8-2021

Low-Oxygen Dry Aging of Beef

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by

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A Thesis

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Master of Science

Major: Animal Science

Under the Supervision of Professor Chris R. Calkins

Lincoln, Nebraska

August, 2021

LOW-OXYGEN DRY AGING OF BEEF

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University of Nebraska, 2021

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Dry aging is known for the development of unique flavors via concentration of flavor compounds through dehydration and the creation of new flavors through oxidation and endogenous enzymatic processes. However, prolonged exposure of meat to the environment in a cooler, which is common practice in dry aging processes, can lead to off-odors and flavors. The objective of this research project was to determine the effects of removing oxygen from the beef dry aging process. Boneless upper 2/3 Choice beef strip loins (n=18) were randomly assigned to three aging treatments: wet aging, traditional (aerobic) dry aging, and low-oxygen dry aging. All treatments were aged for 41 days at 2 ± 1°C. The dry-aged treatments were held at 50% relative humidity (RH) with a fan speed of 2,200 revolutions per minute (RPM). Low-oxygen dry-aged samples were aged in dry aging chambers housed within an oxygen impermeable film bubble. Low-oxygen chambers were flushed with an 80% nitrogen/20% carbon dioxide gas mixture at the beginning of aging and any time the oxygen concentration approached 4%. Food grade oxygen scavengers were also utilized to remove residual oxygen after gas flushing. After the aging process was completed, dry-aged samples were weighed before and after trimming to determine yield. There was a significant difference in yield between the wet-aged (95% yield) and the two dry-aged treatments ($p < 0.05$), but there

was no significant difference ($p > 0.05$) between the low-oxygen (55% yield) and aerobic (54% yield) dry aging treatments. Lipid oxidation was measured using thiobarbituric acid reactive substances (TBARS). There was no significant difference found in TBARS between the wet-aged (1.18 mgs malonaldehyde/kg of tissue) and low-oxygen dry-aged (1.27 mgs malonaldehyde/kg) samples ($p > 0.05$), but there was a significant difference between the aerobic dry-aged (2.46 mgs malonaldehyde/kg) samples and the other two treatments ($p < 0.05$). A paired preference test was conducted to determine consumer flavor preference between low-oxygen and traditionally (aerobic) dry-aged steaks. There were no significant differences found among treatments ($p < 0.05$). In a trained sensory analysis, low-oxygen dry-aged samples had slightly higher numerical values for desirable flavor notes and aerobic dry-aged beef had slightly higher numerical values for undesirable flavor notes. These results suggest that although low-oxygen dry aging significantly reduced lipid oxidation, it is unclear if the differences were detectable to consumers.

Keywords: dry aging, flavor, oxidation

ACKNOWLEDGEMENTS

There are countless individuals that deserve my gratitude for the support they have given me throughout my academic career. I have been incredibly fortunate to be blessed with mentors and friends who have made my time at the University of Nebraska-Lincoln unforgettable. I have learned a considerable amount in my relatively short time at the University and I am thankful for all who have assisted me on my academic and professional journey.

I would first like to thank my family and close friends. My parents instilled in me a passion for animals, a hard work ethic, and the motivation to see jobs through at a young age. Without these early interests and skills, there is no way I would have made it this far in my academic career. I am eternally grateful for the innumerable lessons that I learned on and off the farm. Also, I owe additional thanks to my father for suggesting meat science as a college major when I was unsure about what I wanted to study. The support did not stop at college. My parents supported my mentally and financially throughout college, making sure I never missed a tuition payment and always having a home cooked meal for me when I made it home on the weekends. I also received a lot of academic help from my classmates, fraternity brothers, and close friends who helped me to study, learn, and ultimately maintain my grades throughout college. Though there are too many people to name, I am grateful for the help, advice, and tutoring that each of you gave.

Next, I would like to thank my undergraduate meat science instructors: Dr. Gary Sullivan, Dr. Steve Jones, Dr. Dennis Burson, and Dr. Ty Schmidt. Beyond all of the

knowledge gained in classes, they fueled my passion to learn and taught me about the impact a meat science can have on the world. Dr. Sullivan not only taught me about processed meats, but also how to think about all possible answers when addressing a question and how to use my creativity to develop new meat products. I would also like to thank Dr. Sullivan for giving me my first tour of UNL. From Dr. Jones, I learned how meat is an integral part of our food system. Additionally, I was able to participate in the Animal Science Education abroad trip led by Dr. Jones, which is one of my favorite memories from my undergraduate years. Dr. Schmidt taught me about the conversion of muscle to meat and the nutritional benefits that fresh meat provides. Finally, Dr. Burson taught me about the systems that are utilized to ensure that safe meat products are consistently provided.

I would like to thank the Animal Science Department Head, Dr. Clint Krehbiel, for his commitment to the Animal Science program. As an undergraduate student, I was always amazed at how approachable he was and his willingness to help in any situation. As a graduate student, I admired his dedication to the undergraduate clubs and teams. He was incredibly supportive of the Animal Science Meats Judging Team while I was coaching and even advocated for permission for the judging teams to travel, with necessary precautions, throughout the COVID pandemic. Dr. Krehbiel was also part of the reason I agreed to coach the Meats Judging Team which has been thoroughly enjoyable and an incredible learning experience.

Words cannot express the gratitude that I have for my fellow graduate students: Morgan Henriott, Nick Bland, Nicolas Herrera, Becca Furbeck, Sam Watson, David Velasco, and Felipe Ribeiro, and my food science colleague Soon Lau Kiat. I would like

to thank all of them for making my graduate school experience enjoyable and memorable. The help provided, the advice given, and the time spent together outside of school will forever be remembered. I would like to especially thank David, Nicolas, Becca, Felipe, and Kiat for helping me with my thesis project; it could not have been accomplished without each of you.

I simply could not have completed my research without the help of the support staff at UNL: Calvin Schrock, Sherri Pitchie, and Tommi Jones. I began working with Calvin in the Loeffel Meat Laboratory shortly after starting at UNL which gave me an enormous amount of hands-on experience. This experience benefitted me in both my classes and internships. I consider my experience at Loeffel Meat Lab to be one of the keys to my academic success. Sherri was unbelievably helpful with nearly every aspect of graduate school. The amount of help she provided cannot be quantified. Tommi was crucial in helping me run analyses for my thesis project. I am extremely grateful for all of the help that each of these individuals provided.

Lastly, I would like to thank my graduate advisor, Dr. Calkins. I cannot believe how much I have learned in my two short years in graduate school. I have not only acquired knowledge, but I have also gained critical thinking skills and learned to think differently. I would not have coached the meats judging team had it not been for your encouragement, and it has been one of the most rewarding experiences that I have had in graduate school. Thanks to your mentorship, I have gained confidence in myself as a student, coach, and teacher. You pushed me to be the best I could be and believed in me when I did not believe in myself. I have learned to welcome and embrace new challenges as the rewards have often outweighed any risks. Though my graduate career is ending, I

anticipate that there is still a lot that I will learn from you. I look forward to many more years of mentorship.

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INTRODUCTION

Dry aging has become an ambiguous term, as many food enthusiasts would describe dry aging as an art and those adept in the sciences would recognize it as a scientific process. There is evidence for both parties. From a scientific approach, dry aging is an aging process in which meat, predominately beef primals or subprimals, is exposed to the environment in a refrigerator under controlled time and temperature parameters. It has been suggested that relative humidity should also be controlled. While the general mechanism to dry age meat remains unquestioned, many different approaches can be taken. The literature shows profound variations in aging time, temperature, relative humidity, and even the introduction of molds to impart different and unique flavors into dry-aged products (Kim et al., 2018). Meat and food scientists use different aging parameters to help understand the biochemical pathways that result in the characteristic dry-aged flavor.

Dry aging has a long history, but it has been gaining popularity in the last few years due to its characteristic unique flavors, often described as beefy and roasted (Warren and Kastner, 1992). The development of unique flavors is a consequence of 1) the concentration of flavor through moisture loss and 2) the creation of new flavors from the extended aging. As meat is left unpackaged and exposed to the environment in the refrigerator, it loses moisture, resulting in a concentration of flavor compounds. The side effects are a lighter weight final product, an unpalatable, hard exterior shell or crust that must be trimmed, and ultimately less saleable product. Additionally, differences in aging temperature, time, relative humidity, and the incorporation of molds can produce a wide range of product flavors through countless biochemical pathways. A unique flavor is

almost guaranteed, but not all of the flavors produced during dry aging are positively perceived by consumers. For example, as meat is exposed to atmospheric conditions, the lipids and proteins can oxidize, resulting in off-flavors such as "warmed over flavor" (Adhikari et al., 2011; Kim et al., 2018).

In order to produce a consistent product, there must be a single set of dry aging parameters that are tightly controlled. Additionally, such parameters must produce an optimal product quality. Many studies have investigated different aging temperatures, times, humidity, and molds in the hopes of identifying optimal dry aging parameters, but few have looked at eliminating negative attributes associated with dry-aged meat. During aging, as meat is exposed to the atmosphere, muscle proteins and lipids are oxidized resulting in the development of negative flavor compounds. Oxidation during aging can result from extended exposure to oxygen and light.If oxidation could be limited, or even eliminated, then the final product would possibly have an improved dry-aged flavor. In most dry aging coolers, the lights are turned off during the aging period which makes oxygen the main cause of oxidation in dry-aged meats. Therefore, the removal of oxygen during dry aging may result in a less oxidized product with an improved flavor.

To achieve this goal, a study was designed to look at the effects of low-oxygen conditions on dry-aged beef. The research objectives of this study were to eliminate oxygen exposure from the dry aging system and compare the final level of oxidation and flavor of an low-oxygen product to a traditionally dry-aged control after 41 days of dry aging.

LITERATURE REVIEW

Dry Aging vs. Wet Aging Methods

There are two methods of aging in the meat industry: dry aging and wet aging. Dry aging is defined as aging primals or subprimals exposed the environment in a cooler without packaging under controlled time and temperature parameters (Warren and Kastner, 1992; Ahnström et al., 2006; Savell, 2008; Smith et al., 2014). Wet aging, sometimes referred to as vacuum aging, is defined as prolonged aging in an impermeable vacuum package where the control of time and temperature parameters is also important (Warren and Kastner, 1992; DeGeer et al., 2009). Dry aging was the only method of aging for much of the industry's history, but the invention of the vacuum packaging machine and films, and their introduction into the meat industry in the 1960's resulted in the rapid adoption of wet aging as the main aging method, as it remains today. The main difference between the two aging methods is the presence or absence of a protective film barrier to oxygen entry and moisture loss, as both require some control of time and temperature parameters.

Recent developments in protective film technologies have yielded new "dry aging bags," such as bags from Macpak, Tublin, or Umai, in the attempt of producing a new aging method that results in the best qualities of both wet and dry aging (Ahnström et al., 2006; DeGeer et al., 2009; Dikeman et al., 2013; Li et al., 2013; Li et al., 2014; Stenström et al., 2014; Lee et al., 2017; Prieto et al., 2018). These bags, though they seem similar to the bags utilized in wet aging, are highly moisture permeable which imitates the high moisture loss of dry-aged products through direct exposure to the cooler environment. The purposes of these bags, as stated by the companies that sell them, are

to let moisture out, and to reduce contamination, negative odors, and/or other effects of "outside influences." However, the evidence of these benefits is unclear in the literature. Li et al. (2013) found that the total bacterial count and yeast count for the dry aging bags significantly increased after aging, but others have found little or no differences in total bacterial count (Ahnström et al., 2006; DeGeer et al., 2009; Li et al., 2014; Lee et al., 2017). This technological advancement has led to a new method of differentiation between dry and wet aging, where each is described as the benefits/attributes it provides, rather than the method itself.

Benefits of each Aging Method

Aging is defined as storing primals or subprimals in refrigeration for an extended period of time to increase palatability, specifically tenderness and flavor (Kim et al., 2016; Berger et al., 2018). Both wet and dry aging methods have an impact on tenderness and flavor, among other attributes, but while flavor attributes can differ between the two aging methods, tenderness is similarly impacted by both wet and dry aging (Dashdorj et al., 2016).

There is some disagreement among scientists as to which aging method produces a more tender product, but there is a clear consensus that prolonged aging, regardless of method, results in the tenderization of meat products. Tenderness, defined as the quality of being easily cut or chewed, is arguably the most important sensory attribute to consumers (Miller et al., 1995). Increased tenderness during aging is a result of endogenous proteolytic enzymes which function to break down the muscle proteins (Kemp and Parr, 2012). The two most important factors influencing tenderness

development are aging time and temperature. Smith et al. (1978) suggested that meat be aged above freezing for at least 11 days to improve tenderness, but there is no true biochemical limit on the amount of time a product can be aged. However, prolonged aging may have adverse effects on food quality, specifically microbial spoilage, especially if temperature is not regulated (Minks and Stringer, 1972). Additionally, "tenderization is not a uniformly increasing function with increased aging time" which suggests that aging for increasingly larger times provides a diminishing return in terms of tenderness (Smith et al., 1978). Regardless, both aging methods are capable of producing tender products making both acceptable post-harvest practices. Warren and Kastner (1992) found no differences in tenderness between wet and dry aging and stated that, "preference for one aging treatment over the other based on tenderness was not justified." This differed only slightly with Parrish et al. (1991) in which a trained sensory panel only found slight, yet statistically significant, advantages in tenderness for wet-aged steaks over dry-aged steaks with tenderness scores of 6.36 and 6.20, respectively. Degreer et al. (2009) found no tenderness differences between traditional dry aging methods and dry aging in a bag for both shell loins and strip loins. In 2016, Lepper-Blilie et al. found that wet and dry aging methods only differed by .05 kilograms for Warner-Bratzler Shear Force analysis. Kim et al. (2017) found that dry aging alone resulted in slight tenderness advantages over a stepwise dry/wet aging regime with shear force values of 2.94 kilograms and 2.66 kilograms, respectively. In 2018, both Berger et al. and Kim et al. found no significant differences between wet and dry aging methods. In addition, Berger et al. (2018) found no differences between the in-bag dry aging method and both wet and dry aging methods. The other palatability attribute affected by aging is flavor. Flavor

development is perhaps the most complex process associated with aging, and it is not fully understood. Broadly, during the conversion of muscle to meat, and continuing into storage, thousands of flavor compounds are produced (Van et al., 2012). Flavors can be affected by a myriad of antemortem factors including species, animal breed, animal age, diet, harvesting practices, aging, and cooking method (Khan et al., 2015). Additionally, flavors are produced and impacted during aging, and can be affected by aging parameters (Dashdorj et al., 2016). Aging time, temperature, relative humidity, air flow, and aging method (wet or dry) can all have an impact on the flavors that are developed (Dashdorj et al., 2016).

In meat, there are several classes of molecules that are largely responsible for flavor. These include proteins, lipids, nucleotides, and reducing sugars (Spanier et al., 1997; Mottram, 1998; Van et al., 2012; Dashdorj et al., 2016; Kim et al., 2016; Kim et al., 2019; Setyabrata et al., 2021). The molecules can be further broken down by proteolytic and hydrolytic enzymes, and pyrolysis, or cooking. For example, proteins are broken into free amino acids or small peptides (Spanier et al., 1997). Additionally, further flavor-related changes are made by the oxidation of lipids during storage and the Maillard reaction and Strecker degradation during cooking (St. Angelo et al., 1996). The consequence of these reactions, both enzymatic and cooking-related, is the production of thousands of flavors and flavor-precursor compounds. However, Van et al. (2012) noted that not all flavor compounds are equally important as some are present in levels well below the detection limit for humans. Some of the flavor compounds that are of importance when comparing dry aging to wet aging are inosine 5’-monophosphate (IMP), hypoxanthine, aspartic acid (Asp), glutamic acid (Glu), and lipid oxidation products such

as 2-hexanone, 2-heptanone, 3 methyl butanol, octen-3-ol, E-2-decanal, etc. (Van et al., 2012; Dashdorj et al., 2015; Dashdorj et al., 2016; Kim et al., 2016; Kanokruangrong et al., 2017; Kim et al., 2019). The increase or decrease of the listed compounds are, in part, what results in the differences in flavor between wet and dry-aged products and will be discussed further in later sections.

During dry aging, there are two proposed pathways for flavor development: concentration and creation (Ribeiro, 2020). The first pathway, concentration, is a result of moisture loss during dry aging. Since meat is made up of ~75% water, flavor compounds may be less concentrated in fresh product, when compared to dry aged products. As the meat dries, flavor compounds remain resulting in a concentrated flavor (Dashdorj et al., 2016). The second pathway, creation, is a result of the endogenous enzymes that are still active in the meat long after harvest. As stated previously, glycolysis, proteolysis, and lipolysis all impact the flavor of meat during aging (Khan et al., 2015). Additionally, increased dry aging time increases the oxidation of proteins and lipids resulting in oxidation products that can significantly impact meat flavor (St. Angelo et al., 1996). The biochemical pathways and sensory effects of the "creation" flavor pathways will be discussed in detail in later sections.

The flavor differences between wet and dry aging methods have become a point of contention. Warren and Kastner (1992), Campbell et al. (2001), and Kim et al. (2016) all found that dry aging improves flavor, and therefore consumer acceptability. In contrast, Dikeman et al. (2013), Li et al. (2013), Li et al. (2014), and Smith et al. (2014) all found that consumers could not detect differences between the two aging methods. According to Warren and Kastner (1992), Campbell et al. (2001), and Kim et al. (2016),

the flavor descriptors often associated with dry aging are brown, beefy, roasted, and savory/umami, whereas the flavor descriptors often associated with wet aging are bloody, serumy, and metallic. These flavor descriptors suggest that dry-aged products would tend to have more positive flavor attributes and wet-aged products would tend to have more negative flavor attributes, though the differences may be too small to be perceived by consumers. Regardless, it is agreed that the main reason for dry aging is flavor development, though the pathways in which these unique flavors are developed may be the greatest drawback to dry aging in some instances. As previously stated, there are many parameters that must be regulated to produce dry-aged beef. If parameters are not well regulated, or if different parameters are used each time a product is dry aged, there is likely to be high variability between each "batch" of dry-aged product. When it comes to flavor, this is where wet aging has the upper hand, consistency. While the unique flavors associated with dry aging may be appealing to some consumers, wet aging results in a consistent product that most consumers are already familiar with. Consumers may occasionally favor uniqueness, but consistency is likely to drive the majority of purchases on a day-to-day basis.

Another quality that favors wet aging over dry aging is usable product yield. Moisture loss, though important to flavor development, is detrimental to saleable product yield. Dry aging can result in moisture losses ranging from 6% up to 20% (Li et al., 2014; Lepper-Blilie et al., 2016). Additionally, the hard crust that forms as a result of dry aging can also negatively impact yield since it is unpalatable and must be removed. Trim losses for dry aging can range from 7% to greater than 25% depending on aging time and the skill of the person responsible for trimming the crust (DeGeer et al., 2009; Berger et

al., 2018). Even dry aging in the special dry aging bags can result in high moisture and trim losses ranging from 6% to 15% and 7% to greater than 25%, respectively (DeGeer et al., 2009; Dashdorj et al., 2016; Berger et al., 2018). Wet aging results in minimal moisture loss, which is often negligible, but has not been reported greater than 3%. Also, wet aging results in less trim loss (Dikeman et al., 2013; Smith et al., 2014). In fact, much of the trim loss associated with wet aging is due to trimming excess fat rather than as a result of aging. Together, both moisture and trim losses for dry aging can result in total yield losses of up to 50% whereas wet aging results in minimal losses due to aging and trimming. Some scientists suggest that since aging dehydrates the product so much, there is simply less water to lose resulting in improvements in cooking yield. In fact, some studies have found cooking yields to be higher in dry-aged products (Dikeman et al., 2013; Li et al., 2013; Kim et al., 2019), whereas other studies have reported no such improvements (Stenström et al., 2014; Kim et al., 2016; Berger et al., 2018). The dry aging bags, in agreement with the findings of Stenstrom et al. (2014), Kim et al. (2016), and Berger et al. (2018), do not have an impact on cooking yield. Nonetheless, a consequence of the high moisture and trim losses attributed to dry aging is that low yield is likely the main deterrent from dry aging.

A benefit shared by both aging methods is microbial control. The vacuum packaging used in wet-aged products and the special film used in dry aging bags act as barriers to prevent contamination from the environment (Seideman and Durland, 1983; Ahnström et al., 2006). Similarly, the crust that forms during dry aging, though detrimental to yield, provides some protection from microbial contaminants by preventing microbial penetration (Lee et al., 2017). Hulankova et al. (2018) found that

the total viable count after aging was only slightly higher in dry-aged beef. This agrees with Lee et al. (2017) who found the total aerobic count was marginally larger for dry-aged beef. Furthermore, Hulankova et al. (2018) stated that good microbial quality can be maintained for several weeks in dry-aged beef as long as storage conditions are good. Similarly, Ryu et al. (2018) found that dry-aged samples aged for 60 days had "acceptable food safety." Therefore, neither aging method compromises food quality as long as proper food safety protocols are followed.

Current Research on Dry Aging

There are several parameters that must be considered during aging. Aging time, aging temperature, relative humidity, and air flow all impact the final product (Savell, 2008). In addition, moisture loss is also an important factor to consider when dry aging. Control and monitoring each of these parameters is important because they can influence flavor development and yield (Dashdorj et al., 2016). Research has been conducted to find the optimal dry aging parameters, but no such aging parameters have been identified. There is a multitude of theories on "ideal" aging parameters, but the literature is conflicting.

Time is an important parameter to consider when dry aging. The amount of time a product is aged can impact tenderness (Warren and Kastner, 1992). The more time a product is allowed to age, the more tender it will likely become because endogenous enzymes will have more time to breakdown the proteins in the muscles (Koohmaraie, 1988). However, aging for too long can have negative impacts on dry-aged products. For example, as meat continues to dry age, it loses moisture which negatively impacts

yield. Additionally, prolonged dry aging time, while the meat is exposed to the cooler environment, can result in increased oxidation of lipids and proteins resulting in off flavors (Domínguez et al., 2019). Furthermore, dry aging time determines product throughput. In a smaller processor, restaurant, or store, dry aging may be limited to one or two dry aging cabinets. Longer aging times would therefore result in less dry-aged product being produced. Theoretically, there is an ideal dry aging time where tenderness is maximized, and moisture loss and lipid/protein oxidation are minimized.

There is currently no accepted ideal dry aging time. This is a result of the aforementioned factors. There is disagreement on the ideal amount of moisture that can be lost during dry aging. Since aging time affects moisture loss, if there is no ideal percent moisture loss, it is hard to determine an ideal aging time. Product throughput also influences the time producers are willing to allow for aging. Finally, tenderness and flavor development impact the amount of time that products should be aged. Flavor development during aging is still being investigated so it is hard to determine the preferred aging time based on this attribute. As a result, dry aging time, more specifically the lower bound for dry aging time, is often determined by tenderness. As stated previously, Smith et al. (1978) suggested that meat be aged for at least 11 days to improve tenderness. The upper bound for dry aging, or the longest dry aging time reported in the literature is 60 days (Cho et al., 2018; Ryu et al., 2018). Therefore, most dry aging takes place in the 11 to 60-day range, though an ideal dry aging time is still being investigated.

