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Enhancement of Dry-Aged Beef Quality by Dietary Supplementation of High Levels of Vitamin E

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Enhancement of Dry-Aged Beef Quality by Dietary Supplementation of High Levels of Vitamin E

by

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The objective of this experiment was to determine if dietary supplementation of high levels of vitamin E could delay the development of off-flavors and increase color stability in dry-aged beef strip loins. Crossbred cattle (n = 150; 10/pen) were grain-finished with the dietary addition of 2,200 international units of vitamin E (α-tocopherol) per head per day for 100 days. One low-Choice carcass (n = 12) was selected from 12 of the 15 pens and right (or left) side strip loins were collected. Low Choice control carcasses (n = 12) were randomly selected from commercial, fed-cattle production to serve as controls. Strip loins were randomly assigned to wet or dry aging for 42 days. Lipid oxidation was measured using thiobarbituric acid reactive substances (TBARS) on day 0 (24 h after harvesting) and 42 of aging and after 8 d of retail display (post-aging). Free amino acids were measured on day 0 and day 42 of aging. Percentage discoloration was rated daily during 14 days of simulated retail display. A colorimeter measured L*, a*, and b* values. On day 42, control dry-aged and control wet-aged loins had the highest TBARS values and vitamin E wet and dry-aged loins had lower TBARS values (P = 0.043). After 8 days of retail display post-aging a trend was found. Control dry-aged steaks had the highest TBARS values and vitamin E wet and dry-aged steaks tended to have lower TBARS values (P = 0.085). From the 30 free amino acids analyzed, 27 of them increased during aging for 42 days and 14 free amino acids increased after dry aging compared to wet aging (P < .05).

There was a three-way interaction for discoloration between vitamin E inclusion (control vs high vitamin E), aging type (dry vs wet aging), and retail display day ($P < 0.0001$). Wet-aged controls discolored fastest, followed by dry-aged controls and wet-aged high vitamin E samples. High vitamin E dry-aged samples had the lowest discoloration. There were aging type -by-days of retail display and aging type-by-vitamin E inclusion interactions for a* values ($P < .0001$ and $P = 0.0104$, respectively). Generally, vitamin E inclusion samples maintained higher redness values for longer times. Principal component analysis suggests that control dry-aged samples tend to have higher negative flavor notes. Samples from beef supplemented with high vitamin E (wet and dry) and control wet aged samples, tended to cluster around positive flavor notes such as roasted, umami, and smokey/charcoal. These data indicate the feeding high doses of vitamin E reduces oxidation and improves flavor of dry-aged beef compared to the general population of fed cattle.

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TABLE OF CONTENTS

INTRODUCTION

Muscle is a heterogeneous system that is composed of multiple structures with specific functions. Because of its structural and physiological complexity, muscle can undergo major physiochemical changes postmortem, including pH decline, inhibition, or activation of endogenous proteolytic enzymes, and much more. Storage conditions greatly affect the organoleptic attributes of meat because it directly influences the postmortem rate of reactions. A unique way of modifying the composition of meat is through dietary supplementation. This process allows the control of intrinsic factors and the opportunity to produce a more stable meat product.

Postmortem meat aging is a very efficient way of improving meat tenderness by means of proteolytic activity. There two major types of aging: wet aging and dry aging. During wet aging, meat is stored in a vacuum package for a prolonged amount of time. Dry aging is essentially storing meat without any packaging. During dry aging, meat is exposed to oxygen, air movement, and a variable relative humidity at refrigerated temperatures for an extended amount of time. The result is a product with less water content, improved tenderness, and a complex flavor. Recently, dry aged beef has started to move from high-end restaurants to retail. Consumers are looking for the best eating experience and are willing to pay more for unique quality. Dry aged beef is known for having sophisticated flavor and tender texture. Due to the nature of dry aging (which induces oxidation of lipids, pigments and depletes antioxidants) meat that has been dry-aged does not have a long-lasting shelf life. The environment in retail display is highly oxidative, due to light, temperature changes, and other factors. Oxidation of proteins and lipids is responsible for the unique flavor of dry aged beef; however, excess oxidation can

result in negative flavor profiles, which are unacceptable for consumers. This is a challenge considering the product is more expensive due to the reduced weight due to moisture loss and increased trim loss that occur after dry aging. It is postulated that intervention is needed to suppress the oxidation while maintaining the positive quality attributes of dry-aged beef.

It has been proven that feeding α tocopherol can reduce the rate of oxidation in meat. However, there is not enough evidence to suggest that vitamin E can suppress oxidation of dry-aged beef. The objectives of this research were: 1) to evaluate the lipid oxidation of wet and dry aged beef feed high doses of α tocopherol aged for 42 days; 2) to use α-tocopherol to protect beef flavor during extended aging; 3) to test if high doses of vitamin E can improve color stability during 14 days of retail display of wet or dry-aged beef.

REVIEW OF LITERATURE

Dry Aging History

Meat is highly perishable because of its water, protein, and fat composition. In most cases it requires transportation before it reaches the final consumer. Physical interventions are necessary to avoid bacterial (pathogenic and spoilage) growth. This can be done by controlling the temperature, reducing the water activity in the meat system, increasing the salt content, or adding a protective package. There are many other strategies to reduce bacterial and fungal growth; however, refrigeration has been the most common means for reducing microbial spoilage in meat products worldwide since the late eighteen-hundreds. Although there were refrigerated rail cars before the 1890's, Swift & Company (Chicago, IL, U.S.A.) pioneered the first effective refrigerated rail car for meat transportation in the 1890's. This company was one of the first to utilize the railroad distribution network to transport cold dressed meat (Yeager, 1970). Meat was hung in a specialized wagon that contained ice to keep a constant refrigeration temperature. These were the first recorded cases of what today is known as dry-aged meat. Furthermore, dry aging was done before it was documented, because meat used to be preserved at cold weather conditions before the invention of refrigerating systems (Zhou et al., 2010). The dry aging process occurs when meat is stored in a cold environment without any barrier between the product and the air. The difference in water content between the meat and the air produces a dehydration effect on the product. When primal cuts were stored and distributed in the past, these were subjected to dry, cold air and different environments for a prolonged amount of time, which affected the products' sensory attributes.

