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Advancing the Science of Dry-Aged Beef

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ADVANCING THE SCIENCE OF DRY-AGED BEEF

by

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ADVANCING THE SCIENCE OF DRY-AGED BEEF

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In order to tightly control dry aging environmental conditions, we designed and built a computerized dry aging system that is capable of measuring and precisely controlling relative humidity (RH; ± 1%), temperature (± 0.5 °C), air flow (± 0.015m^3/min), and mass loss (± 5 g). This dissertation addressed the effects of RH and ultimate pH on meat quality and sensory attributes of dry-aged beef. In study one, we learned that wet-aged steaks had higher L* (P = 0.01), a* (P = 0.03), and b* values (P < 0.001), lower discoloration scores (P < 0.05), and lower TBARS (thiobarbituric acid reactive substance) values (P = 0.03) than dry-aged treatments. Under prolonged retail display (RD), dry aging of beef has the potential to reduce color and lipid stability compared to wet aging. In study two, a faster rate of moisture loss was found on the first three days of aging at 50% RH (P < 0.001) when compared to 85% RH. Lower RH results in accelerated moisture loss at the beginning of the aging process without significantly affecting the total amount of moisture loss. Trim loss, yield, and microbial counts were not affected by RH levels (P > 0.05). *Pseudomonadales* dominates the dry-aged loins while *Enterobacteriales* was the most abundant in the wet-aged samples. Lower RH tended to associate with more desirable flavor notes. In study three, meat with high pH (pH = 6.69) had the lowest L*, a*, and b* values (P < 0.05) throughout RD, and also the lowest TBARS

values at 4 and 7 days of RD ($P < 0.001$), regardless the aging method. Ultimate pH did not affect rate ($P = 0.51$), total moisture loss ($P = 0.96$), trim loss ($P = 0.69$) and yield ($P = 0.75$) during dry aging. *Clostridiales* were only observed on WET-DC samples, which also had a higher abundance of *Lactobacillales*. Flavor characteristics of dark cutters were not improved by dry aging. Regarding tenderness, our results showed that aging method (wet or dry), RH level (50, 70 or 85%), and ultimate pH did not have an effect on Warner-Bratzler shear force ($P > 0.05$).

Keywords: dry aging, eating experience, flavor, meat quality, palatability

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INTRODUCTION

There is a growing interest for dry-aged beef primarily driven by upscale restaurants and retailers for the gourmet market due to its enhanced and unique flavor. However, not all dry aging studies have found improved flavor for dry-aged beef (Oreskovich, McKeith, Carr, & Bechtel, 1988; Parrish, Boles, Rust, & Olson, 1991; Smith *et al*., 2008; Dikeman, Obuz, Gök, Akkaya, & Stroda, 2013) and conflicting results regarding the impacts of dry aging on beef palatability have been reported in the scientific literature. These conflicting results may be associated with inconsistent environmental conditions applied during the dry aging process.

Environmental conditions, including the length of aging, temperature, relative humidity (RH), and airflow, are the primary factors to considerer when dry aging because they relate to the development of flavor notes, product shrinkage, shelf-life, microbial spoilage, and economics (Savell, 2008). However, dry aging studies in the literature rarely report all of the environmental conditions tested, and those that do only test specific conditions or limited ranges. Most of these studies have been conducted within a single meat cooler without the precise relative humidity, air speed, and mass loss measurement and control required to provide a clear picture of the drying process.

There are a number of relative humidity parameters reported in the scientific literature. However, there are no published studies that have compared the impact of various RH levels on dry-aged beef. In addition, only a few studies have addressed airflow from a research standpoint. The scientific information is so limited that it cannot be used to support a recommended threshold for RH and airflow from a flavor standpoint. The dry

aging protocols currently used by meat companies were mostly developed by trial and error through many years of experimentation (Savell, 2008).

To address the flavor inconsistency reported in the literature, we have designed and built a computerized dry aging system called Agenator that is capable of measuring and precisely controlling relative humidity (± 1%) and air flow (± 0.015m^3/min). The dry aging chambers (86 cm Length x 47.6 cm Width x 33 cm Height) have built-in weighing scales that can continuously monitor mass loss (± 5 g) and temperature (± 0.5°C). All measured data can be saved on to the connected computer in user-defined intervals, with a minimum of 1 second. This computerized dry aging system allows us to conduct dry aging studies with proper replication. A full description of the Agenator has been previously published by Lau, Ribeiro, Subbiah, and Calkins (2019).

A deeper understanding of the effects of environmental conditions on flavor development in dry-aged beef could provide a conceptual foundation for the development of an effective dry aging guideline that optimizes flavor development and consistency in addition to helping improve consumer eating experience.

Therefore, the objectives of this research were to understand the effects of relative humidity and ultimate pH on moisture loss and on meat quality and sensory attributes of dry-aged beef. More specifically, this study aimed to answer the following questions:

1) Does early hard crust formation affect moisture loss over time?
2) How does moisture loss occur under different RH conditions?
3) Is dry-aged beef flavor dependent upon the rate of moisture loss over time?
4) Could dry aging improve beef flavor of dark cutters and increase yield?
5) What is the effect of dry aging conditions on trim loss and cutting yields?

6) What is the influence of the dry aging process on meat color and lipid stability during retail display life?

Currently, the U.S. does not have a specific guideline for dry aging of beef, nor protocols to ensure dry-aged sensory quality. Therefore, the combination of these studies will address current issues associated with dry-aged beef quality. Moreover, this research could provide the basis for the development of a dry-aging beef guideline to optimize beef flavor and achieve the highest possible value in both domestic and international markets.

LITERATURE REVIEW

Aging

Aging is probably the most widely practiced method to increase meat tenderness and flavor development. There are two methods of aging: wet and dry aging. Wet aging is the most common method used by the meat industry in which meat is aged in a vacuum package under refrigerated storage for a period of time to maximize tenderness, juiciness, and flavor (Oreskovich, McKeith, Carr, & Bechtel, 1988; Parrish, Boles, Rust, & Olson, 1991; Campbell, Hunt, Levis, & Chambers, 2001; Sitz, Calkins, Feuz, Umberger, & Eskridge, 2006). Dry aging, on the other hand, is a process whereby beef carcasses, primals, and/or subprimals are stored unpackaged under controlled temperature, humidity and air flow for a certain period of time to allow the natural enzymatic and biochemical processes that result in improved tenderness and the development of the unique and distinct flavor of dry-aged beef (Savell, 2008).

While wet aging has been extensively studied and is well described in the scientific literature, the science behind the dry aging process has not been the subject of many research studies. This review surveyed dry-aging literature focused on factors that affect meat quality, safety, and yield.

Dry aging of beef

Before the development of vacuum packaging in the 1960s, dry aging was the only option available to age beef (Savell, 2008). Drying is a process in which water is removed from a material by evaporation. During the dry aging process, water is transferred from the interior to the meat surface and is subsequently evaporated to the surrounding environment

(Lewicki, 2004). This results in an enhanced flavor, which has been extensively used to promote dry-aged beef. Therefore, this process has been used by upscale hotels, restaurants, and some food retailers to differentiate their products by enhancing overall palatability while creating a premium price for beef products (Parrish, Boles, Rust, & Olson, 1991; Warren & Kastner, 1992; DeGeer, Hunt, Bratcher, Crozier-Dodson, Johnson, & Stika, 2009).

Savell and Gehring (2018) suggested that the enhanced flavor of dry-aged beef results from both concentration of flavor compounds and creation of flavor. As meat loses water during drying, the flavor compounds are diluted in less water, resulting in higher concentration, which could explain the characteristic stronger flavor of dry-aged beef. With respect to creation, it seems that new flavor compounds are created over time as proteins and fats change their chemical nature, partly through oxidation processes. However, evidence of the benefits of the enhanced dry-aged beef flavor is unclear and some sensory analyses of dry and vacuum-aged beef have revealed inconsistent results (King, Matthews, Rule, & Field, 1995; Laster *et al*., 2008; Sitz, Calkins, Feuz, Umberger, & Eskridge, 2006; Smith *et al*., 2008).

Although dry aging has been used for centuries, there is little information about the impact of environmental conditions and consequently the rate of moisture loss on flavor development in dry-aged beef. Several factors including length of aging, storage temperature, airflow and relative humidity can affect the rate of drying, and consequently impact flavor development, microbial spoilage, and trimming loss. In addition, recently, a high moisture permeable dry-aging bag was introduced to the market intending to produce

dry-aged beef with the same flavor as beef aged using traditional unpackaged dry aging (Ahnström, Seyfert, Hunt, & Johnson, 2006).

The lack of scientific information in addition to the high variability of environmental conditions applied during the dry aging process might explain the inconsistent results regarding the impacts of dry aging on meat quality attributes. Therefore, these parameters need to be carefully balanced and monitored during the process to produce an excellent eating experience from tenderization and enhanced flavor (Savell, 2008).

Dry aging parameters:

Length of aging

It's known that the aging period has a significant effect on the level of beef flavor, tenderness, and juiciness. However, the number of days beef products are dry aged varies extremely among beef programs as well as in the scientific literature. Periods of 14 to 35 days have all appeared to be effective in producing the desired dry-aged results (Savell, 2008).

DeGeer, Hunt, Bratcher, Crozier-Dodson, Johnson, and Stika (2009) dry aged beef for 21 and 28 d and observed that mass and trim losses increased as aging time increased. The authors also reported that the only sensory trait that differed due to aging time was sourness, which was slightly higher at 21 days. Additionally, at the end of the aging period, microbial counts were generally greater at 28 d than 21 d. Overall, it

appears that product dry aged for 21 days may have the same flavor profile but higher yields than product dry aged for 28 days.

Similarly, Smith *et al.* (2008) found that steaks dry aged for 21 d received the highest value for level of beef flavor compared to steaks dry aged for 14, 28, and 35 days. Moreover, steaks dry aged for 28 and 35 d had significantly lower Warner–Bratzler shear force values than those aged for 14 days, but dry aging for 28 days was not different from 21 days. Taken together, these results indicate that dry aging for 21 d appears to be long enough to improve the level of beef flavor, minimize yield losses, and also produce significant improvements in sensory tenderness.

On the other hand, some studies have shown that a longer dry-aging period is required to produce the desired dry-aged results. The U. S. Meat Export Federation suggests that the dry aging process should occur from 28 to 55 d. According to Perry (2012), the length of aging should be between 50 and 80 d. Lida *et al*. (2016) found that tenderness did not change during the first 40 days of dry aging and then gradually increased to day 60. Additionally, Laster *et al*. (2008) reported that consumers preferred 35 d dry-aged ribeye steaks over 14 d dry-aged ribeye steaks. However, the 28 d and 35 d dry-aged ribeye steaks produced the lowest yields with the highest percentage of waste trimmings.

Storage temperature

In addition to the length of aging, the storage temperature is also an important parameter in dry-aging because it directly affects microbial growth and proteolytic enzyme activity in the meat. Although there are no studies that have evaluated the effect of different

storage temperatures on the quality and yield of dry-aged beef, finding the appropriate storage temperature for dry-aged beef is very important. Research has shown that dry-aging has been mostly conducted at temperatures between 0 and 4°C, which is similar to the temperature of regular wet-aging (Savell, 2008).

Laster *et al*. (2008) conducted their dry aging study in a cooler at an average temperature of -0.6°C with a standard deviation of 1.8°C. DeGeer, Hunt, Bratcher, Crozier-Dodson, Johnson, and Stika (2009) dry aged beef loin sections at 2.2°C. Lida *et al*. (2016) and Ryu *et al*. (2018) dry aged beef at 1 to 4°C. Smith *et al*. (2008) reported that short loins were stored at 1.0°C, Ahnström, Seyfert, Hunt, and Johnson (2006) aged beef loin sections at 2.5 and 2.6°C, and Warren and Kastner (1992) used a dry aging room that operated at 3.1 to 3.6°C. The highest storage temperature was reported in the study conducted by Li, Babol, Bredie, Nielsen, Tománková, and Lundström (2014) where the aging room had an average temperature of 5.1°C.

High temperatures can favor the enzymatic processes involved with aging, which will benefit the tenderizing processes and improve palatability. However, high temperatures will also promote microbial growth possibly causing spoilage and/or pathogenic contamination of meat and lipid oxidation. On the other hand, storage temperatures lower than 0°C will slow the enzyme activities, significantly reducing the tenderizing process. Moreover, lower temperatures can retard microbial growth and thus prevent further desired flavor development induced by microbial enzymes. These should be considered when deciding the storage temperature for dry-aged beef.

Air flow

During dry aging, water is transferred from the interior to the meat surface and subsequently evaporates to the surrounding environment. Air flow is an important variable of the drying process because it can affect the water evaporation rate (Lewicki, 2004). This is important because increasing air flow increases evaporation rates and causes faster drying, which may alter the concentration of flavor compounds during dry aging. Economics parameters such as trimming loss and saleable yield may also be affected by changes in rate of drying.

According to Savell (2008), it is important to expose all surfaces to air and cold temperature to ensure uniform drying and minimize spoilage. Supplemental fans, wire racks, perforated shelves, and trees or hooks can be used to optimize air flow ensuring consistency through the drying process. However, keeping air flow evenly distributed throughout the aging chamber is a hard task. Those meat products located close to the fan may undergo faster drying than those located farther from the fan. Another challenge is to define a precise location to measure air velocity, which accurately represents the air flow inside the aging chamber.

Dry aging literature has reported air flow values ranging from 0.2 to 2.5 m/s (Parrish, Boles, Rust, & Olson, 1991; Kim, Kemp, & Samuelson, 2016; Kim, Meyers, Kim, Liceaga, & Lemenager, 2017; Berger *et al*., 2018; Hulànková, Kameníka, Salákováa, Závodskýa, & Borilova, 2018). However, for the most part, studies have not reported air flow values as air flow was not the subject of interest of these studies (Oreskovich, McKeith, Carr, & Bechtel, 1988; Campbell, Hunt, Levis, & Chambers, 2001; Ahnström, Seyfert, Hunt, & Johnson, 2006; Sitz, Calkins, Feuz, Umberger, & Eskridge, 2006; Laster

et al., 2006; Smith *et al*., 2008; DeGeer, Hunt, Bratcher, Crozier-Dodson, Johnson, & Stika, 2009; Li, Babol, Wallby, & Lundström, 2013; Dikeman, Obuz, Gök, Akkaya, & Stroda, 2013; Li, Babol, Bredie, Nielsen, Tománková, & Lundström, 2014; Smith, Harris, Griffin, Miller, Kerth, & Savell, 2014; Stenström, Li, Hunt, & Lundström, 2014; Lida *et al*., 2016; Gudjónsdóttir, Gacutan, Mendes, Chronakis, Jespersen, & Karlsson, 2015; O'Quinn *et al*., 2016; Ryu *et al*., 2018; Lee *et al*., 2017).

Unfortunately, dry aging studies devoted to evaluating the effects of air flow levels on sensorial and economic parameters are very limited in number. To the author's knowledge, the study conducted by Kim, Kemp, and Samuelsson (2016) was the first research project that evaluated the effect of air flow on meat quality attributes of dry-aged beef loins. These authors compared two air velocities (0.2 or 0.5 m/s) and two temperatures (1 or 3°C) between dry aging treatments and found no air velocity effects on waste trimmings, saleable lean, or yield. Based on the sensory test, Kim, Kemp, and Samuelsson (2016) suggested that dry-aging loins at 3°C with 0.2 m/s air-velocity and 49% RH appears to be the best processing regime among the treatments trialed, resulting in enhanced eating quality attributes.

In conclusion, very little research has explored the effects of air flow on sensory attributes and saleable yield. The scientific information is so limited that it cannot be used to support a recommended air velocity setting for dry aging beef products. It is still unclear if increasing air flow would result in lower yield due to excessive trimmings and moisture loss, and if changes in rate of drying could affect flavor development during dry aging.

Relative humidity (RH)

Relative humidity is another important dry aging parameter that has to be controlled because it can affect microbial growth and water evaporation rate. Moreover, economic parameters such as trimming loss and saleable yield may also be affected. In this respect, if the relative humidity is too low, excess product shrinkage will occur, increasing yield losses. On the other hand, if RH is too high, microbial growth will be promoted and off-odors and off-flavors will develop due to spoilage (Savell, 2008).

There are a number of relative humidity parameters reported in the scientific literature. Ryu *et al*. (2018), Kim, Meyers, Kim, Liceaga, and Lemenager (2017), Lida *et al*. (2016), Smith *et al*. (2008), Laster *et al*. (2008), Warren and Kastner (1992), and Parrish, Boles, Rust, and Olson (1991) dry aged beef at 78 to 91% of relative humidity. Moreover, relative humidity below 78% and above 91% have also been reported. Campbell, Hunt, Levis, and Chambers (2001) and Li, Babol, Bredie, Nielsen, Tománková, and Lundström (2014) dry aged beef in a cooler with 75% relative humidity. Lepper-Blilie, Berg, Buchanan, and Berg (2016) used 70%, and the lowest relative humidity has been reported by DeGeer, Hunt, Bratcher, Crozier-Dodson, Johnson, and Stika (2009), who used 50%. On the contrary, 98.1% is the highest RH that has been reported in the scientific literature (Smith, Harris, Griffin, Miller, Kerth, & Savell, 2014)

Although there are many studies about dry-aged beef, there is only one study to the author's knowledge that has compared the effects of different relative humidity levels on dry-aged beef. Lee *et al*. (2017) dry aged sirloins for 28 days in different chambers with 75% and 85% of relative humidity. No significant differences were detected in moisture content, shear force, or total bacterial count. However, the authors reported that flavor and

overall acceptability were significantly higher for steaks dry aged at 75% relative humidity compared to 85% relative humidity.

Dry aging effects on quality attributes:

Flavor

The primary reason for dry-aging beef is to enhance overall palatability, mainly due to the development of unique flavors (Savell, 2008). Savell and Gehring (2018) suggested that the enhanced flavor of dry-aged beef results from both concentration of flavor compounds as meat loses moisture during drying, as well from creation of new flavor compounds caused by changes of chemical nature of proteins and fats through oxidation. Brown roasted, buttery, nutty, and beefy/umami are some of the sensory flavors often associated with dry-aged beef (Campbell, Hunt, Levis, & Chambers, 2001; Savell, 2008; Warren & Kastner, 1992).

The development of these flavors may involve reducing sugars, the release of free amino acids, peptides, and the breakdown of ribonucleotides. Ribonucleotide degradation produces IMP (5′-inosine monophosphate) and GMP (5′-guanosine monophosphate), two compounds that are known to augment the umami flavor in meat (Dashdorj, Amna, & Hwang, 2015). Kim, Kemp and Samuelsson (2016) evaluated the metabolite differences between dry-aged and wet-aged beef and found that tryptophan, phenylalanine, valine, tyrosine, glutamate, isoleucine and leucine, which are known to be associated with meat flavor, were more abundant in the dry-aged beef. However, the factors and the time necessary to develop these flavors are still unclear and thus require further research.

Warren and Kastner (1991) aged strip loins for 11 days and found that dry-aged beef had more intense beefy and brown roasted flavor than wet-aged or unaged samples as determined by trained sensory panels. However, several studies have documented that dry-aged beef flavor begins to develop after 14 days and intensifies thereafter. Campbell, Hunt, Levis, and Chambers (2001) reported that steaks dry aged for 14 and 21 d produced greater dry-aged flavors than steaks dry aged for 7 d. Moreover, Lepper-Blilie, Berg, Buchanan, and Berg (2016) reported that steaks dry aged for 42 and 49 days had the strongest aged flavor compared to 14 and 21 days based on sensory panelists evaluation.

Although these results show that dry aging produces more flavorful beef, conflicting sensory results have been presented throughout many studies, where no differences in dry versus vacuum aged flavors were found (Sitz, Calkins, Feuz, Umberger, & Eskridge, 2006; Laster *et al*., 2008; Smith *et al*., 2008; DeGeer, Hunt, Bratcher, Crozier-Dodson, Johnson, & Stika, 2009; Dikeman, Obuz, Gök, Akkaya, & Stroda, 2013). These inconsistent results between studies might be associated with the unfamiliarity of consumers with the actual dry-aged flavor, lack of developed sense of smell and taste of sensory panelists, and also due to variability in the processing conditions which leads to different outcomes.

Tenderness

Typically, dry aging is not used to promote a tenderness advantage in comparison to wet aging; instead, dry aging is mainly used for enhancing or intensifying flavors. For the most part, published studies have reported no significant differences for shear force values between wet-aged and dry-aged beef (Parrish, Boles, Rust, & Olson, 1991; Sitz,

Calkins, Feuz, Umberger, & Eskridge, 2006; Smith *et al*., 2008; Dikeman, Obuz, Gök, Akkaya, & Stroda, 2013; Li, Babol, Wallby, & Lundström, 2013; Lepper-Blilie, Berg, Buchanan, & Berg, 2016; Lee *et al*., 2017; Berger *et al*., 2018). These studies confirm that improvements in tenderness through the aging process occur regardless of the aging method used (wet or dry).

Tenderness can also be assessed by sensory methods, using untrained consumers or trained expert panelists. Varied results have been found in terms of how aging method affects sensory tenderness. Some sensory analyses have demonstrated that aging method (wet or dry) has minimal to no effects on consumer tenderness perception (Smith *et al*., 2008; Kim, Kemp, & Samuelsson, 2016). Similar results were reported by Lepper-Blilie, Berg, Buchanan, and Berg (2016) who found no differences in tenderness between wet and dry-aged low marbled beef loins by trained panel evaluation.

However, some research has been able to identify benefits to sensory tenderness associated with aging method. Tenderness was significantly greater for wet-aged steaks than dry-aged steaks when evaluated by consumer and trained panels, as reported by Parrish, Boles, Rust, and Olson (1991). Similarly, consumer tenderness rating favored wet-aged steaks when compared to dry-aged counterparts, although WBSF values were similar (Sitz, Calkins, Feuz, Umberger, & Eskridge, 2006). Moreover, Laster *et al*. (2008) found wet-aged steaks had higher scores for “tenderness like” than dry-aged steaks.

Conversely, in some instances tenderness scores determined by trained and untrained beef consumers have favored dry-aged beef. Li, Babol, Bredie, Nielsen, Tománková, and Lundström (2014) found that beef *gluteus medius* aged in a dry aging bag for 14 days was more tender and overall preferred by consumers compared with wet-aged

counterparts. Likewise, Campbell, Hunt, Levis, and Chambers (2001) found that the sensory panelists considered dry-aged meat more tender compared with wet-aged meat. Similar results were reported by Li, Babol, Wallby, and Lundström (2013), who found a trend ($P = 0.06$) for improvement in tenderness for beef *gluteus medius* aged in a dry aging bag in comparison to vacuum-aged meat. Berger *et al*. (2018) found that strip loin steaks dry aged in a bag had higher tenderness compared to the wet-aged steaks.

Water holding capacity

Water is a major component of muscle tissue, which has about 75% water. Around 85% of water is located within the myofibrillar network, held between the thick and thin filaments. The rest of the water, around 15%, is located outside the myofibrillar network, in between myofibrils, muscle fibers, and muscle fascicules (Huff-Lonergan and Lonergan, 2005). The water within muscle is classified in three types: protein associated water, immobilized water, and free water. Protein-associated water has reduced mobility, remaining tightly bound by charged hydrophilic groups. Immobilized water is located within the thick filaments and between the thick and thin filaments and accounts for the majority of the water within the muscle (around 85%). This water is bound by steric effects or by hydrogen bonds and can be mobilized due to alteration of muscle cell structure and changes in pH. Free water is located in the sarcoplasmic area within the muscle cells and can be easily mobilized (Pearce, Rosenvold, Andersen, & Hopkins, 2011). The distribution and mobility of water in meat have a profound influence on meat quality attributes such as juiciness and tenderness.

Water-holding capacity is an important property of fresh meat as it affects the yield and quality of the product, and it is often described as drip loss. The mechanism by which drip is lost from meat is influenced by the pH of the tissue and by the amount of space in the muscle cell (Aberle, Forrest, Gerrard, & Mills, 2012).

During the conversion of muscle to meat, the continual addition of H^+ to the sarcoplasm leads to a reduction in meat pH. Accumulation of H^+ is responsible for a reduction in the net charge of muscle proteins. The net charge of muscle proteins decreases as the pH decreases toward the isoelectric point (pI). When meat proteins are charged, the muscle structure has the ability to expand because of the repulsive nature of these charges. As a result, water can infiltrate into the protein structure, which increases WHC. However, once the pH has reached the pI of the major proteins, the numbers of positive and negative charges on the proteins are equal. Thus, the positive and negative groups within the protein are attracted to each other and can reduce the amount of water that can be attracted and held by that protein network. Since the same charges repel, as the net charge of the muscle proteins reaches zero, repulsion of structures in the myofibril is reduced allowing those structures to pack more closely together, which reduces the space within the myofibril and the capacity of binding water (Puolanne & Halonen, 2010).

Steric effects are physical characteristics that create space within the muscle fiber and facilitate areas for water to occupy. As most of the water is held within myofibrils by capillary forces, any changes to the myofibril can affect WHC. As muscle goes into rigor, cross-bridges form between the thick and thin filaments, thus reducing available space for water to reside. Additionally, during rigor development sarcomeres can shorten; this also

reduces the space available for water within the myofibril (Kristensen & Purslow, 2001; Offer *et al*., 1989).

Rigor shortening also affects WHC. When ATP is depleted, a final binding between actin and myosin is formed, resulting in sarcomere shortening. During rigor, the cells shrink both lengthwise and longitudinally, leaving little room for water. As a result, water is squeezed out of the cell, resulting in increased drip loss (Ertbjerg & Poulanne, 2017).

Proteolytic degradation during aging allows the sarcomere to relax. This degradation allows the inflow of water previously expelled during rigor, increasing meat WHC. Although proteolysis results in increased WHC at early postmortem stages, extended aging has the potential to reduce WHC and increase drip loss. This may be due to the continuous release of protein-associated water resulted from the breakdown of proteins (Huff-Lonergan & Lonergan, 2005).

Kim, Kemp, and Samuelsson (2016) evaluated wet and dry-aged beef loins under different regimes and found that aging types and processing regimes did not affect the drip or cook loss of beef loins. Their results are in line with the results reported by Dikeman, Obuz, Gök, Akkaya, and Stroda (2013) and Warren and Kastner (1992), who found wet and dry-aged strip loins had similar cooking losses. However, Laster *et al*. (2008) reported that dry aging caused less cooking loss than wet aging.

Although Kim, Kemp, and Samuelsson (2016) observed that aging type did not affect drip or cook loss, the authors reported that the total moisture loss, which was calculated by summing initial, drip and cook loss together, was greater in the dry aging treatment compared to wet-aging (36% and 25%, respectively). In addition, in the same study, the authors found that the elevated aging temperature of 3°C resulted in greater total

loss compared to aging at 1°C within the dry-aging treatment (33.7% and 37.7%, respectively).

DeGeer, Hunt, Bratcher, Crozier-Dodson, Johnson, and Stika (2009) compared the effects of two dry-aging methods (unpackaged and in a bag). They observed that mass losses during cooking were 2–3% greater for steaks from loins aged in the dry aging bag than for the steaks from unpackaged dry aging treatment. On the contrary, Ahnström, Seyfert, Hunt, and Johnson (2006) reported similar cooking loss for loins dry aged traditionally or in a highly moisture permeable bag for 14 and 21 days.