The research literature provides a range of dry aging times and experimental approaches. Some studies hold time as a constant to examine other dry aging attributes,

whereas others examine multiple dry aging times to determine the effect of aging on the final product. The shortest dry aging time was reported by Kim et al. (2017) where carcasses were dry aged for only 10 days postmortem. However, in the study the carcasses were then broken down and wet aged for an additional 7 days meeting the suggested 11-day aging time set by Smith et al. (1978). The study found that the shortened dry aging time, along with the additional wet aging, resulted in a tender product with more saleable yield. It was also suggested that the dry/wet aging combination allowed for faster product turnaround which could benefit smaller packers with limited cooler space. Warren and Kastner (1992) aged strip loins for 11 days in either wet or dry aging conditions. This is the shortest dry aging time reported in the literature. In the study, tenderness was found to be similar between the two aging methods and both methods were significantly more tender than unaged samples. Warren and Kastner (1992) also found that dry-aged samples had a beefier flavor and a more brown/roasted flavor suggesting that flavor changes during aging were significant, despite the short dry aging time. Li et al. (2013) dry aged Gluteus medius muscles in special dry aging bags for 14 days after aging for 7 days in a commercial plant for a total of 21 days aging. Li et al. (2013) found that aging for 14 days in the dry aging bags resulted in significantly less saleable yield as compared to wet-aged controls. Both Kim et al. (2016) and Dikeman et al. (2013) dry aged for 21 days after receiving the cuts at 2 days postmortem and 8 days postmortem, respectively. It was determined that dry aging for 21 days impacted tenderness, flavor, and yield. Kim et al. (2016) found that dry aging improved tenderness, but significantly decreased yield. Dikeman et al. (2013) determined that dry aging resulted in higher moisture and trim losses, similar to other

studies. A consumer panel utilized in the study by Kim et al. (2016) found that the dry-aged samples were more flavorful than wet-aged counterparts. This agreed with the metabolite analysis performed by Kim et al. (2016) in which dry-aged samples contained a higher abundance of seven metabolites associated with flavor which were tryptophan, phenylalanine, valine, tyrosine, glutamate, isoleucine, and leucine However, few flavor differences between wet aging and dry aging were found in this study. Dikeman et al. (2013) concluded that wet aging should be preferred over dry aging. Several studies dry aged different cuts for 28 days after 2-4 days postmortem (Lee et al., 2016; Berger et al., 2018; Prieto et al., 2018; Kim et al., 2019). These studies, similar to the 21-day dry aging studies, also found that dry aging had an impact on tenderness, flavor, and yield. Lee et al. (2016) found that aging yield loss resulted from all aging methods utilized in the study: professional dry aging, simplified dry aging, and special bag dry aging. Berger et al. (2018) also found that dry aging resulted in high yield losses. Prieto et al. (2018) found that dry aging in special dry aging bags enhanced flavor profiles of Longissimus lumborum enough for panelists to detect flavor differences but suggested that further investigation of longer aging times should be conducted. Berger et. al (2018) also found that both traditional and in-bag dry aging resulted in significantly higher over-all flavor and tenderness scores than wet-aged counterparts from panelists. Kim et al. (2019) found that the flavor of dry-aged samples was preferred by panelists over wet-aged samples and concluded that dry aging was a better method for improving the quality properties of cuts such as the gluteus medius (butt) and biceps femoris (rump). The gluteus medius (butts), biceps femoris (rumps), and the Longissimus dorsi (loins) examined in the study by Lee et al. (2016) improved in tenderness during aging, and it was also determined that there

was no microbial contamination associated with dry aging. In agreement, Berger et al. (2018) found that microbial counts were similar between wet and dry aging methods. Both O'Quinn et al. (2016) and Sitz et al. (2006) dry-aged strip loins for 30 days. Both studies examined flavor differences between aging methods and the willingness of consumers to purchase dry-aged beef. O'Quinn et al. (2016) dry aged strip loins following 16 or 17 days of wet aging. In this study, consumer panelists found 2 flavor differences between wet and dry-aged samples. Dry-aged samples had a stronger brown/grilled flavor and wet-aged samples tasted less livery. Sitz et al. (2014) first dry-aged strip loins for 30 days and then wet aged them for an additional 7 days during which samples were shipped and stored for sensory analysis. This study found that dry aging resulted in greater yield losses, and that consumers that prefer dry-aged beef are willing to pay the higher prices associated with dry-aged products which compensate for the losses in yield. Smith et al. (1978) dry aged both beef ribs and top sirloin butts for 35 days. It was determined that dry aging resulted in high losses to saleable yield and that dry aging may result in the development of undesirable flavors as found during consumer and trained sensory analyses. Cho et al. (2018) dry aged bone-in loins for a total of 60 days, although temperatures and relative humidity were changed during aging in some of the dry aging treatments. Cho et al. (2018) found that dry aging improved tenderness but resulted in lower yields. Total aerobic plate counts did change during aging, but the changes were likely related to the differences in temperature and relative humidity rather than aging time. The most agreed upon conclusion from all of the aforementioned studies is that dry aging results in yield losses.

Both Ahnstrom et al. (2006) and Parrish et al. (1991) looked at dry aging times of 14 and 21 days. Both studies cited that dry aging resulted in improvements in tenderness but had conflicting conclusions for flavor effects. Ahnstrom et al. (2006) found that tenderness improved for both traditional and in-bag dry aging at both time periods but did not find a significant difference in tenderness between the two time periods. For Parrish et al. (1991) tenderness, determined only after 21 days of aging, was improved, but only slightly, though statistically significant, different, 6.20 and 6.36, for dry and wet aging, respectively. Parrish et al. (1991) also found that aging losses increased with time for both ribs and loins and trim losses after 21 days of aging were significantly higher for dry aging than wet aging. This agreed with Ahnstrom et al. (2006) in which trim losses were similar for the bag dry aging at both time periods, but trim losses for traditional dry aging were slightly higher at 21 days of aging. Ahnstrom et al. (2006) found that both dry aging treatments were rated highly for flavor, especially beefy and brown/roasted flavors. However, for the Parrish et al. (1991) study, consumer panelists found no flavor differences between wet and dry aging treatments. Lastly, Ahnstrom et al. (2006) found that total plate counts were not significantly different for either aging method at either time period. Lee et al. (2019) looked at dry aging times of 14 and 28 days on control and blade tenderized loins. The study found that tenderness increased with additional aging. Also, inosine monophosphate (IMP) content decreased in all samples during aging. Obuz et al. (2014) examined dry aging times of 2 and 23 days. Obuz et al. (2014) found that weight loss during aging and trim loss increased with time for both control dry-aged samples and blade tenderized dry-aged samples. Blade tenderized dry-aged samples lost more weight than control dry-aged samples. Also, the study found that tenderness

increased with time such that there was a significant difference in tenderness between 2 and 23 days. Li et al. (2014) looked at dry aging times of 8 and 19 days. Li et al. (2014) found that aging and trim losses increased with aging time, although samples aged in dry-aged bags had slightly lower losses than traditionally dry-aged samples. Li et al. (2014) also reported that longer aging times led to increased proteolysis and ultimately an increased free amino acid concentration, among other beef flavor precursors. Lower total bacterial counts were found on samples dry aged in bags than traditionally dry-aged samples. DeGeer et al. (2009) examined dry aging times of 21 and 28 days. DeGeer et al. (2009) found that increased aging time resulted in increased aging weight loss and trim losses. The study also found that aging for 28 days does not significantly increase the flavor when compared to 21 days of aging. Finally, DeGeer et al. (2009) found that dry aging in a bag resulted in flavor equal to that of traditional dry aging. Campbell et al. (2001) observed dry aging times of 7, 14, and 21 days and found that dry aging for 14 or 21 days significantly increased the dry-aged flavor when compared to dry aging for 7 days. The study found that tenderness increased for 7- and 14-day aging periods when compared to a day 0 control, but there was no significant improvement in tenderness from 14 to 21 days. Microbial counts were greater for dry-aged samples at all time periods when compared to unaged controls, but aging time did not affect microbial growth. Laster et al. (2008) looked at dry aging times of 14, 21, 28, and 35 days. Laster et al. (2008) determined that dry aging resulted decreased saleable yield with increased aging time. Also, consumers were unable to distinguish between the wet and dry-aged products. Lee et al. (2019) examined the effects of dry aging for 0, 7, 14, 21, and 28 days. Lee et al. (2019) found that moisture content of dry-aged beef decreased as aging

time increased. The study also found that increased dry aging time produced increased the concentration of several free fatty acids and reducing sugars. Ryu et al. (2018) observed aging times of 3, 25, 40, 50, and 60 days and found that total bacterial count, lactic acid bacteria count, and yeast/mold counts increased with increased aging time, although food safety was not compromised. The results of the study suggest that this increase in microorganisms, specifically yeasts and molds, played a role in flavor development as the molds found in the study are major contributors to the sensory properties of dairy foods in cheese manufacturing. Lastly, Lepper-Blilie et al. (2016) examined the effects of dry aging for 14, 21, 28, 35, 42, and 49 days. Lepper-Blilie et al. (2016) found that dry-aged loins lost significantly more weight during aging than wet-aged loins. Additionally, tenderness increased with dry aging time, but no significant improvements in tenderness were found after 28 days of aging. Also, it was determined that the dry-aged loins had a greater aged beef flavor than wet-aged loins.

From the current literature, several conclusions about aging time can be drawn. Increased aging time results in increased moisture loss and trim loss, and aged beef flavor in some studies. Additionally, dry aging in special dry aging bags slightly improved yield in some studies but did not significantly affect yield in other studies suggesting that improvements in yield from in-bag dry aging are minimal. Although microbial counts have been reported to increase with aging time, food quality, as a result of microbial spoilage, is not compromised as a result of prolonged dry aging at least up to 60 days.

Relative humidity is another important parameter to consider when dry aging. If the relative humidity is too high, then meat can spoil or develop rancid odors or flavors as a result of microbial growth. If the relative humidity is too low, bacteria growth will be

restricted, but there will be a higher rate of evaporative moisture loss and total moisture loss could be increased (Savell, 2008; Dashdorj et al., 2016). Reviews of dry aging report that relative humidity in the scientific literature range from 49% to 98%. While relative humidity is often reported in dry aging studies, very few studies have examined the effect of different relative humidity levels on the dry aging process.

DeGeer et al. (2009), Li et al. (2013), and Ribeiro et al. (2021) all reported a relative humidity of 50% during aging. Lepper-Blilie et al. (2016) reported 70% relative humidity. Campbell et al. (2001), Li et al. (2014), Lee et al. (2019), and Lee et al. (2019) all reported a relative humidity of 75% in their studies. O'Quinn et al. (2016) reported a relative humidity of 77%. Warren and Kastner (1992), Laster et al. (2008), Kim et al. (2017), and Berger et al. (2018) all reported a relative humidity of 78% during dry aging, though Warren and Kastner (1992) reported 78% ± 3%. Prieto et al. (2017) reported 80% relative humidity. Parrish et al. (1991) reported a range for the relative humidity of 80%-85%. Both Iida et al. (2015) and Ryu et al. (2018) reported ranges of 80%-90% relative humidity during dry aging. Kim et al. (2018) and Hulankova et al. (2018) reported 85% relative humidity, however Kim et al. (2018) reported the 85% relative humidity ± 10%. Ahnstrom et al. (2006) reported a relative humidity of 87% ± 2.6%. Li et al. (2013) reported a relative humidity of 91% during aging. Smith et al. (2014) reported a relative humidity of 98.1%. Lee et al. (2016) reported two levels of relative humidity depending on aging method. For the professional dry aging treatment, a relative humidity of 85% ± 10% was used, and for the simplified dry aging treatment, a relative humidity of 75% ± 10% was used. However, no comparisons were made between the treatments based on differences in relative humidity.

Cho et al. (2018) utilized four different treatments to examine the effects of different temperatures and levels of relative humidity on dry-aged beef. Treatments 1 and 4 used a relative humidity of 85% for 60 days with the only difference in treatment being temperature, with treatment 1 having a temperature of 2°C and treatment 4 having a temperature of 4°C. Treatment 2 used a stepwise relative humidity regime with 65% for 20 days, 75% for 20 days and 85% for 20 days adding up to a total of 60 days. Treatment 3 also used a stepwise relative humidity regime of 75% for 20 days and 85% for 40 days, again adding up to a total of 60 days. Cho et al. (2018) found that the yield was significantly higher for treatments 1 and 2 at day 20 but found no yield differences between the 4 treatments after 40 and 60 days. Treatment 1 had significantly higher total plate counts than the other three treatments at day 20, 40, and 60. Treatments 2 and 3, which utilized the stepwise, gradual increase in relative humidity, had lower total plate counts than treatments 1 and 4 at 20, 40, and 60 days. The author suggests that this is a result of different levels of surface dehydration at different relative humidity and temperature. Additionally, the study found that treatments 2 and 3 had significantly higher sensory scores for tenderness, juiciness, flavor-likeness, and overall acceptability scores at day 20, 40, and 60. In contrast, treatment 1 had significantly lower tenderness, juiciness, flavor-likeness, and overall acceptability scores at day 20, 40 and 60.

From the current literature, only a few conclusions can be made about the effect of relative humidity on dry aging. Relative humidity can have an impact on moisture loss during aging. At a lower relative humidity, evaporative moisture loss increases. Relative humidity also has an impact on microbial content and sensory scores. Spoilage microorganisms have less trouble growing on dry-aged product at a higher relative

humidity, whereas lower relative humidity levels restrict bacterial growth (Dashdorj et al., 2016).

Temperature during dry aging can have a profound effect on the final product. If aging temperature is too low, meat can freeze, resulting in the reduction of activity forthe proteolytic enzymes responsible for tenderization. If the storage temperature is too high, proteolytic enzymes may be more effective; however, microbial growth will increase resulting in the spoilage of the meat, the development of off-flavors, and the potential that food safety will be compromised. Most dry aging apparatuses, chambers, or shelves are housed in coolers, so temperature is usually relatively easy to control and monitor. Similar to relative humidity, temperature is often reported in dry aging research studies, but few studies have looked at the effect of different aging temperatures on dry-aged products. In the scientific literature, most aging temperatures fall in the range of 0°C to 4°C.

Laster et al. (2008) aged at a temperature of -0.6°C. Parrish et al. (1991) utilized an aging temperature ranging from 0-1°C. Richardson et al. (2008), Lepper-Blilie et al. (2016), and Kim et al. (2017) all used a temperature of 1°C during aging. Lee et al. (2016), Lee et al. (2017), Kim et al. (2018), and Hulankova et al. (2018) dry-aged products at 1±1°C. Stenstrom et al. (2014) used a temperature of 1.6°C during aging. Campbell et al. (2001), Prieto et al. (2018), and Berger et al. (2018) all utilized an aging temperature of 2°C. Both DeGeer et al. (2009) and Dikeman et al. (2013) dry aged at 2.2°C. Li et al. (2013) used a temperature of 2.9°C during aging. Warren and Kastner (1992) dry aged in a dry aging room ranging from 3.1-3.6°C. Ryu et al. (2018) utilized an aging temperature range from 1-4°C. Lee et al. (2019) and Lee et al. (2019) used a dry

aging temperature of 4°C. Smith et al. (2014) dry-aged product at 4±1°C. Li et al. (2014) utilized the highest reported temperature, 5.1°C, during dry aging. O'Quinn et al. (2016) dry aged at a temperature of 1 or 2°C but did not compare or report the effects of the two temperatures used. Likewise, Ahnstrom et al. (2006) used dry aging temperatures of 2.6±.04°C and 2.5±0.3°C for 14 and 21 days of aging respectively but did not compare or report the effects of the two temperatures used.

Kim et al. (2016) utilized an aging temperature of 1 or 3°C depending on treatment. Treatments 1 and 2 were aged at 1°C, whereas treatments 3 and 4 were aged at 3°C. Kim et al. (2016) found that the higher aging temperature of 3°C resulted in a greater total loss when compared to aging at 1°C. Additionally, loins aged at 3°C had lower L* values than those aged at 1°C. It is hypothesized that the decrease in L* value is a result of the lower moisture content in the loins aged at 3°C. There was no significant difference in shear force between loins aged at either aging temperatures. A consumer panel found that the loins aged at 3°C had significantly higher juiciness scores, and treatment 3, which was aged at 3°C, was the most preferred. Cho et al. (2018) utilized 4 different aging regimens with 4 different temperature applications. Treatment 1 was aged for 60 days at 2°C, treatment 2 was aged at 2°C for 40 days followed by 4°C for 20 days, treatment 3 was aged at 2°C for 20 days followed by 4°C for 40 days, and treatment 4 was aged at 4°C for 60 days. There is a gradual increase in temperature during aging for treatments 2 and 3, as opposed to constant temperatures used in treatments 1 and 4. Cho et al. (2018) found that treatments 3 and 4 had significantly lower yields at 20 days, but all 4 treatments had similar yields at 40 and 60 days of aging. The author suggests that the decreased yield in treatments 3 and 4 at 20 days is a result of

the greater shrinkage at the higher aging temperature. Treatment 1 had a significantly higher total plate count than the other three treatments at 20, 40 and 60 days. Treatments 2 and 3 had lower total plate counts and a lower water activity than treatments 1 and 4. It is suggested that this is a result of difference in surface hydration at different temperature and relative humidity. Treatment 1 had a higher moisture content than the other three treatments at 20, 40, and 60 days. Also, treatment 1 had lower scores for tenderness, juiciness, flavor-likeness, and overall acceptability. Treatments 2 and 3 had higher scores for the same sensory attributes.

From the literature, it can be concluded that the control and monitoring of temperature during aging is quite important. More research is needed comparing the effects of different temperatures during dry aging. Regardless, the current literature supports the theory that aging temperature can have profound effects on microbial growth, yield, and sensory attributes. High aging temperatures increase microbial growth and moisture loss, which can affect flavor, color, and other sensory attributes. Low aging temperatures limit proteolytic enzymes and can reduce tenderness.

Air flow, though an important dry aging parameter, is often neglected because it is variable and difficult to measure. Many studies cite the use of perforated shelves or added fans to properly circulate air, but few record or report air flow and even fewer have looked at its effect on final dry-aged products. If air flow is too rapid, products can dry out too quickly. If air flow is too low or not present, microbial growth can be promoted. Important factors to consider when determining proper air flow and air circulation are product location and dry aging shelves. Product placed next to the cooler fan will have a significantly higher air flow than those placed on the opposite side of the dry aging room

or chamber. Additionally, the product placed next to the fan will have higher air flow on the side facing the fan, but a lower air speed on the back side of the product. Multiple fans may be required to properly circulate air. Dry aging shelves are also an important factor to consider when determining air flow. Perforated or grated shelves will allow more air to come in contact with the bottom of the product and will allow moisture to be wicked away. Placing product on solid shelves may increase microbial growth and will not allow the bottom of the product to be exposed to the environment in the cooler that stimulate dry aging.

A range of air flow is reported in the literature. Some studies only report that air flow was present, but did not measure it. Warren and Kastner (1992), Ahnstrom et al. (2006), and Dikeman et al. (2013) all reported that air was circulated in the dry aging room as a result of the normal cooler environment but did not record or report specific air flow speeds. Likewise, Lepper-Blilie et al. (2016) reported the air flow of the air purifying system used during aging, 5.66 m^3/h, but did not record or report the air flow near or around the product. Other studies have recorded and reported air flow speeds but have not looked at the effect of air flow on the final product. Berger et al. (2018) reported an air flow of <0.2 m/s. Prieto et al. (2018) used an air flow speed of 0.5 m/s. Hulankova et al. (2018) reported an air flow of 0.5 ± 0.2 m/s. Parrish et al. (1991) reported a range of air flow from 0.5-2.5 m/s. Lee et al. (2019) used an air flow velocity of 2.5 m/s. Both Lee et al. (2016) and Kim et al. (2018) reported an air flow range of 2-7 m/s. Cho et al. (2018) used an air flow velocity of 3 m/s during dry aging. Lastly, Lee et al. (2017) reported an air flow of 5 ± 3 m/s.

Only two studies have looked at the effect of air flow velocity on final dry-aged product. Kim et al. (2016) used air velocities of 0.2 and 0.5 for treatments 1 and 3 and treatments 2 and 4, respectively. Kim et al. (2016) did not find any significant weight loss differences between treatments as a result of air flow. Lee et al. (2019) utilized air velocities of 0, 2.5, and 5 m/s. Each product at each velocity was aged for 14 or 28 days. There were no differences in mold growth between any of the air velocities at day 14. At day 28, mold and yeast were isolated from the different treatments. The 0 m/s air flow velocity treatment had mold growth which predominately consisted of Pilaira anomala (99.8%). Similarly, the other two air velocity treatments had high P. anomala content, but they also had relatively high Debaryomyces hanseii content (15.9% for 2.5 m/s and 14.7% for 5.0 m/s). Based on these results, the author hypothesized that differences in air flow velocities can change the presence and abundance of different molds and yeasts which may affect sensory attributes. The 0 m/s air velocity had the highest composition of Pseudomonas and Enterobacterium. The 2.5 and 5 m/s treatments had similar Pseudomonas compositions, but the 5 m/s treatment had less Enterobacterium. Also, the 2.5 and 5 m/s treatments had a higher composition of Lactobacillus and Flavobacterium compared to the 0 m/s treatment. The author suggests that the air flow does not necessarily help the bacteria to grow but helps bacteria to get onto the crust via travel through dust or droplets. Additionally, it was found that between days 14 and 28, the 5 m/s air flow velocity treatment has decreased moisture content. The other two treatments did not show the same differences. The dry-aged treatment with 0 m/s air flow velocity had a significantly higher pH than the other treatments, but the cause of it us unknown since most of the bacteria, molds and yeasts were found on samples from all three

treatments. The 0 m/s treatment also had the lowest shear force when compared to the other two air velocities. There was no significant difference in the decrease in shear force between day 0 and 14 for any of the treatments. However, there was a significantly larger decrease in in shear force between days 14 and 28 for the 0 m/s treatment. It is suggested that the difference in shear force is a result of the differing prevalence of different molds and yeasts, which may also explain the pH differences. Regardless of air flow treatment, free fatty acids significantly increased after 28 days of aging. As for flavor, the 2.5 and 5 m/s treatments were shown to be similar, whereas the 0 m/s treatment was distinguishable from the other two. This could be a result of the different mold and yeast compositions between the treatments.

In conclusion, air flow can have several impacts on final dry-aged products. Air flow can affect moisture content during aging. It can also have an impact on the microbial growth and diversity which can ultimately impact sensory attributes. More research is need on the effect of air flow velocities on weight loss rate, total moisture loss, microbial growth and diversity, and flavor and sensory attributes.

