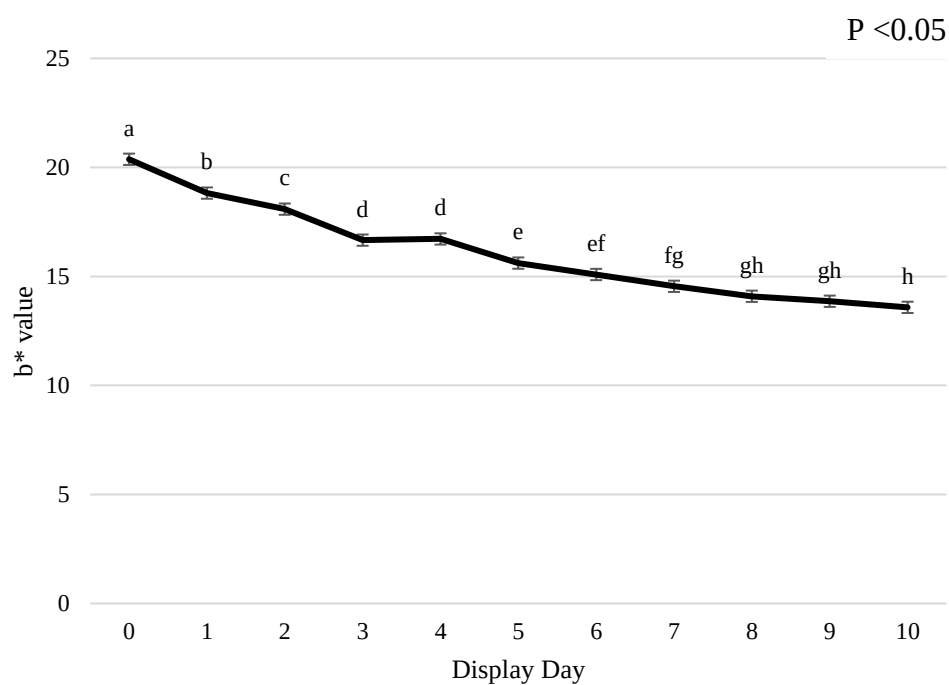


Figure 2.10: Main effect of display day on b* value of beef LLs

Table 2.1: Main effects of EM treatment on total yield, cook loss, aerobic plate counts (APC), Warner-Bratzler Shear Force (WBSF), instrumental color, muscle fiber spacing, ice crystal diameter, thiobarbituric reactive substances assay (TBARS), oxygen radical absorbance capacity (ORAC), desmin and troponin-T degradation, pH, and free calcium content of beef LLs.

	0 kV	2 kV	4 kV	8 kV	SEM ²	P value ¹
Yield (%)	97.01 ^b	97.12 ^b	97.23 ^b	97.69 ^a	0.17	<0.01
Cook Loss (%)	13.87	15.11	14.22	15.28	0.71	0.14
Swab APC (log CFU/mL)	1.78	1.60	1.62	1.35	0.14	0.34
Purge APC (log CFU/mL)	0.76	0.80	0.95	0.80	0.22	0.93
Fat (%)	3.84	3.65	3.55	3.72	0.37	0.89
Moisture (%)	73.11	72.80	72.93	72.79	0.53	0.93
Protein (%)	19.41	20.24	19.98	19.64	0.56	0.48
WBSF (kgf)	3.60	3.52	3.57	3.59	0.20	0.98
L* value	38.36 ^a	38.43 ^a	37.34 ^b	37.67 ^b	0.20	<0.01
a* value	16.60 ^b	16.28 ^{bc}	17.17 ^a	16.15 ^c	0.15	<0.01
b* value	16.18 ^b	16.02 ^{bc}	16.56 ^a	15.77 ^c	0.16	<0.01
Muscle Fiber Spacing (μm)	21.07	22.19	21.35	22.54	1.88	0.85
Ice Crystal Diameter (μm)	35.05	34.59	33.29	34.57	2.21	0.87
Lipid oxidation (mg MDA ³ /kg meat)	1.39	1.63	1.39	1.54	0.12	0.10
Lipophilic ORAC (TE ⁴ /g meat)	0.54 ^a	0.31 ^b	0.36 ^b	0.35 ^b	0.04	<0.01
Hydrophilic ORAC (TE/g meat)	10.28 ^b	10.10 ^b	11.87 ^a	9.19 ^b	0.67	<0.01
Total ORAC (TE/g meat)	10.82 ^{ab}	10.41 ^b	12.23 ^a	9.54 ^b	0.68	<0.01
Degraded Troponin-T (%)	56.51 ^a	54.29 ^a	54.41 ^a	45.39 ^b	2.49	<0.01
Degraded Desmin (%)	37.62 ^a	37.74 ^a	32.92 ^b	31.14 ^b	1.32	<0.01

pH	5.54	5.56	5.54	5.56	0.01	0.42
Free Calcium (nmol/g protein)	2.26 ^b	2.76 ^a	2.65 ^a	2.80 ^a	0.11	<0.01

¹Least-square means within a row without common superscript differ. ($P<0.05$)

²SEM = standard error of least-squares means.

³MDA = malondialdehyde.

⁴TE = Trolox equivalent.

Table 2.2: Main effects of day of display on cook loss, Warner-Bratzler Shear Force (WBSF) thiobarbituric reactive substances assay (TBARS), oxygen radical absorbance capacity (ORAC), desmin and troponin-T degradation, pH, and free calcium content of beef LLs.

	0 d	4 d	7 d	10 d	SEM ²	<i>P</i> value ¹
Cook Loss (%)	15.88 ^a	--	--	13.35 ^b	0.41	<0.01
WBSF (kgf)	4.05 ^a	--	--	3.09 ^b	0.22	0.01
Lipid oxidation (mg MDA ³ /kg meat)	0.97 ^c	0.95 ^c	1.61 ^b	2.42 ^a	0.12	<0.01
Lipophilic ORAC (TE ⁴ /g meat)	0.35	0.34	0.40	0.48	0.06	0.07
Hydrophilic ORAC (TE/g meat)	8.46 ^b	9.48 ^b	11.60 ^a	11.90 ^a	0.75	<0.01
Total ORAC (TE/g meat)	8.81 ^b	9.83 ^b	12.00 ^a	12.37 ^a	0.76	<0.01
Degraded Troponin-T (%)	38.20 ^c	51.05 ^b	58.23 ^a	63.12 ^a	2.49	<0.01
Degraded Desmin (%)	23.65 ^d	35.05 ^c	38.95 ^b	41.77 ^a	1.32	<0.01
pH	5.52 ^b	5.53 ^b	5.55 ^b	5.60 ^a	0.01	<0.01
Free Calcium (nmol/g protein)	1.48 ^d	2.59 ^c	3.02 ^b	3.36 ^a	0.11	<0.01

¹Least-square means within a row without common superscript differ. (*P*<0.05)

²SEM = standard error of least-squares means.

³MDA = malondialdehyde.

⁴TE = Trolox equivalent