Transport of water in the meat being dry aged proceeds in two steps. First, water is transported from the interior to the meat surface by diffusion and capillary flow. Second, water is evaporated at the surface of the meat and is transferred as vapor to the surrounding air. This process is a convective mass transfer (Lewicki, 2004). The rate of convective mass transfer is dependent on RH, air velocity, and temperature. The transport of water in dry-aged beef is dependent upon two mass transfer resistances: internal and external. The internal mass transfer resistance depends on the temperature of the meat and on the effective water diffusion coefficient (diffusion and capillary flow), which can be influenced by a variety of factors, including the pH, proteolysis, and muscle cell structure. Internal mass transfer resistance determines the rate of drying and the time the dry aging process takes. A decrease of water content by a few percentage points can lower the effective diffusion coefficient by two or three orders of magnitude (Lewicki, 2004). The external mass transfer resistance is responsible for the convective mass transfer. The relationship between both external and internal mass transfer resistances affects the rate of drying. If the external resistance is higher than or equal to the internal resistance, the flux of water

reaching the surface of the meat is constant and drying proceeds at a constant rate. If the internal resistance is higher than the external, less water is transported to the surface of the meat (Lewicki, 2004).

In summary, dry-aged meat loses most of the water by convection during the early stage, causing a crust to form as the surface of beef dries out. Once the meat surface has been dried off and the hard crust is formed, the rate of evaporation of water will be driven mainly due to diffusion rate. Hence, the flux of evaporated water in dry-aged beef decreases with time and drying proceeds at a decreased rate.

Juiciness

Juiciness is the amount of perceived juices in the meat during chewing (Miller, 2004). Several studies have reported no differences for juiciness between dry and wet-aged beef (Parrish, Boles, Rust, & Olson, 1991; Sitz, Calkins, Feuz, Umberger, & Eskridge, 2006; Laster *et al*., 2008; Smith *et al*., 2008; Dikeman, Obuz, Gök, Akkaya, & Stroda, 2013; Obuz, Akkaya, Gök, & Dikeman, 2014; Stenström, Li, Hunt, & Lundström, 2014; Smith, Harris, Griffin, Miller, Kerth, & Savell, 2014; Kim, Kemp, & Samuelsson, 2016). Conversely, Richardson, Nute, and Wood (2008) reported that dry-aged beef was juicier than vacuum aged. These findings were also observed by Berger *et al*. (2018) who found greater juiciness of strip loin steaks dry aged in a bag when compared to wet-aged steaks. One possible explanation is that juiciness may have improved by the concentration of the fat through the loss of moisture during the dry aging period (Campbell, Hunt, Levis, & Chambers, 2001).

Campbell, Hunt, Levis, and Chambers (2001) found that increasing dry aging time improved juiciness. Dry aging for 21 days resulted in juicer steaks when compared to steaks dry aged for 14 days, which were juicer than steaks dry aged for 7 days or control steaks (stored in vacuum for 14 days). The authors attributed this finding to the concentration of fat by moisture loss during aging. Similar results were reported by Li, Babol, Bredie, Nielsen, Tománková, and Lundström (2014) who found that strip loin steaks aged for 19 days were juicer than steaks aged for 8 days. On the contrary, several studies have indicated that juiciness was unaffected by dry aging time (Ahnström, Seyfert, Hunt, & Johnson, 2006; Laster *et al*., 2008; Lepper-Blilie, Berg, Buchanan, & Berg, 2016). Lida *et al*. (2016) indicated that juiciness evaluated via trained sensory panel did not change during the 60-day dry aging for highly marbled beef (ranging from 35.8 to 44.9% fat). As explained by Miller (2014) meat with higher fat content will have a longer sustained perception of juiciness.

In some instances, improvements in juiciness were noted with increases in intramuscular fat content (marbling). Parrish, Boles, Rust, and Olson (1991) reported that both trained sensory panel and consumer (untrained) panel rated USDA Prime dry-aged loin steaks juicier than USDA Choice and USDA Select dry-aged steaks. In studies by Laster *et al*. (2008) and Smith *et al*. (2008) the sensory test results also showed differences in juiciness between USDA Choice and Select loin steaks, where the Choice steaks were perceived as juicer. During chewing of meat, the salivary glands are stimulated by fat release. As a result of increased salivation, the meat is perceived as juicer (Miller, 2004). In contrast to all findings mentioned above, Dikeman, Obuz, Gök, Akkaya, and Stroda

(2013) reported no quality grade effect on juiciness of USDA Choice and USDA Select strip loin dry-aged steaks.

Dry aging in a bag did not affect juiciness of beef *Longissimus lumborum* steaks when compared to traditional dry aging or vacuum aging (Dikeman, Obuz, Gök, Akkaya, & Stroda, 2013; Li, Babol, Bredie, Nielsen, Tománková, & Lundström, 2014). This also agrees with Lee *et al*. (2017) who found no differences in juiciness of bag dry-aged cow sirloin steaks compared to traditional dry aging. However, some research has been able to associate the use of dry aging bag with improved juiciness. Trends (P = 0.08) for improvements in juiciness have been observed by Li, Babol, Wallby, and Lundström (2013) where consumers tended to consider the samples aged in dry aging bags juicier than samples aged in vacuum. Likewise, Stenström, Li, Hunt, & Lundström (2014) observed that the consumers who showed a preference between bag dry-aged and vacuum aged, 64% found dry-aged bag steaks juicer than vacuum aged steaks.

Color

Studies focused on the direct comparison of dry aging to wet aging color have not been the subject of much scientific literature and limited information is available regarding the effects of dry aging on beef color attributes. Some studies found no differences in color between dry-aged and wet-aged beef loins (Dikeman, Obuz, Gök, Akkaya, & Stroda, 2013; Brugiapaglia, Destefanis, & Vincenti, 2015). Similar observations were noted by Li, Babol, Wallby, and Lundström (2013) who reported no difference in meat color between beef *gluteus medius* samples aged in vacuum and in dry aging bags for 14 days.

In an aging trial combining two aging methods (dry aging for 10 days followed by wet aging for 7 days), Kim, Meyers, Kim, Liceaga, and Lemenager (2017) reported that stepwise dry/wet aging resulted in similar color characteristics and color stability of beef loins when compared to traditional dry aging. On a similar note, a study revealed similar color stability between dry and wet-aged steaks over 7 days of retail display simulation (Kim *et al*., 2018).

Conversely, lower a* and b* values for dry-aged Holstein cow steaks were reported by Obuz, Akkava, Gök, and Dikeman (2014) after 23 days of aging when compared to their wet-aged counterparts. In accordance, Li, Babol, Bredie, Nielsen, Tománková, and Lundström, (2014) observed a trend ($P = 0.057$) where vacuum aged samples had greater a* values than traditionally dry-aged samples after 19 days of aging. Although Kim, Kemp, and Samuelsson (2016) reported statistically lower L*and a* values for dry-aged loins in comparison to wet-aged loins, they suggested that dry-aging did not result in any substantial adverse impacts on initial meat color, once the dried exterior surface is completely removed in the trimming process.

The general consensus is that dry-aged beef is slightly darker and less red when compared to wet-aged beef. The darker color in dry-aged beef compared to wet-aged might be associated with lower moisture content and surface drying after aging resulting in less light reflection (Kim and Hunt, 2011).

Microbial growth

Bacterial growth

Flavor compounds which have a favorable effect on taste and aroma are concentrated during dry aging. The development of these compounds seems to be proportional to the length of meat aging. Extending dry aging time, on the other hand, creates conditions for the growth of aerobic and psychrotrophic microflora capable of multiplying on the meat at refrigeration temperatures (Blana and Nychas, 2014).

Ahnström, Seyfert, Hunt, and Johnson (2006) indicated that extending aging time from 14 to 21 days did not affect total aerobic count, regardless of dry aging method. Campbell, Hunt, Levis, and Chambers (2001) found that beef *longissimus* muscle steaks that were dry aged for 7, 14 or 21 days had higher aerobic plate counts compared to 14 days of vacuum aging. However, extending dry aging from 7 to 21 days did not affect aerobic counts. These authors suggested that the lack of response to dry-aging time may have been because of growth inhibition caused by surface drying and low storage temperatures.

Li, Babol, Bredie, Nielsen, Tománková, and Lundström (2014) reported lower total bacteria counts for *longissimus* muscle dry aged in a bag than those traditionally dry aged. However, both dry aging methods had greater total bacteria counts when compared to wet aging. In disagreement, Berger *et al*. (2018) found that dry-aged and vacuum aged beef samples had significantly lower total aerobic bacterial populations as compared to dry-aged in bag beef samples. In general, lower total aerobic bacterial counts are expected for vacuum aged samples as the anaerobic environment created by the oxygen impermeable vacuum packaging limits growth of aerobic bacteria.

Parrish, Boles, Rust, and Olson (1991) found that lactic acid bacteria (LAB) counts were higher on vacuum aged beef loins than on their dry-aged counterparts after 21 days of aging. Similarly, Li, Babol, Wallby, and Lundström (2013) reported that beef *gluteus medius* samples aged for 14 days in dry aging bags had lower LAB counts than samples aged in vacuum, both before and after trimming. Berger *et al*. (2018) compared dry aging of beef loins for 21 days in a bag with traditional dry aging and vacuum aging. Their results indicated that dry-aged beef samples had lower LAB concentrations compared to vacuum aging and dry aging bag samples.

In a study comparing traditional dry aging, dry aging bag and wet aging for 8 or 19 days, Li, Babol, Bredie, Nielsen, Tománková, and Lundström (2014) reported that dry aged samples had lower LAB counts on the fat surface than wet-aged samples after 8 and 19 days of aging. In addition, dry-aged samples tended to have lower LAB counts on the meat surface than wet-aged and dry aging bag samples after 19 days of aging. These authors suggested that LAB counts increased with longer aging time regardless of aging method, except for LAB counts on the meat surface of samples that were traditionally dry aged.

Ahnström, Seyfert, Hunt, and Johnson (2006) observed that increasing the aging period from 14 to 21 days decreased LAB on fat and lean tissue of strip loins, regardless of dry aging method (traditional dry aging or dry aging in a highly moisture-permeable bag). Conversely, Campbell, Hunt, Levis, and Chambers (2001) found that counts of LAB increased after 21 days of dry aging in comparison with samples aged for 14 days in vacuum, or dry aged for 7 and 14 days.

In general, the results of dry aging studies indicate that even prolonged dry aging can result in meat of acceptable microbiological quality as values reported in most of the

studies were below the threshold of 7 log CFU per cm^2 or gram, which is associated with off-flavor and spoilage in meat. This may be due to the microbial growth inhibition caused by surface drying in dry-aged samples.

Mold growth

Dry aging encourages the growth of beneficial mold (Dashdorj, Tripathi, Cho, Kim, & Hwang, 2016). The presence of molds from several genera have been reported on the meat surface of dry-aged beef (i.e. Thamnidium, Penicillium, Rhizopus, Debaryomyces and Mucor). Dashdorj, Tripathi, Cho, Kim, and Hwang (2016) suggested that Thamnidium is the most desirable mold as they release proteases, which break down muscle and connective tissue, improving meat tenderness.

Ryu *et al*. (2018) observed that potentially harmful yeasts and molds (*Candida* sp., *Cladosporium* sp., *Rhodotorula glutinis*, and *Rhodotorula mucilaginosa*) were present after 25 days of dry aging. However, these strains disappeared after extending the dry aging period to 60 days. These authors also observed an increase in *Penicillium camemberti* and *Debaryomyces hansenii* (molds used in cheese manufacturing) after 40 up to 60 days of dry aging. In conclusion, Ryu *et al*. (2018) suggested that the change in microorganisms exerts an influence on the quality of dry-aged beef and that fungi may play an important role in the palatability and flavor development of dry-aged beef.

On the other hand, Berger *et al*. (2018) indicated that aging method (wet, dry or dry aging in a bag) did not affect mold counts of beef loins as exterior lean surface was trimmed and discarded. Ahnström, Seyfert, Hunt, and Johnson (2006) reported that mold counts of adipose and lean tissue from strip loins dry aged traditionally or in a bag for 14 or 21 days

did not increase, remaining less than 0.3 log cfu/cm^2 during aging. Similar results were found by Li, Babol, Bredie, Nielsen, Tománková, and Lundström (2014), who found no effect of aging method (wet, dry or dry aging in a bag) or aging time (8 or 18 days) on mold counts of adipose and lean tissue. Likewise, Li, Babol, Wallby, and Lundström (2013) reported no effect of aging method (wet or dry aging in a bag) on mold counts before and after trimming for beef *gluteus medius* dry aged for 14 days.

Perhaps, the contribution of fungi on the flavor development of dry-aged beef is dependent upon the number of days products are dry aged. Dry aging periods of 40 to 60 days have appeared to affect mold count. However, the role some molds play on dry-aged beef flavor development is still unclear.

Dry aging effects on economic parameters:

Shrinkage

Dry aging results in a high percentage of shrink during cooler storage. However, shrinkage is desirable to obtain distinct palatability as moisture is lost over time and the flavor compounds are concentrated, resulting in a characteristic and unique flavor. On the other hand, shrinkage also affects yield; and if these losses are not compensated with higher prices, economic return will be reduced.

As expected, several studies have reported greater shrink loss for dry-aged beef in comparison to wet-aged counterparts (Warren and Kastner, 1992; Laster *et al*., 2018; Dikeman, Obuz, Gök, Akkaya, & Stroda, 2013; Li, Babol, Wallby, & Lundström, 2013; Smith, Harris, Griffin, Miller, Kerth, & Savell, 2014; Li, Babol, Bredie, Nielsen,

Tománková, & Lundström, 2014; Obuz, Akkava, Gök, & Dikeman, 2014; Stenström, Li, Hunt, & Lundström, 2014; Kim, Kemp, & Samuelsson, 2016; Berger *et al*., 2018). In order to decrease cooler shrink and maximize yield, a dry aging bag has been introduced in the meat industry. Hence, recent studies have addressed the differences between beef aged in dry aging bags and under traditional unpackaged dry aging.

Li, Babol, Bredie, Nielsen, Tománková, and Lundström (2014) found that aging loss of beef loins aged in dry aging bags was lower than that traditionally dry aged after 8 days of aging (5.8 vs 6.9%) and 19 days (13.5 vs 15.3%). Likewise, Berger *et al*. (2018) found that dry aging of beef loins for 28 d resulted in greater shrink loss than dry aging in a bag (13.9 vs 9.1%, respectively), which were significantly greater than wet aging (0.9%). In agreement, Dikeman, Obuz, Gök, Akkaya, and Stroda (2013) found greater mass loss for dry-aged boneless loins when compared to loins dry aged in a special bag (15.56 vs 13.48%).

Although there is not a consensus in the literature, for the most part, it seems that mass loss increases as aging time increase (Ahnström, Seyfert, Hunt, & Johnson, 2006; Li, Babol, Bredie, Nielsen, Tománková, & Lundström, 2014; Gudjónsdóttir, Gacutan, Mendes, Chronakis, Jespersen, & Karlsson, 2015; Lepper-Blilie, Berg, Buchanan, & Berg, 2016). Ahnström, Seyfert, Hunt, and Johnson (2006) reported no differences in mass loss between traditional dry aging and dry aging bag after 14 days of aging (6.5 and 6.3%, respectively). However, dry aging in a bag for 21 days decreased mass loss in comparison to traditional dry aging with no effects on sensory attributes (8.8 vs 10.2%). These results contrast with DeGeer, Hunt, Bratcher, Crozier-Dodson, Johnson, and Stika (2009) who reported no differences in aging loss for strip loins dry aged traditionally or in a bag (19.1

vs 17.5%, respectively). DeGeer, Hunt, Bratcher, Crozier-Dodson, Johnson, and Stika (2009) suggested that bone removal from loins accentuates greater moisture movement, regardless of aging method, which could explain the greater aging loss values observed in this experiment. This is in agreement with Lepper-Blilie, Berg, Buchanan, and Berg (2016) who found that bone-in loins lost less mass than boneless (15.7% vs 21.2%).

Some studies have also addressed the effects of USDA Quality Grade on dry aging and shrinkage. Dikeman, Obuz, Gök, Akkaya, and Stroda (2013) found that USDA Select loins had higher shrink loss during aging than USDA Choice loins (11.37% vs. 9.92%). The higher moisture content and the lower intramuscular fat of USDA Select loins might explain the higher mass loss due to evaporation. Conversely, Smith *et al*. (2008) found no differences in cooler shrink between USDA Choice and Select dry-aged short loins aged for 14, 21, 28 or 35 days. Parrish, Boles, Rust, and Olson (1991) also reported no differences in cooler shrink among USDA Prime, Choice and Select loins dry aged for 14 or 21 days.

For the most part, dry aging literature has reported shrinkage values ranging from 2.70 to 19.10%. Such variation among studies would likely be associated with inconsistent processing environment conditions applied during the dry aging process. Kim, Kemp, and Samuelsson (2016) reported a 3% increase in mass loss for dry-aged short loins when the temperature was increased from 1 to 3°C. Additionally, the authors also found that increasing the air velocity from 0.2 to 0.5 m/s resulted in mass loss increments of 1%. In fact, although dry aging has been practiced for decades, there is little information on the impacts of various combined dry aging regimes on mass loss over time.

Retail Yield

A major argument against dry aging involves loss of saleable mass at the retail level due to moisture and trim loss. In general, lower yield with dry aging compared to wet aging has been reported in the literature (Parrish, Boles, Rust, & Olson, 1991; Laster *et al*., 2008; Smith *et al*., 2008; Li, Babol, Bredie, Nielsen, Tománková, & Lundström, 2014; Obuz, Akkava, Gök, & Dikeman, 2014; Kim, Kemp, & Samuelsson, 2016; Lepper-Blilie, Berg, Buchanan, & Berg, 2016; Berger *et al*., 2018). Percent yield varies extremely in the dry aging literature and values ranging from 45 up to 75% have been reported.

A lower percent yield was found by Kim, Kemp, and Samuelsson (2016) for dry-aged beef loins compared to the wet-aged loins (46% and 55%, respectively). Berger *et al*. (2018) found that dry aging beef loins for 28 d resulted in lower total saleable yield than wet aging (56.9 vs 66.6%, respectively). Higher total saleable yield for wet-aged strip loins over dry-aged (71.2% vs 58.3%) was also reported by Laster *et al*. (2008). These results are similar to the findings of Smith, Harris, Griffin, Miller, Kerth, and Savell (2014), who found yield values of 73.64% for wet-aged top sirloin butts and 54.78% for dry-aged.

The influence of aging time on yield has been the subject of several research studies. Laster *et al*. (2008) dry aged beef loins for 14, 21, 28 or 35 days and found decreased total saleable yield for ribeyes as aging period progressed from 14 (72.2%) to 21 (69.3%) and 28 days (64.3%). However, extending aging to 35 days had no detrimental effects on yield (63.5%). A similar pattern for total saleable yield with increasing aging time was observed when top sirloin steaks were fabricated. Smith *et al*. (2008) reported greater saleable yield for short loins dry aged for 14 days (76.5%). Saleable yield was lowest for short loins dry aged 28 and 35 days (71.6 and 69.8%, respectively).

DeGeer, Hunt, Bratcher, Crozier-Dodson, Johnson, and Stika (2009) found that combined losses for strip loins dry aged traditionally or in a moisture permeable bag did not differ (46.9 vs 45.5%, respectively). Ahnström, Seyfert, Hunt, and Johnson (2006) found similar trim loss for traditional dry aging and dry-aging bag (15 and 15.3%, respectively) after 14 days of aging. However, dry aging in the bag decreased trim loss when compared to traditional dry aging after 21 days of aging with no effects on sensory attributes (15.6 vs 17.9%). Similar results were found by Dikeman, Obuz, Gök, Akkaya, and Stroda (2013) who observed combined losses between loins dry aged in a special bag and traditionally dry aged (36.41% versus 35.98%, respectively). Li, Babol, Bredie, Nielsen, Tománková, and Lundström (2014) reported similar total loss between meat aged in dry aging bags (26.7%) and traditional dry aging (27.5%) after 8 days of aging. Extending aging to 19 days resulted in greater total loss when compared to 8 days (37.1 and 40.7% for dry aging bag and traditional dry aging, respectively). However, dry aging methods did not differ from each other. Conversely, Berger *et al*. (2018) found that dry aging in bags minimized yield loss compared to conventional dry aging, increasing by 4% total saleable yield (60.9% vs 56.9%).

Yield can also be affected by presence of bone (Bernardo, 2020). Lepper-Blilie, Berg, Buchanan, and Berg (2016) reported that bone-in beef loins lost less mass than boneless loins (15.7% vs 21.2%), which is in agreement with DeGeer, Hunt, Bratcher, Crozier-Dodson, Johnson, and Stika (2009). One would expect slower rate of drying for bone-in cuts due to the reduced surface area when compared to boneless cuts.

The magnitude of yield loss for dry-aged cuts seems to be dependent upon the dry aging parameters (i.e. temperature, length of drying, air flow, and RH) and products

characteristics (i.g. bone in or boneless cuts, amount of fat cover and intramuscular fat). Therefore, strategies to reduce yield loss without compromising the unique flavor characteristics of dry-aged beef are desirable.

Pricing

Dry aging is a value-adding practice for a niche market. This practice creates additional costs for processors, mainly due to losses in cooler shrink and additional trimming waste. Consequently, lower saleable yields will ultimately lead to a higher price for dry-aged products in the marketplace. Smith *et al*. (2008) suggested that increasing the length of drying continued to decrease margin, forcing even higher retail prices.

Berger *et al*. (2018) conducted a consumer panel survey that aimed to evaluate consumer perception and willingness to pay for dry-aged and grass-fed beef. Among panelists, 42.5% were willing to pay $1.00 more per 0.45 kg for dry-aged beef. When consumers were asked if they were willing to pay $3.00 more per 0.45 kg for premium dry-aged grass-fed beef, only 19.2% of the panelists responded positively. In a similar willingness study conducted by Laster *et al*. (2008), 33.9% of the consumer panelists were willing to spend $2.20 more per 1.00 kg for dry-aged beef. Similar results were found by Smith *et al*. (2008) who reported that 37.68% of the consumers who responded the survey would spend $2.20 more per 1.00 kg for dry-aged beef.

In a study to determine the value consumers place on their preferred product, Sitz, Calkins, Feuz, Umberger, and Eskridge (2006) indicated that those panelists preferring dry-aged beef were willing to bid a higher premium for dry-aged beef as compared to those preferring wet-aged beef ($2.02/0.45 kg vs. $1.76/0.45 kg). Stenström, Li, Hunt, and

Lundström (2014) indicated that 14.8% of consumers were prepared to pay more than the average price for beef that was dry aged, while only 1.6% were prepared to pay more for vacuum-aged beef. These authors suggested that dry-aged meat is recognized as a positive term and provide consumers the feeling of premium class and exclusivity. These results indicate that there is a high value niche market willing to pay premium prices for dry-aged beef.

MATERIALS AND METHODS

Study 1: Color and lipid stability of dry-aged beef during retail display

Sample collection

All animals used in this study were slaughtered humanely, under USDA guidelines. Sixteen USDA low Choice strip loins [Institutional Meat Purchase Specifications number 180] were collected at a commercial beef harvest facility, vacuum-packaged, and transported to the University of Nebraska meat laboratory where they were immediately removed from the vacuum packages. Boneless loins were assigned to one of the four aging treatments: vacuum (WET), dry aging at 50% relative humidity (RH; RH50), dry aging at 70% RH (RH70), or dry aging at 85% RH (RH85). Strip loins were placed in each assigned dry aging chamber (1 loin per chamber) and aged for 42 days at 2°C. The dry aging chambers (86 cm length x 47.6 cm width x 33 cm height) are capable of measuring and precisely controlling relative humidity (± 1%) and air flow (± 0.015m^3/min). The chambers monitor temperature (± 0.5°C) and have built-in sensors that can continuously monitor mass loss (± 5 g). All measured data were saved on the connected computer every 15 s. A full description of this dry aging system called Agenator has been previously published by Lau, Ribeiro, Subbiah, and Calkins (2019). After aging, loins were subjected to retail display (RD) for an additional 7 d. Loins assigned to wet aging were individually vacuum packaged and kept intact over 42 days at 2°C in the same cooler where the dry aged chambers were located.

Dry-aged loins were trimmed of dried surface and subcutaneous fat, and fabricated anterior to posterior into 3 steaks: One 2.54 cm-thick steak for objective color and

subjective discoloration with 7 d RD, and two 1.27 cm-thick steaks for lipid oxidation (one steak for lipid oxidation at 0 d RD and one steak was split in half for lipid oxidation after 4 and 7 d RD).

After fabrication, steaks assigned to lipid oxidation at 0 d of RD were vacuum packaged and frozen at -80°C. Steaks assigned to 4 and 7 d of RD were placed on foam trays (21.6 x 15.9 x 2.1 cm, Styro-Tech, Denver, CO), overwrapped with oxygen-permeable film (Prime Source PSM 18 #75003815, Bunzl Processors Division, North Kansas City, MO; oxygen transmission rate = 2.25 ml/cm^2/24 hr at 23° C and 0% relative humidity; water vapor transfer rate = 496 g/m^2/24 hr at 37.8° C and 90% relative humidity), and placed under RD conditions for 7 d (continuous white fluorescence lighting at 1000 to 1800 lux; F32T8/TL741, 700 series, 32 W, Philips, USA) at 2°C. After 4 or 7 d of RD, steaks were vacuum packaged and stored at -80°C. Steaks were randomly rotated daily to minimize any possible location effects within the display.

Instrumental color

Objective color was measured using the L*, a*, b* scales using a Minolta CR-400 colorimeter (Illuminant D65, 8 mm diameter aperture, 2° standard observer angle; Minolta, Osaka, Japan). Six measurements were made per steak through the overwrap film once a day at a standardized time from d 0 to 7 of RD. Readings were averaged by steak for statistical analysis. Delta E (ΔE) was measured as the numerical total color difference in L*, a*, and b* color space during retail display. Delta E (ΔE) was calculated to measure the magnitude of difference in L*, a*, and b* color space between wet-aged steaks at d 0 of RD and dry aging treatments at d 0 of RD. Delta E was also calculated using the initial

color readings taken on day 0 of RD and the final readings taken on day 7 of RD for each individual steak from each treatment. All calculations were performed using the equations provided by the American Meat Science Association Meat Color Measurement Guidelines (Hunt *et al*., 2012).

Subjective discoloration

Six trained panelists evaluated surface discoloration daily during the 7 d of RD according to the procedure of Senaratne-Lenagala (2012). A reference guide of twelve steak images ranging from 0% to 100% surface discoloration with increments of 10% were provided to panelists to ensure consistent evaluations (Appendix I). A percentage scale was used where 0% meant no discoloration and 100% meant complete surface discoloration.

Lipid oxidation

Steaks used for lipid oxidation were removed from the freezer, cut by hand into small cubes, frozen in liquid nitrogen, powdered in a metal cup blender (Model 51BL32, Waring Commercial, Torrington, CT), and stored at -80°C until further analysis. Thiobarbituric acid reactive substance values (TBARS) were measured at 0, 4 and 7 d of RD according to the procedure of Ahn, Olson, Jo, Chen, Wu, and Lee (1998) with minor modifications (Appendix II). Five grams of powdered meat from each steak were blended with 1 mL of butylated hydroxyanisole solution (10%) and 14 mL of distilled water. Samples were homogenized using a Polytron (Kinmatica AG, Lucern, Sui) for 15 s and centrifuged (2,000 x g for 5 min). After centrifugation, 1 mL of supernatant was collected, mixed with 2 mL of thiobarbituric acid/trichloroacetic acid (TBA/TCA) solution (15%

TCA and 20 mM TBA in deionized distiller water), and placed in a water bath at 70°C for 30 min. Samples were cooled for 10 min in a water bath at 20°C and centrifuged (2,000 x g for 15 min). Two hundred microliters of supernatant were transferred to 96-well plates in duplicate. Absorbance was measured at 540 nm using a microplate spectrophotometer (Model Epoch, Biotek, Winooski, VT). Results were expressed in mg of malonaldehyde per kg of tissue.