The final parameter that must be considered during dry aging is moisture loss. Moisture loss is incredibly important because it directly impacts flavor and yield. Moisture loss is responsible for the concentration of flavors during dry aging which, in part, gives beef its dry-aged flavor (Dashdorj et al., 2016). However, as moisture loss increases, yield is directly, and negatively, impacted. Moisture loss can be affected by a long list of factors. As stated previously, aging time, temperature, relative humidity, and air flow all impact moisture loss rate and total moisture loss (Dashdorj et al., 2016). Additionally, blade tenderizing can result in greater moisture loss during dry aging (Obuz

et al., 2014). Furthermore, fat cover and the presence or absence of bone can influence evaporative moisture losses and impact total moisture loss. Bernardo et al. (2020) found that dry-aged samples in which subcutaneous fat was removed experienced higher evaporation and trimming losses. The theory is that fat provides a protective barrier which prevents some moisture loss. In addition, multiple studies have found that bone-in primals experience less moisture loss than boneless counterparts (DeGeer et al., 2009; Lepper-Blilie et al., 2016; Bernardo et al., 2020). Similar to fat, bone provides a protective barrier which helps to reduce evaporative moisture losses. There is currently no research on the differences in moisture loss between carcasses and primals during dry aging. It is plausible, however, that dry aging on the carcass would result in a higher final yield due to the protective barriers of bone and fat. While some primals may be dry aged without the removal bones and fat, the cut faces on either side of the primal remain exposed to the cooler environment. Moisture loss can be further minimized in carcasses using spray-chilling. Allen et al. (1987) found that spray-chilling, intermittent spraying of carcasses with cooled water during postmortem chilling, resulted in a 1.14% higher yield for spray-chilled carcasses over conventionally chilled carcasses. There is no research comparing the effect of muscle on moisture loss and dry-aged yield. Laster et. Al (2008) reported moisture losses of 11.7 ± 0.3, 5.6 ± 0.2, and 15.0 ± 0.3 for beef ribs, strip loins, and top sirloins, although it is unclear whether these differences were caused by cut or muscle, or if there was another unrelated cause.

There is no reported ideal moisture loss. In fact, there are no studies that look explicitly at the effect moisture loss has on final product yield and flavor. Many studies report moisture loss as a factor of yield, but no studies examine the effects of different

levels of moisture losses. Additionally, reported moisture losses are hard to compare between studies because dry aging parameters are rarely the same.

Warren and Kastner (1992) found that after aging for 11 days, beef strip loins had lost 13.65% of its original yield. Stenstrom et al. (2014) aged the beef Longissimus muscle for 13 days and found that the traditionally dry-aged muscles lost 3.8% and the in-bag dry ages muscles lost 4.9% of the initial weight during aging. Li et al. (2013) observed a 15.2% aging loss in beef Gluteus medius after 14 days of dry aging. Dikeman et al. (2013) found weight losses during 21 days of dry aging to be 15.56% and 13.48% for traditional dry-aged and special bag dry-aged methods, respectively. Kim et al. (2016) found ageing losses of 9%, 10%, 12.5%, and 13.5% during 21 days of aging using different temperature, relative humidity, and air flow parameters. Lee et al. (2016) dry aged butts, loins, and rumps for 28 days using commercial dry aging, simplified dry aging, and specialty bag dry aging. The commercial dry aged treatment was aged in a specialized facility, and the simplified dry aged treatment was aged in an ordinary refrigerator. Lee et al. (2016) found aging losses of 32.76%, 33.53%, and 26.38% in butts, loins, and rumps using the commercial, dry aging method. The simplified aging method resulted in losses of 40.25%, 47.20%, and 37.38% for butts, loins, and rumps, respectively. Finally, the in-bag dry aging resulted in aging losses of 35.90%, 36.69%, and 36.19% for butts, loins, and rumps, respectively. Berger et al. (2018) found that beef strip loins aged 28 days lost 13.9% and 9.1% of initial weight during aging using traditional and in-bag dry aging methods, respectively. Smith et al. (2014) observed aging losses of 15.21% for beef ribs and 13.64% for top sirloins after 35 days of aging. Li et al. (2014) aged beef Longissimus using two aging methods, traditional dry aging

and special bag dry aging, for two time periods, 8 and 19 days. Li et al. (2014) found aging losses for the special bag method to be 5.8% and 13.5% for 8 and 19 days, respectively. Aging losses for traditional dry-aged muscles were 6.9% and 15.3% for 8 and 19 days, respectively. Ahnstrom et al. (2006) traditionally dry-aged beef strip loins for 14 and 21 days resulting in aging losses of 6.5% and 10.2%, respectively. Ahnstrom et al. (2006) also dry-aged beef strip loins in special bags for 14 and 21 days resulting in respective aging losses of 6.3% and 8.8%. Gudjonsdottir et al. (2015) aged beef strip steaks for 7, 14, or 21 days using traditional dry aging and a special chitosan fiber-wrapped dry aging method. The traditional dry aging method resulted in aging losses of 34.3 ± 2.1%, 47.3 ± 1.4%, and 57.6 ± 2.3% for the aging periods of 7, 14, and 21 days. The chitosan fiber-wrapped aging method resulted in aging losses of 26.7 ± 0.1%, 39.8 ± 0.3%, and 50.8 ± 1.4% for the aging periods of 7, 14, and 21 days, respectively.

The importance of moisture loss during aging is clear. It is hypothesized that one of the main causes of dry-aged flavor during aging is the concentration of flavor compounds as a result of evaporative moisture loss. Also, moisture loss during aging directly impacts final yield. It is common knowledge that moisture loss occurs during aging, but more research must be conducted on the effect different moisture losses have on final product flavor.

Oxidation of Meat in Atmospheric Conditions

The exposure of meat to the environment in the cooler during dry aging results in the oxidation of lipids and proteins (Ribeiro et al., 2021). Oxidation of muscle lipids and the myoglobin protein can have negative impacts on product flavor and color. There is a

theory that the oxidation of lipids during dry aging may be partly responsible for the characteristic dry-aged flavor (Khan et al., 2015; Khan et al., 2016). However, since lipid oxidation often results in the deterioration of product quality in fresh and processed products, many scientists believe that lipid oxidation during dry aging may negatively impact the final product flavor. Additionally, oxidation can negatively impact product color and nutritional quality. Muscles have a natural process to deal with oxidation in the body, but postmortem muscle has a limited ability to limit oxidation when meat is exposed to atmospheric conditions.

The process of lipid oxidation is complex. According to Dominguez et al. (2019) and Amaral et al. (2018), lipids are oxidized through three main pathways: autooxidation, enzyme-catalyzed oxidation, and photo-oxidation. Autooxidation is the oxidation of lipids as a result of exposure to oxygen. It is the main cause of oxidation in meat and occurs in three distinct phases: initiation, propagation, and termination. During initiation, an unsaturated fatty acid loses a hydrogen atom at an allylic carbon resulting in the formation of a lipid, or alkyl, free radical. The alkyl free radical can then react with oxygen to form various free radical species such as peroxy radicals. As the concentration of these free radical species increases, they start to react with other unsaturated fatty acids and the propagation phase begins. The reaction between the peroxy radical and the unsaturated fatty acid results in the formation of a hydroperoxide and an alkyl radical. The newly formed alkyl radical can then react with oxygen to form another peroxy radical and the cycle continues. Additionally, the hydroperoxide can decompose, through a reaction with either a metal or another hydroperoxide, to form new hydroxyl, peroxyl, or alkoxyl radicals. Finally, in the termination phase the concentration of radicals

continues to increase to a point in which radicals begin reacting with non-radical compounds or other radicals, to form non-radical products.

The compound responsible for the deterioration of food quality is hydroperoxide. Hydroperoxide is odorless and tasteless, and therefore does not directly impact food quality, but it is also highly unstable. As hydroperoxide decomposes, it creates secondary lipid oxidation products that are responsible for the off odors and flavors associated with oxidized meat. Common secondary degradation products of hydroperoxide include ketones, aldehydes, alcohols, epoxides, and hydrocarbons (Domínguez et al., 2019). Van et al. (2012) listed some of the flavor-active compounds that were found in cooked meat using a gas chromatography-olfactory technique, though it was not stated whether or not these aroma flavor compounds were a result of lipid oxidation. Aldehydes such as E,2-nonenal, E,E,2,4-decadienal, E,E,2,4-nonedienal, nonanal, undecanal, benzenacetaldehyde, decanal, heptanal, E,2-heptenal, and hexanal were all associated with one or more of the following flavors: fatty, sweet, pungent, and green. Some ketones having fruity or musty aroma flavor characteristics included 2-octanone, 2-decanone, 2-dodecanone, and 2-undecanone. 2-ethyl 1-hexanol and 2-octen-1-ol are alcohols with green aroma flavor characteristics. The alcohol 1-pentanol is associated with a fuel oil, fruit, and balsamic aroma and flavor. The hydrocarbon pentane is associated with an oxidized or slightly warmed-over flavor. 1-undecen is a hydrocarbon that has aroma flavor characteristics of fatty, burnt, nutty, and rubbery. Van et al. (2012) identified additional aldehydes, ketones, alcohols, and hydrocarbons as well as pyrazines and sulfur and nitrogen containing compounds that have all been found in cooked meats.

The secondary products produced during lipid oxidation are also important in determining the level of oxidation. For example, malondialdehyde, one of the aldehyde secondary products, is measured to determine the level of lipid oxidation in meat products (Du and McCormick, 2009). Malondialdehyde is used due to its reactivity with thiobarbituric acid (Ayala et al., 2014). The thiobarbituric reactive substances (TBARS) assay is one of the most frequently used tests to determine lipid oxidation in meat; however, other components in the meat can interfere with measurements (Du and McCormick, 2009). Regardless, this assay is still consistently used because it is positively correlated with warmed-over and rancid flavors and aromas (Nolan et al., 1989; Du and McCormick, 2009).

Factors that can affect the susceptibility of meat to oxidize include lipid composition, animal species, diet, prooxidants, antioxidants, and storage and processing conditions. The first is fatty acid composition. Unsaturated fatty acids are highly susceptible to lipid oxidation and the rate of lipid oxidation increases with the level of unsaturation, or the number of double bonds (Ahmed et al., 2016). For example, polyunsaturated fatty acids oxidize at a faster rate than monounsaturated fatty acids.

The species of livestock also affects the oxidation of lipids. Different species of livestock have different levels of unsaturated fatty acids (Wood et al., 2004). Pigs have the most polyunsaturated fatty acids when compared to beef and lamb which are both higher in saturated fatty acids (Wood et al., 2007). Thus, pork fat is more likely to oxidize than beef and lamb fat when in similar environments.

Animal diet can also impact the fatty acid profile of livestock and ultimately lipid oxidation. As a monogastric animal, pigs fed high levels of polyunsaturated fats deposit

fat with similar higher levels of polyunsaturated fatty acids, whereas feeding higher levels of polyunsaturated fatty acids to cattle and sheep has a lesser effect due to the ruminant digestive system (Wood et al., 2004).

Prooxidants, both endogenous and exogenous, can affect lipid oxidation (Domínguez et al., 2019). Iron, whether from heme proteins or free, and prooxidant enzymes can impact oxidation. Metals impact oxidation because they are catalysts for oxidation reactions, specifically the production of reactive oxygen species (Domínguez et al., 2019). In fresh meat, much of the oxidizing metals come from heme proteins such as hemoglobin and myoglobin. Salt, which is a common ingredient used in processed meats, has been found to have prooxidant effects in some studies (Mariutti and Bragagnolo, 2017).

Other processing ingredients possess antioxidant characteristics which help limit or reduce lipid oxidation. Kumar et al. (2015) listed 27 natural antioxidants and 3 synthetic antioxidants that are used to inhibit lipid oxidation, delay the production of off-odors and flavors, and stabilize product color. Two effective and popular antioxidants used in food processing are ascorbic acid and α-tocopherol or Vitamin E (Dey and Neogi, 2019). Antioxidants limit lipid oxidation by reacting with free radicals to produce stable and inactive products (Kumar et al., 2015). They can be classified into two groups, primary and secondary antioxidants. Primary antioxidants interact directly with the free radical compounds to accept the free radical component and delay the onset of lipid oxidation (Mishra and Singh Bisht, 2011). Secondary antioxidants delay lipid oxidation by chelating heavy metals, scavenging oxygen, donating hydrogen to primary antioxidants, absorbing UV radiation and/or deactivating the reactive species (Mishra and

Singh Bisht, 2011). Antioxidants can also be incorporated into the diet of livestock antemortem to limit oxidation in the products the animals. The supplementation of Vitamin E in the diets of beef, pork, and lamb has been found to reduce lipid oxidation in meat products from each of the species (Smith et al., 1996; Macit et al., 2003; Guo et al., 2006).

Finally, storage conditions can have an effect on lipid oxidation. Storage temperature can have a large impact on oxidation. Higher storage temperatures lead to an increase in lipid oxidation since higher temperatures accelerate the breakdown of hydroperoxide which generates free radicals for lipid oxidation (Johnson and Decker, 2015). Exposure to oxygen can also increase lipid oxidation. To prevent this, processors use various packaging technologies, such as gas flushing, modified atmosphere packaging (MAP), vacuum packaging, and the use of oxygen scavengers, to limit or inhibit oxygen from interacting with the product (Johnson and Decker, 2015). Lloyd et al. (2009) found that flushing whole milk powder pouches with nitrogen reduced lipid oxidation during storage. Clausen et al. (2008) studied the effect of various levels of oxygen in modified atmosphere packaging on lipid oxidation and found that packaging in high oxygen concentrations significantly increased lipid oxidation compared to low oxygen and anaerobic packaging systems. Likewise, Kim et al. (2010) found that high oxygen MAP packaging negatively impacted lipid oxidative stability. Kang et al. (2014) found similar results when using high oxygen MAP packaging, and it was also determined that vacuum packaging was the most effective packaging method for limiting lipid oxidation when compared to the MAP packages. Lastly, oxygen scavengers can be utilized to help limit oxidation. Oxygen scavengers limit lipid oxidation by binding

atmospheric oxygen though enzymatic or chemical pathways (Johnson and Decker, 2015). Dey and Neogi (2019) listed the different types of oxygen scavengers: iron, platinum metal group, unsaturated hydrocarbon, α-tocopherol, ascorbic acid, enzyme, and microorganism based. Of the listed oxygen scavengers, all of the mechanisms of lipid oxidation inhibition are the same; they consume or react with oxygen to prevent it from causing lipid oxidation (Dey and Neogi, 2019).

There are many mechanisms which can be used to prevent or reduce lipid oxidation. Lipid composition, animal species, diet, prooxidants, antioxidants, and storage and processing conditions can all impact the rate and the degree to which lipids oxidize. Ultimately, lipid oxidation mitigation is necessary to maintain good product quality.

Lipid oxidation, and its products, can lead to the oxidation of myoglobin (Faustman et al., 2010). Likewise, myoglobin oxidation, specifically metmyoglobin, can result in lipid oxidation (Chaijan, 2008). In living muscle, myoglobin functions as an oxygen storage and transport protein (Ordway and Garry, 2004). In fresh meat, myoglobin is responsible for meat color and the oxidation of myoglobin results in brown discoloration (Suman and Joseph, 2013). According to Mancini and Hunt (2005), color is the most important characteristic considered by consumers when purchasing meat fresh meat and therefore, myoglobin oxidation is of great concern for fresh meat purveyors (Mancini and Hunt, 2005). However, in dry-aged products, the dehydrated and oxidized crust must be removed before selling. Thus, it could be inferred that the oxidation of myoglobin on the meat surface, in regard to color, is not nearly as important in dry aging as the effect that myoglobin oxidation can have on lipid oxidation and the resulting flavor.

Myoglobin is a heme protein found in muscles which is responsible for the storage and transport of oxygen (Wittenberg and Wittenberg, 2003). The heme group in myoglobin is what gives it the ability to bind oxygen (Suman and Joseph, 2013). Myoglobin also has the ability to bind water and carbon monoxide in fresh meat, and nitric oxide in cured meats (Mancini and Hunt, 2005; Suman and Joseph, 2013). The color of meat depends on the molecule that is bound to the heme group and the state of the iron atom in the heme group (Suman and Joseph, 2013). The iron atom can be in a reduced, ferrous Fe^{2+}, or oxidized, ferric Fe^{3+}, state (Suman and Joseph, 2013). When there is no molecule bound to the heme and the iron is in the reduced state, myoglobin is purple in color and referred to as deoxymyoglobin. The binding of oxygen, while the iron remains in the reduced state, produces a bright-red cherry color in beef, which is referred to as oxymyoglobin. Carbon monoxide also produces a bright red color when bound to myoglobin with a reduced iron atom and is referred to as carboxymyoglobin. The oxidation of the iron atom coupled with the binding of water produces a brown color, metmyoglobin, which consumers often associate with poor product quality. In cured meats, nitric oxide binds to myoglobin and produces a pink color after cooking, referred to as nitrosyl-hemochrome,

The oxygen state of myoglobin which is of the most concern is metmyoglobin. Metmyoglobin is formed via the oxidation of oxymyoglobin and results in brown discoloration in fresh meat (Suman and Joseph, 2013). Additionally, the oxidation process that results in the formation of metmyoglobin, referred to as the Fenton reaction, can result in the production of prooxidants and can trigger lipid oxidation (Chaijan, 2008). According to Mancini et al. (2005), there are several factors that can impact the

formation of metmyoglobin: temperature, oxygen partial pressure, pH, bacterial growth, and the ability of muscle to reduce metmyoglobin.

Temperature has an effect on the rate of metmyoglobin formation. According to Suput et al. (2013), higher temperature results in an increase in oxygen consumption by endogenous enzymes, as well as the increased dissociation of oxygen from oxymyoglobin to form myoglobin and an increase in the autoxidation of the myoglobin. Additionally, Suput et al. (2013) stated that low partial pressure results in the formation of metmyoglobin. This is in agreement with George and Stratmann (1952) and Brantley et al. (1993), in which both found that the rate of myoglobin oxidation increased as the partial pressure decreased. Another factor that can impact the oxidation of myoglobin is pH. According to Suput et al. (2013), low pH denatures myoglobin and promotes its oxidation. Similarly, Chen et al. (1992) found that metmyoglobin concentrations were higher in solutions with low pH than solutions with a higher pH. Bacterial growth can impact the oxidation of meat by reducing the oxygen level at the surface of the meat which results in an increase in reduced myoglobin which is then oxidized by metabolic hydrogen peroxide either from the bacteria or the muscle itself (Robach and Costilow, 1961).

The oxidation of oxymyoglobin to form metmyoglobin is a reversible reaction (Seideman et al., 1984). In muscle, there is an enzymatic system which is capable reducing the metmyoglobin and its ferric iron state to a ferrous myoglobin (Bekhit and Faustman, 2005). This reduction of myoglobin, which is a gaining of electrons, is dependent on the donation of an electron from another source (Du and McCormick, 2009). The transfer of electrons is made possible by metmyoglobin reductase, which is a

nicotinamide adenine dinucleotide (NADH), dependent enzyme (Bekhit and Faustman, 2005). Nicotinamide adenine dinucleotide is a coenzyme consisting of nicotinamide and adenine which is capable of donating electrons. However, the NADH pool and the metmyoglobin reducing enzymes are depleted postmortem (Du and McCormick, 2009). Therefore, the ability of muscle to reduce metmyoglobin during aging is not without limit.

Metmyoglobin reducing activity, and ultimately myoglobin oxidation, can also be affected by muscle. According to McKenna et al. (2005), different muscles have different metmyoglobin reducing abilities, as well as different oxygen consumption rates. Oxygen consumption continues in mitochondria at the surface of the muscle postmortem (Ramanathan and Mancini, 2018). The oxygen consumption of mitochondria at the surface of the meat is important for two main reasons i) the consumption of oxygen by mitochondria creates a low partial pressure of oxygen at the surface of meat which can decrease oxidation of myoglobin and ii) mitochondria can reduce metmyoglobin directly (Ramanathan and Mancini, 2018). In fresh meat, a high oxygen consumption rate is less desirable as the mitochondria out-compete the myoglobin for oxygen, preventing the production of oxymyoglobin, and inhibiting the development of the characteristic fresh meat color (Ramanathan and Mancini, 2018). It could then be hypothesized that a high oxygen consumption rate by mitochondria may actually be beneficial to dry-aged products as it could delay the oxidation of myoglobin and thus lipid oxidation. However, according to Ramanathan and Mancini (2018) approximately half of the fatty acids in mitochondria are unsaturated fatty acids. The oxidation of lipids in the mitochondria produces compounds detrimental to mitochondrial function and oxidative stability as a

whole (Ramanathan and Mancini, 2018). Ramanathan and Mancini (2018) stated that 4-hydroxy-2-nonenal (HNE), a secondary product of lipid oxidation, can inactivate enzymes responsible for the production of NADH and limit mitochondrial function. Thus, the effect of oxygen consumption rate by mitochondria, and its effect on dry aging, is worthy of further exploration.

As stated previously, the formation of metmyoglobin occurs when the partial pressure of oxygen is low. This means that metmyoglobin typically forms below the surface of the meat where some, but not all, oxygen has penetrated, resulting in low partial pressure and creating the ideal environment for metmyoglobin formation (McKenna et al., 2005). Consequently, the location of metmyoglobin formation is dependent on the ability of oxygen to penetrate into the muscle tissue (Bendall and Taylor, 1972), which increases with time (McKenna et al., 2005). Therefore, if a low partial pressure of oxygen were to be maintained at the surface or if the product were held in anaerobic conditions, then metmyoglobin would theoretically only form on the surface of the product or would be limited altogether.

To conclude, the exposure of meat to the environment in a cooler, results in lipid and myoglobin oxidation. Lipid oxidation negatively impacts flavor and therefore must be limited during dry aging. To limit lipid oxidation, myoglobin oxidation must also be limited since it can induce the oxidation of lipids. There are many factors that can impact the rate of both lipid and myoglobin oxidation. More research is needed to examine how limiting or inhibiting lipid oxidation could impact the quality of dry-aged products.

Effect of Anaerobic Conditions on Meat

Dry-aged products are generally left unpackaged and exposed to the environment resulting in the formation of an unpalatable crust, the oxidation of lipid and proteins, and the loss of moisture (Warren and Kastner, 1992; Savell, 2008; Khan et al., 2016; Ribeiro et al., 2021). Fresh meat products have the advantage of packaging technologies which can prevent many of the aforementioned dry aging challenges (Dikeman et al., 2013). However, the utilization of these packaging strategies results in the storage of meat in an anaerobic environment. Removing oxygen from meat and meat packages can reduce lipid oxidation, myoglobin oxidation, the metmyoglobin reducing activity (MRA) cycle, and microbial activity (Johnson and Decker, 2015).

Several packaging strategies can be used in fresh meat products. Vacuum and low-oxygen modified atmosphere packaging (MAP), and the use of oxygen scavengers in low-oxygen packaging, are three methods commonly used in the industry to reduce lipid oxidation and prolong shelf life (Johnson and Decker, 2015). Modified atmosphere packaging can contain various levels of several gases to provide a desired effect. Modified atmosphere packaging with high oxygen concentrations are used to promote a bright red color during storage and retail display (Mancini and Hunt, 2005). However, high-oxygen MAP packaging promotes lipid oxidation (Cayuela et al., 2004; Kim et al., 2010). Thus, MAP packaging without oxygen is used to limit lipid oxidation (Berruga et al., 2005). Oxygen scavengers can also be used to remove oxygen from the system ultimately reducing lipid oxidation (Johnson and Decker, 2015). According to Cruz et al. (2012), oxygen scavengers can reduce the oxygen levels in packaging to <0.01%. Finally, vacuum packaging can be used to limit lipid oxidation as it almost completely

removes oxygen from the system (Johnson and Decker, 2015). Several studies have found that vacuum packaging is the most effective packaging method used to prevent lipid oxidation during storage. Nam and Ahn (2003) and Cayuela et al. (2004) found that vacuum packaging resulted in lower TBARS values when compared to other packaging methods.

