DISSERTATION

PROCESSES TO IMPROVE STORAGE SHELF-LIFE AND PALATABILITY OF BEEF

Submitted by

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ABSTRACT

PROCESSES TO IMPROVE STORAGE SHELF-LIFE AND PALATABILITY OF BEEF

Three studies were conducted to evaluate processes to improve the storage shelf-life and palatability of beef. The first two studies evaluated the effects on retail shelf-life and palatability characteristics of beef following Suspended Fresh® storage. Suspended Fresh® (SF) is a patented, proprietary, trademarked process that allows the storage of beef muscles at temperatures at or slightly above their freezing point to slow down microbiological spoilage while maintaining the product's fresh status. These studies evaluated the impact of 60, 75, or 90 d of storage in SF (-2.7±0.3°C) on the retail shelf-life and palatability characteristics of steaks derived from inside rounds (IR), bone-in ribeyes (RE), and striploins (SL) from 10 (n=10) upper two-thirds Choice beef carcasses. Two steaks fabricated from each subprimal were vacuum-packaged, wet-aged for 21 d (3°C), and frozen (-20°C) for Warner-Bratzler shear force (WBSF) and sensory analyses. These steaks served as the control with regard to storage condition and time. The remainder of each subprimal was fabricated into three portions, and after vacuum packaging, were randomly allocated to an SF storage time of 60, 75, or 90 d. After each storage time, five steaks were fabricated from the subprimal pieces, overwrapped, and placed in a retail display case (3°C) under continuous fluorescent light for 7 d. Another two steaks were vacuum-packaged and stored at -20°C until WBSF and consumer sensory evaluations. Consumers (N=238) evaluated each sample for juiciness, tenderness, flavor liking, and overall liking. Instrumental and trained visual color were evaluated daily during retail display, and aerobic bacterial populations (APC), lactic acid bacteria, and *Pseudomonas* spp. were enumerated on days 0, 2, 4, and 7. Data were analyzed in R using a factorial design for the microbial counts or a split-plot for the rest of the analyses. Least-squares means were separated using a significance level of $\alpha=0.05$. For all cuts, initial redness (a^* values) of SF60 steaks were lower ($P < 0.05$) than SF75 and SF90 steaks. In general, irrespective of SF storage time or retail display day, trained panelists did not detect differences in lean color and discoloration of steaks. For all cuts, the APC of SF60 steaks on days 0, 2, and 4 of retail display were lower ($P < 0.05$) than those of SF75 and SF90 samples. The WBSF values decreased ($P < 0.05$) with increased storage time for all the cuts. Similarly, the consumer tenderness rating scores of IR and SL generally increased with the SF storage time. However, storage time did not influence ($P \geq 0.05$) the juiciness, flavor, and overall liking of any cuts. The results of this study suggest it would be feasible to extend the storage time of beef while preserving or improving the sensory quality when held at optimal conditions above the freezing temperature.

The third study was conducted to evaluate the effects of different temperature and time treatment combinations (1A: 56.1°C and 71 min; 1B: 56.1°C and 150 min; 1C: 56.1°C and 240 min; 2A: 61.7°C and 8 min; 2B: 61.7°C and 150 min; 2C: 61.7°C and 240 min) of sous vide cooking on the palatability of beef *biceps femoris*. Beef *biceps femoris* were sliced into 1.6-cm steaks, vacuum packaged as 4.5 kg bags, and randomly assigned to one of the six treatments with 16 packages (n=16) per treatment. Cooked and chilled packages were weighed, and then the weight of the meat was taken to measure cooking loss. Weighed samples were divided into two halves: one was left non-marinated, and the other was assigned to marination. Two 1.6-cm non-marinated steaks were randomly selected and cut in half to measure the internal cooked color. Additionally, non-marinated and marinated steaks were randomly selected for WBSF and sensory analysis by a trained panel. Data were analyzed using a complete randomized design in R with a significance level of $\alpha=0.05$. The cooking loss of samples increased as the temperature and dwell time combinations increased ($P < 0.05$). Internal redness of steaks decreased ($P < 0.05$) with increased temperature and dwell time. The only major difference in WBSF and the trained sensory panel results was between treatment 1C (56.1°C and 240 min) and 2A (61.7°C and 8 min), where 1C samples had lower WBSF values and higher perceived tenderness scores than 2A samples. These results suggest that *biceps femoris* samples can be cooked at conditions examined in this study with minimal impact on palatability, allowing producers more flexibility with cooking time to optimize production time and energy while reducing cooking loss.

Overall, the findings of these studies should be useful to the beef industry as they consider strategies for improving the storage shelf-life and palatability of beef.

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CHAPTER 1: LITERATURE REVIEW

1.1 Introduction

With a fast-growing world population and an increasing food demand, food systems must transform to ensure food security while complying with environmental, economic, and social objectives to meet global sustainability goals (Godfray et al., 2010; FAO, 2018). One way to meet this goal is by extending the shelf-life of fresh meat without any adverse effect on quality attributes. This is critically important for beef-producing and exporting countries such as the United States as it not only reduces the risk of product spoilage and waste but also opens up opportunities for stabilizing supply and facilitating export shipments. With beef production, demand, and price seasonality, stabilizing the supply can allow producers to expand their market reach and improve overall profitability (Lusk et al., 2001; Ardeshiri et al., 2019; USDA-ERS, 2022). Newer approaches such as storing fresh beef below typical chilling temperatures (2 to 4°C) have shown promising results in extending its shelf-life (Small et al., 2012; Chen et al., 2019; Lu et al., 2019; Chen et al., 2020; Yang et al., 2022; Zhang et al., 2023). However, extending the shelf-life of fresh beef can influence several quality characteristics impacting the final retail shelf-life, visual attributes, and palatability of products.

The palatability of beef is essential for consumer eating satisfaction, and it depends on three major components: tenderness, juiciness, and flavor (Smith and Carpenter, 1974). Different beef muscles will differ in their tenderness (Belew et al., 2003; Seggern et al., 2005). For example, muscles such as tenderloin are considered tender, whereas relatively bigger muscles, such as those coming from the round, tend to be tougher and are considered lower-quality cuts. Strategies to improve the tenderness and palatability of beef include extended storage/aging and employing appropriate cooking methods. Tougher and lower-value meat cuts could be transformed into tender cuts through low-temperature long-time cooking methods (Rowe and Kerth, 2013). However, different meat quality results can be achieved depending on the meat cut and the cooking parameters. By effectively improving the shelf-life and palatability of beef, producers and processors can increase their profitability while meeting the growing demand and expectations of consumers for high-quality beef and eating satisfaction.

1.2 Factors affecting the shelf-life of beef

Meat is highly perishable because it contains around 70% water (Arshad, 2018). Fresh meat shelf-life is mainly related to the product processing conditions, which will determine initial microbial load, as well as packaging type and storage temperature (Nethra et al., 2023). Food safety is the principal concern in the meat industry, followed by product quality, and standard practices to reduce the presence of pathogenic bacteria in meat have also been successful at improving its shelf-life. Once produced, the storage conditions, such as packaging type and temperature, will majorly affect beef shelf-life.

1.2.1 Microbial contamination and growth

Beef provides a suitable environment for the growth of microorganisms as it has high water activity, neutral-acidic pH (average 5.4), available protein, and lipids. The interior of healthy muscles is considered sterile, and bacteria are introduced to the meat primarily from the gut during evisceration and the hide through processing and handling. Therefore, initial microbial loads will depend on processing hygiene. Meat is generally considered spoiled when the microbial population reaches 7-8 logs (Vieira et al., 2009).

The shelf-life of beef is determined by the concentration and types of microorganisms, mainly bacteria, initially present and their subsequent growth (Borch et al., 1996). *Pseudomonas* spp. and lactic acid bacteria are the predominant spoilage bacteria associated with fresh red meat stored aerobically or anaerobically, respectively (Borch et al., 1996; Stellato et al., 2016). *Pseudomonas* spp. are gram-negative, psychrotrophic organisms that grow well in high water activity and aerobically stored food, like meat (Dainty and Mackey, 1992; Borch et al., 1996). Meat spoilage by *Pseudomonas* spp. is characterized by adverse effects, such as off-odor, slime formation, and potential greening of meat surfaces (Jackson et al., 1997). Lactic acid bacteria (LAB) are gram-positive bacteria with tolerance to low pH and do not require oxygen for growth (Egan, 1983; Wang et al., 2021). Meat spoilage caused by LAB is characterized by strong off-odors, such as "chemical" and "sour" (Egan, 1983).

1.2.2 Lipid oxidation

Lipid oxidation is a critical factor in meat deterioration from non-microbial sources (Mansour, 2020). Beef contains a considerable amount of lipids, which are susceptible to oxidation, especially when exposed to oxygen, light, and heat (Domínguez et al., 2019). Lipid oxidation in beef refers to the chemical reaction of unsaturated fatty acids with oxygen (Domínguez et al., 2019), resulting in the degradation of lipids and the formation of rancid off-flavors, off-odors, and undesirable changes in meat color (Ross and Smith, 2006; Barden and Decker, 2016). There are intrinsic (meat composition) and extrinsic (light and oxygen exposure) factors that can promote lipid oxidation in beef. As mentioned before, light exposure can induce lipid oxidation and is the fastest source of the oxidative process (Lorenzo et al., 2017). Usually, beef is exposed to light and oxygen at retail to be attractive to the consumer; however, these storage conditions increase oxidative processes (Alirezalu et al., 2019; Domínguez et al., 2019).

1.2.3 Storage conditions

Storage conditions, such as packaging type, packaging atmosphere, and temperature, are extrinsic factors that also influence the shelf-life of meat. Packaging is essential in maintaining fresh quality by protecting the product from external contamination and inhibiting or delaying microbial growth and lipid oxidation (Perna, 2016; Lee, 2018). Different packaging types provide different environments for microbial populations and the different environments will also produce various redox forms of myoglobin, the primary protein responsible for meat color. For example, consumers often expect a bright cherry red color when purchasing fresh beef. For that, myoglobin needs to be oxygenated. Oxygen permeable packaging, or overwrap packaging, is the most common type of packaging in retail establishments since consumers are familiar with it. This type of packaging, however, offers a short shelf-life since oxygen exposure increases the growth rate of aerobic and facultative anaerobic spoilage microflora, and also increases lipid oxidation.

Meat is commonly preserved by storing it at refrigeration temperatures to delay microbial growth and maintain its quality attributes. However, temperature fluctuations during the storage of beef can result in spoilage prior to the expected shelf-life (Nastasijević et al., 2017). The optimum temperature growth range of most spoilage bacteria goes from 12 to 30°C (Nethra et al., 2023). Therefore, typical chilled storage temperatures of meat, which range from 2 to 4°C, slows down bacterial growth and lipid oxidation, improving shelf-life to a few days or weeks depending on the packaging type. For longer storage, freezing is often done, lowering the temperature to -18 to -20°C to stop microbial and enzymatic activity due to the lower temperature and unavailability of water (Nethra et al., 2023).

1.3 Strategies to extend the storage shelf-life of beef

As mentioned in the previous section, storage conditions such as packaging type and temperature play a significant role in preserving meat. Temperatures lower than regular chilling (2-4°C) and different types of packaging can maintain meat quality attributes while slowing down microbial growth and lipid oxidation. Other technologies, such as high-pressure processing and curing, are also used to extend the storage shelf-life of meat, but these can cause major changes in quality attributes, such as color, and therefore, will not be further discussed here.

1.3.1 Temperature control

As previously mentioned, chilled storage (2-4°C) is commonly used to preserve the quality of fresh beef; however, microbial spoilage can limit the storage time (Hopkins and Thompson, 2002; Colle et al., 2015; Coombs et al., 2017). While freezing is an effective method to extend the storage time of beef products, it may result in undesirable changes in beef quality, such as decreased water-holding capacity and color stability (Coombs et al., 2017). Recent studies have demonstrated that use of temperatures lower than regular chilling (i.e., <2-4°C) but above freezing (>-3°C) can significantly extend the storage shelf-life of fresh vacuum-packaged beef to up to 20 weeks, compared to an average of 8-10 weeks in conventional vacuum chilled storage (Small et al., 2012; Chen et al., 2019; Chen et al., 2020). A recently patented, proprietary, and trademarked process called Suspended Fresh® allows the storage of boxed beef subprimals in a controlled environment at temperatures below conventional chilling but above or at their freezing point without ice crystal formation (Lobaugh, 2021). The use of temperatures below typical chilling can slow the growth rate of spoilage microflora and, therefore, extend the storage shelf-life of beef products while maintaining their fresh status.

1.3.2 Vacuum packaging

Vacuum packaging is widely utilized in the food industry to extend the shelf-life of perishable products (Domínguez et al., 2021). Creating an oxygen-free environment around the product effectively inhibits the growth of aerobic bacteria and prevents oxidation (Domínguez et al., 2021). While vacuum packaging is not a novel technology, its utilization in the meat industry is primarily limited to transportation and wholesale cuts (Lee, 2018). Oxygen-permeable packaging continues to dominate retail meat cases (Mancini and Hunt, 2005; McMillin, 2017; Lee, 2018). This preference can be attributed to the fact that when beef lacks oxygen, myoglobin remains in the deoxymyoglobin redox form, resulting in a purple color instead of the bright cherry red that consumers are looking for because it is associated with freshness (Tomasevic et al., 2021; Ramanathan et al., 2022). However, once the beef is removed from the vacuum packaging and exposed to oxygen, myoglobin will oxygenate, and the typical red color of fresh beef will be revealed.

1.3.3 Modified atmosphere packaging

Modified atmosphere packaging (MAP) is a packaging technique that alters the gaseous atmosphere within a food package. It involves using packaging materials with appropriate gas barrier properties to maintain the modified atmosphere throughout the expected shelf-life of the product (Church and Parsons, 1995; Perna, 2016). Vacuum packaging, discussed earlier, can be considered a type of MAP since it modifies the atmosphere by removing all air from the package. However, MAP typically involves introducing new gases such as nitrogen, carbon dioxide, or carbon monoxide into the package to replace the original atmosphere (Domínguez et al., 2021).

In the meat industry, MAP is employed in various ways using different combinations of gases. One popular approach is utilizing an approved carbon monoxide concentration of less than 0.4% along with other filling gases to create low-oxygen packaging. This method ensures a bright cherry red color in beef because carbon monoxide binds with myoglobin, forming a stable red pigment called carboxymyoglobin that retains its color for extended periods compared to oxymyoglobin (McMillin, 2017). However, this type of packaging requires specialized equipment and expensive elemental gases and poses logistical challenges due to increased transportation and display space. Furthermore, it may mask the visual indicators of microbial spoilage due to enhanced color stability.

1.4 Effects of extended storage on beef characteristics

Extended vacuum-packaged storage can significantly impact various quality characteristics of beef. As storage time increases, specific changes in color stability, microbial loads, tenderness, water-holding capacity, and flavor may occur. Understanding these effects is crucial for maximizing the quality of vacuum-sealed beef during extended storage periods.

1.4.1 Color

Meat color plays a crucial role in consumer purchase decisions, as a bright cherry red color is associated with freshness and wholesomeness (Tomasevic et al., 2021, Ramanathan et al., 2022). Deviations from this desired color result in beef being initially discounted and eventually discarded if not sold, which generates food waste and economic losses for the meat industry (Mancini and Hunt, 2005; Ramanathan et al., 2022; King et al., 2023). The color of fresh meat is primarily determined by the redox state of myoglobin, which exists in three primary forms: oxymyoglobin (bright cherry red), deoxymyoglobin (purplish), and metmyoglobin (brown color). Of these, metmyoglobin is the oxidized form of myoglobin, and the brown color is recognized as discoloration. There are endogenous systems in meat that will reduce the state of myoglobin once it has been oxidized. However, these systems are depleted over time, leading to the permanent formation of metmyoglobin and meat discoloration (Ramanathan et al., 2019).

Mitochondria in postmortem muscle remain functional, although their functionality decreases as postmortem time increases (Tang et al., 2005). During postmortem storage, mitochondria continue to utilize oxygen, so mitochondria compete with myoglobin for oxygen when available (Mancini and Ramanathan, 2014). The functionality of mitochondria is crucial for color stability through mitochondria-mediated metmyoglobin-reducing activity (MRA), which is the ability of the muscle to reduce metmyoglobin. Enzymatic processes in the mitochondria produce reducing equivalents (such as NADH) and electrons that can reduce metmyoglobin, contributing to color stability (Echevarne et al., 1990; Joseph et al., 2012). As long as the mitochondria remain functional, the production of reducing equivalents and electrons continues, impacting the color stability of meat during postmortem storage. Therefore, as postmortem age increases, the decrease in mitochondria functionality will minimize competition for oxygen, which consequently can improve myoglobin oxygenation and initial color; however, color stability typically decreases (Mancini and Ramanathan, 2014; Suman et al., 2014; Nair et al., 2018; Ramanathan and Mancini, 2018)

1.4.2 Microbial loads

As the microbial shelf-life of beef is influenced by the initial number and type of microorganisms and their subsequent growth (Borch et al., 1996), extended storage can result in a product with an undesirably high microbial load. It is well documented that at refrigerated conditions, as the storage time of beef increases, microbial loads increase due to growth of coldtolerant spoilage microflora (Hernández-Macedo et al., 2011; Small et al., 2012; Colle et al., 2015; Luzardo et al., 2016; Chen et al., 2019; Lu et al., 2019; Chen et al., 2020). Even when extrinsic factors such as temperature (refrigeration) and environment (vacuum packaging) manage to slow down the growth rate of spoilage microflora, it cannot be totally halted; therefore, initial contamination levels play a key role. Lu et al. (2019) investigated the effect of extended vacuum storage of beef striploin steaks at different storage temperatures (2°C, -4°C, and -18°C) on total aerobic counts. After 4 weeks of storage of steaks that had an initial total aerobic count of 3.3 log CFU/g, counts of samples from all storage temperatures were different, with those samples stored at 2°C having the highest counts, followed by those stored at -4°C and lastly -18°C (Lu et al., 2019). While counts of samples stored at 2°C and -4°C increased after 4 weeks of storage, those at -18°C remained similar to the initial counts (Lu et al., 2019). Following 8 weeks of storage, counts of samples held at 2°C were close to spoilage levels, while those stored below 0°C were lower than 4 log CFU/g (Lu et al., 2019). By the end of the evaluated storage duration (24 weeks), counts of samples stored at -18°C were similar to the initial levels, and counts of samples stored at -4°C stayed below 5 log CFU/g (Lu et al., 2019). Similarly, Colle et al. (2015) analyzed aerobic plate counts of striploin steaks stored at 0°C and reported an increase in counts of steaks as aging time (2, 14, 21, 42, and 63 d) increased.

1.4.3 Tenderness

Tenderness can increase with storage time due to proteolysis of proteins (Koohmaraie, 1992; Lana and Zolla, 2016). Endogenous proteolytic enzymes break down muscle fibers, increasing meat tenderness (Casas et al., 2006). The extent and rate of proteolysis are influenced by intrinsic (e.g., cattle breed and muscle type) and extrinsic (e.g., electrical stimulation, storage time, and temperature) factors (Casas et al., 2006; Bhat et al., 2018, Nair et al., 2019). However, the endogenous proteolytic enzymatic systems do most of their activity in the first 10 days of storage (Nishimura et al., 1998). The benefit of tenderness from extended storage, is primarily attributed to the structural weakening of connective tissue, improving tenderness further, especially in those “lower quality” muscles with more connective tissue content (Nishimura et al., 1998). It is well-reported that extended storage time will improve the tenderness of beef (Gruber et al., 2006; Dixon et al., 2012; Colle et al., 2015; Karney et al., 2022). Nevertheless, storage temperature is a critical extrinsic factor in the rate of tenderization improvement since the proteolytic enzyme activity will cease if the temperature is below freezing and work effectively at higher temperatures (Thomson et al., 2008; Bhat et al., 2018). The previously described Lu et al. (2019) study also assessed quality attributes of the beef striploins, specifically objective tenderness, after extended storage at -4°C (0 to 24 weeks) and 2°C (0 to 8 weeks). The researchers reported that the tenderness of striploin steaks improved after 12 weeks of storage at -4°C compared to those stored for 4 weeks at -4°C (Lu et al., 2019). The authors further reported tenderness improvements in steaks after 8 weeks of chilled storage (2°C) compared to those after 4 weeks of storage at the same temperature (Lu et al., 2019). However, the tenderness of samples after 4 weeks of chilled storage (2°C) was higher than those held for 12 weeks at -4°C.

1.4.4 Water holding capacity

Water holding capacity (WHC) can be defined as the ability of meat and meat products to hold water during processing, storage, and cooking (Hamm, 1986; Pearce et al., 2011). Water released from meat can be described as drip, purge (raw meats), or cook loss (during cooking), and are negatively correlated to WHC (Warner, 2017). As stated before, proteolysis of protein occurs during storage, and this process leads to protein fragmentation and the formation of channels within the muscle fiber structures which can decrease WHC during storage by creating pathways that facilitate the movement of water out of the muscle (Purslow et al., 2016). This can result in a loss of moisture and increased purge loss, negatively impacting cooking loss and the juiciness of meat. However, factors such as storage temperature can influence the purge loss of meat, where meat stored at temperatures below regular chilling but above freezing can have lower purge loss (Warner, 2017).