Statistical Analysis

Objective color data was analyzed as a split-plot repeated measures design with aging treatment as the whole-plot, steak as the slipt-plot, and RD time as the repeated measures, and chamber was considered a random effect. Subjective color data was analyzed as a split-plot repeated measures design with aging treatment as the whole-plot and RD time as the repeated measures. Color panelists were considered a random effect when analyzing subjective discoloration. The TBARS data were analyzed as a split-plot design with aging treatment as the whole-plot, and RD time as the split-plot. In this study, chamber was considered the experimental unit. Interactions between fixed effects were tested and when significant, the interactions were reported. When the interactions were not significant, the main effects of each variable were reported. Data were analyzed using the PROC GLIMMIX procedure of SAS. All means were separated with the LS MEANS and DIFF functions with $\alpha = 0.05$.

Study 2: Effects of relative humidity on dry-aged beef quality

Sample Collection and Fabrication

At the time of grading, 16 USDA low Choice strip loins were collected, vacuum-packaged, and transported to the University of Nebraska Loeffel Meat Laboratory.

At the University of Nebraska meat laboratory, loins were randomly assigned to one of four aging treatments (n = 4 loins/treatment): vacuum (WET), dry-aging at 50% RH (RH50), dry-aging at 70% RH (RH70), or dry-aging at 85% RH (RH85). Initially, three cores (2.54-cm diameter) were removed from the interior surface of lean tissue of each loin for microbiology analysis. Once the initial mass was measured, each strip loin was placed in a dry aging chamber and aged for and aged for 42 d at 2°C and 0.8 m^3/min air speed. Loins assigned to wet aging were individually vacuum packaged and aged in the same cooler for 42 d.

After aging, loins were weighed and immediately trimmed of subcutaneous fat and dehydrated surface, reweighed, and fabricated from anterior to posterior into steaks [one steak for water activity (1.27 cm thick); and nine steaks (2.54 cm thick) - 1 for Warner-Bratzler shear force (WBSF), and eight for sensory analysis].

After fabrication, steaks assigned to pH and water activity measurements were vacuum packaged and frozen at -80°C. Steaks assigned to sensory analysis and WBSF were frozen at -20°C.

Agenator – A computerized dry aging system

To address the flavor inconsistency reported in the literature, we designed and built a computerized dry aging system (the Agenator) that is capable of measuring and precisely controlling relative humidity (± 1%) and air flow (± 0.015m^3/min). The dry aging chambers (86 cm length x 47.6 cm width x 33 cm height) have built-in load cells that can continuously monitor mass loss (± 5 g) and temperature (± 0.5°C). All measured data were saved on the connected computer in user-defined intervals, with a minimum of 1 s. This computerized dry aging system allowed us to conduct dry aging studies with proper replication. A full description of the Agenator has been previously published by Lau, Ribeiro, Subbiah, and Calkins (2019).

pH

Steaks from day 42 of aging were removed from the freezer (-80°C) and thawed at 4°C overnight. Steaks were trimmed of all subcutaneous fat, knife-cut into small cubes, frozen in liquid nitrogen, and powdered in a metal cup blender (Model 51BL32, Waring Commercial, Torrington, CT). Duplicate 10 g samples were placed into 250 mL plastic beakers and placed on a stir plate. Ninety mL of distilled deionized water and a magnetic stir bar were added to ensure constant mixing during the measurement process. The pH was measured using a pH meter (Orion 410Aplus: ThermoFisher Scientific; Waltham, MA) that was calibrated using 4.0, 7.0, and 10.0 standards. The pH measurements of the duplicates were averaged for statistical purposes.

Aging loss (water, trim, and combined losses)

The mass of each strip loin was recorded before and after aging to determine aging loss and yield. After aging, wet-aged beef loins were removed from the vacuum bags, patted dry with paper towels and reweighed. The percentage of moisture loss during wet aging was calculated using the following formula: moisture loss (%) = (initial mass – post aged mass)/initial mass × 100. The percentage daily moisture loss for dry-aged loins was calculated as the difference between the prior day's mass and the current mass divided by the prior day's mass. The percentage total moisture loss for dry-aged loins was calculated as the difference between initial mass and final mass divided by the initial mass and then multiplied by 100. The dry-aged loins were then further processed by trimming dried surfaces and non-edible fat and reweighed to calculate the final saleable yield (%) after aging and trimming.

Water activity (a_w)

Steaks from day 42 of aging were removed from the freezer (-80°C) and thawed at 4°C overnight. Steaks were knife-cut into three horizontal strips of 1 cm each, starting from the dorsal region of the steak (just below the subcutaneous fat) to the ventral region. The strip just below the subcutaneous fat was identified as dorsal, the middle strip was identified as central, and the bottom strip was identified as ventral. Each strip was chopped in an Oster food chopper (Model FPSTMC3321, Sunbeam Products Inc, Boca Raton, FL) for 60 s. Water activity was measured in triplicate using an Aqualab water activity meter (Decagon Devices, Inc., Pullman, WA) at 25°C. Chopped samples were placed in Aqualab water activity cups (Decagon Devices, Inc., Pullman, WA) with enough sample to cover

the entire bottom of the cup. The a_w measurements of the triplicates were averaged for statistical purposes.

Tenderness determination

Steaks for tenderness measurements were removed from the freezer (-20°C) and thawed at 4°C overnight. Steak internal temperatures and mass were recorded before and after cooking. The temperature was recorded for each steak 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). The thermocouples were inserted into the geometric center of each steak with large needles. All steaks were cooked to a target temperature of 71°C on a Belt Grill (TBG60-V3 MagiGril, MagiKitch'n Inc., Quakertown, PA). Belt grill specifications were as follows: preheat = 149°C, top heat = 163°C, bottom heat = 163°C, height of gap = 2.16 cm, and cook time was approximately 6 min. Immediately after cooking, internal temperature and mass were recorded. The cooking loss was calculated using the difference between the pre-cooked mass and final cooked mass divided by the initial mass and multiplied with 100. Steaks were individually bagged and stored overnight at 2°C for WBSF analysis. The following day, six (1.27 cm diameters) cores were removed with a drill press parallel to the orientation of the muscle fibers. Cores were measured using a Food Texture Analyzer (TMS-Pro, Food Technology Corp., Sterling, VA.) with a Warner-Bratzler blade. Peak WBSF values from each core were averaged by steak for statistical analysis.

Microbiology Sample Collection

Microbial analysis of loins was conducted on days 0 and 42. Four individual loins were sampled on day 0 for a baseline understanding of flora present on starting material. On day 42, four loins each from 50% RH, 70% RH, 85% RH and wet-aged treatments were sampled. Three 2.5-centimeter diameter surface cores of the lean surface were aseptically taken from loins and transferred into WhirlPak bags designated for each loin from each treatment as mentioned above (Nasco, Fort Atkinson, WI). Thirty-five mL of sterile BBL Peptone water (Becton, Dickinson and Company, 1057 Franklin Lakes, NJ) was added to sample bags and samples were homogenized for 2 min in a stomacher (bioMerieux Inc., Durham NC). Two 1.75 mL samples were taken directly from the WhirlPak bag and stored in sterile 2 mL microcentrifuge tubes at -20°C until DNA extraction.

Microbial Plating Methods

Sample cores were subjected to microbial plating methods in duplicate. Fifty µl of 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 (PSY) plate counts. DeMan Rogosa Sharpe agar (Thermo Fisher Scientific, Waltham, MA) was used to enumerate lactic acid bacteria (LAB). Plates for APC, AnPC, and LAB were incubated at 37°C and counted at 48 hours. Plates for AnPC were held in an 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). Psychrotrophs were incubated at 4°C and

counted at 10 days.

Microbial Ecology Analysis

Bacterial communities were investigated for each sample using the MiSeq Illumina Sequencing Platform, targeting the bacterial-specific 16s rRNA gene as described by Kozich, Westcott, Baxter, Highlander & Schloss (2013). The DNA was extracted from samples using DNA QuickExtract Solution 1.0 (Epicentre, Madison, WI). Obtained DNA was amplified via the polymerase chain reaction (PCR) in a solution that contained 1X Terra PCR Direct Buffer (Clontech Laboratories Inc., Mountain View, CA), 0.75 U Terra PCR Direct Polymerase Mix (Clontech Laboratories Inc.), approximately 1-5 ng of extracted DNA, and 0.5 μM barcoded universal primers. The PCR reaction was performed alongside negative controls in a Veriti 96 well thermocycler (Thermo Fisher Scientific, Walther, MA), with the following PCR cycle: initial denaturation at 98°C for 3 min, followed by 30 cycles of 98°C for 30 s, 58°C for 30s, and 68°C for 45 s, and a final extension of 68°C for 4 min.

The PCR products were then analyzed on a 1.5% agarose gel to ensure amplification occurred successfully, without contamination of negative controls. Samples were then normalized using the Norgen NGS Normalization 96-Well Kit (Norgen Biotek Corp., Thorold, ON, Canada) according to manufacturer protocol. The pooled sample was then placed in 50 ºC water bath to remove excess ethanol from the normalization kit and run through a spin column. The DNA was found to be lacking in concentration, so samples were subjected to additional PCR using a 5-cycle rendition of the previously described protocol. Products were then separated on a 2% agarose gel, which yielded two bands. The

band corresponding to the bp size of the 16s rRNA V4 subregion was removed with a scalpel and DNA was recovered using the MinElute PCR Purification kit (Qiagen Inc., Germantown, MD). Concentration and bp size of the 16S rRNA libraries were determined using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Library concentration was confirmed with a DeNovix QFX Fluorometer and the Denovix dsDNA High Sensitivity reagent kit (Denovix Inc., Wilmington, DE). The 16S libraries were sequenced using the Illumina MiSeq platform (Illumina, 2457 Inc., San Diego, CA) using the V2 500 cycle kit.

Sequence Processing

An average of 21,754 reads was obtained per sample. Obtained sequence reads were processed with R (version 3.6.0, R Core Team, 2013) and Mothur (Version 1.42.1, Schloss *et al*., 2009). The DADA2 pipeline (Callahan, McMurdie, Rosen, Han, Johnson, & Holmes, 2016a) functioned to prepare sequences and generate amplicon sequence variants (ASVs). Raw reads were quality checked, filtered, trimmed, and merged. Chimeras and non-bacterial sequences were removed. Samples with low biomass were removed, as previously performed in meats studies, due to inherent low cell counts on starting materials (Weinroth *et al*., 2019). Eighteen of the original 20 samples were retained. The ASVs were then assigned and merged with a phylogenetic tree generated from Mothur and metadata file into a phyloseq object (McMurdie & Holmes, 2013). Once ASVs were determined, taxa were assigned based on the Silva (V132, Quast *et al*., 2012) database. The “decontam” package (Davis, Proctor, Holmes, Relman, & Callahan, 2018) was utilized on the dataset to remove possible contaminants. Phyloseq was then utilized to determine relative

abundance of bacterial taxa (Callahan, Sankaran, Fukuyama, McMurdie, & Holmes, 2016b).

Sensory Evaluation

Triangle tests were conducted in two sessions with 32 consumers each. In the first session, panelists were served samples from the RH50% and RH85% treatments. In the second session, panelists were served samples from the WET and RH70% treatments. Each panelist received three 3-digit blind coded samples (1.3 cm x 1.3 cm x 2.54 cm thickness) avoiding the edges and fat kernels of the steaks. Two of these samples were identical and one was different. Panelists were asked to circle the number of the sample that was different in flavor.

Sensory descriptive analysis procedures were performed at Texas A&M University (College Station, Texas) as described by Wall, Kerth, Miller, and Alvarado (2019). Six panelists were trained to scale ten basic flavors and five textural attributes from the beef lexicon on a 16-point intensity scale (where 0 = none and 15 = extremely intense). There were two sample testing sessions. At the beginning of each day, panelists were served two warm-up samples, which were discussed verbally to insure proper scaling and precision of scoring. For sample testing, each panelist was served two cubes (1.3 cm x 1.3 cm x 2.54 cm) assigned a 3-digit blind code, avoiding the edges and fat kernels of the steak, in a plastic cup while in a booth under red lighting. Panelists were given unsalted crackers and double distilled, deionized water for palette cleansing.

Amino acid determination

Steaks from day 42 of aging were removed from the freezer (-20°C) and thawed at 4°C overnight. Steaks were cooked to an internal temperature of 35°C and turned over until they reached a target temperature of 70°C on an electric indoor grill (Hamilton Beach-31605A, Hamilton Beach Brands, Glen Allen, VA). After cooking, steaks were placed on a plate after they were cooked and the juice that seeped onto the plate was captured using a Pasteur pipette. One mL of juice was transferred into a 2mL Eppendorf tube for amino acid determination. Juice samples were kept at -80°C overnight and shipped to Mississippi State University on the following day.

The steak was then bagged (PB-90-C, .85 mil., 15.24x7.62x38.1 cm) and stored overnight at 2°C. The following day, steaks were trimmed of all subcutaneous fat, knife-cut into small cubes, frozen in liquid nitrogen, powdered in a metal cup blender (Model 51BL32, Waring Commercial, Torrington, CT), and bagged (PB-90-C, .85 mil., 15.24x7.62x38.1 cm). Frozen samples were shipped to Mississippi State University overnight.

Amino acids were extracted from 1 g of sample in 5 mL of distilled water. Amino acid derivatives were prepared by using the EZfaast Amino Acid kit (Phenomenex® Inc., Torrance, CA, US), following the propyl chloroformate derivatization procedure developed by Kaspar, Dettmer, Gronwald, and Oefner (2008). The extracted amino acid solution was combined with an internal standard solution (200 µM of norvaline) and deproteinated through solid-phase extraction. The amino acids were then reacted with propyl chloroformate in chloroform, sodium hydroxide, and n-propanol. The derivatives were extracted in isooctane, evaporated, reconstituted in isooctane/chloroform mixture

(4/1, v/v), and transferred to a 2-mL amber glass vial with a fixed insert (Agilent Technologies, Santa Clara, CA) for gas chromatography-mass spectrometry (GC-MS) determination. Amino acid derivatives were injected into an inlet of an Agilent 7890A GC System coupled to an Agilent 5975C inert XL MSD with triple-axis mass detector, an Agilent 7693 Series Autosampler, and a capillary column (Zebron™ EZ-AAA 10 m × 0.25 mm; Phenomenex®, Santa Clara, CA). The inlet was operated at 250°C and 1:15 split ratio. The helium carrier gas was at a 1 mL/min constant flow rate. The temperatures of the transfer line, ion source, and quadrupole were 310, 240, and 180°C, respectively. The oven was programmed initially at 110°C and ramped up to 320°C within 11 min. The solvent delay was 1.30 min. The MS was operated in a SIM (selected ion monitoring) mode and target and qualitative ions were selected according to the manufacturer's recommendation. Amino acids were identified by retention time and target and qualitative ions and quantified by an internal calibration method using authentic standards provided with the kits. The amino acid concentration was expressed as millimole per kg of meat (mmol/kg). The meat juice was processed by being directly combined with the internal standard solution and the amino acid concentration was expressed as millimole per liter of juice (mM).

Statistical Analysis

Rate of moisture loss was analyzed as a split-plot design with treatment as the main plot and days of aging as the repeated measures. Separation of means for rate of moisture loss data was conducted using LS MEANS procedure with SLICEDIFF function at $P < 0.05$. Tenderness, water activity, pH, trim loss, total moisture loss, yield, free amino acids, and microbial plate counts were analyzed as a completely randomized design. Principal

component analysis was applied for ten basic flavors and five textural attributes in wet and dry-aged treatments. In this study, the chamber was considered the experimental unit. Data were analyzed using the PROC GLIMMIX procedure of SAS. All means were separated with the LS MEANS and DIFF functions with $\alpha = 0.05$.

Study 3: Effects of ultimate pH on dry-aged beef quality

Sample Collection and Fabrication

At the time of grading, six USDA low Choice and six matching DC carcasses were selected and boneless strip loins from both sides were obtained and transported to the University of Nebraska Loeffel Meat Laboratory. One steak (1.27 cm thick) for color and pH measurements was obtained from the anterior end of the strip loin. Initially, the objective color was measured using the L*, a*, b* scales with a Minolta CR-400 colorimeter (Illuminant D65, 8 mm diameter aperture, 2° standard observer angle; Minolta, Osaka, Japan). Six measurements of the steak surface were taken through the overwrap film at day 0. Readings were averaged by steak for statistical analysis. Then, steaks were knife-cut into small cubes, trimmed of all subcutaneous fat, frozen in liquid nitrogen, and powdered in a metal cup blender (Model 51BL32, Waring Commercial, Torrington, CT). Then, powdered samples from steaks were weighed out in 10 g duplicates into 250 mL plastic beakers and placed on a stir plate. Ninety mL of distilled deionized water and a magnetic stir bar were added to ensure constant mixing during the measurement process. The pH was measured using a pH meter (Orion 410Aplus: ThermoFisher Scientific; Waltham, MA) that was calibrated using 4.0, 7.0, and 10.0 standards. The pH

measurements of the duplicates were averaged for statistical purposes. *Longissimus* muscle pH was measured, and carcasses were classified as DC (pH = 6.69) or control (pH = 5.47). Then, both strip loins per animal were assigned to 2 aging methods (wet or dry). The 4 treatments included 2 dry aging (DRY and DRY-DC) and 2 wet-aging treatments (WET and WET-DC).

Three cores (2.54-cm diameter) were removed from lean tissue of each loin for microbiology analysis. Once the initial mass was measured, each strip loin was placed in each assigned dry aging chamber and aged for 42 d at 2°C and 0. 8m^3/min air flow. Loins assigned to wet aging were individually vacuum packaged and aged in the same cooler for 42 d.

After aging, loins were weighed and immediately trimmed of dehydrated surface, reweighed, and fabricated anterior to posterior into steaks [one steak for water activity, one for color measurements, and two for lipid oxidation (1.27 cm thick); and eight steaks (2.54 cm thick); 1 for Warner-Bratzler shear force (WBSF), and seven for sensory analysis]. After fabrication, steaks assigned to water activity measurements and lipid oxidation at 0 d of RD were vacuum packaged and frozen at -80°C until further analysis. Steaks assigned to sensory analysis and WBSF were frozen at -20 °C. Steaks assigned to color measurements and 4 and 7 d of RD for lipid oxidation were placed on foam trays (21.6 x 15.9 x 2.1 cm, Styro-Tech, Denver, CO), overwrapped with oxygen-permeable film (Prime Source PSM 18 #75003815, Bunzl Processors Division, North Kansas City, MO; oxygen transmission rate = 2.25 ml/cm^2/24 hr at 23° C and 0% relative humidity; water vapor transfer rate = 496 g/m^2/24 hr at 37.8° C and 90% relative humidity), and placed under RD conditions for 7 d (continuous white fluorescence lighting at 1000 to 1800 lux;

F32T8/TL741, 700 series, 32 W, Philips, USA) at 2°C. After 4 or 7 d of RD steaks were vacuum packaged and stored at -80°C. Steaks were randomly rotated daily to minimize any possible location effects within the display.

Agenator – A computerized dry aging system

The dry aging chambers utilized in this study were designed and built with a computerized dry aging system (the Agenator) that is capable of measuring and precisely controlling relative humidity (± 1%) and air flow (± 0.015m^3/min). The chambers (86 cm Length x 47.6 cm Width x 33 cm Height) have built-in load cells that can continuously monitor mass loss (± 5 g) and temperature (± 0.5°C). All measured data were saved on the connected computer every 15 s. A full description of the Agenator has been previously published by Lau, Ribeiro, Subbiah, and Calkins (2019).

Aging loss (water, trim, and combined losses)

Processing mass loss for the wet-aged loins, percentage daily moisture loss for dry-aged loins, percentage total moisture loss for dry-aged loins, and the final saleable yield (%) were calculated as described in study 2.

Water activity (a_w)

The a_w measurements were done in triplicates as described in study 2.

Instrumental color

Color measures were taken on a daily basis once samples were placed on retail

display as described in study 1.

Subjective discoloration

Subjective discoloration was assessed daily during the 7 d of RD with six trained panelists as described in study 1.

Lipid oxidation

Lipid oxidation was determined with the 2-thiobarbituric acid reactive substances protocol (TBARS) as described by Ahn, Olson, Jo, Chen, Wu, and Lee (1998) with minor modifications and is described in study 1.

Tenderness determination

Tenderness was measured via Warner-Bratzler Shear Force (WBSF) as described in study 2.

Microbiology Sample Collection

Microbial analysis of loins was conducted on d 0 and 42 as described in study 2.

Microbial Plating Methods

Sample cores were subjected to microbial plating methods in duplicates as described in study 2.

Microbial Ecology Analysis

Bacterial communities were investigated for each sample using the MiSeq Illumina Sequencing Platform, targeting the bacterial-specific 16s rRNA according to Kozich, Westcott, Baxter, Highlander & Schloss (2013) protocol and described in study 2.

Sequence Processing

An average of 19,264 reads was obtained per sample. Sequence reads were analyzed as described in study 2.

Sensory Evaluation

Triangle test was conducted in four sessions with 32 consumers. In the first, second, third and fourth sessions, panelists were served samples from the DRY-DC and DRY, DRY-DC and WET-DC, DRY-DC and WET, and WET-DC and WET treatments, respectively. Each panelist received three 3-digit blind coded samples (1.3 cm x 1.3 cm x 2.54 cm thickness) avoiding the edges and fat kernels of the steaks. Two of these samples were identical and one was different. Panelists were asked to identify the sample that was different in flavor.

Statistical Analysis

Rate of moisture loss was analyzed as a split plot design with treatment as the main plot and days of aging as the repeated measures. Separation of means for rate of moisture loss data was conducted using LS MEANS procedure with SLICEDIFF function at $P < 0.05$. Objective and subjective color data were analyzed as a split-plot repeated measures

design with aging treatment as the whole-plot and RD time as the repeated measures. Tenderness, cooking loss, water activity, pH, trim loss, total moisture loss, yield and microbial plate counts were analyzed as a completely randomized design. In this study, chamber was considered the experimental unit. Data were analyzed using the PROC GLIMMIX procedure of SAS. All means were separated with the LS MEANS and DIFF functions with $\alpha = 0.05$.

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STUDY 1: COLOR AND LIPID STABILITY OF DRY-AGED BEEF DURING RETAIL DISPLAY

Abstract

The objective of this study was to determine color and lipid stability of steaks from dry-aged beef loins over 7 d of retail display (RD). Sixteen boneless strip loins were assigned to one of four treatments: wet-aging, dry aging at 50% relative humidity (RH), dry aging at 70% RH, or dry aging at 85% RH and aged for 42 days at 2°C. Dry aging of beef resulted in decreased lightness (L*), redness (a*), and yellowness (b*) values and increased lipid oxidation compared to wet-aged counterparts ($P < 0.05$). Dry-aged steaks had greater discoloration ($P < 0.05$) than wet-aged steaks from d 4 to d 7 of RD. Results suggest that prolonged RD of dry-aged beef has the potential to reduce color and lipid stability compared to wet aging and thus reduce display life. Color and lipid stability were not affected by RH during dry aging.

Keywords: discoloration, dry aging, meat, oxidation, shelf life

Introduction

There has been an increased interest in merchandising dry-aged steaks at the retail consumer level due to its unique flavor (Warren & Kastner, 1992; Campbell, Hunt, Levis, & Chambers, 2001). Although extended dry aging contributes to flavor development, evidence of its effects on color and lipid stability under retail display conditions is incomplete. Previous studies have reported that extended wet aging in vacuum packages negatively impacts color and lipid oxidative stability, resulting in accelerated rate of surface discoloration and oxidized off-flavor development in meat (Ribeiro et al., 2018). In addition, dry-aged beef is darker and less red compared with wet-aged beef due to lower moisture content and surface drying after aging resulting in less light reflection (Kim & Hunt, 2011; Dikeman, Obuz, Gök, Akkaya, & Stroda, 2013; Kim, Kemp, & Samuelsson, 2016). Discoloration due to metmyoglobin formation and darkening due to surface dehydration may result in economic losses since meat purchasing decisions at the retail level are mainly influenced by color (Smith, Belk, Sofos, Tatum, & Williams, 2000; Mancini & Hunt, 2005).

Studies focused on the direct comparison of dry aging to wet aging color have not been the subject of much scientific research. Further understanding of the influence of the dry aging process on meat color and lipid stability is needed to ensure dry-aged beef products can be merchandised without adverse impacts on retail display life. Therefore, this study aimed to determine color and lipid stability of steaks from dry-aged beef loins over 7 d of retail display (RD).

Materials and Methods

Sample collection

All animals used in this study were slaughtered humanely, under USDA guidelines. Sixteen USDA low Choice strip loins [Institutional Meat Purchase Specifications number 180] were collected at a commercial beef harvest facility, vacuum-packaged, and transported to the University of Nebraska Loeffel Meat Laboratory where they were immediately removed from the vacuum packages. Boneless loins were assigned to one of the four aging treatments: vacuum (WET), dry aging at 50% relative humidity (RH; RH50), dry aging at 70% RH (RH70), or dry aging at 85% RH (RH85). Strip loins were placed in each assigned dry aging chamber (1 loin per chamber) and aged for 42 days at 2°C. The dry aging chambers (86 cm length x 47.6 cm width x 33 cm height) are capable of measuring and precisely controlling relative humidity (± 1%) and air flow (± 0.015m^3/min). The chambers monitor temperature (± 0.5°C) and have built-in sensors that can continuously monitor mass loss (± 5 g). All measured data were saved on the connected computer every 15 s. A full description of this dry aging system called Agenator has been previously published by Lau, Ribeiro, Subbiah, and Calkins (2019). After aging, loins were subject to retail display (RD) for an additional 7 d. Loins assigned to wet aging were individually vacuum packaged and kept intact over 42 days at 2°C in the same cooler where the dry-aged chambers were located.

Dry-aged loins were trimmed of dried surface and subcutaneous fat, and fabricated anterior to posterior into 3 steaks: One 2.54 cm-thick steak for objective color and subjective discoloration with 7 d RD, and two 1.27 cm-thick steaks for lipid oxidation (one

steak for lipid oxidation at 0 d RD and one steak was split in half for lipid oxidation after 4 and 7 d RD).

After fabrication, steaks assigned to lipid oxidation at 0 d of RD were vacuum packaged and frozen at -80°C. Steaks assigned to 4 and 7 d of RD were placed on foam trays (21.6 x 15.9 x 2.1 cm, Styro-Tech, Denver, CO), overwrapped with oxygen-permeable film (Prime Source PSM 18 #75003815, Bunzl Processors Division, North Kansas City, MO; oxygen transmission rate = 2.25 ml/cm^2/24 hr at 23° C and 0% relative humidity; water vapor transfer rate = 496 g/m^2/24 hr at 37.8° C and 90% relative humidity), and placed under RD conditions for 7 d (continuous white fluorescence lighting at 1000 to 1800 lux; F32T8/TL741, 700 series, 32 W, Philips, USA) at 2°C. After 4 or 7 d of RD steaks were vacuum packaged and stored at -80°C. Steaks were randomly rotated daily to minimize any possible location effects within the display.