The removal of oxygen (anaerobic) during storage significantly reduces lipid oxidation (Nam and Ahn, 2003; Johnson and Decker, 2015). Johnson and Decker (2015) stated that oxygen level is one of the most important factors affecting lipid oxidation. Similarly, Jo et al. (1999) examined various storage parameters on vacuum packaged pork sausages and claimed that "oxygen availability is the most critical factor in the development of lipid oxidation." In fact, there is evidence that if the concentration of oxygen is drastically reduced, lipid oxidation could be severely limited (Johnson and Decker, 2015). According to Johnson and Decker (2015), oxygen is a primary reactant in lipid oxidation and therefore the reduction or removal of oxygen from a system can greatly affect lipid oxidation. In lipid oxidation, oxygen plays a vital role in the initiation phase as it is responsible for removing hydrogen from the allylic carbon resulting in the formation of alkyl radicals and the onset of lipid oxidation (Domínguez et al., 2019). Therefore, the removal of oxygen from a meat system could result in the reduction or prevention of lipid oxidation.

The effect of oxygen concentration on myoglobin is more complicated. According to George and Stratmann. (1952) and Brantley et al. (1993) the rate of oxidation for myoglobin actually increases as the oxygen concentration decreases peaking at lower partial pressures (1.5 mm of oxygen). This occurs because

metmyoglobin, the oxidized state of myoglobin, is formed at low partial pressures (Šuput et al., 2013). However, the rate of oxidation is null at true anaerobic conditions (0 mm of oxygen) (George and Stratmann, 1952; Brantley et al., 1993). In fact, storing meat in true anaerobic conditions would result in the formation of deoxymyoglobin since there would not be enough oxygen for oxygenation or oxidation (Seideman et al., 1984; Suman and Joseph, 2013). Thus, it is possible to reduce myoglobin oxidation via anaerobic storage, but there still may be a challenge associated with keeping oxygen levels low enough that oxidation rate is not accelerated, such as damaged packaging. Also, myoglobin oxidation can induce lipid oxidation (Chaijan, 2008). Therefore, lipid oxidation could occur at lower oxygen concentrations despite the decrease in rate of lipid oxidation at lower oxygen concentrations.

Myoglobin oxidation is a reversible reaction (Seideman et al., 1984). In fresh meat, metmyoglobin can be reduced to myoglobin via the enzymatic MRA system (Bekhit and Faustman, 2005). Originally, Watts et al. (1966) stated that MRA occurred only in anaerobic conditions. However, several studies have found that the reduction reactions that take place in the MRA cycle can occur in both aerobic and anerobic environments (Shimizu and Matsuura, 1968; Al-Shaibani et al., 1977; Lanier et al., 1978; Hagler et al., 1979). According to Shimizu and Matsuura (1968), the presence of oxygen does not significantly affect the rate of metmyoglobin reduction. Similarly, Hagler et al. (1979) found that the reduction rate was identical in both aerobic and anaerobic conditions, and Lanier et al. (1978) found that there was little or no increase in reduction rate under anaerobic conditions. In contrast, Al-Shaibani et al. (1977) found that the metmyoglobin reduction rate in fish increased as conditions were changed from aerobic

to anaerobic. As previously stated, myoglobin oxidation increases at low oxygen partial pressures (George and Stratmann, 1952; Brantley et al., 1993). Zimmerman et al. (1969) found that although the rates of both MRA and myoglobin oxidation increase at low oxygen partial pressures, the increase in the rate of oxidation is greater than the increase in metmyoglobin reducing activity. Therefore, at low oxygen partial pressures, myoglobin oxidation overwhelms the metmyoglobin reducing system, ultimately resulting in the oxidation of the product. However, in true anaerobic conditions, the MRA system maintains its reducing capacity as myoglobin oxidation is practically null.

Another factor affected by atmospheric conditions is microbial activity. As stated in Gill et al. (1979), meat contains both intrinsic and extrinsic bacteria, where intrinsic bacteria refers to the bacteria naturally found in meat and extrinsic bacteria refers to bacteria from the environment that can sometimes get onto meat during processing or storage. During storage, bacteria may continue to grow compromising the integrity of the meat product (Gill, 1983). Several different methods can be used to help prevent this growth including temperature control, chemical methods, and packaging technologies (Narasimha Rao and Sachindra, 2002; Dave and Ghaly, 2011). Narasimha Rao and Sachindra (2002) described vacuum packaging as the "greatest innovation in meat handling in the last three decades," and stated that the oxygen deficient environment created by the vacuum package can severely reduce or even inhibit the growth of spoilage organisms. According to Seideman et al (1983), the reduction, or rather removal, of oxygen in vacuum packaging results in a decrease in discoloration, off-odors, and off-flavors associated with bacterial spoilage. This agrees with Ercolini et al. (2011) and Mansur et al. (2019) who found that storage in vacuum packaging resulted in a lower

total viable count (TVC) when compared to other packaging strategies. However, Gill et al. (1983) stated that spoilage can still occur if products are improperly packaged or if the vacuum package is defective. During storage, bacterial growth can promote myoglobin oxidation (Robach and Costilow, 1961). Therefore, the use of vacuum packaging, and other anaerobic packaging strategies, is important to the preservation of meat quality.

Biochemistry of Aging

The slaughter of livestock results in a myriad of biochemical changes starting with exsanguination which shifts metabolism from aerobic to anaerobic (Ramanathan et al., 2019). As the animal is exsanguinated, the body loses the ability to transport oxygen to the muscles (Ramanathan et al., 2019). The body quickly uses up residual oxygen and energy production switches from aerobic to anaerobic (Lana and Zolla, 2016). The anaerobic production of ATP is inefficient and relies on glycogen stores which are also quickly depleted (Lana and Zolla, 2016; Ramanathan et al., 2019). The depletion of ATP initiates the onset of rigor mortis which results in the shortening of muscle fibers, the toughening of the muscle/meat, and signals the turning point in the conversion of muscle to meat (Lana and Zolla, 2016).

According to Lana and Zolla (2016), the meat reaches its toughest point during rigor, but gradually decreases in toughness during the "post-rigor or tenderization phase" (aging) resulting in an edible product. Tenderness and flavor are arguably the most important quality traits recognized by consumers (Smith et al., 1978; Laville et al., 2009; Anderson et al., 2012; O'Quinn et al., 2018). The meat industry utilizes aging to achieve acceptable flavor and tenderness in meat products. Aging is simply the prolonged storage

of meat; however, the biochemical processes that take place during aging are complex. During aging, several enzymatic pathways work to breakdown proteins, lipids, nucleotides, and other compounds which affect meat tenderness and flavor (Khan et al., 2016).

The breakdown of proteins via enzymatic processes, which occurs during aging, is referred to as proteolysis. According to Laville et al. (2009), the main effects of proteolysis are seen in the components of the "the extensive system of lateral attachment of myofibrils within Z-lines," including structural proteins such as titin, nebulin desmin, troponin T, and alpha-actinin. Proteolysis is carried out by several enzymes including calpains, cathepsins, proteosome, and caspases (Laville et al., 2009; Huff Lonergan et al., 2010; Kemp and Parr, 2012; Lana and Zolla, 2016). The calpain enzymatic system consists of several isoforms of the calpain enzyme, including the two most well defined calpain isoforms: m-calpain and μ-calpain (Huff Lonergan et al., 2010). Calpains are cited as a major contributor of postmortem tenderization and the calpain isoform μ-calpain is considered to be the primary protease (Huff Lonergan et al., 2010; Kemp and Parr, 2012). In agreement with the statement made by Laville et al. (2009), Ramanathan et al. (2019) stated that calpains "degrade muscle proteins by breaking down muscle structural proteins." Huff-Lonergan et al. (2010) reiterated that the specific role that calpains play in proteolysis is in the breakdown of cytoskeletal proteins such as titin and nebulin and/or intermediate filaments such as desmin. The activity of both m- and μ-calpains relies on the presence of calcium (Huff Lonergan et al., 2010). In fact, the names of the two calpain isoforms reflect the amount of calcium that each enzyme needs to be active with μ-calpain requiring 1-65 μM of calcium and m-calpain requiring 100-

1,000 μM of calcium for activation (Huff Lonergan et al., 2010; Lana and Zolla, 2016; Ramanathan et al., 2019). According to Huff-Lonergan et al. (2010), Kemp and Parr (2012), and Ramanathan et al. (2019), calpain activity is also impacted by pH. Huff-Lonergan et al. (2010) stated that the calpain activity of both isoforms is lower at pH values close to those found in post-mortem muscle and that accelerated pH decline results in accelerated calpain activation. In contrast, Kemp and Parr (2012) stated that rapid pH decline would lead to calpain inactivation and that intermediate pH decline would provide more optimal conditions for calpain activity. Ramanathan et al. (2019) reported that calpains are most effective at a neutral pH. Lastly, calpain activity is affected by calpastatin (Huff Lonergan et al., 2010; Kemp and Parr, 2012; Ramanathan et al., 2019). According to Huff-Lonergan et al. (2010), calpastatin is an endogenous inhibitor of calpains which requires calcium to bind to calpains resulting in an inhibition of calpain activity. Nonetheless, despite extensive studies, there is much that is still unknown about the calpain system, though some conclusions may be drawn (Huff Lonergan et al., 2010). It is clear that calcium availability, pH, and calpastatin activity all have an impact on this proteolytic system, regardless of incongruencies in studies. Ultimately, calpains remain a topic of interest and will continue to be researched as they are likely the primary enzyme system involved in postmortem tenderization.

The role of cathepsins in postmortem tenderization is often debated and is not well understood (Lana and Zolla, 2016). According to Nowak (2011) and Lana and Zolla (2016), cathepsins are endo- and exo- peptidases found in lysosomes. Cheret et al. (2007) suggested that cathepsins promote postmortem proteolysis through a synergistic relationship with the calpain system. However, many authors disagree with the notion

that cathepsins have any impact on postmortem tenderization (Lana and Zolla, 2016). Nowak (2011) stated that the role of cathepsins in tenderization is doubtful, citing a lack of proof that cathepsins are released from the lysosomes postmortem. In the past, Martins et al. (1968) also expressed doubts that cathepsins were involved in meat tenderization citing the results of their study. Sancho et al. (1997) stated that the role of cathepsins in actin degradation was unquestionable but noted that the role of this degradation in the disruption of myofibril structure, and ultimately postmortem tenderization, remains unclear.

The 20s proteasome, or MultiCatalytic Proteinase complex (MCP), is a proteinase complex that is involved in multiple protein degradation pathways (Lana and Zolla, 2016). According to Dutaud et al. (2006), the 20S proteasome is a proteolytic system which is secondary to calpain activity and accounts for the loss of Z-disks during the storage of meat. Similarly, Lamare et al. (2002) found that the 20S proteasome is partly responsible for the "second wave" of proteolysis in meat which occurs after an initial tenderization period by μ-calpain early postmortem. Though it is clear that the 20S proteasome has an effect on postmortem tenderization, the specific action of the 20S proteasome and its role in meat tenderization is still being elucidated.

The last proteolytic system commonly reported in the literature is the caspase system. Caspases are a family of specific cysteine proteases (Nowak, 2011). According to Nowak (2011), caspases create an intracellular network which signals proteolysis. However, similar to cathepsins and proteasome, the contribution of caspases on the postmortem tenderization of meat is not fully understood and requires more research (Huff Lonergan et al., 2010; Nowak, 2011). When discussing proteolysis and

postmortem tenderization, it seems that there is a consensus in the literature such that the calpain system is the primary proteolytic system which synergistically works with and interacts with the other proteolytic systems including cathepsins, proteasome, and caspases.

The breakdown of proteins during aging is not only important for postmortem tenderization, but also for flavor development. Huff-Lonergan et al. (2010) noted that although calpains are not responsible for the complete breakdown of proteins into amino acids, one proposed model is that calpains catalyze the release of myofibrils and myofilaments which can be readily broken down by proteases and lysosomes to into individual amino acids. Regardless of the mechanism, protein breakdown is important to the development of beef flavor. The amino acids glutamic acid and aspartic acid are known to produce a beefy and umami flavor in beef (Kim et al., 2016; Setyabrata et al., 2021). Glutamic acid content increases throughout the aging process (Kim et al., 2016). However, Kim et al. (2016) found that glutamic acid content was clearly higher in dry-aged than wet-aged beef after similar aging periods. This could account, in part, for the some of the differences in flavor between the two aging methods.

Lipolysis is a biochemical pathway in which triacylglycerols (TAGs) are broken down via hydrolytic reactions (Lass et al., 2011). During aging, the oxidation of lipids contributes to the flavor development, but there is also evidence to suggest that the breakdown of lipids through lipolysis could also have an impact on flavor (Dashdorj et al., 2016; Khan et al., 2016; Domínguez et al., 2019). Toldra et al. (1998) stated that the free fatty acid products of lipolysis are more susceptible to lipid oxidation which would likely impact flavor. However, according to Dashdorj et al. (2016), little is known about

the flavor compounds generated by lipolysis, and the impact of these flavor compounds on dry-aged beef palatability is absent from the scientific literature.

Nucleotides are important flavor precursors which interact with free amino acids (Oh et al., 2018; Kim et al., 2019). Among the various nucleotides, those most important to meat are hypoxanthine, inosine, inosine-5'-monophosphate (IMP), and adenosine-50 -monophosphate (AMP) (Dashdorj et al., 2016; Setyabrata et al., 2021). These nucleotides all contribute to the bitter and umami flavors in meat (Kim et al., 2019). During aging, the levels of each of these nucleotides fluctuate resulting in different flavors. As meat is aged, IMP decomposes into inosine and hypoxanthine which can result in an increased bitter flavor (Oh et al., 2018). However, different aging methods can result in different levels of nucleotides. For example, wet aging generally results in a higher level of hypoxanthine, whereas dry aging generally increases IMP and AMP levels (Oh et al., 2018; Kim et al., 2019; Setyabrata et al., 2021). Oh et al. (2018) found that hypoxanthine content increased regardless of aging method, but that the increase was greater in wet than dry-aged products. Kim et al. (2019) found that IMP content decreased in all of the examined cuts regardless of aging method and suggested that this reduction was a result of the continuous dephosphorylation of IMP into inosine during aging. Setyabrata et al. (2021) observed a higher abundance of nucleotides in dry-aged beef when compared to wet-aged beef. These studies may serve as supporting evidence for the argument that dry aging and wet aging each produce inherently different flavors. However, as stated previously, flavor and flavor development are extremely complex topics.

It is clear that there is a plethora of biochemical changes that take place postmortem starting with exsanguination and continuing throughout the aging process. During aging, proteolysis, lipolysis, and other enzymatic pathways result in the breakdown of proteins, lipids, nucleotides, and other flavor precursors resulting in improved tenderness and a range of flavors. These complex processes occur regardless of aging method, though the results may differ. Both dry and wet aging result in increased tenderness; however, the flavor compounds formed through each aging method can vary in kind and amount. Dry aging generally results in higher levels of flavor compounds associated with the umami flavor, whereas wet aging tends to result in higher levels of bitter flavor compounds, dismissing the copious amounts of flavors that result solely from oxidation. Still, similarities in biochemical pathways between both aging methods exist and could explain how some flavor discrepancies are undetectable to consumers.

Impact of Mold Growth

The literature cites a wide range of parameters that are utilized in dry aging. Different times, temperatures, and relative humidity levels all affect the final product, nearly guaranteeing uniqueness in dry aging. Still, some producers use molds to differentiate their products even further. However, the research on the impact of mold on dry-aged products is lacking. In many cases, molds, along with yeasts, are simply identified and enumerated, but implications of mold growth are not explored. DeGeer et al. (2009) and Berger et al. (2018) found that traditional and in-bag dry aging treatments did not affect mold growth. Ahnstrom et al. (2006) also looked at traditional and in-bag

dry aging methods and found that mold counts were lower than 3.0 log cfu/cm^2. Ryu et al. (2018) and Oh et al. (2019) identified yeasts and molds in dry-aged beef samples. Ryu et al. (2018) found the potentially harmful spoilage yeasts and molds *Candida sp.*, *Cladosporium sp.*, *Rhodotorula glutinis*, and *Rhodotorula mucilaginosa* at ca. 25 days of dry aging, but not at 60 days. Ryu et al. (2018) also found that there was an increase in *Penicillium camemberti* and *Debaryomyces hansenii*, which are important in cheese manufacturing, between 40 and 60 days. Oh et al. (2019) found molds belonging to the *Mucoraceae* family and the yeast *Debaryomyces spp.* of the *Saccharomycetaceae* family after dry aging for 28 days. Dashdorj et al. (2016) stated that *Thamnidium*, *Rhizopus*, and *Mucor genera* are all molds associated with dry aging.

Despite the lack of research, many producers still use molds during dry aging to help differentiate their product. However, molds may provide some benefits to dry aging systems. According to Oh et al. 2019, molds play a role in flavor development through the production of proteolytic and/or lipolytic enzymes. This agrees with Dashdorj et al. (2016) who stated that molds are important because their enzymes are able to penetrate into the meat and break down the muscle and connective tissues. However, there are no research papers which document and/or quantify the effect of molds on meat tenderness. Much of what is known about the effect of molds on meat systems comes from studies on fermented meat products, which are easily compared to dry-aged products. For example, Venturini (2019) stated that molds are used in the production of fermented sausage to aid in flavor development through the production of lactic acid and acidification of the product, and through the breakdown of proteins and lipids via the production of proteolytic and lipolytic enzymes. Clearly molds can have an impact in fermented meat

products, but the benefits of molds, or lack thereof, are less documented. Lee et al. (2019) found that air flow velocity impacted microbial and mold growth on the crust of the dry-aged product and suggested that the change in mold/microbial growth could potentially affect the physicochemical traits and flavor compounds of dry-aged beef. More research is required on the impact of molds on flavor development in dry-aged products.

Conclusion

Dry aging science is still relatively new and there are a lot of unknowns. The scientific consensus is that dry aging is a process in which meat, predominately beef primals or subprimals, is exposed to the environment in the refrigerator under controlled time and temperature parameters. It has also been suggested that relative humidity should be controlled. However, the parameters found in the literature are ambiguous and large variations exist in aging time, temperature, relative humidity, and even whether or not molds should be introduced to impart different and unique flavors into dry-aged products. Regardless, dry aging seems to be gaining popularity for its novelty and its characteristic unique flavors, often described as beefy and roasted (Warren and Kastner, 1992). The development of flavors is a complex process that is still being evaluated. In dry-aged products, it is theorized that the unique flavor development is a consequence of 1) the concentration of flavor through moisture loss and 2) the creation of new flavors from the extended aging. As meat is left unpackaged and exposed to the environment in a refrigerator, it loses moisture, resulting in a concentration of flavor compounds. However, extreme moisture loss negatively impacts yield. Additionally, differences in

aging temperature, time, relative humidity, and the incorporation of molds can produce a wide range of product flavors through countless biochemical pathways. Still, not all flavors are positively perceived and as meat is exposed to atmospheric conditions, the lipids and proteins can oxidize, resulting in off-flavors such as “warmed over flavor” (Adhikari et al., 2011).

In order to produce a consistent product, there must be a single set of dry aging parameters that are tightly controlled. Additionally, such parameters must produce an optimal product quality. During aging, as meat is exposed to the atmosphere, and muscle proteins and lipids are oxidized, resulting in the development of negative flavor compounds. If oxidation could be limited, or even eliminated, then the final product would possibly have an improved dry-aged flavor. In dry aging facilities, oxygen is likely the main cause of oxidation in dry-aged meats. Therefore, the removal of oxygen during dry aging may result in a less oxidized product with an improved flavor.

To achieve this goal, a study was designed to look at the effects of low-oxygen conditions on dry-aged beef. The research objectives of this study were to eliminate oxygen exposure from the dry aging system and compare the final level of oxidation and flavor of an low-oxygen product to a traditionally dry-aged control after 41 days of dry aging.

MATERIALS AND METHODS

Sample Collection and Preparation

Experimental Design

The experimental design for this study was a complete, randomized design. The experimental unit for the study was loin. The treatments for the study were: traditional (aerobic) dry aging, low-oxygen dry aging, and wet aging.

Treatments

The treatments for this study were as follows: wet aging, traditional (aerobic) dry aging, and low-oxygen dry aging. The wet-aged treatment was aged in the commercial film packaging from the supplier. These strip loins were aged in the same cooler as the dry-aged strip loins on racks next to the dry aging chambers. The traditional (aerobic) dry-aged treatment was aged in the unsealed chambers, exposed to the environment in the cooler. The low-oxygen dry-aged treatments were aged in an anerobic environment to limit oxygen exposure. The low-oxygen chambers were housed in sealed oxygen impermeable film "bubbles" to inhibit exposure to oxygen. All treatments were aged for 41 days. The two dry aging treatments had the same settings for fan speed and relative humidity. For the two dry aging treatments, weights were recorded every 10 minutes. Oxygen concentration was also measured every 10 minutes for the low-oxygen treatment.

Sample Collection

Eighteen top Choice boneless strip loins, graded after 24 hours, were purchased from Greater Omaha Packing, LLC. in Omaha, NE. The strip loins were collected the

day before the project start date, vacuum packaged, and stored in a cooler overnight at the Loeffel Meat Laboratory at the University of Nebraska-Lincoln. The strip loins were randomly assigned to one of three treatments: traditional (aerobic) dry aging, low-oxygen dry aging, and wet aging.

Sample Preparation

The wet-aged samples were aged in the commercial packaging: Amcor Perflex 64 Series Shrink Bags (Amcor Flexibles North America, Oshkosh, WI) with an oxygen transmission rate = <1.2 cc O_2/645 cm^2/day at 23°C and 0% relative humidity. No further preparation was required for these samples. For the other treatments, further preparation was required. First, cutting tables were sanitized with 90% ethyl alcohol prior to fabrication to provide a sterile environment. Initial weights were recorded for the dry aging treatment samples while in the commercial vacuum packaging. The vacuum packages were then sprayed with 90% ethyl alcohol to sanitize the packages before being opened. The strip loin was then removed from the package and vacuum package bag weight was recorded and purge collected to be weighed. Lastly the trim and purge weights were recorded for the strip loins. These procedures were repeated for the 12 dry-aged strip loins (n = 6 for traditional; n = 6 for low-oxygen). Once all of the strip loins were fabricated and data were recorded, all twelve were transported to the dry aging room in plastic tubs and placed into the dry aging chambers to begin the aging process. Initial weights were recorded for each of the dry aging samples and the system was started.