A large part of the meat in the United States was distributed unpackaged in a refrigerator car or a refrigerated truck until the 1960's. The invention of the vacuum package changed meat distribution abruptly (Savell, 2008). Research was done to evaluate the effect of vacuum packaged meat on consumer acceptance, microbial growth, and weight loss. The usage of vacuum packages resulted in less overall weight loss and reduced microbial plate counts, without a significant difference in tenderness (Minks and Stringer, 1972). Vacuum packages remove the oxygen surrounding the meat, in a process defined as wet aging. Since oxygen is one of the major oxidants, the usage of these vacuum packages extends shelf-life by reducing lipid and myoglobin oxidation. Oxygen is also necessary for aerobic bacterial metabolism; hence, excluding oxygen from the system promotes anaerobic bacterial growth. Wet aging can be considered a hurdle technology because it reduces and controls bacterial proliferation by using a combination of techniques at the same time (Zhou et al., 2010). These include removing oxygen and adding a protective barrier from the environment. Vacuum bags reduce aerobic plate counts; however, many pathogenic bacteria are anaerobes or facultative anaerobes. These organisms do not reproduce rapidly at refrigerated temperatures but can multiply if the product is not properly handled. Common pathogens that require little or no oxygen to reproduce include *Escherichia coli, Listeria monocytogenes* and *Salmonella* (Mor-Mur, 2010).

Current Trends

There has been an increase in interest in dry aging, specifically in the high-end restaurant industry (Technology update, Australia 2010). Additionally, there is a niche market for those individuals that prefer dry aged beef and are willing to pay extra

(Dashdori et al., 2016). Retailers are constantly searching for innovative ways of making their product unique, and dry aging has been successful at meeting consumer needs (Smith et al., 2007).

Parameters of Dry Aging

Dry aging is a process that involves many factors. Critical parameters in dry aging are temperature, time, relative humidity (RH), air flow and light presence/type. The most common ranges for these factors are 1-6 C,14-40 days, 50-98% RH, air flow of 0.2-2.5m/s, and yes or no for light presence, respectively (Dashdorj et al., 2016; Terjung et al., 2020). In contrast with wet aging, dry aging does not include a protective package. This may create the idea that dry-aged products are not safe. However, during the dry aging process, the growth of non-pathogenic external bacterial and fungal populations may reduce pathogenic bacterial growth. Due to the presence of air and moisture from the environment, there is an increase in growth of total bacteria, lactic acid producing bacteria (LAB) and fungi (Ryu et al., 2018). It is known that bacteria and fungi compete for resources and can suppress growth of other bacteria, including pathogenic strains (Saavedra, 1995). Therefore, the increase of total bacteria on the surface of the meat acts as a protection against potential pathogens. Ryu et al (2018) found the absence of B. *cereus*, S. *aureus*, L. *monocytogenes*, E. *coli* O157:H7, and *Salmonella* sp. in beef loins after 60 days of dry aging with 75% relative humidity at 1-4 C. This suggests that although dry-aged beef does not have a protective physical barrier during aging, it contains a biological defense complex. Additionally, the inside of the muscle is essentially sterile.

Aging, by definition, is time dependent. There is no specific number of days that are required for dry aging. It has been shown that beef strip loins dry aged only for 7 days had increased negative organoleptic attributes including metallic and bloody flavors. However, extended dry aging for 14 and 21 days reduced unfavorable flavors, and improved positive sensory attributes such as brown roasted and beefy flavors (Campbell et al., 2001). Yield is a critical aspect to consider in dry aging. Increased dry aging time results in lower yield. This mass reduction is not because of increased trim loss but by means of evaporative water loss (DeGeer et al., 2009). Perry (2012) stated that dry aged loins aged between 50 and 80 days had improved flavor complexity. Loins aged for 120 days had no significant taste differences compared to loins aged 50 and 80 days and had lower juiciness ratings. There is an observable pattern in the discussion of appropriate time for dry aging. This process should not be done for a short period of time nor an exceedingly prolonged amount of time. Laster et al. (2008) found that dry aged loins steadily increased in overall like ratings for 14, 21, 28 and 35 days of aging, while wet aged loins decreased in overall like ratings from day 14 to 21 and remained steady through day 35th. The U.S. Meat Export Federation guidelines (2017) suggest that beef should be dry aged for at least 17 days, and that beef can be dry aged for 35 days without developing any off flavors. Just as any other food product that requires time to develop flavor, there is no exact aging time that works for every specific circumstance. The appropriate time for dry aging can be determined by balancing out yield costs and flavor preference.

Temperature can have a significant effect on many factors, including proteolytic activity, microbiology, and lipid oxidation. Dry aging has been done at low temperatures,

such as -0.05 to 1 C (Smith et al., 2007; Perry, 2011), intermediate temperatures; 2 C (Campbell et al., 2001; Gruber et al., 2006; DaSilva et al., 2020; Ribeiro et al., 2021), and relatively high temperatures such as 3 C (DeGeer et al., 2009). Kim et al. (2016) compared the effects of dry aging beef loins (*M. longissimus lumborum*) at 2 temperatures, 1 C and 3 C with two air velocities; 0.2m/s and 0.5m/s. L* values (which represent lightness in color) were significantly lower in loins aged at 3 C compared to loins aged at 1 C. Additionally, sensory analysis evaluation revealed that loins dry-aged at 3 C and 0.2m/s with 49%RH, resulted in enhanced eating quality attributes.

Besides oxygen presence, temperature and time, another important extrinsic factor that affects dry aged beef is relative humidity. The higher the relative humidity, the more bacteria can grow. At 85% relative humidity, samples can start developing high microbial counts and decay odor after 21 and 42 days of aging (Bernardo et al., 2020). Additionally, with this amount of water in the air, meat does not dry as effectively as with a lower relative humidity (Bernardo et al., 2020). Contradictory to Bernardo et al. (2020), Ribeiro (2020) found that relative humidity only affects the initial drying rate of beef and not the end water content. Although relative humidity can influence initial drying rate, it may not have an impact on visual quality characteristics. Ribeiro et al. (2021) found that relative humidity of 50%, 70% and 85% had no significant effect on percent discoloration of dry-aged beef during 7 days of retail display compared to wet-aged beef. Unlike Bernardo (2020), Ribeiro et al. (2021) did not have to dispose of samples dry-aged for 42 days at 85% relative humidity. This could be due to multiple factors. However, the most prominent difference was that Ribeiro et al. (2021) aged beef in a specialized dry aging chamber called the Agenator, which allows for meticulous control of important

parameters. This aging system not only protects the meat from an unstable refrigerated environment, but it also can record real time weight and temperature while controlling, relative humidity and fan speed (Lau et al., 2019). Very little research has focused on the outcomes of different relative humidity (RH) levels on dry aged beef. Lee et al. (2017) compared the effects of dry aging beef loins with 75%RH and 85%RH and found no significant effects on fat and water content. Nonetheless, the sensory analysis results presented higher flavor, juiciness, and overall acceptability for samples aged at 75%RH. This finding concurs with Ribeiro (2020) in which trained panelists from Texas A&M University (College Station, Texas) performed a descriptive sensory analysis on samples dry aged 42 days at 2◦C with 50%RH, 70%RH, and 85%RH. Positive organoleptic attributes were found in samples with 50%RH, including beefy, umami, buttery and roasted. Negative flavors such as fishy, rancid, metallic, and sour were observed in samples with 70%RH and 85%RH. This indicates that beef dry aging is a delicate process and subtle modifications greatly affect the product. Thus, a combination of different aging conditions will result in a final product with a different flavor and texture profile according to these circumstances.