1.4.5 Flavor

Beef flavor results from the combination of basic tastes (sweet, sour, bitter, salt, umami) and aroma generated during cooking and is influenced by many antemortem (cattle breed, diet, and age) and postmortem (aging process and time, specific muscle, packaging type, cooking method, and seasonings) factors (MacLeod, 1994; Calkins, 2006; Li et al., 2021; Miller et al., 2022). During extended storage of beef, the major impacts on flavor can be derived from storage conditions and time. Increased storage time of beef has been shown to increase lipid oxidation and bacterial population levels, which can contribute to the development and intensity of off-flavors (Stetzer et al., 2008; Hernández-Macedo et al., 2012; Watanabe et al., 2015; Li et al., 2021). Different storage methods of beef, such as wet (using vacuum packaging) or dry aging, will result in the development of distinct volatiles and tastes of cooked beef (Li et al., 2021; Lee et al., 2021; Bischof et al., 2022). In general, the most common practice for extended storage of beef is vacuum packaging due to logistics and yield performance (DeGeer et al., 2009; Li et al., 2014). As postmortem storage time increases, the increase of bacterial fermentation products under vacuum could negatively affect the flavor of beef by intensification of sour notes (Warren and Kastner, 1992; Hernández-Macedo et al., 2012; Shi et al., 2020; Da Silva Bernardo et al., 2021; Li et al., 2021). However, lipid oxidation, a source of rancid flavor, is restrained during vacuum storage due to the absence of oxygen (Kim et al., 2019). Foraker et al. (2020) evaluated sensory attributes, using trained panels for striploins aged (3, 14, 28, 35, 49, and 63 d) in chilled storage (2°C) and reported a shift in flavor between 35 d and 49 d of storage time that was characterized by more intense sour notes. In agreement, Garmyn et al. (2020) evaluated consumer flavor and overall liking of striploins aged in chilled storage (2°C) every 7 d from 21 to 84 d and reported a significant decrease in both attributes as postmortem aging time increased. In contrast, Colle et al. (2015) assessed consumer flavor and overall liking of striploins steaks aged (2, 14, 21, 42, and 63 d) at 0°C, and reported no differences in either attribute across the different aging periods. The different findings between these two studies might be due to the lower temperature of storage (0°C) used by Colle et al. (2015). Juarez et al. (2010) demonstrated that storage temperature could affect the off-flavor intensity, with striploins and inside rounds steaks stored at 5°C having a greater off-flavor than steaks stored at 1°C.

1.5 Factors influencing the palatability of beef

Palatability refers to the overall sensory characteristics that contribute to the eating experience of beef. The palatability of beef depends on three major components: tenderness, juiciness, and flavor (Smith and Carpenter, 1974), and it is a crucial factor in determining consumer satisfaction, preferences, and repurchase decisions.

1.5.1 Tenderness

Tenderness refers to the ease with which muscle fibers can be chewed and broken down in the mouth (Miller et al., 2022). Tenderness has been widely recognized as a major determinant of overall eating satisfaction and consumer acceptability of beef (Miller et al., 2001; Shackelford et al., 2001; Miller, 2020). It is influenced by factors such as the cut of meat, age of the animal and breed, postmortem storage time and temperature, marbling (intramuscular fat), cooking method, and degree of doneness.

1.5.2 Flavor

The flavor of beef is a combination of taste and aroma. Several studies have demonstrated that flavor is of great importance when tenderness is acceptable (Goodson et al., 2002; Killinger et al., 2004; Behrends et al., 2005), and recent studies have indicated that it could have greater influence than tenderness on overall consumer eating satisfaction of beef (O'Quinn et al., 2018; Felderhoff et al., 2020; Miller, 2020). Beef flavor is influenced by many factors, such as cattle breed, diet, marbling grade, aging process and time, packaging type, cooking method, and any added seasonings or marinades (Miller et al., 2022). Also, beef flavor is highly subjective to individual preferences and varies across countries more than preferences for beef based on tenderness (Miller, 2020). Differences in flavor preference are most likely influenced by the flavor derived from locally produced and consumed beef (Miller, 2020).

1.5.3 Juiciness

Juiciness refers to the sensation produced by meats with higher levels of juices and is closely associated with consumer preference (Maughan et al., 2012). Consumers' perceptions of juiciness and tenderness are positively correlated and, thus, contribute to the overall sensory experience (Miller, 2020). Juiciness can be influenced by factors such as the level of marbling, cooking method, and degree of doneness.

1.5.4 Marbling

Marbling in beef refers to intramuscular fat, which is the fat that is dispersed within the lean muscle tissue (Morales et al., 2013). The presence and distribution of intramuscular fat can impact the palatability of beef, and it is often associated with increased tenderness, juiciness, flavor intensity, and buttery flavor leading to a satisfactory eating experience. In fact, it is so important that the U.S. Department of Agriculture (USDA) quality grade estimates the palatability of carcasses based on the marbling score and animal maturity. The marbling score represents the amount of intramuscular fat found between the 12th and 13th rib; from the highest to the lowest, the USDA quality grades are Prime, Choice, Select, Standard, Commercial, Utility, Cutter, and Canner (AMS, 2018). A study that reviewed the influence of different attributes on consumers beef eating satisfaction reported that 91%, 87%, 83%, and 75% of consumers that eat Prime, top Choice, low Choice, and Select steaks have a positive eating experience, respectively (O'Quinn et al., 2018).

1.6 Strategies to enhance the palatability of beef

Enhancing the palatability of beef is a key objective for producers, processors, and even chefs seeking to provide a positive eating experience. As mentioned before, the palatability of beef comprises factors such as tenderness, flavor, and juiciness. To achieve this, several strategies can be employed. One can be the selection of cuts of known tenderness and marbling. However, aging techniques, including dry and wet aging, can further improve tenderness and produce the precursor of favorable beef flavor. The appropriate cooking method per muscle type is also important to maximize the palatability of a specific beef cut. When utilized effectively, these strategies can improve consumer eating satisfaction.

1.6.1 Aging

Aging refers to the postmortem storage of meat at refrigeration temperatures, typically between 2 and 4°C, for an average of 14 days. Aging of beef is an effective way to improve the tenderness and other palatability characteristics of beef (Bhat et al., 2018). As referred to in the extended storage changes of beef (section 1.4.3), the tenderization during aging is primarily from endogenous proteolytic enzyme activity. The extent of tenderness improvement and development of favorable flavors of beef after aging depends on the aging type, aging time, and intrinsic factors such as breed, sex, and muscle type (Campbell et al., 2001; Dashdorj et al., 2015). There are two standard aging methods, wet and dry, and the benefits in palatability will depend on which one is implemented (Bischof et al., 2022). Both types of aging will improve tenderness and flavor since the taste of cooked non-aged beef has been described as "metallic" (Epley, 1992). The products from protein degradation during aging include free amino acids, which along with the reducing sugars, are reactants for the Maillard reaction in cooked beef, producing positive color, flavors, and aroma (Martins et al., 2000; Koutsidis et al., 2008). However, wet-aged (vacuum-packaged) beef could develop negative taste attributes such as "bloody" and "acidic" (Warren and Kastner, 1992). Dry aging is the aging of beef in an aging cabinet with constant refrigerated temperature and controlled relative humidity with no protective packaging, thus allowing an aerobic environment (Savell 2008; Bischof et al., 2022). This type of aging is mostly associated with the favorable development of beef flavors, such as "meaty," "earthy," and "brown-roasted" (Warren and Kastner, 1992). Still, it can also be associated with bitterness due to lipid-oxidized volatiles (Dashdorj et al., 2015). Dry-aged beef is dominated by aerobic microorganisms like bacteria, yeasts, and molds (Bischof et al., 2022), which may influence the flavor (Oh et al., 2019).

1.6.2 Cooking method

The appropriate cooking method for the selected meat cut is crucial since each cut has intrinsic characteristics in terms of marbling, connective tissue content, and tenderness (Ramsey, 1984; Rowe and Kerth, 2013). Furthermore, cooking methods influence the quality characteristics of meat, such as palatability and yield, by causing physical and biochemical changes in protein, carbohydrates, and lipids (Lee et al., 2014). There are two fundamental cooking methods for meat; (i) dry heat, such as grilling, pan-searing, hot air in an oven, and (ii) moist heat, where the meat is in high humidity or in a liquid in a closed container, like stewing, boiling, smoking (Ramsey, 1984; Rowe and Kerth, 2013). Moist heat cooking is mainly used when the meat cuts are not naturally tender and have more connective tissue that can benefit from breaking down during prolonged heating (Rowe and Kerth, 2013; Dominguez-Hernandez et al., 2018). A moist heat method called sous vide, which consists of vacuum packaging the raw meat in a heat-stable package and immersing it in a water bath under controlled temperature and time (Schellekens, 1996), has gained interest in the food service industry (Dominguez-Hernandez et al., 2018; Bhat et al., 2020). This cooking method poses different benefits from the quality and shelf-life standpoint of meat, which will be further discussed in section 1.7.

1.7 Effects of sous vide cooking on beef characteristics

Sous vide cooking is a remarkable technique that can provide consistency in doneness, improve tenderness, reduce cooking loss, and limit the risks of microbial contamination by controlled cooking temperature and time under an anaerobic environment (Baldwin, 2012a; Ismail et al., 2022; Thathsarani et al., 2022). However, the different characteristics of meat, such as microbial contamination, cooked color, cooking loss, and tenderness, following sous vide cooking can be influenced by the temperature and time used.

1.7.1 Microbial loads

Sous vide cooking is an optimal option for cooked meat products to enhance the shelf-life by reducing the microbial load (Baldwin, 2012a; Bıyıklı et al., 2020; Yang et al., 2020; Haghighi et al., 2021; Ismail et al., 2022; Thathsarani et al., 2022). However, when low cooking temperatures are used, longer dwell times are needed to achieve the lethality of certain microorganisms. In the U.S., the Food Safety and Inspection Service (FSIS) provides cooking guidelines for the safe production of ready-to-eat meat and poultry products; these guidelines are known as Appendix A (FSIS, 2021). For final internal temperatures lower than 70°C, additional dwell time is needed, and this dwell time increases with the cooking temperature reduction (FSIS, 2021). For instance, sous vide processing is commonly performed with temperatures as low as 55°C. At this temperature, dwell time must be at least 89 min (almost 1.5 h), with cooking time being the time it takes the product to hit 55°C of internal temperature plus the dwell time. As the cooking temperature increases, dwell time decreases. For example, with a cooking temperature of 60°C, the dwell time is 12 min (FSIS, 2021). Therefore, it is crucial to consider the total cooking time when using temperatures below 70°C to ensure safety and benefit from shelf-life extension when using sous vide.

1.7.2 Cooked meat color

The internal color of cooked beef indicates its degree of doneness. The degree of doneness depends on the final or highest internal temperature reached for the cooked meat (Baldwin, 2012; Ismail et al., 2022). As previously mentioned, myoglobin is the main protein responsible for meat color. Although distinct from fresh meat, the brown color formed in cooked meat results from myoglobin denaturation. Denaturation of myoglobin begins at around 55°C and 65°C, and most denaturation happens at 75°C (Varnam and Sutherland, 1995; Hunt et al., 1999). The three forms of myoglobin, briefly explained in section 1.4.1, differ in their sensitivity to thermal denaturation (King and Whyte, 2006). Deoxymyoglobin is the least sensitive to heat, followed by oxymyoglobin and metmyoglobin (Van Laack et al., 1996; Hunt et al., 1999). As myoglobin denatures, the different redox forms denature to a final brown color known as ferrihemochrome, the characteristic color of cooked meats (Varnam and Sutherland 1995). Therefore, higher temperatures will produce faster denaturation and extend the brown color of cooked meats.

1.7.3 Cooking loss

When cooking meat, the water-holding capacity of proteins decreases due to protein degradation that results in water being expelled from the muscle (Lepetit et al., 2000; Kondjoyan et al., 2013). Slow-cooking conditions, such as sous vide, can minimize fluid loss, as meat is cooked in moist conditions (James and Yang, 2012; Roldán et al., 2014). Still, as the temperature and cooking time increase, the cooking loss percentage also increases (Vaudagna et al., 2002; García-Segovia et al., 2007; Christensen et al., 2011; Roldán et al., 2013; Ismail et al., 2019; Naqvi et al., 2021c; Naqvi et al., 2021b). For example, Niqvi et al. (2021c) evaluated the effects of sous vide cooking temperature (55°C, 65°C or 75°C) and time (1 h, 8 h, or 18 h) on the cooking loss of beef *biceps femoris* and reported that as cooking temperature and time increased, cooking loss increased. More specifically, the lowest cooking loss percentages were for samples cooked at 55°C for 1 h, and the highest cooking loss percentages were those from samples cooked at 75°C for 8 and 18 h (Naqvi et al., 2021c).

1.7.4 Tenderness

One of the major advantages of sous vide cooking of meats is to improve low-value tougher cuts by increasing their tenderness (Alahakoon et al., 2018; Ismail et al., 2019). In sous vide cooking, the improvement in the tenderness of the meat is primarily due to the impact of temperature and time on connective tissue within the muscle (Purslow, 2018). Prolonged heating causes the breakdown of connective tissue as a result of protein denaturation or collagen solubilization (Dominguez-Hernandez et al., 2018). Several studies have found an improvement in beef tenderness when cooking time increases at the same cooking temperature (Alahakoon et al., 2018; Bhat et al., 2020; Naqvi et al., 2021a; Karki et al., 2022). Niqvi et al. (2021c) evaluated the effects of sous vide cooking temperature (55°C, 65°C or 75°C) and time (1 h, 8 h, or 18 h) on the tenderness of beef *biceps femoris* and *semitendinosus* from carcasses of older animals (30 to 42 months) and reported that as cooking time increased within the same temperature, tenderness increased. Specifically, at 55°C, *biceps femoris* samples cooked for 18 h were more tender than those cooked for 1 and 8 h (Naqvi et al., 2021c). For the 65°C cooking temperature, *biceps femoris* samples cooked for 18 h were the most tender, followed by those cooked for 8 h, which were more tender than those cooked for 1 h (Naqvi et al., 2021c). Additionally, *biceps femoris* samples cooked at 55°C or 65°C for 1 h had similar tenderness; however, those cooked for 8 h at 65°C were more tender than those samples cooked at 55°C for the same period (Naqvi et al., 2021c). Higher temperatures during prolonged cooking time increase collagen solubility, decreasing the connective tissue strength (Laakkonen et al., 1970; Christensen et al., 2011; Naqvi et al., 2021c). Thus, the cooking temperature and time for a specific beef product need to be investigated to get an optimal combination.

1.8 Final remarks

Beef production, demand, and price are seasonal (Lusk et al., 2001; Ardeshiri et al., 2019; USDA-ERS, 2022). By extending the storage period of fresh beef, meat processors can better manage fluctuations in demand and supply, ensuring a steady availability of beef in the market. Recent studies have shown that storage of fresh beef at temperatures lower than the usual chilling range of 2 to 4°C can extend the shelf-life of beef (Small et al., 2012; Chen et al., 2019; Lu et al., 2019; Chen et al., 2020; Yang et al., 2022; Zhang et al., 2023). Nonetheless, as discussed in section

1.4, extending the storage of fresh beef can impact various quality aspects, including the final retail shelf-life, visual characteristics, and overall palatability of the products.

The palatability of beef relies on three key factors: tenderness, juiciness, and flavor (Smith and Carpenter, 1974) with tenderness being the major determinant of overall eating satisfaction and consumer acceptability of beef (Miller et al., 2001; Shackelford et al., 2001; Miller, 2020). Variations in tenderness can be observed among different muscles of beef (Belew et al., 2003; Seggern et al., 2005). Improving the tenderness and overall palatability of beef can be achieved through extended storage/aging and the use of low-temperature, long-duration cooking methods as discussed in sections 1.4 and 1.7. Enhancing the shelf-life and palatability of beef in a successful manner allows processors to satisfy the rising consumer demand and expectations for high-quality beef. Therefore, research on strategies to extend the storage time and improve the palatability of beef is essential to the meat industry. Accordingly, the primary goal of this dissertation is to evaluate processes that could improve storage shelf-life and palatability of beef.

CHAPTER 2: EVALUATION OF BEEF RETAIL SHELF-LIFE FOLLOWING EXTENDED SUSPENDED FRESH® STORAGE

2.1 Summary

The storage of meat at temperatures below regular chilling and above freezing can extend the storage shelf-life of fresh beef; however, its retail shelf-life after extended storage has not been thoroughly investigated. Suspended Fresh® (SF) is a patented process where meat is stored at temperatures at or slightly above their freezing point to increase the storage shelf-life while maintaining the product's fresh status. This study evaluated the retail shelf-life of steaks derived from 10 paired (n = 10) upper two-thirds Choice beef inside rounds (IR), bone-in ribeyes (RE), and striploins (SL) that had been stored in SF (-2.7±0.3°C) for different periods of time. The subprimals were fabricated into three portions, and after vacuum packaging, were randomly allocated to an SF storage time of 60, 75, or 90 d. After each storage time, subprimal portions were fabricated into steaks, overwrapped, and placed in a retail display case (3°C) for 7 d. Steaks were evaluated daily for instrumental and visual color and microbial levels (aerobic plate counts [APC], lactic acid bacteria counts, and *Pseudomonas* spp. counts) on days 0, 2, 4, and 7. For all cuts, initial redness (a^* values) of SF60 steaks were lower ($P < 0.05$) than SF75 and SF90 steaks. In general, irrespective of SF storage time or retail display day, trained panelists did not detect differences in lean color and discoloration of steaks. For all cuts, APC of SF60 steaks on days 0, 2, and 4 of retail display were lower ($P < 0.05$) than those of SF75 and SF90 samples. Samples from SF60 presented a longer microbial retail shelf-life than those from SF75 and SF90, due to lower initial microbial loads following SF storage. However, the retail shelf-life of samples from SF75 and SF90 was

similar. This study suggested the possibility of extending the storage life of fresh beef by holding it just above freezing temperature.

2.2 Introduction

As the demand for fresh beef continues to grow worldwide, the extension of shelf-life has become very important for beef-producing and exporting countries such as the United States (Gonzalez et al., 2022). Although chilled storage (2-4°C) is commonly used to preserve the quality of fresh beef, microbial spoilage can limit the storage time (Hopkins and Thompson, 2002; Colle et al., 2015; Coombs et al., 2017). While freezing is an effective method to extend the storage time of beef products, it may result in undesirable changes, such as decreased water-holding capacity and color stability (Coombs et al., 2017). However, recent studies have demonstrated that using temperatures lower than regular chilling (i.e., <2-4°C) but above freezing (>-3°C) can significantly extend the storage shelf-life of fresh beef to up to 20 weeks, compared to an average of 8-10 weeks in conventional chilled storage (Small et al., 2012; Chen et al., 2019; Chen et al., 2020). These findings suggest that the use of lower storage temperatures could be a promising approach for maintaining the quality of fresh beef during extended storage and transportation. Suspended Fresh® (SF) is a patented, proprietary, and trademarked process that stores boxed beef subprimals in a controlled environment at temperatures below conventional chilling but above or at their freezing point without ice crystal formation (Lobaugh, 2021). The use of temperatures below typical chilling can slow down the growth rate of spoilage microflora and, therefore, extend the storage shelf-life of beef products while maintaining their fresh status.

Meat color is a crucial quality attribute that heavily influences consumers' purchase decisions at the retail level (Tomasevic et al., 2021, Ramanathan et al., 2022). A bright cherry red color in beef is preferred by consumers as it is associated with freshness and wholesomeness.

Deviations from this desired color result in beef products at retail being initially discounted and eventually discarded if not sold, which generates food waste and economic losses for the meat industry (Mancini and Hunt, 2005; Ramanathan et al., 2022; King et al., 2023). External factors such as storage time and temperature can affect meat color and the growth rate of spoilage bacteria. A recent study evaluating the retail color of steaks fabricated from beef striploins after extended vacuum-packaged storage at -1°C reported that the steaks from striploins stored for 12 weeks had a retail shelf-life of at least 5 days, whereas it was shorter (3 days) after 16 to 20 weeks of storage (Zhang et al., 2023). However, the color stability and microbial shelf life of beef subprimal cuts after SF storage, which uses a slightly lower temperature (-2°C) has not been investigated. Therefore, the objective of this study was to evaluate the color and microbial shelf-life during retail display of beef inside round (IR), bone-in ribeye (RE), and striploin (SL) cuts following 60, 75, and 90 d of SF storage.

2.3 Materials and Methods

2.3.1 Sample collection and preparation

Beef subprimals (IR, RE, and SL) were collected from 10 (n = 10) paired upper two-thirds Choice beef carcasses in a commercial beef processing facility. The subprimals were transported under refrigeration to the Department of Animal Sciences Global Food Innovation Center (GFIC) at Colorado State University (Fort Collins, CO). Upon arrival, each subprimal was portioned, individually vacuum-packaged, and randomly assigned to an SF storage period (60, 75, or 90 d; -2.7±0.3°C). The packaged portions were then separated by cut and SF storage time, boxed, and transported overnight under refrigeration to a certified SF storage facility. Following each storage period, the products were shipped overnight to the GFIC. Immediately after arrival, each vacuum-packaged cut was weighed and then aseptically removed from its packaging and placed on a sanitized tray to obtain the weight of the meat without the packaging and any product purge. Five 1.27-cm steaks were cut from each portion and were placed on individual white Styrofoam trays lined with absorbent pads and were overwrapped with oxygen-permeable polyvinyl chloride (PVC) packaging film (O_2 transmission = 23,250 mL x m^2 x d^{-1}, 72 gauge; Resinite Packaging Films, Borden, Inc., North Andover, MA). Overwrapped steaks were placed in a commercial multi-deck retail display case (Hussman Model No. M3X8GEP) under continuous, cool-white fluorescent lighting (2,200 to 2,500 lx) at a temperature of 3°C (±1°C) for 7 d (the first day of retail display was designated as d-0). Each sample was identified with a random four-digit number for visual and instrumental color evaluation. Trays were rotated within the display case once a day to account for light intensity and temperature variation within the display case.

2.3.2 Purge loss

Purge loss for each subprimal portion after SF storage was determined by taking the weight of the vacuum-packed subprimal cut portion (total weight), the weight of the meat without packaging (meat weight), and the weight of the dry package. Purge loss (PL) was expressed as a percentage relative to total weight using the following formula: %PL = [(total weight-(meat weight+package weight))/(total weight)]×100

2.3.3 Color evaluations

Instrumental lean color measurements were obtained with a portable HunterLab MiniScan LabScan EZ4500 colorimeter (Hunter Associates Laboratory, Reston, VA) equipped with a 6 mm measurement port (2.54-cm diameter aperture, illuminant A, and 10° standard observer). The instrument was standardized before each use, using standard tiles covered with overwrap film. Color measurements (6 technical replicate readings), CIE L^* (lightness), a^* (redness), and b^*

(yellowness) were obtained once every day from the same set of steaks, which included 10 per cut within each SF storage time. The color measurements of each steak were taken at random locations for the duration of retail display, through the overwrap film. Averages of the L^*, a^*, and b^* values were used for statistical analysis. In addition, a minimum of six trained panelists evaluated the percent of metmyoglobin formation (lean discoloration) and lean color daily using a continuous 8-point scale; where 1 = extremely bright cherry-red, 2 = bright cherry-red, 3 = moderately bright cherry-red, 4 = slightly bright cherry-red, 5 = slightly dark cherry-red, 6 = moderately dark-red, 7 = dark red, 8 = extremely dark-red. Panelists were selected and trained following the American Meat Science Association Meat Color Measurement Guidelines (King, 2023). Ratings of individual panelists were collected using Qualtrics software and averaged to obtain a single panel rating for each sample and visual attribute.