Dry Aging System Preparation

Low-oxygen Chamber Development

The Agenator dry aging system was used to dry age the strip loins in this project. A full description of the system can be found in "Agenator: An open-source computer-controlled dry aging system for beef" (Lau et al., 2019). For this project, six of the dry aging systems were updated to be able to run at low oxygen levels, see Appendix 1. All of the tubing used for these six chambers was replaced with oxygen impermeable, EJ Beverage Tubing (TM BFX6-10 3/8" ID x 5/8" OD; Eldon James Corp., Denver, CO). In addition, the pumps used to circulate air needed to be sealed. This was accomplished by sealing the intake and outflow valves with Loctite epoxy plastic bonder (Henkel Corp., Dusseldorf, Germany). The two compartments of the pump were then sealed with DAP Clear Window, Door & Siding Silicone (DAP Products Inc., Baltimore, MD) so that maintenance could still be performed on the pumps if needed. A rubber seal was added to the caps of the drying columns and the intake and outflow valves were sealed with LA-CO Slic-tite Heavy Duty Thread Sealing Compound (LA-CO Industries Inc., Elk Grove, IL). Reverse Osmosis Canister 1.27 cm Ports (Bulk Reef Supply, Golden Valley, MN) were added to all twelve of the chambers as oxygen scavenger columns to remove residual oxygen via Multisorb FreshPax CR oxygen absorber (Multisorb Filtration Group, Buffalo, NY). Five 4.5 cm x 10 cm oxygen scavengers were put into columns attached to the low-oxygen chambers and replaced each time the oxygen concentration rose to 4%. The 6 low-oxygen chambers were housed inside a High Barrier Clear Roll Stock film with a 5 mil gauge x 341 mm width (oxygen transmission rate for 24 hours at 23°C and 0% relative humidity = 0.20 cc/6451 cm^2/24 hours) (Bunzl,

Omaha, NE) “bubble”. The film “bubbles” were created out of individual film sheets that were joined together using a Mophorn Heat Crimp Sealer FKR-400 Portable Hand Held Heat Crimper Sealer (Mophorn, Huaian City, Jiangsu Province, China) and folded to form a rectangular shape. The electrical wires needed to connect the computer to the system were threaded through SKINTOP Non-Metallic Strain Relief Cord Connectors (LAPP North America, Florham Park, NJ). Additionally, the top end of the “bubble” remained unsealed until the project was started. Finally, a LuminOx optical oxygen sensor (Oxygen range = 0-25%, LuminOx, Coatbridge, United Kingdom) was added to all 12 chambers to measure the concentration of oxygen in the closed system.

Chamber Preparation and Maintenance

To start the aging process, the interior of the aging chambers, as well as the wire-rack scale, were sanitized using 90% ethanol solution. The scales were calibrated prior to the start of the project. After the day 0 fabrication was completed, strip loins were placed into the dry aging chambers and initial weights were recorded. The lids were placed on the traditionally (aerobic) dry-aged strip loins and the computer program was started. For the low-oxygen dry-aged strip loins, the lids were placed on the chamber, and then the “bubble” was sealed, closing the system. Next, the bubbles were flushed with an 80% nitrogen/20% carbon dioxide gas mixture via a two-step process. First, the bubbles were inflated with the gas mixture, and then the pumps were used to pull out as much air as possible before flushing with the gas mixture. This process was repeated until the oxygen concentration in each of the chambers was less than or equal to 2%. At this point, the bubbles were connected to all components of the system and oxygen absorbers

were inserted into the oxygen scavenger columns to remove residual oxygen. Lastly, the dry aging computer program was started. Throughout the aging period, if the oxygen concentration reached 4% in a chamber, the gas flush process was repeated, and oxygen scavengers were replaced for that chamber. For all of the chambers, fan speed was set at 2,000 rpm and the target relative humidity was set at 50%.

Dry Aging System

The software for the dry aging chambers, namely the program for the Arduino (Somerville, MA), microcontroller is split into multiple classes. Some classes were derived from open-source libraries, however, most of the classes were written in Visual Studio 2017 (Microsoft Corporation, Redmond, WA) with the Visual Micro add-on (version 1902.15.0) for integration with Arduino. An additional Arduino program for setting the I2C addresses of the HIH8131-021-001 modules was included in the same repository. The source code and compiled executable files of the C# program that runs on the computer are located in a separate repository. The code can be found in "Agenator: An open-source computer-controlled dry aging system for beef" (Lau et al., 2019). The software allows for the relative humidity and fan speed for the chamber to be set for the dry aging chambers. After starting the program, the software monitors the relative humidity and fan speed, and records the weight every 10 minutes. For the low-oxygen dry aging chambers, the oxygen concentration is also recorded. The software controls the relative humidity by cycling air through the drying columns when the relative humidity sensor detects a relative humidity that is higher than the pre-set value.

Fabrication

After the samples aged for 41 days, final weights were collected, and the dry aging system was stopped. All of the tables in the fabrication room were sanitized before samples were retrieved from the dry aging cooler. After sanitation, the aged strip loins were transported to the fabrication room and final weights were again recorded using the fabrication room scale. Wet-aged strip loins were weighed packaged and unpackaged. Vacuum bags were also weighed to calculate purge. Prior to cutting, the knives were disinfected with 90% ethyl alcohol. The strip loins were then faced on the anterior end using the disinfected knife and three 2.5 cm cores were aseptically collected from the surface of the face piece and transferred into WhirlPak bags designated for each material (Nasco, Fort Atkinson, WI). After all microbiological samples were collected, the dry-aged loins were trimmed of the hardened external crust. All of the trimmed material was collected and weighed to determine yield.

Once all of the dry-aged strip loins were trimmed, steaks were sliced starting at the anterior end of the strip loins and designated to different analyses (See Appendix II). The analyses and steak thicknesses were as follows: 2.4 cm steak for Warner-Bratzler shear force (WBSF), 1.25 cm steak for water activity, 1.25 cm steak for thiobarituric acid reactive substances analysis (TBARS) and other lab analyses, an extra 2.4 cm steak for lab analyses, and the rest of the strip loin was portioned into 2.4 cm steaks for sensory analysis. The TBARS samples were trimmed of all subcutaneous fat before freezing. The 2.4 cm steak used for WBSF was also used for color after 30 minutes of bloom time. All of the steaks were then individually vacuum packaged and stored until further analysis. Water activity, lab, and TBARS steaks were stored at -80°C. Warner-Bratzler

shear force and sensory steaks were stored in one of two freezers at -20°C. For the various lab analyses, the excess powdered sample from the TBARS steak was used. The TBARS steak yielded more powdered sample than required for the TBARS analysis, therefore the excess powdered sample was used for the proximate composition analyses as well and the lab steaks were retained intact in the -80°C freezer for potential future analyses.

Color

Color was recorded using a Minolta CR-400 colorimeter (Illuminant D65, 8 mm diameter aperture, 2◦ standard observer angle; Minolta, Osaka, Japan). Five to ten minutes prior to color measurement, the Minolta was placed in the environment where samples were to be measured to become equilibrated with the temperature. The colorimeter was then turned on. The settings for the colorimeter were set by pressing the "Index Set" key on the measurement screen and are as follows: Printer = on, Color space = off, Protect = on, Auto Average = 6, Illuminant = D65 (normal daylight), Back light = off, Buzzer = on. Following colorimeter set up, the D65 settings were set using the "Calibrate" key on the measurement screen and are as follows: Y = 93.13, x = 0.3164, y = 0.3330. Next, the measurement head "eye" was set on white calibration tile (Calibration Plate, Serial No. 14933058, Konica Minolta, Japan) and the measurement button on the measuring head was pressed to initiate calibration. The head was not moved during the calibration process. After calibration, the "Color Space" key was used to navigate to the L*, a*, b* screen on the measurement screen. The calibration process was completed, and samples were ready to be measured. For sample measurement, 6

readings were taken from the Warner-Braztler shear force steak from each strip loin, moving the measuring head between each measurement. The steaks were allowed to bloom for 30 minutes prior to measuring. The measuring head was wiped using a soft, dry, clean cloth between samples. Color data were printed by the colorimeter and recorded.

Lipid Oxidation

For the TBARS analysis, the procedure originally outlined in Buege and Aust (1978) and modified by Ahn et al. (1998) was used. Thiobarbituric acid reactive substance samples still in vacuum packaging from fabrication were thawed overnight in a refrigerator at 4°C. The sample was then diced into small pieces and submerged in liquid nitrogen. The frozen sample was then blended in a metal cup blender (Model 51BL32, Waring Commercial, Torrington, CT) until it was reduced to a fine powder. Powder not used for the analysis was stored at -80°C. Powdered samples used for the analysis were stored in liquid nitrogen when not in use. All of the reagents required for the TBARS assay were mixed prior to beginning the assay. Tetraethoxypropane (TEP) Solution (1,1,3,3-Tetraethoxypropane) stock solution was created by diluting 99 μL TEP (97%) (Sigma TEP- T9889) and bringing it to a volume of 100 mL distilled deionized water (ddH_2O). The stock solution was then diluted to a 1:3 (TEP Solution: ddH_2O) ($1x10^{-3}$ M). One liter of TBA/TCA (2-thiobarbituric acid/trichloroacetic acid) stock solution was made by mixing 15% TCA (weight by volume) (Sigma TCA- T9159) and 20 mM TBA (molecular weight 144.5) (Sigma TBA- T5500) reagent in ddH_2O. This was accomplished by dissolving 2.88 g TBA in warm ddH_2O and then adding TCA (150 g)

and ddH_2O to bring the volume to 1 L. Next a 10% BHA (butylatedhydroxyanisole) stock solution was produced. Ten grams of BHA (Sigma BHA- B1253) were dissolved in 90 mL ethanol (90%) and 5 mL of ddH_2O. After mixing the stock solutions, standards (in duplicate) were produced for the analysis and were as follows: Blank = 1 mL ddH_2O, Standard 5 = 100 µl working TEP + 1.90 mL ddH_20 ($5x10^{-5}$M), Standard 4 = 1 mL Std. 5 + 1 mL ddH_20 ($2.5x10^{-5}$M), Standard 3 = 1 mL Std. 4 + 1 mL ddH_20 ($1.25x10^{-5}$M), Standard 2 = 1 mL Std. 3 + 1 mL ddH_20 ($.625x10^{-5}$M), and Standard 1 = 1 mL Std. 2 + 1 ml ddH_20 ($.3125x10^{-5}$M). One milliliter of Standard 1 was removed and discarded, leaving 1 mL behind. After mixing all of the reagents and standards, the analysis was started. Five grams of powdered sample were transferred into a 50 mL conical tube, and 14 mL of ddH_2O and 1.0 mL of BHA Stock Solution was added. The solution was homogenized for 15 seconds using a Polytron (Kinmatica AG, Lucern, Sui). The homogenized sample was then centrifuged at 2,000 x G for 5 minutes. Next, 1 mL of homogenate was transferred to a 15 mL conical tube. Two milliliters of TBA/TCA solution were then added, and the solution was vortexed. The vortexed solution was incubated in a water bath at 70°C for 30 minutes to develop color. The samples were then cooled in a cold-water bath at 21°C for 10 minutes. The tubes were centrifuged at 2,000 x G for 15 minutes. Lastly, duplicate aliquots of 200 µL were transferred from each tube to a 96 well plate. The samples were read using a microplate spectrophotometer (Model Epoch, Biotek, Winooski, VT) at 540 nm. Milligrams of malonaldehyde per kilogram of tissue were then calculated with the equation K(extraction)=(S/A) x MW x (106/E) x 100, where S=Standard concentration (1x10-8 moles 1,1,3,3-tetraethoxypropane)/5 ml, A=Absorbance of standard, MW=MW of

malonaldehyde (72.063 g/mole), E= sample equivalent (1), and P=Percent recovery. The final calculation was 0.012 x concentration x 72.063 x 106 = mg malonaldehyde/kg of tissue.

Tenderness

Slice Shear Force

Frozen steaks (-20°C) in vacuum packaging from fabrication were thawed overnight in a refrigerator at 4°C. Raw weights and temperatures were recorded for each of the steaks. Temperatures were recorded using an insulated T thermocouple (5SC-TTT-30-120, OMEGA Engineering, Inc., Stamford, CT) connected to a handheld thermometer (OMEGA 450-ATT, Engineering, Inc., Stamford, CT) with the probes being inserted into the geometric center of the steak. The steaks were then cooked on a belt grill (TBG60-V3 MagiGril, MagiKitch'n Inc., Quakertown, PA). The belt grill was preheated to 149°C with both top and bottom elements heated to 163°C. The height of the gap in the belt grill was 2.16 cm. The steaks were cooked to an internal temperature of 70°C and cooked weights and temperatures were recorded. For the slice shear force analysis, the procedure outlined in "Standardizing collection and interpretation of Warner-Bratzler shear force and sensory tenderness data" (Wheeler et al., 1997) was followed. After cooking, the steaks were then placed into a sizing box for the slice shear force analysis and the lateral end of each of the cooked steaks was removed to obtain a 5 cm long section of steak. After sizing, the steaks were placed into the slice box so that a slice could be taken from the center of the sample. The samples sliced at a 45° angle, parallel with the muscle fibers, using a double-bladed knife. The cut sample was

centered and sheared using a Food Texture Analyzer (TMS-Pro, Food Technology Corp., Sterling, VA.) with a shear force blade. The remaining sample was placed in a bag and placed in a cooler to be cooled for Warner-Bratzler shear force the following day (Lorenzen et al., 2010). The shear force blade was run at 200 mm/min. The maximum force, in kilograms, required to shear the sample was recorded.

Warner-Bratzler Slice Shear Force

The next day, the 6 round cores, 1.27 cm in diameter, were drilled out of the cooled samples. The cores were placed on the Food Texture Analyzer (TMS-Pro, Food Technology Corp., Sterling, VA.) with a Warner-Bratzler blade so that the center of the core was perpendicularly aligned 60° "V-shaped" portion of the blade. The blade was run at 200 mm/min. The maximum force, in kg, required to shear each core was recorded. The shear force values for the 6 cores were then averaged to give a final Warner-Bratzler value for each steak.

Water Activity

Water activity steaks were thawed overnight in a refrigerator at 4°C. To prepare the steaks, 1 cm was first trimmed off the ventral end of the steak. Next, three 1 cm strips were cut and collected, discarding the trimmings and the subcutaneous fat. The three samples corresponded to the ventral, medial, and dorsal portions of each steak. The steak strips were then chopped using an Oster food processor (Model FPSTMC3321, Sunbeam Products Inc., Boca Raton, FL) and placed into Aqualab water activity cups (Decagon Devices, Inc., Pullman, WA) so that the bottom of the well was completely covered.

Water activity measurements were run in duplicate. The water activity was measured using an Aqualab water activity meter (Decagon Devices, Inc., Pullman, WA) at 25 ◦C. The values from each location were then averaged to establish a water activity value for each steak, and ultimately treatment averages were determined. Data were recorded and averaged for each location on the steak.

Proximate Composition

Moisture and Ash

Powdered sample from the TBARS analysis was removed from the ultra-low freezer (-80°C). This assay was performed on a Thermogravimetric Analyzer (TGA-701 Leco Corp., St. Joseph, MO.). To begin the analysis, sample identification numbers were entered into the computer. Next the method of operation was selected. Both moisture and ash were analyzed in this assay. The moisture settings were as follows: Covers = off, Ramp rate = 6 d/m, Ramp time = 17 min, Start temp = 25°C, End temp = 130°C, Atmosphere = N, Flow rate = high, Hold time = 00 min, Constant weight = 0.05%, and Constant weight time = 09 min. The ash settings were as follows: Covers = off, Ramp rate = 20 d/m, Ramp time = 30 min, Start temp = 130°C, End temp = 600°C, Atmosphere = O, Flow rate = high, Hold time = 00 min, Constant weight time = 0.05%, and Constant weight time = 09 min. The general settings for this assay were as follows: crucible density = 3.00, cover density = 3.00, and sample density = 1.00. After selecting the operation method, “analysis” was selected and the “collect” button was clicked. Empty crucibles were entered into the selected furnace so that they could be weighed to obtain a tare weight. After obtaining a tare weight, 1 g of powdered sample was loaded into the

machine. Once all of the samples were loaded, the machine automatically started. When the analysis was completed, data were exported to a flash drive. Crucibles were removed from the machine after 30 minutes to allow them to cool down. Upon removal, crucibles were cleaned and dried in a drying oven for 1.5 hours. Dried crucibles were transported to a desiccator for future use.

Fats

For the fat analysis, the extra powdered sample from the TBARS analysis was utilized. Before beginning the assay, the analytical balance checked to make sure it was clean and level. After zeroing the balance, the weighing paper and paper clip used for the analysis were weighed and recorded. Next, 2 grams of sample were added to the weighing paper with a spatula. The total sample weight was recorded. The weighing paper was then folded to contain the sample and secured with a paper clip. Samples were weighed in triplicate. After completing the weighing process, samples were transported to the ultra-low freezer (-80°C) until they were ready to be analyzed. For fat extraction, the Soxhlet method (AOAC, 1990) was used. First, the ground glass connections were checked, wiped clean with a dry paper towel, and a thin coating of Dow Corning® high vacuum grease (Sigma-Aldrich, St. Louis, MO) was applied. Each boiling flask was checked to make sure that it contained boiling stones to prevent violent solvent boiling. The samples were then loaded into the Soxhlet tubes and arranged so that none of the samples were above the level of the top bend in the narrow tubing on the outside of the Soxhlet. Next, large, 500 mL boiling flasks were filled with 400 mL of ethyl ether solvent, and the small, 125 mL flasks were filled with 100 mL of solvent under the fume

hood. The Soxhlet was then fitted onto the boiling flask. The assembly was moved to the extraction room and placed on the condenser. The glass ground connections were again checked, and the boiling flasks were checked to make sure that each was resting on the heating element. The bare metal surface of the burners was covered with a ceramic fiber sheet. The water supply to the condenser was turned on. The heating elements control dials were turned so that they rested between three and four. Once again, fittings and boiling stones were checked. Fat extractions for beef generally take ~48 hours to run. When completed, the burners were turned off and the solvent was allowed to cool completely before removing. Once the burners had cooled, the flask and Soxhlet tube were slowly uncoupled from the condenser. The Soxhlet was covered to prevent ether vapors from escaping while being transported to the fume hood. The samples were then air dried in the fume hood for 2 hours to get rid of the remaining ether. The samples were then placed in a drying oven at 105°C and left overnight. The samples were then weighed, and fat percent was calculated using the equation ((Original weight including filter paper and paper clip-fat extracted sample weight)/Sample weight)*100) - % Moisture = % Fat.

Microbial Analysis

Sample cores from day 0 and day 42 samples were subjected to microbial plating methods in duplicate. Fifty µL of the homogenized steak and BBL Peptone water sample was administered to 100 mm agar plates utilizing an Eddy Jet spiral plater (IUL, S.A., Barcelona, Spain). Brain, Heart, Infusion agar (Thermo Fisher Scientific, Waltham, MA) was used to conduct aerobic plate counts (APC), anaerobic plate counts (AnPC), and

psychrotrophic (PPC) plate count. Both the APC and AnPC were incubated at 37°C and counted at 48 hours. Anaerobic plate counts were held in anaerobic chamber (BD GasPak EZ Large Insulation Container; Becton, Dickinson, and Company, Sparks, MD) with three oxygen absorbent packs (BD GasPak EZ sachet ; Becton, Dickinson, and Company, Sparks, MD). Psychrotrophic plate counts were incubated at 4°C and counted at 10 days.

Consumer Sensory Analysis

This consumer sensory analysis was approved by an Institutional Review Board (project identification 18497). A preference test was used to compare the two dry aging treatments: traditional (aerobic) dry aged and low-oxygen dry aged. Frozen steaks were thawed in a refrigerator overnight at 4°C. The steaks were then cooked on electric grills (Hamilton Beach-31605A, Hamilton Beach Brands, Glen Allen, VA) to 30°C, then flipped and cooked to a final temperature of 70°C. The steaks were set on a cutting board and allowed to cool for approximately 2 minutes; until they could be handled. Each steak was trimmed of excess fat and connective tissue so that only lean sample remained. The samples were cut into 1 cm x 2 cm x 2.5 cm portions and placed in a TMPT Food Warmer (Smithville, TN) until being served, but for no longer than 30 minutes. Each sample was given a unique 3-digit number and served to faculty, students, and professors at the University of Nebraska-Lincoln with no prior training. Each panelist was given a cup of water and a receptacle for waste. Panelists were asked to rinse their mouths with the provided water to cleanse their palettes before starting and between samples. The samples were served simultaneously in two separate sample cups. Red colored lighting was utilized in the sensory room to minimize color effects. The panelists were asked to

circle the 3-digit code corresponding to the sample they preferred. Sensory sheets were collected and tallied.

Trained Sensory Analysis

Preparation

The trained sensory analysis was conducted at Texas A&M University with the approval of the Institutional Review Board for Use of Humans in Research at Texas A&M University (IRB2018-0958M). Twenty-four hours prior to cooking, meat was placed in a 4˚C cooler to thaw. Sample raw weights were obtained and raw temperatures were recorded using a hand-held thermometer (model HH-72T, Omega Engineering Inc., Stanford, CT). A copper constantan thermocouple (Omega Engineering, Stamford, CT) was placed in the geometric center of the samples to monitor cooking temperature.

Cooking

Steaks were cooked on a stainless steel, electric stove top grill (StarMax 536GF 36 inch Countertop Electric Griddle, Star Manufacturing International, Inc., St. Louis, MO) set at 177˚C. Grill temperatures were verified prior to initiation of cooking. Initial internal temperatures and time were recorded. The steaks were cooked until reaching an internal temperature of 71˚C. The steaks were flipped when internal temperature reached 35˚C. Final internal temperature, time, and cooked weight were recorded. Samples were wrapped in aluminum foil and placed in a Bain Marie warmer (APW Wyott W-3Vi 30.5x50.8cm, Alan,TX) with water held at 63°C in warmer pans and lids (Royal Industries, 15.2 x 25.4 cm, Brooklyn, NY) for no more than 20 minutes prior to being

served to the trained descriptive flavor and texture panel. Steaks were cut into 1.27 cm by 2.5 cm with no visible connective tissue or fat. Panelists were served two of the 1.27 cm x 2.5 cm random samples for evaluation.

Descriptive Flavor and Texture Attribute Panel

Six expert descriptive flavor and texture attribute panelists with more than 400 hours of experience were used. Panelists were trained for 13 days reintroducing flavor and texture attributes from the beef lexicon (Adhikari et al., 2011) and AMSA (2015). Forty-three flavor and texture attributes were used. Texture attributes for steaks were as follows: juiciness, connective tissues, and muscle fiber tenderness. References were continually provided to panelists during training and testing. The panelists were given an expectorant cup, double-distilled, deionized water, napkins, and saltless saltine crackers (Premium Unsalted Tops Saltine Crackers, Nabisco, East Hanover, NJ). Panelists were required to use the crackers followed by water as palate cleansers between samples.

Samples were tested within a two-hour period with a ten-minute break approximately after 1 h to prevent fatigue. Fifteen minutes before each testing session, a "warm up" sample was provided to panelists and the group leader. Each attribute was discussed and given a score (0 = none and 15 = extremely intense) for daily calibration.