Instrumental color

Objective color was measured using the L*, a*, b* scales using a Minolta CR-400 colorimeter (Illuminant D65, 8 mm diameter aperture, 2° standard observer angle; Minolta, Osaka, Japan). The calibration process was done on a daily basis using a white ceramic tile provided by the manufacturer (Calibration Plate, Serial No. 14933058, Konica Minolta, Japan) and the D65 settings were set as follows: Y = 93.13, x = 0.3164 and y = 0.3330. Six measurements were made per steak through the overwrap film once a day at a standardized time from d 0 to 7 of RD. Readings were averaged by steak for statistical analysis. Delta E (ΔE) was calculated to measure the magnitude of difference in L*, a*, and b* color space between wet-aged steaks at d 0 of RD and dry aging treatments at d 0 of RD. Delta E was

also calculated using the initial color readings taken on day 0 of RD and the final readings taken on day 7 of RD for each individual steak from each treatment. All calculations were performed using the equations provided by the American Meat Science Association Meat Color Measurement Guidelines (Hunt *et al*., 2012).

Subjective discoloration

Six trained panelists evaluated surface discoloration daily during the 7 d of RD according to the procedure of Senaratne-Lenagala (2012). A reference guide of ten steak images ranging from 0% to 100% surface discoloration with increments of 10% were provided to panelists to ensure consistent evaluations. A percentage scale was used where 0% meant no discoloration and 100% meant complete surface discoloration.

Lipid oxidation

Steaks used for lipid oxidation were removed from the freezer, cut by hand into small cubes, frozen in liquid nitrogen, powdered in a metal cup blender (Model 51BL32, Waring Commercial, Torrington, CT), and stored at -80°C until further analysis. Thiobarbituric acid reactive substance values (TBARS) were measured at 0, 4 and 7 d of RD according to the procedure of Ahn, Olson, Jo, Chen, Wu, and Lee (1998). Five grams of powdered meat from each steak were blended with 1 mL of butylated hydroxyanisole solution (10%) and 14 mL of distilled water. Samples were homogenized using a Polytron (Kinmatica AG, Lucern, Sui) for 15 s and centrifuged (2,000 x g for 5 min). After centrifugation, 1 mL of supernatant was collected, mixed with 2 mL of thiobarbituric acid/trichloroacetic acid (TBA/TCA) solution (15% TCA and 20 mM TBA in deionized

distiller water), and placed in a water bath at 70°C for 30 min. Samples were cooled for 10 min in a water bath at 20°C and centrifuged (2,000 x g for 15 min). Two hundred microliters of supernatant were transferred to 96-well plates in duplicate. Absorbance was measured at 540 nm using a microplate spectrophotometer (Model Epoch, Biotek, Winooski, VT). Results were expressed in mg of malonaldehyde per kg of tissue.

Statistical Analysis

Objective color data was analyzed as a split-plot repeated measures design with aging treatment as the whole-plot and RD time as the repeated measures, and chamber was considered a random effect. Subjective color data was analyzed as a split-plot repeated measures design with aging treatment as the whole-plot, steak as the split-plot, and RD time as the repeated measures. Color panelists were considered a random effect when analyzing subjective discoloration. The TBARS data were analyzed as a split-plot design with aging treatment as the whole-plot, and RD time as the split-plot. In this study, chamber was considered the experimental unit. Interactions between fixed effects were tested and when significant the interactions were reported. When the interactions were not significant, the main effects of each variable were reported. Data were analyzed using the PROC GLIMMIX procedure of SAS. All means were separated with the LS MEANS and PDIFF or SLICEDIFF functions with $\alpha = 0.05$.

Results and Discussion

Instrumental color

For all three color readings, a RD effect was found ($P < 0.001$). In general, a* and b* values decreased as RD time increased, regardless of the aging treatment. The L^* values were greater at day 0 RD in comparison to all other days of RD, regardless of aging treatment (Table 1). However, no differences in L* values among days were found during 1 to 7 d of RD ($P > 0.05$). For a* values, differences were found among all days as shown in Table 1. The b* values were greater at day 0 and 1 RD, followed by day 2 and 3, while no significant difference was observed on day 4 to 7 of retail display (Table 1).

In addition to retail display effect, aging treatment also had an effect on all three color readings. Wet-aged steaks had higher L* ($P = 0.01$), a* ($P = 0.03$), and b* values ($P < 0.001$) than any other dry-aged treatment (Table 2). No differences in L*, a*, and b* values among dry-aged treatments were found ($P < 0.05$). Similar results were also observed in other studies, where dry-aged beef steaks were darker and less red compared with wet-aged steaks (Dikeman, Obuz, Gök, Akkaya, & Stroda, 2013; Kim, Kemp, & Samuelsson, 2016) due to moisture loss (Kim & Hunt, 2011). The darker color in the dry-aged loins compared to the wet-aged loins may be explained by less light reflection associated with lower moisture content.

Delta E was calculated to measure the change of color space between wet and dry-aged treatments at d 0 of RD. No differences were found among dry aging treatemtns (*P*-value = 0.83; SEM = 0.903). Delta E values for RH50, RH70, and RH85 were 6.82, 6.17, and 6.13, respectively. Larger delta E values reflect a larger change in overall color and

according to Mokrzycki and Tatol (2011) clear difference in color is noticed when delta E is greater than 3.5. Therefore, our results suggest that there is a detectable difference in overall color between wet and dry aging treatments at the beginning of the RD. Delta E was also calculated to measure the change of color space from the beginning to the end of RD. Although no significant differences were found for delta E values among treatments (P = 0.39; Table 2), our results suggest that color differences can be observed as retail display progressed from d 0 to d 7.

Detection of color by the human eye is influenced by light reflection. When light strikes meat, it can be absorbed, reflected, or scattered. Light must reflect off the object being viewed and return to the eye in order to be detected. The reflected light is perceived by the eye, captured and transmitted to the brain, where color is interpreted. Therefore, the wavelengths of light that are absorbed by the meat are not perceptible to the eye (AMSA, 2012). Dry aging results in shrinkage during cooler storage as moisture is lost over time. Both shrinkages in height and ribeye area have been observed (Calkins & Ribeiro, 2019). Shrinkage of myofibrils increases the space available for light absorption, thus decreasing the light scattering power of the meat and contributing to darkening of color intensity.

Holman, van de Ven, Mao, Coombs, and Hopkins (2017) reported the relationship between a* and consumer acceptance of beef color. Beef color was considered acceptable (with 95% acceptance) when a* values were equal to or above 14.5. In this study, the days required for each sample to reach an a* value of 14.5 was calculated by interpolating between the two days with values above and below 14.5. The 14.5 color threshold value was met by steaks from the dry aging treatments RH50, RH70, and RH85 after 2.4, 2.7, and 2 days of RD, respectively. Steaks from the WET group were statistically different

from steaks of dry aging group and reached the color threshold after 5.2 days of RD ($P \leq 0.05$; SEM = 1.11). A similar pattern was found in our discoloration data using the 20% surface discoloration threshold. Perhaps, a* values can be used as an objective and practical tool by meat retailers to predict meat discoloration and accurately predict color display life.

Subjective Color (Discoloration)

A 2-way interaction between treatment and RD for discoloration was observed ($P = 0.03$; Figure 1). No differences were found among treatments over the first 2 d of RD ($P > 0.05$). Samples began to diverge on day 3 of RD. Dry-aged steaks had greater discoloration scores ($P < 0.05$) than wet-aged steaks from day 4 onward. No differences in discoloration scores among dry-aged treatments were found.

A 50% decline in purchasing decisions with 20% surface discoloration on RD beef has been reported by Hood and Riordan (1973). Even at lower levels of discoloration, consumers begin to discriminate against discolored meat and will select non-discolored products if both packages are viewed in retail display. According to Smith, Belk, Sofos, Tatum, and Williams (2000), nearly 15% of retail beef is discounted in price due to surface discoloration which corresponds to annual revenue losses of $1 billion.

The same strategy used to calculate the days required for each sample to reach an a* value of 14.5 was used to calculate the days required for each sample to reach 20% discoloration. If the value of 20% was never reached then a value of 7.0 d was used. The 20% discoloration threshold was met by steaks from the dry aging treatments RH50, RH70, and RH85 after 4.1, 3.9, and 4.7 days of RD, respectively. Steaks from the WET group reached the color threshold after 6.1 days of RD ($P = 0.06$; SEM = 0.55). The faster

discoloration observed for dry-aged steaks when compared to wet-aged steaks could be attributed to the extent of exposure to oxygen and depletion of reducing compounds due to the number of times the meat went through the color cycle.

Discoloration is caused by an accumulation of metmyoglobin on the meat surface due to the oxidation of myoglobin. Muscle ability to convert metmyoglobin (ferric state) to reduced ferrous state through metmyoglobin reducing activity (MRA) is limited and is continually depleted as time postmortem progresses (Mancini & Hunt, 2005). Reduction of metmyoglobin is crucial to meat color life and greatly depends on muscle's oxygen scavenging enzymes, reducing enzyme systems, and the nicotinamide adenine dinucleotide hydride (NADH) pool (Renerre, 1990; Mancini & Hunt, 2005). Once NADH is depleted, MRA is limited and accumulation of metmyoglobin on the meat surface occurs (Kim & Hunt, 2011).

Lipid oxidation

There was an RD effect on TBARS values ($P < 0.001$). Greater TBARS values were seen as RD progressed from d 0 to d 4 and d 7, regardless of the aging treatment (Table 3). A treatment effect was observed for lipid oxidation ($P = 0.03$). Dry-aged steaks had higher TBARS values than wet-aged steaks. No differences in TBARS values among dry aging treatments were found (Table 3).

Lipid oxidation is a three-step chemical process responsible for the deterioration of meat quality (Descalzo et al., 2005; Pradhan, Rhee, & Hernández, 2000). The first step, initiation, begins with the removal of hydrogen from a carbon chain of fatty acid (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). After initiation, a chain reaction is

triggered where the free radicals react with oxygen to form peroxy radicals, which then react with unsaturated lipids, forming hydroperoxides. This step is known as propagation (Wong, 1989). Finally, in the termination step, radicals react among one other, resulting in radical combinations ultimately terminating in non-radical products (Wong, 1989).

In live muscle, enzymes are capable of eliminating free radicals formed during oxidation through the formation of water (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). However, after slaughter, the balance of prooxidants and antioxidants of living muscle is disrupted, favoring oxidation (Gray, Gomma, & Buckley, 1996; Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). Consequently, muscle cells become damaged and overall meat quality can be affected (Kanner, 1994). The larger detriment of lipid oxidation is perceived in meat color and flavor (Greene, 1969). Lipid and myoglobin oxidation in meat often appear to be linked and the oxidation of one leads to the formation of chemical species that can exacerbate oxidation of the other (Faustman, Sun, Mancini, & Suman, 2010). This is in agreement with our study, where greater TBARS values and discoloration scores were observed for dry-aged treatments in comparison with wet-aged counterparts.

The rate of lipid oxidation depends on several conditions including duration of storage and packaging. The importance of packaging on lipid oxidation has been well described in the literature. Faustman, Sun, Mancini, and Suman (2010) indicated that the presence of oxygen catalyzes the formation of primary oxidative products that propagate to form secondary oxidative products that continue the oxidative chain reaction. Therefore, the elimination of oxygen from meat packaging is a critical factor preventing lipid oxidation during storage. As expected, lipid oxidation was favored by long time storage

under aerobic conditions (dry aging) and increased TBARS values were observed for dry-aged treatments in comparison with wet-aged counterparts.

Lipid oxidation increases beef flavor deterioration during aging, and this deterioration can be closely related to TBARS. Campo, Nute, Hughes, Enser, Wood, and Richardson (2006) considered TBARS values exceeding 2.28 mg of malonaldehyde per kg as unacceptable for beef because at this level rancid flavor overpowers beef flavor. Conversely, Hughes, McPhail, Kearney, Clarke, and Warner (2015) considered levels between 2.60 and 3.11 mg of malonaldehyde per kg as acceptable to consumers In this study, the limiting threshold of 2 mg of malonaldehyde per kg was met by steaks from RH50 and RH85 dry-aged treatments at day 4 of RD, suggesting that dry-aged steaks aged for 42 days can be merchandised in the retail level for 3 d without detrimental effects on lipid oxidation.

Conclusion

Dry aging of beef resulted in decreased lightness and redness values and increased lipid oxidation compared to wet aging. With prolonged RD, dry aging of beef has the potential to reduce color and lipid stability compared to wet aging and thus reduce display life. In this study, the 14.5 color threshold for a* value was met by steaks from the RH70 and RH85 treatments after 2.7 days of RD, and after 2.4 days for the RH50 treatments, while the 20% discoloration threshold was met by steaks from the RH50 after 4.1 days of RD, and after 3.9 and 4.7 days for the RH70 and RH85 treatments, respectively. Dry-aged steaks from RH50 and RH85 treatments overtook the consumer thresholds for acceptable levels of lipid oxidation at d 4 of RD. Results suggest that dry-aged steaks aged for 42 days

can be merchandised at retail level for 2 days without detrimental effects on color and lipid oxidation.

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Table 1. Objective lightness (L*), redness (a*) and yellowness (b*) values of strip loin steaks (*Longissimus lumborum)* wet aged or dry aged for 42 days at 50, 70 or 85% relative humidity through 7 days of retail display.

Days on retail display	L*	a*	b*
0	46.32[a]	17.94[a]	7.93[a]
1	44.97[b]	16.99[b]	7.70[a]
2	44.98[b]	15.46[c]	6.97[b]
3	43.94[b]	14.35[d]	6.83[b]
4	44.01[b]	13.36[e]	6.43[c]
5	44.07[b]	12.12[f]	6.35[c]
6	44.23[b]	10.82[g]	6.30[c]
7	43.98[b]	9.28[h]	6.32[c]
SEM	0.6844	0.6516	0.1560
P-value	< 0.001	< 0.001	< 0.001

[a-h] Means in the same column with different superscripts differ ($P < 0.001$).

Table 2. Color measurements and lipid oxidation value (TBARS; mg malonaldehyde /kg of meat) of strip loins steaks (*Longissimus lumborum*) wet aged (WET) or dry aged for 42 days at 50, 70 or 85% relative humidity (RH).

Quality trait	Treatments				SEM	*P*-value
	WET	RH50%	RH70%	RH85%		
L*	46.40[a]	43.64[b]	43.74[b]	43.87[b]	0.790	0.01
a*	16.79[a]	12.51[b]	12.87[b]	12.15[b]	1.599	0.03
b*	8.22[a]	6.35[b]	6.44[b]	6.42[b]	0.306	< 0.001
ΔE	11.22	9.27	7.31	9.91	1.563	0.39
TBARS	2.03[a]	2.97[b]	2.81[b]	3.17[b]	0.355	0.03

[a,b] Means in the same row with different superscripts differ ($P < 0.05$).

Table 3. Lipid oxidation value (TBARS; mg malonaldehyde /kg of meat) of strip loin steaks (*Longissimus lumborum)* wet or dry aged for 42 days at 50, 70 or 85% relative humidity with 0, 4 and 7 days retail display.

Days on retail display	TBARS	SEM	*P*-value
0	1.01[a]	0.39	< 0.001
4	2.22[b]		
7	3.53[c]		

[a-c] Means in the same column with different superscripts differ ($P < 0.001$).

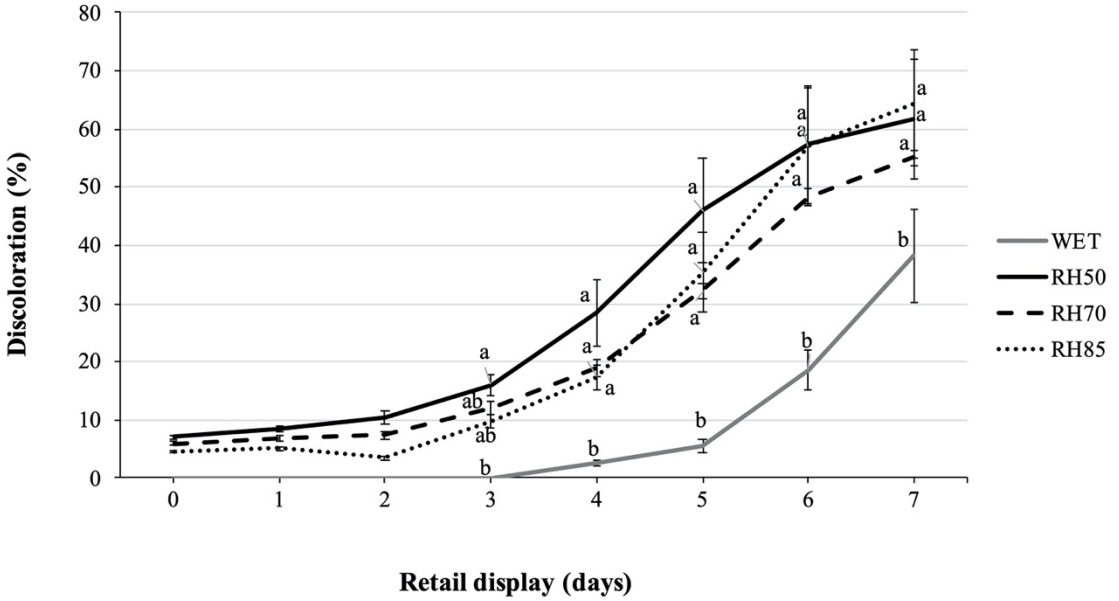

Figure 1. Discoloration scores (%) of strip loins steaks wet or dry aged for 42 days at 50, 70 or 85% relative humidity (RH) through 7 days of retail display. [a,b] Means in the day with different superscripts differ ($P < 0.05$).

STUDY 2: EFFECTS OF RELATIVE HUMIDITY ON DRY-AGED BEEF QUALITY

Abstract

This study was conducted to evaluate the effects of relative humidity (RH) on moisture loss and flavor in dry-aged beef. Sixteen strip loins were assigned to one of the four aging treatments: vacuum (WET), dry-aging at 50% RH, dry-aging at 70% RH, or dry-aging at 85% RH and aged for 42 days at 2°C. Loins were evaluated for evaporation loss, trim loss, tenderness, sensory, and microbiological characteristics. Results suggest that lower RH results in accelerated moisture loss at the beginning of the aging process without significantly affecting the total amount of moisture loss. Yield, tenderness, and microbial counts were not affected by RH levels. *Pseudomonadales* dominates the aerobically dry-aged loins while *Enterobacteriales* was the most abundant in the wet-aged samples. Dry-aged samples had increased content of free amino acids in the beef juice compared to the wet-aged counterpart. Dry-aging at 50% RH tended to associate with more desirable flavor notes.

Keywords: dry-aging, flavor, meat, moisture loss, sensory, palatability.

Introduction

There has been a growing interest in dry-aged beef due to its enhanced and unique flavor. The altered flavor characteristics of dry-aged beef are often attributed to two primary factors: concentration and creation of flavor (Savell & Gehring, 2018). The concentration of flavor compounds occurs as a consequence of moisture loss through drying, which makes the flavor more intense. The creation of flavor is more complex. It seems that some of the flavor changes in dry-aged beef are the result of the concentration of flavor precursors (especially amino acids) that can react with compounds found in the Maillard reaction to produce meat odorants (Kim, Kemp, & Samuelsson, 2016; Kim *et al*., 2018; Lee, Yoon, Kim, Oh, Yoon, & Jo, 2019). In addition, an increase in glutamate-based compounds, which contribute to umami/savory flavor, has also been observed with dry aging (Kim, Kemp, & Samuelsson, 2016).

Intriguingly, not all dry aging studies have found improved flavor for dry-aged beef (Oreskovich, McKeith, Carr, & Bechtel, 1988; Parrish, Boles, Rust, & Olson, 1991; Smith *et al*., 2008; Dikeman, Obuz, Gök, Akkaya, & Stroda, 2013; Smith, Harris, Griffin, Miller, Kerth, & Savell, 2014; Kim, Meyers, Kim, Liceaga, & Lemenager, 2017; Hulànková, Kameníka, Salákováa, Závodskýa, & Borilova, 2018) and conflicting results regarding the impacts of dry aging on beef palatability have been reported in the scientific literature (Warren & Kastner, 1992; Campbell, Hunt, Levis, & Chambers, 2001; Ahnström, Seyfert, Hunt, & Johnson, 2006; Li, Babol, Wallby, & Lundström, 2013; Stenström, Li, Hunt, & Lundströma, 2014; O'Quinn *et al*., 2016; Kim, Kemp, & Samuelsson, 2016; Lepper-Blilie, Berg, Buchanan, & Berg, 2016; Kim, Meyers, Kim, Liceaga, & Lemenager, 2017; Berger

et al., 2018). These conflicting results may be associated with inconsistent environmental conditions applied during the dry-aging process.

Environmental conditions including temperature, airflow, length of aging, and relative humidity (RH) are the primary factors to consider when dry aging because they are related to the development of flavor notes, product shrinkage, shelf-life, microbial spoilage, and economics (Savell, 2008). However, dry aging studies in the literature rarely report all of the environmental conditions tested, and those that do only test specific conditions or limited ranges.

Although RH has been repeatedly cited as one of the most important parameters during dry aging, the ideal RH that should be used for dry aging is still unclear. Relative humidity levels applied during dry-aging studies vary extremely in the literature. Dry aging literature has reported RH parameters from 49% to 98% (Parrish, Boles, Rust, & Olson, 1991; Warren & Kastner, 1992; Campbell, Hunt, Levis, & Chambers, 2001; Ahnström, Seyfert, Hunt, & Johnson, 2006; Smith *et al*., 2008; DeGeer, Hunt, Bratcher, Crozier-Dodson, Johnson, & Stika, 2009; Li, Babol, Wallby, & Lundström, 2013; Smith, Harris, Griffin, Miller, Kerth, & Savell, 2014; Lepper-Blilie, Berg, Buchanan, & Berg, 2016; Kim, Kemp, & Samuelsson, 2016). Most of these studies have been conducted within a single meat cooler and/or without the accurate RH measurement and control required to provide a clear picture of the drying process.

There is a commonly held belief that if RH is too low, excess product shrinkage and crust formation will occur due to rapid evaporation of water from the meat surface and that high RH causes slow drying and prevents crust formation on the surface of the meat. If true, meat dried with a low RH should lose less moisture than meat dried at a higher RH

due to formation of hard surface crust on the former. The perception is that surface over-drying should hinder moisture loss. Conversely, if RH is too high, spoilage bacteria can grow and result in off-flavors (Savell, 2008). All of these problems occur because of improper handling of moisture. Thus, it seems that proper dry aging entails a controlled rate of moisture loss.

A critical question is the influence of RH on moisture loss over time in dry-aged beef. The working hypothesis is that rapid drying due to low RH at the beginning of the dry aging process will create a hard crust on the meat surface that will reduce moisture release over time. As a result, the enhanced flavor characteristic of dry-aged beef will be affected negatively. This hypothetical phenomenon can be likened to "case hardening" where the creation of a hard crust limits moisture loss during the drying process in sausages (Feiner, 2006).

Although the idea of enhanced flavor has been extensively used to promote dry-aged beef, a better understanding of ideal dry aging conditions could substantiate these claims. Therefore, the objective of this research was to understand the effects of RH on moisture loss over time in dry-aged beef. More specifically, this study aimed to answer the following questions: 1) How does moisture loss occur under different RH conditions? 2) Does early hard crust formation affect moisture loss over time? 3) What is the effect of RH on trim loss and yield? 4) Could RH affect dry-aged beef flavor and tenderness? 5) What is the effect of RH on microbial growth and diversity of microflora?

Materials and Methods

Sample Collection and Fabrication

At the time of grading, 16 USDA low Choice strip loins were collected, vacuum-packaged, and transported to the University of Nebraska Loeffel Meat Laboratory. Subsequently, loins were randomly assigned to one of four aging treatments (n = 4 loins/treatment): vacuum (WET), dry-aging at 50% RH (RH50), dry-aging at 70% RH (RH70), or dry-aging at 85% RH (RH85). Initially, three cores (2.54-cm diameter) were removed from the anterior surface of lean tissue of each loin for microbiology analysis. Once the initial mass was measured, each strip loin was placed in a dry aging chamber and aged for 42 d at 2°C and 0.8 m^3/min air flow. Loins assigned to wet aging were maintained, individually, in the original vacuum packaging from the plant and kept intact over 42 days at 2°C in the same cooler where the dry-aged chambers were located.

After aging, loins were weighed and immediately trimmed of subcutaneous fat and dehydrated surface, reweighed, and fabricated from anterior to posterior into steaks [one steak for water activity (1.27 cm thick); one steak for pH (1.27 cm thick); and nine steaks (2.54 cm thick) - one for Warner-Bratzler shear force (WBSF) measurements, and eight for sensory analysis].

After fabrication, steaks assigned to pH and water activity measurements were vacuum packaged and frozen at -80°C. Steaks assigned to sensory analysis and WBSF were vacuum packaged and frozen at -20°C.

Agenator – A computerized dry aging system

To address the flavor inconsistency reported in the literature, we designed and built a computerized dry aging system (the Agenator) that is capable of measuring and precisely controlling relative humidity (± 1%) and air flow (± 0.015m^3/min). The dry aging chambers (86 cm length x 47.6 cm width x 33 cm height) have weighting scales with load cells that can continuously monitor mass loss (± 5 g) and temperature (± 0.5°C). All measured data were saved on a connected computer in user-defined intervals, with a minimum of 1 s. This computerized dry aging system allowed us to conduct dry aging studies with proper replication. A full description of the Agenator has been previously published by Lau, Ribeiro, Subbiah, and Calkins (2019). In this study, the measured data were acquired in 5 min intervals.

pH

Steaks from day 42 of aging were removed from the freezer (-80°C) and thawed at 4°C overnight. Steaks were knife-cut into small cubes, frozen in liquid nitrogen, and powdered in a metal cup blender (Model 51BL32, Waring Commercial, Torrington, CT). Duplicate 10 g samples along with 90 mL of distilled deionized water were placed into 250 mL plastic beakers. The contents were stirred with a magnetic stir bar and stir plate to ensure constant mixing during the measurement process. The pH was measured using a pH meter (Orion 410Aplus: ThermoFisher Scientific; Waltham, MA) that was calibrated using 4.0, 7.0, and 10.0 standards. The pH measurements of the duplicates were averaged for statistical purposes.

Aging loss (water, trim, and combined losses)

The mass of each strip loin was recorded before and after aging to determine aging loss and yield. After aging, wet-aged beef loins were removed from the vacuum bags, patted dry with paper towels and reweighed. The percentage of moisture loss during wet aging was calculated using the following formula: moisture loss (%) = (initial mass – post aged mass)/initial mass × 100. The percentage daily moisture loss for dry-aged loins was calculated as the difference between the prior day's mass and the current mass divided by the prior day's mass. Since mass measurements were acquired continuously by the Agenator system, the mass readings used for these calculations were extracted around the same time every day. The percentage total moisture loss for dry-aged loins was calculated as the difference between initial mass and final mass divided by the initial mass and then multiplied by 100. The dry-aged loins were then further processed by trimming dried surfaces and non-edible fat, and reweighed to calculate the final saleable yield (%) after aging and trimming.