Dry Aging Expenses

Meat products are sold by weight. Drying the meat can greatly compromise the revenue collected. Dry aging not only reduces the weight of the meat, but it also creates an outer dry crust on the product that needs to be removed before the product can be consumed. Removing this crust subtracts more mass from the product and takes longer time to process than regular wet-aged beef (Laster et al., 2008). It has been estimated that for dry-aged beef to cover the cost of weight loss, USDA Choice dry-aged beef would

have to cost about 18.8% more than USDA Choice wet-aged beef (Smith et al., 2008). However, this may be an underestimate as the price of dry-aged beef is directly related to moisture and trim loss. The lower the total yield, the greater the cost. This increase in price is mainly because of drying time, weight loss (from drying and crust removal) and increased processing time. Another prospect to consider is that not all muscles dry at the same rate, and their spatial position greatly affects the rate of dehydration. It has been reported that dry aging of muscle groups can result in the over-drying of outer muscles, which lowers consumer acceptance (Smith et al., 2014). Moreover, trained panelists have found the presence of putrid flavor profiles closer to the outer dry crust (Smith et al., 2014). This not only reduces the number of beef primals that can be successfully aged, but it also makes it difficult to know exactly how much to trim from a dry-aged product. Another disadvantage of dry aging is that it reduces the a* values (which represent redness) during retail display, and increases the lipid oxidation (Kim et al., 2016; Ribeiro et al., 2021). However, Mancini and Ramanathan (2014) found that extended aging reduces mitochondrial oxygen consumption rate, resulting in increased initial a* values. Although dry aging increases lipid oxidation and reduces a* values during retail display, the a* changes are not perceived by consumers for two or more days of retail display (Ribeiro et al., 2021). This may aid dry-aged beef appearance during its initial blooming.

Flavor

Beef dry aging is often described as an art more than a science because of all the complexity involved in this process. Additionally, detailed information about dry aging is specific to the parameters applied in the experiments. For these reasons, processors tend to decide the optimal conditions based on previous batch experiences and outcomes

rather than scientific data. Dry aging can be compared in many ways to artisanal cheese or wine making. There are many important factors to consider, and producers will not know if their production parameters were adequate until the product is finished. Similarly, dry aging is a complex process applied to a complex system. There is evidence of positive and negative outcomes of dry aging (Dashdorj et al., 2016). Flavor rather than tenderness is the main reason for dry-aging, and it is the most important element that separates wet-aged from dry-aged beef (Kim et al., 2016).

Although there is evidence suggesting the presence of off-flavors in dry-aged beef (Smith et al., 2014), it also has flavor profiles that consumers enjoy. Meats that undergo this process are appreciated because they have enhanced beefy flavor and juiciness compared to wet-aged beef (Campbell et al., 2001). Dry-aged beef is more expensive than wet-aged beef, making it a luxury good. Although not all consumers are familiar with aged beef flavor, consumers that prefer dry-aged beef are willing to pay more for those specific flavor notes (Sitz et al., 2005). Because the dry-aged beef is not consumed as much as wet-aged beef, it is challenging to objectively analyze consumer acceptance. Many elements can influence beef flavor, such as breed, sex, reproductive status, diet, processing and many more. Additionally, multiple dry aging factors can also affect dry-aged beef flavor.

To further understand the importance of flavor in dry-aged beef, it is necessary to analyze flavor and the flavor compounds present in meat. Because the brain can interpret multiple stimuli at a given time, flavor can be defined as the sensation created by chemical compounds in the mouth in combination with sight and smell inputs (Reineccius, 2005). However, texture also creates impressions in the brain that start in the

mouth. Therefore, it is complicated to analyze flavor without considering texture. The process of creating a certain perception in the mouth and how it is translated into an idea or flavor is not fully understood, but it can be hypothesized (Deibler and Delwiche, 2004). Scientists have isolated compounds using gas chromatography and mass spectrometry to create models of what flavors are associated with specific foods or smells. Flavor is a significant factor in meat sensory interpretation. Because flavor compounds are created by chemicals, it is necessary to analyze beef composition. Raw meat (top loin steak) on average is composed of ~70% water, ~ 22% protein, ~7% fat and ~1% ash. (Smith et al., 2011). The most variable component is fat; higher quality grades will contain more fat. Meat products are consumed cooked. This is mainly because of food safety and for flavor development. During the cooking process, the proportion of meat components change due to the loss of water and fat solubilization. Reducing the water content inherently increases the fat present in the system. Thus, the flavor of cooked meat is more intense that raw meat. Additionally, thermal energy induces chemical reactions between proteins and fats that create many different flavor compounds.

Flavor Compounds in Meat

Hot foods have a greater number of volatile aggregates in comparison to cold foods, which greatly influence overall flavor. Chemical reactions tend to occur rapidly under high heat conditions. A distinctive reaction that happens in cooked meat is the Maillard reaction. This non-enzymatic browning happens when amino compounds adhere to reducing sugars under high heat conditions (Calkins and Hodgen, 2007). Furanthiols and their disulphides are produced by the Maillard reaction or by thermal degradation of

thiamin. Important furanthiols and disulphides in meat flavor are 2-methyl-3-furyl disulphide, 2,5-dimethyl-3-furanthiol, and bis 2-methyl-3-furyl disulphide (Farmer, 1994). Pyrolysis requires high temperatures; this is the chemical decomposition by high temperatures of organic compounds. When meat is cooked, water loss, oxidation, Maillard reaction, pyrolysis decomposition, and many other reactions create the ideal conditions for the formation of many flavors and aromatic compounds. Meat composition can be divided into polar and non-polar compounds. Under heat, polar compounds including sugars and some amino acids tend to produce volatile compounds that are related to meaty flavors. In contrast, volatile compounds that develop when heating non-polar compounds such as fats and oils tend to be associated with species-specific characteristics (Kramlich and Pearson, 1958; Wasserman and Gray, 1965; Mottram, 1998).