Each panelist was seated in a separate breadbox-style booth that contained red lights (44.2 lux) to mask any color effects. Samples were identified with random three-digit codes and served at least 5 minutes apart in soufflé cups that would not impact flavor. Panelists recorded their scores using a 15-point scale from 0= none to 15=extremely intense.

Statistical Analysis

Yield, Color, TBARS, Tenderness, Water Activity, Proximate, and Untrained Sensory Analysis

In this study, the data from the listed analyses were analyzed as a completely randomized design, with loin as the experimental unit for the study. Data were analyzed using the PROC GLIMMIX procedure of SAS (version 9.4, SAS Institute, Cary, NC). All means were separated with the LS MEANS and PDIFF functions with $\alpha = 0.05$.

Microbial Analysis

Microbial plate counts were analyzed as a completely randomized design. In this study, the loin was considered the experimental unit. Data were analyzed using the PROC GLIMMIX procedure of SAS (version 9.4, SAS Institute, Cary, NC). All means were separated with the LS MEANS and PDIFF or SLICEDIFF functions with $\alpha = 0.05$.

Trained Sensory Analysis

Data were analyzed using SAS (version 9.4, SAS Institute, Cary, NC) with $\alpha<0.05$. For descriptive attributes data, the PROC GLIMMIX procedure was used. For this analysis, loin was considered the experimental unit. Least square means were calculated using the LS MEANS and PDIFF functions in SAS only when significance was found in the Analysis of Variance. To understand relationships between trained descriptive flavor and texture attributes, XLSTAT (v2020, Addinsoft, New York, NY) was used. Principle component analysis (PCA) was conducted by Texas A&M

University and used to analyze the aforementioned relationships, and the descriptive flavor and texture attributes results were presented as biplots. In the PCA analysis, factors are generated to describe the relationship between flavors. Each factor consists of the same flavor attributes, but with differing levels of contribution to the factor. After the factors are generated, a biplot is produced which plots the flavor attributes on a scatterplot. Once the flavor attributes are plotted, the samples are entered into the analysis. The PCA function then plots the samples on the scatterplot in proximity to the flavors that have the largest impact on each sample.

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Low-Oxygen Dry Aging of Beef

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The author would like to thank and acknowledge all of the graduate students, faculty, and professors that helped with this project.

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ABSTRACT

Dry aging is known for the development of unique flavors via concentration of flavor compounds through dehydration and the creation of new flavors through oxidation and endogenous enzymatic processes. However, prolonged exposure of meat to the environment in a cooler, which is common practice in dry aging processes, can lead to off-odors and flavors. The objective of this research project was to determine the effects of a low-oxygen atmosphere in a dry aging system. Boneless upper 2/3 Choice beef strip loins (n=18) were randomly assigned three aging treatments: wet aging, traditional (aerobic) dry aging, and low-oxygen dry aging. All treatments were aged for 41 days at 2 ± 1°C. The dry aged treatments were held at 50% relative humidity (RH) with a fan speed of 2,200 revolutions per minute (RPM). Low-oxygen dry-aged samples were aged in dry aging chambers housed within an oxygen impermeable film bubble. Low-oxygen chambers were flushed with an 80% nitrogen/20% carbon dioxide gas mixture at the beginning of aging and any time the oxygen concentration approached 4%. Food grade oxygen scavengers were also utilized to remove residual oxygen after gas flushing. After the aging process was completed, dry-aged samples were weighed before and after trimming to determine yield. There was a significant difference in yield between the wet-aged (95% yield) and the two dry-aged treatments ($p < 0.05$), but there was no significant difference between the low-oxygen (55% yield) and aerobic (54% yield) dry aging treatments. Lipid oxidation was measured using thiobarbituric acid reactive substances (TBARS). There was no significant difference found in TBARS between the wet-aged (1.18 mgs malonaldehyde/kg of tissue) and low-oxygen dry-aged (1.27 mgs malonaldehyde/kg) samples ($p < 0.05$), but there was a significant difference between the

aerobic dry-aged (2.46 mgs malonaldehyde/kg) samples and the other two treatments ($p < 0.05$). A paired preference test was conducted to determine consumer flavor preference between low-oxygen and traditionally (aerobic) dry-aged steaks. There were no significant differences found among treatments ($p < 0.05$). In a trained sensory analysis, low-oxygen dry-aged samples had slightly higher numerical values for desirable flavor notes and aerobic dry-aged beef had slightly higher numerical values for undesirable flavor notes. Further analysis via principle component analysis was able to show the flavor differences more clearly. These results suggest that although low-oxygen dry aging significantly reduced lipid oxidation and influenced the flavor, although it is unclear if the flavor differences were detectable to consumers.
Keywords: dry aging, flavor, oxidation

INTRODUCTION

Dry aging has become an ambiguous term, as many food enthusiasts would describe dry aging as an art and those adept in the sciences would recognize it as a scientific process. There is evidence for both parties. From a scientific approach, dry aging is an aging process in which meat, predominately beef primals or subprimals, is exposed to the environment in a refrigerator under controlled time and temperature parameters (Savell, 2008). It has been suggested that relative humidity should also be controlled. While the general mechanism to dry age meat remains unquestioned, many different approaches can be taken. The literature shows profound variations in aging time, temperature, relative humidity, and even the introduction of molds to impart different and unique flavors into dry-aged products. Dry aging has been around for a long time, but it has been regaining popularity recently due to the characteristic unique flavors it produces, often described as beefy and roasted (Warren and Kastner, 1992). The development of unique flavors is a consequence of 1) the concentration of flavor through moisture loss and 2) the creation of new flavors from the extended aging. As meat is left unpackaged and exposed to the environment in a refrigerator, it loses moisture, resulting in a concentration of flavor compounds. The side effects are a lighter weight final product, an unpalatable, hard exterior shell or crust that must be trimmed, and ultimately less saleable product. Additionally, differences in aging temperature, time, relative humidity, and the incorporation of molds can produce a wide range of product flavors through countless biochemical pathways. However, not all of the flavors produced during dry aging are positively perceived by consumers. For example, as meat is exposed to atmospheric conditions, the lipids and proteins can oxidize, resulting in off-

odors and flavors (St. Angelo et al., 1996). In order to produce a consistent product, there must be a single set of dry aging parameters that are tightly controlled. Additionally, such parameters must produce an optimal product quality. During aging, as meat is exposed to the atmosphere, muscle proteins and lipids are oxidized resulting in the development of negative flavor compounds. If oxidation could be limited, or even eliminated, then the final product would possibly have an improved dry-aged flavor. Oxidation during aging can result from extended exposure to oxygen. Therefore, the removal of oxygen during dry aging may result in a less oxidized product yielding an improved flavor. To achieve this goal, an experiment was designed evaluate the effects of low-oxygen conditions on dry-aged beef. The research objectives of this study were to limit oxygen exposure from the dry aging system and compare the final level of oxidation and flavor of an low-oxygen product to a traditionally dry-aged control after 41 days of dry aging.

MATERIALS AND METHODS

Sample Collection and Preparation

The experimental design for this study was a complete, randomized design. The experimental unit for the study was loin. The treatments for the study were: traditional (aerobic) dry aging, low-oxygen dry aging, and wet aging. The wet-aged treatment was aged in the commercial film packaging from the supplier. These strip loins were aged in the same cooler as the dry-aged strip loins on racks next to the dry aging chambers. The traditional (aerobic) dry-aged treatment was aged in the unsealed chambers, exposed to atmospheric oxygen and the cooler's temperature. The low-oxygen dry-aged treatments

were aged in an anerobic environment to limit oxygen exposure. The low-oxygen chambers were housed in sealed oxygen impermeable film "bubbles" (oxygen transmission rate for 24 hours at 23°C and 0% relative humidity = 0.20 cc/645 cm^2/24 hours) to limit exposure to oxygen. All treatments were aged for 41 days. The two dry aging treatments had the same settings for fan speed and relative humidity. For the two dry aging treatments, weights were recorded every 10 minutes. Oxygen concentration was also measured every 10 minutes for the low-oxygen treatment.

Eighteen USDA upper 2/3 Choice boneless strip loins, graded after 24 hours, were purchased from Greater Omaha Packing, LLC. in Omaha, NE. The wet-aged samples were aged in the commercial Amcor Perflex 64 Series Shrink Bags (Amcor Flexibles North America, Oshkosh, WI) with an oxygen transmission rate = <1.2 cc O_2/645 cm^2/day at 23°C and 0% relative humidity. No further preparation was required for these samples. Dry aging treatment samples were weighed packaged and unpackaged, and package weights were recorded to determine purge. Lastly, the trim weights were recorded for the strip loins. Once all of the strip loins were fabricated and data were recorded, all twelve were transported to the dry aging room in plastic tubs and placed into the dry aging chambers to begin the aging process. Initial weights were recorded for each of the dry aging samples and the system was started.

Dry Aging System Preparation

The Agenator dry aging system, described in "Agenator: An open-source computer-controlled dry aging system for beef" published by Lau et al. (2019), was used to dry age the strip loins in this project. For this project, six of the dry aging systems

were updated to be able to run at reduced oxygen levels, see Appendix 1. All of the tubing used for these six chambers was replaced with oxygen impermeable, EJ Beverage Tubing (TM BFX6-10 3/8" ID x 5/8" OD; Eldon James Corp., Denver, CO). In addition, the pumps used to circulate air were made air tight by sealing the intake and outflow valves with Loctite epoxy plastic bonder (Henkel Corp., Dusseldorf, Germany). The two compartments of the pump were then sealed with DAP Clear Window, Door & Siding Silicone (DAP Products Inc., Baltimore, MD). A rubber seal was added to the caps of the drying columns and the intake and outflow valves were sealed with LA-CO Slic-tite Heavy Duty Thread Sealing Compound (LA-CO Industries Inc., Elk Grove, IL). Reverse Osmosis Canister 1.27 cm Ports (Bulk Reef Supply, Golden Valley, MN) were added to all twelve of the chambers to serve as oxygen scavenger columns, which removed residual oxygen via Multisorb FreshPax CR oxygen absorber (Multisorb Filtration Group, Buffalo, NY). Five 4.5 cm x 10 cm oxygen scavengers were put into columns attached to the low-oxygen chambers and replaced each time the oxygen concentration rose to 4%. The 6 low-oxygen chambers were housed inside a "bubble" composed of High Barrier Clear Roll Stock film with a 5 mil gauge x 341 mm width and an oxygen transmission rate for 24 hours at 23°C and 0% relative humidity of 0.20 cc/645 cm^2/24 (Bunzl, Omaha, NE). The film "bubbles" were created out of individual film sheets that were joined together using a Mophorn Heat Crimp Sealer FKR-400 Portable Hand Held Heat Crimper Sealer (Mophorn, Huaian City, Jiangsu Province, China) and folded to form a rectangular shape. The electrical wires needed to connect the computer to the system were threaded through SKINTOP Non-Metallic Strain Relief Cord Connectors (LAPP North America, Florham Park, NJ). Additionally, the top end of the "bubble"

remained unsealed until the project was started. Finally, a LuminOx optical oxygen sensor (Oxygen range = 0-25%, LuminOx, Coatbridge, United Kingdom) was added to all 12 chambers to measure the concentration of oxygen in the closed system.

To start the aging process, the interior of the aging chambers were sanitized using 90% ethanol solution. The scales were calibrated prior to the start of the project. After the day 0 fabrication was completed, strip loins were placed into the dry aging chambers and initial weights were recorded. The lids were placed on the traditionally (aerobic) dry-aged strip loins and the computer program was started. For the low-oxygen dry-aged strip loins, the lids were placed on the chamber, and then the "bubble" was sealed, closing the system. The bubbles were flushed with an 80% nitrogen/20% carbon dioxide gas mixture via a two-step process until the oxygen concentration in each of the chambers was less than or equal to 2%. The bubbles were connected to all components of the system and oxygen absorbers were inserted into the oxygen scavenger columns to remove residual oxygen. Lastly, the dry aging computer program was started. Throughout the aging period, if the oxygen concentration reached 4% in a chamber, the gas flush process was repeated, and oxygen scavengers were replaced for that chamber, see Figure 1. For all of the chambers, fan speed was set at 2,000 rpm and the target relative humidity was set at 50%.

Fabrication

After the samples aged for 41 days, final weights were collected, and the dry aging system was stopped. All of the tables in the fabrication room were sanitized before samples were retrieved from the dry aging cooler. After sanitation, the aged strip loins

were transported to the fabrication room and final weights were again recorded using the fabrication room scale. Wet-aged strip loins were weighed packaged and unpackaged. Vacuum bags were also weighed to calculate purge. Prior to cutting, the knives were disinfected with 90% ethyl alcohol. The strip loins were then faced on the anterior end using the disinfected knife and three 2.5 cm cores were aseptically collected from the 0.5 cm thick faced piece and transferred into WhirlPak bags designated for each sample (Nasco, Fort Atkinson, WI). After all microbiological samples were collected, the dry-aged loins were trimmed of the hardened external crust. All of the trimmed material was collected and weighed to determine yield.

Once all of the dry-aged strip loins were trimmed, steaks were sliced starting at the anterior end of the strip loins and designated to different analyses, see Appendix 2. The analyses and steak thicknesses were as follows: 2.4cm steak for Warner-Bratzler shear force (WBSF), 1.25 cm steak for water activity, 1.25 cm steak for thiobarituric acid reactive substances analysis (TBARS) and lab analyses, an extra 2.4 cm steak for lab analyses, and the rest of the strip loin was portioned into 2.4 cm steaks for sensory analysis. The TBARS samples were trimmed of all subcutaneous fat before packaging and freezing. The 2.4 cm steak used for WBSF was also used for color after 30 minutes of bloom time. All of the steaks were then individually vacuum packaged and stored until further analysis. Water activity, extra lab, and TBARS steaks were stored at -80°C. Warner-Bratzler shear force and sensory steaks were stored in one of two freezers at -20°C.

Color

The steaks used for the color analysis were allowed to bloom for 30 minutes prior to measuring. Color was recorded using a Minolta CR-400 colorimeter (Illuminant D65, 8 mm diameter aperture, 2° standard observer angle; Minolta, Osaka, Japan), see Appendix 3. Five to ten minutes prior to color measurement, the Minolta was placed in the environment where samples were to be measured to become equilibrated with the temperature. The Minolta was calibrated on a white calibration tile (Calibration Plate, Serial No. 14933058, Konica Minolta, Japan). For sample measurement, 6 readings were taken from the Warner-Braztler shear force steak from each strip loin, moving the measuring head between each measurement. Color values were recorded as L*, a*, and b*.

Lipid Oxidation

For the TBARS analysis, the procedure originally outlined in Buege and Aust (1978) and modified by Ahn et al. (1998) was used (Appendix IV). Thiobarbituric acid reactive substance samples still in vacuum packaging from fabrication were thawed overnight in a refrigerator at 4°C. The sample was then diced into small pieces and submerged in liquid nitrogen. The frozen sample was then blended in a metal cup blender (Model 51BL32, Waring Commercial, Torrington, CT) until it was reduced to a fine powder. All of the reagents required for the TBARS assay were mixed prior to beginning the assay. Five grams of powdered sample were transferred into a 50 mL conical tube, and 14 mL of ddH_2O and 1.0 mL of BHA stock solution, 10g butylatedhydroxyanisole dissolved in 90 mL ethanol (90%) + 5mL ddH_20, was added.

The solution was homogenized for 15 seconds using a Polytron (Kinmatica AG, Lucern, Sui). The homogenized sample was then centrifuged at 2,000 x G for 5 minutes. Next, 1 mL of homogenate was transferred to a 15 mL conical tube. Two milliliters of TBA/TCA solution were then added, and the solution was vortexed. The vortexed solution was incubated in a water bath at 70°C for 30 minutes to develop color. The samples were then cooled in a cold-water bath at 21°C for 10 minutes. The tubes were centrifuged at 2,000 x G for 15 minutes. Lastly, duplicate aliquots of 200 µL were transferred from each tube to a 96 well plate. The samples were read using a microplate spectrophotometer (Model Epoch, Biotek, Winooski, VT) at 540 nm. Milligrams of malonaldehyde per kilogram of tissue were then calculated.

Slice Shear and Warner-Bratzler Shear Force

Frozen steaks (-20°C) in vacuum packaging from fabrication were thawed overnight in a refrigerator at 4°C. Raw weights and temperatures were recorded for each of the steaks. Temperatures were recorded using an insulated T thermocouple (5SC-TTT-30-120, OMEGA Engineering, Inc., Stamford, CT) connected to a handheld thermometer (OMEGA 450-ATT, Engineering, Inc., Stamford, CT) with the probes being inserted into the geometric center of the steak. The steaks were then cooked on a belt grill (TBG60-V3 MagiGril, MagiKitch'n Inc., Quakertown, PA). For the slice shear force analysis, the procedure as outlined by Wheeler et al. (1997) was used. After cooking, the steaks were then placed into a sizing box, sized, and a slice was taken from the center of the sample. The samples were sliced at a 45° angle, parallel with the muscle fibers, using a double-bladed knife. The cut sample was centered and sheared using a

Food Texture Analyzer (TMS-Pro, Food Technology Corp., Sterling, VA.) with a shear force blade. The remaining sample was placed in a bag and placed in a cooler to be cooled for Warner-Bratzler shear force the following day (Lorenzen et al., 2010). The maximum force, in kilograms, required to shear the sample was recorded. The next day, the 6 round cores, 1.27 cm in diameter, were removed from the cooled samples. The cores were placed on the Food Texture Analyzer (TMS-Pro, Food Technology Corp., Sterling, VA.) with a Warner-Bratzler blade so that the center of the core was perpendicularly aligned 60° "V-shaped" portion of the blade. The maximum force, in kg, required to shear each core was recorded. The shear force values for the 6 cores were then averaged to give a final Warner-Bratzler value for each steak. The blade was run at 200 mm/min for both shear force analyses.

Water Activity

Water activity steaks were thawed overnight in a refrigerator at 4°C. Three 1 cm strips were cut and collected, discarding the trimmings and the subcutaneous fat. The three samples corresponded to the ventral, medial, and dorsal portions of each steak. The steak strips were then chopped using an Oster food processor (Model FPSTMC3321, Sunbeam Products Inc., Boca Raton, FL) and placed into Aqualab water activity cups (Decagon Devices, Inc., Pullman, WA) so that the bottom of the cup was completely covered. Water activity measurements were run in duplicate. The water activity was measured using a Aqualab water activity meter (Decagon Devices, Inc., Pullman, WA) at 25°C. The values from each location were then averaged to establish a water activity

value for each steak, and ultimately treatment averages were determined. Data were recorded and averaged for each location on the steak.

Proximate Composition

Powdered sample was removed from the ultra-low freezer (-80°C). The moisture and ash were measured using a Thermogravimetric Analyzer (TGA-701 Leco Corp., St. Joseph, MO.). After obtaining a tare weight for the TGA, 1 g of powdered sample was loaded into the machine and analyzed. When the analysis was completed, data were exported to a flash drive.

For the fat analysis, two grams of sample were placed onto weighing paper and weighed. The paper was folded to contain the sample and secured with a paper clip. Samples were analyzed in triplicate. For the fat extraction, the Soxhlet method (AOAC, 1990) was used. The samples were then loaded into the Soxhlet apparatus and fats were subjected to an ether extraction. When the extraction step was completed, the samples were then air dried in the fume hood for approximately 2 hours. The samples were then placed in a drying oven at 105°C and left overnight. The samples were then weighed, and fat percent was calculated.

Microbial Analysis

After the cores were collected from the steaks, they were transferred into WhirlPak bags (Nasco, Fort Atkinson, WI). Next, 35 mL of sterile BBL™ Peptone water (Becton, Dickinson and Company, 1057 Franklin Lakes, NJ) was added to sample bags and samples were agitated for 120 seconds in a stomacher (bioMerieux Inc., Durham

NC). Sample cores from day 42 samples were subjected to microbial plating methods in duplicate. Fifty µL of the agitated steak and BBL™ Peptone water sample was administered to 100 mm agar plates utilizing an Eddy Jet spiral plater (IUL, S.A., Barcelona, Spain). Brain, Heart, Infusion agar (Thermo Fisher Scientific, Waltham, MA) was used to conduct aerobic plate counts (APC), anaerobic plate counts (AnPC), and psychrotrophic (PPC) plate count. Both the APC and AnPC were incubated at 37°C and counted at 48 hours. Anaerobic plate counts were held in anaerobic chamber (BD GasPak EZ Large Insulation Container; Becton, Dickinson, and Company, Sparks, MD) with three oxygen absorbent packs (BD GasPak EZ sachet ; Becton, Dickinson, and Company, Sparks, MD). Psychrotrophic plate counts were incubated at 4°C and counted at 10 days.

Consumer Sensory Analysis

This consumer sensory analysis was approved by an Institutional Review Board (project identification 18497). A preference test was used to compare the two dry aging treatments: traditional (aerobic) dry aged and low-oxygen dry aged. Frozen steaks were thawed in a refrigerator overnight at 4°C. The steaks were then cooked on electric grills (Hamilton Beach-31605A, Hamilton Beach Brands, Glen Allen, VA) to 30°C, then flipped and cooked to a final temperature of 70°C. The steaks were set on a cutting board and allowed to cool for approximately 2 minutes; until they could be handled. Each steak was trimmed of excess fat and connective tissue so that only lean sample remained. The samples were cut into 1 cm x 2 cm x 2.5 cm portions and placed in a TMPT Food Warmer (Smithville, TN) until being served, but for no longer than 30 minutes. Each sample was given a unique 3-digit number and served to faculty, staff, and students at the

University of Nebraska-Lincoln with no prior training. Each panelist was given a cup of water and a receptacle for waste. Panelists were asked to rinse their mouths with the provided water to cleanse their palettes before starting and between samples. The samples were served simultaneously in two separate sample cups. Red colored lighting was utilized in the sensory room to minimize color effects. The panelists were asked to circle the 3-digit code corresponding to the sample they preferred. .

Trained Sensory Analysis

The trained sensory analysis was conducted at Texas A&M University with the approval of the Institutional Review Board for Use of Humans in Research at Texas A&M University (IRB2018-0958M). Twenty-four hours prior to cooking, meat was placed in a 4˚C cooler to thaw. Sample raw weights were obtained, and raw temperatures were recorded using a hand-held thermometer (model HH-72T, Omega Engineering Inc., Stanford, CT). A copper constantan thermocouple (Omega Engineering, Stamford, CT) was placed in the geometric center of the samples to monitor cooking temperature. Steaks were cooked on a stainless steel, electric stove top grill (StarMax 536GF 36 inch Countertop Electric Griddle, Star Manufacturing International, Inc., St. Louis, MO) set at 177˚C. Grill temperatures were verified prior to initiation of cooking. Initial internal temperatures and time were recorded. The steaks were cooked until reaching an internal temperature of 71˚C. The steaks were flipped when internal temperature reached 35˚C. Final internal temperature, time, and cooked weight were recorded. Samples were wrapped in aluminum foil and placed in a Bain Marie warmer (APW Wyott W-3Vi 30.5x50.8cm, Alan,TX) with water held at 63°C in warmer pans and lids (Royal

Industries, 15.2 x 25.4 cm, Brooklyn, NY) for no more than 20 minutes prior to being served to the trained descriptive flavor and texture panel. Steaks were cut into 1.27 cm by 2.5 cm with no visible connective tissue or fat. Panelists were served two of the 1.27 cm x 2.5 cm random samples for evaluation.

