Understanding the impacts of cooking and freezing processes on meat quality and physiochemical properties of beef steaks

by

Erin Beyer

B.S., Texas Tech University, 2017
M.S., Texas Tech University, 2019

AN ABSTRACT OF A DISSERTATION

Submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Animal Sciences and Industry
College of Agriculture

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ABSTRACT

This project aimed to understand 1) the physiochemical changes that occur throughout the cooking process by determining myoglobin denaturation percentages between three degrees of doneness and three muscles and 2) the impact of freezing on eating quality, perception of quality, and physiochemical properties of beef *Longissimus lumborum* steaks aged for 21, 28, or 35 d.

The objective of the first study was to determine the change in myoglobin denaturation through three degrees of doneness (medium rare, medium, and well-done) within the *Longissimus lumborum, Gluteus medius,* and *Biceps femoris* muscles. Beef strip loins (*n=12*), and top butts (*n=12*) were collected at a Midwest beef processing plant and brought to Kansas State University for processing. The strip loins (LL) were denuded and the top butts were denuded and separated into the *Biceps femoris* (BF) and *Gluteus medius* (GM). The muscles were sliced into 2.5-cm steaks and assigned to one of the following treatments: raw, medium rare (MR), medium (MED), or well-done (WD). All three muscles were aged for 28 d at 4°C and then frozen and held at -20°C. The steaks assigned for lab assays were cooked to the appropriate degree of doneness (DOD) using a Thermopen to monitor peak temperatures to reach 62.8°C, 71°C or 76.7°C for each DOD, respectively. *L*,a* , b** color readings were taken using a Hunter Lab Miniscan, and samples were powdered immediately for moisture, fat, pH, myoglobin denaturation, metmyoglobin-reducing activity, and lipid oxidation. Myoglobin denaturation was determined using a modified protocol provided in the AMSA color guidelines. The change in absorbance was used to calculate the myoglobin denaturation,% of each sample. The metmyoglobin-reducing activity was performed following a modified protocol provided in the

AMSA color guidelines to determine the change from metmyoglobin to oxymyoglobin using absorbance at 525 nm. Lastly, lipid oxidation was determined by finding the concentration of malondialdehyde per kg of meat tissue. Data were analyzed using SAS Proc GLIMMIX with a split plot design and an α of 0.05 was considered significant. Myoglobin was denatured 29.08%, 48.34% or 70.17% at each DOD, respectively. The metmyoglobin-reducing activity was the highest ($P < 0.05$) for the raw treatment and decreased ($P < 0.05$) with each DOD. As expected, the a^* values decreased ($P < 0.05$) with each different DOD, however, the pH was not impacted ($P > 0.05$). Similarly, the cooking loss percentages increased ($P < 0.05$) with each DOD and the Warner-Brazler shear force values were higher ($P < 0.05$) for well-done steaks in comparison to the other treatments. Lastly, there was an interaction ($P < 0.05$) for lipid oxidation as the DOD impacted the level of oxidation of the three muscles. As expected, the myoglobin denaturation percentage increased with increasing DOD and behaved similarly to changes in the a^* and metmyoglobin-reducing activity values. This research gives more insight to the impacts of cooking and the changes that proteins, especially myoglobin, undergoes between different DOD.

The objective of the second study was to determine the impact of freezing on eating quality, perception of quality, and physiochemical properties of beef *Longissimus lumborum* steaks aged for 21, 28, or 35 d. Beef carcasses ($N = 72$; $n = 18$ per collection; 6 per aging period) were selected from a Midwestern beef processing plant on two different kill dates 1 week apart. The strip loins were cut into 2.5-cm steaks, given a random four-digit code, and assigned to either 21 , 28, or 35 d aged with one of the following designations: trained sensory panels, consumer sensory panels, shear force, or lab assays. All 18 loins from the first kill date represented the frozen samples while the later kill date represented the fresh samples. All steaks were aged to their appropriate aging period at 2-4°C in the absence of light. After aging, the

frozen samples were blast frozen at -20°C for 24 h before being placed in a 2-4°C refrigerator to thaw. At exactly 21, 28, and 35 d aged, the steaks were fed in trained and consumer sensory panels to eight panelists at a time for a total of three trained and consumer panel sessions per day. Trained sensory panelists were asked to rate initial juiciness, sustained juiciness, myofibrillar tenderness, connective tissue amount, overall tenderness, beef flavor intensity, and off-flavor intensity. Consumer panelists were asked to rate the liking of juiciness, tenderness, flavor, and overall liking while determining the acceptability of each factor. The consumers were fed two of each treatment with no additional information and two of each treatment with the statement "previously frozen", or "fresh, never frozen". One pairing correctly described the sample and one pairing did not. This data was used to determine the perception of quality for a fresh or frozen beef steak. Internal color, Warner-Brazler shear force, slice shear force, purge loss, and cook loss were measured on the same day as the sensory panels. Also, on the same day, an additional steak was powdered to determine proximate analysis as well as the determination of metmyoglobin-reducing activity, and lipid oxidation.

Overall, there were no interactions between the aging period and freezing treatment. There were only minute differences between the trained and consumer sensory data. Trained panelists determined the fresh samples were juicier ($P < 0.05$), but the frozen samples were more tender ($P < 0.05$) overall. Similarly, the consumer panelists determined the frozen samples were more tender ($P < 0.05$) but did not distinguish a difference ($P > 0.05$) in overall liking. Additionally, 89.3% of consumers found the frozen samples to be acceptable for tenderness, compared to only 83.9% of consumers for the fresh samples ($P < 0.05$). However, there were no differences ($P > 0.05$) found within the percentage of fresh and frozen samples characterized as "unsatisfactory", "everyday quality", "better than everyday quality", or "premium quality", with

the majority of samples falling within the “better than everyday quality” category. These results were supported by the physiochemical properties evaluated. The frozen samples had lower ($P < 0.05$) shear force values, higher ($P < 0.05$) cook loss, purge loss, L^* values, and a greater ($P < 0.05$) metmyoglobin-reducing activity. However, no differences ($P > 0.05$) were found within a^* values and lipid oxidation amounts. Similarly, the consumer ratings were not altered when given additional information about the preservation method. Therefore, the consumer’s perception of eating quality was not impacted when the statements “previously frozen” or “fresh, never frozen” were included. Based on this study, the actual eating quality and perception of quality are not impacted by freezing beef steaks.

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Major Professor
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Dedication

This dissertation is dedicated to my parents, Kevin and Lynette, for always supporting my dreams and helping me make them a reality.

Chapter I- Review of Literature

The effect of multiple degrees of doneness on cooked color, tenderness, and eating quality characteristics

Introduction:

The mechanisms that comprise the changes a steak undergoes from the raw state to the time it is sitting on a consumer's plate are vast. While evaluating factors within the raw product such as quality grade or muscle have been a priority of most research for decades, the factors affecting the cooked product such as the degree of doneness (DOD) have been largely ignored. Therefore, the objective of this review of literature was to determine the mechanism of meat color, and the impact of cooking on meat color, tenderness, and the overall eating quality.

Raw Meat Color

Understanding raw meat color is imperative to explore the changes that occur within meat color through different DODs. Raw meat color is one of the most saturated fields in meat science. Rightfully so, as raw meat color is one of the main factors impacting consumers purchasing decisions (Mancini and Hunt, 2005; Seidman et al., 1984). Most consumers associate meat color with wholesomeness even though changes in meat color are not primarily caused by bacterial growth (Seideman et al., 1984). Discoloration at the retail level creates issues of food waste and significant economic loss with the U.S. losing $3.73B in 2020 (Mancini and Hunt, 2005; Seidman et al., 1984). Therefore, since meat color is used as an indicator of shelf-life and freshness, identifying mitigations to extend meat color and shelf-life has been an area of interest for multiple decades. While huge improvements have been made in this field, there are still gaps in research, mostly around color stability.

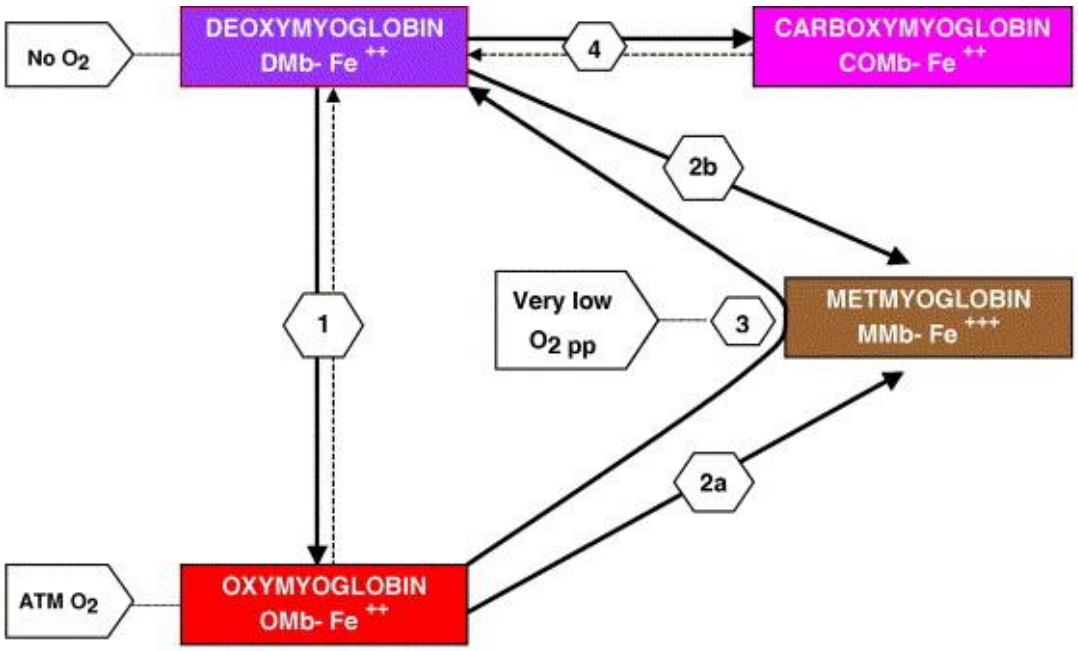
Meat color is driven mostly by one molecule, myoglobin (Mb) (Mancini and Hunt, 2005). Myoglobin is the primary protein responsible for storing oxygen in a living system while also being responsible for meat color and all variations of pigments within meat products (Mancini and Hunt, 2005). In a living system, hemoglobin brings oxygen from the lungs to the muscles, and due to the differences in oxygen affinity, oxygen is transferred and stored within myoglobin (Seideman et al., 1984). However, in fresh meat, myoglobin binds atmospheric oxygen. While the role of myoglobin in a living system is different than in post-mortem systems, the basics of oxygen storage is similar.

In fresh meat, Mb can exist in multiple forms depending on the ligand bound to the sixth coordination site of the heme iron and the oxidative state of the heme iron (Suman and Joseph, 2013; Bekhit and Faustman, 2005). Myoglobin is most commonly found in one of the following states: deoxymyoglobin (DMb), oxymyoglobin (OMb), carboxymyoglobin (CMb), or metmyoglobin (MetMb) (Mancini and Hunt 2005; Suman and Joseph, 2013; Seideman et al., 1984). These pigments are transient and can be influenced by a variety of factors such as packaging, metmyoglobin-reducing activity (MRA), the affinity of oxygen, the availability of ligands, the structure of Mb, and other extrinsic factors (Mancini and Hunt 2005). The complexity of these factors and their interactions as well as the overall complexity of the Mb molecule makes raw meat color a difficult field.

The complexity of myoglobin starts with the primary sequence. Myoglobin is a highly conserved molecule within red meat species for the overall function however, minor differences in the primary sequencing heavily dictate changes in stability (Suman and Joseph, 2013). While changes in the primary structure mainly occur between different species, they can also be linked to different breeds (Bekhit et al., 2019; Suman and Joseph, 2013). Additionally, different species

have differing concentrations of Mb due to differences in muscle fiber metabolic needs, altering the final pigment (Seideman et al., 1984). However, the main color difference between species is attributed to the primary sequencing as well as the Mb concentration (Suman and Joseph, 2013). These differences between species and breeds affect thermal stability, oxygen affinity, heme retention, and oxidation, therefore, playing an integral role in the overall pigment, and self-life of the raw product (Suman and Joseph, 2013).

Mb has a unique biological role due to its tertiary structure. It contains a heme ring bound to an iron atom which can form six other bonds depending on the environment (Mancini and Hunt, 2005). While the first five coordination sites are irreversible with four pyrrole nitrogen atoms and a proximal histidine, the sixth coordination site changes based on the molecules in proximity and the relative affinities (Suman and Joseph, 2013; Mancini and Hunt, 2005). Two main factors dictate the pigment of raw meat: the molecule or ligand bound at the sixth coordination site, and the state of the iron (Suman and Joseph, 2013; Bekhit and Faustman, 2005). Iron can exist in two forms based on the valence state of the element. It can be reduced or oxidized to form ferrous (Fe^{2+}) or ferric (Fe^{3+}), respectively. From the first time meat is exposed to oxygen, expected color changes will occur following the well-studied color triangle (Mancini and Hunt, 2005).



Rx 1 (Oxygenation): $DMb + O_2 \rightarrow OMb$

Rx 2a (Oxidation): $OMb + [\text{oxygen consumption or low } O_2 \text{ partial pressure}] - e^- \rightarrow MMb$

Rx 2b (Oxidation): $[\text{DMb – hydroxyl ion – Hydrogen ion complex}] + O_2 \rightarrow MMb + O_2^-$

Rx 3 (Reduction): MMb + Oxygen consumption + metmyoglobin reducing activty → DMb

Rx 4 (CarboxyMb): DMb + carbon monoxide → COMb

Mancini and Hunt, 2005

The color triangle, as shown above from Mancini and Hunt (2005), explains the mechanism of color change. The first step, oxygenation, will occur once the meat is exposed to oxygen. Before exposure, the meat will exist in the DMb state visually appearing as a purple pigment (Mancini and Hunt, 2005). After an appropriate “bloom” time, the oxygen will bind to the sixth coordination site, creating a bright cherry-red color or OMb pigment (Mancini and Hunt, 2005). This diatomic oxygen molecule will not immediately cause the oxidation of the Fe atom; however, it will interact with the distal histidine, slightly changing the function of the Mb molecule (Mancini and Hunt, 2005). Resonance within OMb allows it to prevent oxidation of the heme iron, leaving DMb as the more vulnerable pigment (Behkit et al., 2019). While OMb is the desired pigment to be present at the time of purchase and consumption, it is not an infinite

pigment. Oxidation of the bound Fe atom is inevitable, converting Fe^{2+} to Fe^{3+} to form a brown pigment or MetMb (Bekhit et al., 2019). MetMb can be reduced to DMb through MRA (Bekhit and Faustman, 2005). The ability to reduce MetMb to DMb is also a finite system depending on the inherent rate of autooxidation, availability of NADH, other reducing equivalents, or the enzymatic activity needed for reduction (Behkit et al., 2019; Bekhit and Faustman, 2005). Therefore, improving MRA through the activation of the mitochondria or the addition of other constituents could be a successful pathway to improve meat color and shelf-life (Behkit et al., 2019; Bekhit and Faustman, 2005). While color chemistry within raw meat has been studied heavily within scientific literature, color stability and the role of the mitochondria in meat color are still not fully understood.

Mitochondrial Role in Meat Color

A common misconception in meat science is that the mitochondria lose activity or become inactive without oxygen from the circulatory system, however, the mitochondria are active for up to 60 d in postmortem meat (Ramanathan et al., 2018). There are two main mechanisms in which the mitochondria affect raw meat color: oxygen consumption and MRA.

MetMb- reducing activity is a complex pathway crucial to extend the shelf-life of fresh meat color. There are two main pathways for MRA to reduce MetMb to DMb, either through electron-transported non-enzymatic MRA, or NADH- dependent reductase MRA (Zhu et al., 2009). Therefore, the mitochondria are mostly responsible for a successful MRA in a post-mortem system. However, there are many other MRA systems including anaerobic MRA which could occur exclusively without the mitochondria (Zhu et al., 2009). The flexibility of the MRA system provides the potential to optimize color stability without activating the mitochondria.

The most common route for MRA to occur is through the reduction within the electron transport chain with NADH- cytochrome b_5 reductase (Bekhit and Faustman, 2005). NADH-cytochrome b_5 reductase uses two electrons from NADH to reduce cytochrome b_5 allowing cytochrome b_5 to reduce MetMb to DMb (Bekhit and Faustman, 2005; Shirabe et al., 1992). Other non-enzymatic pathways exist such as using the reduction of ferricytochrome b_5 to ferrocytochrome b_5 to transfer two electrons to MetMb without the aid of cytochrome b_5 reductase; however, the exact mechanism is unlcear (Bekhit and Faustman, 2005; Livingston et al., 1985). The flexibility of MRA pathways makes it challenging to fully understand the factors that impact MRA within muscles, but there are known factors that correlate with higher MRA.

Inherently, muscle type and the dominant fiber type play a significant role in MRA activity (Bibber- Krueger et al., 2020; Bekhit et al., 2019; Mancini et al., 2018). With a greater amount of more active mitochondria, MRA will be more active for a longer period of time, thus increasing MRA for the muscle (Suman et al., 2014). Therefore, MRA is directly impacted by the dominant muscle fiber type (Suman et al., 2014). However, more oxidative muscles are not more color stable, because color stability relies on other factors as well. The *Longissimus dorsi* (LD) has been shown to have higher color stability and a* values throughout multiple d of display in comparison to the *Psoas major* (PM) even though the PM is a more oxidative muscle (Mancini et al., 2018). A more active MRA system is positively correlated with color stability, but color stability is also dependent on oxygen consumption, making the relationship complex.

Oxygen consumption rate (OCR) is the amount of oxygen taken up by the mitochondria in fresh meat (Mancini et al., 2018). Since the mitochondria are active for such an extended period of time, they will be competing with Mb for atmospheric oxygen (Mancini et al., 2018). A higher OCR will leave Mb in the DMb state making it more vulnerable to be oxidized to MetMb

(Bibber- Krueger et al., 2020; Mancini et al., 2018). A very high OCR will result in a shortened shelf-life leaving Mb with two options, DMb or MetMb which both could result in a consumer bias by producing a brown or dark purple pigment.

Similar to MRA, fiber type and muscle type play a significant role in dictating OCR (Bibber- Krueger et al., 2020; Mancini et al., 2018). A more oxidative muscle will inherently have a greater number of more active mitochondria, increasing the OCR and leading to a more color labile muscle (Bibber- Krueger et al., 2020; Mancini et al., 2018; Suman et al., 2014). Additionally, Bibber- Kruger et al., (2020) demonstrated even small fiber type differences within a muscle can dictate a difference in OCR, leading to changes in color stability within the posterior end of the LD vs the anterior end.

An additional mechanism is regulated by the pH of the muscle. A higher pH activates the mitochondria, thus increasing OCR and MRA proportionally (Kiyimba et al., 2021; Ramanathan et al., 2014; Tang et al., 2005). Overall, this relationship has a negative relationship with fresh beef color. Not only will the increased OCR leave Mb in a more vulnerable state forcing DMb to be the dominate pigment, but a higher pH will also result in a higher water-holding capacity (WHC), resulting in darker colored meat (Kiyimba et al., 2021; Tang et al., 2005). This relationship is most obvious in dark-cutting beef. Kiyimba et al. (2021) illustrated this relationship by determining the mitochondrial activity of dark-cutting beef vs normal beef and found that dark-cutting beef had a higher mitochondrial activity as shown by a higher OCR and MRA. While it is well known that the high pH in dark-cutting beef is dictated by a limited or depleted amount of glycogen at the time of exsanguination, the connection with the mitochondria has been less studied but plays an integral role in the final meat quality.

Combining OCR and MRA tell very different stories on the role of the mitochondria within fresh meat color and stability. While an increase in mitochondrial activity will increase MRA, it will also increase OCR. Therefore, most muscles classified as color stable, are considered moderate for mitochondrial activity. The LD is considered color stable, and while it is primarily glycolytic, it is a more moderate muscle in terms of metabolism with a mixture of glycolytic and oxidative fibers (Suman et al., 2014). By definition, a color-stable muscle has high MRA and low OCR while a color-labile muscle has low MRA and high OCR (Suman et al., 2014). While these combinations seem counterintuitive, they could be possible due to the many mechanisms of MRA, however, this combination has not been found with current methodology. Due to the relationship between MRA and OCR, this field is still being studied to understand how to improve color stability in fresh meat. Additionally, any research on color stability within cooked meat has only been briefly researched, leaving a significant gap for future research.

Other Factors Impacting Raw Meat Color and Stability

Improving meat color and stability has been a focus for the meat science industry for decades due to its impact on the consumer's perception of quality. Therefore, there are many studies that have evaluated interventions to improve meat color and stability. As previously discussed, muscle, ultimate pH, and muscle fiber type heavily affect color stability. However, other factors such as electrical stimulation, rigor temperature, packaging type, antioxidants, general storage conditions, and certain additives like lactate and succinate also impact the raw color stability of a meat product (Bekhit et al., 2019).