There more than 1000 compounds found in meat, and many interact with each other. Many experiments have been done to identify what polar compounds influence beef taste and aroma. Morton et al. (1960) created a patent for a formula that generated meat-like flavor. A combination of sugars and amino acids was boiled to develop meat flavor and aroma. Cysteine (two cysteine molecule attached by a disulfide bond) was determined as an important factor in meat flavor. Extensive research has been done to produce, isolate, and analyze sulfur-containing compounds found in cooked meat. By means of simultaneous steam distillation and liquid chromatography, important sulfur-containing molecules that have been related to positive meat organoleptic attributes such as brothy, gravy and roasted are: methylthiomethanethiol, 1-methithioethanethiol, and 1-

(-2-methyl-3-thienylthio)-ethanethiol. Additionally, 1-(2-methyl-3-furyithio)-ethanethiol has been identified as a potent good-meat aromatic compound (Werkhoff et al., 1990).

Fatty Acid Effect on Flavor

Nonpolar and amphiphile compounds such as fats and phospholipids serve a critical role in flavor development. Species have different digestive systems and are capable of absorbing nutrients in different manners. Monogastric animals tend to incorporate and store fats with the same degree of saturation as when ingested. Ruminants on the other hand, can saturate their fats. Unsaturated fats tend to be fluid at room temperature while saturated fat remains firm at room temperature. The difference in saturation also affects the rate of oxidation and the products produced up on heating. Saturated fatty acids take more time and energy to oxidize, and unsaturated fatty acids oxidize at lower temperatures. This is because double bonds are less stable and require less energy to break. Therefore, species-specific flavors arise from fatty acid composition and degree of saturation (Mottram, 1998; Calkins and Hodgen, 2007). Products derived from fatty acid oxidation induced by heating include aldehydes, ketones, esters hydrocarbons and more. The two main types of hydrocarbon chains found in meat are triglycerides and phospholipids. Triglycerides are essentially an energy reservoir, and phospholipids generally compose cell and organelle membranes. While there is evidence that both triglycerides and phospholipids influence chemical reactions occurring in cooked meat, the most prominent flavor and odor compounds arise from phospholipids interacting with other molecules (Mottram and Edwards, 1982).

Research has been done to separate and analyze both polar and nonpolar volatile compounds by themselves or in the case of amino acids in combination with reducing

sugars (Kramlich and Pearson, 1959; Wasserman and Gray, 1965; Werkhoff et al., 1990; Mottram, 1998). However, when meat is cooked all chemicals are present at the same time in an environment ideal for multiple reactions to occur. The Maillard reaction is the origin for multiple desired meat flavor compounds. Although this reaction can occur only with amino acids and reducing sugars, the presence of lipids can greatly impact the development of other compounds. Whitfield et al. (1988) investigated the effects of adding lecithin (phospholipid) into mixture solutions containing glycine, lysine, cystine and ribose to understand the effect of lipids present in the Maillard reaction process. Lecithin reduced the number of heterocyclic compounds. Additionally, it produced more carbonyl compounds which differ in composition and rate of production of volatile versus nonvolatile compounds. Alkylbenzenes are other volatile compounds that have been isolated and related to roast beef flavor. They are created by the peroxide free radical decomposition of long chain fatty acids. The degree of unsaturation of fatty acids determines which alkylbenzenes are formed during thermal oxidative cyclization (Min et al., 1977). This suggests that the presence and nature of fats can affect the creation of volatile and nonvolatile compounds, acting as conditioners for meat taste and aroma. Thus, the creation of flavor is a combination of many chemical reactions between polar and nonpolar groups that produce unique meat profiles when they interact.

Flavor Development During Dry Aging

Meat aging only expands the complexity involved in flavor and aromatic compound development during cooking. Muscles contain a specialized degradation system composed of many different enzymes. Endogenous proteolytic enzymes found in muscle are considered calcium dependent, meaning they are activated in the presence of

calcium. During aging, the reduced activity of the sarcoplasmic endoplasmic reticulum ATPase allows for the release of calcium via natural diffusion from the sarcoplasmic reticulum. The calpain system is then activated and cytoskeletal proteins are degraded. During aging there is an increase in tenderization due to post-mortem proteolysis (Taylor et al., 1995; Bhat et al., 2018). Protein degradation during aging produces byproducts that are involved in flavor development. It has been shown that beef biceps femoris wet aged for 1 to 30 days and cooked at 120 C increased the number of compounds associated with positive cooked meat aroma and flavor. These compounds are heterocyclic compounds and benzeneacetaldehydes such as 2,3-dimethylthiophene, 2-formyl-3-methylthiophene and pyrazine (Watanabe et al., 2015). Because of the different physical conditions applied to dry aged beef, the meat produces different compounds during aging. There is evidence of significant difference of metabolites present after dry aging when compared to wet aging. Dry aging increases the concentrations of tyrosine, glutamate, tryptophan, phenylalanine, valine isoleucine and others when compared to wet aging of beef. This could be due to differences in rate of protein degradation after concentration due to water loss (Kim et al., 2016). Water evaporation during aging concentrates the meat system This can potentially influence the type of physico-chemical reactions in meat. Lee et al. (2019) investigated the relationship between water loss and free amino acids of wet and dry-aged beef. They found that wet and dry-aged beef contained the same amount of moisture until day 28 when the dry-aged loins started losing moisture significantly. Additionally, dry-aged loins had greater free amino acids and reducing sugars after day 21 compared to wet-aged loins. Using an electronic tongue, dry-aged beef presented higher sourness, saltiness, umami flavors, taste active compounds and volatile

compounds when compared to wet-aged loins. Another ramification of water loss may be the presence of yeasts and molds in dry-aged beef. Dry aged beef foments the growth of fungi. These organisms are capable of proteolytic and lipolytic activities (Kim et al., 2018). Cultures of *Pilaira anomala* (mold) and *Debaryomyces hansenii* (yeast) isolated from dry-aged beef were inoculated in agar plates containing proteins and fats according to the methods of Atanassova et al. (2016). The results from the experiment presented a higher amount of free fatty acids and free amino acids on dry aged beef (Kim et al., 2018).

Oxidation

Dry aged meat is exposed to oxygen. This element can react spontaneously with multiple substances. Oxygen is a highly reactive species. Because of its two unpaired electrons, this molecule is in a high-energy state. Due to entropy, oxygen is constantly attempting to react with other compounds. According to the international union of pure and applied chemistry (IUPAC) Compendium of Chemical Terminology "Gold Book" (2014) oxidation has 3 definitions 1) The removal of one or more electrons from a molecule 2) An increase in the oxidation number of an atom 3) The gain of an oxygen molecule or the loss of hydrogen atoms from an organic substrate. Lipid oxidation is an important type of oxidation that occurs in meat products. In these cases, phospholipids and triglycerides can lose hydrogen molecules thereby losing electrons.