Six expert descriptive flavor and texture attribute panelists with more than 400 hours of experience were used. Panelists were trained for 13 days reintroducing flavor and texture attributes from the beef lexicon (Adhikari et al., 2011) and AMSA (2015). Forty-three flavor and texture attributes were used. Texture attributes for steaks were as follows: juiciness, connective tissues, and muscle fiber tenderness. References were continually provided to panelists during training and testing. The panelists were given an expectorant cup, double-distilled, deionized water, napkins, and saltless saltine crackers (Premium Unsalted Tops Saltine Crackers, Nabisco, East Hanover, NJ). Panelists were required to use the crackers followed by water as palate cleansers between samples. Samples were tested within a two-hour period with a ten-minute break approximately after 1 h to prevent fatigue. Fifteen minutes before each testing session, a “warm up” sample was provided to panelists and the group leader. Each attribute was discussed and given a score (0 = none and 15 = extremely intense) for daily calibration. Each panelist was seated in a separate breadbox-style booth that contained red lights (44.2 lux) to mask any color effects. Samples were identified with random three-digit codes and served at least 5 minutes apart in soufflé cups that would not impact flavor. Panelists recorded their scores using a 15-point scale from 0= none to 15=extremely intense.

Statistical Analysis

In this study, the data from the yield, color, TBARS, tenderness, water activity for both location and treatment, proximate composition, and the untrained sensory analyses were analyzed as a completely randomized design, with loin as the experimental unit for the study. Data were analyzed using the PROC GLIMMIX procedure of SAS (version 9.4, SAS Institute, Cary, NC). All means were separated with the LS MEANS and PDIFF functions with $\alpha = 0.05$.

Microbial plate counts were analyzed as a completely randomized design. In this study, the loin was considered the experimental unit. Data were analyzed using the PROC GLIMMIX procedure of SAS (version 9.4, SAS Institute, Cary, NC). All means were separated with the LS MEANS and PDIFF or SLICEDIFF functions with $\alpha = 0.05$.

Data from the trained sensory analysis were analyzed using SAS (version 9.4, SAS Institute, Cary, NC) with $\alpha<0.05$. For descriptive attributes data, the PROC GLIMMIX procedure was used. For this analysis, loin was considered the experimental unit. Least square means were calculated using the LS MEANS and PDIFF functions in SAS only when significance was found in the Analysis of Variance. To understand relationships between trained descriptive flavor and texture attributes, XLSTAT (v2020, Addinsoft, New York, NY) was used. Principle component analysis (PCA) was conducted by Texas A&M University and used to analyze the aforementioned relationships between the descriptive flavor and texture attributes. In the PCA analysis, factors are generated to describe the relationship between flavors. Each factor consists of the same flavor attributes, but with differing levels of contribution to the factor. After the factors are generated, a biplot is produced which plots the flavor attributes on a

scatterplot. Once the flavor attributes are plotted, the samples are entered into the analysis. The PCA function then plots the samples on the scatterplot in proximity to the flavors that have the largest impact on each sample.

RESULTS

Yield

There was a significant difference in moisture loss between the wet-aged and dry-aged treatments ($P<0.05$), however there was no significant difference between the two dry aging treatments ($P>0.05$; Table 1). On average, the low-oxygen and aerobic dry-aged treatments both lost 24.1% moisture during aging. The wet-aged samples lost no moisture during aging. Likewise, there was a significant difference in trim loss between the wet-aged and dry-aged treatments ($P<0.05$), but there was no significant difference between the dry-aged treatments ($P>0.05$). The average trim losses for the low-oxygen and aerobic dry-aged samples were 27.4% and 28.8%, respectively. Wet-aged samples lost an average of 5.0% due to trimming. Finally, there was a significant difference in total yield between the wet-aged treatment and the dry-aged treatments ($P<0.05$), but there was no significant difference between the two dry aging treatments. The final total yield for the wet-aged treatment was 95%, whereas the low-oxygen and aerobic dry-aged treatments were 55% and 54%, respectively.

Color

There was no significant difference in L* for any of the treatments ($P>0.05$; Table 2). For both a* and b*, there was a significant difference between the dry-aged

treatments and the wet-aged treatment ($P<0.05$), but there was no significant difference between the two dry-aged treatments ($P>0.05$; Table 2).

Lipid Oxidation

For the TBARS analysis, there was no significant difference found between the low-oxygen dry-aged and wet-aged treatments ($P>0.05$), but both were less than steaks from the aerobic dry aging treatment ($P<0.05$; Table 3). The average mg of malonaldehyde/kg of tissue for the aerobic dry-aged treatment was 2.56, whereas the averages for the wet-aged and low-oxygen dry-aged treatments were 1.20 and 1.28, respectively.

Tenderness

There were no significant differences between any of the treatments for Warner-Bratzler Shear Force ($P>0.05$) Additionally, no significant differences were found for any of the treatments for Slice Shear Force ($P>0.05$; Table 4).

Water Activity

There was no significant location effect on water activity within treatment for any of the treatments ($P>0.05$). The values from each location were then averaged to establish a water activity value for each steak, and ultimately treatment averages were determined. No significant differences were found between any of the aging treatments for water activity ($P>0.05$), see Table 5.

Proximate Composition

No significant differences were found between any of the aging treatments for percent moisture ($P>0.05$). There was no significant difference in fat percent between the two dry aging treatments ($P>0.05$). However, a significant difference in fat percent was found between the wet-aged treatment and the two dry-aged treatments in which the wet-aged treatment had a higher percentage of fats ($P<0.05$; Table 6).

Microbial Analysis

There were no significant differences found between the low-oxygen and aerobic dry-aged treatments for aerobic plate count ($P<0.05$), anaerobic plate count ($P<0.05$), and psychrotrophic plate count ($P<0.05$). The wet-aged treatment was significantly higher than the two dry-aged treatments for all microbial analyses ($P<0.05$; Table 7).

Sensory

For the consumer sensory preference test, no significant differences were found between the two dry aging treatments ($P<0.05$), see Table 8.

For the trained panel sensory test, no significant differences were found between any of the treatments for any of the flavors except for sweet and refrigerator/stale ($P<0.05$), see Table 9. For the sweet flavor descriptor, aerobic dry-aged samples were found to have a significantly larger value ($P<0.05$) when compared to the wet-aged and the low-oxygen dry-aged treatments. However, the aerobic dry-aged samples also had a significantly higher value for the refrigerator/stale flavor descriptor ($P<0.05$) when compared to the other two treatments. It is of note that the low-oxygen dry-aged samples

had slightly higher numerical values for positive flavor descriptors, whereas aerobic dry-aged samples had slightlyhigher numerical values for negative flavor descriptors.

These subtle flavor impacts can be more clearly seen in the PCR analysis (Figure 3). For the PCR analysis, Factors 1 and 2 accounted 41.27% of the cumulative variability. Factor 1 accounted for 25.55% and Factor 2 accounted for 15.73% of the cumulative variability (Figure 4). The main flavor descriptors that contributed to Factor 1 were Beef ID, Brown/Roasted, Bloody/Serumy, Sour, Metallic, Cardboardy, Sour Milk, and Smokey. The main flavor descriptors that contributed to Factor 2 were Fat-Like, Bitter, Sweet, Buttery, Warmed-Over Flavor, and the texture descriptors Juiciness and Muscle Fiber Tenderness. By looking at the PCR biplot (Figure 4), 6 flavor clusters were identified, they are: Cluster 1- Rancid, Refrigerator/Stale, Musty, Metallic, Liver, Fishy, and Warmed-Over Flavor, Cluster 2- Green, Salty, Umami, and Heated Oil, Cluster 3- Bloody/Serumy, Sour, and Sour Milk, Cluster 4- Sweet, Juiciness, and Fat, Cluster 5- Buttery and Muscle Fiber Tenderness, and Cluster 6- Beef ID, Brown/Roasted, and Smokey. By grouping the flavors into clusters, it can be seen that the low-oxygen dry-aged samples tend to aggregate near clusters 5 and 6, which contain more positive flavor descriptors, or cluster 2 which contains both positive and negative flavor descriptors. The other two treatments do not fit into clusters as easily. Both the wet-aged and aerobic dry-aged treatments tend to more random flavor descriptors.

DISCUSSION

The main concern when it comes to dry aging is yield loss. Therefore, the high yield loss that was recorded for the dry-aged treatments are not surprising. The yields

found in this study, 55% for low-oxygen and 54% for aerobic, agree with those found in the literature for dry-aged products. Moisture losses alone, as reported in the literature, can account for 6% to 20% of the total weight loss (Li et al., 2014; Lepper-Blilie et al., 2016). Additionally, trim losses, as reported in the literature, can account for 7% to greater than 25% of the total weight loss with higher trim losses for bone-in product than boneless product (DeGeer et al., 2009; Berger et al., 2018). Thus, a range of 13% to 55% yield loss could be seen in a dry-aged product depending on the parameters used and the ability of the person responsible for trimming. The yields from this study are well within the range cited in the literature, however the moisture losses for this project are on the higher end of the range which is likely due to the low relative humidity coupled with the above average aging time. Many of the dry aging times cited in the literature are less than or equal to 28 days (Campbell et al., 2001; Dashdorj et al., 2016; Khan et al., 2016; Kim et al., 2018). Regardless, the yields in this study, though low, are not of concern.

The color differences found in this study agree with Ha et al. (2019), who found significant differences between a* and b* when comparing wet and dry aging methods. The a* values for the wet-aged treatment were much higher than the low-oxygen and aerobic dry aging treatments (26.45 versus 22.50, 21.90, , respectively). Some dry aging studies have found significant differences between wet-aged and dry-aged treatments, but the effect of differences on consumer acceptance are not well defined.

It is well established that the amount of time a product is aged can impact tenderness (Warren and Kastner, 1992). Smith et al. (1978) suggested that meat be aged for at least 11 days to improve tenderness. However, there is no upper limit to how long meat can be aged. In this study, meat was aged for 41 days, and no differences were

found between the treatments. This agrees with the literature such that aging has been proven to improve tenderness regardless of the aging method used (Dashdorj et al., 2016).

The water activity results for this study agree with Ribeiro et al. (2020) such that aging method, wet aging or dry aging, does not have a significant effect on the water activity for the final product samples. Additionally, the results agree with Ribeiro et al. (2021) in which no significant location effect was found for any of the dry aging treatments.

The proximate compositions of the strip loins after aging are of interest. Wet-aged strip loins had a significantly higher percent of fat when compared to the dry-aged samples. Dikeman et al. (2013) and Berger et al. (2018) reported that dry-aged beef had a lower moisture content than wet-aged counterparts. As seen with the yield, dry-aged products lose a large amount of water during aging, whereas moisture is constant in the wet-aged products due to the packaging it is sealed in. Therefore, it is surprising that the wet-aged samples did not have a significantly lower percent fat at the end of aging. Perhaps the loins selected for dry aging had higher marbling scores (intra-muscular fat content) than the dry-aged samples.

Microbial differences found in this project are interesting, but not surprising. As meat ages, a hardened crust forms which could provide some protection from microbial growth (Lee et al., 2017) as the reduced water activity of the crust likely makes it a poor environment for microbial growth. Hulankova et al. (2018) found that the total viable count after aging was only slightly higher in dry-aged beef, which agreed with Lee et al. (2017) who found the total aerobic count was marginally larger for dry-aged beef.

However, the results from this experiment are in slight contradiction to the two aforementioned studies as wet-aged products in this study had significantly higher microbial counts than both of the dry-aged treatments. It is hypothesized that this difference in microbial counts between the two aging methods, wet and dry, could likely be a result of the low water activity found in the dry-aged product crust. Additionally, the low oxygen environment may have contributed to the lower microbial counts, though the effects were not significant.

The main objective of this research study was to limit oxidation. As seen in the TBARS values, this objective was clearly demonstrated. Still, the impact of the lower TBARS values were not clearly seen in the sensory analyses. It is clear that dry aging in low-oxygen conditions significantly reduces oxidation, such that oxidation values for wet-aged samples were not significantly different. Lipid oxidation is often responsible for product quality deterioration in fresh and processed products, and many scientists believe that lipid oxidation during dry aging may negatively impact the final product flavor. With the reduction of lipid oxidation in this experiment, and the likely reduction of negative flavors typically produced during oxidation, it would be expected that the two aging methods would result in clearly different flavors. This may be due to the relatively low levels of lipid oxidation in all treatments. However, a consumer sensory panel was unable to distinguish between the dry aging treatments, and trained panelists were only able to find subtle differences between treatments. In the trained sensory analysis, low-oxygen dry-aged samples had slightly higher numerical values for desirable flavor notes such as beef ID, brown/roasted, and umami, and aerobic dry-aged beef had slightly higher numerical values for undesirable flavor notes such as bloody/serumy, sour,

metallic, burnt, sour milk, and warmed-over. Therefore, a PCA analysis was conducted to evaluate the flavor differences found between the aging treatments. It is interesting that the reduction in lipid oxidation had flavor impacts that were detectable by the flavor profile panel but undetectable in the consumer sensory analysis. This new information raises questions about the impact of these flavor differences to consumers.

CONCLUSION

From this study, it can be concluded that dry aging in low-oxygen conditions has a significant effect on lipid oxidation. However, despite having significantly lower lipid oxidation values, consumers were unable to detect differences between low-oxygen and aerobic dry-aged samples. Trained sensory panelists were able to detect subtle flavor differences which suggested that beef dry aged under aerobic conditions were associated with negative flavor notes and beef dry aged in low-oxygen conditions was associated with positive flavor notes. The impact of these subtle flavor differences on consumers bears further study.

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Tables

Table 1. Mean moisture loss, trim loss, final weight, and final yield of wet and dry-aged loins aged for 41 days. (n=18)

	Aging Treatment				
Trait	Low-oxygen Dry Aging	Traditional (Aerobic) Dry Aging	Wet Aging	SEM[c]	P-Value
Moisture loss (%)	24.1%[a]	24.1%[a]	1.6%[b]	0.6749	<0.0001
Trim loss (%)	27.4%[a]	28.8%[a]	5.0%[b]	0.8714	<0.0001
Total weight loss (%)	45.1%[a]	46.0%[a]	5.0%[b]	0.8996	<0.0001
Final weight (kg)	3.61[a]	3.40[a]	6.07[b]	0.1775	<0.0001
Final yield (%)	55.0%[a]	53.9%[a]	95.0%[b]	0.0090	<0.0001

[a-b] Means in the same row with different superscripts are different (P<.05)
[c] Standard error of the means.
Moisture loss for wet aged loins was attributed to purge loss
Moisture loss was calculated as:
moisture loss = (initial weight prior to aging - final weight after only aging)/initial weight prior to aging
Trim loss was calculated as: trim loss = (trimmed weight/aged weight)
Total weight loss was calculated as:
total loss = (initial weight prior to aging - final weight after aging & trimming)/initial weight prior to aging

Table 2. Mean L*, a*, and b* values for wet and dry-aged loins aged for 41 days. (n=18)

	Aging Treatment				
Color Measurement	Low-oxygen Dry Aging	Traditional (Aerobic) Dry Aging	Wet Aging	SEM[c]	P-Value
L*	42.25[a]	40.76[a]	41.77[a]	1.02	0.5819
a*	22.50[a]	21.90[a]	26.45[b]	0.73	0.0010
b*	11.47[a]	10.98[a]	12.97[b]	0.32	0.0015

[a-b] Means in the same row with different superscripts are different (P<.05)
[c] Standard error of the means.

Table 3. Mean thiobarbituric acid reactive substances values in mgs malonaldehyde/kg of sample for wet and dry-aged loins aged for 41 days. (n=18)

	Aging Treatment				
	Low-oxygen Dry Aging	Traditional (Aerobic) Dry Aging	Wet Aging	SEM[c]	P-Value
TBARS values (mgs malonaldehyde/kg of tissue)	1.28[a]	2.56[b]	1.20[a]	0.19	0.0002

[a-b] Means in the same row with different superscripts are different (P<.05)
[c] Standard error of the means.

Table 4. Mean Warner-Bratzler Shear Force and Slice Shear Force values in kg for wet and dry-aged strip loins aged for 41 days. (n=18)

	Aging Treatment				
Tenderness Analysis (kg)	Low-oxygen Dry Aging	Traditional (Aerobic) Dry Aging	Wet Aging	SEM[a]	P-Value
Warner-Bratzler Shear Force	2.89	2.99	2.80	0.17	0.7352
Slice Shear Force	12.54	13.10	14.00	1.00	0.5930

[a] Standard error of the means.

Table 5. Mean water activity values for wet and dry-aged strip loins aged for 41 days. (n=18)

	Aging Treatment				
	Low-oxygen Dry Aging	Traditional (Aerobic) Dry Aging	Wet Aging	SEM[a]	P-Value
Water Activity	0.99	0.99	0.99	1.00	0.3266

[a] Standard error of the means.

Table. 6. Mean moisture and fat percentages for wet and dry-aged strip loins aged for 41 days. (n=18)

	Aging Treatment				
	Low-oxygen Dry Aging	Traditional (Aerobic) Dry Aging	Wet Aging	SEM[c]	P-Value
Moisture %	68.27[a]	68.86[a]	68.81[a]	0.64	0.7738
Fat %	6.91[b]	6.45[b]	9.42[a]	0.01	0.0209

[a-b] Means in the same row with different superscripts are different (P<.05)
[c] Standard error of the means.

Table 7. Mean aerobic plate counts, anaerobic plate counts, and psychrotropic plate counts (Log_{10} CFU/cm^2)for wet and dry-aged strip loins aged after 41 days of aging. Bacterial plate counts on all media for were below the detection limit (1.21 Log_{10} CFU/cm^2) on Day 0. (n=18)

	Aging Treatment				
	Low-oxygen Dry Aging	Traditional (Aerobic) Dry Aging	Wet Aging	SEM[c]	P-Value
APC	1.98[a]	1.56[a]	3.27[b]	0.32	<0.001
AnPC	1.56[a]	1.70[a]	3.19[b]	0.17	<0.001
PPC	1.97[a]	1.60[a]	4.63[b]	0.36	<0.001

APC: aerobic plate count, AnPC: anaerobic plate count, PPC: psychrotrophic plate count.
[a-b] Means in the same row with different superscripts differ P < 0.001.
[c] Standard error of the means.

Table 8. Number of consumer panelists preferring low-oxygen or traditional (aerobic) dry-aged loins by day of sensory test.

	Sensory day				
Preference	Day 1	Day 2	Total	SEM[c]	P-Value
Low-oxygen dry aged	12[a]	14[a]	31[a]	2.85	0.4768
Traditional (Aerobic) dry aged	19[a]	10[a]	24[a]	2.85	

[a] Means in the same column with different superscripts are different (P < .05)
[c] Standard error of the means.

Table 9. Mean flavor descriptor values for the trained sensory panel conducted at Texas A&M.

	Aging Treatment				
Flavor Descriptor	Low-oxygen Dry Aging	Traditional (Aerobic) Dry Aging	Wet Aging	SEM[c]	P-Value
Beef Flavor ID	8.89	8.64	8.81	0.3268	0.8609
Brown/Roasted	9.44	9.08	9.53	0.3598	0.6590
Bloody/Serumy	2.00	2.25	2.11	0.2588	0.7966
Fat-Like	2.83	2.92	2.75	0.2243	0.8698
Bitter	2.19	2.39	2.36	0.1695	0.6862
Salty	1.86	2.08	1.78	0.1422	0.3207
Sweet	0.72[a]	0.33[b]	0.42[b]	0.0978	0.0317
Sour	2.42	2.69	2.39	0.2278	0.5864
Umami	4.69	4.56	4.58	0.1331	0.7441
Metallic	2.56	2.81	2.47	0.2023	0.4960
Musty-Earth/Hummus	0.50	0.53	0.61	0.1946	0.9160
Cardboardy	1.89	1.72	1.47	0.2763	0.5763
Burnt	0.47	0.72	0.61	0.2419	0.7710
Buttery	0.14	0.28	0.22	0.0813	0.4952
Sour-Milk/Dairy/ Cooked Milk	0.72	0.83	0.72	0.2256	0.9242
Fishy	0.28	0.31	0.03	0.1278	0.2708
Green	0.14	0.06	0.00	0.0610	0.3005
Heated Oil	0.00	0.06	0.14	0.0610	0.3005
Liver-Like	0.92	1.06	1.50	0.2706	0.3096
Rancid	0.03	0.00	0.08	0.0508	0.5143
Refrigerator Stale	0.06[a]	0.42[b]	0.00[a]	0.0908	0.0112
Smokey Charcoal/Wood	0.14	0.14	0.14	0.0904	0.9999
Warmed Over	0.08	0.28	0.14	0.0854	0.2865
Texture Descriptors					
Juiciness	8.64	8.75	8.81	0.4125	0.9579
Muscle Fiber Tenderness	9.56	8.94	9.81	0.4243	0.3615
Connective Tissue Amount	11.25	10.94	11.17	0.3215	0.7898

[a] Means in the same row with different superscripts are different ($P < 0.05$)

Superscripts were only assigned to values that were significant. Non-significant values do not have superscripts.

[c] Standard error of the means.

Table 10. Factor loading table for the PCA analysis for factors 1 and 2.