Water activity (a_w)

Steaks from day 42 of aging were removed from the freezer (-80°C) and thawed at 4°C overnight. Steaks were knife-cut into three horizontal strips of 1 cm each, starting from the dorsal region of the steak (just below the subcutaneous fat) to the ventral region. The strip just below the subcutaneous fat was identified as dorsal, the middle strip was identified as central, and the bottom strip was identified as ventral. Each strip was chopped in an Oster food chopper (Model FPSTMC3321, Sunbeam Products Inc, Boca Raton, FL) for 60 s. Water activity was measured in triplicate using an water activity meter (AquaLab

4TE, Decagon Devices, Inc., Pullman, WA) at 25°C. Chopped samples were placed in Aqualab water activity cups (Decagon Devices, Inc., Pullman, WA) with enough sample to cover the entire bottom of the cup. The a_W measurements of the triplicates were averaged for statistical purposes.

Tenderness determination

Steaks for tenderness measurements were removed from the freezer (-20°C) and thawed at 4°C overnight. Steak internal temperatures and mass were recorded before and after cooking. The temperature was recorded for each steak 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). The thermocouples were inserted into the geometric center of each steak with large needles. All steaks were cooked to a target temperature of 71°C on a Belt Grill (TBG60-V3 MagiGril, MagiKitch'n Inc., Quakertown, PA). Belt grill specifications were as follows: preheat = 149°C, top heat = 163°C, bottom heat = 163°C, height of gap = 2.16 cm, and cook time was approximately 6 min. Immediately after cooking, internal temperature and mass were recorded. The cooking loss was calculated using the difference between the raw mass and final cooked mass divided by the initial mass and multiplied with 100. Steaks were individually bagged and stored overnight at 2°C for WBSF analysis. The following day, six 1.27 cm diameter cores were removed with a drill press parallel to the orientation of the muscle fibers. Cores were measured using a Food Texture Analyzer (TMS-Pro, Food Technology Corp., Sterling, VA.) with a Warner- Bratzler blade. Peak WBSF values from each core were averaged by steak for statistical analysis.

Microbiology Sample Collection

Microbial analysis of loins was conducted on days 0 and 42. Four individual loins were selected as representative samples on day 0 for a baseline understanding of microflora present on starting material. On day 42, four loins each from 50% RH, 70% RH, 85% RH and wet-aged treatments were sampled. Three 2.54 cm diameter surface cores of the lean surface were aseptically taken from loins and transferred into WhirlPak bags designated for each loin from each treatment as mentioned above (Nasco, Fort Atkinson, WI). Thirty-five mL of sterile BBL Peptone water (Becton, Dickinson and Company, 1057 Franklin Lakes, NJ) was added to sample bags and samples were homogenized for 2 min in a stomacher (bioMerieux Inc., Durham NC). Two 1.75mL samples were taken directly from the WhirlPak bag and stored in sterile 2 mL microcentrifuge tubes at -20°C until DNA extraction.

Microbial Plating Methods

Sample cores were subjected to microbial plating methods in duplicate. Fifty µl of 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 (PSY) plate counts. DeMan Rogosa Sharpe agar (Thermo Fisher Scientific, Waltham, MA) was used to enumerate lactic acid bacteria (LAB). Plates for APC, AnPC, and LAB were incubated at 37°C and counted at 48 hours. Plates for AnPC were held in an 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). Plates for PSY were incubated at 4°C and counted after 10 days. Plate counts were determined and converted to $\log_{10}$ CFU/cm^2.

Microbial Ecology Analysis

Bacterial communities were investigated for each sample using the MiSeq Illumina Sequencing Platform, targeting the bacterial-specific 16s rRNA gene as described by Kozich, Westcott, Baxter, Highlander & Schloss (2013). The DNA was extracted from samples using DNA QuickExtract Solution 1.0 (Epicentre, Madison, WI). Obtained DNA was amplified via the polymerase chain reaction (PCR) in a solution that contained 1X Terra PCR Direct Buffer (Clontech Laboratories Inc., Mountain View, CA), 0.75 U Terra PCR Direct Polymerase Mix (Clontech Laboratories Inc.), approximately 1-5 ng of extracted DNA, and 0.5 μM barcoded universal primers. The PCR reaction was performed alongside negative controls in a Veriti 96 well thermocycler (Thermo Fisher Scientific, Walther, MA), with the following PCR cycle: initial denaturation at 98°C for 3 min, followed by 30 cycles of 98°C for 30 s, 58°C for 30 s, and 68°C for 45 s, and a final extension of 68°C for 4 min.

The PCR products were then analyzed on a 1.5% agarose gel to ensure amplification occurred successfully, without contamination of negative controls. Samples were then normalized using the Norgen NGS Normalization 96-Well Kit (Norgen Biotek Corp., Thorold, ON, Canada) according to manufacturer protocol. The pooled sample was then placed in 50ºC water bath to remove excess ethanol from the normalization kit and run through a spin column. The DNA was found to be lacking in concentration, so samples were subjected to additional PCR using a 5-cycle rendition of the previously described

protocol. Products were then separated on a 2% agarose gel, which yielded two bands. The band corresponding to the bp size of the 16s rRNA V4 subregion was removed with a scalpel and DNA was recovered using the MinElute PCR Purification kit (Qiagen Inc., Germantown, MD). Concentration and bp size of the 16S rRNA libraries were determined using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Library concentration was confirmed with a DeNovix QFX Fluorometer and the Denovix dsDNA High Sensitivity reagent kit (Denovix Inc., Wilmington, DE). The 16S libraries were sequenced using the Illumina MiSeq platform (Illumina, 2457 Inc., San Diego, CA) using the V4 500 cycle kit.

Sequence Processing

An average of 21,754 reads was obtained per sample. Obtained sequence reads were processed with R (version 3.6.0, R Core Team, 2013) and Mothur (Version 1.42.1, Schloss *et al*., 2009). The DADA2 pipeline (Callahan, McMurdie, Rosen, Han, Johnson, & Holmes, 2016a) functioned to prepare sequences and generate amplicon sequence variants (ASVs). Raw reads were quality checked, filtered, trimmed, and merged. Chimeras and non-bacterial sequences were removed. Samples with low biomass were removed, as previously performed in meats studies, due to inherent low cell counts on starting materials (Weinroth *et al*., 2019). Eighteen of the original 20 samples (4 samples from d 0 and 16 from d 42 being 4 from each aging treatment) were retained. The ASVs were then assigned and merged with a phylogenetic tree generated from Mothur and metadata file into a phyloseq object (McMurdie & Holmes, 2013). Once ASVs were determined, taxa were assigned based on the Silva (V132, Quast *et al*., 2012) database. The “decontam” package (Davis,

Proctor, Holmes, Relman, & Callahan, 2018) was utilized on the dataset to remove possible contaminants. Phyloseq was then utilized to determine the relative abundance of bacterial taxa (Callahan, Sankaran, Fukuyama, McMurdie, & Holmes, 2016b).

Sensory Evaluation

Triangle tests were conducted in two sessions with 32 consumers each. In the first session, panelists were served samples from the RH50% and RH85% treatments. In the second session, panelists were served samples from the WET and RH70% treatments. Each panelist received three 3-digit blind coded samples (1.3 cm x 1.3 cm x 2.54 cm thickness) avoiding the edges and fat kernels of the steaks. Two of these samples were identical and one was different. Panelists were asked to circle the number of the sample that was different in flavor.

Sensory descriptive analysis procedures were performed at Texas A&M University (College Station, Texas) as described by Wall, Kerth, Miller, and Alvarado (2019). Six panelists were trained to scale ten basic flavors and five textural attributes from the beef lexicon on a 16-point intensity scale (where 0 = none and 15 = extremely intense). There were two sample testing sessions. At the beginning of each day, panelists were served two warm-up samples, which were discussed verbally to ensure proper scaling and precision of scoring. For sample testing, each panelist was served two cubes (1.3 cm x 1.3 cm x 2.54 cm) assigned a 3-digit blind code, avoiding the edges and fat kernels of the steak, in a plastic cup while in a booth under red lighting. Panelists were given unsalted crackers and double distilled, deionized water for palette cleansing.

Amino acid determination

Steaks from day 42 of aging were removed from the freezer (-20°C) and thawed at 4°C overnight. Steaks were cooked to an internal temperature of 35°C and turned over until they reached a target temperature of 70°C on an electric indoor grill (Hamilton Beach-31605A, Hamilton Beach Brands, Glen Allen, VA). After cooking, steaks were placed on a plate and the juice that seeped onto the plate was captured using a Pasteur pipet. One mL of juice was transferred into a 2mL Eppendorf tube for amino acid determination. Juice samples were kept at -80°C overnight and shipped on dry ice to Mississippi State University on the following day.

The steak was then bagged (PB-90-C, .85 mil., 15.24x7.62x38.1 cm) and stored overnight at 2°C. The following day, steaks were trimmed of all subcutaneous fat, knife-cut into small cubes, frozen in liquid nitrogen, powdered in a metal cup blender (Model 51BL32, Waring Commercial, Torrington, CT), and bagged (PB-90-C, .85 mil., 15.24x7.62x38.1 cm). Frozen samples were shipped to Mississippi State University overnight.

Amino acids were extracted from 1 g of sample in 5 mL of distilled water. Amino acid derivatives were prepared by using the EZfaast Amino Acid kit (Phenomenex® Inc., Torrance, CA, US), following the propyl chloroformate derivatization procedure developed by Kaspar, Dettmer, Gronwald, and Oefner (2008). The extracted amino acid solution was combined with an internal standard solution (200 µM of norvaline) and deproteinated through solid-phase extraction. The amino acids were then reacted with propyl chloroformate in chloroform, sodium hydroxide, and n-propanol. The derivatives were extracted in isooctane, evaporated, reconstituted in isooctane/chloroform mixture

(4/1, v/v), and transferred to a 2-mL amber glass vial with a fixed insert (Agilent Technologies, Santa Clara, CA) for gas chromatography-mass spectrometry (GC-MS) determination. Amino acid derivatives were injected into an inlet of an Agilent 7890A GC System coupled to an Agilent 5975C inert XL MSD with triple-axis mass detector, an Agilent 7693 Series Autosampler, and a capillary column (Zebron™ EZ-AAA 10 m × 0.25 mm; Phenomenex®, Santa Clara, CA). The inlet was operated at 250°C and 1:15 split ratio. The helium carrier gas was at a 1 mL/min constant flow rate. The temperatures of the transfer line, ion source, and quadrupole were 310, 240, and 180°C, respectively. The oven was programmed initially at 110°C and ramped up to 320°C within 11 min. The solvent delay was 1.30 min. The MS was operated in a SIM (selected ion monitoring) mode and target and qualitative ions were selected according to the manufacturer's recommendation. Amino acids were identified by retention time and target and qualitative ions and quantified by an internal calibration method using authentic standards provided with the kits. The amino acid concentration was expressed as millimole per kg of meat (mmol/kg). The meat juice was processed by being directly combined with the internal standard solution and the amino acid concentration was expressed as millimole per liter of juice (mM).

Statistical Analysis

Rate of moisture loss was analyzed as a split-plot design with aging treatment as the main plot and days of aging as the repeated measures. Separation of means for the rate of moisture loss data was conducted using LS MEANS procedure with SLICEDIFF function at $P < 0.05$. Tenderness, water activity, pH, trim loss, total moisture loss, yield, free amino acids, and microbial plate counts were analyzed as a completely randomized

design. Principal component analysis was applied for ten basic flavors and five textural attributes in wet and dry-aged treatments. In this study, the chamber was considered the experimental unit. Data were analyzed using the PROC GLIMMIX procedure of SAS. All means were separated with the LS MEANS and DIFF functions with $\alpha = 0.05$.

Results and Discussion

pH

Neither the aging method (dry or wet) nor the RH level (50, 70, or 85%) had an effect on pH of strip loins aged for 42 days ($P > 0.05$; Table 1). Similar results were reported by Stenström, Li, Hunt, and Lundströma (2014) who found no significant differences in pH between dry- and wet-aged beef samples. Berger *et al*. (2018) also found no differences in the final pH between dry and wet-aged samples.

A few studies are suggesting that changing microbial composition on the dried crust surface of dry-aged beef exerts an influence on product quality. Lee, Yoon, Kim, Oh, Yoon, and Jo (2019) found that different airflow velocities changed microbial composition on the surface of dry-aged beef, resulting in significant changes in pH and flavors compounds. They suggested that the presence of *Pilaira anomala* and *Debaryomyces hansenii* on the surface crust of dry-aged beef may affect proteolytic activity, inducing an increase in pH due to the production of amine/ammonia. Conversely, Kim, Kemp, and Samuelsson (2016) reported minimal differences in pH when comparing different aging types (dry or wet) and processing regimes (different temperatures, airflow velocities, and relative humidity

levels). This observation is in agreement with our results, as no differences in final pH among RH levels were found.

Aging loss (moisture, trim, and combined losses)

Dry-aged samples had higher total moisture loss, trim loss, and lower saleable yield than wet-aged counterparts due to surface dehydration and the amount of trimming required to remove the dried exterior surface from these products ($P < 0.05$; Table 2). However, there were no differences among RH treatments for total moisture loss (Figure 1), trim loss, and saleable yield ($P > 0.05$; Table 2).

There was a RH treatment by day of aging interaction for the rate of moisture loss ($P < 0.001$; Figure 2). A faster rate of moisture loss was found for RH50 when compared to RH85 on the first day of dry aging (4.31% versus 2.30%), while RH70 was intermediate (2.92%; $P < 0.05$). On day 2, RH50 and RH70 had higher rates of moisture loss (1.95% and 1.88%, respectively) than RH85 (1.55%; $P < 0.05$). Samples began to converge on day 3 of aging (1.44%, 1.34% and 1.26% for RH50, RH70, and RH85, respectively). From day 4 onward, no differences in the rate of moisture loss among high, intermediate and low RH levels were found ($P > 0.05$; Figure 2).

Water is the primary component of fresh beef (around 75%) and beef loses a substantial amount of water during the dry-aging process. Removal of water from meat being dry aged proceeds in two steps. First, water is transported from the interior to the meat surface. Different mechanisms exist for water movement within the meat, but diffusion and capillary flow prevail in foods (Lewicki, 2004). Second, water is evaporated from the surface of the meat and is transferred as a vapor to the surrounding air. This

process is known as convective mass transfer. The transport of water in dry-aged beef is dependent upon two mass transfer resistances: internal and external (Lewicki, 2004). The internal mass transfer resistance depends on the temperature of the meat and on the effective water diffusion coefficient (diffusion and capillary flow). The external mass transfer resistance is responsible for the convective mass transfer. The relationship between both external and internal mass transfer resistances affects the rate of drying. If the external resistance is higher than or equal to the internal resistance, the flux of water reaching the surface of the meat is constant and drying proceeds at a constant rate. If the internal resistance is higher than the external, less water is transported to the surface of the meat (Lewicki, 2004). Our results indicate that the flux of evaporated water in dry-aged beef decreases with time. Moisture diffusion within the meat during the final stages of dry aging is more difficult than at the beginning of the dry aging process, thus drying proceeds at a decreased rate. Therefore, the internal mass transfer resistance determines the rate of drying, which is not affect by RH. Instead, RH only affected convective mass transfer at the beginning of the dry aging process. In summary, moisture loss is affected by convection (RH) during the early stage of dry aging, causing a crust to form as the surface of beef dries out. Once the meat surface has been dried off and the hard crust is formed, the rate of evaporation of water will be driven mainly by diffusion rate.

There is a common belief in the meat industry that rapid drying creates a hard crust on the meat surface, which would act as a protective barrier against moisture loss, holding moisture inside the product. In sausage, this phenomenon is called case hardening (Feiner, 2006). Once case hardening occurs, the hypothesis suggests moisture loss from the surface is essentially suspended. If this hypothesis is correct, in the case of dry aging beef, drying

at low relative humidity would be recommended to get case hardening and avoid excessive moisture loss. In this study, however, no case hardening effects occurred during dry aging, even when RH was kept very low (50%). Instead, the results suggest that lower RH resulted in more rapid moisture loss only at the beginning of the aging process without significantly affecting the total amount of moisture loss at the end of the aging process.

Water activity (a_w)

Steaks from dry-aging treatments had lower a_w values ($P < 0.001$; Table 1) than steaks from the wet-aged group. No differences in a_w values among RH treatments were found. There was a location effect for a_w values. Samples from the ventral region of the steak had lower a_W values than samples from the central and dorsal regions ($P < 0.001$; Table 3). During the dry aging process, the surface of the beef dries out causing a crust to form and creating a moisture gradient between the meat surface and the inner region. According to Fick's first law of diffusion, the flow goes from regions of high concentration to regions of low concentration, with a magnitude that is proportional to the concentration gradient (Tyrrell, 1964). Once the crust is formed, water is transferred from the interior to the meat surface and is subsequently evaporated to the surrounding environment (Lewicki, 2004).

The higher a_w values found for samples from the dorsal region are likely due to the protective role of fat against moisture loss. Samples from the dorsal region (Figure 3) were protected by the subcutaneous fat layer, while samples from the ventral region had no fat protection. Perhaps the moisture gradient across the steak results in flavor variation within

a steak, a potential consequence of the concentration of flavor compounds. This possible phenomenon has yet to be studied.

Tenderness determination and cooking loss

Neither aging method (wet or dry) nor RH level (50, 70 or 85%) had an effect on WBSF (P = 0.66; Table 4). These results were expected as the mechanism of beef tenderization is independent of atmospheric oxygen. Moreover, tenderness differences are minimized after prolonged aging. In this project, meat was aged for 42 days, which may explain the similarity in shear force values among treatments found in this project. Dry aging resulted in lower cooking loss when compared to wet aging, regardless of the RH level applied during drying ($P < 0.01$; Table 4). However, no differences were found in cooking loss across RH treatments ($P > 0.05$; Table 4).

For the most part, dry aging studies have reported no significant differences for shear force values between wet and dry-aged beef (Parrish, Boles, Rust, & Olson, 1991; Sitz, Calkins, Feuz, Umberger, & Eskridge, 2006; Smith *et al*., 2008; Dikeman, Obuz, Gök, Akkaya, & Stroda, 2013; Lepper-Blilie, Berg, Buchanan, & Berg, 2016; Berger *et al*., 2018). These studies confirm that improvements in tenderness through the aging process occurs regardless of the aging method used (wet or dry).

Kim, Kemp, and Samuelsson (2016) compared two aging temperatures (1 or 3°C) and two air velocities (0.2 or 0.5 m/s) for dry aging. The treatments resulted in a wide range of RH (49, 55, 73 or 76%). No differences in shear force values between dry-aged loins were found, irrespective of different dry aging treatment. Lee *et al*. (2017) dry-aged sirloins from low-marbled Hanwoo cows for 28 days with different aging methods and no

differences in shear force were observed for samples dry aged at 85% RH and 1°C when compared to samples dry aged at 75% RH and 2°C. These results are in agreement with our findings and indicate that cooler conditions such as RH have no impact on beef tenderness. Although aging improves beef tenderness, dry aging is mainly used for intensifying flavors.

Microbial Analysis

Day 0 bacterial plating methods determined that the starting loin APC, AnPC, LAB, and PSY counts were less than 2 log cfu/cm^2. Dry-aged treatments of all RH had significantly lower ($P < 0.001$, Table 5) final APC, AnPC, and LAB plate counts than the wet-aged treatment. Final APC, AnPC, and LAB between dry-aged treatments were not significantly different ($P > 0.05$). These findings suggest that dry-aging has the potential to reduce bacterial counts. Tittor, Tittor, Brashears, Brooks, Garmyn, and Miller (2011) observed a notable decrease in *Salmonella* and *Escherichia coli* O157:H7 in beef fat and lean tissues dry aged for 28 days, affirming the notion that dry-aged meat is likely not a food safety risk.

Outside of pathogen control, dry-aging could protect against spoilage bacteria outgrowth, as observed by the suppression of the LAB. Berger *et al*. (2018) observed dry-aged beef loins had fewer APC and LAB than their wet-aged counterparts. Other researchers have observed a plateau or decrease of aerobic plate counts in dry-aged beef over time. While aerobic plate counts initially increased for Ryu *et al*. (2018), counts decreased from 50 to 60 days of aging. Hulànková, Kameníka, Salákováa, Závodskýa, and Borilova (2018) used LOESS (local polynomial regression fitting by weighted least

squares) and Baranyi microbial growth models to plate count data from beef loins aged 36 days, predicting plateau or decrease in growth after two weeks of aging. Our results, however, are in contrast of those of Li, Babol, Bredie, Nielsen, Tománková, and Lundström (2014) where wet-aged beef *longissimus* had lower final APC and LAB than their dry-aged counterparts.

Differences in microbial counts could potentially be attributed to differences in microbial ecology and manufacturing practices; different microbiomes may be more or less adapt to growing in dry aging conditions. *Pseudomonadales* dominated the initial composition of beef samples utilized in our study (Table 6). *Pseudomonas*, belonging to this order, is a major taxon associated with aerobic meat spoilage, due to its ability to readily utilize glucose and amino acids at refrigeration temperature (Ercolini, Russo, Torrieri, Masi, & Villani, 2006). *Pseudomonadales* continued to dominate the aerobically dry-aged loins across all RH. *Enterobacteriales* initially were the second most abundant taxon, however, this group grew to 70.3% of the abundance in the wet-aged sample. The lack of oxygen imparted by vacuum-packaging on the wet treatment gives *Enterobacteirales* and lactic acid bacteria more opportunity to grow. Lee, Yoon, Kim, Oh, Yoon, and Jo (2019) observed similar ubiquity of *Pseudomonas* in beef dry aged at differing airflow velocities, however, *Enterobacteriaceae* were not as abundant on initial samples. De Filippis, La Storia, Villani, and Ercolini (2013) traced final spoilage bacterial of beef steaks back to initial carcass flora, and the processing environment's residential microbiome, enforcing the idea that differences in manufacturing practices are responsible for differences in bacterial populations. The relationship between the residential

microbiome of the processing environment, carcass contamination, and dry-aged meat products merits further investigation.

Sensory analysis

Results from the triangle test indicated that there was a detectable difference between WET and RH70 ($P = 0.02$). There was no detectable difference between RH50 and RH85 ($P = 0.14$). In contrast, no differences among treatments were found for flavor notes identified by the descriptive flavor analysis panel using analysis of variance ($P > 0.05$). However, the PCA of the sensory profiling data showed a separation among the aging methods (Figure 4). Using PCA, two factors explained 83% of the variation in sensory attributes. The RH50 treatment tended to be associated with relatively positive flavor notes, including beef flavor ID, roasted, umami, smoky/charcoal, heated oil, and brown flavors. The RH70 treatment tended to associate with sour milk, sour aromatics, rancid, and fishy flavors, while RH85 tended to associate with oxidized flavors like cardboard, warmed-over, metallic, green, liver-like and sour flavor notes. Wet-aged steaks were fairly neutral in flavor notes (Figure 4). These results suggest that RH may modify sensory attributes in dry-aged beef.

Kim, Kemp, and Samuelsson (2016) compared various processing regimes for dry aging including temperature, RH, and air velocity. The given 2 × 2 (temperature × air velocity) combinations resulted in various RH regimes, such as 76% (at 1°C and 0.2 m/s), 73% (at 1°C and 0.5 m/s), 49% (at 3°C and 0.2 m/s), and 55% (at 3°C and 0.5 m/s). These authors found that loins dry aged at 3°C with 0.2 m/s and 49% RH for 3 weeks produced meat with the greatest acceptable overall sensory traits compared to the other regimes

trialed that had higher RH. This observation is in agreement with our study, where dry aging at 50% RH was associated with more desirable flavor notes when compared to 70% and 85% RH counterparts.

There are several RH levels reported in the scientific literature. The scientific information is still limited and cannot be used to support a recommended threshold for RH from a flavor standpoint. However, in a review, Kim *et al*. (2018) noted that, for the most part, no significant dry aging effects on palatability attributes were observed when the RH was higher than 80%. At the same time, positive results from dry-aging on palatability attributes have been observed when RH was lower than 78% (Kim *et al*., 2018).

Free amino acids

Amino acid values for all aging treatments are presented in Tables 7 and 8, for cooked meat and juice, respectively. Differences ($P < 0.05$) were found in the amount of alanine (ALA) and glycine (GLY) for cooked meat samples among aging treatments (Table 7). Both ALA and GLY were highest for strip loin steaks from dry-aged treatments and lowest for steaks from the wet-aged group. However, no differences among RH levels for free amino acid content of cooked meat were found, possibly because the final moisture content was similar among the three dry-aged treatments.

Differences ($P < 0.05$) were found in the amount of alanine (ALA), valine (VAL), lysine (LYS), glutamate (GLU), leucine (LEU), isoleucine (ILE), threonine (THR), proline (PRO), methionine (MET), phenylalanine (PHE) tyrosine (TYR) for juice samples among aging treatments (Table 8). In general, higher content of free amino acids was found for dry-aged samples when compared to wet-aged samples. This is important as the

concentration of free amino acids can directly increase flavor intensity (Frank et al., 2016) and also serve as substrates for aroma volatiles through the Maillard reaction and Strecker degradation (Mottram, 1998). Specifically, LEU and ILE can react with dicarbonyl compounds formed in the Maillard reaction to produce the meat odorants 2- and 3-methylbutanal (Koutsidis et al., 2008a, 2008b). Kim et al. (2016) identified that TRP, PHE, VAL, TYR, GLU, ILE and LEU were more abundant in the dry-aged beef samples than in the wet-aged samples. In particular, GLU is associated with a savory/beefy (umami) flavor in meat (Nishimura, 1998). The abundance of compounds such as ILE, LEU, PHE, and TYR may promote bitterness (Dinh, Legako, Miller, and Brooks, 2018).

Our results could suggest that the juices from cooked beef may play an important role in influencing consumer preference in beef flavor, as dry-aged samples had increased content of free amino acids in the beef juice compared to the wet-aged counterpart (Table 8).

Conclusion

Results suggest that no case hardening effect occurs during dry aging of beef, even when the RH was kept very low (50%) and the total mass loss was 23%. Instead, the lower RH resulted in more rapid moisture loss at the beginning of the aging process without significantly affecting the total amount of moisture loss. Trim loss, yield, tenderness and microbial counts were not affected by RH levels. *Pseudomonadales* dominates the aerobically dry-aged loins while *Enterobacteriales* was the most abundant in the wet-aged samples. Dry-aged samples had increased content of free amino acids in the beef juice

compared to the wet-aged counterpart. Dry-aging at 50% RH tended to associate with more desirable flavor notes.

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Table 1. Final pH and water activity of strip loins (*Longissimus lumborum*) wet aged (WET) or dry aged for 42 days at 50, 70 or 85% relative humidity (RH).

	Treatment					
	WET	RH50%	RH70%	RH85%	SEM	*P*-value
pH	5.58	5.55	5.53	5.60	0.015	0.29
a_w	0.992[a]	0.980[b]	0.979[b]	0.981[b]	0.001	< 0.001

[a,b] Means in the same row with different superscripts differ ($P < 0.05$).

Table 2. Moisture loss (%), trim loss (%) and yield (%) of strip loins (*Longissimus lumborum*) wet aged or dry aged for 42 days at 50, 70 or 85% relative humidity.