Lipid oxidation in food is the main cause of quality degradation (Shahidi and Zhong, 2010). Meat arises from a living organism that naturally evolved to control oxidative stress in order to maintain homeostasis. After the animal is slaughtered, the muscle system does not have the same means for oxidative control. Oxidation of meat

products can create the development of unpleasant odors and flavors leading to consumer rejection (Lima et al., 2013). Because consumers purchase meat products based on their appearance and smell, oxidation prevention strategies can be implemented to sustain quality and reduce economic loss. Interventions such as diet and animal handling can be done when the animal is alive and common post-harvest strategies applied to meat products to avoid lipid oxidation include the control of oxygen exposure, temperature, light and more (Dominguez, 2019). Controlling oxidation in dry aged meat is complicated. During dry aging conditions such as presence of oxygen, light exposure and time are catalyzers for oxidative reactions.

Lipid peroxidation occurs in a series of complex autoxidation reactions involving free radicals. These reactions can be classified as occurring during initiation, propagation, and termination phases. In the initiation phase, also known as Fenton chemistry, free radicals are created. These compounds can be produced by multiple factors. The most common are atmospheric oxygen, light, and metals. These molecules (and photons) are capable of either charging polyunsaturated fatty acids with energy, affecting their affinity to hydrogen, or abstracting hydrogen atoms, creating a highly reactive free radical with one or more unpaired electrons (Min and Ahn, 2005). Additionally, hydrogens bound to allylic or bis-allylic carbons on polyunsaturated fatty acids are very reactive, and easily abstracted due to the electron resonance between double bonds. The next step in oxidation is the propagation reaction in the presence of oxygen and free radicals form lipid peroxyl radicals. During propagation, polyunsaturated fatty acids react with lipid radicals and oxygen to produce a hydroperoxide and another lipid peroxyl radical. The reaction is autocatalytic because radical products become reactants. Oxidation reactions

break down fatty acid chains into multiple fragments capable of undergoing oxidative chemistry. The extent of the propagation reaction is directly proportional to the amount of double bonds present in the system (Ahmed et al., 2016). The final reaction of autoxidation is termination. During this stage, lipid peroxyl radicals react with each other, producing a final hydroperoxide and oxygen molecule. With no further catalytic products (free radicals) the autoxidation reaction ceases (Kubow, 1992; Min and Ahn, 2005; Ahmed et al., 2016).

Oxidative Quantification

There are many ways to measure oxidation in meat products. These techniques require machinery capable of detecting oxidative substances and byproducts of oxidation. The most common tools include gas chromatography, capillary electrophoresis and high-performance liquid chromatography. A relatively cost-effective and very common assay used by meat and food scientists is thiobarbituric acid reactive substances (TBARS). This analysis measures the amount of malondialdehyde (which is a byproduct of lipid peroxidation) present in a given sample. Two molecules of thiobarbituric (TBA) acid react with a molecule of malondialdehyde (MDA). This reaction produces a pink chromogen which has a greater absorbance at a wavelength of 532 nm. After the reaction between the TBA and MDA, the supernatant is centrifuged and read in a spectrophotometer. The approximate amount of malondialdehyde can be found with a standard curve with known concentrations of MDA, and the units for TBARS are in mg of malondialdehyde per kg of sample (Greenwald, 1985; Ghani et al., 2017).

Peroxide value (PV) is another analysis often used in foods and fats to detect oxidation. It involves dilution of fats or oils in an organic solvent with excess potassium

iodide. The amount of peroxides present is calculated based on the color disappearance (iodide formed) that occurs during a titration reaction with sodium thiosulfate. This assay has considerable limitations. Because peroxides are mostly found in the propagation stage of oxidation, a low (PV) value can indicate both low oxidation and oxidation that has already occurred (Yildiz et al.,2003). Additionally, this method requires a large quantity of organic solvents, making it expensive. During the oxidation of mono and polyunsaturated fatty acids, hydrocarbon chains are broken down. Compounds that have been identified as responsible for off-flavor in fats and oils are aldehydes. These by-products that arise from secondary oxidation can be measured by the anisidine value (AV). This procedure involves the reaction of α-and β-aldehydes with ρ-anisidine in an acetic acid solution which produces a yellow derivative with a maximum light absorbance at 350 nm wavelength (White, 1995). Analyzing fat and oil oxidation is a difficult process. This is because what is being analyzed with TBARS, PV and AV are by-products of primary or secondary oxidation, making it difficult to determine the extent lipid oxidation. A new instrument used today to effectively quantify lipid oxidation is nuclear magnetic resonance (NMR). With this technology scientists are able to detect hydroperoxides, n-alkanals, and aldehydes with a detection limit of 0.01 mmol/kg in less than 5 minutes. Because NMR detects quantity, the ratios between primary and secondary oxidation by-products effectively describe the oxidation status (Merkx et al., 2018).

Antioxidant Classes

Fats and oils are indispensable for the human diet. Many foods contain nutritious lipids that are susceptible to oxidation in the presence of oxygen and light. Avoiding oxidation of foods results in increased shelf life and sustains the quality attributes of the

product. Oxidation occurs in a series of steps. To avoid these, it is necessary to intervene, inducing termination of the oxidative reaction. Antioxidants are synthetic or natural substances that inhibit or delay oxidation. Primary antioxidants are free radical scavengers. Free radicals are necessary to start and propagate oxidation. Reducing the number of reactive lipid peroxyl radicals reduces the autocatalysis of the oxidation reaction. Primary antioxidants work via three mechanisms: hydrogen atom transfer (HAT), single electron transfer-proton transfer (ET), and sequential proton loss. Phenolic compounds are naturally occurring antioxidants. Because of their internal electron resonance, their affinity to both the hydrogen on the hydroxyl group and the electrons is low. In the presence of a free radical, phenolic compounds can deprotonate or donate a hydrogen atom to the free radicals, greatly reducing their reactivity and terminating the chain reaction (Galano and Alvarez-Idaboy, 2013; Michalik et al., 2019). Secondary antioxidants, often referred to as preventive antioxidants, are substances that can indirectly reduce oxidation. The most common modes of action of secondary antioxidants are decomposition of hydroperoxides, chelation of transition metals, oxygen scavengers, primary antioxidant regenerators and more (Karadag et al., 2009; Gupta, 2014).