2.3.4 Microbiological analysis

On days 0, 2, 4, and 7 of retail display, 10 randomly selected IR, RE, and SL steaks were analyzed for bacterial population levels. A 4×4 cm^2 sample was aseptically excised from the center of each steak using a disposable scalpel. The excised samples were placed into a Whirl-Pak filter bag (710 mL; Nasco, Modesto, CA) with 50 mL of maximum recovery diluent (MRD; Acumedia-Neogen, Lansing, MI) and then mechanically pummeled for 2 min (Masticator, IUL Instruments, Barcelona, Spain). Samples were serially diluted in MRD, and aliquots of appropriate dilutions were surface-plated, in duplicate, onto Tryptic Soy Agar (TSA; Acumedia-Neogen) to enumerate aerobic bacterial populations (aerobic plate counts; APC), and on *Pseudomonas* Agar Base with *Pseudomonas* CFC Selective Agar Supplement (PSA; Oxoid Ltd., Basingstoke, UK) to obtain *Pseudomonas* spp. counts. Samples were also analyzed for lactic acid bacteria (LAB) counts using the pour plate method with an overlay. Specifically, 1 mL of appropriate sample dilutions were mixed in 10 mL of molten (<45°C) Lactobacilli MRS Agar (Becton, Dickinson and Company [BD], Sparks, MD); this was done in duplicate. After the agar had set, a 10-mL overlay of molten Lactobacilli MRS Agar was added to each plate to generate an anaerobic environment. All plates were incubated at 25°C, and colonies were counted after 72 ± 1 h (PSA plates), or 72 to 96 h ± 1 h (TSA and Lactobacilli MRS plates) of incubation.

2.3.5 Statistical analysis

All statistical analyses were conducted using R statistical software version 4.0.3 (R Core Team, 2020). A split-plot design was used to evaluate the effects of SF storage time and retail display days on instrumental and visual color, and SF storage time on purge loss within each subprimal. Data analysis was performed using the *lme4* package (Bates et al., 2015) as a mixed model, where SF storage time (SF60, SF75, and SF90), retail display day, and their interactions were fixed effects, and the individual carcass was set as a random effect. For the microbiological analysis, the experiment was designed as a 3 (SF storage times) × 4 (sampling times) factorial for each subprimal cut and bacterial count type (APC, LAB count, *Pseudomonas* spp. count). Bacterial populations were expressed as least squares means for log CFU/cm^2 under the assumption of a log-normal distribution of plate counts. All least-square means were calculated using the *emmeans* package (Length, 2020). The differences between least-square means are reported using a significance level of $\alpha = 0.05$ with Tukey's multiple comparison adjustment.

2.4 Results

2.4.1 Purge loss

The percentage of PL by cut and SF storage time is shown in Figure 2.1. Differences ($P < 0.05$) in PL with duration of SF storage were only found in IR, where the PL of SF60 samples

(4.9%) was lower than that of SF90 samples (8.8%). For RE and SL, even when the SF storage time was not significant ($P \geq 0.05$), a numerical increase in purge loss was observed as SF storage time increased.

2.4.2 Meat color

Instrumental color values, lightness (L^*), redness (a^*), and yellowness (b^*) for IR, RE, and SL steaks for all SF storage times are presented in Tables 2.1, 2.2, and 2.3, respectively. For IR (Table 2.1), the interaction between SF storage time and display day was significant ($P < 0.05$) for a^* and b^* values. No ($P \geq 0.05$) effect of SF storage time or display day was observed for L^* values of IR steaks. On day 0 of retail display, the IR steaks in SF storage for 60 d had lower ($P < 0.05$) a^* values than those in SF storage for 75 d and 90 d. However, by day 1, the a^* values of SF75 and SF90 steaks were similar ($P \geq 0.05$) to those of SF60 samples. The initial (day 0) b^* values of SF60 IR steaks were also lower ($P < 0.05$) than SF75 and SF90 samples. All b^* values of IR steaks decreased ($P < 0.05$) during retail display, with samples from all SF storage times having similar ($P \geq 0.05$) values by the end of retail display.

An interaction between SF storage time and display day was observed ($P < 0.05$) for L^* values of RE steaks (Table 2.2). The L^* values of SF90 and SF75 steaks were greater ($P < 0.05$) than those of SF60 steaks at the beginning of the display. There was no ($P \geq 0.05$) change in L^* values of SF60 steaks during display, while values of SF75 and SF90 samples decreased ($P < 0.05$) over the retail display period. After 7 days of display, SF75 steaks had the highest ($P < 0.05$) L^* value. Storage time in SF and display day influenced ($P < 0.05$) a^* and b^* values of RE steaks, but there was no interaction ($P \geq 0.05$). Steaks from SF75 and SF90 RE had a higher ($P < 0.05$) initial and final a^* value than steaks from SF60 RE. Overall, a^* values of all RE samples decreased ($P < 0.05$) over time regardless of the SF storage time. Yellowness (b^* values) of SF90 RE samples were higher ($P < 0.05$) than SF60 steaks at the beginning of the retail display, but by day 2 and until the end of retail display, b^* values of steaks from all SF storage times were generally similar ($P \geq 0.05$).

For SL steaks (Table 2.3), there was an interaction between SF storage time and display day ($P < 0.05$) for L^* and b^* values, while only the main effects were significant ($P < 0.05$) for redness (a^* values). The L^* values of SL SF60 steaks were lower ($P < 0.05$) than those of SF75 and SF90 samples on day 0 of retail display, but these values increased ($P < 0.05$) slightly during retail display. There were no ($P \geq 0.05$) changes over time in the L^* values of SF75 steaks. Although there were some minor changes in L^* values of SF90 samples during retail display, they were numerically very small. The a^* values of all SL samples decreased ($P < 0.05$) as display day increased, irrespective of the SF storage time. Similar to IR and RE steaks, the initial redness of SF60 SL steaks was lower ($P < 0.05$) than that of SF75 and SF90 samples. Additionally, the initial b^* values of SL steaks were all different ($P < 0.05$) by SF storage time, with SF60 having the lowest and SF90 the highest. All b^* values of SL steaks decreased ($P < 0.05$) during retail display.

Visual lean color and percentage of discoloration results for IR, RE, and SL steaks from all SF storage times are presented in Tables 2.4, 2.5, and 2.6, respectively. For IR (Table 2.4), there was no SF storage time × display day interaction ($P \geq 0.05$), nor SF storage time effect ($P \geq 0.05$) on lean color evaluation. Only a display day effect ($P < 0.05$) was observed with regards to IR lean color. On day 2 of retail display, lean color scores of samples, regardless of SF storage time, were higher ($P < 0.05$) than their day 0 scores, going from moderately bright cherry-red to slightly bright cherry-red. The interaction between the fixed effects was significant ($P < 0.05$) for the IR steak discoloration percentage. From day 0 to day 2, samples from all SF storage times had similar ($P \geq$

0.05) discoloration percentages. However, from day 4 through the end of display, SF90 steaks had the highest (*P* < 0.05) percentage of discoloration.

There was no interaction (*P* ≥ 0.05) between SF storage time and display day on lean color and discoloration percentage of RE steaks (Table 2.5) and only the display day was significant (*P* < 0.05). Similar to the IR samples, on day 2 of display, irrespective of SF storage time, the lean color scores of steaks were higher (*P* < 0.05) compared with their corresponding day 0 scores, declining from moderately bright cherry-red to slightly bright cherry-red. However, from day 5 of retail display, samples from SF60 had a lower (*P* < 0.05) lean color score (i.e., less dark red) than that of SF90 steaks. The discoloration percentage of all RE steaks increased (*P* < 0.05) gradually until day 3 of retail display, but on day 4 the discoloration percentage was more than double compared to the previous day.

For the SL samples (Table 2.6), an interaction between SF storage time and display day was observed (*P* < 0.05) for the percentage of discoloration. However, neither the interaction nor the SF storage time effect was significant (*P* ≥ 0.05) for lean color and only the display day was significant (*P* < 0.05). On day 3 of retail display, lean color scores of SL steaks were greater (*P* < 0.05) when compared with their corresponding day 0 scores, declining from moderately bright cherry-red to slightly bright cherry-red. The percentage discoloration of SL steaks from all SF storage times increased (*P* < 0.05) during retail display, with no differences (*P* ≥ 0.05) observed between the SF storage times up to day 5 of display. On the last two days, the SF90 steaks had a lower (*P* < 0.05) percentage of discoloration than the SF60 and SF75 samples.

2.4.3 Microbial populations

Microbial populations (APC, LAB counts, and *Pseudomonas* spp. counts) recovered from the IR, RE, and SL steaks on days 0, 2, 4, and 7 days of retail display are presented in Tables 2.7, 2.8, and 2.9, respectively. For IR samples (Table 2.7), an interaction between SF storage time and display day was observed ($P < 0.05$) for the APC, whereas for the LAB and *Pseudomonas* spp. counts, only the main effects were significant ($P < 0.05$). For SF60, SF75, and SF90 IR samples, APC increased ($P < 0.05$) from 1.8 to 6.3, 4.6 to 7.7, and 5.1 to 7.6 log CFU/cm^2, respectively, during the retail display period (Table 2.7). Regardless of retail display day, APC of SF60 IR steaks were lower ($P < 0.05$) than those of SF75 and SF90 IR samples. Likewise, LAB counts of SF60 IR samples were lower ($P < 0.05$) than those of SF75 and SF90 IR steaks but only until day 4 of retail display. Counts of LAB recovered from IR samples were initially (day-0) numerically similar or higher than the recovered APC. Regardless of the retail display day, *Pseudomonas* spp. counts of SF60 IR samples were lower ($P < 0.05$) than those of SF75 and SF90 IR steaks. Initially recovered *Pseudomonas* spp. counts from all IR samples were numerically lower than the recovered APC and LAB; however, they were numerically similar by day 7 of retail display.

For RE samples (Table 2.8), SF storage time × display day was significant ($P < 0.05$) for the APC but not for the LAB and *Pseudomonas* spp. counts. Both fixed effects (SF storage time and display day) were significant ($P < 0.05$) for the LAB counts, but only display day was significant ($P < 0.05$) for the *Pseudomonas* spp. counts. Aerobic bacterial populations (APC) of SF60, SF75, and SF90 RE steaks increased ($P < 0.05$) from 4.1 to 8.3, 5.3 to 8.6, and 5.4 to 8.5 log CFU/cm^2, respectively from day 0 to day 7 of display. The APC and LAB counts of SF60 RE steaks were lower ($P < 0.05$) than those of the SF75 and SF90 samples until day 4 of retail display. In contrast, there was no initial (day-0) or final (day-7) display differences ($P \geq 0.05$) in

Pseudomonas spp. counts of RE steaks regardless of the SF storage time. Overall, *Pseudomonas* spp. counts of RE samples increased ($P < 0.05$) by 4.6 (SF75) to 5.1 (SF60) log CFU/cm^2 by the end of the display period.

Similar to the other cuts, there was a SF storage time and display day interaction ($P < 0.05$) for the APC of SL steaks (Table 2.9). Initial APC of SF60, SF75, and SF90 SL samples were 3.1, 4.8, and 4.8 log CFU/cm^2, respectively, and these increased ($P < 0.05$) to 7.3, 8.1, and 7.6 log CFU/cm^2, respectively, by the end of retail display. The APC of SF60 SL steaks on days 0, 2, and 4 were lower ($P < 0.05$) than the APC of corresponding SF75 and SF90 samples. SF storage time and display day influenced ($P < 0.05$) the LAB and *Pseudomonas* spp. counts of SL steaks, but there was no interaction ($P \geq 0.05$) between display day and storage time. On days 0 and 2, LAB counts from SF60 SL steaks were lower ($P < 0.05$) than SF75 and SF90 samples. For *Pseudomonas* spp. counts, samples of SF60 were lower ($P < 0.05$) than SF90 only on day 0. The *Pseudomonas* spp. counts were similar ($P \geq 0.05$) on days 4 and 7 regardless of the SF storage time.

2.5 Discussion

Temperatures lower than regular chilling have been used to extend the storage shelf-life of meat (Chen et al., 2019; Lu et al., 2019; Chen et al., 2020; Zhang et al., 2023). However, most of these studies only evaluated microbial population levels and meat quality characteristics immediately following the storage times. In the current study, we evaluated shelf-life during retail display following extended storage for three economically important beef subprimal cuts (IR, RE, and SL).

An increase in purge is considered an economic loss to the meat industry and is unappealing to consumers (Kim et al., 2014; Van Rooyen et al., 2018). Purge loss is likely an accumulative effect of changes in the water holding capacity (Zhu et al., 2017) where an increased storage time could increase PL (Warner, 2017; Gagaoua et al., 2022). In the current study, the effect of SF storage time impacted PL ($P < 0.05$) only for IR (Figure 2.1). Similarly, Lu et al. (2019) reported an increase in PL from week 4 to week 8 in beef SL stored at 2°C and -4°C. The samples stored at -4°C were also evaluated for extended storage for up to 24 weeks, but the PL did not further increase after week 8 (Lu et al., 2019).

Meat color is commonly associated with freshness and wholesomeness by consumers, which makes it a critical quality attribute (Tomasevic et al., 2021). In this study, both instrumental and visual color attributes were evaluated. In general, retail display day was the critical component in the color stability of the three muscles, while SF storage time played a role but was not as significant as display day, probably because all SF storage times could be considered extended storage. Differences in L^* values among SF storage times were observed for RE and SL steaks (Tables 2.2 and 2.3), where L^* values of SF60 steaks were lower ($P < 0.05$) throughout the whole retail display period for RE steaks and days 0 to 3 for SL samples. With more time in storage, greater proteolysis is expected, which could increase the amount of surface water and, consequently, the lightness (Hughes et al., 2020). Likewise, English et al. (2016b) reported a higher L^* value in beef SL aged 62 d compared to 21 d. Colle et al. (2015) assessed the color of aged (2, 14, 21, 42, 63 d; 0°C) SL steaks during four days of retail display and found an interaction between aging time and display day. These researchers observed lower L^* values in SL steaks aged for 2 d as compared to the rest of the aging periods during the first two days of retail display.

Initial (day 0) surface redness (a^*) of SF60 steaks for all cuts was lower ($P < 0.05$) than their corresponding SF75 and SF90 samples. As postmortem age increases, there is decreased competition from mitochondria for oxygen, consequently improving myoglobin oxygenation and initial color; however, color stability typically decreases due to a decrease in mitochondria functionality (Mancini and Ramanathan, 2014; Suman et al., 2014; Nair et al., 2018; Ramanathan and Mancini, 2018). In contrast, English et al. (2016a) reported a lower initial a^* value in beef SL aged for 62 d compared with 21 d. Also, Karney et al. (2022) observed lower initial a^* values for beef SL steaks aged for 63 d compared to 14, 21, 28, and 49 days of aging. On the other hand, a recent study (Zhang et al., 2023) evaluating the retail shelf-life of beef SL after storage at -1°C reported that samples stored for 4 weeks had greater initial a^* values (day-1) than those held for 8, 12, and 16 weeks, but were similar to those stored 20 weeks. However, the a^* values decreased during the display days as storage time increased indicating a lower color stability during display (Zhang et al., 2023). Moreover, in another study that evaluated beef SL stored at -1°C up to 20 weeks, initial a^* values gradually increased with an increase in storage time (Chen et al., 2020).

The visual lean color of the samples was not affected ($P \geq 0.05$) by SF storage time for any of the cuts we examined (Tables 2.4 to 2.6). On day 2 of retail display, the lean color scores of samples were higher ($P < 0.05$) compared with their corresponding day 0 scores, going from moderately bright cherry-red to slightly bright cherry-red for all the muscles. These results matched our instrumental redness values, where no major differences were observed between the SF storage times, but a^* values declined over the display period. Similarly, English et al. (2016a) also observed a rapid increase in muscle color scores for SL aged 62 d, where in 24 h, score averages changed from bright cherry-red to slightly bright cherry red. Moreover, lean muscle color scores from 62 d aged products were higher than 21 d but were similar to 42 d aged samples (English et al., 2016a).

In the current study, steaks from the three SF storage times discolored similarly within each of the evaluated cuts. The slightest discoloration in beef can generate consumer discrimination or an initial discount by the retailer, and extensive beef discoloration could be rejected by the consumer, causing a significant loss of resources and value for the meat industry (Mancini and Hunt, 2005; Suman et al., 2014; Ramanathan et al., 2022). With increased postmortem time, mitochondrial functionality decreases, leading to a decline in metmyoglobin-reducing activity and faster discoloration (Ramanathan and Mancini, 2018; Ramanathan et al., 2019). In agreement, Colle et al. (2015), reported that SL steaks aged for 21, 42, and 63 d had more surface discoloration compared to 2 and 14 d aged samples. According to Hood and Riordan (1973), consumers are less likely to purchase beef steaks when the surface discoloration reaches 20%. In this study, the discoloration percentages stayed lower than 20% for RE and SL until day 3 and 4 of retail display, respectively, regardless of SF storage time. Although IR steaks discolored faster, with more than 20% discoloration present by day 2 of retail display, this was expected since it is comprised of less stable color muscles (*adductor and semimembranosus*; McKenna et al., 2005).

Steaks from the different muscles and SF storage times in the current study, were analyzed for APC, LAB counts, and *Pseudomonas* spp. counts on days 0, 2, 4, and 7 of retail display. Day-0 APC of SF60, SF75, and SF90 RE steaks were numerically higher than those recovered from day-0 samples of IR and SL. This could be due to the additional handling that is involved in processing bone-in products. Overall, APC of SF60 steaks were lower ($P < 0.05$) than those of SF75 and SF90 samples up to day 4 of retail display for all cuts. However, there were no differences ($P \geq 0.05$) between APC of samples from SF75 and SF90, regardless of the display day or cut evaluated. Chen et al. (2019) evaluated the initial (day 0) APC of SL steaks stored at -1 ± 0.5°C for up to 20 weeks and, similarly, reported an increase in APC of samples up to week 5 of storage, but there were no differences between APC of samples stored for 9, 12, 15, and 20 weeks. Colle et al. (2015) analyzed APC of SL steaks on day 0 and 4 of retail display after storage at 0°C and reported an increase in the APC of SL steaks as aging time (2, 14, 21, 42, and 63 d) increased.

In general, recovered initial (day-0 of retail display) LAB counts of samples, regardless of cut, were numerically similar or higher than the recovered APC of their corresponding SF storage time. After 7 days of retail display, counts of LAB were similar to or lower than the APC. With a prolonged storage period under vacuum-packaged conditions, as in the case of SF storage, it is anticipated that bacterial populations will be dominated by LAB at the start of retail display. After exposing the samples to oxygen, other bacteria will grow and potentially slow down the growth rate of the LAB. Other studies have found similar results after extended vacuumed storage of beef (Chen et al., 2019; Luzardo et al., 2016; Small et al., 2012). Moreover, the previously mentioned Chen et al. (2019) study also reported that LAB counts were higher or similar to the APC. They also observed similar APC and LAB initial counts of samples after 9 weeks (63 d) of storage at -1 ± 0.5°C (Chen et al., 2019).

Pseudomonas spp. are considered important spoilage organisms of chilled meat stored under aerobic conditions (Hilgarth et al., 2019; Pennacchia et al., 2011). In the current study, initial *Pseudomonas* spp. counts on steaks were lower than those of APC and LAB counts regardless of SF storge time or cut. This was an expected finding since SF storage occurred under vacuum-packaged conditions which inhibited the growth of *Pseudomonas* spp. (Gill, 1996). Once the steaks were placed in retail display (aerobic packaging), *Pseudomonas* spp. populations increased rapidly reaching levels of ca. 6 to 8 log CFU/cm^2 on day 7 of display.

Taking into account a threshold of 7 log CFU/cm^2 for APC as an indicator of microbial spoilage in beef, our study indicated that SF60 IR, RE, and SL steaks had a longer retail shelf-life than the SF75 and SF90 steaks. This outcome was expected since the SF60 samples had a lower initial microbial load than the SF75 and 90 samples. However, the retail microbial shelf-life of SF75 and SF90 steaks was similar.

2.6 Conclusions

Shelf-life performance of beef products can affect consumer purchase decisions. Therefore, evaluating shelf-life performance (color and microbial) after extended storage periods such as SF storage is essential. Of the cuts evaluated, the PL of only the IR steaks was affected by the different SF storage times. The initial redness (a^* value) of SF60 steaks were lower than SF75 and SF90 steaks for all the cuts. In general, trained panelists did not find differences in lean color and discoloration percentage with the different SF storage times, irrespective of the cut evaluated. Additionally, microbial retail shelf-life depended on initial contamination levels of cuts following SF storage, with a longer retail shelf-life for samples from SF60 and similar for SF75 and SF90 samples. The findings of this study can be useful for the meat industry when considering extending the storage shelf-life of boxed beef subprimals by storing them in a controlled low-temperature environment.

Table 2.1: Effect of Suspended Fresh® (SF) storage times (60, 75, or 90 days) on surface lightness (L^* value), redness (a^* value), and yellowness (b^* value) of inside round steaks (IR; n = 10) during retail display (3°C) for 7 d under aerobic packaging.