The addition of succinate has been in focus for many of these studies due to its usage in the electron transport chain (ETC) (Mancini et al, 2011; Ramanathan et al., 2013; Ramanathan et al., 2011; Zhu et al., 2009). Succinate dehydrogenase converts succinate to fumarate in complex

II of the ETC, therefore, a higher content of succinate should activate the downstream complexes of the ETC by producing a greater amount of reducing equivalents (Ramanathan et al., 2013). However, Ramanathan et al., (2011) did not find this relationship. Succinate increased pH and MRA, but had no impact on raw a* values; however, succinate did increase cooked a* values in a similar study (Ramanathan et al., 2011 & Ramanathan et al., 2013). Therefore, succinate has applications to prevent premature browning of ground meat products, but not necessarily to increase the shelf-life of raw meat color. Understanding color stability and ways to help preserve it are still being researched in raw meat. However, color stability within cooked proteins is almost completely unevaluated at this time. Future studies should focus on the role of color stability within cooked color by investigating the impact of the raw MRA and OCR on the final cooked color.

Cooked Color

Cooked color is the visual external and internal appearance of a steak which drives the consumer's perception of quality and food safety. Cooked color is driven by changes in functionality in the sarcoplasmic proteins, changing the pigment from a red color to a grayish-brown color over time (Hunt et al., 1999). While consumers are always advised to use a meat thermometer to determine the appropriate DOD, the visual appearance of the steak is most commonly used (Prill et al., 2019; King and Whyte, 2006). Additionally, meat color is one of the strongest driving forces for consumers' purchasing motivators while impacting their perception of quality (Prill et al., 2019; Bekhit and Faustman, 2005). While cooked color may seem simple by definition, changes in cooked color are complex and require further investigation.

While the mechanisms of raw color change and the color triangle cycle are very well studied, cooked color has been accepted to be a direct result of cooking and intrinsic factors and

therefore, not studied at the same level. Similarly, consumers typically do not buy cooked meat products where the color is visible (besides in food service), reducing the economic impact of cooked meat color. However, the other changes that occur during cooking have been studied more extensively such as impacts on juiciness, tenderness, flavor, oxidation, and overall eating acceptability (Yoo et al., 2020; Dinh et al., 2018; Drey et al., 2019; O'Quinn et al., 2016; Walsh et al., 2010). While raw color is one of the top drivers for the consumer's purchasing habits, the cooked color impacts the overall quality perception at the time of consumption, thus impacting the overall eating experience (Henchion et al., 2017; Prill et al., 2019). Therefore, cooked color should be included in more consumer-based research to increase the understanding of the impact of cooked color on eating quality.

Additionally, through the development of degrees of doneness, consumers expect a steak to visually appear the way they envision and if it doesn't, they will have a lower perception of the quality (Cox et al., 1997). With a factor so impactful, more research into the mechanism can be helpful for improving meat quality and consistency in the future.

Degrees of Doneness

The most common application of cooked color is demonstrated within the varying DODs. The DODs are centered in science around the end-point temperature during the cooking process, but to the consumers, it relates to the internal appearance of the steak after cooking (Cross et al., 1976; Prill et al., 2019; Drey et al., 2019 & Cox et al., 1997). Roasts and other muscles suggested for a moist heat cookery are not included within DODs, due to high amounts of collagen, reducing the impact of cooked internal color. The premise of DODs are centered around providing a parameter of quality consistency for the consumer while ensuring the product is still

wholesome to consume. The infographic below illustrates the color differences between six different DODs.

These color changes are the main indicators for a consumer to expect a certain eating experience (Prill et al., 2019). It is even frequent for consumers to be asked to cut into a steak to ensure it is cooked the way they envisioned because the connection between the visual aspect of a steak is one of the most important quality cues (Prill et al., 2019; Glitsch, 2000; Imram, 1999). Other than cases with premature browning , the internal color can be an important indicator for DOD and known quality changes that arise between different cooking levels (Prill et al., 2019). The visual cues associated with varying DODs alter the consumer's perception of the eating experience, being a very powerful influencer for the overall perceived palatability (Prill et al., 2019).

Until a few studies recently from Drey et al. (2019), and Prill et al., (2019), cooked color and the impact of varying DODs on eating quality have remained unsaturated fields. However, as the amount of literature increases in this area, it will become obvious that this is a very complex field of study. The science behind the multitude of cooked color pigments between DODs is highly dependent on a multitude of intrinsic and extrinsic factors including the denaturation of Mb, and the oxidative state of the heme iron.

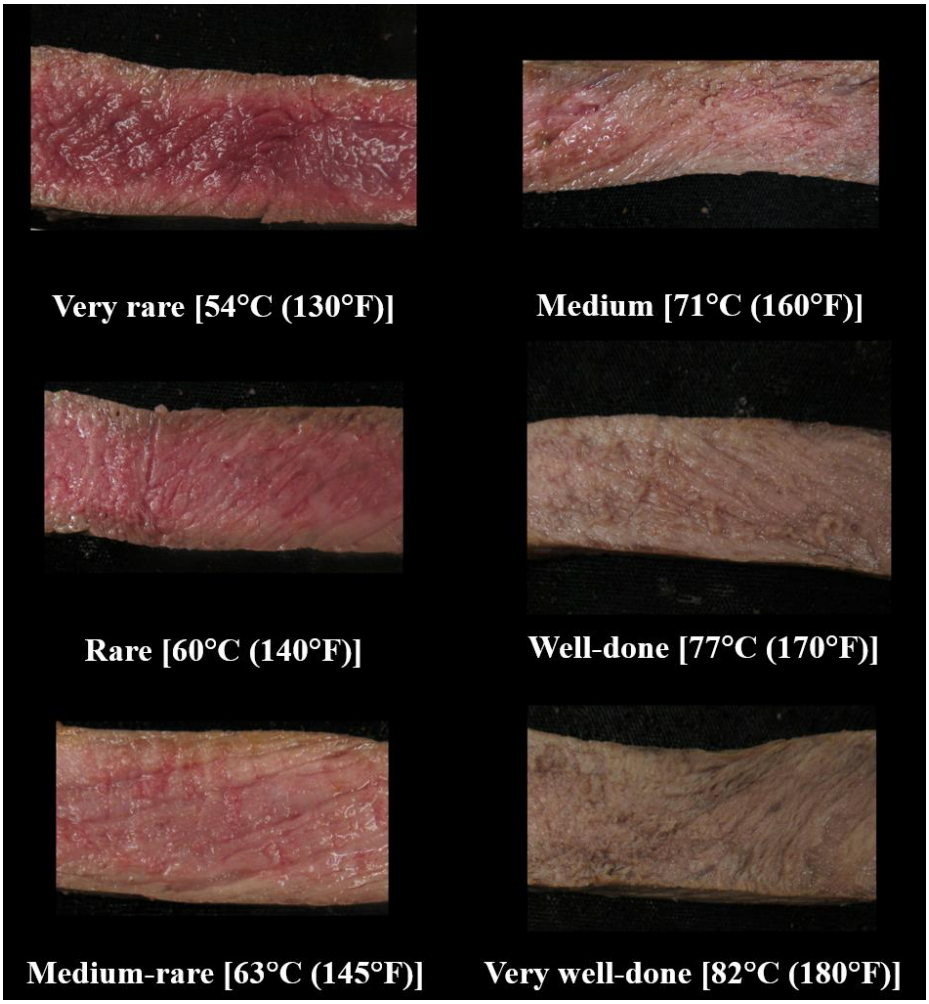


Figure 1: Visual representation of the different degrees of doneness of beef steaks Prill et al., 2019

Thermal Stability of Myoglobin and Cooked Color

The structure, form, and thermal stability of Mb, and MRA greatly affect the raw color and can translate into differences in the cooked state. Additionally, the structure and form mostly affect the final cooked color by impacting the thermal stability of Mb (Suman and Joseph, 2013). The primary structure dictates differences in thermal stability, heme retention, and oxygen affinity all affecting the raw and cooked color (Suman et al. 2013). The different forms of Mb have different thermal stabilities, greatly affecting the final cooked color (Hunt et al., 1999).

While MRA is still less understood in cooked meat, it also impacts the thermal stability of Mb. Therefore, thermal stability has the overwhelming largest impact on the final cooked color.

On average, Mb begins to denature around 55°C and continues to denature up to 80°C (King and Whyte, 2006; Hunt et al., 1999; Varnam et al., 1995). However, each Mb form denatures at different temperatures, so the relative ratio of OMb, DMb, and MetMb at the time of cooking will have the greatest impact on the denaturation of Mb and the final cooked color (Ramanathan et al., 2019; Hunt et al., 1999). One of the first papers to establish this relationship was a study by Hunt et al. (1999) who evaluated the denaturation of DMb, OMb, and MetMb of ground beef patties. The study determined oxidized forms of Mb had a lower thermal stability and resulted in a greater percentage of denaturation (Hunt et al., 1999). While this study was pivotal for the ground beef industry, it was never repeated in whole muscles, leaving a significant gap for cooked meat color.

The thermal stability of Mb is also affected by the pH as shown by the Hunt et al. (1999) study. A higher pH led to less denaturation of each form of Mb at the same end-point temperature (Hunt et al., 1999). Other studies have found similar results when a pH over 6 was used (Hague et al., 1994; Marksberry, 1990; Trout, 1989; Mendenhall, 1989). However, when more moderate pHs were used such as 5.5-5.75, no significant effect was found (Hague et al., 1994; Marksberry, 1990; Trout, 1989; Mendenhall, 1989). While this could be a result of confounding factors such as the pH impacting which Mb form was dominant prior to cooking, it could also have other mechanisms.

MetMb-reducing activity can also impact the final cooked color. However, this relationship is less understood. As outlined in figure 1, this system is limited to the amount of substrates required for the reduction, therefore, without the mitochondria active, it is a finite

system, especially in cooked meat (Bekhit and Faustman, 2005). Potentially, a greater MRA can lead to greater thermal stability through the cooking process (Warren et al., 1996), but this relationship has not been exclusively studied and remains a gap in research.

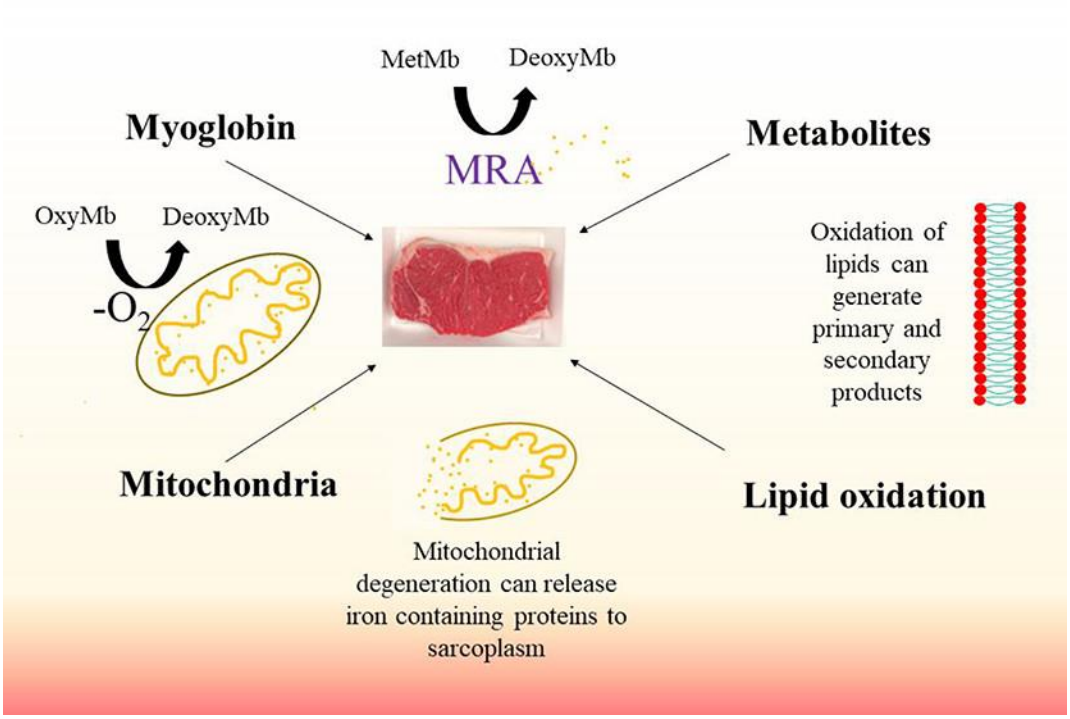


Figure 2: MetMb Reducing Activity
Ramanathan et al., 2020

Another factor influencing differences in the internal appearance after cooking is premature browning. Premature browning is described as meat turning brown internally prior to 71°C, giving consumers a false perception of the DOD (Ramanathan et al., 2019; Hague et al., 1994). While the direct cause of premature browning is still unknown, some intrinsic and extrinsic factors have been linked. The most frequent factor leading to premature browning is linked to the oxidative state of the heme iron prior to cooking (Ramanathan et al., 2019). The Hunt et al., (1999) study also alluded to this phenomenon, but it was not fully understood until years later. Now it is known that factors that impact thermal stability, also impact premature browning such as muscle type, packaging type, aging, and the use of antioxidants (Ramanathan

et al., 2019; Suman et al., 2009; Suman et al., 2004; Philips et al., 2001). With many factors influencing the cooked color, it is imperative to lead consumers to the usage of a food thermometer while cooking.

Structural Changes of Myoglobin through Cooking

The heat through the cooking process adds many new mechanisms impacting the overall appearance and eating experience. Heat is a common method to denature proteins, changing their solubility and functionality (Tornberg, 2005). These changes can impact the water-holding capacity, tenderness, and potentially the pigment for optically active proteins such as myoglobin (Tornberg, 2005). The changes in color through the cooking process occur through post-translational changes and denaturation of Mb by forming a hemochrome or hemichrome pigment (Mancini et al., 2011). The cooked meat pigment exists in two forms, ferro or ferri, indicating the oxidative state of the iron (Mancini et al., 2011). In a ferrous state, the heme iron will form a dull grey color as hemochrome, however, as the heme iron becomes oxidized, it will become brown as the ferric hemichrome pigment (Suman and Joseph, 2013). As previously discussed, these changes are highly related to the relative ratio of OMb, DMb, and MetMB at the time of cooking, especially in ground meat products. However, the denaturation process also produces structural changes to Mb making it more challenging to track and understand.

As the temperature increases, the Mb molecule goes through a series of post-translation changes that have the same molecular weight but different isoelectric points (pI) and functionality (Wang et al., 2021; Salim et al., 2019). Salim et al. (2019) ran 2-D gels of the sarcoplasmic proteins of steaks cooked to two DODs and found a different Mb spot for a steak cooked to 60°C and 71°C indicating the role of the post-translational changes were driven by the protein's thermal stability. In a follow-up study by Wang et al. (2021), the post-translational

changes were determined to be phosphorylation, methylation, carboxymethylation, acetylation, and alkylation. These changes occurred through the aging process and decreased the redox stability leading to less thermal stability (Wang et al., 2021). These changes have made Mb difficult to track through the cooking process in previous studies. The cooked color changes therefore can be dictated by a wide variety of factors impacting the thermal stability of Mb such as the oxidative state, packaging type, pH, added ingredients, and cooking method (King and Whyte, 2006; Hunt et al., 1999). Understanding the modifications that occur through the treatment of different cooking levels will only help to further understand Mb, how it functions and how to ensure consistency from one steak to the next. Post-translational changes, thermal stability, and other factors relating to cooked meat color are still being heavily researched and drive many research questions for future projects.

Degree of Doneness Impact on Eating Quality

Similar to cooked color, understanding eating quality differences within different DODs has been in focus more recently. In general, cooking steaks to different degrees of doneness can impact the moisture content, juiciness, and objective and subjective tenderness through protein hardening and aggregation (Mckillip et al., 2017; Drey et al., 2019; O'Quinn et al., 2015; Lorenzen et al., 2005). Understanding the impact of different degrees of doneness on the overall eating experience for consumers helps ensure consumers have a positive eating experience every time they cook or order a steak. While it is documented that lower degrees of doneness have improved juiciness and overall liking ratings and, in some studies, tenderness ratings, the majority of consumers still prefer a medium DOD (McKillip et al., 2017). The physiochemical changes that occur through the cooking process drive changes in the eating experience and

therefore understanding these changes is imperative for improving eating quality and providing a consistent product to the consumer.

Tenderness

Tenderness is one of the three pillars that combine for the overall eating experience and it is considered by some to be the most important palatability factor (Hammond et al.; 2022, Miller et al., 2001). Tenderness is a combination of the myofibrillar tenderness and background toughness consisting of the amount and type of connective tissue present (Purhcas et al., 2014). Tenderness is the most widely studied palatability factor, having the first widespread audit to evaluate tenderness across the United States (Miller et al., 2001; Morgan et al., 1991). While there is extensive research around the effects of marbling, muscle, aging periods, chilling methods, animal age, and other intrinsic factors on tenderness cooked to the same DOD, less is known for impacts within cooked muscles for different DOD (Nair et al., 2019; Killinger et al., 2004; King et al., 2003; Shorthose et al., 1990). It is widely accepted that meat increases in toughness as it cooks using a high-heat cookery method, through multiple intrinsic pathways (He et al., 2018; Gatellier et al., 2010; Sante-Lhoutellier et al., 2008). Even though tenderness has been studied the most extensively, there still remain gaps in the research around the mechanisms of protein hardening and the changes that occur throughout the cooking process.

Tenderness of a steak is negatively impacted by the DOD (Drey et al., 2019). While a higher degree of marbling can limit the negative impact of cooking, each increase of DODs negatively impacts the overall sensory scores, including tenderness (Drey et al., 2019). The mechanism behind the decreasing sensory scores have been attributed to higher cook losses, increased disulfide bond formation, and protein aggregation leading to protein hardening (He et al., 2018; Gatellier et al., 2010; Sante-Lhoutellier et al., 2008). Figure 3 illustrates the effect of

cooking on particle size by demonstrating the aggregation of proteins in image c (Promeyrat et al., 2010). This study evaluated the impact of cooking on the protein aggregation of ground pork loin (Promeyrat et al., 2010). These changes are driven by an increase in protein oxidation and denaturation through the cooking process thus increasing the susceptibility of the formation of disulfide bonds and hydrophobic interactions between protein side chains (He et al., 2018; Promeyrat et al., 2010). Figure 4 demonstrates the decrease in thiols or SH functional groups and therefore demonstrates the formation of disulfide bonds by adding heat to sausage, and other cooked meats (He et al., 2018). The increase of disulfide bonds and aggregation combine to produce protein hardening throughout a dry heat cooking process (Bertola et al., 1994).

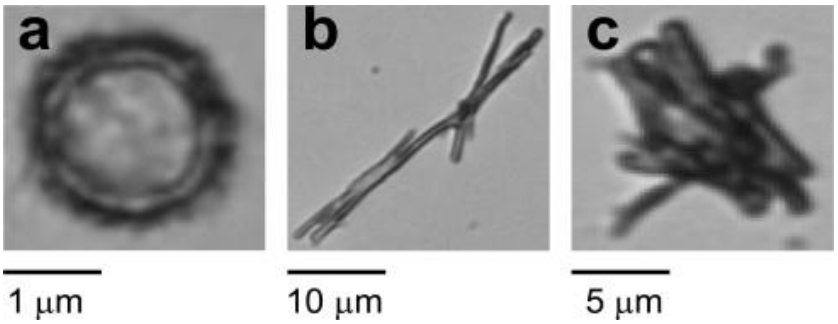


Figure 3: Examples of particles detected with the granulometer FPIA-3000. (a) Small vesicles of emulsified lipids; (b) myofibrillar fragment observed in raw meat; (c) protein aggregate observed in cooked meat.
Figure: Promeyrat et al., 2010

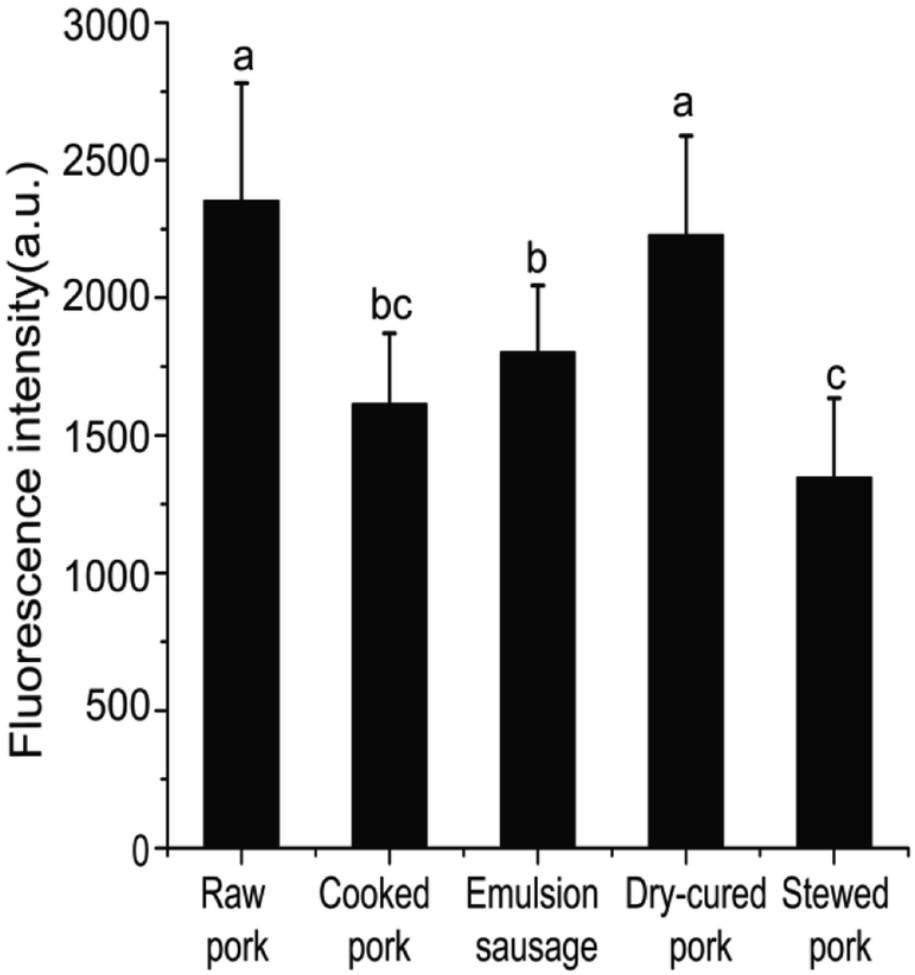


Figure 4: Florescence of SH groups indicating the formation of disulfide bonds
Figure: He et al., 2018

Factors Impacting Tenderness

Other factors that impact tenderness include the location of muscle, the age of the animal, the aging period, the cooking method, and others (Nair et al., 2019; Eilers et al., 1996; Shorthose et al., 1990). The relationship between these factors and tenderness have been widely studied; however, understanding which tenderness factors are the most important for eating quality is still not fully understood (Hammond et al., 2022). Of these factors, collagen content, the type of collagen, and its impact on eating quality are still popular areas of research.