There are many types of antioxidants, and they can be categorized in many ways. The biological antioxidants (all non-synthetic antioxidants) can be divided into two groups: enzymatic and non-enzymatic. Enzymatic antioxidants break down reactive free radicals. Important enzymes that reduce oxidation (oxidative stress) in mammals are superoxide dismutase and glutathione peroxidase. Non-enzymatic antioxidants include primary and secondary antioxidants which are considered dietary antioxidants.

Antioxidants can also be categorized based on polarity. For example, both vitamin C and vitamin E are non-enzymatic dietary antioxidant vitamins, however, vitamin C is water-soluble and vitamin E is liposoluble (Huang et al., 2005; Nimse and Pal, 2015). Because foods systems vary in structure and composition there is not a single antioxidant that can work for all foods. Primary and secondary antioxidants are often used together to produce a synergistic antioxidant effect. Marinova et al. (2007) studied the effect of α-tocopherol in combination with myricetin on the autoxidation of triacylglycerols of sunflower oil, finding that the combination of both substances delayed autoxidation more effectively than when the compounds were applied by themselves. Another important consideration for antioxidants in foods is flavor. Reducing oxidation in a product needs to occur without negatively affecting the organoleptic attributes of food.

Vitamin E - α Tocopherol

The discovery of vitamins and antioxidants is achieved by the careful observation of natural processes. Evans and Bishop (1922) noticed that rats fed only purified casein, corn starch, lard, and low amounts of dried whole yeast, became sterile without any other significant changes to their body and behavior. They determined that a dietary "factor X" was necessary for reproduction. Rat fertility was regained when the rats were fed fresh green lettuce leaves. In a subsequent experiment to understand the dietary requirements for reproduction, Sure (1923) was the first one to propose the name "vitamin E" to describe the new dietary factor that influences reproduction. In a paper titled "The Unsaponifiable Lipids of Lettuce" Olcott and Mattill (1931) were the first ones to identify the antioxidant capabilities of vitamin E. The name *tocopherol* was recommended to Evans by George Calhoun, a professor of Greek from the University of

California. The name arises from the Greek word *tokos*, meaning offspring and *phero* representing to bear (Bell, 1987).

Vitamin E is a term that includes tocopherols α, β, γ, and δ and tocotrienols α, β, γ, and δ (Brigelius-Flohe and Traber, 1990). The most abundant liposoluble chain-breaking antioxidant from the vitamin E group present in nature is α tocopherol. It can be found in phospholipid membranes of mammalian cells (Webster, 2012). Due to its polarity, α tocopherol is an antioxidant stored with lipids and phospholipids (where oxidation starts). In comparison with commonly used food commercial antioxidants such as rosemary extract and butylated hydroxytoluene (BHT), α tocopherol reacts with peroxyl radicals 200 times faster (Burton and Traber, 1990) than synthetic (BHT). Additionally, α tocopherol reacts much quicker with peroxyl radicals than polyunsaturated fatty acids. Therefore, a single molecule of α tocopherol can protect multiple polyunsaturated fatty acid chains (Burton and Traber, 1990).

Early experiments on muscle-based products suggest the antioxidant effect of vitamin E produced by dietary supplementation of α tocopherol (Marusich et al., 1975; Tsai et al., 1978). Adding α tocopherol in a diet form is an alternative to protect meat products from lipid oxidation. Vitamin E is sold in different chemical forms. Some are naturally occurring denoted by a "d" before their name and some are racemic synthesized, denoted by a "*rac*". The most common in the market include d-α tocopherol, d-α-tocopheryl, all-*rac*-α-tocopherol and all-*rac*-α-tocopheryl (Liu et al., 1995). The National Research Council (NRC; 1984) recommendation for cattle of vitamin E supplementation is 15-60 international units (IU) of vitamin E per kg of dry matter (DM) per day. However, improvements in performance, immunity, and lipid antioxidation have

been observed in cattle fed higher doses than the ones alluded by the NRC (Hill and Williams, 1993). Vitamin E is absorbed in the tissue via complex digestive mechanisms.

Due to its liposoluble nature, vitamin E is solubilized by bile acids and absorbed by the small intestine epithelial cells. Then, it is attached into chylomicrons and transported to the liver via the lymphatic system. From there, the liver re-secretes vitamin E to the blood plasma in the form of very-low-density lipoproteins. Finally, Vitamin E is incorporated in multiple tissues including skeletal muscle, pancreas, liver, adipose tissue and more (Liu et al., 1995). The dietary supplementation of vitamin E needs to be calculated carefully according to the desired outcome in meat products. Hill and Williams (1993) observed rapid depletion of α tocopherol in blood plasma during finishing from cattle that was grass fed and transitioned to a grain diet without vitamin E supplementation. Additionally, cattle that were fed 500 IU/Day and 1,000 IU/Day of vitamin E with their grain diets during finishing steadily increased levels of α tocopherol in blood plasma (Liu et al., 1995). High amounts of α tocopherol in the blood plasma will lead to deposition of α tocopherol in multiple tissues, including adipose tissue and skeletal muscle (Liu et al., 1995). An increase in intramuscular α tocopherol levels has proven to reduce lipid oxidation in meat (Juarez et al., 2012). Lipid oxidation in meat occurs differently based on the extrinsic conditions applied. Steaks are normally sold in an oxygen permeable package that is exposed to light. Therefore, retail display conditions are highly oxidative. Vitamin E has proven to be effective at delaying lipid oxidation during retail display when fed in relatively high amounts. Sanders et al. (1997) found that feeding 1,000 IU/day and 2,000 IU/day reduces MDA concentrations during simulated retail display using an oxygen permeable packaging and exposure to light.

Delaying lipid oxidation by means of antioxidant dietary addition may also delay discoloration in meat because myoglobin oxidation is believed to be closely related to lipid oxidation (Greene et al., 1971). Arnold et al. (1992) analyzed the effects of dietary supplementation of vitamin E on retail display of longissimus lumborum steaks. They found that feeding 300 IU/day for 266 days, 1,140 IU/day for 67 days or 1,200 IU/day for 38 days, successfully delayed meat discoloration, increasing retail display days. Vitamin E affects some of the most important quality attributes of meat, including color and lipid oxidation. It has been found that feeding 500 IU/day for 123 days produces increased α tocopherol in subprimal cuts which creates a product with less lipid oxidation, reduced discoloration and increased acceptable shelf life during retail display when compared to controls (Sherbeck et al., 1995). An important consideration when feeding high levels of α tocopherol is depletion rate. Arnold et al., (1993) found that feeding beef 2,080 IU/d and 3,520 IU/d of α tocopherol for 126 days resulted higher α tocopherol concentration in longissimus muscle and subcutaneous fat compared to beef that were fed 300 IU/d. However, α tocopherol depletion in tissue from beef fed 2,080 and 3,520 IU/d occurred between 196-266 days and depletion of α tocopherol form beef fed 300 IU/d happened 231-309 days. This suggests that excess supplementation of α tocopherol increases deposition of α tocopherol that can endure for a substantial amount of time during postmortem storage before being depleted.