	SF storage	**Days of retail display**								**SE**
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	
L^*	60	35.4^{ax}	33.7^{ay}	33.6^{ax}	33.4^{ax}	33.6^{ax}	34.2^{ax}	34.6^{ax}	34.9^{ax}	0.9
	75	37.6^{ax}	35.7^{axy}	34.7^{ax}	34.5^{ax}	34.8^{ax}	34.2^{ax}	34.6^{ax}	35.0^{ax}	0.9
	90	36.7^{ax}	37.0^{ax}	35.6^{ax}	35.6^{ax}	35.2^{ax}	35.5^{ax}	35.3^{ax}	34.9^{ax}	1.0
a^*	60	19.3^{ay}	17.7^{ax}	15.7^{bx}	14.6^{bcxy}	13.3^{cdxy}	12.2^{dxy}	10.5^{exy}	9.5^{ex}	0.5
	75	22.1^{ax}	18.9^{bx}	16.9^{cx}	15.6^{cdx}	14.4^{dex}	13.4^{efx}	11.7^{fx}	9.9^{gx}	0.5
	90	22.0^{ax}	18.8^{bx}	16.0^{cx}	14.0^{dy}	12.8^{dey}	11.1^{ey}	10.1^{fy}	8.9^{gx}	0.5
b^*	60	15.6^{ay}	15.4^{ay}	14.6^{abcy}	14.8^{abxy}	13.9^{bcdy}	13.6^{bcdy}	12.9^{dy}	13.2^{cdx}	0.4
	75	17.7^{ax}	16.5^{abxy}	15.7^{bcx}	15.4^{bcx}	15.1^{bcx}	15.2^{bcx}	14.9^{cx}	14.4^{cx}	0.4
	90	18.2^{ax}	17.5^{ax}	15.3^{bxy}	14.0^{bcy}	13.8^{bcy}	13.4^{cy}	13.7^{cy}	13.8^{bcx}	0.4

SE: standard error of the mean

$^{a\text{-}g}$ Least squares means with different superscripts within a row are different ($P < 0.05$).

$^{x\text{-}y}$ Least squares means with different superscripts within a column and color parameter are different ($P < 0.05$).

Table 2.2: Effect of Suspended Fresh® (SF) storage times (60, 75, or 90 days) on surface lightness (*L** value), redness (*a** value), and yellowness (*b** value) of bone-in ribeye steaks (RE; n = 10) during retail display (3°C) for 7 d under aerobic packaging.

	SF storage	**Days of retail display**								**SE**
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	
L*	60	34.5^{ay}	33.6^{ay}	33.6^{ay}	32.0^{ay}	33.6^{ay}	33.8^{ay}	33.4^{ay}	33.3^{ay}	0.7
	75	39.2^{ax}	38.3^{abx}	36.8^{bcdx}	37.4^{abcdx}	37.7^{abcx}	36.3^{bcdx}	35.4^{dx}	35.8^{cdx}	0.6
	90	39.5^{ax}	38.0^{abx}	36.8^{bcx}	36.7^{bcx}	36.7^{bcx}	36.8^{bcx}	35.0^{cdxy}	33.9^{dy}	0.6
a*	60	17.6^{ay}	16.2^{abx}	14.1^{bx}	13.5^{bcx}	11.0^{cdx}	8.7^{dey}	7.0^{ey}	7.7^{ey}	0.8
	75	19.9^{ax}	17.5^{bx}	15.4^{bcx}	14.1^{cdx}	12.8^{dex}	11.0^{efx}	10.1^{fx}	10.3^{fx}	0.7
	90	20.0^{ax}	18.0^{abx}	15.8^{bcx}	14.1^{cdx}	12.3^{dex}	10.4^{exy}	10.1^{ex}	10.7^{ex}	0.7
b*	60	14.2^{ay}	13.2^{aby}	11.9^{bcx}	12.1^{bcx}	11.0^{cdx}	10.7^{cdx}	9.3^{dy}	9.4^{dx}	0.5
	75	15.0^{axy}	13.6^{aby}	12.6^{bcx}	12.2^{bcdx}	12.0^{bcdx}	11.4^{cdex}	10.8^{dex}	9.9^{ex}	0.5
	90	15.8^{ax}	15.1^{ax}	13.2^{bx}	12.0^{bcx}	11.2^{cdx}	10.4^{cdx}	10.0^{dxy}	9.7^{dx}	0.5

SE: standard error of the mean

$^{a-f}$ Least squares means with different superscripts within a row are different ($P < 0.05$).

$^{x-y}$ Least squares means with different superscripts within a column, and color parameter are different ($P < 0.05$).

Table 2.3: Effect of Suspended Fresh® (SF) storage times (60, 75, or 90 days) on surface lightness (L^* value), redness (a^* value), and yellowness (b^* value) of striploin steaks (SL; n = 10) during retail display (3°C) for 7 d under aerobic packaging.

	SF storage	Days of retail display								SE
		0	1	2	3	4	5	6	7	
L^*	60	31.6^{by}	32.7^{aby}	32.8^{aby}	31.9^{by}	33.4^{abx}	33.6^{ax}	33.9^{ax}	33.8^{ax}	0.6
	75	35.0^{ax}	35.2^{ax}	34.4^{ax}	34.9^{ax}	34.4^{ax}	34.4^{ax}	34.4^{ax}	34.0^{ax}	0.6
	90	35.7^{ax}	34.1^{abxy}	34.6^{abx}	34.5^{abx}	33.8^{bx}	34.9^{abx}	35.1^{abx}	35.0^{abx}	0.6
a^*	60	17.3^{ay}	16.8^{ay}	15.7^{abxy}	14.6^{bcx}	13.1^{cdx}	11.8^{dx}	9.0^{ey}	7.2^{ex}	0.5
	75	19.0^{ax}	16.7^{by}	14.7^{cy}	13.6^{cdx}	12.2^{dex}	10.7^{efx}	8.8^{fgy}	8.4^{gx}	0.5
	90	19.9^{ax}	18.5^{abx}	16.3^{bcx}	15.1^{cdx}	13.7^{dex}	12.3^{efx}	11.0^{fx}	8.6^{gx}	0.6
b^*	60	13.8^{az}	13.8^{ay}	13.2^{ay}	12.9^{abxy}	11.9^{bcy}	11.3^{cy}	10.7^{cy}	10.7^{cy}	0.4
	75	14.8^{ay}	13.5^{by}	12.5^{bcy}	12.2^{cdy}	11.6^{cdy}	11.6^{cdxy}	11.2^{dy}	11.3^{dxy}	0.4
	90	16.2^{ax}	16.1^{ax}	14.3^{bx}	13.6^{bcx}	13.0^{bcdx}	12.5^{cdx}	12.5^{cdx}	12.3^{dx}	0.4

SE: standard error of the mean

$^{a\text{-}g}$ Least squares means with different superscripts within a row are different ($P < 0.05$).

$^{x\text{-}z}$ Least squares means with different superscripts within a column and color parameter are different ($P < 0.05$).

Table 2.4: Effect of Suspended Fresh® (SF) storage times (60, 75, or 90 days) on visual lean color[1] and percentage of discoloration (metmyoglobin formation) evaluation, by a trained panel (n = 6), of inside round steaks (IR; n = 10) during retail display (3°C) for 7 d under aerobic packaging.

	SF storage	**Days of retail display**								**SE**
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	
Lean color[1]	60	3.1^{dx}	3.9^{dx}	4.8^{cx}	5.4^{bcx}	5.7^{abx}	5.7^{abx}	6.1^{abx}	6.4^{ax}	0.3
	75	3.4^{dx}	4.2^{cdx}	4.8^{bcx}	4.9^{bcx}	5.4^{abx}	5.6^{abx}	5.9^{ax}	6.1^{ax}	0.3
	90	3.6^{fx}	4.2^{efx}	4.8^{dex}	5.4^{cdx}	5.8^{bcx}	6.1^{abcx}	6.6^{abx}	6.8^{ax}	0.3
%Dis	60	1.6^{ex}	7.2^{ex}	25.7^{dx}	38.2^{cdx}	43.5^{cxy}	49.8^{bcxy}	67.4^{aby}	74.3^{ay}	5.6
	75	0.1^{fx}	7.5^{efx}	20.9^{dex}	23.9^{dey}	32.0^{cdy}	45.9^{bcy}	59.2^{by}	79.6^{ay}	5.7
	90	4.0^{dx}	10.7^{cdx}	20.8^{cdx}	28.4^{cxy}	48.6^{bx}	61.4^{bx}	84.4^{ax}	94.5^{ax}	5.9

[1] Panelists scored each steak to assess lean color using a continuous 8-point scale (1 = extremely bright cherry-red, 2 = bright cherry-red, 3 = moderately bright cherry-red, 4 = slightly bright cherry-red, 5 = slightly dark cherry-red, 6 = moderately dark red, 7 = dark red, 8 = extremely dark red).

SE: standard error of the mean

$^{a\text{-}f}$ Least squares means with different superscripts within a row are different ($P < 0.05$).

$^{x\text{-}y}$ Least squares means with different superscripts within a column and color parameter are different ($P < 0.05$).

Table 2.5: Effect of Suspended Fresh® (SF) storage times (60, 75, or 90 days) on visual lean color[1] and percentage of discoloration (metmyoglobin formation) evaluation, by a trained panel (n = 6), of bone-in ribeye steaks (RE; n = 10) during retail display (3°C) for 7 d under aerobic packaging.

	SF storage	**Days of retail display**								**SE**
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	
Lean color[1]	60	3.3^{fx}	3.4^{efx}	4.2^{dex}	4.4^{cdy}	4.8^{cdx}	5.1^{bcy}	5.9^{aby}	6.5^{ay}	0.2
	75	3.0^{fx}	3.8^{ex}	4.4^{dex}	4.9^{cdxy}	5.1^{cx}	5.8^{bx}	6.4^{abxy}	6.6^{ay}	0.2
	90	3.4^{ex}	4.0^{dex}	4.5^{cdx}	5.1^{cx}	5.2^{cx}	5.9^{bx}	6.7^{ax}	7.2^{ax}	0.2
%Dis	60	1.0^{cx}	3.1^{cx}	8.1^{cdx}	14.3^{cdx}	29.9^{bcx}	53.8^{abx}	78.1^{ax}	76.2^{ax}	6.3
	75	0.1^{cx}	1.9^{cx}	8.3^{cx}	15.7^{cdx}	35.1^{bcx}	42.1^{bx}	74.4^{ax}	81.0^{ax}	5.5
	90	0.8^{ex}	2.0^{ex}	6.3^{ex}	13.0^{dex}	33.7^{cdx}	53.3^{bcx}	66.7^{abx}	85.4^{ax}	5.5

[1] Panelists scored each steak to assess lean color using a continuous 8-point scale (1 = extremely bright cherry-red, 2 = bright cherry-red, 3 = moderately bright cherry-red, 4 = slightly bright cherry-red, 5 = slightly dark cherry-red, 6 = moderately dark red, 7 = dark red, 8 = extremely dark red).

SE: standard error of the mean

$^{a\text{-}f}$ Least squares means with different superscripts within a row are different ($P < 0.05$).

$^{x\text{-}y}$ Least squares means with different superscripts within a column and color parameter are different ($P < 0.05$).

Table 2.6: Effect of Suspended Fresh® (SF) storage times (60, 75, or 90 days) on visual lean color[1] and percentage of discoloration (metmyoglobin formation) evaluation, by a trained panel (n = 6), of striploin steaks (SL; n = 10) during retail display (3°C) for 7 d under aerobic packaging.

	SF storage	**Days of retail display**								**SE**
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	
Lean color^{1}	60	3.0^{fx}	3.3^{efx}	3.9^{dex}	4.4^{cdx}	4.4^{cdx}	4.8^{cx}	5.7^{bx}	6.4^{ax}	0.2
	75	3.1^{cx}	3.5^{cx}	3.7^{cx}	4.4^{bx}	4.6^{bx}	4.9^{bx}	5.6^{ax}	5.9^{ax}	0.2
	90	3.5^{ex}	3.5^{ex}	4.0^{dex}	4.4^{cdx}	4.9^{bcx}	5.4^{abx}	5.6^{abx}	6.1^{ax}	0.2
%Dis	60	0.4^{ex}	0.4^{ex}	5.8^{ex}	9.9^{dex}	20.6^{cdx}	33.1^{cx}	64.4^{bx}	88.7^{ax}	3.5
	75	0.3^{ex}	1.3^{ex}	3.9^{ex}	6.9^{dex}	17.9^{cdx}	31.4^{cx}	64.5^{bx}	85.0^{ax}	3.5
	90	0.3^{ex}	2.2^{dex}	5.7^{dex}	7.1^{dex}	17.5^{cdx}	30.8^{bcx}	45.8^{by}	72.2^{ay}	3.8

[1] Panelists scored each steak to assess lean color using a continuous 8-point scale (1 = extremely bright cherry-red, 2 = bright cherry-red, 3 = moderately bright cherry-red, 4 = slightly bright cherry-red, 5 = slightly dark cherry-red, 6 = moderately dark red, 7 = dark red, 8 = extremely dark red).

SE: standard error of the mean

$^{a-f}$ Least squares means with different superscripts within a row are different (*P* < 0.05).

$^{x-y}$ Least squares means with different superscripts within a column and color parameter are different (*P* < 0.05).

Table 2.7: Mean (n = 10) bacterial counts (log CFU/cm^2 ± standard deviation) of inside round (IR) steaks in retail display (3°C, 7 days). Steaks were fabricated from cuts that were previously held under vacuum-packaged Suspended Fresh® (SF) storage conditions (60, 75, or 90 days).

Bacterial count	**Days of SF storage**	**Days of retail display**			
		0	**2**	**4**	**7**
Aerobic bacterial populations	60	1.8 ± 0.3^{dy}	2.7 ± 0.4^{cy}	4.1 ± 0.6^{by}	6.3 ± 0.8^{ay}
	75	4.6 ± 1.1^{cx}	6.0 ± 0.8^{bx}	6.5 ± 0.6^{bx}	7.7 ± 0.5^{ax}
	90	5.1 ± 0.8^{cx}	6.3 ± 0.5^{bx}	6.7 ± 1.0^{abx}	7.6 ± 0.6^{ax}
Lactic acid bacteria	60	3.0 ± 1.0^{cy}	3.7 ± 1.2^{cy}	5.1 ± 0.7^{by}	6.4 ± 0.8^{ay}
	75	4.8 ± 0.9^{cx}	5.8 ± 0.7^{bx}	6.0 ± 0.5^{bx}	7.3 ± 0.8^{ax}
	90	4.9 ± 0.7^{cx}	5.8 ± 0.3^{bcx}	6.6 ± 1.0^{abx}	7.1 ± 0.7^{axy}
Pseudomonas spp.	60	$<1.4 \pm 0.3^{cy*}$	1.6 ± 0.7^{cy}	3.2 ± 0.6^{by}	6.1 ± 0.9^{ay}
	75	2.0 ± 0.5^{dxy}	2.8 ± 0.5^{cx}	4.1 ± 0.5^{bx}	7.4 ± 0.4^{ax}
	90	2.2 ± 0.8^{dx}	3.4 ± 0.5^{cx}	4.7 ± 0.5^{bx}	7.4 ± 0.8^{ax}

[*] Four of the 10 samples analyzed had a *Pseudomonas* spp. count that was below the microbial analysis detection limit of 1.2 log CFU/cm^2 (15 CFU/cm^2); therefore, the mean is reported as < (less than)

[a-d] Least squares means with different superscripts within a row are different ($P < 0.05$).

[x-y] Least squares means with different superscripts within a column and bacterial count type are different ($P < 0.05$).

Table 2.8: Mean (n = 10) bacterial counts (log CFU/cm^2 ± standard deviation) of bone-in ribeye (RE) steaks in retail display (3°C, 7 days). Steaks were fabricated from cuts that were previously held under vacuum-packaged Suspended Fresh® (SF) storage conditions (60, 75, or 90 days).

Bacterial count	**Days of SF storage**	**Days of retail display**			
		0	**2**	**4**	**7**
Aerobic bacterial populations	60	4.1 ± 0.6^{cy}	4.6 ± 0.6^{cy}	6.2 ± 0.5^{by}	8.3 ± 0.3^{ax}
	75	5.3 ± 0.3^{dx}	6.2 ± 0.7^{cx}	7.4 ± 0.9^{bx}	8.6 ± 0.3^{ax}
	90	5.4 ± 0.4^{cx}	5.9 ± 0.3^{cx}	7.3 ± 0.5^{bx}	8.5 ± 0.3^{ax}
Lactic acid bacteria	60	4.2 ± 0.6^{cy}	4.6 ± 0.6^{cy}	6.1 ± 0.6^{by}	7.8 ± 0.5^{ax}
	75	5.3 ± 0.3^{dx}	6.2 ± 0.8^{cx}	6.9 ± 0.7^{bx}	8.2 ± 0.4^{ax}
	90	5.4 ± 0.3^{cx}	6.0 ± 0.3^{cx}	7.3 ± 0.4^{bx}	8.3 ± 0.3^{ax}
Pseudomonas spp.	60	3.0 ± 0.4^{cx}	3.5 ± 0.6^{cy}	5.6 ± 0.6^{by}	8.1 ± 0.2^{ax}
	75	3.7 ± 0.3^{dx}	4.8 ± 0.8^{cx}	6.6 ± 0.8^{bx}	8.3 ± 0.4^{ax}
	90	3.5 ± 0.7^{dx}	4.5 ± 0.7^{cx}	6.6 ± 0.8^{bx}	8.4 ± 0.4^{ax}

$^{a\text{-}d}$ Least squares means with different superscripts within a row are different ($P < 0.05$).
$^{x\text{-}y}$ Least squares means with different superscripts within a column and bacterial count type are different ($P < 0.05$).

Table 2.9: Mean (n = 10) bacterial counts (log CFU/cm^2 ± standard deviation) of striploins (SL) steaks in retail display (3°C, 7 days). Steaks were fabricated from cuts that were previously held under vacuum-packaged Suspended Fresh® (SF) storage conditions (60, 75, or 90 days).

Bacterial count	**Days of SF storage**	**Days of retail display**			
		0	**2**	**4**	**7**
Aerobic bacterial populations	60	3.1 ± 0.6^{cy}	3.7 ± 0.7^{cy}	5.5 ± 0.5^{by}	7.3 ± 0.4^{ay}
	75	4.8 ± 0.6^{dx}	5.7 ± 0.6^{cx}	6.5 ± 0.5^{bx}	8.1 ± 0.6^{ax}
	90	4.8 ± 0.6^{cx}	5.7 ± 0.6^{bx}	6.4 ± 0.7^{bx}	7.6 ± 0.3^{axy}
Lactic acid bacteria	60	3.8 ± 0.7^{cy}	4.3 ± 0.9^{cy}	5.6 ± 0.6^{bx}	6.4 ± 0.4^{ay}
	75	4.7 ± 0.6^{cx}	5.8 ± 0.7^{bx}	6.1 ± 0.4^{bx}	7.4 ± 0.4^{ax}
	90	4.7 ± 0.5^{cx}	5.7 ± 0.7^{bx}	6.3 ± 0.7^{abx}	7.0 ± 0.6^{axy}
Pseudomonas spp.	60	2.0 ± 0.6^{cy}	2.8 ± 0.5^{cy}	5.0 ± 0.6^{bx}	7.3 ± 0.5^{ax}
	75	2.7 ± 0.6^{dxy}	3.8 ± 0.9^{cx}	5.4 ± 0.9^{bx}	7.8 ± 0.5^{ax}
	90	2.8 ± 0.8^{cx}	3.4 ± 0.7^{cxy}	5.0 ± 0.6^{bx}	7.5 ± 0.4^{ax}

$^{a-d}$ Least squares means with different superscripts within a row are different ($P < 0.05$).

$^{x-y}$ Least squares means with different superscripts within a column and bacterial count type are different ($P < 0.05$).

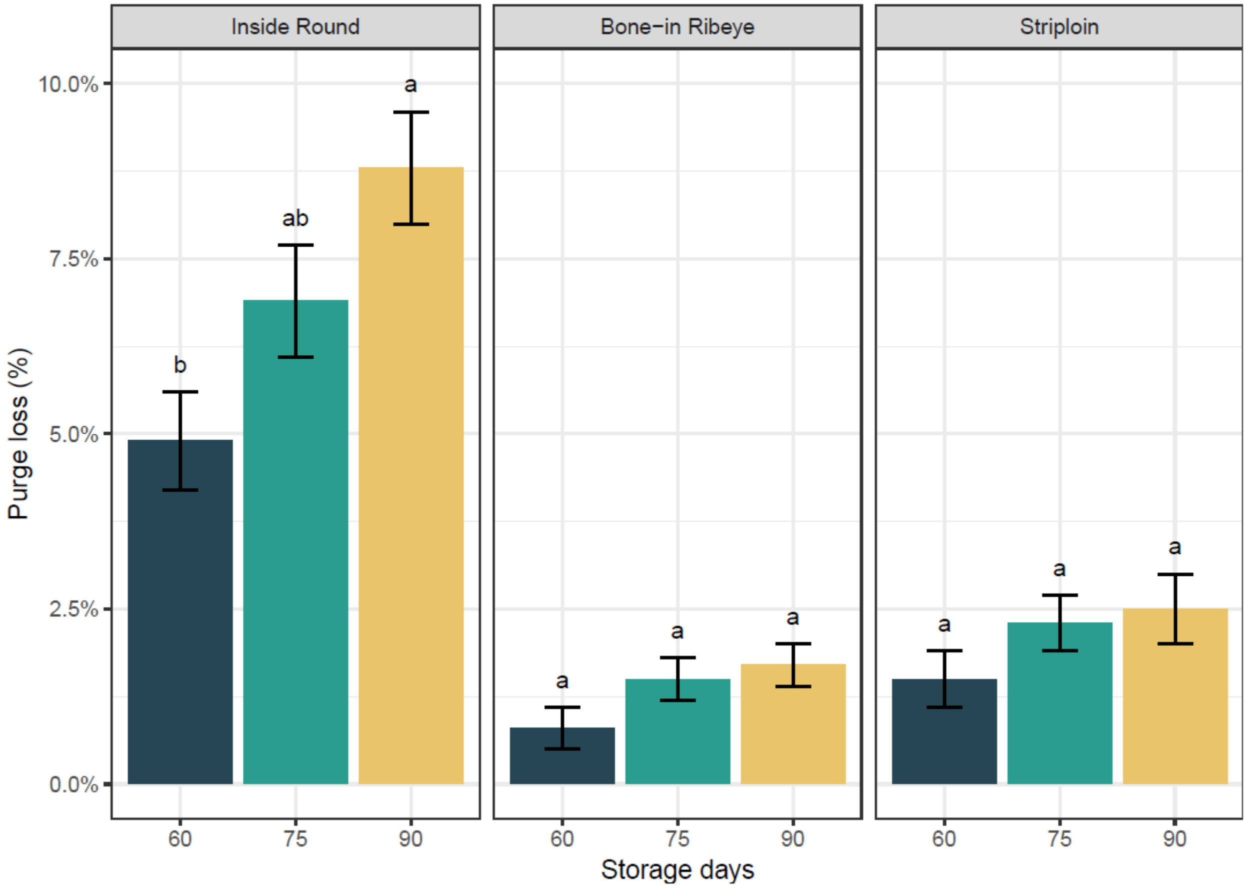

Figure 2.1. Effect of Suspended Fresh® storage times (60, 75, or 90 days) on the percentage of purge loss of inside round (IR), bone-in ribeye (RE), and striploin cuts (SL; n = 10). Different letters (a-b) indicate significant differences ($P < 0.05$). Error bars represent the standard error of the mean.