Flavor Descriptor	Factor 1	Factor 2
BeefID	-0.739	0.160
BrownRoasted	-0.761	-0.019
BloodySerumy	0.812	0.350
Fat	0.366	0.763
Bitter	0.098	-0.620
Salty	0.235	0.045
Sweet	0.081	0.632
Sour	0.729	0.154
Umami	0.271	0.155
Metallic	0.671	-0.189
Musty	0.582	-0.180
Cardboardy	0.748	-0.191
Burnt	-0.561	-0.403
Buttery	-0.143	0.557
SourMilk	0.788	0.158
Fishy	0.339	-0.452
Green	0.118	0.082
HeatedOil	0.351	0.062
Liver	0.544	-0.187
Rancid	0.273	-0.268
RefrigeratorStale	0.382	-0.398
Smokey	-0.769	-0.139
WOF	0.490	-0.637
Juiciness	0.228	0.807
MFT	-0.218	0.506
CT	-0.208	-0.333

Figures

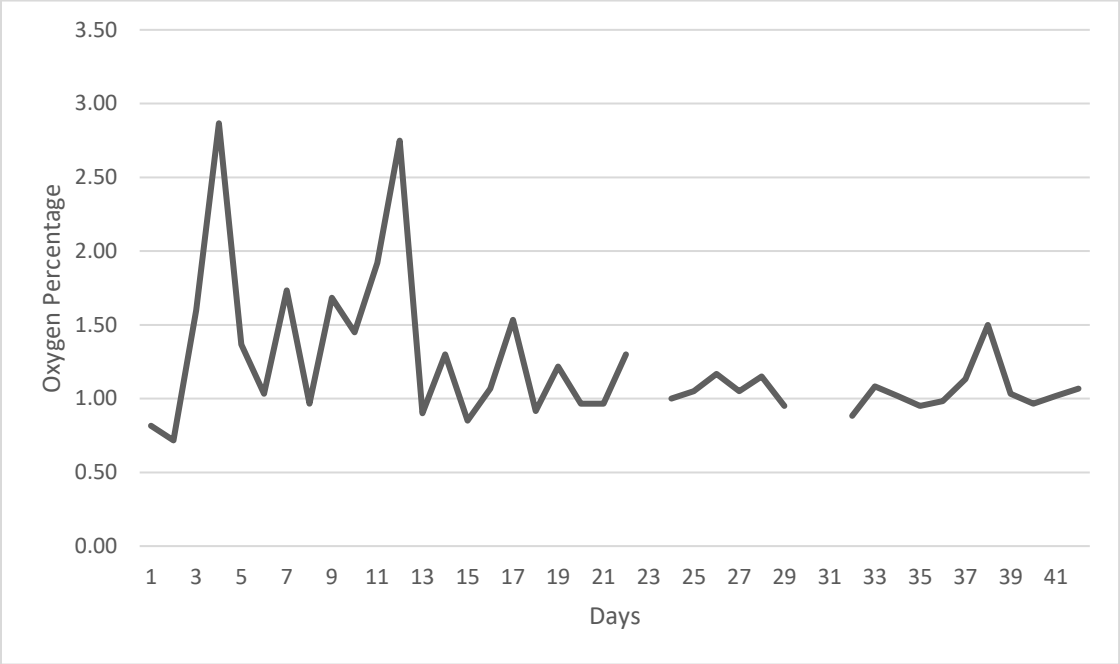


Figure 1. Average recorded oxygen concentration for low-oxygen chambers during 41 days of aging. Gaps in the data were due to recording technical errors for the computer used to run the dry aging system. Peaks in the data are due to mechanical issues for some, not all, of the chambers.

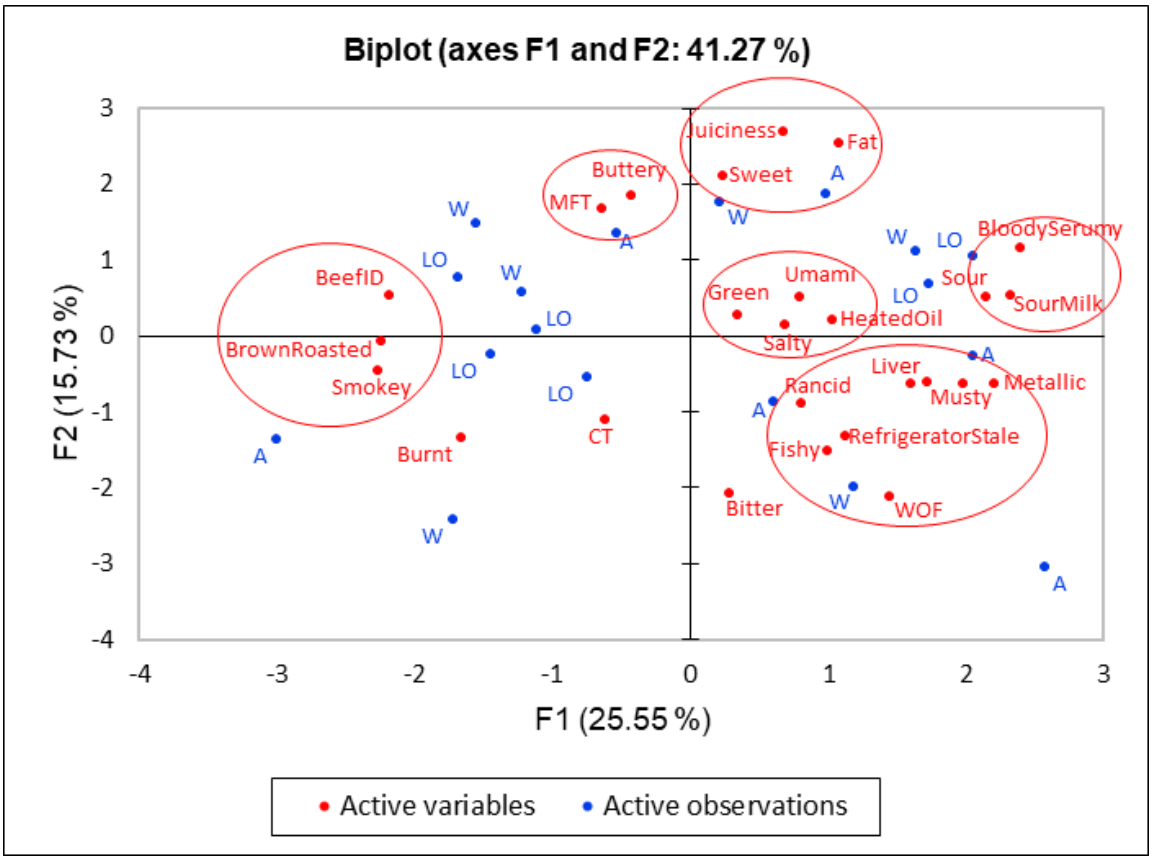


Figure 2. PCA Biplot- For the PCR analysis, Factors 1 and 2 accounted 41.27% of the cumulative variability. Factor 1 accounted for 25.55% and Factor 2 accounted for 15.73% of the cumulative variability. The main flavor descriptors that contributed to Factor 1 were Beef ID, Brown/Roasted, Bloody/Serumy, Sour, Metallic, Cardboardy, Sour Milk, and Smokey. The main flavor descriptors that contributed to Factor 2 were Fat-Like, Bitter, Sweet, Buttery, Warmed-Over Flavor, and the texture descriptors Juiciness and Muscle Fiber Tenderness.

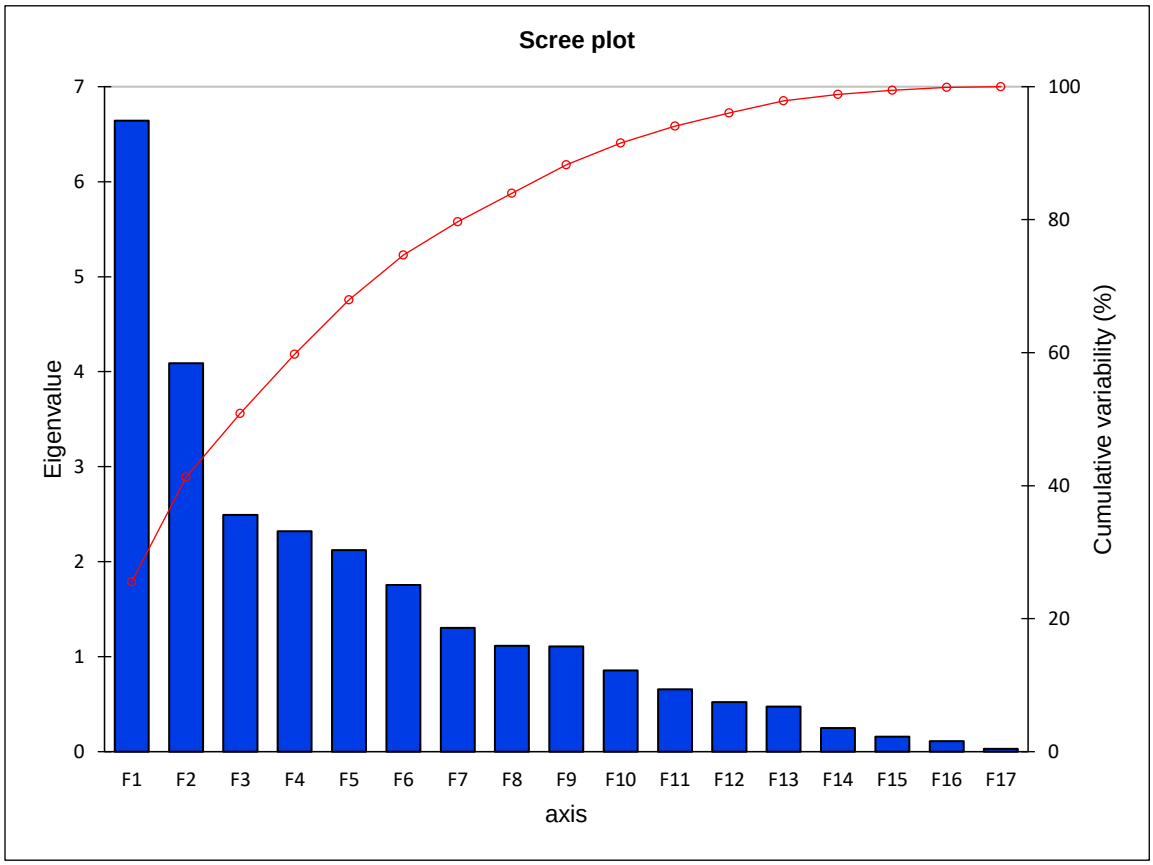


Figure 3. Scree plot of the cumulative variability for each factor. As shown in the plot, factors 1 and 2 account for a large amount of the variability.

RECOMMENDATIONS FOR FUTURE RESEARCH

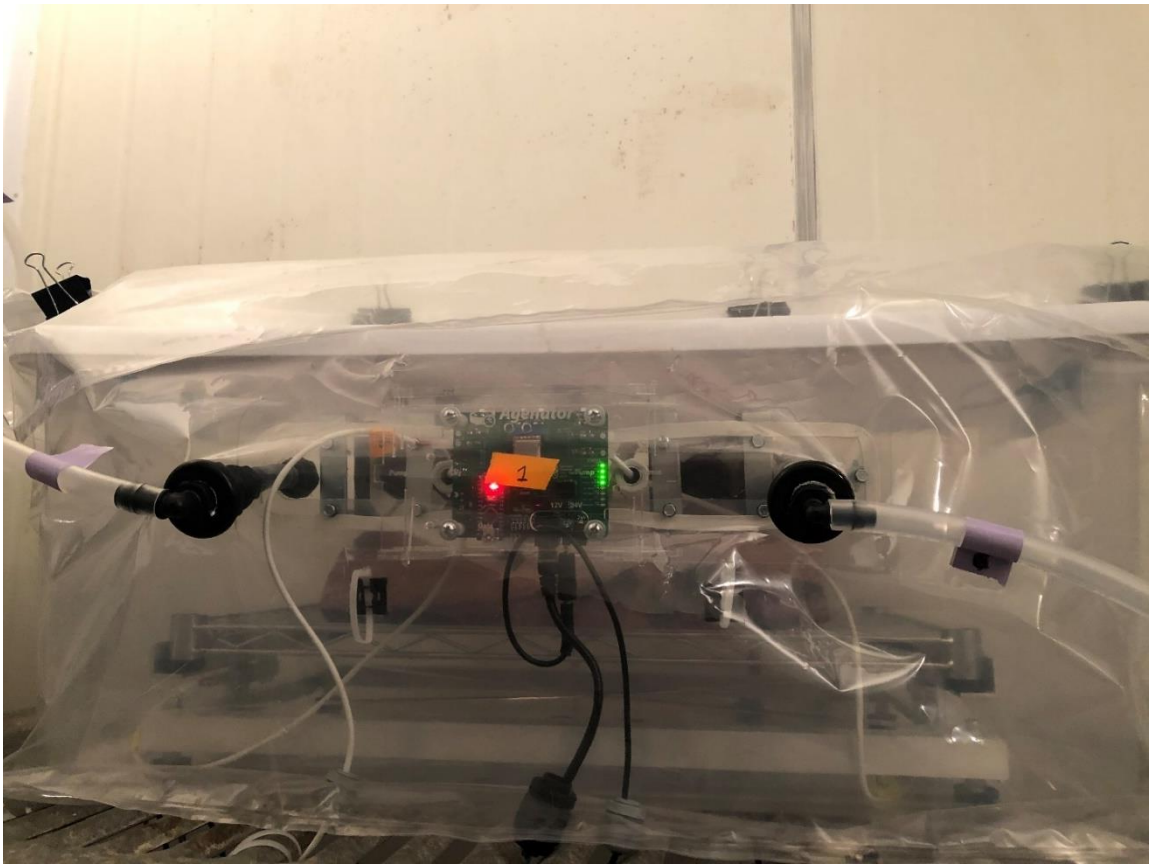
This research study has raised more questions than it has answered. While it is intriguing that lipid oxidation can be limited by dry aging in low-oxygen conditions, it is equally interesting that this significant difference in lipid oxidation had minimal effect on final product flavor. Flavor development is a complex process, but it must be understood for scientists to be able to identify the how the unique characteristics of dry-aged beef are produced. It is also important that the impact of each method of flavor development during dry aging, either through concentration of flavor compounds as a result of moisture loss or the creation of new flavor compounds during aging, be investigated. Therefore, further exploration of the dry-aged flavor development process is recommended. To accomplish this, a study could be designed in which the concentration and creation effects can be examined individually. Such studies would need to produce products that have different moisture losses aged for similar times and similar moisture losses aged for different lengths of time. The suggested study would hold aging time constant, reducing the effects of the flavor creation pathway, which would allow for the examination of the impact that concentration of flavor compounds has on final product flavor. The second suggested study would hold moisture loss constant, reducing concentration effects, so that the impact of flavor creation during aging could be examined. In addition to the aforementioned recommend research, I would suggest that additional research be conducted on limiting oxidation during aging by means other than oxygen removal. Such research could produce new evidence as to whether or not lipid oxidation affects dry-aged beef flavor, and to what extent. I suggest that antioxidants, either through incorporation in diet or as a topical agent, by applied to dry-aged products

to determine if there are other means in which lipid oxidation can be reduced in dry-aged products, as well as the effect that it has on final product flavor.

APPENDIX

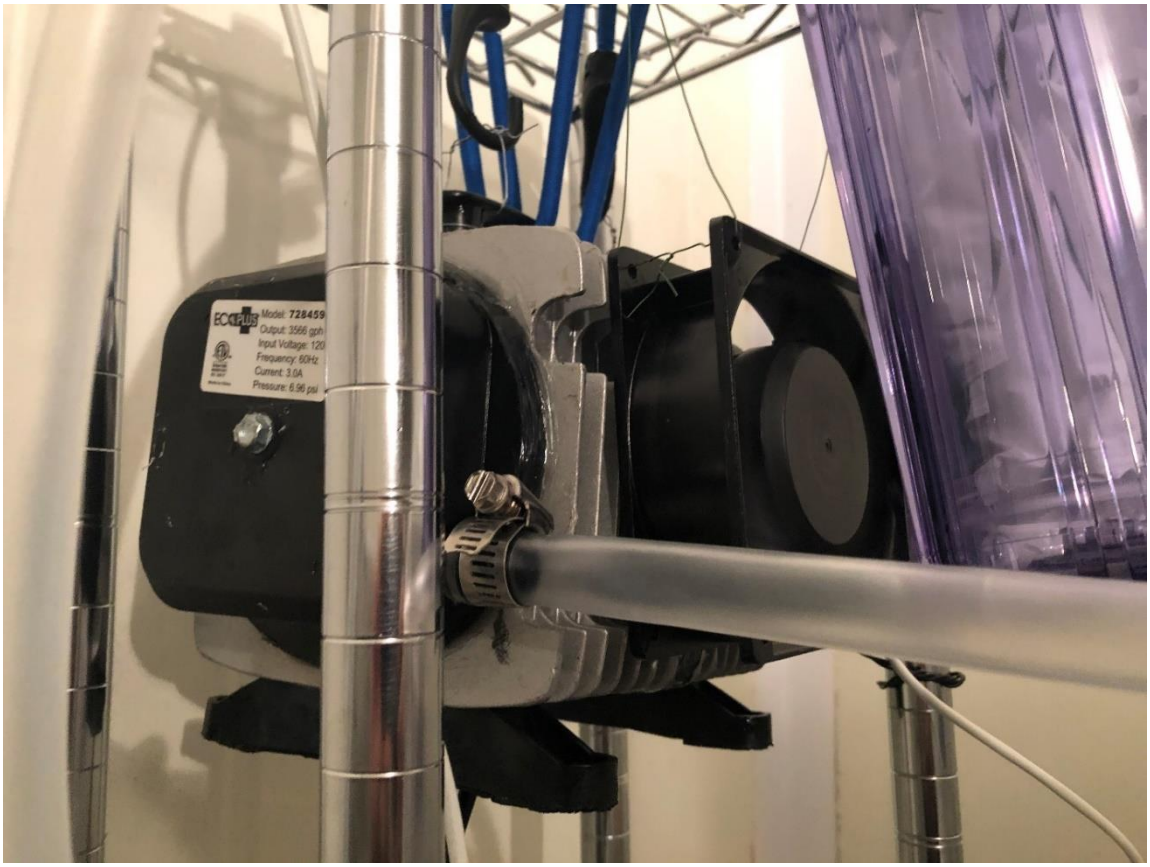
Appendix I: Low-oxygen Chamber Components

Component 1. Chamber inside film “bubble”



Component 2. Oxygen Absorber Column (with oxygen absorbers inside)

Component 3. Sealed Pump



Component 4. Sealed Drying Columns

Appendix II: Fabrication Map

1”	**WBSF**	Anterior
½”	**Water Activity (a_w)**	
1”	**LAB**	
½”	**TBARS**	
	Sensory	
	Sensory	
	Sensory	
	Sensory	
	Sensory	
	Sensory	
	Sensory	
	Sensory	
	Sensory	
	Sensory	
	Extra	Posterior

Appendix III: Colorimeter Calibration

Objective color (L* a* b*) calibration instructions- Minolta Calibration Procedures

1. Before Calibration:

Calibrate Minolta in the same temperature conditions as the measurements being taken.

-Place the Minolta in the environment where samples will be measured about 5 to 10 minutes before calibrating so it can become equilibrated with the temperature.

Calibrate with the same materials as you will be taking measurements.

-If the measurement will not be taken directly on the meat surface, you must calibrate the Minolta with the same material it will be measuring through. For example, if you want to take readings from samples that are wrapped in overwrap, you must put some overwrap around the measuring head "eye" while calibrating using the white tile.

2. Turn the power to the measuring head ON.

3. Turn the power to the data processor ON while holding down the [DELETE/UNDO] key at the same time.

-Release the [DELETE/UNDO] key when you hear a BEEP. (This action deletes any previous data that might still be stored in the data processor)

4. When the screen turns on, the question "Initial set ok?" appears, press the [Measure Enter] key.

5. Once you get to the measurement screen, press the [Index Set] key.

    a. Use the arrows and the [Measure Enter] key to adjust all the following settings:

        i. Printer: On

        ii. Color Space: Off

        iii. Protect: On

        iv. Auto Average: However many readings wanted per sample (1- 30)

        v. Illuminant: D65

        vi. Back Light: Off vii. Buzzer: On viii. Disp. Limit

    b. Press the [Esc] key to return to the measurement screen. 177

6. Press the [Calibrate] key while in the measurement screen.

7. Enter in the numbers listed on the calibrating white tile for the D65 setting using the following:

    a. [<>] keys and the numeric pad

    b. (The [<>] keys move the cursor)

    c. D65 settings: Y: 93.13 x: 0.3164 y: 0.3330

8. Set up the measuring head so that it is resting on the LCD screen and the “eye” is facing up.

    a. Place the while calibration tile on the measuring head, near the middle of the tile.

9. Press either the measurement button on the measuring head OR the [Measure Enter] key on the data processor after making sure the ready lamp is ON.

    a. Make sure the white tile is completely on the measuring head “eye”.

    b. The calibration is complete after the lamp flashes 3 times and the screen returns to the measurement screen.

    c. Do not move the measuring head during calibration.

10. Press the [Color Space] key until the L* a* b* screen shows up.

11. Calibration is finished and the Minolta is ready.

    a. To save battery life, turn both the measurer and data collector off after calibration is finished until you need it for measuring. The calibration and setting will not be erased.

    b. When turning back on for measurements, ONLY turn on the power buttons. DO NOT hold down the [DELETE/UNDO] key at the same time. This will delete the calibration and settings and all of the steps will have to be repeated.

Cleaning:

- Wipe machine down with a soft, clean dry cloth. Never use a solvents such as thinner or benzene.
- If the white calibration tile becomes dirty, wipe it gently with a soft, clean dry cloth. If dirt is difficult to remove, wipe it with lens cleaner and cloth, then dry.

Appendix IV: Thiobarbituric Acid Reactive Substances Assay

Buege and Aust (1978), Modified by Ahn et al. 1998

TEP Solution (1,1,3,3-Tetraethoxypropane) (Make new weekly)

Stock Solution: Dilute 99 □l TEP (97%) bring volume to 100 mL ddH20

Working Solution: Dilute stock solution to 1:3 (TEP Solution:ddH20) (1x10-3M)

TBA/TCA (2-Thiobarbituric Acid/Trichloroacetic Acid) Stock Solution: 1L

15% TCA (w/v) and 20 mM TBA (MW 144.5) reagent in ddH20.

Dissolve 2.88 g TBA in warm ddH20 first, then add TCA (150g) and ddH20 to 1L

BHA (ButylatedHydroxyAnisole) Stock Solution:

Make 10% stock solution by dissolving in 90% ethanol.

10g BHA dissolved in 90 mL ethanol (90%) + 5mL ddH20

Standards: In duplicate

Blank:	1 ml ddH20	Moles of TEP
Standard 5:	100 mL working TEP + 1.90 mL ddH20	(5x10-5M)
Standard 4:	1 mL Std. 5 + 1 mL ddH20	(2.5x10-5M)
Standard 3:	1 mL Std. 4 + 1 mL ddH20	(1.25x10-5M)
Standard 2:	1 mL Std. 3 + 1 mL ddH20	(.625x10-5M)
Standard 1:	1 mL Std. 2 + 1 ml ddH20	(.3125x10-5M)

Remove 1 mL of Standard 1 and discard it, leaving 1 mL behind.

Procedure

- Mix all reagents and standards before beginning.
- Transfer 5 g of powdered sample into a 50 ml conical tube, add 14 ml of ddH2O and 1.0 mL of BHA (Butylated hydroxyanisole).
- Homogenize for 15 sec with a polytron
- Centrifuge for 2000xg for 5 minutes.
- Transfer 1 ml of homogenate or standard to 15 ml conical tube
- Add 2 ml of TBA/TCA solution, vortex.
- Incubate in a 70ºC water bath for 30 min to develop color.
- Cool samples in a cold water bath at 21°C for 10 min.
- Centrifuge tubes at 2000×g for 15 min.
- Transfer duplicate aliquots of 200 □l from each tube into wells on a 96 well plate.
- Read absorbance at 540nm.

Calculations: mgs of malonaldehyde/kg of tissue

K(extraction)=(S/A) x MW x (106/E) x 100

Where S=Standard concentration (1x10-8 moles 1,1,3,3-tetraethoxypropane)/5ml.

A=Absorbance of standard MW=MW of malonaldehyde (72.063 g/mole)

E= sample equivalent (1) P=Percent recovery

Final calculation: .012 x concentration x 72.063x106 = mgs malonaldehyde/kg of tissue

Reagents (Sigma): TBA- T5500; TCA- T9159; TEP- T9889; BHA- B1253