The relationship between tenderness and cooking varies from muscle to muscle, depending on the amount and type of collagen among other factors (Hammond et al., 2022). For

muscles that warrant a moist heat cookery, the relationship between cooking, time, and tenderness is very different from the relationship between a steak requiring a dry heat cookery due to the collagen content (Reid et al., 1971). Collagen is a stromal protein found in one of the three connective tissue layers generally adding to the background toughness (Weston et al., 2002). Connective tissue hinders the overall eating experience for the consumer by adding to the background toughness (Dubost et al., 2013; Purslow 2013; Purslow, 2005). Connective tissue is deposited due to the location, and function of the muscle, as well as the age of the animal (Purslow, 2005; Boccard et al., 1979). Collagen contains either mature or immature cross-linkages which determine the ability of the collagen to solubilize through cooking (Weston et al., 2002). Immature collagen can turn to gelatin through a time and temperature relationship by structurally changing the alpha-helices into random coils (Chen et al., 2014). The solubilization of collagen or gelatinization greatly increases the tenderness of the product (Chen et al., 2014). This conversion takes significant time usually warranting a "low and slow" cooking method for muscles with high collagen contents or high amounts of mature collagen contents (Reid et al., 1971). Understanding the role of gelatinization and different collagen breakdown systems is still being heavily researched with gaps in the current knowledge.

In a correlation study done by Hammond et al. (2022), the relationship between tenderness and different factors is highly dependent on the specific muscle. While tenderness for the LD, *Gluteus medius* (GM), *Triceps Brachii* (TB), and *Semitendinosus* (ST) was mostly driven by proteolytic degradation, tenderness for *Pectoralis profundus* (PP) was driven by collagen amount (Hammond et al., 2022). Understanding muscle-specific influencers of tenderness can help the industry customize interventions to maximize the whole carcass. While

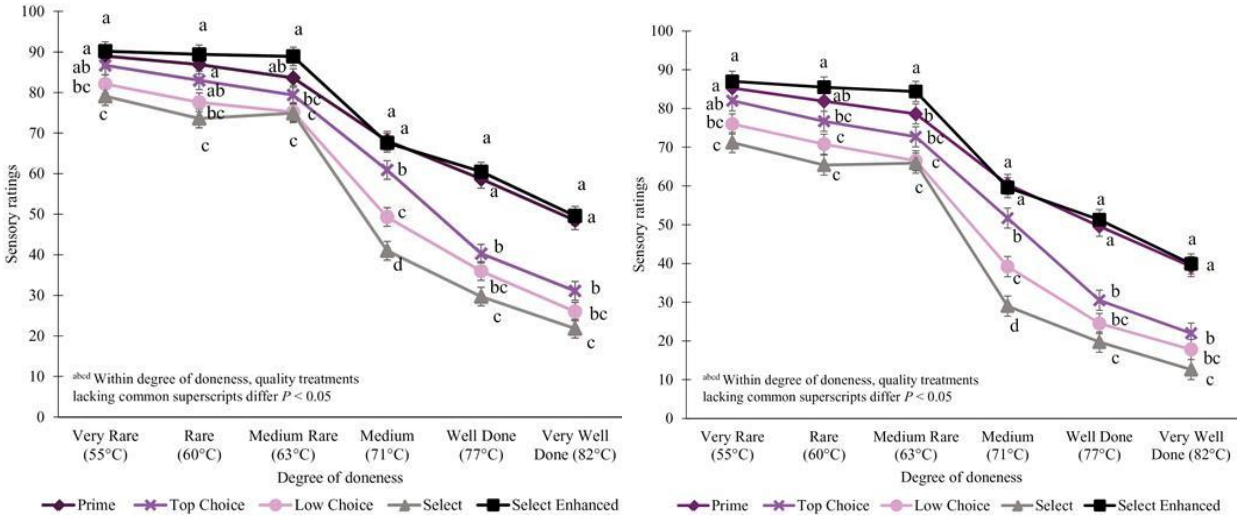
tenderness has been the focus of research for decades, there are still areas where research is needed to continue to optimize beef quality.

Juiciness

Juiciness is most directly impacted by the DOD. Moisture content or the perceived juiciness of a steak is a combination of the moisture content left after cooking aiding in the overall mouthfeel. Juiciness can be broken into initial juiciness which is mostly dictated by the DOD, or sustained juiciness which is mostly dictated by marbling (Lucherk et al., 2017). Since juiciness is directly related to the DOD and the amount of marbling present at the end of cooking, it can be tied to the quality grade (Drey et al., 2019). While tenderness has been studied the most extensively, and flavor has increased in research within the past decade, juiciness has remained an unpopular research focus with limited studies explicitly studying juiciness (Yoo et al., 2020; Lucherk et al., 2017; Bertram et al., 2005).

Each increase in DOD negatively impacts the overall sensory scores, especially for juiciness (Savell et al., 1999; Lorenzen et al., 1999; Drey et al., 2019). In general, as steaks are cooked to a higher DOD, they lose moisture and the perceived juiciness is reduced (Drey et al., 2019 & Lucherk et al., 2016). This general concept and others were vetted in a series of papers determining "beef customer satisfaction" within one muscle and multiple quality grades and DODs (Goodson et al., 2002; Savell et al. 1999; Lorenzen et al., 1999; Neely et al., 1999). While this concept seems cut and dry, Drey et al. (2019) determined another relationship that impacts how severe the change in juiciness will be at higher DODs. A higher degree of marbling can limit the impact of cooking (Drey et al., 2019; Lucherk et al., 2016). This quality grade and DOD interaction found by Drey et al. (2019) and Lucherk et al. (2016) indicates a relationship between quality grade and DOD to protect against the loss of moisture through the cooking process,

adding extra insurance for overcooking a steak. Figures 5 and 6 show sensory scores for varying DODs from vary rare to very well-done illustrating the quality grade and DOD interaction and the decrease in trained panelist ratings for initial juiciness and sustained juiciness with the increased DODs (Drey et al., 2019). The USDA Prime and enhanced strip steaks were able to maintain higher juiciness scores across multiple DODs. While the Prime, enhanced, and Top Choice strips were similar within the very-rare DOD, the Prime and enhanced treatments were significantly juicier at a very well-done DOD for both initial and sustained juiciness ratings. Additionally, Lucherk et al., (2016) found a similar trend as the Prime treatment decreased 19% in juiciness ratings versus a 30% deficit for the Standard treatment. This data clearly outlines a previously messy concept. Even though juiciness is most directly tied to DOD, as indicated by Drey et al. (2019), the relationship is more complex than previously thought.



Figures 5 and 6: Drey et al., 2019

Flavor

Flavor is the most complex palatability trait comprised of the production of volatile compounds and the detection through the retronasal olfaction system (Legako et al., 2015; Kerth

et al., 2015). Overall, the volatile compounds produced through cooking attribute to the overall beef flavor including sulfur-containing compounds, ketones, disulfides, aldehydes, and many others (Legako et al., 2015; Kerth et al., 2015). The development of these volatile compounds occurs through two main pathways: thermal lipid degradation and Maillard browning (Kerth et al., 2015). Figure 7 outlines the different pathways for volatile compounds to be produced.

The Maillard reaction requires an amino group, reducing sugar, and heat, while producing hundreds of volatile compounds through the steps of dehydration, Strecker degradation, and condensation (Kerth et al., 2015). On the other hand, lipid degradation occurs through the removal of the glycerol backbone and further oxidation of the fatty acid chains (Kerth et al., 2015). Overall, the lipid degradation products are produced first, followed by the Maillard products, producing a complicated system of volatile compounds working together to produce aroma and flavor (Kerth et al., 2015).

Amplified flavor profiles can develop through higher DODs as the Maillard reaction occurs at higher temperatures than thermal lipid degradation (Kerth et al., 2015; Lorenzen et al., 2005). Vierck et al. (2019) conducted a study with three DODs, quality grades, and enhancements and determined the biggest impact on the volatile compound profile was the DOD. The Maillard products were correlated with the very well-done DOD while the lipid degradation products like Hexanal were correlated more with the rare DOD (Vierck et al., 2019). Gardener and Legako (2018) found a three-way interaction between four Strecker aldehydes, four pyrazines, and one ester between the DOD, quality grade, and product type. Steaks cooked to a medium DOD and lower exhibited 4-heptanolide whereas medium-well and well-done DODs were associated with pyrazines and hexanol (Lorenzen et al., 2005). Hexanol is the primary lipid degradation and oxidation by-product of oleic acid, the most prominent fatty acid in the body

(Kerth et al., 2015). As steaks cook longer, more fatty acids are degraded, and their by-products can accumulate. Similarly, the longer a steak is exposed to high heat, the greater development of volatile compounds produced from the Maillard reaction. While the Maillard reaction is constant from species to species, the thermal lipid degradation is highly species-dependent due to the different fatty acid profiles (Kerth et al., 2015). The production and interaction of volatile compounds makes understanding flavor very complex and still a heavily researched field.

Overall, many factors can impact the flavor of a steak including the DOD, fatty acid profile, muscle, quality grade, and cooking method (Vierck et al., 2019; Gardner and Legako, 2018; O'Quinn et al., 2016; Legako et al., 2015; Kerth et al., 2015). Out of all three pillars of eating quality, flavor is the only one to have positive attributes with increasing DODs. Lucherk et al. (2016) found flavor liking scores to increase for the Prime treatment, but the other quality grades resulted in an increase from a rare DOD to a medium DOD, but a decline to a well-done DOD. Therefore, beef flavor is still a heavily researched field due to the complexity and interactions of the flavor compounds. While flavor can become more intense with increasing DODs by the production of certain volatile compounds, it does not overcome the decrease in juiciness, and tenderness, therefore, increasing DODs, will result in a decline in overall liking (Drey et al., 2019). This confirms the findings from O'Quinn et al. (2018) which determined that the failure of even one pillar of palatability drastically increases the chance of failure for overall liking indicating all three pillars are significant to the overall eating experience.

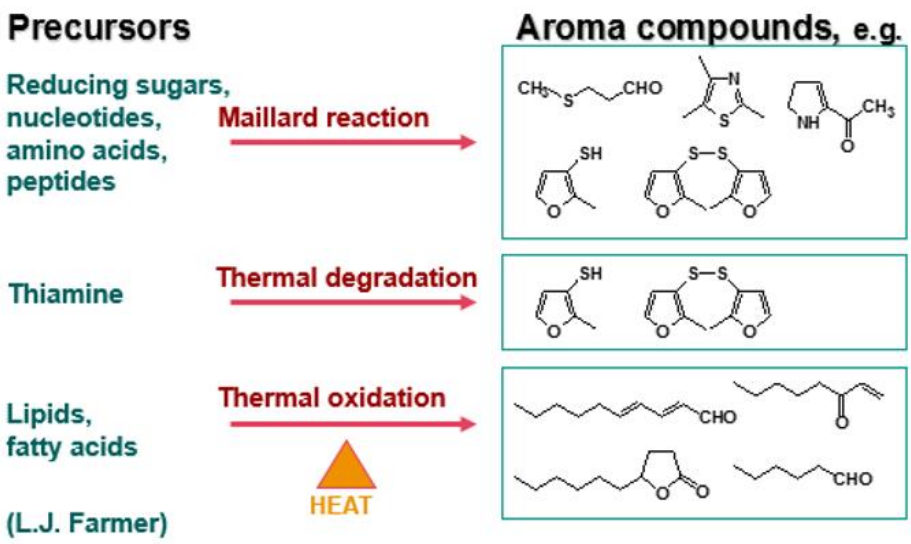


Figure 7: Mechanisms in flavor development for meat

Kerth et al., 2015

Conclusion:

The mechanisms that occur through the cooking process are influenced by a multitude of intrinsic and extrinsic factors such as the state of Mb, thermal stability, cooking method, quality grade, muscle, and species. Cooking proteins impacts cooked color by changing the pigment to either hemichrome or hemochrome, denaturing Mb, and producing multiple post-translational changes. Additionally, the cooking process causes protein hardening through the aggregation of proteins and the formation of disulfide bonds. Lastly, as the DODs increase, juiciness, tenderness, and overall liking decrease, deteriorating the eating experience. Further research to continue to understand each factor that attributes to the differences found between different DODs is imperative to continue to provide consumers with a high quality and consistent eating experience.

While evaluating factors within the raw product such as quality grade or muscle have been a priority of research for decades, the factors only affecting the cooked product such as the DOD have been out of focus. Out of all the meat quality areas highlighted above, the mechanism of cooked color remains the least understood. Therefore, one objective of the current work was to determine the changes in cooked color and myoglobin denaturation by cooking three different muscles to medium rare, medium, or well-done DOD.

The mechanism of freezing and its impact on the eating experience and physiochemical properties of beef steaks

Introduction:

Freezing meat has been a long-standing process for preservation allowing for more flexibility for consumption and movement through the food chain by decreasing enzymatic activity, microbial growth, and other forms of deterioration (Dang et al., 2021; Leygonie, Britz and Hoffman, 2012). Due to the vast necessity of freezing meat, it was a widely accepted practice until more recently (Pietrasik and Janz, 2009). A shift in consumer opinions has created a market for a "fresh, never frozen" product (Lambooji et al. 2019; Buckley et al., 1977). This shift was caused by unsupported claims that fresh beef has a greater eating experience than frozen beef (Pietrasik and Janz, 2009). Due to the demand, the label "fresh, never frozen" can be marketed at a higher price, motivating industry leaders to determine ways to keep beef fresh. While some physical and chemical changes in freezing proteins such as ice crystals and purge loss have been researched, the elicited quality impacts have not been fully vetted, leaving gaps in the research.

Consumer Perception of Fresh vs Frozen Beef

Freezing meat has been a long-standing debate with consumers for decades within the retail setting (Lambooji et al. 2019; Buckley et al., 1977). The perception that meat quality and particularly, eating quality, are reduced through freezing started with the consumer; however, this claim has never been supported in the scientific literature (Pietrasik and Janz, 2009). Consequently, the use of frozen meat in a consumer-facing setting has not been very common due to these negative perceptions around quality. Regardless, consumer opinions are the most

important to any industry, so being able to discuss differences between fresh and frozen beef with consumers is imperative moving forward.

While consumers have continued doubts about the quality of frozen beef, few studies have done an exact comparison of fresh and frozen beef. Additionally, it has never been determined whether consumers prefer a fresh steak over one that has been frozen and thawed in a blind panel (Lagerstedt et al., 2008). These two big questions could help uncover the impact of freezing from a quality or perceived quality standpoint, allowing the industry to make more informed decisions around freezing.

Advantages of Freezing Meat

The main advantage of freezing meat is the added shelf-life and flexibility of storage and transportation by minimizing bacterial growth and slowing the deterioration of quality (Dang et al., 2021; Lagerstedt et al., 2008). Due to the chemical composition of meat, microbial spoilage is always a potential outcome if held in a fresh state leaving few options to extend the shelf-life such as freezing or processing (Dang et al., 2021). Therefore, freezing meat is not an option for the meat science industry, but a necessity.

Freezing is imperative to ensure meat can reach all parts of the world (Iskandar et al., 2019; Leygonie et al., 2012). The U.S. beef export market is essential for the U.S. economy and to provide other countries with high-quality protein (USDA, 2022). In 2022, the U.S. exported $6.6 B worth of beef to east-Asian countries alone, a 22% increase from any previous year, and over $10 B in total (USDA, 2022). Transport to east-Asia can take weeks leaving few options for preservation including extended aging, freezing, or deep chilling. Freezing can increase the shelf-life to up to 12 months in the freezer without the known quality changes that accompany

extended aging (USDA, 2013). The added flexibility for exported meat has been a necessity. However, even domestic beef uses a freezing step to improve the flexibility of transport while keeping quality consistent.

Freezing is advantageous to domestic suppliers by allowing the flexibility to hold products in a frozen warehouse before shipping them to retailers or food services without any added pressure to meet a specific aging specification (Iskandar et al., 2019). This flexibility aids in product consistency. Logistically, it is very challenging to process, age, and ship meat all over the country with targeted aging parameters. Domestic cold chain management is complex and should be controlled from the time of chilling or freezing to the time of cooking (Ren et al., 2022; Nastasijevic et al., 2017). The easiest solution to ensure the product arrives in a consistent manner is to freeze it once it reaches the targeted aging period.

While transport can be unpredictable and lengthy, freezing meat alleviates all unpredictable variables. Even though the shipping time is significantly shortened for domestic products, the USDA still recommends consumers to use raw beef products within 5 d of purchasing, leaving little room within the commerce or retail for extra storage (USDA, 2020). Some suppliers ship frozen products directly to retailers or food service to slack out or thaw, or they ship previously frozen products to retailers to thaw while in transport for immediate display (Ren et al., 2022). These extra options for cold chain management can be beneficial for multiple layers in the food chain providing a time buffer and preventing inconsistencies in aging periods. Therefore, a form of freezing is imperative in the food chain to allow for extended shelf-life and quality protection to allow for easier commerce systems or storage for suppliers, retailers, food service, research, consumers, or any other end user.

Disadvantages of Freezing Meat

While the advantages of freezing meat are obvious, there are a few factors contributing to the negative perceptions of freezing. Consumer perceptions have played a large role in dictating this market. However, scientific literature has shown quality deficits including increased purge loss, cook loss, protein denaturation, oxidation, and discoloration, especially through retail display (Kim et al., 2018; Aroeira et al., 2016; Leygonie et al., 2012). However, the economic loss of freezing has been a much more important factor than decreasing eating quality. Increased purge loss decreases yield and therefore value and the potential discoloration and oxidation could impact shelf-life. While freezing has been typically described as causing "quality defects", "quality" has mostly been used to describe these factors impacting storage and not necessarily eating quality (Kim et al., 2018; Aroeira et al., 2016; Leygonie et al., 2012). While water-holding capacity factors such as cooking loss and purge loss are important for the overall eating quality, it is not always an indicator of juiciness or overall liking. Therefore, linking a decrease in eating quality with a decrease in water-holding capacity is not always accurate. Furthermore, using these factors to state freezing negatively impacts meat quality completely ignores a sector of meat quality: the consumer's eating experience. This void in research has attempted to be addressed, however, gaps still remain in this field.

Ice Crystal Formation

In order to understand the quality impacts of freezing, understanding ice crystal formation is imperative. The basic mechanism of change from fresh to frozen meat is the development of extracellular and intracellular ice crystals (Rahelic et al., 1985). The mechanisms around ice crystal formations in frozen beef have been widely studied (Mulot et al., 2019; Do et al., 2004; Martino et al., 1988; Rahelic et al., 1985). Therefore, it is known that ice crystals form

while meat is freezing, and the size, location, and morphology of the ice crystals can play a huge role in meat quality (Aroeira et al., 2016 ; Botinestean et al, 2016). Since the formation of ice crystals is inevitable, the size, shape, and location of the crystals are the most important factors impacting freezing quality (Li et al., 2022; Aidani et al., 2014).

Freezing quality is commonly tied to the freezing rate and holding temperature of the product as both factors have a thermodynamic relationship with the conversion of liquid to solid water molecules (Li et al., 2022). Overall, ice crystals have a generally negative impact on meat quality, potentially leading to freezer burn, increased purge, decreased color stability, and increased rate of oxidation; however, the extent of these factors are dictated by the inherent characteristics of the ice crystal (Sanchez et al., 2012). Therefore, understanding their formation within frozen products becomes pivotal to mitigating any negative impacts.