CHAPTER 3: EVALUATION OF BEEF PALATABILITY FOLLOWING SUSPENDED FRESH® STORAGE

3.1 Summary

Extending the shelf-life of fresh meat without having an adverse effect on its quality attributes is critical to the meat industry to reduce waste, stabilize supply, and facilitate export. Suspended Fresh® (SF) is a trademarked process that allows the storage of beef muscles at temperatures at or slightly above their freezing point to slow down microbiological spoilage while maintaining the product's fresh status. This study evaluated the impact of 60, 75, or 90 d of storage in SF (-2.7±0.3°C) on the palatability characteristics of steaks derived from inside rounds (IR), bone-in ribeyes (RE), and striploins (SL) from 10 (n=10) upper two-thirds Choice beef carcasses. Two steaks fabricated from each subprimal were vacuum-packaged, wet-aged for 21 d (3°C), and frozen (-20°C) for Warner-Bratzler shear force (WBSF) and sensory analyses. These steaks served as the control with regard to storage condition and time. The remainder of each subprimal was fabricated into three portions, and after vacuum packaging, were randomly allocated to an SF storage time (60, 75, or 90 d). After each storage time, subprimals were fabricated into steaks, vacuum-packaged, and stored (-20°C) for WBSF and consumer sensory analyses. Consumers (N=238) evaluated cooked samples for juiciness, tenderness, flavor liking, and overall liking. Data were analyzed using a mixed model with storage time as the fixed effect and individual carcasses as the random effect. The WBSF values decreased ($P < 0.05$) with increased storage time for all the cuts. Similarly, the consumer tenderness rating scores of IR and SL generally increased with the SF storage time. However, storage time did not influence ($P \geq 0.05$) the juiciness, flavor, and

overall liking of any cuts. The results of this study suggest it would be feasible to extend the storage time of beef while preserving or improving the sensory quality when held at optimal conditions above the freezing temperature.

3.2 Introduction

With a fast-growing world population, and an increasing food demand, food systems must undergo transformations to ensure food security while complying with environmental, economic, and social objectives to meet global sustainability goals (Godfray et al., 2010; FAO, 2018). One way to meet this increasing demand is by extending the shelf-life of fresh meat without any adverse effect on quality attributes. This is critically important to reduce waste, stabilize supply, and facilitate export shipments. Meat is commonly preserved by storing it at chilled temperatures (2-4°C). However, its shelf-life under regular chilling conditions can be limited (on average 8-10 weeks under vacuum packaging) primarily due to microbial spoilage (Coombs et al., 2017; Lu et al., 2019). Previous research has shown that the shelf-life of fresh beef can be increased to up to 20 weeks (140 d) when the product is vacuum-packaged and stored below regular chilling temperatures (around -0.5 to -1.5°C) (Small et al., 2012; Chen et al., 2019). Furthermore, extended refrigerated storage/aging times can improve beef tenderness (Ramanathan et al., 2020).

The 2015 National Beef Tenderness Survey reported that the average length of aging for steaks in retail and food service settings was 25.9 and 31.5 d, respectively (with a range of 3 to 102 d), and 11% of the steaks had less than 14 d of aging (Martinez et al., 2017). Such a variation in postmortem aging time could lead to inconsistency in tenderness between products (Dixon et al., 2012; Marino et al., 2013; Nair et al., 2019). In addition, during the peak steak consumption times of the year, such as the summer season (Lusk et al., 2001; USDA-ERS, 2022), not all retailers and restaurants might have the supply of tender steaks needed to meet the demand, which could result in the use of steaks with low postmortem aging days or use of frozen beef. Additionally, beef market prices are seasonal, becoming highly inelastic during the summer (Lusk et al., 2001), since consumers are willing to pay a higher price for specific cuts during this cookout season (Ardeshiri et al., 2019). Even when frozen beef can be used to stabilize the supply during the peak demand periods of the year, negative quality attributes associated with frozen beef, such as high purge, low color stability, poor tenderness, and high off-flavors, need to be considered (Wheeler et al., 1990; Lu et al., 2019; Wang et al., 2020).

A trademarked process known as Suspended Fresh® (SF) allows the storage of boxed beef subprimals in a temperature-controlled environment, keeping the meat slightly above or at its freezing point without the formation of ice crystals (Lobaugh, 2021). This process could slow down the growth of spoilage microflora and continue postmortem tenderization while maintaining the product's fresh status (Lobaugh, 2021). Thus, it enables beef buyers to have a product held for weeks or even months (≤ 3 months) delivered to them, equivalent to a fresh product in terms of storage temperature. Previous studies have shown that extended postmortem aging of samples for more than 42 d can adversely affect beef flavor (Juárez et al., 2010; Garmyn et al., 2020; Karney et al., 2022). However, the specific palatability characteristics of beef following SF extended storage are still unknown. Therefore, the objective of this study was to evaluate tenderness and palatability characteristics of beef inside round (IR), bone-in ribeye (RE), and striploin (SL) cuts following 60, 75, and 90 d of SF storage.

3.3 Materials and Methods

3.3.1 Collection and sample processing

Beef subprimals (IR, RE, and SL) were collected from 10 (n = 10) paired upper two-thirds Choice beef carcasses from a commercial beef processing facility. All individually identified subprimals were transported under refrigeration to the Department of Animal Sciences Global Food Innovation Center (GFIC) at Colorado State University (Fort Collins, CO). Upon arrival, two 2.5-cm steaks fabricated from each subprimal were vacuum-packaged, wet-aged for 21 d (3°C), and then frozen (-20°C) for Warner-Bratzler shear force (WBSF), cooking loss, and consumer sensory analyses. These samples served as the control treatment. The remainder of each intact subprimal was cut into sections, individually vacuum-packaged, and randomly assigned to 60, 75, or 90 d of SF storage (SF60, SF75, or SF90 d; -2.7±0.3°C). The packaged pieces were separated by subprimal cut and SF storage time, boxed, and transported overnight under refrigeration to a certified SF storage facility. Following each SF storage period, the products were transported overnight to the GFIC. Immediately after arrival, the sections were removed from their vacuum package and two steaks of 2.5-cm were fabricated from each. These steaks were individually vacuum-packaged and stored at -20°C for WBSF, cooking loss, and consumer sensory evaluations.

3.3.2 Cooking method

Before cooking, the frozen steaks were tempered for 24 h at 3°C to attain a raw internal temperature of 0 to 4°C at the time of cooking. All excess external fat was trimmed off the raw steaks and then cooked in a combi-oven (Model SCC WE 61 E; Rational, Landsberg am Lech, Germany) set at 204°C, until a peak internal temperature of 71°C was achieved. A thermometer

(Thermapen Mk4, Thermoworks Inc., American Fork, UT) placed in the geometric center of each steak was used to determine the peak internal temperature of the cooked steaks.

3.3.3 Cooking loss determination

The trimmed raw steaks were weighed on a food scale (V22XWE3T, Ohaus Corporation, Parsippany, NJ) prior to cooking. After cooking, the steaks were weighed again to calculate the percentage of cooking loss (CL%) with the following formula: cook loss percentage = [(raw weight – cooked weight)/raw weight] × 100.

3.3.4 Warner-Bratzler Shear Force

Cooked steaks destined for WBSF determination were placed on trays, avoiding any overlap, and were covered with plastic wrap and refrigerated (3°C) overnight. The next day, cooked steaks were trimmed of visible connective tissue to expose muscle fiber orientation. Hand-held coring devices were used to remove at least six 1.2-cm diameter cores parallel to the muscle fibers from each steak. Cores were sheared once, perpendicular to the muscle fibers, using a universal testing machine (Instron Corp., Canton, MA) fitted with a Warner-Bratzler shear head (crosshead speed: 200 mm/min, load cell capacity: 100 kg). Peak shear force was recorded, and values from the cores taken from each steak were averaged to obtain a single WBSF value for each steak. The average peak shear force of the cores was used for statistical analysis.

3.3.5 Sensory Evaluation

The Colorado State University Institutional Review Board approved the procedures used in this study (IRB exemption #2784, October 28, 2021). A total of 120 steaks were randomly assigned, by subprimal cut and storage time (i.e., 21 d wet-aged control, SF60, SF75, and SF90), to one of 30 panels with four steaks evaluated per panel. Steaks were also randomly assigned a serving order within each panel, and eight panelists evaluated each steak. Frozen steaks were tempered for 18 to 24 h at 3°C before cooking, and the samples were cooked (as described previously) in the serving order. Cooked steaks were trimmed of all external fat and connective tissue and cut into cuboidal pieces (1-cm^2 × cooked steak thickness). Samples were served warm to panelists, and portions comprised mainly of fat or heavy connective tissue were not utilized.

Panelists were recruited (N=238) from Fort Collins, CO, and surrounding communities. Panels took place at Colorado State University in groups of 24 panelists. At the beginning of each panel, participants were given a brief orientation to provide sample evaluation and ballot completion instructions. Then, participants signed a consent form, and demographic and beef consumption information was collected (Table 3.1). Each panelist evaluated a total of four samples, with each sample representing a different storage time (i.e., 21 d wet-aged, SF60, SF75, and SF90). Two cuboidal portions per sample were given to each participant. Panelists were provided unsalted saltine crackers (Nabisco Unsalted Tops Premium; Mondelez Global LLC, East Hanover, NJ), water, and 25% diluted unsweetened apple juice to use as palate cleansers between samples. Samples were rated using a 100-point scale for juiciness (0 = extremely dry, 50 = neither juicy nor dry, 100 = extremely juicy), tenderness (0 = extremely tough, 50 = neither tough nor tender, 100 = extremely tender), flavor (0 = dislike extremely, 50 = neither like nor dislike, 100 = like extremely), and overall liking (0 = dislike extremely, 50 = neither like nor dislike, 100 = like extremely). Additionally, consumers evaluated these four attributes for acceptability (juiciness acceptance, tenderness acceptance, flavor acceptance, and overall liking acceptance), including the presence or absence of off-flavors, with a "yes" or "no" answer, leaving perceived acceptance levels to consumers' interpretation. All data were collected using Qualtrics software.

3.3.6 Statistical analysis

A split-plot design was used to evaluate the effects of the storage times on cooking loss, WBSF, and consumer sensory score evaluations within each subprimal cut since it is known that different subprimals have different quality characteristics. Data analysis was performed in R version 4.0.3 (R Core Team, 2020) using the *lme4* package (Bates et al., 2015) as a mixed model, where storage time (i.e., 21 d wet-aged and SF storage time [SF60, SF75, SF90]) was set as a fixed effect and individual carcass as a random effect. Least-square means were calculated using the *emmeans* package (Length, 2020), and their differences are reported using a significance level of $\alpha = 0.05$ with Tukey's multiple comparison adjustment. Additionally, a mixed-effects binomial logistic regression model was used to estimate the probability of sensory trait acceptance and absence of off-flavor based on the storage conditions and times, where the carcass was set as a random effect and storage time as a fixed effect.

3.4 Results

3.4.1 Cooking loss

The percentage of cooking loss by cut and storage days is shown in Figure 3.1. The different storage conditions (i.e., SF or 21 d wet-aged) and times had no effect ($P \geq 0.05$) on cooking loss irrespective of subprimal cut.

3.4.2 Warner-Bratzler shear force (WBSF)

The WBSF values of all three cuts generally decreased with an increase in storage time, as shown in Figure 3.2. However, WBSF values of SF60 steaks were similar ($P \geq 0.05$) to the control steaks subjected to 21 d of wet aging, regardless of the cut. WBSF values for SF90 RE and SL steaks were lower ($P < 0.05$) than WBSF values of SF60 and 21 d wet-aged RE and SL samples.

For IR, SF75 and SF90 samples had lower ($P < 0.05$) WBSF values than steaks subjected to 21 d of wet aging, but were similar ($P \geq 0.05$) to those of SF60 steaks.

3.4.3 Sensory evaluation

Consumer sensory ratings for juiciness, tenderness, flavor, and overall liking are presented in Figures 3.3, 3.4, and 3.5 for IR, RE, and SL, respectively, and the probability of acceptance of these sensory traits and absence of off-flavor for all cuts, are shown in Table 3.2. As presented in Figure 3.3, IR sample scores for juiciness, flavor, and overall liking were similar ($P \geq 0.05$) between the storage conditions and days. Juiciness scores of IR samples (Figure 3.3A) ranged between 52.3 (SF90) and 57.2 (SF75), while flavor liking (Figure 3.3C) presented a slight numerical increase as storage time increased. Tenderness scores for IR SF75 steaks were higher ($P < 0.05$) than those of 21 d wet-aged samples, but were not different ($P \geq 0.05$) from the tenderness scores of SF60 and SF90 steaks (Figure 3.3B). The overall liking scores of all IR samples were similar ($P \geq 0.05$; Figure 3.3D). Additionally, all the sensory traits' acceptance probabilities were similar ($P \geq 0.05$) for IR steaks within storage condition and days (Table 3.2). However, the tenderness acceptability ($P = 0.1$) of SF75 steaks had a probability of 0.92, which is more than 7 points higher than the rest of the treatments. This result for the IR steaks follows the same pattern as the tenderness score, where SF75 steaks had the highest tenderness rating.

The results of the consumer sensory evaluation of the RE steaks are shown in Figure 3.4. Consumers could not detect ($P \geq 0.05$) any difference in the sensory attributes of RE steaks, regardless of the storage time or condition (Figure 3.4). Likewise, there were no differences ($P \geq 0.05$) in the probability of the absence of off-flavor and acceptability for juiciness, tenderness, flavor, and overall liking within each storage period (Table 3.2). As expected, the tenderness acceptability of RE steaks presented a probability higher than 0.91, regardless of the storage time (Table 3.2).

For the SL samples, there was no ($P \geq 0.05$) storage time effect on juiciness, flavor, and overall liking (Figure 3.5). However, consumers detected differences among the storage times for tenderness ratings of SL steaks. More specifically, tenderness scores of SF90 samples were greater ($P < 0.05$) than those of SF60 steaks but similar ($P \geq 0.05$) to tenderness scores of SF75 and 21 d wet-aged samples. Additionally, the probability of acceptance of all sensory traits evaluated and the absence of off-flavor on SL steaks did not ($P \geq 0.05$) differ by storage time (Table 3.2).

3.5 Discussion

Previous research has demonstrated that storage of beef below typical chilling temperatures (-0.5 to -1.5°C) can extend its shelf-life by up to 20 weeks (Small et al., 2012; Chen et al., 2019; Yang et al., 2022). Beef quality attributes such as WBSF, cooking loss, and color, following storage at temperatures below 0°C without freezing have been evaluated previously (Chen et al., 2019; Lu et al., 2019; Lu et al., 2020; Yang et al., 2022; Zhang et al., 2023). These studies have demonstrated that storage of vacuum-packaged beef below typical chilling temperatures can extend its shelf-life while improving postmortem tenderization. However, the effect of this extended storage on palatability traits has not been examined extensively. Palatability traits such as tenderness, juiciness, and flavor are critical for consumer acceptance and repurchase decisions (Smith and Carpenter, 1974; Lyford et al., 2010). In the current study, palatability characteristics, including cooking loss, WBSF, and sensory evaluations of three major beef cuts (IR, RE, and SL) following SF storage were assessed. In general, as storage time increased, WBSF values decreased, and tenderness rating increased, with no differences in cooking loss or juiciness, flavor, and overall liking.

Most of the weight loss that occurs during cooking is the result of water being expelled by protein degradation and muscle fiber contraction (Lepetit et al., 2000; Kondjoyan et al., 2013; Hughes et al., 2014). With extended storage time, a decrease in water holding capacity is expected, which can lead to an increase in cooking loss. However, in the current study, the storage condition and time did not have an effect on the cooking loss of the IR, RE, and SL steaks (Figure 3.1). Cao et al. (2022) evaluated the cooking loss of beef *longissimus dorsi* and reported that samples stored at 4°C had higher cooking loss than samples held at -1.5°C for the same length of time. Interestingly, under both storage conditions, cooking loss initially increased from 0 to 4 d and then decreased during the remainder of the storage time (Cao et al., 2022). In contrast, Lu et al. (2019) assessed the effect of extended storage at -4°C (0 to 24 weeks) and 2°C (0 to 8 weeks) on SL. Samples stored at -4°C had greater cooking loss than those held at 2°C (Lu et al., 2019). However, the cooking loss of samples held at -4°C for 8, 12, and 24 weeks were similar, suggesting that changes in cooking loss might not be significant after 8 weeks (56 d) of storage (Lu et al., 2019).

Tenderness is considered the most critical factor in overall eating satisfaction and consumers' acceptability of beef (Miller et al., 2001; Shackelford et al., 2001). In the present study, WBSF values of all three cuts tended to decrease with an increase in storage time, as shown in Figure 3.2. In general, initial tenderness (21 d wet-aged) for all the cuts met the Minimum Tenderness Threshold Value of less than 4.4 kg WBSF (ASTM, 2011). Furthermore, WBSF values of RE and SL steaks after 21 d of wet aging could be considered "very tender" (ASTM, 2011), and WBSF values of IR steaks achieved this threshold after SF60. The WBSF results of this study agree with previous studies that have observed a decrease in shear force with an increase in aging time (Gruber et al., 2006; Dixon et al., 2012; Colle et al., 2015; Karney et al., 2022). However, the results can vary depending on parameters such as storage temperature and muscle evaluated (Lu et al, 2019; Nair et al., 2019; Lu et al., 2020). For example, Lu et al. (2019) reported that WBSF values of SL steaks after 12 weeks of storage at -4°C were lower than those stored for 4 weeks at -4°C. The authors further reported that WBSF values of steaks after 8 weeks of chilled storage (2°C) were lower than those after 4 weeks of storage at the same temperature (Lu et al., 2019). However, WBSF values of samples after 4 weeks of chilled storage (2°C) were significantly lower than those held for 12 weeks at -4°C, indicating that the time and temperature of storage can have an impact on tenderness. Similarly, Karney et al. (2022) performed WBSF analyses on SL that had been wet-aged at 0°C for 14, 21, 28, 35, 49, or 63 d and reported that 49 and 63 d aged samples had the lowest WBSF values, while 21, 28, and 35 d aged steaks had similar values.

Consumers' eating satisfaction is crucial when evaluating new technologies to extend beef shelf-life since it is documented that consumers are willing to pay more for beef that meets their eating expectations (Lyford et al., 2010). Generally, beef palatability is attributed to juiciness, tenderness, and flavor (Smith and Carpenter, 1974), and these primary sensory traits were evaluated in the current study, along with overall liking. Juiciness refers to the sensation produced by meats with higher levels of juices, and is positively correlated with consumer preference (Maughan et al., 2012). The changes in the juiciness of steaks from the different SF storage periods varied depending on the cut evaluated. However, consumers could not detect differences ($P \geq 0.05$) in juiciness among any of the storage treatments. These results agreed with the cooking loss results (Figure 3.1), where no differences ($P \geq 0.05$) were observed, and also with the juiciness acceptability results, where there was no significant ($P \geq 0.05$) change with storage time (Table 3.2). Laster et al. (2008) performed consumer sensory evaluations on RE and SL steaks that were wet-aged at -0.6±1.8°C for 14, 21, 28, and 35 d (after initial aging for 9 d during shipment) and reported no differences in the juiciness levels of RE and SL steaks with the aging period.

In the current study, consumers were able to detect differences in tenderness with the storage time for IR and SL samples. However, they could not detect differences in tenderness for RE steaks, regardless of the storage time. This result is also in agreement with Laster et al. (2008) where consumers were unable to detect the differences in tenderness of RE after wet aging. The probability of acceptance of tenderness did not ($P \geq 0.05$) vary by storage time, irrespective of the cut (Table 3.2). Moreover, these observations in consumer evaluations were consistent with the WBSF results, where WBSF values of SF90 IR and SL steaks were more than 1 kg lower compared to IR and SL steaks aged for 21 days. Specifically, the WBSF values of SF75 and SF90 IR steaks were lower ($P < 0.05$) than those of 21 d wet-aged samples, and WBSF values of SF90 SL steaks were lower ($P < 0.05$) than those of SF60 samples. Although there was a statistical difference between WBSF values of SF90 RE steaks and WBSF values of SF60 and 21 d of wet-aged RE samples, the change was less than 1 kg which could explain why consumers could not perceive the difference (Miller et al., 1995).

Several studies have demonstrated that flavor is of great importance when tenderness is acceptable (Goodson et al., 2002; Killinger et al., 2004; Behrends et al., 2005), and a recent study indicated that it could have more influence than tenderness on overall consumer eating satisfaction of beef (O'Quinn et al., 2018). In the current study, as storage time increased, flavor scores tended to numerically increase for IR and SL steaks (Figures 3.3C and 3.4C), whereas flavor scores numerically decreased for RE steaks (Figure 3.4C); however, none of these changes were statistically significant ($P \geq 0.05$). The overall liking scores of samples were similar ($P \geq 0.05$) for all storage times, regardless of the cut evaluated (Figure 3.3D, 3.4D, and 3.5D). Similar to the present study, Colle et al. (2015) assessed flavor and overall liking of SL steaks aged (2, 14, 21, 42, and 63 d) at 0°C, and reported no differences in either attribute across the different aging periods. In contrast, Garmyn et al. (2020) evaluated flavor and overall liking every 7 d of SL aged at 2°C from 21 to 84 d and reported a significant decrease as postmortem aging time increased. Differences in results from the current study compared to others, where overall liking decreased with aging time, could be due to the lower temperature used during SF storage. Previously, Juarez et al. (2010) demonstrated that storage temperature could affect the off-flavor intensity with SL and IR steaks stored at 5°C having a greater off-flavor compared to steaks stored at 1°C.