Conclusion

Taking multiple factors into consideration, the dietary supplementation of vitamin E to cattle can potentially reduce economic loss and maintain meat quality through retail conditions. Numerous experiments have found positive effects of vitamin E in meat

products (Marusich et al., 1975; Tsai et al., 1978; Arnold et al., 1992; Sherbeck et al., 1995; Sanders et al., 1997; Faustman et al., 1998; Juarez et al., 2012). However, limited research has been done analyzing the effects of dietary supplementation of vitamin E in dry-aged beef. During dry aging, lipid oxidation is fomented due to aerobic conditions, producing a wide variety of desired flavors. However, dry aging also produces undesired flavors due to over-oxidation of lipids (Terjung et al., 2020). Dietary supplementation of vitamin E could potentially reduce negative flavors generated during dry aging while maintaining the desired flavors of dry-aged beef.

MATERIALS AND METHODS

Experimental Design

This experiment was conducted as a completely randomized design with a 2 x 2 factorial arrangement of treatments design between vitamin E inclusion (control vs vitamin E) and aging type (wet vs dry aging). The experimental unit of the experiment were the beef strip loins (n = 24). Each treatment combination had 6 replicates.

Vitamin E Supplementation

Crossbred Angus cattle were grain finished with the supplementation of 2,200 international units (IU) of vitamin E (α-tocopheryl-acetate) per head per day for the last 100 days of the finishing period. A total of 150 animals (10 animals/pen) were grain finished with vitamin E supplementation.

Sample Collection and Preparation

Longissimus lumborum (beef loins) from USDA low-Choice A-maturity cattle were obtained from Greater Omaha Packing, LLC 24 h post-slaughter (day zero represents samples acquired 24 h after harvest). Twelve of the selected loins were from cattle fed a diet supplemented with high vitamin E content (2,200 IU per head per day) and 12 loins were obtained from the general population of fed cattle at the plant to serve as controls. Commercial cattle in the U. S. are typically fed ca. 50 IU per head per day. Loins were collected and transported to the University of Nebraska-Lincoln Loeffel meat lab. At the meat lab excess subcutaneous fat was removed, so all loins had about 2.0 ± 0.2 cm, and a specific treatment ID was randomly assigned to all 24 loins. From the 12 vitamin E inclusion loins, six were randomly assigned for dry aging and six were for wet aging. From the 12 control loins, six were randomly designated for dry aging and six

were for wet aging. For each loin, two 1.27 cm steaks were taken on day zero, one from the anterior end and one from the posterior end, for laboratory analysis. Steaks were vacuum packaged and frozen at -80 C until further analysis. From the rest of all loins, a 2.40 cm steak was taken from the anterior end for Warner-Bratzler Shear force analysis (WBSF) performed on that same day. However, these data were not used in the experiment due to lack of sample required for future analyses. Thus, a comparison between d 0 and d 42 WBSF was not achieved. All loins chosen for wet aging were re-vacuum packaged with a nylon/polyethylene vacuum bag (Bunzl Processor, Clarity 3-mil, Riverside, MO) on a vacuum packager (Multivac, C 500, Morgan Hill, CA) and the loins chosen for dry aging were placed in the previously sanitized aging chambers.

Wet and Dry-Aging

The 12 loins assigned to wet-aged were vacuum packaged and stored at refrigerated temperatures (3-6 C). Wet aged loins were placed besides the dry aging chambers to ensure equal temperature distribution between wet and dry-aged treatments. Loins assigned for dry aging were placed in the aging chambers created by Lau et al. (2019) with the following conditions: temperature 3-6 C, 50% relative humidity, and 2,200 revolutions per minute fan speed. The spatial orientation of all the control and all the vitamin E inclusion loins in the aging chambers were consistent. The posterior ends of the loins were facing the same sides of their respective aging chambers to ensure equal aging conditions. The lights in the refrigerator were off to prevent photooxidation. Both wet and dry-aged loins were aged for 42 days.

Fabrication

On Day 42, wet aged loins were taken out of the vacuum bags and purge and loin weights were recorded. Right afterwards, dry-aged loins were taken out from the aging chambers, and the hardened outer crust of the loin was removed. Weights of the trim and the loins were recorded. A slicer was used to cut all loins (controls first) into the following steaks anterior to posterior: one 1.27 cm steak to face-off the loin; three 2.40 cm steaks for lab analyses, trained sensory analysis, and retail display analysis; three 2.40 cm steaks for UNL sensory analysis; and one 1.27 cm steak for thiobarbituric acid reactive substances (TBARS) analysis. Subcutaneous fat from the TBARS steak was trimmed off and the longissimus muscle was split into two equal rectangular 2.40 cm by 5.08 cm halves. One of them was immediately vacuum packaged and the other one was placed on a tray covered with oxygen permeable polystyrene film to analyze oxidation after retail display. The remaining portions of the loins were vacuum packaged and frozen at -80 C.

Retail Display and Color Measurements

On day 42 of aging, a 2.40 cm steak from each loin for all treatment combinations (vitamin E inclusion-dry-aged, vitamin E inclusion-wet-aged, control dry-aged and control wet-aged) was placed on a Styrofoam tray (220 mm x 170 mm x 30 mm, Styro-Tech, Denver, CO) and subjected to 14 d of simulated retail display. Originally the retail display was set to 8 d, however, because of the color stability results the retail display simulation was extended to 14 d. This explains the difference between TBARS retail display days and color retail display d. All steaks were wrapped with an oxygen permeable polystyrene film with an oxygen transmission rate of 2.25 ml/cm2/24 h at 23

C (Prime Source, PSM 18 #75003815, Kansas City, MO). The retail display simulation conditions were the same as described by Ribeiro (2020): refrigerated temperatures (3-6 C) and a constant 24 hour 32-W white fluorescent light exposure of 1,000 to 1,800 Lux of illuminance. During this period, an objective color measurement was taken from all steaks at 11 AM every day. The measurement was made with a Minolta Chromameter CR-400 (Minolta Camera Company, Osaka Japan) spectrophotometer taking the average of 6 readings across the steaks through the oxygen permeable film. The measurement diameter was 8 mm with an 11 mm illumination diameter. The assigned illuminant was D65 and the standard observer was 2 degrees. The Minolta Chromameter was calibrated before the daily readings using a blank calibration plate with the plastic film used to package the steaks. This objective color measurement obtained the following readings: L* = psychometric lightness; a* = positive values represent redness and negative values represent greenness; and b* = positive values represent yellowness and negative values represent blueness. Additionally, five graduate students were asked to evaluate the subjective percent discoloration of the steaks daily. All students were asked to arrive at the same time every day and rank all steaks for discoloration from 0 to 100%. Images representing examples of the percent discoloration from 0% to 5% and 10% increments until 100% discoloration were available for students to serve as a baseline.