The effects of freezing are primarily due to the freezing of the aqueous portion of the muscle and the consolidation of the remaining constituents (Leygonie et al., 2012). Ice crystals are formed in two pivotal steps, nucleation of the water molecules and crystallization of the water molecules (Mulot et al., 2019). The rate of nucleation and crystal growth are tied to the rate of the water molecule reaching the thermodynamic equilibrium; therefore, they are dependent on the freezing rate (Mulot et al., 2019; Kashchiev and Van Rosmalen, 2003). The longer the water molecules do not reach the thermodynamic equilibrium, the larger and more abnormal the crystals can become (Mulot et al., 2019). In simplified terms, the faster meat can freeze, the smaller and less damaging the ice crystals are, however, there are other factors that impact overall freezing quality.

Generally, freezing parameters greatly impact the quality of the ice crystals such as temperature, airflow, freezing time as well as the inherent properties of the meat or product.

Lower air temperatures and higher airflow velocities correlate to smaller, less damaging ice crystal formation (Do et al., 2004; Rahelic et al., 1985). The speed of freezing has one of the largest impacts on the ice crystal formation quality (Kim et al., 2015). Fast-frozen steaks develop smaller ice crystals leading to less cell membrane damage and therefore, less water loss as purge or during cooking (Kim et al., 2015). A study evaluated the 3-D structure and size of ice crystals from a -15°C and -120°C freezing system and found 100µm and 10µm spherical ice crystals respectively (Do et al., 2004). The size and morphologies of these ice crystals are shown below in Figure 8 Similarly, beef stored at -18°C compared to 4°C, -1°C, -6°C and -9°C resulted in less cellular damage and water migration as indicated by less drip loss and surface hydrophobicity (Li et al., 2017). Therefore, it is widely accepted that holding meat at a lower temperature not only prolongs the shelf-life significantly but also results in less cellular damage. However, freezing and holding meat at very low temperatures require enormous energy inputs and infrastructure which could be limiting factors.

Storage time can also impact freezing quality, but the exact impact is less clear based on research. An increased storage time is associated with larger ice crystals or the development of oxidation in the form of freezer burn (Bao et al., 2021). The risk of freezer burn can increase over time due to the increased risk of oxidation or surface dehydration mostly caused by fluctuations in temperature (Bao et al., 2021). Additionally, the longer the frozen storage period, the larger the ice crystals can become (Choi et al., 2015). However, Li et al. (2022) found most of the damage to the cell membranes occurs within the initial freezing step and does not continue to significantly deteriorate throughout various holding times if the temperature remains constant. These discrepancies indicate the need for additional research to understand the true impact of storage time on ice crystal formation.

Since ice crystal formation is mostly dictated by freezing parameters, improved freezing techniques have been a focus for many years. This shift has created new freezing techniques aimed to minimize or reduce the impacts of freezing solely based on the reduction of ice crystal size. The effect of freezing on ice crystal formation has dominated most space in the research field leaving other areas wildly under-researched. While freezing techniques have changed and improved within the last few decades, new research has been conducted on the impacts of freezing in limited areas. Consequently, the current research mostly exists around the impacts of tenderness and ice crystal formation of freezing beef and other proteins, without looking at other confounding factors such as calpastatin inactivation, leaving gaps in the research (Aroeira et al., 2016 ; Grayson et al., 2014). Additionally, these newer studies have not evaluated eating quality, leaving the effect of freezing on palatability as a continued void in the literature.

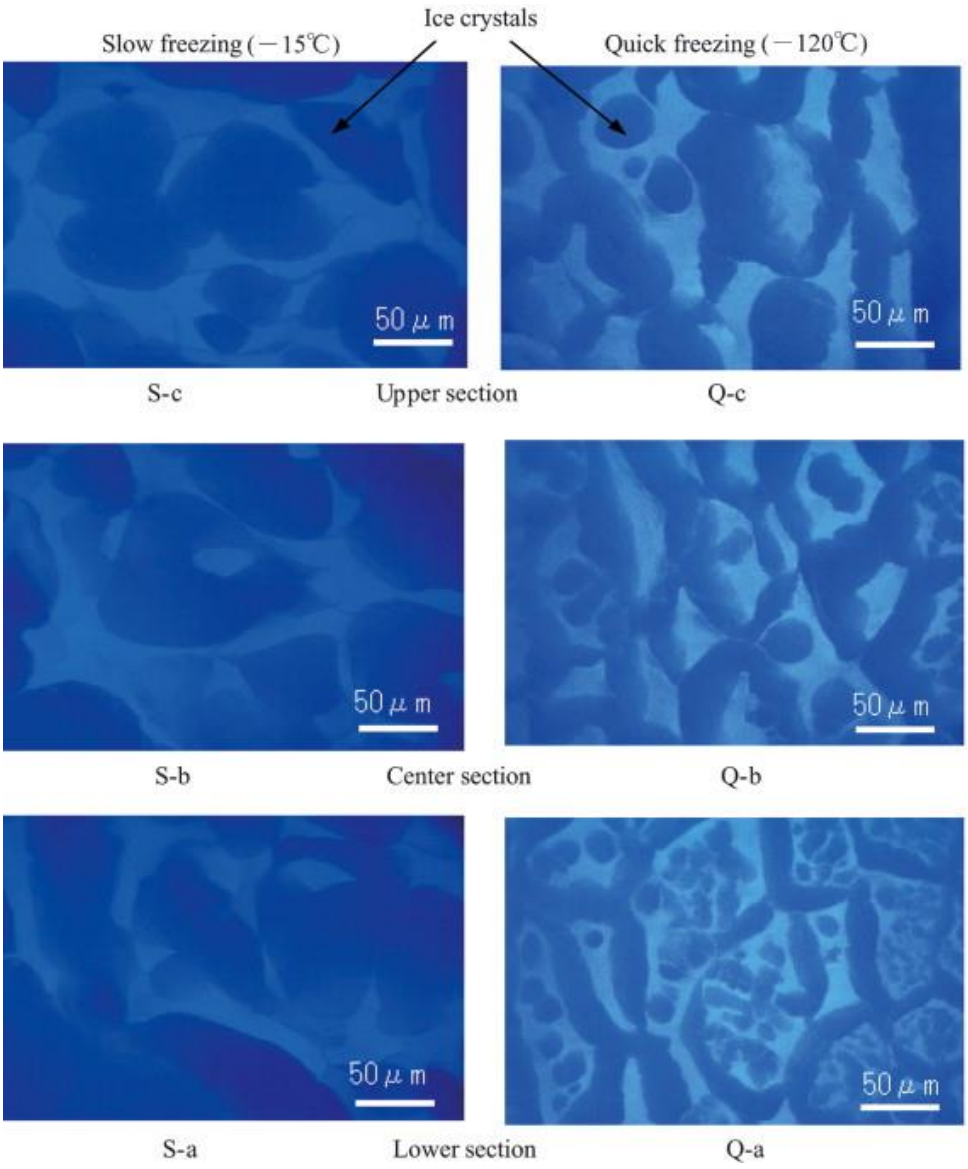


Figure 8: Do et al., 2004

Freezing Methods on Ice Crystal Formation

The temperature, airflow, and time of freezing all impact ice crystals formation and therefore the meat quality. The easiest way to control for different freezing parameters is to change the freezing method. Common freezing methods include blast freezing, cryogenic freezing, plate freezing, individual quick freezing (IQF), or refrigerator freezing (Lu et al., 2022).

The most common freezing practice today is blast freezing due to ease and efficiency to prevent large ice crystal formation, therefore, decreasing purge loss and other negative impacts associated with freezing (Lu et al., 2022; Dempsey et al., 2012). Blast freezing is a faster freezing method than conventional techniques such as at-home freezing (Hergenreder et al., 2013). However, there are faster freezing methods that have increased in research within the past decade, but not necessarily in industry applicability.

When plate freezing, immersion freezing, or conventional at-home freezing were compared, the immersion freezing method produced the smallest and most compact ice crystals (Wang et al., 2020). However, immersion freezing might not be the most practical freezing method for the industry or consumers. Similarly, a study compared a pressurized freezing method adding 140MPa to the freezing chamber and compared it to a traditional blast frozen method held at the same temperature and found the ice crystals to be 10x smaller with the added pressure (Chevalier et al., 2000). Similar to immersion freezing, a pressurized chamber might not be possible to implement on a large scale right now. Overall, there is still room for improvement within the current freezing technologies. As newer technologies continue to be researched, including information on the overall eating quality, should be imperative.

Freezing Impact on Tenderness

Similar to ice crystal formation, the impacts of freezing on tenderness have been heavily researched. However, the impacts of freezing on tenderness have resulted in conflicting results (Grayson et al., 2014). Typically, the positive attributes of freezing have been centered around the obvious increase in shelf-life and inconsistent improvement in tenderness (Botinestean et al, 2016 ; Grayson et al., 2014). These discrepancies in tenderness have been explained through differences in muscles, and freezing parameters (Grayson et al., 2014). While an impact on

tenderness has mostly been found, the extent of these differences has varied widely. To understand this discrepancy, the mechanism of tenderness changes through the freezing process must be explored.

Ice Crystal Formation on Tenderness

The freezing effect on tenderness has been attributed to two main phenomena: ice crystal formation and inactivation of the calpastatin system (Aroeira et al., 2016 ; Botinestean et al, 2016; Grayson et al., 2014). Ice crystal formation typically increases tenderness. As the ice crystals form, they can rupture the muscle cell membrane, leading to some negative and positive quality effects after thawing including an improvement in tenderness (Aroeira et al., 2016; Botinestean et al, 2016). Ice crystals impact tenderness by two mechanisms: decreasing structural integrity and releasing proteolytic enzymes and calcium during the thawing process (Vieira et al., 2009). While it is mostly accepted that freezing and thawing steaks decrease the shear force values of certain muscles; there are limited studies determining the impact of subjective tenderness (Grayson et al., 2014; Farouke et al., 2003; Wheeler et al., 1990). Additionally, the differences in shear force values are lessened with increased aging periods, muscles with higher collagen contents, and smaller ice crystal formation (Vieira et al., 2009). Since tenderization is not infinite, extended aging decreases the improvement. Additionally, smaller, less damaging ice crystals cause less structural damage and release fewer proteolytic enzymes during the thawing process, lessening the tenderization impacts (Vieira et al., 2009). However, it is difficult to make bold statements about the tenderness impacts without consumer data. Since the elicited subjective tenderness is the most important parameter impacting palatability, a gap in the literature remains.

Calpastatin Inactivation on Tenderness

This less-researched route of calpastatin inactivation impacts WBSF values, acting as a potential intervention for historically myofibrillarly tough muscles. This route of calpastatin inactivation could lead to industry processing changes including an aging period after freezing. Calpastatin and not the calpains are sensitive to frozen temperatures and become irreversibly damaged over time (Grayson et al., 2014). At -20°C and -80°C, m-calpain and μ-calpain maintained the same activity for 123 d of storage, whereas calpastatin decreased in activity after two weeks of storage regardless of the frozen temperature (Kristensen et al., 2006). This relationship is shown in the figure below (Kristensen et al., 2006). Similarly, in a study comparing fresh and frozen SSF values, the frozen then aged combination elicited the greatest change in tenderness with similar changes in Desmin degradation (Grayson et al., 2014). This secondary impact on tenderness could lead to industry implications of using freezing to help add value to tougher muscles before the aging process.

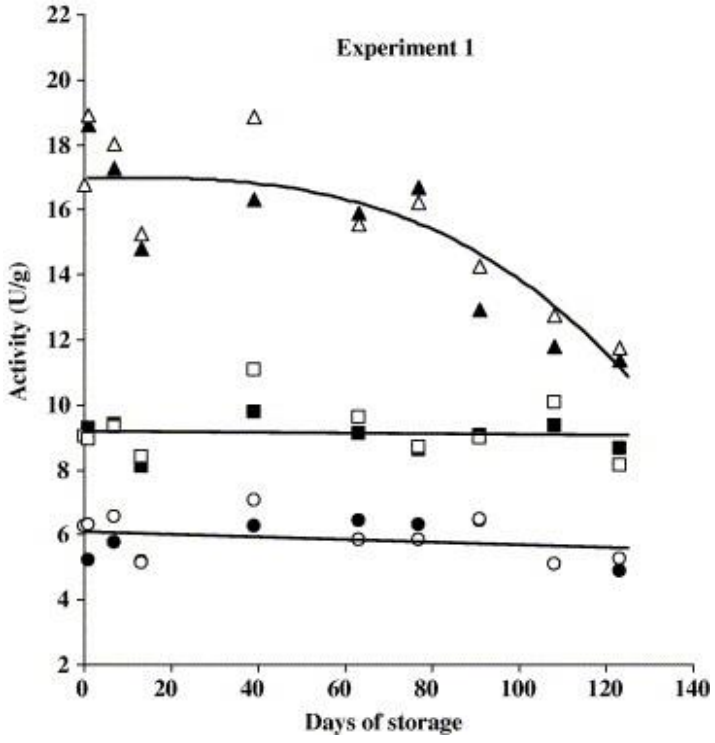


Figure 9. Measured activity of μ-calpain (circles), m-calpain (squares) and calpastatin (triangles) in meat stored at −20 °C (closed marks) and −80 °C (open marks). A polynomial regression line is added the calpastatin data (R^2 = 0.75) and a linear regression the calpain data

Kristensen et al., 2006

Freezing Impact on Protein Oxidation and Denaturation

While the impacts of freezing on tenderness and water-holding capacity traits have been evaluated for decades, protein oxidation and denaturation are still hot topics in meat science with ongoing research. Protein oxidation and denaturation can decrease color stability and cause quality defects through structural changes that occur through the thawing process.

Most structural damage that leads to protein oxidation and denaturation occurs early in the freezing process but is not seen until after the thawing process. However, added damage can occur throughout freezing due to changes in the freezing conditions (Bao et al., 2021). While freezing halts most activity within the muscle, small changes can still occur throughout the storage time, especially if temperature fluctuations occur (Xia et al., 2009). These fluctuations can occur for a multitude of reasons, but most commonly throughout transport. Wavering between a frozen state and a thawed state causes amplified damage primarily within protein oxidation and denaturation leading to less desirable meat quality attributes due to flavor and textural changes (Bao et al., 2021; Xia et al., 2009).

Protein oxidation is caused by two main mechanisms. The sublimation of the ice crystals can increase the release of prooxidants such as metals and heme proteins by disrupting the cell membrane stability, thereby, increasing the rate of protein and lipid oxidation (Zhang et al., 2023). In addition to this mechanism, protein oxidation is attributed to the conversion of

sulfhydryl groups to disulfides during the freezing process or the formation of cross-linkages between sulfur groups within protein side chains (Soyer et al., 2010). While the basic mechanism behind an increase in protein oxidation has been determined, the pro-oxidants precisely causing this oxidation is still not fully understood, especially in red meat species.

Protein oxidation can increase by many freezing factors such as changes in temperature, freezing method, storage time and temperature, or the number of freezing cycles. The lower the freezing temperature, the less oxidation will occur after thawing (Soyer et al., 2010). Oxidation can increase throughout multiple freeze-thaw cycles leading to changes in flavor, color, and potentially overall acceptability (Xia et al., 2009). Xia et al. (2009) found the carbonyl and malondialdehyde content to increase linearly with an increase in freeze-thaw cycles in addition to a significant decrease in sulfhydryl content and a* values. The study hypothesized that repeated cycles caused the release of certain prooxidants able to interact with the proteins after the cellular structure was compromised (Xia et al., 2009). Similarly, another study found faster freezing methods such as ultra-sound freezing resulted in a decreased carbonyl content due to less cellular damage (Zhang et al., 2023).

Most research in this area is for aquatic species and some poultry species as protein oxidation in red meat is less significant (Baron et al., 2007; Soyer et al., 2010). These fields did most of the work to understand the mechanism of protein oxidation during freezing. It has been fully vetted within aquatic species that most protein oxidation production occurs through the conversion of thiol groups to disulfide groups and later to carbonyl groups due to the release of the heme pigments and other prooxidants (Bao et al., 2021; Baron et al., 2007; Soyer et al., 2010). However, a study of similar depth has not been conducted on red meat.

Protein denaturation can occur throughout the freezing process as well. Denaturation is typically seen as an increase in surface hydrophobicity or hydrophobic aggregation of myofibrillar proteins (Lee et al., 2022). These changes are driven by changes in solute concentration by the freezing of the extracellular spaces, coined “cold denaturation” (Lee et al., 2022). Similar to the relationship with protein oxidation, protein denaturation increases with slower freezing times and higher storage temperatures as shown by an increase in surface hydrophobicity (Zhang et al., 2019). Zhang et al. (2019) proposed in addition to the general changes in solute concentration, freezing the extracellular water concentrates the protons decreasing the pH, therefore denaturing the proteins in proximity upon thawing. The same research team completed a follow-up study to further test this hypothesis by creating high and low pH conditions and measuring surface hydrophobicity and water holding capacity (Zhang et al., 2021). This study confirmed pH is affecting the mechanism of protein denaturation within a freeze-thaw system. This area of research is relatively unsaturated leaving room for future research to further understand the mechanism of protein denaturation and its impact on tenderness and eating quality.

Intrinsic Factors Impacting the Effects of Freezing

Intrinsic factors such as pH, moisture content, muscle metabolism, or muscle location can impact the severity of the freezing impacts. More oxidative fibers exhibited greater damage from freezing leading to a decrease in water-holding capacity and color stability (Cheng et al., 2020). On the other hand, as previously discussed, the relative pH can impact how severe the freezing impacts will be with a lower overall pH resulting in a significant change in protein oxidation and denaturation (Zhang et al., 2021). These studies resulted in conflicting data as more oxidative fibers typically have a higher ultimate pH due to the change in pH decline, and pH decline

cessation (England et al., 2016). These relationships have been minimally researched and future studies should be conducted to further understand how muscle metabolism, muscle location, and pH impact the effects of freezing. Most freezing studies determined the impact of external factors on freezing quality while the intrinsic factors have been less studied.

Deep Chill

Deep chill is a processing technique used to prevent the usage of the "previously frozen" label on meat products sold at retail (Cargill, 2018). Due to the loose definition of "frozen" by USDA, meat can be held above 0°F and be considered fresh, even though the freezing point of meat is 28°F (USDA, 2007; USDA, 2013). While techniques like blast freezing and other newer freezing technologies reduce the size of the ice crystals, therefore, minimizing some negative impacts of freezing, it is still believed that consumers deprioritize products labeled as "previously frozen" (Lambooji et al. 2019; Buckley et al., 1977). In the retail world, it is commonly believed to be a deterrent for consumers. This strong consumer trend of wanting fresh, local, and natural products continues to be a driver for producers and suppliers in all commodities, especially in the meat industry (Lambooji et al. 2019; Buckley et al., 1977). Therefore, retailers have pushed suppliers to provide options for fresh, never-frozen meat without losing the flexibility of the added shelf-life. This push has expedited the use of a deep chill method.

The deep chill method holds meat between 5°F and 10°F without allowing it to pass below 0°F (Cargill, 2018). Marketing frozen meat that has been held under 0°F must be labeled "previously frozen", therefore, deep chill allows a temperature buffer without the added labeling pressure (USDA, 2007; Cargill, 2018). Meat freezes at 28°F so even with a deep chill, it will still partially freeze leaving the same research gap of understanding the palatability differences

between fresh and frozen beef steaks but minimizing any perceived quality effect from the consumers. Additionally, little research has been conducted and published on the quality impacts of deep chill within the retail setting or for palatability traits. However, following the trends established by the research done around freezing, holding the product between 0°F to 28°F would result in larger, more damaging ice crystals in comparison to any other freezing method. This high freezing temperature increases the risk of fluctuating between a frozen and thawed state and could lead to amplified meat quality issues. Due to these concerns, this field needs to be further researched. While this area of research needs to be vetted, it requires an additional infrastructure of cold storage held above 0°F, limiting the feasibility within the academic realm. Regardless, deep chill remains a huge gap in research for the meat science industry.

New Emerging Freezing Techniques

Due to the negative freezing quality characteristics associated with freezing, novel freezing methods have emerged to mitigate additional negative quality impacts. These technologies include high-pressure freezing, immersion freezing, electrically and magnetically assisted freezing, and ultrasound-assisted freezing, among others (Zhang et al., 2023; Zhan et al., 2018). In a study comparing immersion freezing, ultra-sonic freezing, and traditional blast freezing, ultra-sonic freezing resulted in the least significant changes to the protein structure as measured by less surface hydrophobicity, and lower carbonyl content (Zhang et al., 2023). Similarly, high hydrostatic pressure freezing was compared to blast freezing and resulted in less purge, reduced discoloration, and deceased thaw time (Massaux et al., 1999). However, Fernandez et al. (2007) hypothesized differing results and claimed high hydrostatic pressure causes quality defects including discoloration and increased purge. However, their results aligned with Massaux et al. (1999), resulting in improved color when high hydrostatic pressure

was combined with freezing (Fernandez et al., 2007). Overall, these newer technologies need to be further researched before beginning to make a plan to implement wide scale. Additionally, while some of these new methods diminish some discussed quality issues, they do not all provide tangible alternatives for the meat industry to implement with the current limitations of technology.