	Treatment					
	WET	RH50%	RH70%	RH85%	SEM	*P*-value
Moisture loss	1.14[a]	23.87[b]	23.20[b]	22.64[b]	0.90	< 0.01
Trim loss	0.0[a]	14.86[b]	14.58[b]	14.99[b]	1.12	< 0.01
Yield	98.86[a]	61.27[b]	62.22[b]	62.37[b]	1.85	< 0.01

[a,b] Means in the same row with different superscripts differ ($P < 0.05$).

Table 3. Water activity of strip loins (*Longissimus lumborum*) wet aged or dry aged for 42 days at 50, 70 or 85% relative humidity.

Sampling location	a_w	SEM	*P*-value
Dorsal	0.985[a]	0.0008	< 0.001
Central	0.984[a]		
Ventral	0.980[b]		

[a,b] Means in the same column with different superscripts differ ($P < 0.001$).

Table 4. Warner-Bratzler shear force (WBSF) and cooking loss (%) of strip loin steaks (*Longissimus lumborum*) wet aged (WET) or dry aged for 42 days at 50, 70 or 85% relative humidity (RH).

	Treatment					
	WET	RH50%	RH70%	RH85%	SEM	*P*-value
WBSF (N)	25.59	25.10	22.46	22.26	0.246	0.66
Cooking loss (%)	15.14[a]	7.93[b]	8.04[b]	8.30[b]	0.776	< 0.01

[a,b] Means in the same row with different superscripts differ ($P < 0.05$).

Table 5. Bacterial plate counts (Log_{10} CFU/cm^2) of beef *Longissimus lumborum* surface after 42 days of wet aging (WET) or dry aging at 50, 70 or 85% relative humidity (RH).

	Treatment				
	WET	RH50%	RH70%	RH85%	*P*-value
APC	3.47[a]	1.41[b]	1.51[b]	1.43[b]	< 0.001
AnPC	3.10[a]	1.52[b]	1.45[b]	1.45[b]	< 0.001
LAB	2.81[a]	1.60[b]	1.41[b]	1.66[b]	< 0.001
PPC	5.45	3.39	3.12	3.41	0.105

APC: aerobic plate count, AnPC: anaerobic plate count, LAB: lactic acid bacteria, PPC: psychrotrophic plate count.

[a,b] Means in the same row with different superscripts differ ($P < 0.001$).

Table 6. The relative abundance (%) of bacterial orders isolated from exterior surface of beef *Longissimus lumborum* prior to aging, and after 42 days of wet aging (WET) or dry aging at 50, 70 or 85% relative humidity (RH).

	Day 0	Day 42			
		WET	RH50%	RH70%	RH85%
Ardenticatenales	5.6	ND	ND	ND	17.0
Bacteroidales	2.1	ND	ND	ND	ND
Enterobacteriales	6.8	70.3	27.8	52.3	5.2
Pseudomonadales	75	27.8	69.8	47.1	72.2
Rhizobiales	6.6	ND	ND	ND	ND
< 1% Abundant Orders	3.9	1.9	2.4	0.6	5.6

ND: not detected.

Table 7. Free amino acid contents of cooked strip loin steaks (μmol/kg) wet aged (WET) or dry aged for 42 days at 50, 70 or 85% relative humidity (RH).

Amino acid	Treatment				SEM	*P*-value
	WET	RH50%	RH70%	RH85%		
ALA	4.18[b]	6.16[a]	6.59[a]	7.06[a]	0.653	0.021
GLY	2.18[b]	2.99[a]	3.28[a]	3.23[a]	0.286	0.040
VAL	2.28	3.07	3.23	3.31	0.373	0.207
LYS	1.63	2.06	2.18	1.83	0.218	0.314
LEU	2.19	2.73	2.81	2.61	0.242	0.293
ILE	1.49	1.86	1.93	1.96	0.181	0.246
THR	1.15	1.78	1.73	1.67	0.190	0.098
SER	1.83	2.66	2.47	2.56	1.297	0.212
PRO	0.65	0.82	0.88	0.65	0.086	0.264
ASN	0.53	0.72	0.73	0.68	0.072	0.225
ASP	0.24	0.27	0.38	0.27	0.054	0.290
MET	0.91	0.96	1.07	1.03	0.119	0.795
PHE	1.28	1.50	1.61	1.48	0.113	0.230
GLU	2.19	2.51	2.72	2.45	0.214	0.389
4HYP	0.06	0.10	0.09	0.18	0.047	0.341
3HYP	0.22	0.43	0.36	0.85	0.228	0.264
TYR	1.04	1.24	1.23	1.11	0.101	0.443

[a-b] Means in the same row with different superscripts differ ($P < 0.05$).

Table 8. Free amino acid contents of meat juice (μM) from strip loins (*Longissimus lumborum*) wet aged (WET) or dry aged for 42 days at 50, 70 or 85% relative humidity (RH).

Amino acid	Treatment				SEM	*P*-value
	WET	RH50%	RH70%	RH85%		
ALA	13.14[b]	18.74[a]	18.02[a]	20.61[a]	1.651	< 0.001
GLY	6.12	8.14	5.57	8.69	1.088	0.148
VAL	6.65[b]	10.27[a]	10.67[a]	12.87[a]	1.002	0.004
LYS	5.46[b]	16.90[ab]	8.00[b]	28.86[a]	4.834	0.006
LEU	3.90[c]	7.73[ab]	5.52[b]	10.37[a]	1.355	0.018
ILE	3.47[b]	6.05[a]	6.33[a]	7.85[a]	0.656	0.002
THR	2.18	4.10	3.60	4.18	0.676	0.072
SER	3.33	6.01	4.62	5.16	1.215	0.369
PRO	1.72[b]	2.51[ab]	2.40 [ab]	3.16[a]	0.333	0.049
ASN	1.25	2.09	1.94	2.42	0.342	0.138
ASP	0.30	0.41	1.16	0.81	0.244	0.136
MET	2.17[b]	4.32[a]	3.96[ab]	4.51[a]	0.642	0.034
PHE	2.30[c]	5.12[ab]	3.68[bc]	6.38[a]	0.836	0.019
GLU	4.34b[c]	4.91[bc]	8.35[a]	7.19[ab]	1.091	0.027
4HYP	0.33[b]	0.97[a]	1.07[a]	1.32[a]	0.122	< 0.001
3HYP	1.77[b]	5.39[a]	6.22[a]	7.08[a]	0.737	< 0.001
TYR	1.91[b]	4.14[ab]	2.42[b]	5.73[a]	1.651	0.004

[a-c] Means in the same row with different superscripts differ ($P < 0.05$).

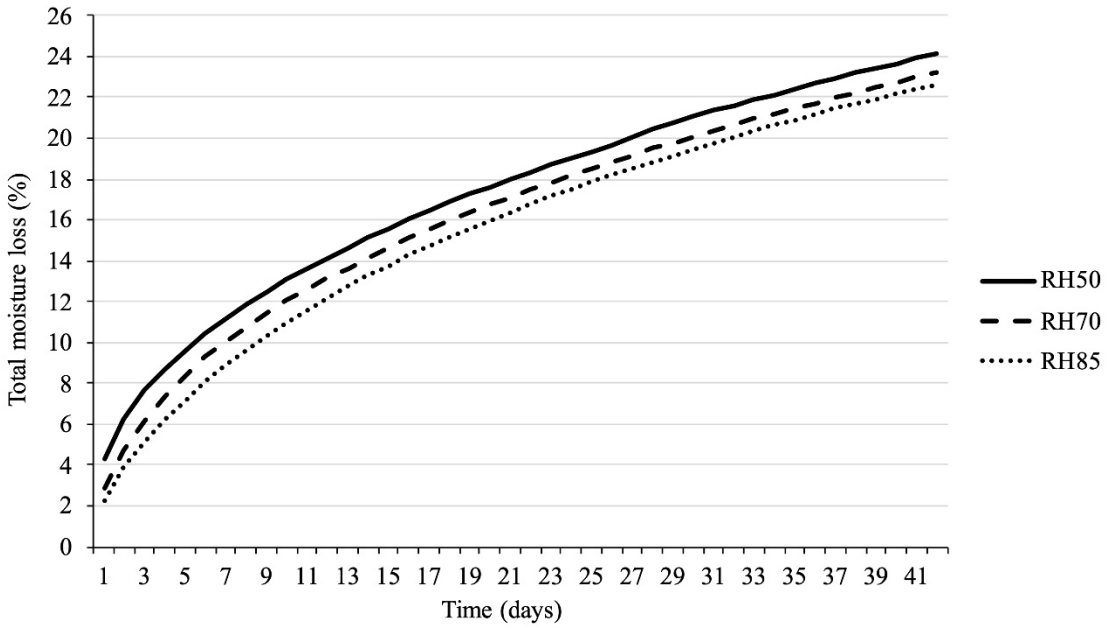

Figure 1. Total moisture loss (%) of boneless strip loins (*Longissimus lumborum*) dry aged for 42 days at 50, 70 or 85% relative humidity (RH; $P = 0.75$; SEM = 0.9022).

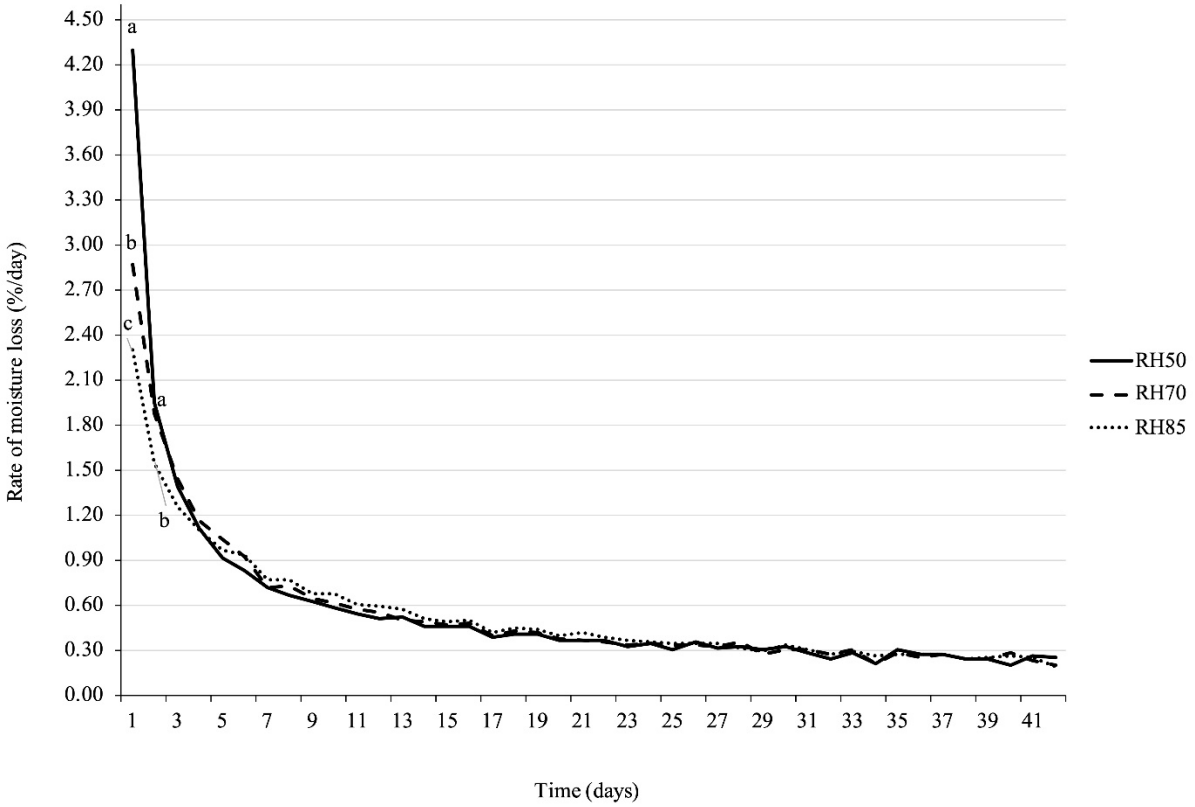

Figure 2. Rate of moisture loss (% per day) of boneless strip loins (*Longissimus lumborum*) dry aged for 42 days at 50, 70 or 85% relative humidity (RH; $P < 0.001$; SEM = 0.0544).

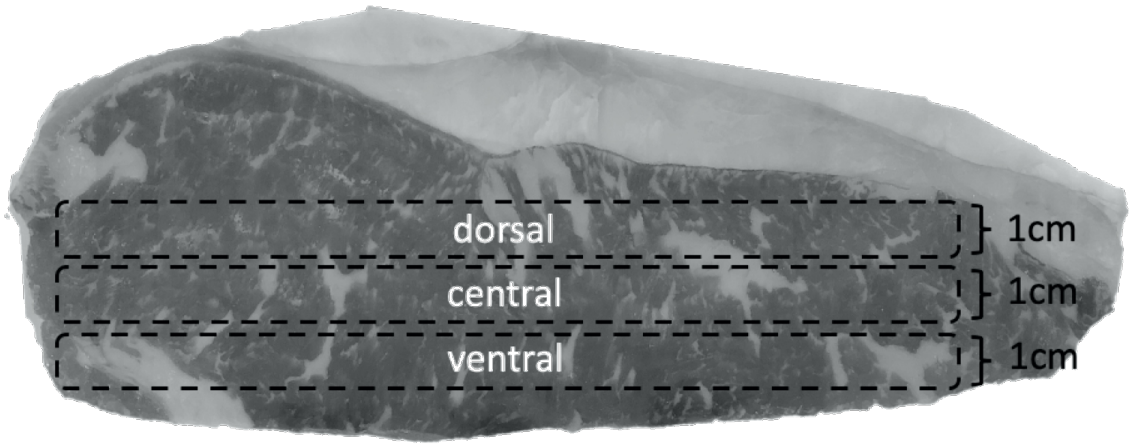

Figure 3. Sampling location for water activity of strip loins wet or dry aged for 42 days at 50, 70 or 85% relative humidity.

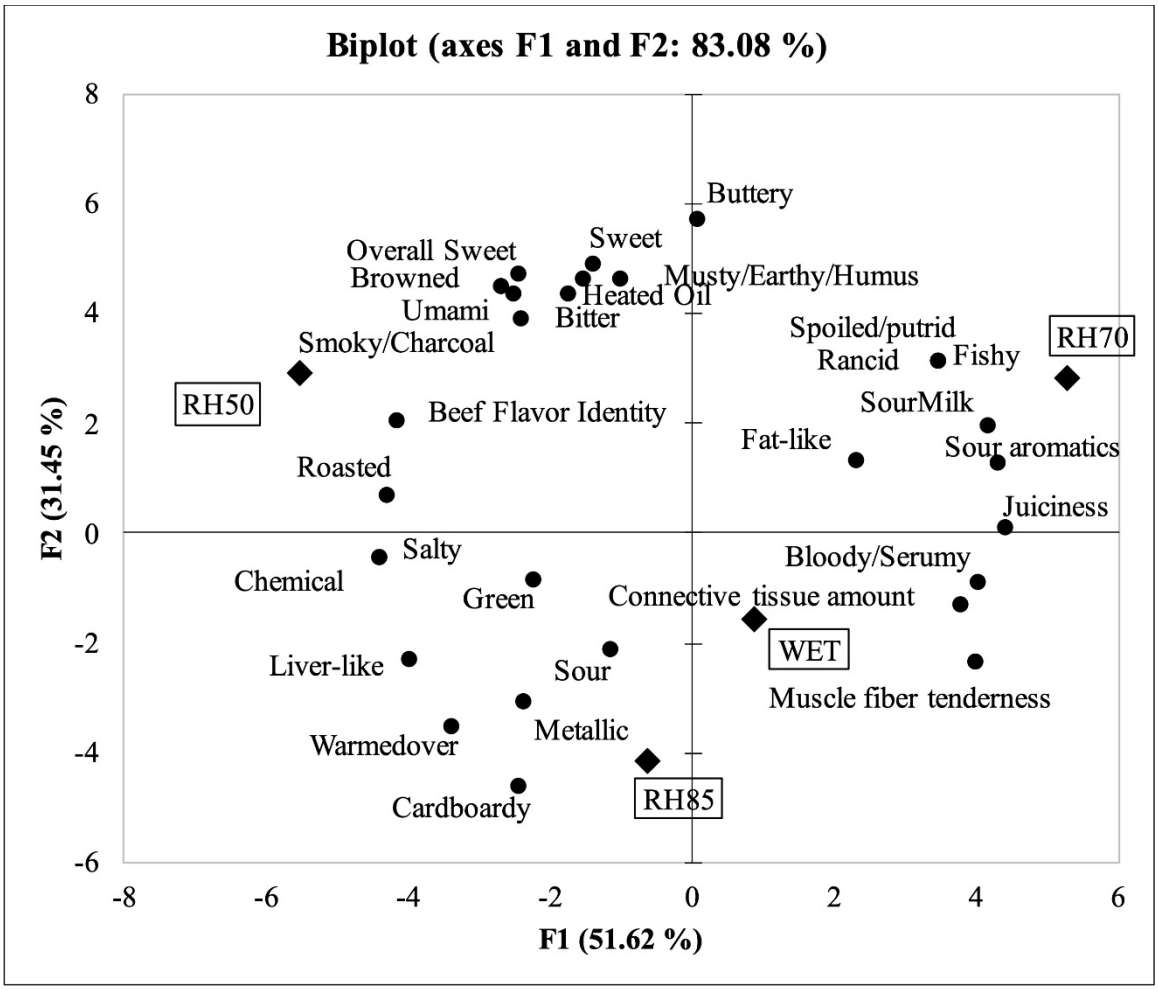

Figure 4. Principal component biplot of sensory attributes where RH50 = dry-aged loins at 50% relative humidity (RH), RH70 = dry-aged loins at 70% RH, RH85 = dry-aged loins at 85% RH, and WET = wet-aged loins for trained sensory panel.

STUDY 3: EFFECTS OF ULTIMATE PH ON DRY-AGED BEEF QUALITY

Abstract

This study aimed to evaluate pH effects on moisture loss and meat quality characteristics of dry-aged beef. Twelve strip loins from six normal pH carcasses (pH = 5.47) and twelve dark cutting (DC) strip loins from six high pH carcasses (pH = 6.69) were obtained. One strip loin from each carcass was dry aged and one was wet aged, giving four treatments: (DRY, DRY-DC, WET, and WET-DC. Loins were aged for 42 d. Ultimate pH did not affect the rate and total moisture loss in dry-aged beef. Trim loss, yield, and tenderness were not affected by ultimate pH during dry aging. In general, DC steaks had the lowest lightness (L*), redness (a*), and yellowness (b*) values, regardless of aging method. Discoloration scores and TBARS values for DC steaks remained low throughout retail display. Dry aging significantly reduced bacterial counts mitigating the microbial damages associated with DC. Flavor characteristics of DC were not improved by dry aging.

Keywords: dry aging, flavor, meat, moisture loss, water-holding capacity.

Introduction

There has been an increased interest in the dry aging process due to the unique flavor developed throughout the process. During dry aging, water is transferred by diffusion from the interior to the meat surface and is subsequently evaporated to the surrounding environment (Lewicki, 2004). As meat loses water, the flavor compounds are concentrated, resulting in a stronger flavor. Temperature, relative humidity, air flow and storage time are often cited as the primary variables to consider when dry aging because they can affect the water evaporation rate and other issues of quality and economics. The effects of ultimate pH on the quality, palatability, and shrinkage of dry-aged beef should be determined. Meat pH may be important during dry aging as it relates to the ability of the muscle to bind water.

Muscle pH drops as H^+ accumulates during the conversion of muscle to meat. The net charge of muscle proteins decreases as the pH decreases toward the isoelectric point. When a protein has a charge, the protein structure has the ability to expand because of the repulsive characteristics of the charges. As a result, water can infiltrate, which will increase muscle water holding capacity (WHC). However, when the pH reaches the isoelectric point the number of positive and negative charges is equal and repulsion is reduced, allowing proteins to pack together, which reduces the space available for water within the myofibril and the amount of water that can be held (Huff-Lonergan & Lonergan, 2005).

According to the 2016 National Beef Quality Audit (Boykin *et al*., 2017), 1.9% of cattle sampled were determined to be dark cutters (DC). Currently, cattle that are sold using carcass grid-based pricing are discounted on average US$0.74/kg (USDA, 2020) when classified as DC. Dark cutter carcasses are known to have differences in tenderness

(Bouton, Carrol, Fisher, Harris, & Shorthose 1973; Dransfield, 1981; Dutson, 1983; Purchas, 1990), lean color that is undesirable consumers, and flavor (Wulf, Emnett, Leheska, & Moeller, 2002). Grayson, Shackelford, King, McKeith, Miller, and Wheeler (2016) investigated the specific flavor notes of dark cutting beef and suggested that detriments to flavor are associated with the severity of the DC. Undesirable flavor notes such as fat-like, rancid, heated oil, chemical, and musty/earthy/hummus flavors increased whereas metallic, sour, and salty flavors decreased as severity of DC increased.

Dry aging has been used to upgrade low quality beef. Several studies have found that dry-aging enhanced palatability attributes of meat, especially flavor. Brown-roasted, beefy, buttery, nutty, roasted-nut, and sweet flavor are some of the flavor notes often associated with dry aging (Campbell, Hunt, Levis, & Chambers, 2001; Corbin *et al*., 2015; Warren & Kastner, 1992). Perhaps dry-aging could be an effective post-harvest value-adding strategy to provide improved palatability characteristics of DC carcass. Therefore, this study aimed to evaluate pH effects on moisture loss when dry aging and the effects on meat quality characteristics. Dry aging of dark cutters (DC) beef may improve flavor and increase yield.

Materials and Methods

Sample Collection and Fabrication

At time of grading, six USDA low Choice and six matching DC carcasses were selected and boneless strip loins from both sides were obtained and transported to the University of Nebraska Loeffel Meat Laboratory. Strip loins from normal pH carcasses

were collected 48 hours after harvest. Due to the fact that DC carcasses are only fabricated once a week, DC strip loins from were collected in a range of 48 and 96 hours after harvest. One steak (1.27 cm thick) for color and pH measurements was obtained from the anterior end of the strip loin. Initially, objective color was measured after 30 min on the bloomed, freshly-cut surface using the L*, a*, b* scales with a Minolta CR-400 colorimeter (Illuminant D65, 8 mm diameter aperture, 2° standard observer angle; Minolta, Osaka, Japan). Six measurements of the steak surface were taken through the overwrap film at day 0. Readings were averaged by steak for statistical analysis. Then, steaks were trimmed of all subcutaneous fat, knife-cut into small cubes, frozen in liquid nitrogen, and powdered in a metal cup blender (Model 51BL32, Waring Commercial, Torrington, CT). Powdered samples from steaks were weighed in 10 g duplicates into 250 mL plastic beakers and placed on a stir plate. Ninety mililiters of distilled deionized water and a magnetic stir bar were added to ensure constant mixing during the measurement process. The pH was measured using a pH meter (Orion 410Aplus: ThermoFisher Scientific; Waltham, MA) that was calibrated using 4.0, 7.0, and 10.0 standards. The pH measurements of the duplicates were averaged for statistical purposes. *Longissimus* muscle pH was measured, and carcasses were classified as DC (pH = 6.69±0.09) or control (pH = 5.47±0.02). One strip loin from each carcass was dry aged and one was wet aged, giving four treatments: (DRY, DRY-DC, WET, and WET-DC.

Three cores (2.54-cm diameter) were removed from lean tissue of each loin for microbiology analysis. Once the initial mass was measured, each strip loin was placed in each assigned dry aging chamber and aged for 42 d at 2 °C and 0.8 m^3/min air speed. Loins

assigned to wet aging were individually vacuum packaged and aged in the same cooler for 42 d.

After aging, loins were weighed and immediately trimmed of dehydrated surface, reweighed, and fabricated anterior to posterior into steaks [one steak for water activity (1.27 cm thick), one for color measurements (1.27 cm thick), two for lipid oxidation (1.27 cm thick); and eight steaks (2.54 cm thick); 1 for Warner-Bratzler shear force (WBSF), and seven for sensory analysis]. After fabrication, steaks assigned to water activity measurements and lipid oxidation at 0 d of RD were vacuum packaged and frozen at -80 °C until further analysis. Steaks assigned to sensory analysis and WBSF were frozen at -20 °C. Steaks assigned to color measurements and 4 and 7 d of RD for lipid oxidation were placed on foam trays (21.6 x 15.9 x 2.1 cm, Styro-Tech, Denver, CO), overwrapped with oxygen-permeable film (Prime Source PSM 18 #75003815, Bunzl Processors Division, North Kansas City, MO; oxygen transmission rate = 2.25 ml/cm^2/24 hr at 23° C and 0% relative humidity; water vapor transfer rate = 496 g/m^2/24 hr at 37.8° C and 90% relative humidity), and placed under RD conditions for 7 d (continuous white fluorescence lighting at 1,000 to 1,800 lux; F32T8/TL741, 700 series, 32 W, Philips, USA) at 2°C. After 4 or 7 d of RD steaks were vacuum packaged and stored at -80°C. Steaks were randomly rotated daily to minimize any possible location effects within the display.

Agenator – A computerized dry aging system

The dry aging chambers utilized in this study were designed and built with a computerized dry aging system (the Agenator) that is capable of measuring and precisely controlling relative humidity (± 1%) and air flow (± 0.015m^3/min). The chambers (86 cm

Length x 47.6 cm Width x 33 cm Height) have built-in load cells and sensors that can continuously monitor mass loss (± 5 g), temperature (± 0.5°C), and RH. All measured data were saved on the connected computer every 15 s. A full description of the Agenator has been previously published by Lau, Ribeiro, Subbiah, and Calkins (2019).

Aging loss (water, trim, and combined losses)

After aging, wet-aged beef loins were removed from the vacuum bags, patted dry with paper towels and reweighed. The processing mass loss for the wet-aged loins during aging was calculated by the difference between initial mass and remaining mass, which was calculated by the subtraction of purge loss from the initial mass. . The percentage daily moisture loss for dry-aged loins was calculated as the difference between the prior day's mass and the current mass divided by the prior day's mass. The percentage total moisture loss for dry-aged loins was calculated as: (initial mass – post-aging mass)/initial mass x 100. The dry-aged loins were then further processed by trimming dried surfaces and non-edible fat and reweighed to calculate the final saleable yield (%) after aging and trimming.

Water activity (a_w)

Steaks from d 42 of aging were removed from the freezer (-80°C) and thawed at 4°C overnight. Steaks were knife-cut into three horizontal strips of 1 cm each, starting from the dorsal region of the steak (just below the subcutaneous fat) to the ventral region. The strip just below the subcutaneous fat was identified as dorsal, the middle strip was identified as central, and the bottom strip was identified as ventral. Each strip was chopped in an Oster food chopper (Model FPSTMC3321, Sunbeam Products Inc, Boca Raton, FL)

for 60 s. Water activity was measured in triplicate using an Aqualab water activity meter (Decagon Devices, Inc., Pullman, WA) at 25°C. Chopped samples were placed in Aqualab water activity cups (Decagon Devices, Inc., Pullman, WA) until the entire bottom of the cup was covered. The a_w measurements of the triplicates were averaged for further statistical analyses.

Instrumental color

Objective color measures were collected using the L*, a*, b* scales with a Minolta CR-400 colorimeter (Illuminant D65, 8 mm diameter aperture, 2° standard observer angle; Minolta, Osaka, Japan). The calibration process was done on a daily basis using a white ceramic tile provided by the manufacturer (Calibration Plate, Serial No. 14933058, Konica Minolta, Japan) and the D65 settings were set as follows: Y = 93.13, x = 0.3164 and y = 0.3330. The colorimeter was set to record and print an average of 6 readings per steak and measurements were taken through the overwrap film once a day at a standardized time from d 0 to 7 of RD. Readings were averaged by steak for statistical analysis.