3.6 Conclusions

Tenderness and palatability performance are important factors when evaluating new technologies as they affect consumers' eating satisfaction and repurchase decisions. In the current study, the WBSF values of IR, RE, and SL steaks decreased over SF storage time (up to 90 d), and perceived tenderness by consumers increased with no adverse effect on juiciness, flavor, and overall liking. These results suggested that storage temperatures lower than typical chilling temperatures, such as the one used in SF, could extend the storage life of beef while preserving or even improving sensory performance. Furthermore, with seasonality in beef price and consumption, these results can be useful to the meat industry to stabilize supply.

Table 3.1: Demographic characteristics of consumers (N = 238) who participated in the consumer sensory panels.

Characteristic	Response	Percentage of consumers
Gender	Male	50%
	Female	48%
	Other	2%
Age group	Under 20 years old	11%
	20 to 29 years old	49%
	30 to 39 years old	21%
	40 to 49 years old	7%
	50 to 59 years old	8%
	Over 60 years old	4%
Ethnic origin	African-American	1%
	Asian	6%
	Caucasian/white	65%
	Hispanic	21%
	Native American	1%
	Mixed race	2%
	Other	5%
Marital status	Single	65%
	Married	35%
Household size	1 person	29%
	2 people	36%
	3 people	17%
	4 people	10%
	5 people	4%
	6 people	3%
Annual household income	Under $25,000	37%
	$25,000 to $34,999	5%
	$35,000 to $49,999	10%
	$50,000 to $74,999	12%
	$75,000 to $99,999	10%
	$100,000 to 149,999	16%
	$150,000 to $199,999	4%
	> $199,999	5%
Education level	Non-high school graduate	2%
	High school graduate	7%
	Some college/technical school	26%
	College graduate	34%
	Post-college graduate	31%
Preferred beef degree of doneness	Very rare	2%
	Rare	6%
	Medium rare	49%
	Medium	26%
	Medium well	12%

	Well done	4%
	Very well done	2%
Beef consumption per week	Every other week	4%
	Weekly	15%
	2 to 3 times a week	37%
	4 to 5 times a week	19%
	Daily	25%

Table 3.2: Probability of absence of off-flavor and acceptability for juiciness, tenderness, flavor, and overall liking of inside round (IR), bone-in ribeye (RE), and striploin (SL) steaks (n = 10) that were in Suspended Fresh® storage (60, 75, or 90 d) or wet-aged for 21 d.

Cut	**Days of storage**	**Juiciness acceptability**	**Tenderness acceptability**	**Flavor acceptability**	**Absence of off-flavor**	**Overall acceptability**
IR	**21**	0.78	0.79	0.82	0.81	0.81
	60	0.82	0.85	0.84	0.84	0.78
	75	0.85	0.92	0.90	0.83	0.89
	90	0.71	0.81	0.91	0.76	0.82
	SE	0.08	0.07	0.05	0.06	0.05
	***P*-value**	**0.28**	**0.10**	**0.33**	**0.67**	**0.33**
RE	**21**	0.89	0.93	0.96	0.96	0.93
	60	0.83	0.92	0.94	0.87	0.83
	75	0.81	0.94	0.91	0.93	0.92
	90	0.76	0.91	0.89	0.94	0.87
	SE	0.05	0.04	0.04	0.06	0.05
	***P*-value**	**0.27**	**0.92**	**0.48**	**0.25**	**0.35**
SL	**21**	0.82	0.90	0.89	0.88	0.92
	60	0.82	0.91	0.91	0.89	0.92
	75	0.87	0.95	0.91	0.86	0.93
	90	0.85	0.95	0.84	0.85	0.87
	SE	0.05	0.04	0.05	0.05	0.05
	***P*-value**	**0.80**	**0.43**	**0.53**	**0.85**	**0.55**

SE: standard error, largest of the probabilities in the same column.

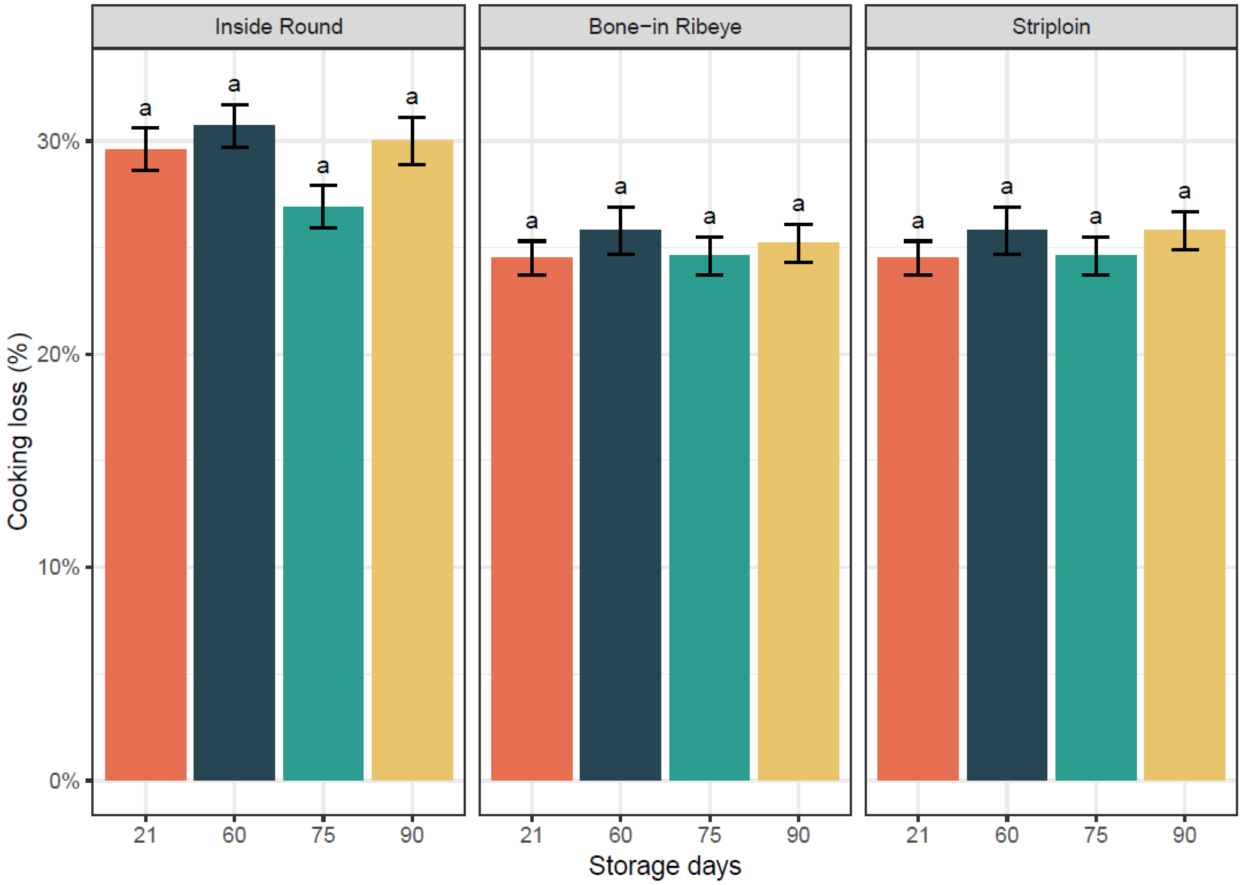

Figure 3.1: Effect of Suspended Fresh® storage times (60, 75, or 90 d) or 21 d of wet aging on the percentage of cooking loss of inside round (IR), bone-in ribeye (RE), and striploin (SL) steaks (n = 10). Same letters (a) above the error bars indicate similarity ($P \geq 0.05$). Error bars represent the standard error of the mean.

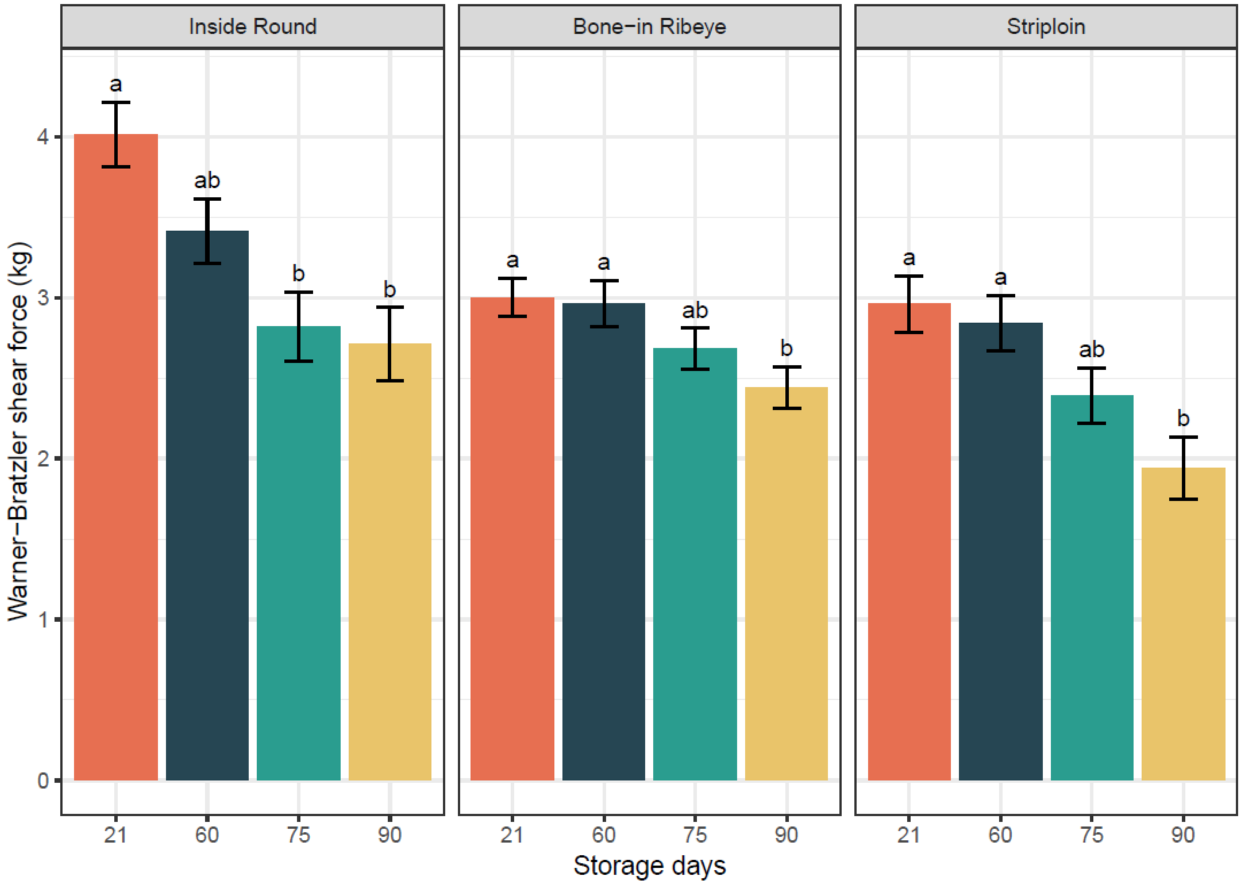

Figure 3.2: Effect of Suspended Fresh® storage times (60, 75, or 90 d) or 21 d of wet aging on Warner-Bratzler shear force (kg) of inside round (IR), bone-in ribeye (RE), and striploin (SL) steaks (n = 10). Different letters (a-b) above the error bars indicate significant differences ($P < 0.05$). Error bars represent the standard error of the mean.

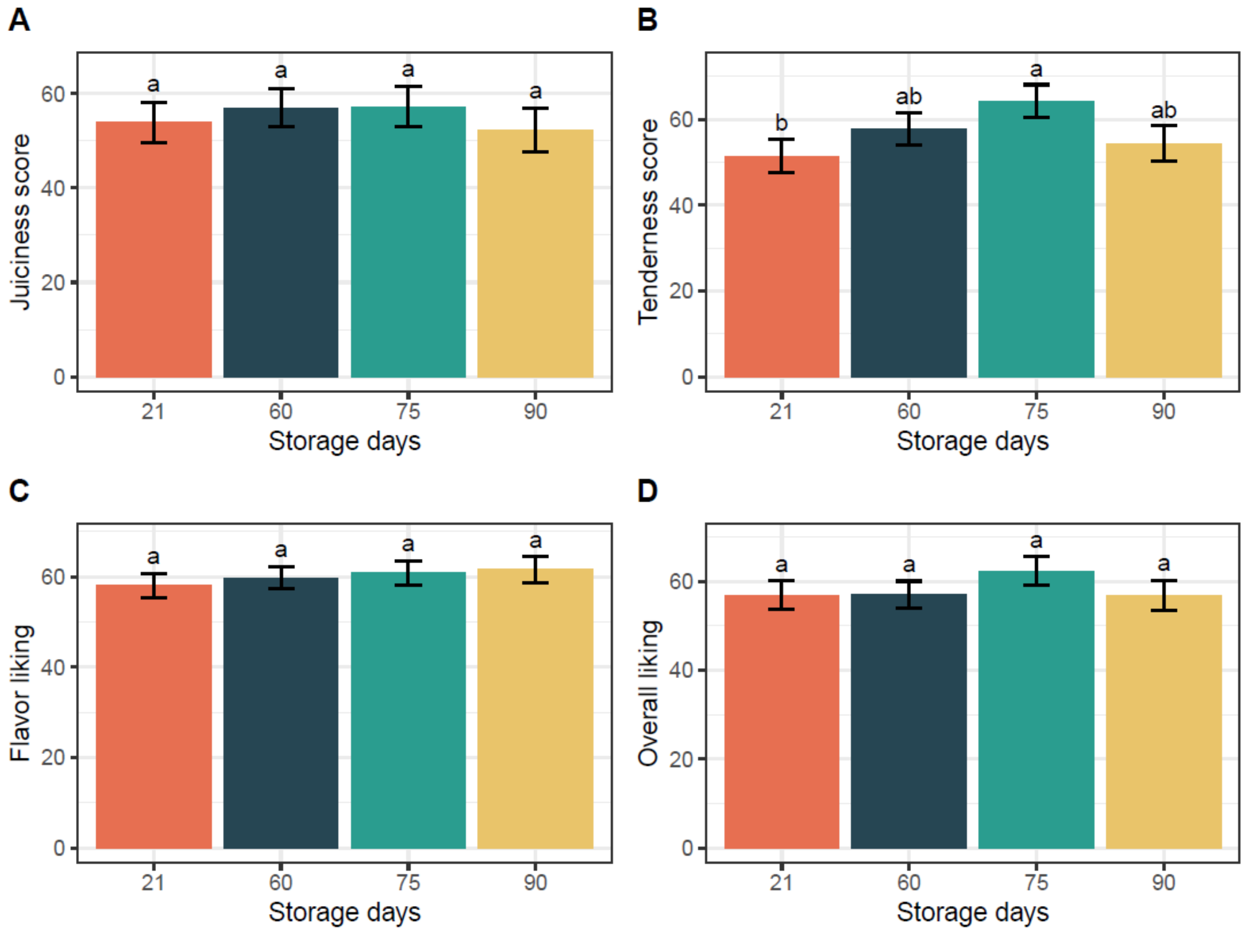

Figure 3.3: Consumer panelists (N = 79) sensory evaluation of inside round steaks (IR; n = 10) that were in Suspended Fresh® storage (60, 75, or 90 d) or were wet-aged for 21 d. A: juiciness with a scoring scale from 0 to 100 was used, with 0 being labeled as extremely dry, 50 neither juicy nor dry, and 100 extremely juicy; B: tenderness with a scoring scale from 0 to 100 was used, with 0 being labeled as extremely tough, 50 neither tough nor tender, and 100 extremely tender; C: flavor liking and D: overall liking using scoring scale from 0 to 100 with 0 being labeled as dislike extremely, 50 neither like nor dislike, and 100 like extremely. Different letters (a-b) above the error bars indicate significant differences ($P < 0.05$). Error bars represent the standard error of the mean.

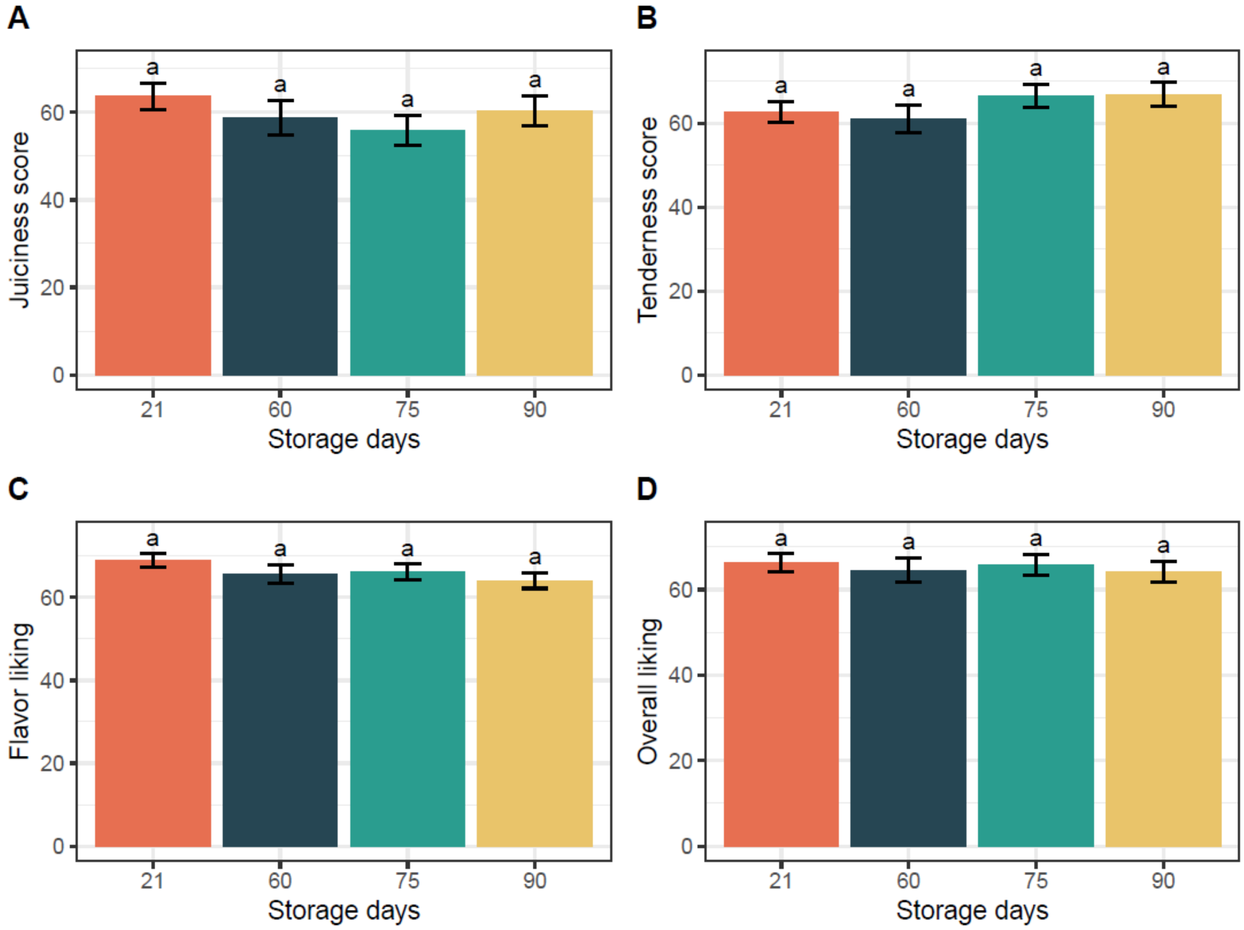

Figure 3.4: Consumer panelists (N = 79) sensory evaluation of ribeye steaks (RE; n = 10) that were in Suspended Fresh® storage (60, 75, or 90 d) or were wet-aged for 21 d. A: juiciness with a scoring scale from 0 to 100 was used, with 0 being labeled as extremely dry, 50 neither juicy nor dry, and 100 extremely juicy; B: tenderness using a scoring scale from 0 to 100 was used with 0 being labeled as extremely tough, 50 neither tough nor tender, and 100 extremely tender; C: flavor liking and D: overall liking using scoring scale from 0 to 100 was used with 0 being labeled as dislike extremely, 50 neither like nor dislike, and 100 like extremely. Same letters (a) above the error bars indicate similarity ($P \geq 0.05$). Error bars represent the standard error of the mean.