New freezing technologies seem to be premature since the basic understanding of the impact of freezing on consumer acceptability has not yet been researched before the current study. If the impact of freezing has little to no impact on the overall eating experience, the research around freezing could shift to newer freezing methods that require fewer energy inputs. As the energy crisis continues to grow, finding freezing technologies with a smaller carbon footprint with increased efficiencies could be imperative. Therefore, research with newer technology is important, even if it is not tangible in the industry right now.

Conclusion:

Since the impacts of freezing described above are strongly driven by the size, shape, and location of the ice crystal, with the current technologies, the negative and positive impacts of freezing have almost all been eliminated. As the industry moves to new forms of chilling, deep chilling, and newer freezing methods, continuing to understand the impact of freezing and ice crystal formation on the eating quality of red meat remains a priority.

Most past studies have only evaluated different freezing methods, storage temperatures, storage time, and airflow velocities without comparing a frozen steak to a fresh steak of equal aging periods, leaving a gap in the research. A study by Grayson et al. (2014), evaluated fresh beef steaks versus frozen and thawed beef steaks of unequal aging periods, therefore, not being a

true comparison of fresh and frozen beef steaks. The current project will therefore fill in the voids of the current research around freezing beef to provide a platform for new innovation to continue to improve freezing techniques to optimize meat quality and consumer satisfaction. Additionally, this study will illuminate any perceived quality differences when consumers are fed labeled fresh or frozen beef steaks to understand the impact of the consumer perception on the overall eating experience. A further understanding of consumer motivators should always be a priority for the meat industry. Therefore, the objectives of this study were to determine the impact of freezing by comparing fresh and frozen beef strip loins of three aging periods for palatability traits, tenderness, and physiochemical properties affecting the overall quality attributes.

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Chapter 2- Change in myoglobin denaturation and physiochemical properties among three degrees of doneness and three muscles

INTRODUCTION

The mechanisms that comprise the changes a steak undergoes from the raw state to the time it is sitting on a consumer's plate are vast. The color change associated with cooking is largely due to complex changes in myoglobin (Suman and Joseph, 2013; Mancini and Hunt, 2005). Myoglobin is the primary protein responsible for raw and cooked meat color (Suman and Joseph, 2013; Mancini and Hunt, 2005). Myoglobin undergoes a series of intricate changes through the aging and cooking processes altering the pigment (Salim et al., 2021; Hughes et al., 2020). While the mechanisms of color change within raw meat have been intensely studied, the same depth has not been achieved for cooked meat color.

Cooked meat color is the visual external and internal appearance of a steak which drives the consumer's perception of quality and food safety (Prill et al., 2019; Cox et al., 1997). Through the development of different degrees of doneness (**DOD**), consumers expect a steak to visually appear the way they envision the characteristics for the specific DOD, and if it does not, they will have a lower perception of the quality (Prill et al., 2019; Cox et al., 1997). While consumers are always advised to use a meat thermometer to determine the appropriate DOD, the visual appearance of the steak is most commonly used for DOD determination (Prill et al., 2019; King and Whyte, 2006).

The heat of the cooking process changes the overall appearance and eating experience of a steak (Tornberg, 2005). While heat is necessary for cooking, it is also a common method to denature proteins, changing their solubility and functionality (Tornberg, 2005). These changes

can impact the water-holding capacity, tenderness, and potentially the pigment for optically active proteins such as myoglobin (Tornberg, 2005). Salim et al. (2021) determined myoglobin undergoes post-translational changes through the cooking process, but the basic question of myoglobin denaturation has yet to be answered in whole muscles.

Within raw meat, muscle variation has been shown to make a drastic difference in meat color based on location, muscle metabolism, and inherent color stability (Mancini & Hunt, 2005; Park et al., 2018). Therefore, there could be inherent cooked color and color stability differences found within cooked steaks of different muscles. Since the final cooked color of a steak has been shown to be one of the most important factors for the consumer's eating experience, an improved understanding of how DOD differs among muscles could provide valuable information for food service, processors, and even consumers.

The current level of understanding of cooked color leaves numerous gaps in the understanding and control of the impact of DOD on consumer eating experience. While evaluating factors within raw product such as quality grade or muscle have been a priority of research for decades, the factors affecting the cooked product such as the DOD have been largely ignored. Therefore, the objective of this study was to determine the changes in myoglobin denaturation and physiochemical properties of three different muscles cooked to a medium rare, medium, or a well-done DOD.

MATERIALS AND METHODS

Product collection and fabrication

Beef strip loins (n = 12, IMPS # 180), and top butts (n = 12, IMPS # 184) grading USDA Select were collected from a Midwestern beef processor and transported to Kansas State

University. Marbling scores, ribeye area, backfat thickness, and ossification were recorded for each carcass in Appendix A. Subprimals were aged for 28 d in covered cardboard boxes at 2-4°C before being sliced into 2.54-cm thick steaks. Eight steaks were randomly assigned a DOD by numbering the steaks from the anterior end. Based on the randomization, each steak was assigned one of the following DOD: raw, medium rare (**MR**), medium (**MED**), or well-done (**WD**). Two steaks from each DOD were reserved for Warner-Brazler shear force (WBSF) or lab assays. Once labeled and vacuum sealed, the steaks were frozen and held at -20°C.

Cooking procedures and internal color

Each steak was thawed 24 h prior to cooking at 2-4°C. Once thawed, a thermometer (Thermopen mk4, Salt Lake City, UT) was inserted into the thickest part of the steak and added to a clamshell grill (Cuisinart Griddler Deluxe, East Windsor, NJ) set to 175°C. The steaks were removed 5-7°C below the designated DOD and the peak internal temperatures were recorded. The MR, MED, and WD samples were cooked to an end-point temperature of 63°C, 71°C, and 77°C, respectively. The steaks were allowed to rest for three minutes before being sliced in the geometric center from cooked surface to cooked surface to evaluate internal color. Three color readings were taken at different internal locations. An average of three readings for CIE *L**, *a**, and *b** were taken using a Hunter Lab Spectrophotometer (Illuminant A, 1.27 cm aperture, 10° observer, Hunter Lab Associates Laboratory, Reston, VA). After the color readings, the steaks were chopped and frozen in liquid nitrogen and blended (Waring Products, New Hartford, CT) into powder for future assays and held in a -80°C freezer.

Warner-Brazler shear force

The WBSF samples were cooked using the same protocol as described above and followed the approved method from the American Meat Science Association (AMSA, 2015). Briefly, samples were cooled for at least 12 h at 2-4°C before cores were taken. Six cores were taken parallel to the muscle fibers and sheared perpendicular to the muscle fiber orientation using an Instron (Model 5569, Instron Corp., Canton, MA) with a cross-head speed of 250 mm/min and a load cell of 100 kg. An average of all six measurements were used to represent each sample.

Myoglobin denaturation

Myoglobin denaturation was determined using the protocol in the AMSA Color Guidelines (King et al., 2023). A 0.3 g powdered sample of each steak was weighed into a 2 ml microcentrifuge tube with six ceramics beads pre-added. Immediately after weighing, 1.3 ml of 20 mM potassium phosphate buffer was added to each tube. The samples were homogenized for 30 s using the bead homogenizer and transferred to 1.5 ml microcentrifuge tubes to be centrifuged for 30 min at 10,000 x g. The supernatant was poured into a 50 ml beaker and added to a 5 ml syringe. A filter was added to the end of the syringe and the supernatant was filtered into a new 1.5 ml centrifuge tube as the final sarcoplasmic extraction. A 200 µl sample was plated in duplicate on a 96-well plate. Sodium hypochlorite (50 µl) was added to each well to reduce all forms of myoglobin to the deoxymyoglobin form. The absorbance was read at 433 nm and converted to the percentage denatured using the following formula: % myoglobin denaturation = (Raw Absorbance (A) – [1- (Raw A – Cooked A)]) x 100.

Metmyoglobin-reducing activity

The metmyoglobin-reducing activity **(MRA)** was determined using the protocol in the AMSA Color Guidelines and modified to use 0.3 g instead of 5 g of sample (King et al., 2023). Briefly, 0.3 g of the powdered sample was weighed and 1.2 ml phosphate buffer was added before being centrifuged at 14,000 rpm for 30 min. A 96-well plate was prepared by adding 50 µl of 0.75 mM metmyoglobin, 25 µl of 3.0 mM potassium ferrocyanide, 50 µl of deionized water, 25 µl of 5 mM EDTA, and 25 µl 3 mM sodium citrate buffer. Next, 50 µl of each sample was added in duplicate. After the sample was added to each well, 25 µl of 1 mM NADH was quickly added, and the cells were agitated before being read at 525nm every 60 seconds for 180 seconds. Beer's law was used to calculate the change in absorbance as the change from metmyoglobin to oxymyoglobin.

MRA = change in absorbance*$12{,}000^{-1}$ *path length^{1} *minutes^{-1} *g of sample^{-1} *volume of reagents.

Lipid oxidation

Lipid oxidation was determined through the thiobarbituric acid reactive substances (TBARS) assay following the procedures described by Ahn et al., 1998, and similar procedures used at Kansas State University (Dahmer et al., 2022). Briefly, 0.3 g sample of powdered sample was weighed and added to 1.5 ml bead tubes with 1.4 ml of deionized water and 0.1 ml of butylated hydroxy anisole. The samples were centrifuged at 2,000 x g for 5 min and 0.4 ml of the supernatant was transferred into pre-labeled glass tubes. Next, 0.8 ml of thiobarbituric acid and trichloroacetic acid was added to each tube. After vortexing, the tubes were covered in aluminum foil and incubated in a 70°C water bath for 30 min. Then the samples were cooled and centrifuged at 3,000 x g for 15 min. Lastly, 0.2 ml of the supernatant was pipetted into a 96-well

plate in duplicate. The plate was run at 532 nm absorbance and a standard curve was used to determine the concentration of MDA.

Proximate analysis

The fat content was measured using the Folch method (Folch, et al 1957). A 5 g sample was weighed in duplicate into 50 ml centrifuge tubes. Water, cholorofom, and methanol were added to each tube using a repeating pipetter. The mixture was shaken by a mechanical shaker for 4 min. The sample was centrifuged for 10 min at 5000 rpm and the resulting supernatant was removed using a pipette. Next, a 4 ml sample of the chloroform layer under the hard solid film was added to a pre-dried and weighed glass tube. The samples were evaporated using heating stones and nitrogen gas. The resulting sample was dried for 24 h at 100°C and reweighed for a fat percentage measurement. Fat content was determined by the amount of sample left in the tube after drying as a percentage of the initial weight.

Moisture was determined by the approved AOAC drying oven method (950.46 and 934.01; AOAC, 1995). A 4-g sample was weighed in duplicate into pre-dried and weighed aluminum pans. The samples were added to a drying oven for 24 h at 100°C and re-weighed to determine moisture loss. Moisture content was determined as the difference between the initial weight and dried weight as a percentage subtracted from 100.

The pH was determined by weighing 5 g of each sample in duplicate with 50 ml of deionized water. The sample was homogenized with a medium head homogenizer for 20 sat 10,000 x g. The mixture was read with a calibrated InLab Solids Pro-ISM probe (Part #51344155; Mettler-Toledo, Schwerzenbach, Switzerland) connected to a Seven Compact pH meter (Mettler-Toledo, Columbus, Ohio).

Statistical analysis

The statistical analysis was conducted using SAS (Version 9.4; SAS Inst., Inc., Cary, NC) PROC GLIMMIX. Carcass served as the experimental unit. Fixed effects were muscle type and DOD. Data were analyzed as a split-plot design with the whole plot factor as muscle and the subplot factor of DOD. An α of 0.05 was set for a level of significance. The Kenward-Roger adjustment was used in all analyses.

RESULTS AND DISCUSSION

Cooked internal color

It is well documented that internal color changes throughout the cooking cycle; however the relationship between the factors impacting cooked color remains unknown (Schwartz et al., 2022; Salim et al., 2020; Prill et al., 2019; Hunt et al., 1999). This study aimed to evaluate all factors impacting cooked color, especially myoglobin. Consumers expect DODs to appear a certain way when cooked or ordered at a restaurant, and if they do not, their eating experience could be altered (Prill et al., 2019; Cox et al., 1997). While consumer acceptance and expectations of each DOD have been established, the mechanisms behind the color change have remained unexplored (Schwartz et al., 2022; Prill et al., 2019).

Color acceptability is mostly linked to the *a** and *L** values, with *a** having the largest changes throughout shelf-life and during the cooking cycle (Prill et al., 2019; Dikeman et al., 2015; Mancini & Hunt, 2005). As expected, in the current study, DOD impacted the internal color values, especially the *a** value. *L**, *a**, and *b** values are shown in Table 2.1. *L** values were not impacted ($P > 0.05$) by the different DODs. Dikeman et al., (2013) found the same relationship for *L** values between the MR and MED DODs for the LL. However, Prill et al., (2019) found a time and DOD interaction where the MR samples lightened over time and the

WD samples darkened. While *L** and *a** values impact raw meat color, these studies indicate *L** values might have less of an impact on cooked meat color compared to raw meat color (Prill et al., 2019; Dikeman et al., 2013). Additionally, the LL resulted in the highest ($P < 0.05$) *L** value followed by the GM with the BF having the lowest ($P < 0.05$) *L** value. Lightness is highly dependent on the muscle fiber type, muscle metabolism, moisture content, pH, and color stability, with differences commonly found among raw muscles (Park et al., 2018; Suman et al., 2014; Mancini & Hunt, 2005). Our results would indicate such muscle-to-muscle differences in lightness are not only present in raw muscles, but in the cooked form as well. Overall, the current study illustrates *L** values have the potential to represent other intrinsic differences between various cooked muscles, while not being impacted by different DODs.

As expected, the *a** values decreased ($P < 0.05$) with each increase in DOD. The current study and others provide evidence that the *a** value is the best representation of the DOD (Salim et al., 2021; Prill et al., 2019). However, *a** values were not different ($P > 0.05$) among muscles, but there was a numerical difference with the BF having the highest *a** value numerically. These similarities are surprising as *a** values are also linked to muscle metabolism and functions in the body and therefore color stability (Park et al., 2018; Suman et al., 2014; Mancini & Hunt, 2005), though such differences were not observed in the current study in cooked samples.

Previous studies support the idea that consumers associate certain internal color patterns with each DOD, and these changes can be measured by changes in *a** values (Salim et al., 2020; Prill et al., 2019; Yancey et al., 2016; Yancey et al., 2011). Two studies done by Yancey et al., (2016), and Yancey et al., (2011) found similar relationships between DOD and *a** values. Regardless of the dry heat cookery method, Yancey et al. (2016; 2011) found the *a** values to decrease from a MR, MED, and WD DOD. However, Yancey et al. (2016) observed a muscle

effect where this relationship was not expressed within the *infraspinatus* muscle, but it was within the *Longissimus thoracis,* and *Semimembranosus* muscles (Yancey et al., 2016; Yancey et al., 2011). While the current study did not find differences among muscles for *a** values, the *infraspinatus* and other muscles evaluated by Yancey et al. (2016) were not included. Taken together, these results indicate additional muscles may produce differences in *a** values that were not observed within the muscles of the current work. This potential interaction between muscle and DOD could potentially be explained by inherent differences in intrinsic factors such as pH, moisture content, and oxidation levels of different muscles at the time of cooking.

Dikeman et al. (2013) found a similar trend of DOD and *a** values within the LL; however, their study found an interaction between DOD and aging method of wet versus dry-aging. The dry-aged steaks did not have a significant difference in *a** values between the MR and MED treatments, but the wet-aged steaks produced similar results as those found in the current study. While not explored in that paper, this may provide evidence that differences in oxidation from dry-aging or other processes could be impacting cooked color or cooked color stability of whole muscles and warrants further investigation. The current study and the previously cited studies provide support for a strong relationship between *a** values and DOD, while highlighting some unknown factors between the relationship between *a** values and different cooked muscles and aging methods.

Myoglobin denaturation

Myoglobin denaturation has been briefly researched within ground beef but remains unexplored for cooked whole muscles before the current study. In general, myoglobin begins to denature at 55°C and continues to become fully denatured at 80°C (King and Whyte; 2006; Hunt et al., 1999). Within a ground product, the level of myoglobin denaturation is dependent on the

oxidation state of myoglobin at the time of cooking (Hunt et al., 1999). Ground beef also has a much higher recommended end-point temperature (71.1°C) to ensure food safety compared to whole muscle cuts (62.8°C) (Mancini and Hunt, 2005, USDA, 2016). This elevated and required end-point temperature for food safety prevents the same variation within internal color and myoglobin denaturation for ground products in comparison to whole muscle products that are commonly to cooked to a range of DOD spanning from rare to WD. Most previous work focusing on ground beef cooked color development focused on premature browning (Hunt et al., 1999). Hunt et al. (1999) determined myoglobin denaturation of ground beef with varying percentages of deoxymyoglobin, oxymyoglobin, and metmyoglobin. The authors determined the relationship between end point temperature and myoglobin denaturation was highly dependent on the relative percentages of each myoglobin form at the time of cooking (Hunt et al., 1999). Because of the inherent differences of a ground product versus a whole muscle cut, these denaturation percentages and the relationship with temperature could be different between ground beef and whole muscles.

Myoglobin denaturation, shown in Table 2.1, was hypothesized to increase as *a** values decreased, potentially providing an explanation to the changes in *a** values across DOD. As expected, myoglobin denaturation increased ($P < 0.05$) in a similar fashion to the observed decrease in *a** values. Myoglobin denaturation increased ($P < 0.05$) for each DOD. Myoglobin was denatured 29.08%, 48.34%, or 70.17% at MR, MED, and WD DODs, respectively. However, muscle did not ($P > 0.05$) have an impact on myoglobin denaturation percentage. Myoglobin denaturation percentages help explain the lack of differences found across muscles for the *a** values, with each muscle evaluated having a similar amount of myoglobin denatured at each DOD. These results differ from those of Yancey et al., (2016), who reported a* values to be

dependent on the muscle evaluated. This differences is likely the result of the muscles used in each study, but provide an indication that myoglobin denaturation may differ across other muscles of the carcass not evaluated in the current work.

This same trend of increased myoglobin denaturation at increased DOD has been shown in ground beef products as well (Ryan et al., 2006; Mancini and Hunt, 2005; Hunt et al., 1999). Ryan et al., (2006) suggested an 80% denaturation must be reached to achieve a WD DOD appearance for ground beef, while the current study suggests only a 70% denaturation is required for whole muscles. This difference could be due to the inherent differences of a ground meat product compared to a whole muscle cut. This key difference provides further evidence that the previous work in ground beef cannot be fully translated into whole muscle cuts. While ground beef and whole muscles are similar, understanding the differences between the two products and how myoglobin changes through cooking in each is pertinent to the overall understanding of cooked meat color development.

Understanding the baseline myoglobin denaturation level for certain degrees of doneness, as was established in the current study, can be used for future studies evaluating differences in cooked color stability while looking at other factors such as aging, processing techniques, pH, or cookery methods. Even though myoglobin undergoes post-translational changes through cooking to increase thermal stability as described by Salim et al., (2020), it still significantly denatures at each DOD and directly corresponds to the visual changes associated with each DOD. Myoglobin denaturation helps explain most of the color differences between differing DODs, but cooked color stability can help explain further color differences after cooking and slicing.

Metmyoglobin-reducing activity

Metmyoglobin-reducing activity is shown in Table 2.1 and illustrates a component of color stability. While MRA is normally associated with raw meat color stability, it can also impact the amount a cooked sample can bloom after cooking, potentially playing a role in consumer acceptability. The MRA assay was used in the current work to determine the ability of the cooked pigment to bloom after cooking, or as a measure of cooked color stability (Bekhit and Faustman, 2005). MRA decreased ($P < 0.05$) with increasing DODs. The raw treatment resulted in the highest MRA with 3.03 nmol*min^{-1}*g^{-1} of sample. The WD sample resulted in minimal activity. Muscle type also impacted MRA. The BF and GM resulted in a greater ($P < 0.05$) MRA than the LL. While all three muscles are typically considered moderate to stable for color stability of raw product, cooked color stability has not been previously investigated for the same muscles (Suman et al., 2014).

Cooked color stability is imperative to understand as it impacts the last pigment seen before consumption. It explains differences in the internal appearance of a steak a few minutes after slicing compared to the initial appearance. This phenomenon was illustrated by Prill et al., (2019) who took internal color readings at 0 min up to 12 min after slicing. Prill et al., (2019) illustrated the relationship between DOD and the ability of the a^* to recover after cooking. Their research supports the findings of the present study as the MR DOD resulted in a greater a^* change compared to the MED or WD DODs (Prill et al., 2019). The current study found MRA to gradually decline with increasing DOD, but even at the WD DOD, there was a marginal level of MRA remaining. These results indicate the ability for myoglobin to be reduced even after an extensive cooking process, further indicating an intimate link between MRA and DOD up to a certain point. Prill et al., (2019) found all DODs to recover some a^* values, besides the very

well-done DOD, indicating the irreversible damage done to myoglobin is between the WD (77°C) and very well-done (82°C) DODs for whole muscles. This was supported by Moriyama et al., (2010) who determined for cardiac muscle, myoglobin was irreversibly denatured at 75°C. While Prill et al., (2019) did not directly measure color stability, they did measure the visual impact of color stability, further emphasizing the results found in the current study. Our study serves as the first to investigate MRA of cooked whole muscles and may help explain differences in cooked color and the associated color stability of cooked beef within future studies.