Subjective discoloration

Surface discoloration was assessed daily during the 7 d of RD with six trained panelists according to the procedure of Senaratne-Lenagala (2012). A reference guide of ten steak images ranging from 0% to 100% surface discoloration with increments of 10% was provided to panelists to ensure consistent evaluations. A percentage scale was used where 0% meant no discoloration and 100% meant complete surface discoloration.

Lipid oxidation

Lipid oxidation was determined with the 2-thiobarbituric acid reactive substances protocol (TBARS) as described by Ahn, Olson, Jo, Chen, Wu, and Lee (1998), with minor modifications, on steaks after 0, 4 and 7 d of RD. Steaks used for lipid oxidation were removed from the freezer, cut by hand into small cubes, frozen in liquid nitrogen, powdered in a metal cup blender (Model 51BL32, Waring Commercial, Torrington, CT), and stored at -80°C until further analysis. Five grams of powdered meat from each steak were blended with 1 mL of butylated hydroxyanisole solution (10%) and 14 mL of distilled water. The samples were then homogenized using a Polytron (Kinmatica AG, Lucern, Sui) for 15 s and centrifuged (2,000 x g for 5 min). After centrifugation, one milliliter of supernatant was collected, mixed with 2 mL of thiobarbituric acid/trichloroacetic acid (TBA/TCA) solution (15% TCA and 20 mM TBA in distilled, deionized water), and placed in a water bath at 70°C for 30 min. After cooling for at least 10 min in a water bath at 20°C, samples were centrifuged (2,000 x g for 15 min) and 0.2 mL of supernatant were transferred to 96-well plates in duplicates. Absorbance was measured at 540 nm using a microplate spectrophotometer (Model Epoch, Biotek, Winooski, VT). Results were expressed in mg of malonaldehyde per kg of tissue.

Tenderness determination

Tenderness was measured via Warner-Bratzler Shear Force (WBSF). Steaks were removed from the freezer (-20°C) and thawed at 4°C overnight. Steak internal temperatures and mass were recorded prior and after cooking. Temperature was recorded for each steak 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). The thermocouples were inserted into the geometric center of each steak with large needles. All steaks were cooked to a target temperature of 71°C on a Belt Grill (TBG60-V3 MagiGril, MagiKitch'n Inc., Quakertown, PA). Belt grill specifications were as follows: preheat = 149°C, top heat = 163°C, bottom heat = 163°C, height of gap = 2.16 cm, and cook time was approximately 6 min. Immediately after cooking, internal temperature and mass were recorded. The percentage cook loss was calculated using the difference between the pre-cooked mass and final cooked mass divided by the initial mass (x 100). The steak was individually bagged and stored overnight at 2°C for WBSF analysis. The following d, six (1.27 cm diameters) cores were taken parallel to the muscle fiber with a drill press. Tenderness of the cores was measured using a Food Texture Analyzer (TMS-Pro, Food Technology Corp., Sterling, VA.) with a Warner-Bratzler blade. Peak WBSF values from each core were averaged by steak for statistical analysis.

Microbiology Sample Collection

Microbial analysis of loins was conducted on d 0 and 42. Six individual loins of both normal and DFD animals were surveyed for baseline measurements on d 0. On d 42, six loins from each treatment (DRY, DRY-DC, WET, WET-DC) were sampled for microbial analysis. Three 2.5-cm diameter surface cores of lean tissue were aseptically taken from loins and transferred into WhirlPak bags (Nasco, Fort Atkinson, WI). Cores were homogenized for 2 min with 35 mL of sterile BBL Peptone water (Becton, Dickinson and Company, 1057 Franklin Lakes, NJ) in the WhirlPak bags in a stomacher (bioMerieux Inc., Durham NC). Duplicates of 1.75 mL samples were taken directly from the WhirlPak

bag and stored in sterile 2 mL microcentrifuge tubes at -20°C until DNA extraction.

Microbial Plating Methods

Sample cores were subjected to microbial plating methods in duplicates. Fifty µL of each sample were platted 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 plate counts (PPC). DeMan Rogosa Sharpe agar (Thermo Fisher Scientific, Waltham, MA) was used to enumerate lactic acid bacteria (LAB). Plates for APC, AnPC, and LAB were incubated at 37°C and microbial colonies were counted after 48 h. Plates for AnPC were held in an 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). Plates for PPC were incubated at 4°C and counted after 10 d.

Microbial Ecology Analysis

Bacterial communities were investigated for each sample using the MiSeq Illumina Sequencing Platform, targeting the bacterial-specific 16s rRNA gene as described by Kozich, Westcott, Baxter, Highlander & Schloss (2013). The DNA was extracted from samples using DNA QuickExtract Solution 1.0 (Epicentre, Madison, WI). Obtained DNA was amplified via polymerase chain reaction (PCR) containing 1X Terra PCR Direct Buffer (Clontech Laboratories Inc., Mountain View, CA), 0.75 U Terra PCR Direct Polymerase Mix (Clontech Laboratories Inc.), approximately 1-5 ng of extracted DNA,

and 0.5 μM barcoded universal primers. The PCR reaction was performed alongside negative controls in a Veriti 96 well thermocycler (Thermo Fisher Scientific, Walther, MA), with the following PCR cycle: initial denaturation at 98°C for 3 min, followed by 30 cycles of 98°C for 30 s, 58°C for 30 s, and 68°C for 45 s, and a final extension of 68°C for 4 min.

After amplification, PCR products were analyzed on a 1.5% agarose gel to ensure amplification occurred successfully and without contamination of negative controls. Samples were then normalized using the Norgen NGS Normalization 96-Well Kit (Norgen Biotek Corp., Thorold, ON, Canada) according to manufacturer protocol. Pooled sample was then placed in a 50°C water bath to remove excess ethanol from the normalization kit and run through a spin column. As DNA was found to be lacking in concentration, samples were subjected to additional PCR using a 5-cycle rendition of the previously described protocol. Products were then separated on a 2% agarose gel, which yielded two bands. The band corresponding to the bp size of the 16s rRNA V4 subregion was removed with a scalpel and DNA was recovered using the MinElute PCR Purification kit (Qiagen Inc., Germantown, MD). Concentration and bp size of the 16S rRNA libraries were determined using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Library concentration was confirmed with a DeNovix QFX Fluorometer and the Denovix dsDNA High Sensitivity reagent kit (Denovix Inc., Wilmington, DE). The 16S libraries were sequenced using the Illumina MiSeq platform (Illumina, 2457 Inc., San Diego, CA) using the V4 500 cycle kit.

Sequence Processing

An average of 19,264 reads was obtained per sample. Obtained sequence reads were processed with R (version 3.6.0, R Core Team, 2013) and Mothur (Version 1.42.1, Schloss *et al*., 2009). The DADA2 pipeline (Callahan, McMurdie, Rosen, Han, Johnson, & Holmes, 2016a) functioned to prepare sequences and generate amplicon sequence variants (ASVs). Raw reads were quality checked, filtered, trimmed, and merged. Chimeras and non-bacterial sequences were removed. Samples with low biomass were removed, as previously performed in meats studies, due to inherent low cell counts on starting materials (Weinroth *et al*., 2019). Twenty-six of the original 36 samples were retained. The ASVs were then assigned and merged with a phylogenetic tree generated from Mothur and metadata file into a phyloseq object (McMurdie & Holmes, 2013). Once ASVs were determined, taxa were assigned based on the Silva (V132, Quast *et al*., 2012) database. The "decontam" package (Davis, Proctor, Holmes, Relman, & Callahan, 2018) was utilized on the dataset to remove possible contaminants. Phyloseq was then utilized to determine the relative abundance of bacterial taxa (Callahan, Sankaran, Fukuyama, McMurdie, & Holmes, 2016b).

Sensory Evaluation

Triangle tests were conducted in four sessions with a total of 32 consumers per session. In the first, second, third and fourth sessions, panelists were served samples for DRY-DC versus DRY, DRY-DC versus WET-DC, DRY-DC versus WET, and WET-DC versus WET treatments, respectively. Each panelist received three 3-digit blind coded samples (1.3 cm x 1.3 cm x 2.54 cm thickness) avoiding the edges and fat kernels of the

steaks. Two of these samples were identical and one was different. Panelists were asked to identify the sample that was different in flavor.

Statistical Analysis

Rate of moisture loss was analyzed as a split plot design with treatment as the main plot and days of aging as the repeated measures. Separation of means for rate of moisture loss data was conducted using the LSMEANS procedure with SLICEDIFF function at $P < 0.05$. Objective and subjective color data were analyzed as a split-plot repeated measures design with aging treatment as the whole-plot and RD time as the repeated measures. Color panelists were considered a random effect when analyzing subjective discoloration. Tenderness, cooking loss, water activity, pH, trim loss, total moisture loss, yield and microbial plate counts were analyzed as a completely randomized design. In this study, the chamber was considered the experimental unit. Data were analyzed using the PROC GLIMMIX procedure of SAS. All means were separated with the LS MEANS and DIFF functions with $\alpha = 0.05$.

Results and Discussion

Initial pH and color measurements

Prior to aging, the normal pH and DC loins had mean pH values of 5.47 and 6.69, respectively ($P < 0.001$; Table 1). Higher L*, a*, and b* values were found for the normal pH loins when compared to DC loins ($P < 0.001$; Table 1). These results may be explained by greater reflectance associated with more moisture on the meat surface (Bertram,

Engelsen, Busk, Karlsson, & Andersen, 2004; Dikeman, Obuz, Gök, Akkaya, & Stroda. 2013).

Aging loss (moisture, trim, and combined losses)

Strip loins from WET-DC and WET treatments had higher yields, and lower moisture losses, and trim losses when compared to DRY and DRY-DC ($P < 0.001$; Table 2). No differences in total moisture loss ($P = 0.96$; Figure 1), rate of moisture loss ($P = 0.51$; Figure 2), trim loss ($P = 0.69$) or yield ($P = 0.75$) between DRY-DC and DRY were found (Table 2).

Studies have shown that in meat with higher ultimate pH, water-holding capacity is increased as the structure of protein is more open, allowing for more water to be trapped in the myofibril (Dransfield, 1981; Purchas, 1990). Water-holding capacity is an important property of fresh meat as it affects the yield and quality of the product, and it is often described as drip loss.

Meat pH may be important during dry aging as it relates to the ability of muscle to bind water. We hypothesized that dry aging of DC beef would reduce moisture loss, resulting in increased yield. However, moisture loss data from this study revealed an intriguing phenomenon, in which DRY-DC loins lost the same amount of water, at the same rate, as DRY loins. One explanation for such a phenomenon is that free water, which is located in the sarcoplasmic area within the muscle cells, can be easily mobilized during dry aging. Surface dehydration due to dry aging creates a region of low concentration of water in the meat surface. As a result, the dry hardened surface changed the diffusion rate

of moisture through the meat. Our results suggest the internal mass transfer resistance determines the rate of drying.

Following Fick's first law of diffusion, water flows from regions of high concentration to regions of low concentration, with a magnitude that is proportional to the concentration gradient (Tyrrell, 1964). Once the crust is formed, water is transferred from the interior to the meat surface and is subsequently evaporated to the surrounding environment (Lewicki, 2004).

Transport of water in the meat being dry aged proceeds in two steps. First, water is transported from the interior to the meat surface by diffusion and capillary flow. Second, water is evaporated at the surface of the meat and is transferred as vapor to the surrounding air. This process is a convective mass transfer (Lewicki, 2004). The rate of convective mass transfer is dependent on RH, air velocity, temperature, and shape of the product. The transport of water in dry-aged beef is dependent upon two mass transfer resistances: internal and external. The internal mass transfer resistance depends on the temperature of the meat and on the effective water diffusion coefficient (diffusion and capillary flow), which can be influenced by a variety of factors, including the pH, proteolysis, and muscle cell structure. Internal mass transfer resistance determines the rate of drying. A decrease of water content by a few percentage points can lower the effective diffusion coefficient by two or three orders of magnitude (Lewicki, 2004). The external mass transfer resistance is responsible for the convective mass transfer. The relationship between both external and internal mass transfer resistances affects the rate of drying. If the external resistance is higher than or equal to the internal resistance, the flux of water reaching the surface of the

meat is constant and drying proceeds at a constant rate. If the internal resistance is higher than the external, less water is transported to the surface of the meat (Lewicki, 2004).

In summary, dry-aged meat loses most of the water by convection during the early stage, causing a crust to form as the surface of beef dries out. Once the meat surface has been dried off and the hard crust is formed, the rate of evaporation of water will be driven mainly due to diffusion. Hence, the flux of evaporated water in dry-aged beef decreases with time and drying proceeds at a decreased rate. Our results suggest that diffusion was not affected by ultimate pH during dry aging. This may explain the similar water lost found for DC beef when compared to normal pH beef after 42 days of dry aging.

Water activity (a_w)

Steaks from WET-DC and WET treatments had the highest a_w values, DRY had the lowest, while DRY-DC was intermediate ($P < 0.001$; Table 3). There was no location effect for a_w values ($P = 0.12$). the interaction between fixed effects was tested, but no significance was found ($P = 0.33$). Therefore, only the main effect was reported.

These results were expected as higher ultimate pH increases the meat water-holding capacity, while dry aging reduces moisture content (Huff-Lonergan & Lonergan, 2005; Berger, Kim, Legako, Martini, Lee, Ebner, & Zuelly, 2018). This finding is in agreement with other similar studies, where dry-aged beef had a lower moisture content than wet-aged counterparts (Dikeman, Obuz, Gök, Akkaya, & Stroda. 2013; Berger, Kim, Legako, Martini, Lee, Ebner, & Zuelly, 2018).

Instrumental color

Instrumental color data indicate that the aging method had an effect on L* values ($P < 0.0001$). DRY-DC and WET-DC steaks had the lowest lightness (L*) values ($P < 0.05$) throughout RD. No differences in L* values were observed for WET and DRY steaks until d 1 of RD. After that, WET and DRY samples started to diverge and WET had the highest L* values, while DRY was intermediate (Figure 3). Similarly, aging method also had an effect on b* values ($P < 0.0001$). DRY-DC and WET-DC steaks had lower b* values ($P < 0.05$) throughout RD, WET had greater, while DRY was intermediate (Figure 4).

A significant interaction between aging method and RD on a* values (an indication of redness) was observed (Figure 5, $P < 0.0001$). WET steaks had greater redness (a*) values ($P < 0.05$) than any other treatment until d 5 of RD. DRY-DC and WET-DC had the lowest a* values ($P < 0.05$) from d 0 to d 3, while DRY was intermediate. No differences in a* values among DRY-DC, WET-DC, and DRY were found ($P > 0.05$) on d 4 and 5. At d 7, DRY had lower a* values than ($P < 0.05$) all other treatments.

There is a general agreement that dry-aged beef steaks are slighter darker and have lower redness values compared with wet-aged steaks (Dikeman, Obuz, Gök, Akkaya, & Stroda, 2013; Kim, Kemp, and Samulesson, 2016). In agreement with that, in our study, DRY steaks had lower L* and a* values when compared with WET. The lighter color in the WET steaks compared to the DRY counterpart is mostly due to greater moisture content after aging resulting in more light reflection (Kim & Hunt, 2011).

Holman, van de Ven, Mao, Coombs, and Hopkins (2017) reported the relationship between a* values and consumer acceptance of beef color. The authors considered that beef color was acceptable (with 95% acceptance) when a* values were equal to or above

14.5. In this study, the 14.5 color threshold value was reached by steaks from the WET treatment between 5 and 6 d of RD and steaks from the DRY group reached the color threshold between 3 and 4 d of RD. The a* values of DC were always below 14.5, regardless of aging method since d 0 of RD, confirming the visually unappealing lean color of DC samples.

In general, DC steaks darkened more slowly in terms of L*, a*, and b* when compared with WET and DRY steaks. Purchas, Yan, and Hartley (1999) reported that meat samples darkened at a decreasing rate in terms of L*, a*, and b* values as pH increased. Similar results have also been reported by López-Campos, Zawadski, Landry, Aalhus, and Uttaro (2014) and Purchas (1990).

Subjective discoloration and lipid oxidation

A 2-way interaction between treatment and RD for discoloration was observed ($P < 0.001$; Figure 6). As expected, discoloration increased as RD time increased, regardless of the aging method (wet or dry). No differences were found among treatments on d 0 and d 1 of RD. Samples began to diverge on d 2 of RD and DRY steaks had greater discoloration ($P < 0.05$) than any other treatment from d 2 to d 7 of RD. DRY-DC and WET-DC steaks had lower discoloration ($P < 0.05$) than WET steaks at d 5, 6 and 7 of RD. Discoloration scores for DC steaks remained low throughout the RD period.

Color is the first criterion used by the consumer when deciding on a meat purchase. Consumers usually discriminate against discolored meat and select non-discolored products if both packages are viewed in retail display. Hood and Riordan (1973) reported that a 20% surface discoloration on retail displayed beef can result in sale reductions of up

to 50%. In this study, the 20% discoloration threshold was met by steaks from the DRY treatment after 4 d of RD, and after 6 d of RD for steaks from the WET treatment. Although they may have been rejected due to their dark color, DC steaks did not reach the 20% discoloration threshold even after 7 d of RD. López-Campos, Zawadski, Landry, Aalhus, and Uttaro (2014) reported that color stability of meat with pH between 6.0 and 6.4 deteriorated more slowly under conventional overwrap. Purchas, Yan, and Hartley (1999) have also observed that the rate of discoloration of beef decreases as ultimate pH increases.

The exact mechanisms by which aging influences color and oxidative stability of muscles has not been fully determined, however, the faster discoloration observed for DRY steaks when compared to WET steaks may be attributed to the extent of exposure to oxygen during aging and depletion of endogenous reducing compounds or antioxidants (Bekhit, Hopkins, Fahri, & Ponnampalam, 2013). Discoloration is caused by an accumulation of metmyoglobin on the meat surface due to the oxidation of myoglobin. Muscle's ability to convert metmyoglobin (ferric state) to the reduced ferrous state through metmyoglobin reducing activity (MRA) is limited and is continually depleted as time postmortem progresses (Mancini & Hunt, 2005).

Evidence for an interaction between the processes of myoglobin oxidation and lipid oxidation in meat has been demonstrated by a number of model studies (Faustman, Sun, Mancini, & Suman, 2010). In agreement with that, a 2-way interaction between treatment and RD was observed for lipid oxidation ($P < 0.001$). DRY steaks had greater TBARS values than any other treatment at 0 d RD. As expected, lipid oxidation increased as RD progressed. At 4 and 7 d of RD, DRY steaks had the highest TBARS values, followed by WET steaks, and DRY-DC and WET-DC steaks had the lowest TBARS values (Table 4).

The rate of lipid oxidation depends on several conditions including length of aging and presence of oxygen. Faustman, Sun, Mancini, and Suman (2010) indicated that the presence of oxygen catalyzes the formation of primary oxidative products that propagate to form secondary oxidative products that continue the oxidative chain reaction. Therefore, lipid oxidation was favored by extended storage under aerobic conditions (dry aging) and increased TBARS values were observed for DRY steaks in comparison with WET counterparts. DRY-DC and WET-DC steaks had the lowest TBARS values. Previous research also noted less lipid oxidation in dark-cutting beef than normal pH beef (Sawyer, Apple, Johnson, Baublits, & Yancey, 2009; English, Wills, Harsh, Mafi, VanOverbeke, & Ramanathan, 2016).

The biochemical reactions directly responsible for lipid oxidation and myoglobin oxidation generate primary and secondary products capable of enhancing both processes in a reciprocal manner. In dark-cutting meat, greater muscle pH can enhance mitochondrial oxygen consumption making myoglobin less susceptible to oxidation (more stable). Consequently, lipid oxidation is delayed (English et al., 2016; Faustman, Sun, Mancini, & Suman, 2010).

Lipid oxidation increases beef flavor deterioration during aging, and this deterioration can be closely related to TBARS. Campo, Nute, Hughes, Enser, Wood, and Richardson (2006) considered TBARS values exceeding 2.28 mg of malonaldehyde per kg as unacceptable for beef because at this level rancid flavor overpowers beef flavor. In this study, the limiting threshold of 2 mg of malonaldehyde per kg was met by DRY steaks since d 0 of RD, while DC steaks met this threshold only after 7 d of RD.

Shear force and cooking loss

There were no differences among treatments for WBSF (Table 5; $P = 0.67$). This observation is in agreement with other dry-aging studies that also reported no significant differences for shear force values between wet and dry-aged beef (Parrish, Boles, Rust, & Olson, 1991; Ahnström, Seyfert, Hunt, & Johnson, 2006; Smith *et al*., 2008; Dikeman, Obuz, Gök, Akkaya, & Stroda, 2013; Lepper-Blilie, Berg, Buchanan, & Berg, 2016; Berger *et al*., 2018; Bernardo *et al*., 2020). Evidence from these studies confirms that improvements in tenderness through the aging process occurs regardless of the aging method used (wet or dry).

Regarding the effect of ultimate pH in tenderness, many researchers have shown that tenderness is dependent on the severity of DC. Tenderness tends to increase with increasing ultimate pH. However, carcasses with intermediate pH (5.9 to 6.2) are usually the toughest (Bouton, Carrol, Fisher, Harris, & Shorthose 1973; Wulf, Emnett, Leheska, & Moeller, 2002; Grayson, Shackelford, King, McKeith, Miller, & Wheeler, 2016). A possible cause of shear force differences could be related to increased water-holding capacity and greater activity of proteases such as calpains in meat with a higher pH (Purchas, 1990; Grayson, Shackelford, King, McKeith, Miller, & Wheeler, 2016; Kendall, Koohmaraie, Arbona, Williams, & Young, 1993). Among these studies, it should be noted that significant differences in tenderness were found for aging periods of 7 to 23 days. Perhaps, the extended aging period utilized in this study (42 days) might have minimized tenderness differences among treatments.

Cooking losses were the lowest for DRY-DC steaks, WET had the greatest cooking loss, while WET-DC and DRY were intermediate (Table 5; $P < 0.001$). Similarly, Laster

et al. (2008) reported that dry aging caused less cooking loss than wet aging. Similar to tenderness, reduced cooking loss of dry-aged beef could be related to increased water-holding capacity and reduction of moisture content during dry aging. However, multiple studies have reported that wet and dry-aged steaks had similar cooking losses (Warren & Kastner, 1992; Dikeman, Obuz, Gök, Akkaya, & Stroda, 2013; Kim, Kemp, & Samuelsson, 2016; Berger *et al*., 2018).

Grayson, Shackelford, King, McKeith, Miller, and Wheeler (2016) evaluated the effect of ultimate pH on cooking loss and observed that cooking losses decreased as pH increased, which is in line with the findings of this study. McClain and Mullins (1969) also observed that higher pH resulted in lower cook loss; however, they disagree with Purchas and Aungsupakorn (1993), who found no difference in cooking loss of meat with differing pH.

Microbial Analysis

The WET-DC samples had significantly higher APC and AnPC than WET counterparts ($P < 0.001$; Table 6). Past research has documented dark cutters having reduced shelf-life, as the high pH of DC meat allows quicker outgrowth of different species (Gill & Newton, 1979). Additionally, low levels of glucose found in DC meat force bacteria to use amino acids for growth, generating putrid spoilage associated odors at lower microbial loads. (Garcia-Lopez, Prieto, & Otero, 1998). Interestingly, dry aging mitigated the microbial damages associated with DC. APC, AnPC, and LAB of DRY steaks were not significantly different from and DRY-DC and were significantly lower ($P < 0.001$) than their wet-aged counterparts.

The microbial community of all samples evaluated contained *Enterobacteriales* and *Pseudomonadales* (Table 7). The observed ubiquity of pseudomonads is consistent with observations by Capouya, Mitchell, Clark, Clark, and Bass (2020), where *Pseudomonas fragi* was identified as a core taxon, occurring in more than ninety percent of dry-aged beef bone-in strip loins. *Pseudomonadales* and *Enterobacteriales* are recognized as common spoilage organisms in aerobically stored beef (Ercolini, Russo, Torrieri, Masi, & Villani, 2006). WET-DC had *Clostridiales* not observed in the other treatments, and a higher abundance of *Lactobacillales*. These taxa have anaerobic metabolisms that allow for proliferation in vacuum packaging. Clostridia sequences in this study mapped to spoilage-associated taxa, which are known to contribute to gas production giving rise to "blown-packs" (Odeyemi, Alegbeleye, Strateva, & Stratev, 2020). While *Lactobacillales* are acid producers, many lactic acid bacteria have optimal growth at pH between 5.8 and 6.9, possibly explaining their abundance in DC samples (Rault, Bouix, & Béal, 2009).

Sensory analysis

Results from the triangle test indicated a detectable difference between DRY-DC and DRY ($P = 0.01$), DRY-DC and WET-DC ($P = 0.01$), DRY-DC and WET ($P = 0.01$) and WET-DC and WET ($P < 0.01$). A high number of panelists rated inferior eating satisfaction associated with DC flavor, although they were not asked questions regarding preference.

Grayson, Shackelford, King, McKeith, Miller, and Wheeler (2016) suggested that detriments to flavor are associated with the severity of DC. They reported that differences in undesirable flavor notes found across DC classes were mostly in severe (pH = 6.89) and

moderate (pH = 5.59) DC. As severity of DC increased, undesirable flavor notes such as rancidity, musty/earth/hummus, fat-like, and metallic increased. Conversely, as severity of DC increased, brown/roasted, sour, umami and salty flavor intensity decreased. Similarly, association of DC with "off-flavors" has been reported by other studies (Wulf, Emnett, Leheska, & Moeller, 2002; Yancey, Dikeman, Hachmeister, Chambers IV, & Milliken, 2005).

In our study, DC samples were classified as moderate DC (pH = 6.69). Severe and moderate DC tended to be on the extreme end of differences in flavor (Grayson, Shackelford, King, McKeith, Miller, & Wheeler, 2016). Perhaps, dry aging of moderate DC beef concentrated undesirable flavor notes, which could explain the difference found between DRY-DC and WET-DC. These extreme differences in flavor notes would likely be undesirable to the consumer.

Conclusions

Ultimate pH did not affect the rate of moisture loss, total moisture loss, trim loss, yield, or tenderness in dry-aged beef. Discoloration scores and TBARS values for DC steaks remained low throughout retail display; however, DC steaks were darker than the control counterparts. Dry aging significantly reduced bacterial counts mitigating the microbial damages associated with DC. *Enterobacteriales* and *Pseudomonadales* were present in all samples evaluated. *Clostridiales* were only observed on samples of WET-DC, which also had a higher abundance of *Lactobacillales*. Flavor characteristics of DC were not improved by dry aging.

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Table 1. Initial pH and color measurements of normal and dark-cutting (DC) strip loins (n = 6 per treatment) before aging.

	Normal	DC	SEM	*P*-value
pH	5.47[a]	6.69[b]	0.0314	< 0.001
L*	45.19[a]	38.87[b]	0.5807	< 0.001
a*	18.23[a]	8.97[b]	0.6269	< 0.001
b*	7.89[a]	2.95[b]	0.4217	< 0.001

[a,b] Means in the same row with different superscripts differ ($P < 0.001$).