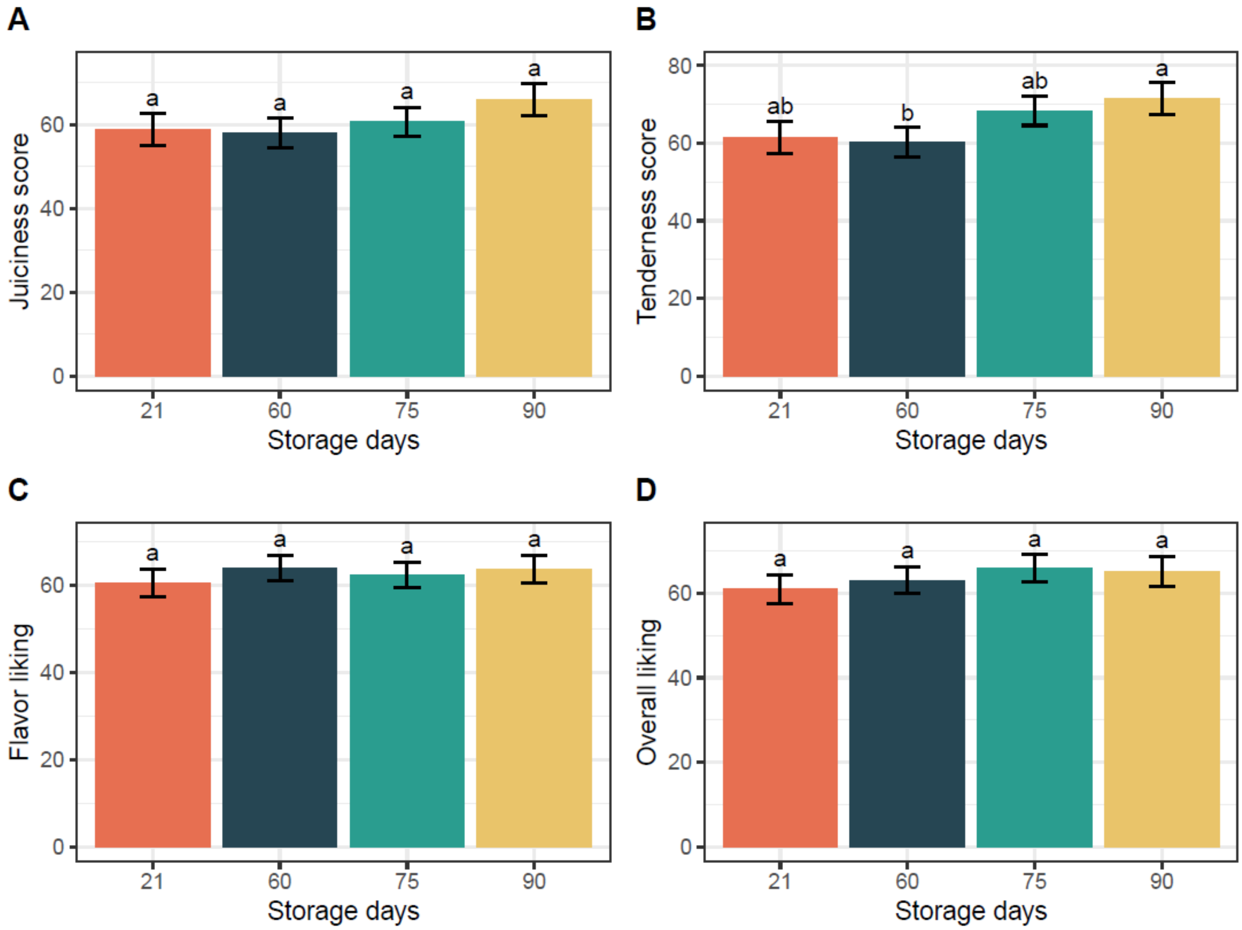

Figure 3.5: Consumer panelists (N = 80) sensory evaluation of striploin steaks (SL; n = 10) that were in Suspended Fresh® storage (60, 75, or 90 d) or were wet-aged for 21 d. A: juiciness with a scoring scale from 0 to 100 was used, with 0 being labeled as extremely dry, 50 neither juicy nor dry, and 100 extremely juicy; B: tenderness using a scoring scale from 0 to 100 was used with 0 being labeled as extremely tough, 50 neither tough nor tender, and 100 extremely tender; C: flavor liking and D: overall liking using scoring scale from 0 to 100 was used with 0 being labeled as dislike extremely, 50 neither like nor dislike, and 100 like extremely. Different letters (a-b) above the error bars indicate significant differences ($P < 0.05$). Error bars represent the standard error of the mean.

CHAPTER 4: OPTIMIZING SOUS VIDE COOKING TEMPERATURE AND DWELL TIME FOR BEEF PALATABILITY

4.1 Summary

Sous vide processing is a low-temperature long-time cooking method that allows the improvement of sensory attributes of tough meat cuts. However, beef palatability following sous vide can depend on the muscle used, cooking temperature, and time. This study evaluated the effects of different temperature and time treatment combinations (1A: 56.1°C and 71 min; 1B: 56.1°C and 150 min; 1C: 56.1°C and 240 min; 2A: 61.7°C and 8 min; 2B: 61.7°C and 150 min; 2C: 61.7°C and 240 min) of sous vide cooking on the palatability of beef *biceps femoris*. Beef *biceps femoris* were sliced into 1.6-cm steaks, vacuum packaged as 4.5 kg bags, and randomly assigned to one of the six treatments with 16 packages (n=16) per treatment. Cooked and chilled packages were weighed, and then the weight of the meat was taken to measure cooking loss. Weighed samples were divided into two halves: one was left non-marinated, and the other half was assigned to marination. From the non-marinated samples, two 1.6-cm steaks were randomly selected and cut in half for internal cooked color assessment. Additionally, non-marinated and marinated steaks were randomly selected for Warner Bratzler shear force (WBSF) and sensory analysis by a trained panel. Data were analyzed using a complete randomized design in R. Cooking loss of samples increased as the temperature and dwell time combinations increased ($P < 0.05$). Internal redness of cooked steaks decreased ($P < 0.05$) with increased temperature and dwell time. The only major difference in WBSF and the trained sensory panel results was between treatment 1C and 2A, where 1C samples had lower WBSF values and higher perceived tenderness scores

than 2A samples. These results suggest that *biceps femoris* samples can be cooked at conditions examined in this study with minimal impact on palatability, allowing producers more flexibility with cooking time to optimize production time and energy while reducing cooking loss.

4.2 Introduction

Consumer preference and willingness to pay more for beef that meets their eating expectations directly correlate with the satisfaction derived from cooked meat (Lyford et al., 2010; Polkinghorne and Thompson, 2010). The palatability of beef depends on three major components: tenderness, juiciness, and flavor (Smith and Carpenter, 1974). Tenderness has been widely recognized as a major determinant of overall eating satisfaction and consumer acceptability of beef (Miller et al., 2001; Shackelford et al., 2001; Miller, 2020). The cooking method and muscle used highly influence beef tenderness, where typically tough muscles are suggested to be cooked at relatively low temperatures, in moist environments (addition of water), for a longer time for maximum tenderness improvement. Moist heat cooking methods can reduce the amount of cook loss (Rowe and Kerth, 2013) and directly impact juiciness, as it is the positive sensation produced by meats with higher levels of juices (Maughan et al., 2012). In addition, beef flavor can be equally or more important than tenderness for overall consumer eating satisfaction (O'Quinn et al., 2018; Felderhoff et al., 2020; Miller, 2020), and it can be influenced by many factors, including the cooking method, and any added seasonings or marinades (Miller et al., 2022).

One cooking method gaining popularity in the food service industry is sous vide processing, which involves vacuum-sealing raw meat in a heat-stable package and immersing it in a temperature-controlled water bath for an extended period (Schellekens, 1996). Sous vide cooking offers numerous advantages over conventional methods, including precise temperature control, improved shelf-life, potential cost savings in labor and materials, and reduced cooking loss due to the moist environment (Baldwin, 2012; James and Yang, 2012; Roldán et al., 2014; Alahakoon et al., 2018; Ismail et al., 2022; Thathsarani et al., 2022). Additionally, several studies have demonstrated that the low-temperature long-time combination can improve the tenderness and different sensory attributes of low-value or tougher meat cuts (Mortensen et al., 2012; Christensen et al., 2013; Sun et al., 2019; Bhat et al., 2020; Naqvi et al., 2021c; Naqvi et al., 2021b; Gámbaro et al., 2023). Typical sous vide processing temperatures range from 50°C to 65°C (Ismail et al., 2022) since tenderness tends to increase in this range (Baldwin, 2012b). However, the impact of sous vide cooking on tenderness and collagen solubility varies based on the specific muscle used and the temperature-time combination (Shackelford et al., 1995; Baldwin, 2012; Purslow, 2018; Ismail et al., 2022).

In addition to its culinary benefits, sous vide cooking is advantageous for enhancing the shelf-life of cooked meat products by reducing microbial loads and the risk of external contamination during storage (Baldwin, 2012; Bıyıklı et al., 2020; Yang et al., 2020; Haghighi et al., 2021; Ismail et al., 2022; Thathsarani et al., 2022). However, when low temperatures are used, longer dwell times are needed to achieve the lethality of certain microorganisms. In the U.S., the Food Safety and Inspection Service (FSIS) provides cooking guidelines for the safe production of ready-to-eat meat and poultry products, known as Appendix A (FSIS, 2021). For final internal temperatures lower than 70°C, additional dwell time is needed, and this dwell time increases as the cooking temperature decreases (FSIS, 2021).

As the improvement of palatability and shelf-life of meat using sous vide cooking can depend on specific muscle, temperature, and time use, the current study was designed to evaluate the palatability characteristics of beef *biceps femoris* following different sous vide temperature and time treatment combinations.

4.3 Materials and Methods

4.3.1 Sample collection and preparation

Vacuum packaged and aged (37 days) beef *biceps femoris* subprimal packages were opened and sprayed with 875 ppm of sodium chlorite (Keeper Professional, Bio-Cide, Norman, OK) in a commercial sous vide facility. Each subprimal was fabricated into 1.6-cm slices using a vertical slicer (Graselli Model NSL600.BI). The sliced *biceps femoris* were weighed to a target weight of 4.5 kg, and packaged into thermal resistance bags (O_2 transmission 24 h/23°C 1.1 ss/sq. m, Winpak, Winnipeg, MB, Canada) and sealed using a Multivac R245 thermoforming machine (MULTIVAC Sepp Haggenmüller SE & Co. KG, Wolfertschwenden, Germany). Each package represented one sample, and 16 packages (n=16) were randomly assigned to one of six treatments (i.e., 96 samples total). Samples were cooked in custom-made sous vide tanks at the commercial production facility until they reached the target temperature (56.1 or 61.7°C) and held for the specific dwell time assigned to each treatment (1A: 56.1°C and 71 min; 1B: 56.1°C and 150 min; 1C: 56.1°C and 240 min; 2A: 61.7°C and 8 min; 2B: 61.7°C and 150 min; 2C: 61.7°C and 240 min). Shorter times for each temperature used were selected based on Appendix A for minimum lethality dwell time (i.e., 56.1°C – 71 min and 61.7°C – 8 min; FSIS 2021). After completion of each treatment dwell time, samples were chilled to < 4°C in custom-made sous vide tanks. Chilled packages were boxed and transported under refrigeration to the Department of Animal Sciences Global Food Innovation Center at Colorado State University (Fort Collins, CO). After arrival, samples were stored under refrigeration for at least 20 days, not exceeding 35 days, to simulate transportation and storage time in a food service establishment. Following storage, on each processing day, two packages (i.e., samples) per treatment were randomly used for analysis. Each sample was weighed and then steaks were aseptically removed from the packaging and placed in a sanitized bowl to obtain the weight of the meat without the packaging for determination of product cook loss. Weighed samples were divided into two halves: one was left non-marinated, and the other half was assigned to marination. From the non-marinated samples, two 1.6-cm steaks were randomly selected and cut in half for internal cooked color assessment. Additionally, another two non-marinated steaks were randomly selected and saved for WBSF and sensory analysis.

4.3.2 Cooking loss

Cooking loss for each sample was determined by taking the weight of the whole package (total weight), the weight of the meat without packaging (meat weight), and the weight of the dry package. The cooking loss was expressed as a percentage relative to total weight using the following formula: % Cooking loss = [(total weight - (meat weight + package weight)) / (total weight)] × 100.

4.3.3 Internal cooked color evaluation

Samples designated for color evaluation were cut in half and allowed to oxygenate for at least 30 min. The instrumental color measurements were obtained with a portable HunterLab MiniScan LabScan EZ4500 colorimeter (Hunter Associates Laboratory, Reston, VA) equipped with a 12.5-mm measurement port (2.54-cm diameter aperture, illuminant A, and 10° standard observer). The instrument was standardized before each use, using standard tiles. Color measurements (3 technical replicate readings) for CIE L^* (lightness), a^* (redness), and b^* (yellowness) were obtained at the internal section. The technical replicate readings were averaged for statistical analysis.

4.3.4 Marination process and reheating of marinated samples

A commercial marination paste (water, chipotle chili, rice bran oil, cumin, garlic, black pepper, and oregano) was added at 6.4% (64 g per kilogram of meat) to the sample halves assigned for marination. The meat samples were thoroughly mixed and coated with the marinade and then placed in a pan liner inside of a metal pan (32.38 cm length x 17.78 cm width x 10.16 cm height). Samples were marinated under refrigeration for 20 ± 1 h before reheating. Following marination, samples were reheated to an internal temperature of 60°C using a flap-top grill. A thermometer (Thermapen Mk4, Thermoworks Inc., American Fork, UT) placed in the geometric center of each sample was used to determine the internal temperature of the reheated sample.

4.3.5 Warner-Bratzler shear force (WBSF)

Both marinated and non-marinated samples were randomly assigned for Warner Bratzler shear force (WBSF) analysis. The reheated marinated samples were allowed to cool down to 4°C under refrigeration before shear force evaluation. The samples were trimmed of visible connective tissue to expose muscle fiber orientation and cut into six strips (1 cm^2 × cooked steak thickness) parallel to the muscle fiber. Strips were sheared once, perpendicular to the muscle fibers, using a universal testing machine (Instron Corp., Canton, MA) fitted with a Warner-Bratzler shear head (crosshead speed: 200 mm/min, load cell capacity: 100 kg). Peak shear force was recorded, and values from the strips taken from each sample were averaged to obtain a single WBSF value for each sample. The average peak shear force of the strip was used for statistical analysis.

4.3.6 Trained sensory evaluation

The Colorado State University Institutional Review Board (IRB) reviewed the procedures (IRB #4408) used in this study and determined it as not human subjects research (§45

CFR46.102(l)). Each sample was identified with a random three-digit number for sensory evaluations. For the non-marinated and marinated meat samples, 96 samples for each were randomly assigned to one of 8 panels, with 12 samples evaluated per panel and two samples representing a different sous vide cooking temperature and time combination treatment. Samples were also randomly assigned a serving order within each panel. Before panel evaluations, panelists were trained to evaluate beef flavor, tenderness, juiciness, sour, and metallic flavor on a fifteen-point continuous scale. Panel anchors for these attributes were trained with references set by Adhikari et al. (2011). Each sample was evaluated by a trained sensory panel of at least six qualified panelists with attribute training specifications indicated in Table 4.1. All data were collected using Qualtrics software.

For non-marinated samples, steaks designated for sensory analysis were vacuum packaged and placed for 30 min in a circulator water bath (Isotemp 6200 H24, Fisher Scientific, Waltham, MA) set at 55°C to reheat them. These samples were kept in the water bath (55°C) to maintain temperature throughout the sensory panel. Samples were cut into 1.9-cm^2 squares and served in the predetermined serving order. Panelists were provided unsalted saltine crackers (Nabisco Unsalted Tops Premium; Mondelez Global LLC, East Hanover, NJ), deionized water, and unsweetened apple juice to use as palate cleansers between samples.

For sensory evaluation of the marinated samples, steaks were reheated (as described previously under section 4.3.5) according to the serving order. Samples were cut into 1.9-cm^2 squares and kept warm in a holding cabinet (1000-UP, Alto-Shaam, Menomonee Falls, WI, USA) at 55°C during the panel sessions. Panelists were served 2-3 pieces of the sample and allowed 3 min between samples to cleanse their palate with a plain bagel (Thomas' Plain; Bimbo Bakeries, Horsham, PA, USA), deionized water, and unsweetened apple juice.

4.3.7 Statistical analysis

All statistical analyses were conducted in R statistical software version 4.0.3 (R Core Team, 2020). A complete randomized design was used where the effect of each treatment was evaluated. All marginal means were calculated using the *emmeans* package (Length, 2020). The differences between means were reported using a significance level of $\alpha = 0.05$ with Tukey's multiple comparison adjustment.

4.4 Results

4.4.1 Cooking loss

The percentage cooking loss by treatment (i.e., sous vide cooking temperature and dwell time) is shown in Figure 4.1. The different cooking temperatures and dwell times affected cooking loss ($P < 0.05$). The cooking loss percentage increased as temperature and dwell time increased ($P < 0.05$). More specifically, treatment 2C (61.7°C and 240 min) samples had the highest ($P < 0.05$) percentage of cooking loss (22.8%), while those cooked following treatment 1A (56.1°C and 71 min) had the lowest ($P < 0.05$) percentage of cooking loss (14.5%). Within the three dwell times of the 56.1°C treatment, the percentage of cooking loss of samples with a dwell time of 150 and 240 min was similar ($P \geq 0.05$) but greater ($P < 0.05$) than that of the 71 min dwell time samples. However, for those samples cooked at 61.7°C, as dwell time increased (8, 150, 240 min), cooking loss percentage of samples increased ($P < 0.05$).

4.4.2 Internal cooked color

Internal cooked surface lightness (L^*), redness (a^*), and yellowness (b^*) for all treatments are presented in Table 4.2. No treatment effect was observed ($P \geq 0.05$) for L^* values of samples.

However, treatment influenced a^* and b^* values of the samples ($P < 0.05$). The internal surface redness (a^* values; Figure 4.2) of samples decreased ($P < 0.05$) with increase in temperature and dwell time. Samples cooked at 61.7°C had lower ($P < 0.05$) a^* values than those cooked at 56.1°C, regardless of the dwell time. For samples cooked at 56.1°C there was a gradual decrease in a^* values with increase in dwell time. The 71 min dwell time samples (treatment 1A) had greater ($P < 0.05$) redness than the 240 min dwell time samples (1C). However, the 150 min dwell time samples (treatment 1B) had similar ($P \geq 0.05$) redness to both the 71 min and 240 min dwell time samples. Like surface redness, yellowness (b^* values) decreased ($P < 0.05$) as temperature and dwell time increased (Table 4.2). For instance, samples from treatments 2B and 2C had lower ($P < 0.05$) b^* values than those from treatments 1A and 1B, but similar ($P \geq 0.05$) to those of treatments 1C and 2A.

4.4.3 Warner-Bratzler shear force (WBSF)

The WBSF values of the non-marinated and marinated steaks are shown in Table 4.3. No ($P \geq 0.05$) treatment effect was observed for the WBSF values of the non-marinated steaks. The average WBSF value for all non-marinated samples was 4.13 kg, with the highest numerical WBSF value being 4.47 kg and the lowest 3.64 kg for treatments 1A and 1C, respectively. In contrast, treatment had a significant ($P < 0.05$) effect on the WBSF values of the marinated steaks. Marinated steaks from treatment 1C had the lowest ($P < 0.05$) WBSF values; however, they were only different ($P < 0.05$) from treatment 2A (61.7°C and 8 min) steaks.

4.4.4 Trained sensory evaluation

Trained sensory ratings for the non-marinated and marinated steaks on beef flavor, tenderness, juiciness, sour, and metallic flavors are presented in Table 4.4. For non-marinated samples, the treatment effect was significant ($P < 0.05$) only for tenderness and juiciness. Tenderness scores for treatment 1C steaks were slightly higher ($P < 0.05$) than those of treatment 2A steaks but were similar ($P \geq 0.05$) to the other treatments (1A, 1B, 2B, and 2C). Juiciness scores of treatment 1B and 1C steaks were higher ($P < 0.05$) than steaks subjected to the 2A treatment; however, they were not different ($P \geq 0.05$) from treatments 1A, 2B, and 2C. All sample scores for sour and metallic flavors were below 2, described as "barely detectable" irrespective of treatment.

Regardless of the evaluated attribute, there was no ($P \geq 0.05$) treatment effect for the marinated steaks. Tenderness scores of steaks ranged between 10.2 (2A and 2B) and 11.7 (2C), and juiciness scores from 4.8 (2A) to 5.9 (2C), with an average value of 5.3. Similar to non-marinated steaks, the sour and metallic flavor scores of marinated steaks were all below "barely detectable."

4.5 Discussion

Several studies have evaluated the effect of sous vide cooking on physicochemical and sensory characteristics of beef (Mortensen et al., 2012; Christensen et al., 2013; Alahakoon et al., 2018; Ismail et al., 2019; Naqvi et al., 2021c; Naqvi et al., 2021b; Naqvi et al., 2021a; Gámbaro et al., 2023). These studies have shown that sous vide cooked meat at low temperatures and for a long time improves sensory attributes of lower-quality meat muscles. However, results can vary with the cooking parameters (temperature and time) and the meat cut used. The current study assessed sensory characteristics, including cooking loss, internal cooked color, WBSF, and trained sensory evaluations of beef *biceps femoris* following sous vide cooking. In general, cooking loss increased as cooking temperature and time increased, while internal surface redness decreased.

Overall, sous vide cooked steaks at a lower temperature for a longer time had better performance in tasting sensory evaluations.

Cooking loss is of great economic importance to the food service industry since it is a loss of weight. When cooking meat, the water-holding capacity of proteins decreases due to protein degradation that results in water being expelled from the muscle (Lepetit et al., 2000; Kondjoyan et al., 2013). Slow-cooking conditions, such as sous vide, can minimize fluid loss, as meat is cooked in moist conditions (James and Yang, 2012; Roldán et al., 2014). Still, as the temperature and cooking time increases, the cooking loss percentage also increases (Vaudagna et al., 2002; García-Segovia et al., 2007; Christensen et al., 2011; Roldán et al., 2013; Ismail et al., 2019; Naqvi et al., 2021c; Naqvi et al., 2021b). In agreement with findings of previous studies, an increase in percentage cooking loss was observed in our study as the treatment temperature and time increased. Specifically, samples from the 1A treatment, cooked at the low temperature and with the shortest dwell time, had less ($P < 0.05$) cooking loss than samples from the rest of the treatments. Additionally, samples from treatment 2C, cooked at the highest temperature and for the longest dwell time, had the highest ($P < 0.05$) percentage cooking loss. Likewise, Niqvi et al. (2021c) evaluated the effects of sous vide cooking temperature (55°C, 65°C or 75°C) and time (1 h, 8 h or 18 h) on cooking loss of beef *biceps femoris* and reported that as cooking temperature and time increased, cooking loss increased (Naqvi et al., 2021c). For example, the lowest cooking loss percentages were for samples cooked at 55°C for 1 h, and the highest cooking loss percentages were those from samples cooked at 75°C for 8 and 18 h (Naqvi et al., 2021c).