While other studies have not looked at the MRA of cooked whole muscles, some studies have determined differences in the thermal stability of cooked proteins, which is intimately linked to color stability (Suman et al., 2014; Suman and Joseph, 2013). Previous differences in the cooked color of ground beef have been associated with the thermal stability of the primary form of myoglobin at the time of cooking (Suman and Joseph, 2013; Hunt et al., 1999). It is understood that the relative ratio of the myoglobin pigments at the time of cooking heavily drives the thermal and color stability of ground beef, and thus, potentially whole muscles (Hunt et al., 1999). This link between the relative ratio of the myoglobin pigments at the time of cooking to the elicited color stability previously evaluated in ground product needs to be similarly established for whole muscles.

The present study found muscle to impact MRA, with the GM and BF having a higher ($P < 0.05$) MRA compared to the LL. Conversely, the raw color stabilities of the three muscles have been determined in previous works, with the LL having the highest color stability in raw product (Suman et al., 2014). Therefore, our study indicates the raw color stability does not completely translate to cooked color stability. This could be due to differences in thermal stability or the relative ratios of myoglobin at the time of cooking, but needs to be investigated further.

Additionally, the relationship between muscles and *L** values align with the MRA data providing some evidence for a further relationship between myoglobin oxidation state and MRA. While the MRA among the different DODs was expected, the difference between muscles was surprising, potentially highlighting the role of intrinsic factors that differ between muscles such as pH, metabolism, moisture content, and oxidation on cooked color and their role in cooked color stability.

Since it is well known that this visual component of the cooked steak is crucial to satisfy the eating experience for the consumer, and the final internal appearance is dictated by color stability, understanding cooked color stability warrants further investigation especially around factors impacting the oxidation state of myoglobin at the time of cooking (Prill et al., 2019; Bekhit and Faustman, 2005). MRA could be used to help explain differences in cooked color stability, especially when evaluating other factors impacting cooked color including the oxidation state of myoglobin, aging method, differing muscles, display period, freezing techniques, and aging periods.

pH

The pH data is shown in Table 2.1. Even though color and pH are related, only muscle impacted pH, with the BF having a higher ($P < 0.05$) pH value compared to the other muscles. Typically, pH and *a** values have a significant correlation in raw meat, but the relationship overall has been shown to be weaker for cooked products (Mahmood et al., 2017). While pH did not align with the *a** differences or myoglobin denaturation in the current study, it did provide some support for the differences found among muscles for the other color attributes tested. Potentially, pH could help explain differences in color stability and cooked *L** values among muscles.

Cooking concentrates solutes in meat, typically increasing the pH (Fletcher et al., 2000), but the impact of cooking on pH has not been widely studied. While the current study aimed to look at the impact of DOD and muscle on pH, other studies have evaluated the cooked color stability differences within set ranges of raw meat pH (Fletcher et al., 2000; Cox et al., 1994). As pH increased, the color stability increased allowing a steak to be overcooked for a specific DOD, but still have the appropriate *a** value (Cox et al., 1994). This relationship with color stability and pH is long-standing for ground beef (Schoenbeck et al., 2000). A higher pH within the raw materials has been linked to persistent pinking and ground beef with a higher ratio of deoxymyoglobin compared to the lower pH groups (Schoenbeck et al., 2000). Our study only found pH differences among muscles, but the observed difference did not elicit any color differences within the *a** values as hypothesized. However, these results did align with the differences found for cooked color stability for the different muscles, as the BF had a higher pH, MRA, and *L** value in comparison to the LL, potentially supporting a theory of pH impacting myoglobin oxidation and therefore color stability.

Cooked lipid oxidation

While typically associated with off odors and flavors of meat, lipid oxidation has a complex relationship with meat flavor (Kerth et al., 2015). Heat is a known oxidizer and contributes positively to the development of flavor (Kerth et al., 2015). Additionally, different muscles can have different levels of lipid oxidation based on fat content and antioxidant capacity (Kerth et al., 2015). Cooked lipid oxidation could positively correlate with flavor development or lead to potential off-flavors such as warmed-over flavor (Kerth et al., 2015). Cooked lipid oxidation is not the most common assay due to the complex relationship with flavor, however, the current study aimed to determine any connection between myoglobin denaturation, color

stability, or the other factors impacting cooked color, including the amount of cooked lipid oxidation. As previously discussed, oxidation of myoglobin can play a role in cooked color, therefore, there could be a connection with lipid oxidation and cooked color as well.

Lipid oxidation is shown in Table 2.3. There was an interaction ($P < 0.05$) for lipid oxidation between muscle and DOD. Within each DOD, there were differences between the three muscles. There were no oxidation differences ($P > 0.05$) within the raw treatment between the three muscles. However, for both the MR and WD treatments, the BF had greater ($P < 0.05$) lipid oxidation compared to the LL. Within the MED DOD, the BF resulted in the greatest ($P < 0.05$) MDA concentration. The WD BF sample resulted in the highest ($P < 0.05$) lipid oxidation amount compared to all other treatments, while the raw LL and raw GM resulted in the lowest ($P < 0.05$) oxidation compared to all treatments besides the raw BF. Overall, within each DOD, the BF resulted in more ($P < 0.05$) lipid oxidation than the LL, while the GM resulted in intermediate oxidation. These results mirror differences found within pH, L^* values, and MRA, highlighting the relationship between pH, lipid oxidation, and cooked color stability.

Proximate analysis and cooking characteristics

Cooked proximate analysis results are shown in Table 2.2. The attributes evaluated for proximate analysis can provide further insight into the physical changes that occur throughout cooking between different DODs. The differences found within the proximate analysis data match previous studies evaluating different DODs (Drey et al., 2019; Lucherk et al., 2016; Yancey et al., 2011; Smith et al., 2011). The elicited impact of heat on meat products occurs through a few basic mechanisms such as structural changes, denaturation, and aggregation of myofibrillar and sarcoplasmic protein and expulsion of water molecules previously bound to the

proteins (Schwartz et al., 2022). Additionally, these factors could help explain differences seen among muscles for the objective color measurements.

Cook loss and moisture content are inherently linked and are a predictor of water-holding capacity and perceived juiciness during sensory analysis (Yancey et al., 2011). While moisture content has been used as an indicator of water-holding capacity, it could have an additional relationship with color attributes such as L^* values (Yancey et al., 2011). As expected, cook loss increased ($P < 0.05$) with each DOD, while the LL had the lowest ($P < 0.05$) cook loss compared to the other muscles. Similarly, moisture content decreased ($P < 0.05$) with each DOD while the BF and GM resulted in a higher ($P < 0.05$) moisture content compared to the LL. As cooking loss increases, inherently, the moisture content and perceived juiciness decreases as shown by our study and others (Schwartz et al., 2022; Drey et al., 2019; Yancey et al., 2011; Smith et al., 2011). However, Drey et al. (2019) did not find the same relationship between cooked moisture content and DOD as found in our study. USDA Select, and Choice graded steaks did not decrease in moisture content with increasing DOD (Drey et al., 2019). However, Drey et al. (2019) study found an interaction between DOD and QG, illustrating the moisture content change was driven more by QG than it was by DOD. However, because only one QG was used in the present study, the moisture content decreased and cook loss increased with each DOD.

Interestingly, moisture content is an intrinsic factor that could help explain the results previously seen among muscles. The BF and GM had a higher moisture content than the LL, following the trend established by the pH, and the objective color attributes. Potentially, a higher pH can lead to more bound water leading to a higher moisture content, and more light reflectance before and after cooking (Dikeman et al., 2013).

On the other hand, the fat content had an inverse relationship to moisture content, as demonstrated by previous studies (Drey et al., 2019; Yancey et al., 2011; Smith et al., 2011). The WD samples had a higher ($P < 0.05$) percentage of fat compared to the raw samples due to the expulsion of moisture during cooking; however, the WD and raw treatments were similar ($P > 0.05$) to MR and MED for fat content. Additionally, the LL and BF had a greater ($P < 0.05$) fat content than the GM. The change in fat content seen in the current study and others is the result of the decrease in moisture content (Drey et al., 2019). During cooking, it is well documented that cook loss and fat percentages increase, while moisture content decreases, with an increase in cook time, temperature, or DOD (Drey et al., 2019; Yancey et al., 2011; Smith et al., 2011).

While powdering cooked steaks is uncommon, it did highlight the differences in proximate analysis between the different DODs, and cooked muscles, serving as a better indicator of the factors potentially impacting cooked color, while helping to highlight known changes in the physical properties of meat.

Objective tenderness

Lastly, tenderness has a well-documented relationship with DOD (Lucherk et al., 2016; Smith et al., 2011; Yancey et al., 2011). In the current study, only the DOD impacted the WBSF values, with MR having the lowest ($P < 0.05$) value, being the most tender, while the MED and WD samples were similar ($P > 0.05$). There were no differences ($P > 0.05$) among muscles. This contradicts previous studies when compared within the same DOD. However, there could be a relationship between muscle and the impact of DOD on and tenderness. For DODs, the current study further supported the findings from previous research (Lucherk et al., 2016; Smith et al., 2011; Yancey et al., 2011). As the end-point temperature increases, WBSF values and subjective tenderness ratings are negatively impacted through protein oxidation, denaturation, and

aggregation (Lucherk et al., 2016; Smith et al., 2011; Yancey et al., 2011). Further, there are many mechanisms that play a role in impacting tenderness changes with varying DODs including the expulsion of moisture, the aggregation of the hydrophobic regions of protein side chains due to denaturation, and the formation of disulfide bonds (Bertola et al., 1994). The loss of water holding capacity as illustrated through the changes of cook loss help explain the decrease in tenderness due to the expulsion of water occurring with increasing DODs. In understanding the mechanism of cooked meat tenderness, there is a potential connection with protein oxidation, aggregation, and cooked meat color.

CONCLUSION

Even though changes in cooked color are widely accepted and expected, few studies have taken a comprehensive look at the factors impacting these changes. A baseline is needed to further investigate the changes that are observed within the cooked color of whole muscles. Overall, the data in the current work supported the hypothesis that an increase in DOD would decrease cook yield, moisture content, a^* values, color stability, and increase perceived toughness, lipid oxidation, and myoglobin denaturation. However, an impact of muscle type on cooked color was not expected. The differences observed between the three muscles outlined the potential relationships between the intrinsic properties of meat and cooked meat color and color stability. Comprehensively, these changes that contribute to the overall visual alterations experienced from cooking a steak are more complex than previously thought.

The primary objectives of this study was to evaluate the factors impacting cooked meat color within three whole muscle cuts. Of these factors, the impact of DODs on myoglobin was of the highest priority. Myoglobin is a complicated molecule that has been shown to undergo post-translational changes in previous research, but the question of how much of myoglobin is

denatured at certain DODs has remained unexplored. As expected, the myoglobin denaturation percentage increased with increasing DOD and behaved similarly to changes in the *a** values and MRA. These changes were accompanied by expected changes in internal color, moisture content, WBSF, lipid oxidation, and cook loss. Additionally, a connection between *L** values, pH, color stability, lipid oxidation, and moisture content emerged through the evaluation of three muscles. This research gives additional insight to the impacts of cooking and the changes that proteins, especially myoglobin, undergo between different DOD. Lastly, it can serve as a baseline for changes in myoglobin denaturation and cooked color for future research evaluating other processing steps such as aging periods, cooking methods, different muscles, or freezing methods and their impacts on cooked color.

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Table 2.1. Cooked color, pH, myoglobin denaturation, and color stability of three degrees of doneness and three muscles

Degree of doneness	*L**[1]	*a**[2]	*b**[3]	pH	Myoglobin denaturation[4], %	MRA[5]
Raw	-	-	-	5.68	-	3.03[a]
Medium rare, 62.8°C	50.04	25.32[a]	20.69[a]	5.82	29.08[c]	2.49[b]
Medium, 71.1°C	50.90	21.90[b]	20.68[a]	5.83	48.34[b]	1.76[c]
Well-done, 76.7°C	50.33	17.68[c]	19.49[b]	5.75	70.17[a]	0.85[d]
SEM[6]	0.36	0.59	0.34	0.06	2.08	0.18
P - Value	0.06	<0.01	<0.01	0.09	<0.01	<0.01
Muscle[6]						
LL	53.03[a]	20.07	20.17	5.74[b]	48.85	1.58[b]
BF	48.21[c]	21.89	20.40	5.87[a]	49.65	2.17[a]
GM	50.03[b]	21.60	20.28	5.71[b]	49.08	2.34[a]
SEM[7]	0.83	0.80	0.40	0.06	3.17	0.18
P - Value	<0.01	0.05	0.84	0.01	0.97	<0.01

[abc] Means within the same column without a common superscript differ ($P < 0.05$).

[1] *L**: 0 = black, 100 = white

[2] *a**: -60 = green, 60 = red

[3] *b**: -60 = blue, 60 = yellow

[4] Myoglobin Denaturation, %=(1-[Raw-Cooked)/Raw]) x 100

[5] Metmyoglobin reducing activity; nmol/min/g of muscle

[6] LL: *Longissimus lumborum*, BF: *Biceps femoris*, GM: *Gluteus medius*

[7] SE (largest) of the least squares means

Table 2.2. Fat, moisture, cook loss, and Warner-Brazler shear force (WBSF) of three degrees of doneness and three muscles

Degree of doneness	Fat,%	Moisture,%	Cook loss[1],%	WBSF, kg[2]
Raw	4.41[b]	72.69[a]	-	-
Medium rare, 62.8°C	4.69[ab]	66.52[b]	14.13[c]	3.39[b]
Medium, 71.1°C	4.97[ab]	64.61[c]	20.00[b]	4.29[a]
Well-done, 76.7°C	5.25[a]	62.93[d]	24.71[a]	4.63[a]
SEM[3]	0.30	0.25	1.46	0.18
P - Value	0.04	< 0.01	< 0.01	< 0.01
Muscle[3]				
LL	5.23[a]	65.88[b]	16.64[b]	4.16
BF	5.32[a]	67.01[a]	21.86[a]	3.95
GM	3.94[b]	67.18[a]	20.34[a]	4.22
SEM[4]	0.53	0.41	1.74	0.22
P - Value	0.02	< 0.01	< 0.01	0.44

[abcd] Means within the same column without a common superscript differ ($P < 0.05$).

[1]Cooking loss = 1 - (cooked weight / raw weight) × 100

[2]Warner-Bratzler shear force; kg.

[3]LL: *Longissimus lumborum*, BF: *Biceps femoris*, GM: *Gluteus medius*

[4]SE (largest) of the least squares means

Table 2.3. Lipid oxidation of three muscles within raw, medium rare, medium, and well-done degrees of doneness

Degree of doneness	Muscle[1]	Lipid oxidation[2]
Raw		
	BF	1.70^{gh}
	GM	1.35^{h}
	LL	1.17^{h}
Medium rare		
	BF	3.42^{ef}
	GM	3.11^{efg}
	LL	2.12^{fgh}
Medium		
	BF	6.87^{b}
	GM	5.11^{cd}
	LL	3.91^{de}
Well-done		
	BF	8.73^{a}
	GM	6.54^{bc}
	LL	4.12^{de}
SEM[3]		0.81
P - Value		< 0.01

^{abcdefgh} Means within the same column without a common superscript differ ($P < 0.05$).
[1]LL: *Longissimus lumborum*, BF: *Biceps femoris*, GM: *Gluteus medius*
[2]Lipid Oxidation; MDA/kg protein
[3]SE (largest) of the least squares means

Chapter 3- Evaluation of fresh and frozen beef strip loins of equal aging periods for palatability traits, and physiochemical properties

INTRODUCTION

Freezing meat has been a long-standing debate with consumers within the retail setting (Lambooji et al. 2019; Buckley et al., 1977). The strong trend of preferring fresh, local, and natural products continues to be a driver for consumers in all commodities, especially in the meat industry. Marketing frozen meat that has been held under -17.8°C must be labeled "previously frozen" (USDA, 2007; Cargill, 2018). In retail, this call-out is commonly believed to be a deterrent for some consumers, affecting their purchasing habits (Pietrasik and Janz, 2009). Therefore, retailers have pushed suppliers to provide options for fresh, never-frozen meat only, limiting cold storage and shelf-life. The perception that meat quality, particularly eating quality, are reduced through freezing started with the consumer; however, this claim has never been supported in scientific literature, leaving a significant gap in understanding (Pietrasik and Janz, 2009).

The largest advantage of freezing meat is the added shelf-life and flexibility of storage and transportation through minimizing bacterial growth and slowing the deterioration of quality (Dang et al., 2021; Lagerstedt et al., 2008). Due to the chemical composition of meat, microbial spoilage is always a potential outcome if meat is held in a fresh state, leaving few options to extend the shelf-life (Dang et al., 2021). Thus, the freezing of meat is not only an option for the meat industry, but a necessity.

Previous freezing research mostly evaluated the impacts of tenderness and ice crystal formations of frozen beef alone (Aroeira et al., 2016 ; Grayson et al., 2014). The impact of ice

crystal formation has been widely studied and accepted, impacting current perceptions of frozen beef quality (Aroeira et al., 2016; Grayson et al., 2014; Martino et al., 1988). It is known that ice crystals form while meat is freezing, and the size, location, and morphology of the ice crystal can play a large role in meat quality (Aroeira et al., 2016 ; Botinestean et al, 2016). Additionally, the freezing temperature, air velocity, and storage time all impact the ice crystal size and morphology and can alter meat quality (Mulot et al., 2019; Do et al., 2004; Martino et al., 1988; Rahelic et al., 1985). As the ice crystals form, they can rupture the muscle cell membrane, potentially leading to the release of prooxidants, metals, and water molecules (Zhang et al., 2023). However, the magnitude of these effects are strongly driven by the size and shape of the ice crystals, therefore, with the current freezing technologies, the impacts of freezing have almost been eliminated.

The effects of freezing on the quality of beef have been studied widely in reference to ice crystal formation, purge loss, tenderness changes, and oxidation; however, no study has directly compared an equally aged fresh product against a frozen product to determine palatability changes. Other studies have primarily focused on comparing storage methods of refrigerated versus freezing conditions with unequal aging periods at refrigerated conditions (Grayson et al., 2014; Hergenreder et al., 2013; Lagerstedt et al., 2008; Farouke et al., 2003; Wheeler et al., 1990). By comparing unequal aging periods, the palatability effects of freezing, especially tenderness, have been inconsistent (Grayson et al., 2014; Hergenreder et al., 2013). While Grayson et al., (2014), and Aroeira et al., (2016) found tenderness to improve after freezing, the magnitude of the impact was drastically different due to inconsistent methods. Even more so, Hergenreder et al., (2013) found the frozen samples to be tougher than the fresh control with a

significantly longer aging period. This gap in research prevents any global statements to be made about the effect of freezing on meat quality or eating quality.

The lack of a comprehensive study evaluating equally aged fresh and frozen steaks prevents a true understanding of the impact of freezing. Since freezing is imperative for the meat industry, this comparison needs to be made. Additionally, while it is widely accepted in the meat industry that consumers deprioritize frozen meat, no study has evaluated such claims (Pietrasik & Janz, 2009). Therefore, the objectives of this study were to determine the eating quality and consumer perception differences between fresh and frozen beef steaks of three equal aging periods and to evaluate consumer perceptions of fresh vs. frozen beef.

MATERIALS AND METHODS

The Kansas State University (**KSU**) Institutional Review Board approved all procedures for use of human subjects in sensory panel evaluations (IRB #7440.8, October 2022).

Sample collection and fabrication

Beef carcasses (N= 72; n = 18/ collection; 6 per aging period) were selected from a Midwestern beef processing plant on two different kill dates 1 week apart. The carcasses all graded USDA Low Choice and were A maturity. The collection team from Kansas State University collected data on ribeye area, fat thickness, percentage of kidney, pelvic, and heart fat, marbling scores, lean maturity, and skeletal maturity (Appendix A). Trimmed strip loins (IMPS # 180, NAMP, 2010) were collected from the right side of the carcasses. The collection was repeated twice for a total of 72 strip loins from two different kill dates as shown in Appendix A. The strip loins were transported to KSU and were processed the day following the last collection. The strip loins were sliced into 12 steaks at a 2.5-cm thickness. Each steak was given

a random four-digit code and assigned to either 21 d, 28 d, or 35 d aged with one of the following designations: trained sensory panels, consumer sensory panels, shear force, or lab assays. All 18 loins from the first kill date represented the frozen samples while the later kill date represented the fresh samples. All steaks were aged to their appropriate aging period at 2-4°C in the absence of light. After aging, the frozen samples were blast frozen at -20°C for 1 week before being placed in a 2-4°C refrigerator to thaw 24 h before the time of use. At the time of thawing, the fresh samples were at the exact same aging period as the frozen samples at the time of freezing. This served as the direct comparison of fresh and frozen beef steaks for palatability traits, and lab assays.