Table 2. Effect of ultimate pH (Dark cutters [DC] vs. Normal) and aging method (WET vs. DRY) on moisture loss, trim loss and saleable yield of strip loins (*Longissimus lumborum*) aged for 42 days.

	Treatment					
	WET	WET-DC	DRY	DRY-DC	SEM	*P*-value
Moisture loss (%)	0.59[b]	0.02[b]	21.56[a]	21.51[a]	0.660	< 0.001
Trim loss (%)	0.0[b]	0.0[b]	27.88[a]	28.78[a]	1.119	< 0.001
Yield (%)	99.31[a]	99.95[a]	50.56[b]	49.71[b]	1.506	< 0.001

[a,b] Means in the same row with different superscripts differ ($P < 0.05$).

Table 3. Effect of sampling location, ultimate pH (Dark cutters [DC] vs. Normal) and aging method (WET vs. DRY) on water activity of strip loins (*Longissimus lumborum*) aged for 42 days.

Sampling position	a_w			
	WET	WET-DC	DRY	DRY-DC
Dorsal	0.990	0.992	0.985	0.987
Central	0.991	0.991	0.985	0.989
Ventral	0.991	0.992	0.986	0.989
Average of all samples	0.990^{ab}	0.992^{a}	0.985^{c}	0.988^{b}

[a,b] Means in the same column with different superscripts differ ($P < 0.001$).

SEM = 0.001

Table 4. Effect of ultimate pH (Dark cutters [DC] vs. Normal) and aging method (WET vs. DRY) on lipid oxidation (TBARS; mg malonaldehyde/kg of meat) of strip loin steaks aged for 42 days with 0, 4 and 7 days retail display.

	Treatment					
Day	WET	WET-DC	DRY	DRY-DC	SEM	*P*-value
0	0.98[b]	0.37[b]	3.94[a]	0.58[b]	0.490	< 0.001
4	4.98[b]	1.93[c]	7.31[a]	1.37[c]		
7	8.28[b]	2.23[c]	11.22[a]	2.89[c]		

[a,b] Means in the same row with different superscripts differ ($P < 0.05$).

Table 5. Effect of ultimate pH (Dark cutters [DC] vs. Normal) and aging method (WET vs. DRY) on Warner-Bratzler shear force (WBSF) and cooking loss of strip loin steaks (*Longissimus lumborum*) aged for 42 days.

	Treatment					
	WET	WET-DC	DRY	DRY-DC	SEM	*P*-value
WBSF (kg)	2.21	2.25	2.21	2.04	0.132	0.67
Cooking loss (%)	10.50[a]	6.82[b]	7.01[b]	4.76[c]	0.570	< 0.001

[a-c] Means in the same row with different superscripts differ ($P < 0.05$).

Table 6. Bacterial plate counts (Log_{10} CFU/cm^2) of normal and dark-cutting (DC) beef *Longissimus lumborum* surface after 42 days of wet aging (WET) or dry aging (DRY).

	Treatment				
	WET	WET-DC	DRY	DRY-DC	*P*-value
APC	3.51[b]	5.57[a]	2.77[c]	2.86[c]	<.0001
AnPC	3.23[b]	5.52[a]	2.82[bc]	2.44[c]	<.0001
LAB	2.90[a]	3.69[a]	1.44[b]	1.36[b]	<.0001
PPC	5.75[a]	5.75[a]	3.39[c]	4.12[b]	<.0001

APC: aerobic plate count, AnPC: anaerobic plate count, LAB: lactic acid bacteria, PPC: psychrotrophic plate count. [a,b] Means in the same row with different superscripts differ ($P < 0.001$).

Table 7. The relative abundance (%) of bacterial orders isolated from exterior surface of normal and dark-cutting (DC) beef longissimus prior to aging, and post 42 days aging in vacuum packagings (WET) and dry aging chambers (DRY)

	Day 0		Day 42			
	CON	DC	WET	WET-DC	DRY	DRY-DC
Actinomycetales	5.6	ND	ND	ND	ND	ND
Aeromonadales	ND	ND	5.7	ND	2.4	10.9
Bacteriodales	ND	ND	17.1	ND	ND	ND
Clostridiales	ND	ND	ND	7.1	ND	ND
Enterobacteriales	38.2	49.6	55.2	48.7	42.2	65.9
Lactobacillales	11.8	ND	ND	14.2	ND	2.2
Pseudomonadales	40.4	47.0	12.5	25.0	42.2	8.3
< 1% Abundant Orders	4.0	3.4	9.5	5.0	13.2	12.7

ND: not detected.

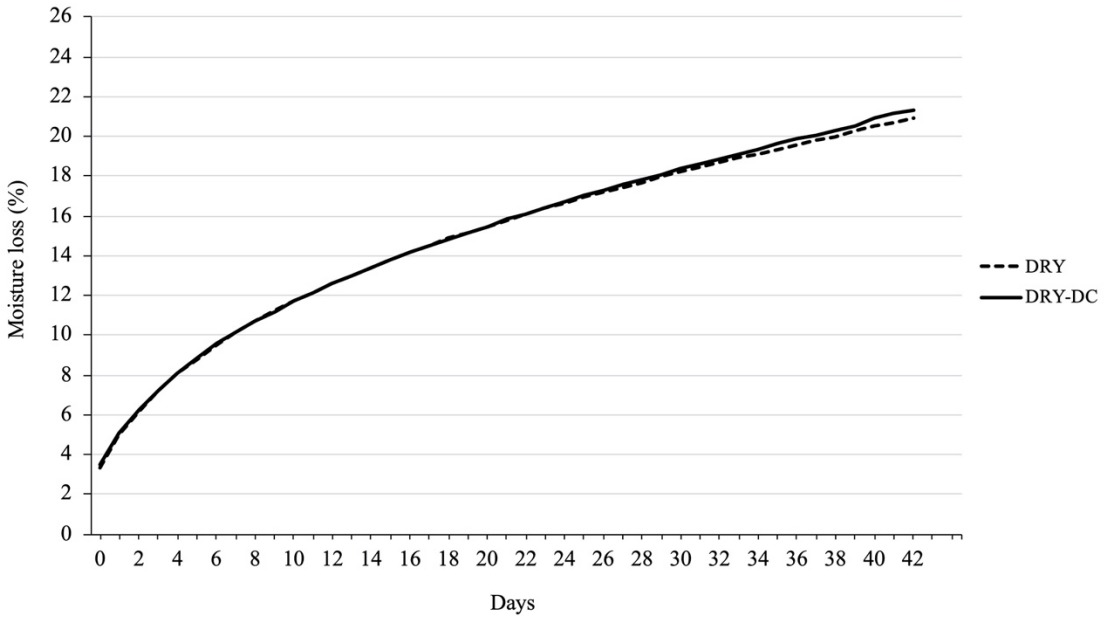


Figure 1. Effect of ultimate pH (Dark cutters [DC] vs. Normal) on total moisture loss of strip loins dry aged for 42 days (*P*-value = 0.96; SEM = 0.72).

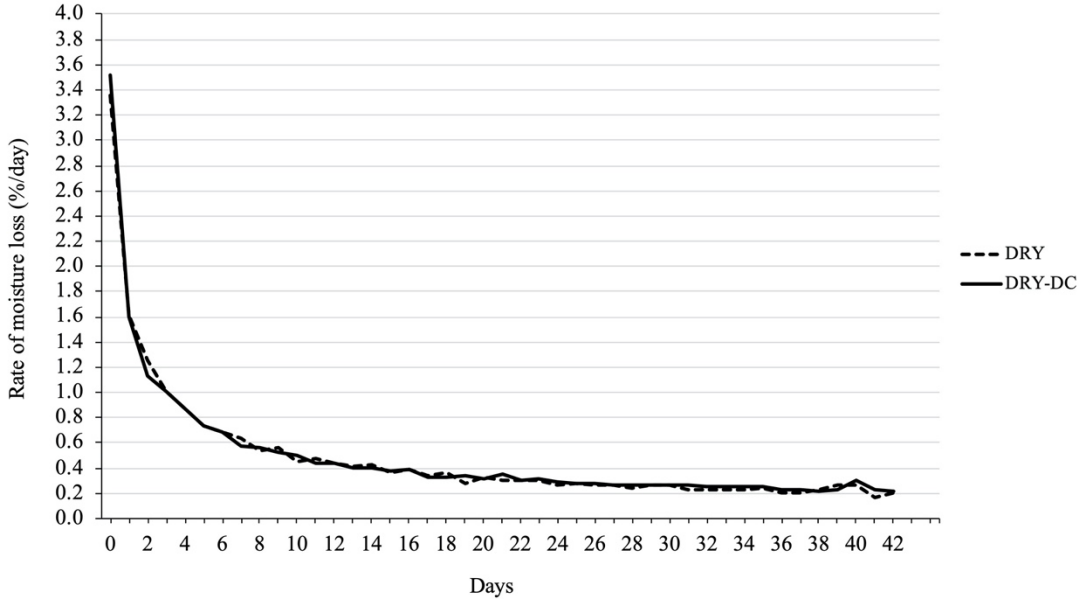


Figure 2. Effect of ultimate pH (Dark cutters [DC] vs. Normal) on rate of moisture loss of strip loins dry aged for 42 days (*P*-value = 0.51; SEM = 0.08).

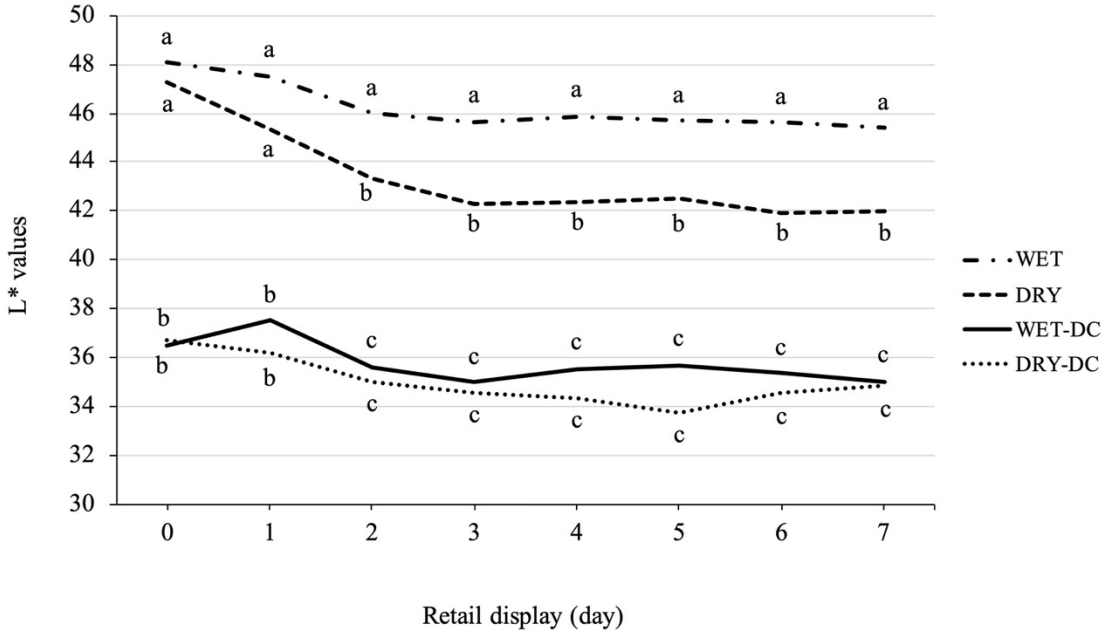


Figure 3. Effect of ultimate pH (Dark cutters [DC] vs. Normal) and aging method (Wet vs. Dry) on lightness (L*) values of strip loins steaks aged for 42 days through 7 d of retail display. [a-c]Means within a day with different superscripts are different ($P < 0.05$).

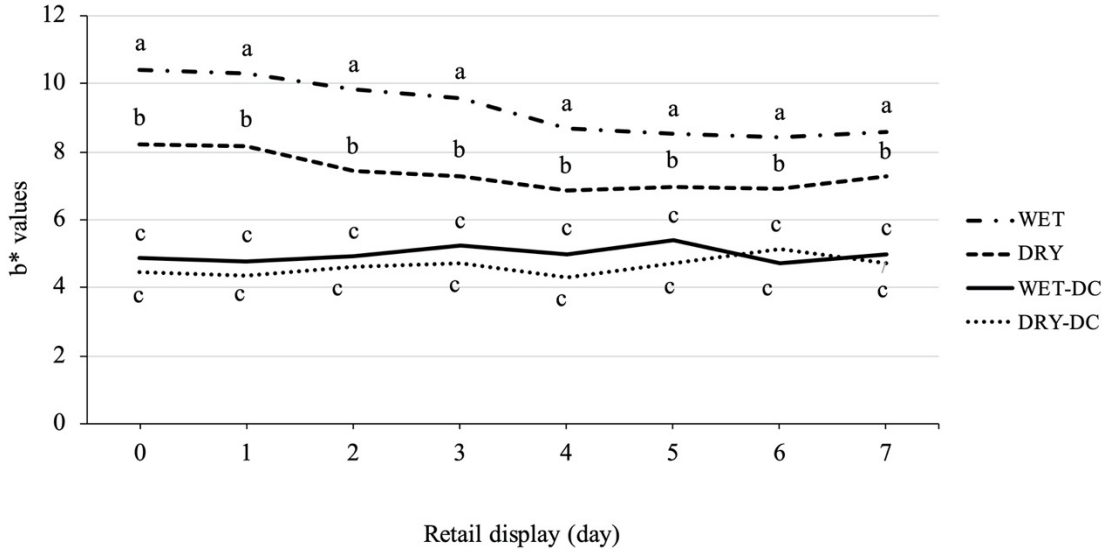


Figure 4. Effect of ultimate pH (Dark cutters [DC] vs. Normal) and aging method (Wet vs. Dry) on yellowness (b*) values of strip loins steaks aged for 42 days through 7 d of retail display. [a-c]Means within a day with different superscripts are different ($P < 0.05$).

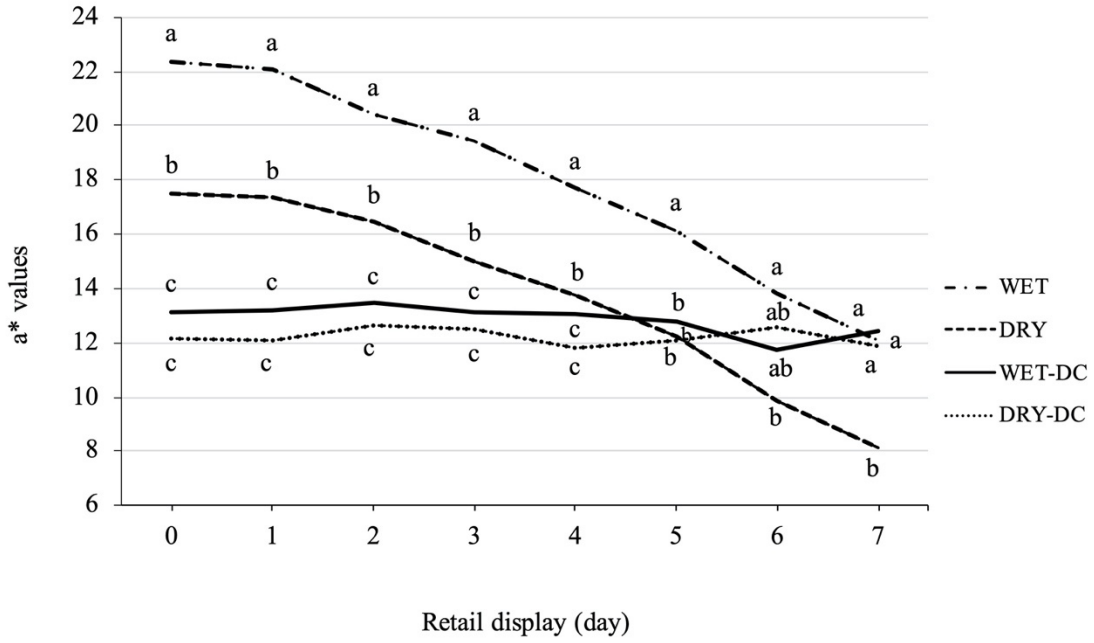


Figure 5. Effect of ultimate pH (Dark cutters [DC] vs. Normal) and aging method (Wet vs. Dry) on redness (a*) values of strip loins steaks aged for 42 days through 7 d of retail display. [a-c]Means within a day with different superscripts are different ($P < 0.05$).

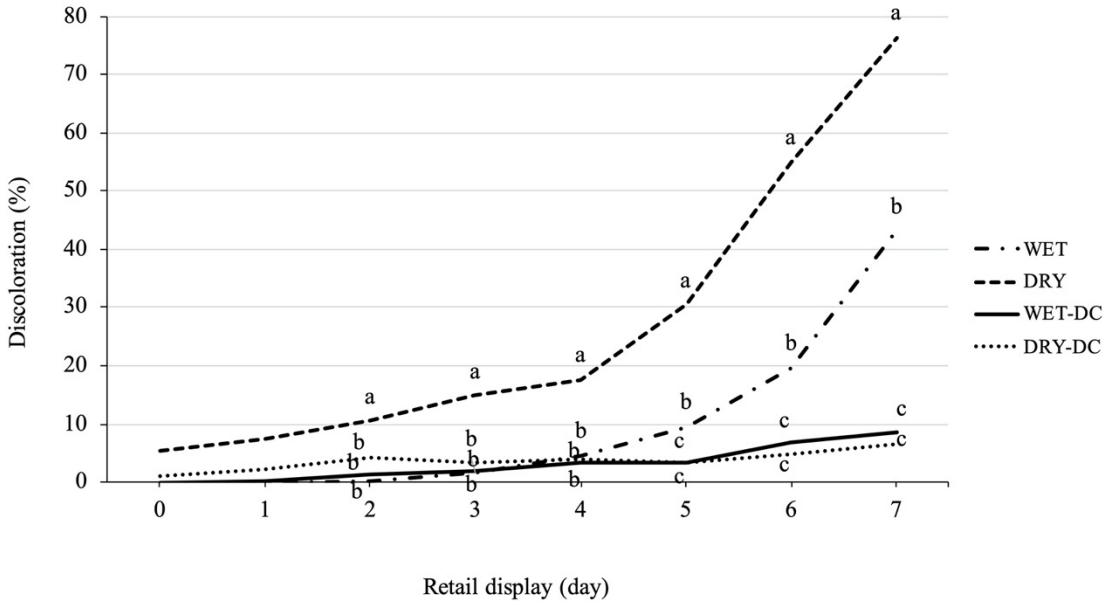


Figure 6. Effect of ultimate pH (Dark cutters [DC] vs. Normal) and aging method (Wet vs. Dry) on discoloration (%) of strip loins steaks aged for 42 days (d) through 7 d of retail display. [a-c]Means within a day with different superscripts are different ($P < 0.05$).

RECOMMENDATIONS FOR FUTURE RESEARCH

The altered flavor characteristics of dry-aged beef are often attributed to two primary factors: concentration and creation of flavor. Concentration of flavor compounds occurs as a consequence of moisture loss through drying, which makes the flavor stronger and more intense. Creation of flavor is more complex. It seems that some of the flavor changes in dry-aged beef are the result of concentration of flavor precursors (especially amino acids) that can react with compounds found in the Maillard reaction to produce meat odorants. Part of the flavor enhancement in dry-aged beef has been attributed to oxidation of proteins and fats (creation of flavor) during aging. Of course, the very nature of dry aging exposes beef to oxygen and increases in oxidized compounds also occur. Under normal conditions, consumers typically perceive oxidized flavors in a negative way. Imagine if anaerobic dry aging would promote desirable flavor development with minimal negative notes and a more desirable microbiome. What if you could achieve dry aging without the oxidation? Wouldn't that improve flavor desirability of dry-aged beef? And, if it's just a matter of concentrating flavor precursors, couldn't you dramatically reduce aging time by accelerating moisture loss? Unfortunately, there are no published studies that have compared the effects of aerobic and anaerobic conditions on dry-aged beef flavor. Additionally, other dry aging regimes with different fan speeds still need to be tested to investigate the effects of accelerated moisture loss on flavor development in dry-aged beef.

In summary, based on the findings of the current studies some suggestions for future research can include:

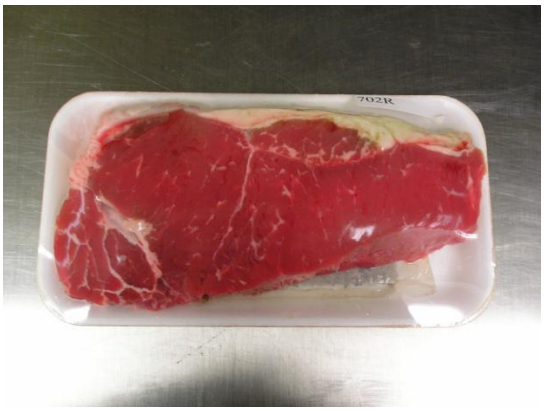
1. Conduct experiments to evaluate the effects of anaerobic and aerobic dry aging on sensory traits of dry-aged beef to obtain a deeper understanding of the effects of oxygen exposure on flavor development in dry-aged beef.
2. Determine which flavor compounds are associated with anaerobic and aerobic dry aging to investigate how the flavor development process occurs in dry-aged beef. This project could provide a conceptual foundation for development of flavor in dry-aged beef under anaerobic and aerobic conditions. Therefore, this could lead to an optimized dry aging guideline for flavor development and consistency, in addition to helping improve the consumer eating experience.
3. Evaluate the microbial communities, including bacterial and mold species, of aerobic and anaerobic dry-aged beef to understand how their growth on the crust can contribute to the unique flavors of dry-aged beef.
4. Explore the effects of degree of doneness on perception of dry-aged beef flavor as the reduced moisture in dry-aged beef may make perception of flavor differences more difficult to detect.
5. Alter the dry aging conditions so that moisture loss can be accelerated, in an attempt to determine if speed of moisture loss impacts flavor development.

The findings of these studies could provide the basis for development of an effective dry aging beef guideline to address flavor inconsistency and alter the way beef is dry aged to improve eating satisfaction of consumers.

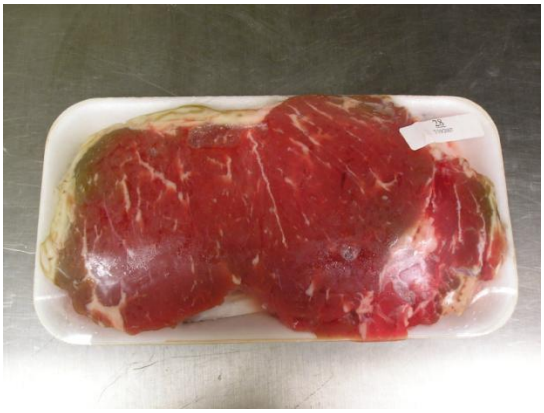
APPENDIX I

Visual guide for percentage surface discoloration (Senaratne-Lenagala, 2012)

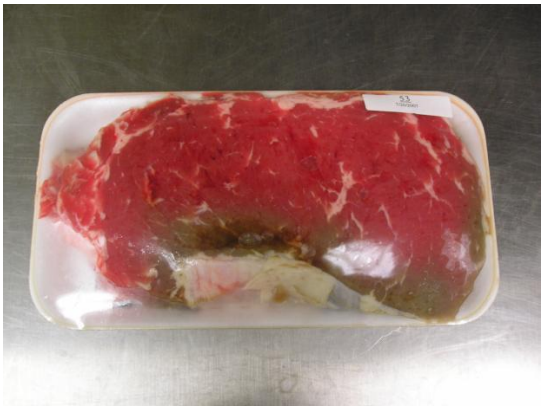
0%



5%



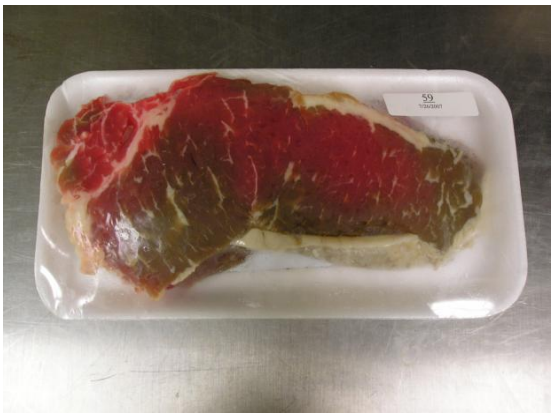
10%



20%

30%

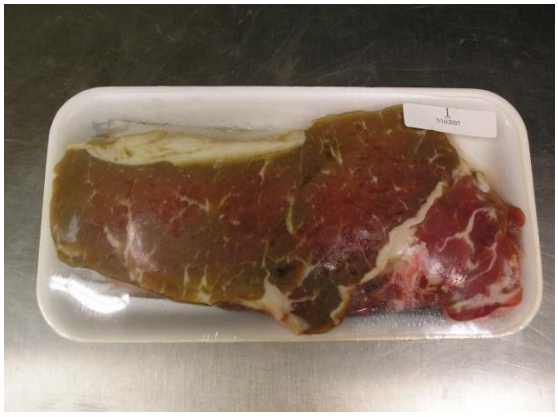
40%



50%

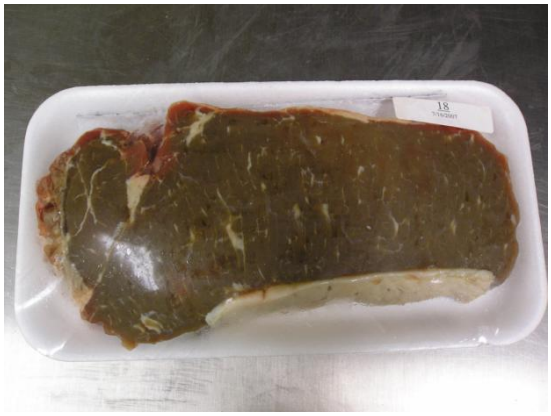
60%

70%



80%

90%



100%

APPENDIX II

Lipid Oxidation Thiobarbituric Acid Assay Protocol

(Ahn, Olson, Jo, Chen, Wu, and Lee, 1998)

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

Stock Solution: Dilute 99 µl TEP (97%) bring volume to 100 mL ddH_20

Working Solution: Dilute stock solution to 1:3 (TEP Solution:ddH_20) ($1x10^{-3}M$)

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

15% TCA (w/v) and 20 mM TBA (MW 144.5) reagent in ddH_20.

Dissolve 2.88 g TBA in warm ddH_20 first, then add TCA (150g) and ddH_20 to 1L

BHA (ButylatedHydroxyAnisole) Stock Solution:

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

10g BHA dissolved in 90 mL ethanol (90%) + 5mL ddH_20

Standards: In duplicate

		Moles of TEP
Blank:	1 ml ddH_20	
Standard 5:	0.1 mL working TEP + 1.90 mL ddH_20	($5x10^{-5}M$)
Standard 4:	1 mL Std. 5 + 1 mL ddH_20	(2.5x10-5M)
Standard 3:	1 mL Std. 4 + 1 mL ddH_20	($1.25x10^{-5}M$)
Standard 2:	1 mL Std. 3 + 1 mL ddH_20	($.625x10^{-5}M$)
Standard 1:	1 mL Std. 2 + 1 ml ddH_20	($.3125x10^{-5}M$)

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 ddH_20

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 for 10 min.
- Centrifuge tubes at 2000×g for 15 min.
- Transfer duplicate aliquots of 200 µl 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 (10^6/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 $72.063x10^6$ = mgs Malonaldehyde/kg of tissue

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