The internal color of cooked beef is an indicator of its degree of doneness. The degree of doneness is dependent on the final or highest internal temperature reached for the cooked meat (Baldwin, 2012b; Ismail et al., 2022). Myoglobin is the main protein responsible for meat color.

Although distinct from fresh meat, the brown color formed in cooked meat results from myoglobin denaturation due to increased temperature. In general, in sous vide cooking, the cooking temperature will play a more prominent role than the cooking time when evaluating the changes in the color of cooked meat (Ismail et al., 2022). In the current study, the a^* (redness) and b^* (yellowness) values of cooked steaks were influenced by treatment, where the cooking temperature played more of a role than time. In contrast, L^* values (lightness) of samples were not affected by the treatments evaluated. Other studies have reported either increased or decreased L^* values of sous vide cooked meat with different cooking temperatures and times (García-Segovia et al., 2007; Roldán et al., 2013; Alahakoon et al., 2018). For example, Alahakoon et al. (2018) observed a decrease in L^* value of beef *deep pectoralis* samples when temperatures increased (60, 65, and 70°C) and an increase in L^* value when cooking time increased (24, 48, and 72 h) within the same temperature. This variation in L^* observed might be because other intrinsic and extrinsic factors, such as muscle type and aging time, are more influential than cooking time (Ismail et al., 2022). On the other hand, in the current study, redness (a^*) decreased as treatment temperature and time increased. As temperature rises, myoglobin denaturation increases; however, the thermal denaturation rate depends on the myoglobin redox form (Hunt et al., 1999; King and Whyte, 2006). Since the internal cooked color of vacuum-packaged steaks was evaluated in this study, myoglobin could be in the deoxymyoglobin form during the thermal process, which is the least sensitive to heat denaturation (Hunt et al., 1999; King and Whyte, 2006). Therefore, high a^* values were observed in steaks from treatments cooked using 56.1°C. Additionally redness decreased as dwell time increased because the longer meat is held at a particular temperature, it becomes paler (Charley and Weaver, 1998; Baldwin, 2012). Even at 61.7°C there was relatively high redness, because even though myoglobin denaturation starts at 55°C, deoxymyoglobin (the predominant redox form in vacuum packaged meat) is not fully denatured until 75°C (Hunt et al., 1999). In agreement with these results, Ismail et al. (2019) found that beef *semitendinosus* steaks that were sous vide cooked at a lower temperature (60°C) and shorter time (6 h) presented a higher internal surface redness than those samples cooked at the same temperature for 12 h or at higher temperatures (65, 70, and 75°C). Furthermore, Gambaro et al. (2023) evaluated the interior color of beef hind shank sous vide cooked at different temperatures (55 and 65°C) and times (5, 8, 12, and 24 h), and reported that the $a*$ values of samples cooked at 55°C decreased with an increase in cooking time, but were higher than all samples cooked at 65°C, regardless of the cooking time. Similar observations were also reported by Vaudagna et al. (2002) for beef *semitendinosus* sous vide cooked at 50, 60, or 65°C up to 390 min, and Roldan et al. (2013) for lamb loins sous vide cooked at 60, 70, or 80°C for 6, 12, or 24 h. However, there were no significant changes in $a*$ values of samples held for a longer time at the same temperature in these studies.

The internal surface yellowness ($b*$) of samples followed a similar pattern as redness, where $b*$ values decreased with treatment temperature and time increment. Likewise, Gambaro et al. (2023) found similar observations for beef shank. However, other authors (García-Segovia et al., 2007; Alahakoon et al., 2018; Ismail et al., 2019) saw an increase in $b*$ values of beef cooked samples as sous vide temperature (60°C, 65°C, 70°C, 75°C) or time (0.5 h, 0.75 h, 1 h, 6 h, 12 h, 24 h, 48 h, 72 h) increased. These authors attributed the increase in yellowness to a rise in myoglobin denaturation, which results in ferrihemochrome formation, the brown color of cooked beef. Differences in results with our study can be due to higher temperature and time combinations used in these mentioned studies.

One of the major advantages of sous vide cooking of meats is to improve low-value tougher cuts by increasing their tenderness (Alahakoon et al., 2018; Ismail et al., 2019). In this study, WBSF values of non-marinated steaks were similar regardless of the cooking treatment, even though there were some numerical differences between the treatments. One contributing factor to the lack of statistical significance could be the relatively large variation within samples (SEM of 0.27) for *biceps femoris* muscle. Even so, WBSF values of steaks that were marinated following treatment and grill reheated were influenced by treatment. The lowest ($P < 0.05$) WBSF value for marinated samples was from treatment 1C (56.1°C for 240 min); in accordance, non-marinated samples from the same treatment had numerically the lowest WBSF values. Several studies have found significant changes in WBSF values of beef muscles depending on cooking temperature and time (Vaudagna et al., 2002; Christensen et al., 2013; Alahakoon et al., 2018; Ismail et al., 2019; Bhat et al., 2020; Naqvi et al., 2021a; Naqvi et al., 2021c; Naqvi et al., 2021b; Karki et al., 2022; Gámbaro et al., 2023), promoted from the breakdown of connective tissue as a result of protein denaturation or collagen solubilization caused by prolonged heating (Dominguez-Hernandez et al., 2018).

Beef palatability is of great importance when evaluating cooking methods, and as mentioned before, it is generally attributed to juiciness, tenderness, and flavor (Smith and Carpenter, 1974). In this study, trained panelists evaluated the intensity of beef flavor, tenderness, juiciness, and sour and metallic flavors of beef samples. The different treatments did not affect the intensity of beef, sour, and metallic flavors for non-marinated steaks. Similarly, Naqvi et al. (2021b) evaluated the beef flavor and metallic flavor of *biceps femoris* steaks sous vide cooked at 65°C for 8 or 12 h using trained panels and reported no differences between the treatments. Tenderness is of great importance in overall eating satisfaction and consumers' acceptability of beef (Miller et al., 2001; Shackelford et al., 2001). The tenderness scores of non-marinated samples generally increased with longer dwell time and were higher for the lower temperature. The highest tenderness score was for samples from treatment 1C (56.1°C and 240 min), and this finding agreed with the WBSF results. Likewise, the previously mentioned Naqvi et al. (2021b) study observed an increased tenderness rating of beef samples cooked for 12 h compared to those cooked for 8 h at 65°C. Moreover, Gambaro et al. (2023) reported that the tenderness scores within the same temperature significantly increased as cooking time (5, 8, 12 or 24 h) increased, with samples cooked for 24 h at 55 and 65°C having the highest rating (Gámbaro et al., 2023). However, tenderness scores between cooking temperatures of samples were only different following 12 h of cooking time, where samples cooked at 65°C had a higher tenderness rating than those cooked at 55°C (Gámbaro et al., 2023). Furthermore, Mortensen et al. (2012) reported an increase in tenderness scores with an increase in cook time (3, 6, 9, or 12 h) for veal *semitendinosus* sous vide at different temperatures (56, 58, and 60°C), with the highest rating observed for those samples cooked at 56°C for 12 h.

Juiciness is an essential sensory attribute positively correlated with consumer preference, and it is defined as the sensation produced by meats with higher levels of juices (Maughan et al., 2012). Similar to the tenderness scores, juiciness of non-marinated samples tended to increase with an increase in dwell time, with samples from treatment 1C having the highest score, but their score was statistically different only from the treatment 2A samples. These results contrast with the cooking loss results (Figure 4.1), where a cooking loss percentage increase was observed as the treatment temperature and time increased. These results, where perceived juiciness increased as perceived tenderness increased, could be due to the halo effect of tenderness and the correlation between these sensory attributes (Miller, 2020). Similarly, Gambaro et al. (2023) observed a numerical increase in juiciness when cooking time increased for those samples cooked at 55°C. However, samples cooked at 65°C had lower juiciness scores than those cooked at 55°C.

Moreover, Naqvi et al. (2021b), that also evaluated beef *biceps femoris* did not find any differences in the juiciness rating of samples cooked at 65°C for 8 or 12 h.

Sensory evaluations for marinated and grilled reheated steaks did not differ, regardless of the treatment temperature and time. The average tenderness and juiciness scores for all samples were 10.8 and 5.3, respectively, numerically similar to those of non-marinated steaks. These results could be due to marination masking any potential differences in the sensory attributes, which could benefit food service restaurants that typically marinate the meat before reheating them.

4.6 Conclusions

Cooking loss, tenderness, and palatability are critical factors when evaluating different sous vide cooking temperatures and times as they affect consumers' eating satisfaction as well as food service profits. In the current study, the percentage of cooking loss increased with sous vide temperature and time. However, the internal surface redness decreased with increased sous vide temperature and time. Additionally, the only major difference in WBSF and trained sensory panel evaluations was between treatment 1C (56.1C and 240 min) and 2A (61.7C and 8 min), where treatment 1C samples had lower WBSF values and higher perceived tenderness scores than treatment 2A samples. Moreover, there was no off-flavor (sour or metallic) detected with any of the cooking temperature-time combinations. These results suggest that *biceps femoris* samples can be cooked at conditions examined in this study with minimal impact on palatability. This will give producers more flexibility with cooking time and enables them to optimize production time and energy while reducing cooking loss.

Table 4.1: Definitions and references for beef flavor attributes and intensities[1].

Attribute	Definition	Reference
Beef flavor	Amount of beef flavor identity in the sample	Swanson® beef broth = 5.0 (aroma and flavor) 80% lean ground beef = 7.0 (aroma and flavor) Beef brisket = 11.0 (aroma and flavor)
Metallic	The impression of slightly oxidized metal, such as iron, copper, and silver spoons	USDA choice strip steak = 4.0 (aroma and flavor) Dole® canned pineapple juice = 6.0 (aroma and flavor)
Sour	Fundamental taste factor associated with citric acid	0.015% citric acid solution = 1.5 (flavor) 0.050% citric acid solution = 3.5 (flavor)
Flavor Intensities	Universal scale for flavor intensities	2.0 - Soda flavor in saltine crackers 5.0 - Apple flavor in Motts® apple sauce 10.0 - Grape flavor in Welch's® grape juice 12.0 - Cinnamon flavor in Big Red® chewing gum
Tenderness	Refers to the ease perceived during masticating	9.0 - Eye of round 160°F 14.0 - Tenderloin 150°F
Juiciness	Refers to the sensation produced by meats with higher levels of juices	2. 0 - Carrot 8.0 - Cucumber 10.0 - Apple 15.0 - Watermelon

[1] Intensity scale: 0 = none; 2 = barely detectable; 4 = identifiable but not very intense; 6 = slightly; 8 = moderately intense; 10 = intense; 15 = extremely intense

Table 4.2: Effect of sous vide cooking temperature and dwell time on internal surface lightness (L^* value) and yellowness (b^* value) of *biceps femoris* steaks (n = 16).

Cook temperature	**56.1°C**			**61.7°C**			**SE**
Dwell time (min)	**71 (1A)**	**150 (1B)**	**240 (1C)**	**8 (2A)**	**150 (2B)**	**240 (2C)**	
L^*	52.3^{a}	50.5^{a}	50.9^{a}	51.3^{a}	51.1^{a}	50.6^{a}	0.7
b^*	21.9^{a}	21.0^{ab}	19.7^{abc}	19.0^{bc}	18.4^{c}	18.3^{c}	0.6

SE: standard error of the mean
$^{a\text{-}c}$ Least squares means with different superscripts within a row are different ($P < 0.05$).

Table 4.3: Effect of sous vide cooking temperature and dwell time on Warner-Bratzler shear force (kg) of *biceps femoris* steaks (n = 16).

Cook temperature	**56.1°C**			**61.7°C**			**SE**
Dwell time (min)	**71 (1A)**	**150 (1B)**	**240 (1C)**	**8 (2A)**	**150 (2B)**	**240 (2C)**	
Non-marinated	4.47^{a}	3.70^{a}	3.64^{a}	4.41^{a}	4.50^{a}	4.07^{a}	0.27
Marinated	3.88^{ab}	3.83^{ab}	3.43^{b}	4.57^{a}	3.93^{ab}	4.15^{ab}	0.21

SE: standard error of the mean

$^{a\text{-}b}$ Least squares means with different superscripts within a row are different ($P < 0.05$).

Table 4.4: Effect of sous vide cooking temperature and dwell time on trained sensory panelists attributes from *biceps femoris* steaks (n = 16).

Steak marination	**Cook temperature**	**56.1°C**			**61.7°C**			**SE**
	Dwell time (min)	**71 (1A)**	**150 (1B)**	**240 (1C)**	**8 (2A)**	**150 (2B)**	**240 (2C)**	
Non-marinated	Beef flavor[1]	7.2^{a}	7.0^{a}	7.4^{a}	7.2^{a}	7.4^{a}	6.7^{a}	0.4
	Tenderness[1]	10.2^{ab}	11.0^{ab}	11.4^{a}	9.1^{b}	11.0^{ab}	10.6^{ab}	0.5
	Juiciness[1]	5.1^{ab}	5.9^{a}	6.4^{a}	4.4^{b}	5.8^{ab}	5.6^{ab}	0.4
	Sour[1]	0.9^{a}	0.9^{a}	0.5^{a}	0.6^{a}	0.7^{a}	0.7^{a}	0.2
	Metallic[1]	1.2^{a}	0.9^{a}	0.6^{a}	0.8^{a}	1.0^{a}	1.1^{a}	0.2
Marinated	Beef flavor[1]	7.6^{a}	7.0^{a}	6.9^{a}	7.5^{a}	7.5^{a}	7.1^{a}	0.4
	Tenderness[1]	10.8^{a}	10.7^{a}	10.9^{a}	10.2^{a}	10.2^{a}	11.7^{a}	0.5
	Juiciness[1]	5.4^{a}	5.2^{a}	5.4^{a}	4.8^{a}	5.1^{a}	5.9^{a}	0.4
	Sour[1]	0.6^{a}	0.6^{a}	0.8^{a}	1.4^{a}	0.8^{a}	1.0^{a}	0.2
	Metallic[1]	1.0^{a}	0.5^{a}	1.2^{a}	1.3^{a}	1.4^{a}	1.5^{a}	0.3

SE: standard error of the mean

$^{a-b}$ Least squares means with different superscripts within a row are different ($P < 0.05$).

[1] 0 = none; 2 = barely detectable; 4 = identifiable but not very intense; 6 = slightly; 8 = moderately intense; 10 = intense; 15 = extremely intense

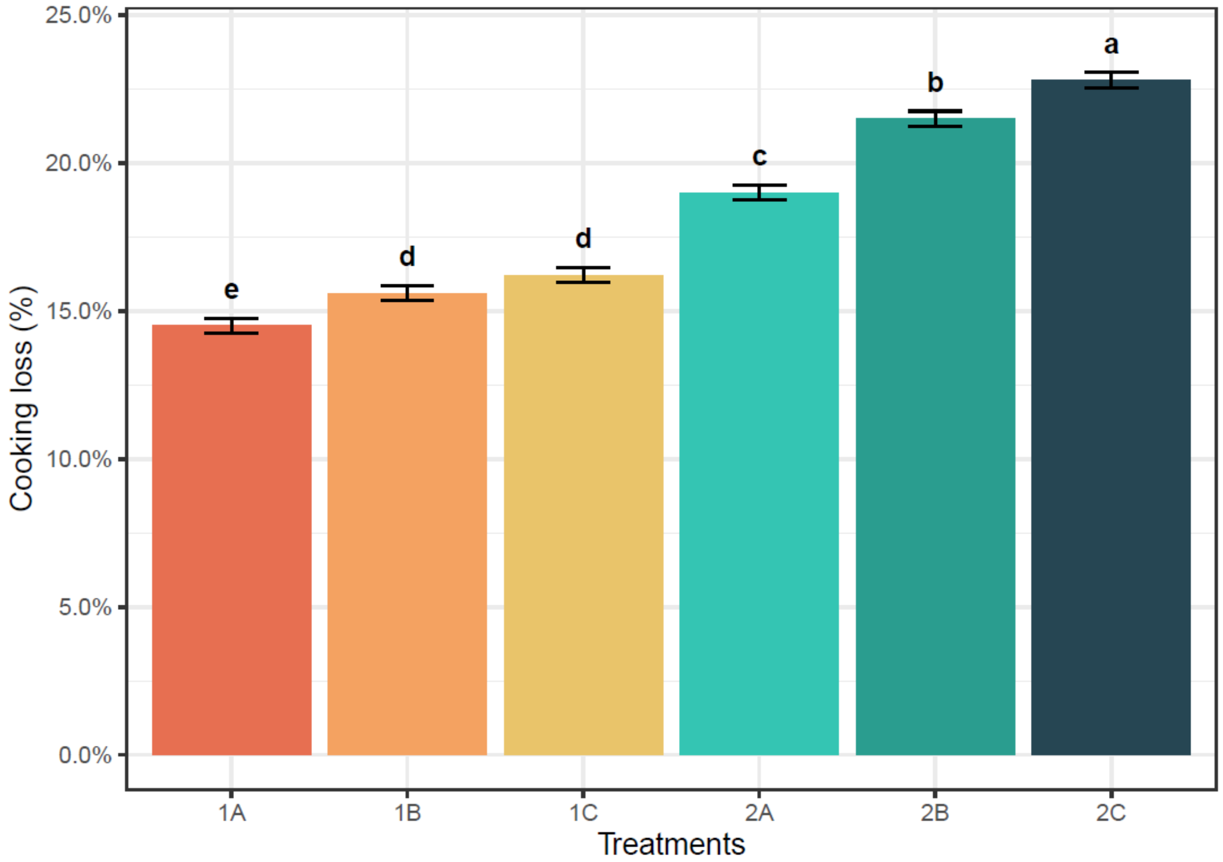

Figure 4.1: Effect of sous vide cooking temperature and dwell time (1A: 56.1°C and 71 min; 1B: 56.1°C and 150 min; 1C: 56.1°C and 240 min; 2A: 61.7°C and 8 min; 2B: 61.7°C and150 min; 2C: 61.7°C and 240 min) on the percentage of cooking loss of *biceps femoris* steaks (n = 16). Different letters (a-e) indicate significant differences ($P < 0.05$). Error bars represent the standard error of the mean.

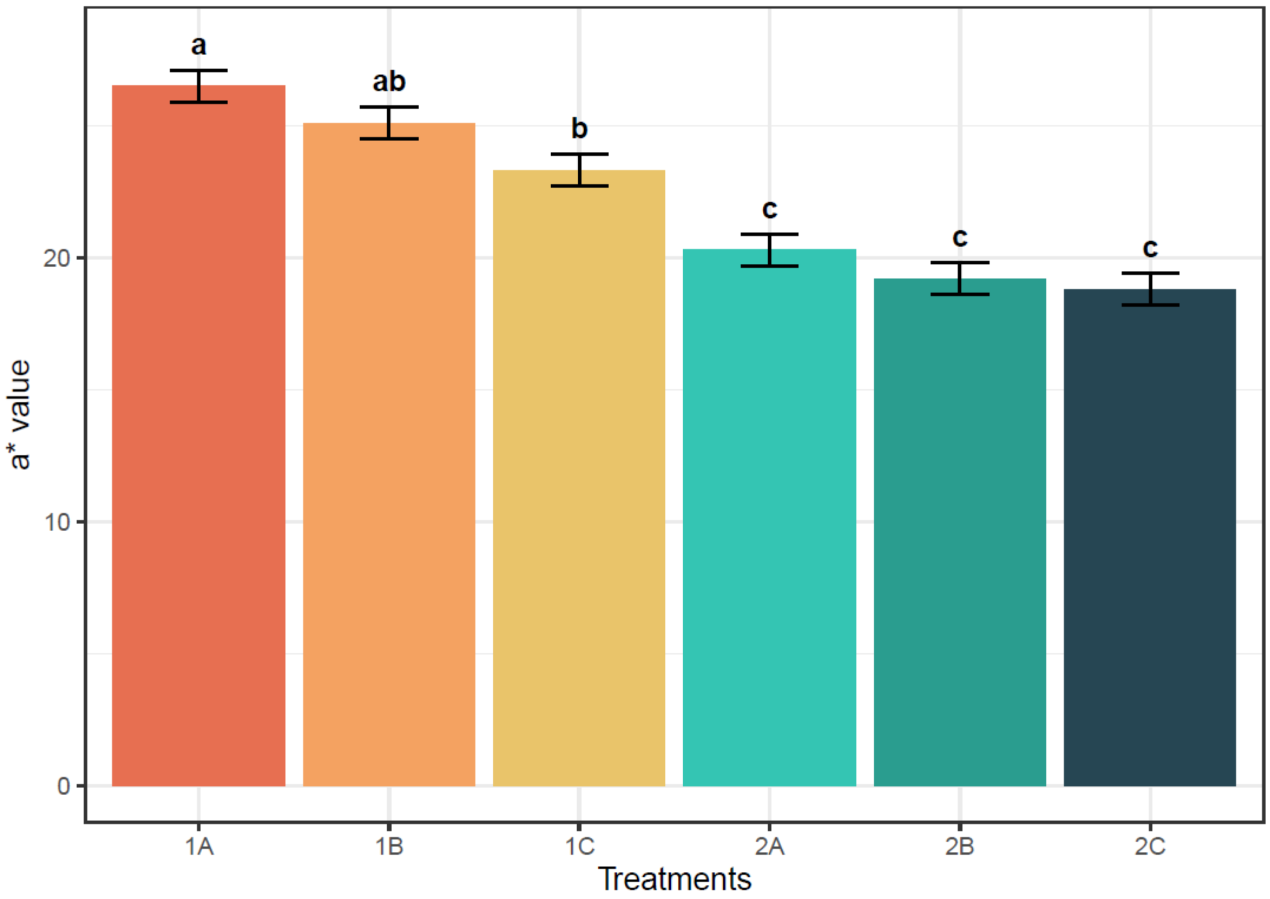

Figure 4.2: Effect of sous vide cooking temperature and dwell time (1A: 56.1°C and 71 min; 1B: 56.1°C and 150 min; 1C: 56.1°C and 240 min; 2A: 61.7°C and 8 min; 2B: 61.7°C and 150 min; 2C: 61.7°C and 240 min) on the internal surface redness (a* value) of biceps femoris steaks (n = 16). Different letters (a-c) indicate significant differences ($P < 0.05$). Error bars represent the standard error of the mean.

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