Trained sensory panels

At exactly 21 d, 28 d, and 35 d aged, the steaks were fed in trained sensory panels to eight trained panelists at a time for a total of three-panel sessions per day. Trained descriptive panelists were trained and used anchors similar to previous sensory panels conducted at KSU (Farmer et al., 2022; Beyer et al., 2021; Drey et al., 2019; Olson et al., 2019; Prill et al., 2019; Rice et al., 2019). The steaks were cooked to a final peak end-point temperature of 71°C on clamshell grills (Cuisinart Griddler Deluxe, East Windsor, NJ). The temperature was monitored using a thermometer and the end-point temperature was recorded (Thermapen mk4, Salt Lake City, UT). Upon cooking, each steak was sliced into 1 × 1-cm cubes for serving. A warm-up sample was served before the treatment samples and discussed to prevent panelist drift. Each panelist received two cubes of each sample to eliminate variation within the sample. Additionally, each panelist was given unsalted crackers, water, and apples as palate cleansers. The sensory panelists were asked to evaluate each sample for initial juiciness, sustained juiciness, myofibrillar tenderness, connective tissue amount, overall tenderness, beef flavor

intensity, and off-flavor intensity. These attributes were evaluated on 0-100 line scales with 0 being anchored as extremely dry, tough, or bland, and 100 being extremely juicy, tender, and flavorful with 50 serving as a neutral midpoint. The sensory panel data were collected on electronic tablets (Lenovo TB-8505F, Morrisville, NC) using Qualtrics (Version 2417833; Qualtrics Software, Provo, UT).

Consumer sensory panels

On the same day as the trained descriptive panels, consumers (n = 48 aging/collection, N = 144 total) were fed eight samples, four from each treatment. Consumer sensory panels were conducted and fed similar to previous panels held at KSU (Farmer et al., 2022; Beyer et al., 2021; Drey et al., 2019; Olson et al., 2019; Prill et al., 2019; Rice et al., 2019). Samples were cooked as described above. Briefly, steaks were cooked to an internal peak temperature of 71°C, sliced into 1 × 1-cm cubes, and served to each group of consumers. The consumers were given unsalted crackers, water, and apple juice as palate cleansers. The consumers were asked to evaluate the samples for tenderness, juiciness, flavor liking, and overall liking as well as the indicate if each sensory trait was acceptable (yes/no). The palatability traits were measured on a 0 to 100 line-scale with 0 representing not tender, not juicy, or dislike extremely and 100 representing very tender, very juicy, or like extremely. The consumers were also asked to classify the samples into various perceived quality levels (premium quality, better than everyday quality, everyday quality, and unacceptable). The consumer sensory panel data was collected similar to descriptive panel data, on the same electronic tablets (Lenovo TB-8505F, Morrisville, NC) using Qualtrics (Version 2417833; Qualtrics Software, Provo, UT).

Consumers were given eight total samples either with no information or with the label "Previously Frozen", or "Fresh, Never Frozen". The first four samples were served without any

additional information while the last four samples were given with information about four steaks before consumption. Within the identified samples, one steak from each treatment was labeled correctly, and the other was labeled with the opposite treatment to help understand the consumers' perception of fresh versus frozen beef steaks.

Shear force, cooking characteristics, and internal color

On the same day as the descriptive and consumer sensory panels, a steak from each treatment and from each strip loin was cooked for shear force and prepared for all other lab assays. Before cooking, purge loss was determined. First, the full package was weighed, opened, and the packaging was rinsed with water, and dried with paper towels before being reweighed. The raw sample was weighed for purge loss and cook loss. Next, the steaks were cooked to an end-point temperature of 71°C and immediately prepared and sliced for slice shear force using the protocol of Shackleford et al. (1999). A 2.54-cm wide cut was made parallel to the muscle fibers in in the lateral portion of the steak to measure the slice shear force (SSF) and internal color using a Hunterlab Miniscan Spectrophotometer (Illuminant A, 2.54 cm aperture, 10° observer, Hunter Lab Associates Laboratory, Reston, VA). After a 3-min bloom time measured with a timer, 3 readings were taken in different locations within the internal cut surface, and an average of *L**, *a**, and *b** values were recorded and the relative percentages of metmyoglobin, oxymyoglobin, and deoxymyoglobin were determined using spectral data according to the AMSA Color Guidelines (King et al., 2023).

The samples were sheared for SSF immediately following the color readings and then the remaining steak was cooled for Warner- Bratzler Shear Force using the protocol of the American Meat Science Association (AMSA, 2015). Both measures of tenderness were conducted using an INSTRON Model 5569 testing machine (Instron, Canton, MA). The slice was inserted and

sheared with a flat, blunt-end blade using the Instron testing machine described above with a crosshead speed of 500 mm/minute. The shear force measurement for each sample was recorded as the peak force (kg). Lastly, the steaks for WBSF were cooled for 24 h at 2-4°C before cores were taken. Six cores were taken parallel to the muscle fibers and sheared perpendicular to the muscle fibers using an Instron with a cross-head speed of 250 mm/min and a load cell of 100 kg. An average of all six measurements were used to represent each sample and data were presented as average kg force.

Sample preparation and proximate analysis

The assigned lab assay steak from each treatment was cut into cubes, dipped in liquid nitrogen, and ground into a fine powder using a blender (Waring Products, New Hartford, CT) before being stored at -80°C until use. The fat content was determined using the Folch method (Folch et al., 1957) and the moisture content was determined using the drying oven method (950.46 and 934.01; AOAC, 1995). The additional powdered sample was stored for surface hydrophobicity, lipid oxidation, and metmyoglobin-reducing activity.

Surface hydrophobicity

Surface hydrophobicity is a measure used to determine the amount of hydrophobic groups exposed due to denaturation or damage to the muscle fiber. Surface hydrophobicity was determined using the protocol described by Dominguez-Hernandez et al. (2021). Briefly, 0.3 g of meat powder and 1.5 ml of sodium phosphate buffer was added to a 1.5 ml tube with glass beads. The sample was homogenized for 30 s and centrifuged at 4000 x g for 5 min. The supernatant was discarded and 1 ml of buffer was added and recentrifuged following the parameters above to wash away the remaining sarcoplasmic proteins. Myofibrillar proteins were extracted with SDS

and standardized to 2,000 µg/ml of protein. An aliquot of protein stock was added to 1mg/ml concentration of bromophenol blue and incubated for 10 min in the absence of light. The resulting sample was diluted to a 1/10 ratio with deionized water and added to a 96-well plate in duplicate. The absorbance was taken at 595 nm and was used to calculate the BPB bound/mg of protein.

Metmyoglobin-reducing activity

The metmyoglobin-reducing activity was determined using the protocol from the AMSA Color Guidelines (King et al., 2023) modified to reduce the sample to a 0.3 g sample instead of a 5 g sample. Briefly, 0.3 g of the powdered sample and 1.2 ml sodium phosphate buffer was added and centrifuged 14,000 rpm for 30 min. A 96-well plate was prepared by adding 50 µl of 0.75 mM metmyoglobin, 25 µl of 3.0 mM potassium ferrocyanide, 50 µl of deionized water, 25 µl of 5 mM EDTA, and 25 µl 3 mM sodium citrate buffer. Next, 50 µl of each sample was added in duplicate. After the sample was added, 25 µl of 1 mM NADH was quickly added, and the cells were agitated before being read at 525nm every 60 seconds for 180 seconds. Beer's law was used to calculate the change in absorbance as the change from metmyoglobin to oxymyoglobin.

MRA = change in absorbance*$12{,}000^{-1}$ *path length^{1} minutes^{-1} *g of sample^{-1} *volume of reagents.

Lipid oxidation

Lipid oxidation was determined using the thiobarbituric acid reactive substances (TBARS) assay following the procedures described by Ahn et al., 1998, and similar procedures done at KSU (Dahmer et al., 2022). Briefly, 0.3 g sample of powdered sample was weighed and added to 1.5 ml bead tubes with 1.4 ml of deionized water and 0.1 ml of butylated hydroxy

anisole. The samples were centrifuged at 2,000 × g for 5 min and 0.4 ml of the supernatant was transferred into pre-labeled glass tubes. Next, 0.8 ml of thiobarbituric acid and trichloroacetic acid was added to each tube. After vortexing, the tubes were covered in aluminum foil and incubated in a 70°C water bath for 30 min. Then the samples were cooled and centrifuged at 3,000 × g for 15 min. Lastly, 0.2 ml of the supernatant was pipetted into a 96-well plate in duplicate. The plate was ran at an 532 nm absorbance and a standard curve was used to determine the concentration of MDA.

Statistical analysis

The statistical analysis was conducted using SAS (Version 9.4; SAS Inst., Inc., Cary, NC) PROC GLIMMIX. Carcass served as the experimental unit. Data were analyzed as a 2 × 3 factorial design with the fixed effects of aging period and the fresh vs frozen state. There were no interactions found between the aging periods and the freezing method. Peak temperature was used a covariate when applicable. To determine the impact of "previously frozen", or "fresh, never frozen" labels, all combinations of information and freezing treatments were combined and evaluated as a completely randomized design. An α of 0.05 was set for a level of significance. The Kenward-Roger adjustment was used in all analyses.

RESULTS

Trained sensory evaluation

The sensory evaluation scores from the trained sensory panels are presented in Table 3.3. Trained panelists determined the fresh samples were juicier ($P < 0.05$) within initial and sustained juiciness ratings compared to the frozen samples, while no differences ($P > 0.05$) were found between the different aging periods. The frozen samples were rated as more tender ($P <$

0.05) for overall tenderness in comparison to the fresh samples, while the treatments did not differ ($P > 0.05$) for myofibrillar tenderness. However, the frozen samples showed a marginal increase in myofibrillar tenderness but it was not significant. There was not a difference ($P > 0.05$) for myofibrillar or overall tenderness among the aging periods. However, the frozen samples had a reduced ($P < 0.05$) connective tissue amount rating in comparison to the fresh samples, while the 21 d and 35 d aged samples resulted in lower ($P < 0.05$) connective tissue amount ratings compared to the 28 d samples. Lastly, there were no differences ($P > 0.05$) for beef flavor intensity between the freezing methods or among the aging periods.

Consumer sensory evaluation

Consumer demographic information is displayed in Table 3.1. Both genders were equally represented. The majority of the consumers came from a 1 or 2 person household and were single (59.4%). Most of the participants either fell into the 20-29 year old or over 60 year old categories. A vast majority (83.9%) were Caucasian or of white ethnic origin, but African-American, Asian, Latino, and Native American races were all represented. The majority (50.4%) of the consumers indicated they lived in a household earning $50,000 or more. Most of the consumers used for this study reported flavor to be the most important palatability trait, while tenderness was identified as the most variable by 42.6% of consumers, more than the other traits. The vast majority (78.1%) of consumers preferred a medium or a medium rare degree of doneness, and consumed beef up to 5 d a week (74%). Moreover, purchasing motivators are presented in Table 3.2. Overall, price played a larger ($P < 0.05$) role in purchasing decisions compared to all other options besides meat color. The fresh or frozen claim ranked in the lowest 7 traits for importance to consumers when purchasing meat at retail and was similar ($P > 0.05$) in importance to traits including many animal production claims.

The consumer sensory results found in Table 3.4 are similar to the trends found by the trained descriptive panelists. The consumers found no differences ($P > 0.05$) between the fresh and frozen samples for juiciness, but did rate the frozen samples more tender ($P < 0.05$). However, this tenderness difference did not impact ($P > 0.05$) the overall liking. Unlike the trained sensory results, the consumers found a difference ($P < 0.05$) in juiciness and tenderness for the different aging periods, with the 21 d aged samples having lower ($P < 0.05$)juiciness and tenderness ratings in comparison to the other aging periods. Also, the 28 d aged samples had the highest ($P < 0.05$) flavor and overall liking scores, while the 21 d aged steaks having the lowest ($P < 0.05$) scores for the same traits.

Results for the percentage of samples rated as acceptable for each palatability trait are presented in Table 3.5. A greater ($P < 0.05$) percentage of consumers (89.3%) identified frozen samples as acceptable for tenderness, in comparison to the fresh counterpart (83.9%). No other differences ($P > 0.05$) in the percentage of samples rated acceptable were found between the fresh and frozen samples. Among aging treatments, the 21 d aged samples resulted in the lowest ($P < 0.05$) percentage of samples rated acceptable for juiciness, tenderness, flavor, and overall liking. Additionally, consumers were asked to determine the quality level of each sample (Table 3.6). An equal ($P > 0.05$) percentage of fresh and frozen samples were classified as "unsatisfactory", "everyday quality", "better than everyday quality", and "premium quality". The 21 d aged samples resulted in the highest ($P < 0.05$) percentage of steaks rated as "unsatisfactory quality" and the lowest ($P < 0.05$) percentage of steaks rated as "premium quality".

Following blinded sample evaluation, consumers were given additional samples with information about the preservation method for the final four samples. Two samples were correctly identified, and two samples were identified with the incorrect information. As reported

in Table 3.7, these labels did not impact ($P > 0.05$) the eating quality of any attribute. There were numerical differences found with the frozen sample labeled as "previously frozen" resulting in the highest average rating for juiciness, tenderness, flavor liking, and overall liking; however, these differences were not significant.

Warner-Brazler shear force and cooking characteristics

Objective measures of tenderness, moisture, oxidation, and color were used to further understand the physiochemical changes that occur from the freezing process (Table 3.8). Supporting the sensory data, the frozen samples resulted in a lower ($P < 0.05$) WBSF and SSF value than the fresh samples. Additionally, the 21 d aged samples were the toughest ($P < 0.05$) as shown by the highest WBSF values. The fresh samples had lower ($P < 0.05$) cook loss and purge loss than the frozen samples. The 21 d aged samples had the lowest ($P < 0.05$) cook loss of all aging periods; however, the same trend was not observed for purge loss, as the 35 d aged samples resulted in the least purge loss ($P < 0.05$).

Internal color, color stability, lipid oxidation, and surface hydrophobicity

Internal color, color stability, and oxidation measurements are presented in Table 3.9. The fresh samples had higher ($P < 0.05$) L^* values in comparison to the frozen samples, appearing visually lighter; however, the two treatments did not differ ($P > 0.05$) in a^* values. The relative percentages of MetMb, OMb, and DMb were similar ($P > 0.05$) for both cold storage treatments. However, the frozen steaks had significantly higher ($P < 0.05$) color stability as indicated by a higher MRA. The 21 d aged treatment had the highest ($P < 0.05$) a^* value and percentage of DMb, while having a higher ($P < 0.05$) L^* value compared to the 35 d aged treatment and a lower ($P < 0.05$) MetMb percentage as compared to the 28 d aged treatment.

Lastly, there were no ($P > 0.05$) differences in lipid oxidation and surface hydrophobicity for the fresh vs frozen treatments or among the aging periods.

DISCUSSION

Sensory comparisons of fresh and frozen beef are uncommon, potentially due to the logistical issues of comparing fresh and frozen product of equal aging periods. Other studies have compared the two cold storage methods, but did so by aging the fresh treatment for substantially longer or by including aging periods after freezing (Grayson et al., 2014; Hergenreder et al., 2013). The wide range of methods within freezing studies has made it difficult to determine the true impacts of freezing on palatability and meat quality traits. Grayson et al., (2014) compared seven fresh or frozen treatments ranging from 2 d fresh or frozen up to 28 d fresh or frozen, with two treatments including a 12 or 14 d aging period after freezing. The storage days listed for the frozen treatments were frozen storage times rather than aging periods (Grayson et al., (2014). Similarly, Hergedreder et al., (2013) outlined the objectives of their study were to compare typical processing methods for freezing and thawing and therefore included a 14 d, and 21 d fresh treatments and blast frozen or conventionally frozen treatments with a 3 to 10 d freezing period. Aroeira et al., (2016) compared the impact of freezing on eating quality traits between two breeds and four aging periods for fresh and frozen steaks with equal storage time, not aging periods. While these studies provide insight into the effects of freezing, they do not answer the basic question of determining the impact of freezing on the palatability and physiochemical properties of beef steaks. Therefore, the current study serves as the best comparison to date of fresh vs frozen beef of equal aging periods. With equal aging periods, the

current study found only a few differences within the consumer and trained sensory ratings, specifically in only tenderness and juiciness.

The effects of freezing on meat quality proved to be much less substantial within the current study in comparison to previous works (Kim et al., 2018; Aroeira et al., 2016; Leygonie et al., 2012). While marginal differences were found for juiciness and tenderness within the consumer and trained sensory data, the overall liking ratings were similar for the two treatments. These results were supported by the physiochemical assays evaluated. Additionally, the consumers' ratings were not changed when given information about the cold storage method used within the samples evaluated. This is in direct contradiction to the dogma that consumers deprioritize frozen meat. However, it has been previously noted that this dogma has never been evaluated in scientific literature (Pietrasik and Janz, 2009). The current study can serve as the foundation for the comparison of palatability traits and the consumers' perception of fresh vs frozen beef of equal aging periods.

It is well documented that freezing impacts tenderness in some capacity (Aroeira et al., 2016; Grayson et al., 2014; Lagerstedt et al., 2008). The impact of tenderness occurs through two mechanisms. First, the rupturing of the cell membrane, causing a physical improvement by decreasing the shear force (Rahelic et al., 1985) or from a post-freezing aging period (Aroeira et al., 2016). Our study illustrated freezing improved tenderness with an 18.9% decrease in SSF values and an 8.8% decrease in WBSF values as well as an increase of overall tenderness ratings within both the trained panel and consumer panel ratings by 6.3% and 6.0%, respectively. Other studies have researched the impact of freezing on tenderness with similar results (Aroeira et al., 2016; Grayson et al., 2014; Lagerstedt et al., 2008). Grayson et al., (2014) found a 42.3% decrease in SSF values from a 14 d fresh sample in comparison to a 14 d frozen sample with a 14

d aging period after freezing. However, a post-freezing aging period could also impact tenderness by the deactivation of calpastatin through the freezing process potentially attributing to the greater percentage of change in shear force from the fresh sample (Grayson et al., 2013). Freezing deactivates calpastatin activity, therefore, allowing the calpains to be more active in a post-freezing system (Aroeira et al., 2016; Grayson et al., 2014; Kristenson et al., 2006). In every study that included a comparable aging period to the fresh sample, the frozen treatment resulted in decreased shear force values, just like in this study (Grayson et al., 2014; Aroeira et al., 2016).

While it is mostly accepted that freezing and thawing steaks decreases the shear force values of certain muscles; there have been conflicting reports (Aroeira et al., 2016; Grayson et al., 2014; Lagerstedt et al., 2008; Farouke et al., 2003; Wheeler et al., 1990). Hergenreder et al., (2013) found a 43.5% increase in WBSF values using a blast freezing treatment and a 35.5% increase in WBSF values using a conventional freezing treatment in comparison to a 21 d aged fresh control. Similarly, due to the difference in design described above, Aoreira et al. (2016) found WBSF values to decrease when freezing samples for 0 d and 7 d of storage, but the fresh samples in their study resulted in lower WBSF values for 14 d and 21 d of storage. These discrepancies were most likely the result of unequal refrigerated aging periods due to differences in the project design (Grayson et al., 2013; Grujic et al., 1993). This latter approach makes it challenging to compare the properties changed by freezing, but does represent what is typically found in the industry and retail. Due to the large discrepancies in methods, the impact of freezing even within the published scientific literature remains unclear. Consequently, this wide variety of project designs have made it challenging to fully understand the impact of freezing on meat quality, tenderness especially. However, from the current study, it can be concluded, that within equal aging periods, the tenderness of beef steaks is improved with freezing.

Juiciness was also impacted by freezing within the trained sensory data of the present study, but it was not identified by the consumers. This may indicate the observed difference was too marginal to be detected by an untrained group of panelists. This was supported by Hergedreder et al., (2013) who used consumer panelists to evaluate palatability traits between the treatments described above and found no differences for juiciness. Additionally, the attributes that are correlated to juiciness ratings such as cook loss and purge loss were also increased by freezing. These impacts have been of the highest scrutiny within the industry due to the economic loss related to moisture loss (Kim et al., 2018; Aroeira et al., 2016; Leygonie et al., 2012). However, the increase in purge found in this study was 0.8%, having little to no practical impact. In contrast, Aroeira et al., (2016) found up to a 6.6% increase in purge for a 21 d aged vs frozen steak. While the two studies have differing freezing methods, the purge loss difference is substantial, illustrating the importance of the freezing method. It is also worth noting that in the current study and the Aroeira et al. (2016) study, purge loss was measured on a steak basis rather than a wholesale basis, which would be more common in industry. Moreover, cook loss for frozen samples resulted in around a 3% or less increase compared to the fresh counterpart in this study and other others (Aroeira et al., 2016; Hergedreder et al., 2013). The decrease in water-holding capacity attributes can be tied back to the well-vetted mechanism of ice crystal formation (Kim et al., 2018; Aroeira et al., 2016; Leygonie et al., 2012). The rupturing of the cell membranes has been shown to release water, ions, enzymes, metals, and other molecules typically protected by the cell membrane (Sanchez et al., 2012). This loss of cellular integrity can be improved with improved freezing techniques, as potentially seen in the current study. Similarly, the changes in the water-holding capacity attributes, cook loss, and purge loss, were not severe enough to elicit an impact on the juiciness liking ratings for the consumers.

The physiochemical properties evaluated supported the sensory data, finding few differences between the fresh and frozen samples. Freezing decreased *L** values, but had no impact on *a** values, or the relative percentages of MMb, OMb, or DMb for cooked color. As expected, an increased aging period decreased *L** and *a** values due to the gradual decline in color stability throughout aging (Ramanathan et al., 2020; English et al., 2016). However, cooked color has not been measured between different aging periods, but the same relationship has been found while using raw steaks (English et al., 2016). While cooked color has also not been evaluated within fresh and frozen steaks, larger color differences have been found in previous studies evaluating the raw product (Jeong et al., 2011). These results indicated while raw color might be affected by freezing, it does not translate to changes in cooked color or potentially the thermal stability of myoglobin.

The MRA assay was used in the current work to determine the ability of the pigment to bloom after being oxidized, or as an objective measure of raw color stability (Bekhit and Faustman, 2005). Surprisingly, the frozen samples resulted in a greater MRA than the fresh samples, indicating the frozen sample would have a greater ability to reduce MMb to OMb. This is the opposite of the previous hypothesis from Suman et al., (2014) stating freezing meat decreases color stability. It is documented that freezing typically decreases color stability in a retail display starting at 4 d of the display, but the impact is dependent on the previous aging period (Hergenreder et al., 2013). However, a different methodology was used in our study in comparison to others due to the need to use powdered samples (Nair et al., 2018; Jeong et al, 2011). A potential explanation could be that the inevitable rupturing of the cell membranes through the thawing process could release a surge of enzymes able to perform enzymatic MRA initially, but potentially depleting the longevity of the MRA system. However, this hypothesis

should be further tested in an additional study. Between cooked color and color stability, our results indicate that freezing meat does not negatively impact meat color.

Lipid oxidation was not impacted by freezing or the aging parameters used. Lipid oxidation has produced conflicting results based on the protein source, time of freezing, number of freeze cycles, and the freezing temperature (Dalali et al., 2022; Setyabrata et al., 2019; Rahman et al., 2015). Consistently, multiple freeze-thaw cycles can cause a significant increase of lipid oxidation and deterioration of other quality parameters (Setyabrata et al., 2019; Rahman et al., 2015). The sublimation of the ice crystals can increase the release of prooxidants such as metals and heme proteins by disrupting the cell membrane stability, thereby, increasing the rate of protein and lipid oxidation (Zhang et al., 2023). Protein oxidation, and denaturation have also been tied to freezing if storage or a display period was included after thawing (Bao et al., 202; Xia et al., 2009). Oxidation can increase throughout multiple freeze-thaw cycles leading to changes in flavor, color, and potentially overall acceptability within sensory evaluations (Xia et al., 2009). Regardless, the MDA concentration in our study was far too low to be perceived by trained panelists (Zhang et al., 2019). Therefore, the freezing parameters chosen for the current study could have eliminated enough cellular damage to prevent detectable oxidation.

CONCLUSION

Overall, the impact of freezing on eating quality and physiochemical properties of beef steaks resulted in minimal differences. The consumer panelists found minimal differences between the fresh and frozen treatments. Even though the frozen sample was considered more tender, and the fresh sample was considered juicier, and the overall liking was not impacted. This final assessment of eating quality is the most important. While it was previously believed that the eating quality of frozen beef is lower than its fresh counterpart, especially as viewed by

consumers, the current study fails to support this claim. While some meat quality factors are impacted by freezing, the overall eating quality is not negatively impacted. Similarly, informing consumers of the frozen/fresh state of the product prior to evaluation did not alter their assessment, providing evidence that for consumers, “fresh, never frozen” labeling may not be impactful. Based on this study, the actual eating quality and perception of quality is not impacted by freezing beef steaks of equal aging periods. Therefore, frozen meat should not be discounted due to the eating quality or perception of the quality of beef steaks. This study can provide guidance for the industry to make supported decisions on cold chain management strategies.

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Table 3.1. Demographic characteristics of consumers (N = 144) who participated in consumer sensory panels

Characteristic	Response	Percentage of consumers
Gender	Male	50.3
	Female	49.7
Household Size	1 person	25.8
	2 people	38.7
	3 people	8.4
	4 people	13.5
	5 people	3.9
	6 or more people	5.8
Educational Level	High school graduate	20.6
	Non-high school graduate	3.2
	Some college/technical school	45.2
	College graduate	18.1
Marital Status	Married	50.6
	Single	59.4
Age	Under 20	16.8
	20-29	32.3
	30-39	2.6
	40-49	7.1
	50-59	11.6
	Over 60	29.7
Ethnic Origins	African-American	2.6
	Asian	1.3
	Caucasian/White	83.9
	Latino	1.9
	Mixed Race	4.5
	Native American	2.6
Income	Under $25,000	37.4
	$25,000-$34,999	8.4
	$35,000-$49,999	3.2
	$50,000-$74,999	16.8
	$75,000-$99,999	12.9
	$100,000-$149,999	9.7
	$150,000-$199,999	5.2
	Over $199,999	5.8
Most important palatability trait when consuming beef	Tenderness	32.9
	Juiciness	11.6
	Flavor	54.8
Most variable palatability trait when consuming beef	Tenderness	42.6
	Juiciness	29.0
	Flavor	27.7
Preferred degree of doneness when consuming beef	Very Rare	2.6
	Rare	3.9
	Medium rare	43.9
	Medium	34.2
	Medium well	11.6
	Well-done	3.9
Weekly beef consumption	1 to 5 times	74.0
	6 to 10 times	23.9
	11 or more times	4.5

Table 3.2. Fresh beef steak purchasing motivators of consumers (N = 144) who participated sensory panels

Characteristic	Importance of each trait[1]
Price	73.0[a]
Color	72.6[ab]
USDA Grade	66.3[bc]
Nutrient Content	64.5[cd]
Size, weight, and thickness	64.9[cd]
Marbling	59.9[de]
Familiarity with cut	58.1[e]
Animal Welfare	56.6[ef]
Eating satisfaction claims	56.6[e]
Antibiotic use in animals	50.1[fg]
Fresh or frozen claim	49.4[g]
Growth hormone used in animals	49.4[g]
Animals fed a grass-based diet	48.2[gh]
Organic claim	44.8[ghi]
Animals fed a grain-based diet	42.7[hi]
Brand of product	40.2[i]
Packaging	39.9[i]
SEM[2]	3.2
P - value	< 0.01

[abcdefghi] Means within the same column without a common superscript differ ($P < 0.05$).

[1]Purchasing motivators: 0 = extremely unimportant, 100 = extremely important.

[2]SE (largest) of the least square means.

Table 3.3. Least squares means (n = 12/ age/ treatment) of trained sensory panelist palatability ratings[1] for fresh and frozen beef steaks of three aging periods.

Treatment	Initial juiciness	Sustained juiciness	Myofibrillar tenderness	Connective tissue mount	Overall tenderness	Beef flavor intensity
Freezing						
Frozen	60.7[b]	54.5[b]	70.6	4.0[b]	69.3[a]	33.4
Fresh	64.0[a]	58.5[a]	67.3	5.4[a]	65.2[b]	33.7
SEM[2]	1.42	1.60	1.70	0.45	1.75	0.65
P - value	0.02	0.01	0.06	< 0.01	0.02	0.64
Aging						
21 d	61.6	55.8	67.2	4.2[b]	66.2	34.1
28 d	62.1	56.3	68.5	5.7[a]	65.5	32.4
35 d	63.3	57.4	71.2	4.3[b]	69.9	34.2
SEM[2]	1.74	1.91	2.05	0.55	2.15	0.79
P - value	0.63	0.67	0.15	< 0.01	0.10	0.06

[abc] Means within the same section of the same column without a common superscript differ ($P < 0.05$).

[1]Sensory scores: 0 = extremely dry/tough/bland/none, 50 = neither juicy/dry/tough/tender, 100 = extremely juicy/tender/abundant/intense.

[2]SE (largest) of the least square means.

Table 3.4. Least squares means (n = 12/ age/ treatment) of consumer sensory panelist palatability ratings[1] for fresh and frozen beef steaks of three aging periods.

Treatment	Juiciness	Tenderness	Flavor liking	Overall liking
Freezing				
Frozen	64.5	65.1[a]	62.7	63.8
Fresh	66.0	61.4[b]	61.7	61.6
SEM[2]	1.46	1.65	1.42	0.45
P - value	0.30	0.03	0.47	0.16
Aging				
21 d	61.6[b]	58.0[b]	55.3[c]	56.5[c]
28 d	68.3[a]	67.6[a]	68.6[a]	68.4[a]
35 d	66.0[a]	64.0[a]	62.8[b]	63.2[b]
SEM[2]	1.82	2.05	2.05	1.95
P - value	< 0.01	< 0.01	< 0.01	< 0.01

[abc] Means within the same section of the same column without a common superscript differ ($P < 0.05$).

[1]Sensory scores: 0 = extremely dry/tough/dislike; 50 = neither dry nor juicy, neither tough nor tender, neither like nor dislike; 100 = extremely juicy/tender/like extremely.

[2]SE (largest) of the least square means.

Table 3.5. Least squares means (n = 12/ age/ treatment) of the percentage of consumers who rated each palatability trait as acceptable

Treatment	Juiciness acc.	Tenderness acc.	Flavor acc.	Overall liking acc.
Freezing				
Frozen	86.6	89.3[a]	84.0	83.5
Fresh	87.4	83.9[b]	84.5	86.9
SEM[1]	0.18	0.19	0.17	0.17
P - value	0.70	0.01	0.85	0.13
Aging				
21 d	81.1[b]	78.1[b]	76.1[c]	76.8[b]
28 d	91.2[a]	88.4[a]	89.7[a]	89.4[a]
35 d	87.0[a]	91.3[a]	84.6[b]	87.4[a]
SEM[1]	0.22	0.25	0.22	0.23
P - value	< 0.01	< 0.01	< 0.01	< 0.01

[abc] Means within the same section of the same column without a common superscript differ ($P < 0.05$)

[1]SE (largest) of the least squares means

Table 3.6. Least squares means (n = 12/ age/ treatment) of the percentage of consumers who rated samples as each of the quality levels

Treatment	Unsatisfactory	Everyday quality	Better than everyday quality	Premium quality
Freezing				
Frozen	11.4	50.5	26.7	8.6
Fresh	14.2	53.5	23.7	5.5
SEM[1]	0.19	0.12	0.14	0.28
P-value	0.18	0.31	0.24	0.09
Aging				
21 d	22.1[a]	54.7	20.3[b]	2.6[c]
28 d	9.6[b]	48.4	26.9[a]	14.9[a]
35 d	9.4[b]	52.9	28.9[a]	8.2[b]
SEM[1]	0.25	0.15	0.17	0.39
P-value	< 0.01	0.21	0.02	< 0.01

[ab] Means within the same section of the same column without a common superscript differ ($P < 0.05$)

[1]SE (largest) of the least squares means

Table 3.7. Least square means (n = 12/ age/ treatment) of consumer sensory panelist palatability ratings[1] for fresh and frozen beef steaks of three aging periods when given no information, true information or false information about the cold storage method.

Treatment-Information	Juiciness	Tenderness	Flavor liking	Overall liking
Fresh[2]				
None[3]	65.6	60.8	59.7	60.3
Fresh[3]	66.4	61.2	65.0	63.6
Frozen[3]	66.6	62.6	62.4	62.3
Frozen[2]				
None[3]	63.1	65.3	60.9	62.3
Fresh[3]	64.4	64.2	63.0	63.5
Frozen[3]	67.4	65.6	66.2	66.6
SEM[4]	2.95	3.40	2.81	3.16
P-value	0.54	0.38	0.08	0.34

[abc] Means within the same section of the same column without a common superscript differ ($P < 0.05$).

[1]Sensory scores: 0 = extremely dry/tough/dislike; 50 = neither dry nor juicy, neither tough nor tender, neither like nor dislike; 100 = extremely juicy/tender/like extremely.

[2] Actual product type

[3] Information given to consumers

[4]SE (largest) of the least square means.

Table 3.8. Least square means of Warner-Brazler shear force (WBSF), slice shear force (SSF), cook loss, purge loss and surface hydrophobicity for fresh and frozen beef steaks of three aging periods.

Treatment	WBSF, kg	SSF, kg	Cook loss, %	Purge loss, %	Surface hydrophobicity[1]
Frozen	3.1[b]	15.4[b]	17.1[a]	1.6[a]	4.6
Fresh	3.4[a]	19.0[a]	14.9[b]	0.8[b]	4.6
SEM[2]	0.14	0.86	0.45	0.12	0.78
P - value	< 0.01	< 0.01	<0.01	< 0.01	0.97
21 d	3.6[a]	18.9[a]	14.7[b]	1.3[a]	5.1
28 d	3.2[b]	17.0[ab]	16.4[a]	1.3[a]	4.7
35 d	3.0[b]	15.6[b]	16.9[a]	1.0[b]	4.0
SEM[2]	0.17	1.05	0.55	0.14	0.97
P – value	< 0.01	< 0.01	< 0.01	0.03	0.51

[abc] Means within the same section of the same column without a common superscript differ ($P < 0.05$).

[1]Surface Hydrophobicity: µg BPB/ mg protein

[2]SE (largest) of the least square means.

Table 3.9. Least square means of *CIE L*,a**, MRA, and relative percentages of oxymyoglobin (OMb), deoxymyoglobin (DMb), metmyoglobin (MetMb), and metmyoglobin-reducing activity (MRA) for bloomed internal color of fresh and frozen steaks of three aging periods.

Treatment	*L**[1]	*a**[2]	MetMb, %	OMb, %	DMb, %	MRA[3]	Lipid oxidation[4]
Freezing							
Frozen	56.8[b]	20.6	33.3	60.7	6.0	3.67[a]	0.66
Fresh	59.2[a]	20.2	33.1	61.3	5.6	3.08[b]	0.62
SEM[5]	0.57	0.73	1.29	1.37	0.91	0.27	0.06
P - value	< 0.01	0.62	0.89	0.66	0.63	0.03	0.56
Aging							
21 d	59.0[a]	22.7[a]	31.0[b]	61.0	8.0[a]	3.64	0.67
28 d	58.2[ab]	18.7[b]	35.3[a]	61.0	3.8[b]	3.21	0.68
35 d	56.8[b]	19.7[b]	33.3[ab]	61.0	5.6[b]	3.26	0.58
SEM[5]	0.70	0.89	1.58	1.68	1.11	0.33	0.07
P – value	< 0.01	< 0.01	0.03	0.99	< 0.01	0.37	0.30

[abc] Means within the same section of the same column without a common superscript differ ($P < 0.05$).

[1]*L*:* 0 = black, 100 = white

[2]*a*:* -60 = green, 60 = red

[3]MRA: Metmyoglobin-reducing activity; nmol/min/g

[4]Lipid Oxidation: MDA/mg meat tissue

[5]SE (largest) of the least square means.

Appendix A-Supplemental Data

Table 4.1- Carcass characteristics of both studies

Trait[1]	Study 1[2]	Study 2[3]
HCW	940.9	980.8
PYG	2.8	3.1
APYG	2.9	3.2
KPH[4]	3.5	3.5
REA	16.9	15.1

[1] HCW: Hot carcass weight; PYG: Preliminary yield grade; APYG: Adjusted preliminary yield grade; KPH: Kidney, pelvic, and heart fat; REA: Ribeye area
[2] USDA Select carcasses (Slight 00-90); A-maturity
[3] USDA Choice carcasses (Small 20-90); A-maturity
[4] KPH was standard for all carcasses

Table 4.2- Collection information to compare fresh and frozen beef steaks of equal aging periods

Collection day[1]	Freeze- 21 d	Evaluation- 21 d	Freeze- 28 d	Evaluation- 28 d	Freeze- 35 d	Evaluation- 28 d
August 23[2]	Sept. 13	Sept. 20	Sept. 20	Sept. 27	Sept. 27	Oct. 4
August 30[3]	NA	Sept. 20	NA	Sept. 27	NA	Oct. 4

[1] Collection at Nebraska Beef, Omaha, NE
[2] All samples used for frozen treatment
[3] All samples used for fresh treatment

Appendix B - Sensory panel evaluation forms

INFORMED CONSENT STATEMENT

1. I volunteer to participate in research involving Sensory Evaluation of Meat. This research will be conducted by personnel in the Department of Animal Sciences and Industry at Kansas State University.

2. I fully understand the purpose of the research is for the evaluation of beef steaks, pork chops, lamb chops, goat meat, poultry meat, ground meat, and processed meat products from the previously mentioned species for the sensory traits of tenderness, juiciness, flavor intensity, connective tissue amount, off flavor presence, odor, and color and sensory evaluation will last approximately one hour.

3. I understand that there are minimal risks associated with participating and that those risks are related to possible food allergies. All meat products will be USDA inspected and all ingredients are GRAS (generally accepted as safe) by FDA.

4. I understand that my performance as an individual will be treated as research data and will in no way be associated with me for other than identification purposes, thereby assuring confidentiality of my performance and responses.

5. My participation in this study is purely voluntary; I understand that my refusal to participate will involve no penalty or loss of benefits to which I am otherwise entitled and that I may discontinue participation at any time without penalty or loss of benefits to which I am otherwise entitled.

6. If I have any questions concerning my rights as a research subject, injuries or emergencies resulting from my participation, I understand that I can contact the Committee on Research Involving Human Subjects, 203 Fairchild Hall, Kansas State University, Manhattan, KS 66506, at (785) 532-3224.

7. If I have questions about the rationale or method of the study, I understand that I may contact, Dr. Travis O'Quinn, 247 Weber Hall, Kansas State University, Manhattan, KS 66506, at (785) 532-3469 or Sally Stroda, 107 Weber Hall, at 785-532-1273.

I have read the Subject Orientation and Test Procedure statement and signed this informed consent statement, this _____ day of _____, _____.

_____ _____

Printed name Signature

Please sign and return one copy. The second copy is for your records.

Consumer Ballot Samples 1-4

Sample Number

1156

Juiciness

Extremely Dry
0

Neither Juicy nor Dry
50

Extremely Juicy
100

Juiciness

Was the sample acceptable for juiciness?

Acceptable

Unacceptable

Tenderness

Extremely Tough
0

Neither Tough nor Tender
50

Extremely Tender
100

Tenderness

Was the sample acceptable for tenderness?

Acceptable

Unacceptable

Flavor

Dislike Extremely
0

Neither Like nor Dislike
50

Like Extremely
100

Flavor

Flavor

Dislike Extremely
0

Neither Like nor Dislike
50

Like Extremely
100

Flavor

Was the sample acceptable for flavor?

Acceptable

Unacceptable

Overall Liking

Dislike Extremely
0

Neither Like nor Dislike
50

Like Extremely
100

Overall

Was the sample acceptable overall?

Acceptable

Unacceptable

Please choose one of the following to rate the quality of the beef sample you have eaten.

Unsatisfactory

Everyday Quality

Better than everyday quality

Premium Quality

Consumer Ballot Samples 5-8

Sample Number- Previously Frozen

8519

Juiciness

Extremely Dry	Neither Juicy nor Dry	Extremely Juicy
0	50	100

Juiciness

Was the sample acceptable for juiciness?

- Acceptable
- Unacceptable

Tenderness

Extremely Tough	Neither Tough nor Tender	Extremely Tender
0	50	100

Tenderness

Was the sample acceptable for tenderness?

- Acceptable
- Unacceptable

Flavor

Dislike Extremely	Neither Like nor Dislike	Like Extremely
0	50	100

Flavor

Flavor

Dislike Extremely
0

Neither Like nor Dislike
50

Like Extremely
100

Flavor

Was the sample acceptable for flavor?

Acceptable

Unacceptable

Overall Liking

Dislike Extremely
0

Neither Like nor Dislike
50

Like Extremely
100

Overall

Was the sample acceptable overall?

Acceptable

Unacceptable

Please choose one of the following to rate the quality of the beef sample you have eaten.

Unsatisfactory

Everyday Quality

Better than everyday quality

Premium Quality

Trained Panelist Ballot

Panelist

Sample Number

Initial Juiciness

Extremely Dry
0

Neither Dry nor Juicy
50

Extremely Juicy
100

Sustained Juiciness

Extremely Dry
0

Neither Dry nor Juicy
50

Extremely Juicy
100

Myofibrillar Tenderness

Extremely Tough
0

Neither Tough nor Tender
50

Extremely Tender
100

Connective Tissue Amount

None
0

Extremely Abundant
100

Overall Tenderness

Extremely Tough
0

Neither Tough nor Tender
50

Extremely Tender
100

Beef Flavor Intensity

Extremely Bland
0

Extremely Intense
100

Off Flavor Intensity

Extremely Bland
0

Extremely Intense
100

☐ Not Applicable

Off-Flavor Description

→