Oxidative Rancidity

On day zero (24 h after harvest), a 1.27 cm steak was sliced from the anterior end of all loins. The subcutaneous fat was trimmed off and longissimus muscle was isolated while the sample was still cold. Samples were then vacuum packaged with a nylon/polyethylene vacuum bag (Bunzl Processor, Clarity 3-mil, Riverside, MO) and

frozen at -80 C. At the time of the analysis, the isolated longissimus samples were thawed in their respective bags for 8 h at 3-6 C. Once the samples were thawed, the longissimus was cut into cubes and quickly frozen in liquid nitrogen. Then, the samples were pulverized, with a heavy-duty food blender (Waring Commercial, 700S, Torrington, CT). All samples were bagged, tagged and frozen at -80 C. On day 42 (after wet or dry aging), 1.27 cm steaks were sliced from the anterior side from all loins to remove the outer surface. The other 6 steaks were sliced (as described above) and a 1.27 cm steak was sliced for TBARS. One half of the 1.27 cm steak was designated to analyze oxidation on day 42 after wet or dry aging and the other was for oxidation analysis after an additional 8 days of retail display. The longissimus muscle was isolated from the 1.27 cm steak and two rectangles of 2.40 x 5.08 cm were cut from them. For TBARS on day 42 the steak was frozen and pulverized. For the TBARS analysis following retail display, the samples were placed on a tray covered with oxygen permeable polystyrene film and exposed to light during retail display days (same retail display materials and conditions as samples shown above). After the retail display, all samples were pulverized. All samples were analyzed using an adaptation of the TBARS protocol presented by Buege and Aust (1978). Five grams of the pulverized sample were transferred into 50 mL conical tubes and 14 mL of double-deionized (dd) water were added with 1.0 mL of butylated hydroxyanisole to avoid oxidation during the procedure. The samples were then homogenized for 15 seconds using a polytron (Kinmatica, AG, Lucern, Sweeden). The solution was centrifuged at 2,000 x G for 5 min. One mL of the supernatant was transferred into 15 mL conical tubes. Two mL of thiobarbituric acid/trichloroacetic acid were added to the conical tubes. The samples were then incubated at 70 C for 30 min in a

water bath. Samples were cooled after incubation in a cold-water bath at 0 C for 10 minutes. Then, the samples were centrifuged at 2,000 x G for 15 minutes. A volume of 200 μL of the supernatant solution were transferred into 96 well plates and read in a spectrophotometer (Epoch, Biotek, Winooski, VT) with a wavelength of 540 nm. The concentration of malonaldehyde was calculated using the absorbance of a standard curve with known concentrations of malonaldehyde.

Water Activity

Steaks from day zero (1.27 cm from the posterior side), and 2.40 cm steaks from day 42 were frozen at -80 C. Samples were thawed at 3-6 C for 8 h in their respective vacuum bags. Once all samples were thawed, the longissimus muscle was isolated, and hand cut into 0.5 by 0.5 cm squares. Thus, the dimensions were 0.5 cm x 0.5 cm x 1.27 cm. The resulting block of meat were cut on the 1.27 cm side, making pieces close to 0.5 cm x 0.5 cm x 0.63 cm. Particles were placed in water activity cups covering the bottom area and maintaining the volume to a specific level denoted on the disposable cups. All steaks were analyzed triplicate (three different samples per steak) with an Aqualab water activity meter (Decagon Devices, Inc., Pullman, WA). Day 42, 2.40 cm steaks were thawed in their respective vacuum bags. Once thawed the samples were cut in into squares of 0.5 by 0.5 cm, making a rectangle prisms 0.5 cm x 0.5 cm by 2.40 cm. The longitudinal 2.40 cm side was hand cut in half twice (in 1/4ths) producing particles with a volume of about 0.5 cm x 0.5 cm x 0.63 cm. The average of the triplicates was used to estimate the water activity. Left over samples from day 0 and 42 were vacuum packaged and stored at -80 C for possible future laboratory analysis.

Sensory Analysis

Sensory analysis was conducted at Texas A&M University (College Station, Texas). A Group of 6 trained sensory panelists analyzed a 2.40 cm steak from all loins/treatment combinations. There were 6 control dry-aged steaks, 6 control wet-aged steaks, 6 vitamin E inclusion dry-aged steaks, and 6 vitamin E inclusion wet-aged steaks. An independent random number ID tag was placed to all steaks to avoid bias. Samples were cooked to an internal temperature of 30 C and then flipped to and cooked to a final temperature of 71 C on a stainless steel, electric stove top grill (Manufacturing International, StarMax, 536GF, St. Louis, MO). Raw and cooked weights and temperatures were recorded. Samples were wrapped in aluminum foil and placed in a Bain Marie warmer (APW, Wyott W-Vi, Alan, TX) at 63 C for no longer than 20 minutes before being served to the trained panel. Steaks were cut into 1.27 x 1.27 x 2.40 cm cubes (the 2.40 dimension was the natural thickness of the steak). Samples had no visible fat nor connective tissue.

The institutional review board of use of humans in research at Texas A&M approved the research. Panelists (with more than 400 hours of experience) were asked to rank a variety of flavors from 0 to 15 (0 = none and 15 = extremely intense). These flavors include beef flavor, brown/roasted, bloody, fat-like, bitter, salty, sweet, umami, metallic, burnt, buttery, cardboardy, animal hair, green, fishy, panty, rancid, stale, soapy, putrid, and juiciness. References were provided to panelists continually during training and testing.

Free Amino Acid Analysis

Amino acids were extracted from 1 g of sample in 5 mL of distilled water, followed by a purification through a 0.2 μm and a 3 kDa membrane filter. Amino acids were combined with 200 nmol/mL of norvaline to serve as an internal standard and derivatized by propyl chloroformate (Kaspar et al., 2008). Isooctane was used to extract the derivatives and the solution was transferred to a 2 mL glass vial with a fixed insert (Agilent Technologies, Santa Carla, CA) for gas chromatography-mass spectrometry determination. The 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). Using helium as a gas carrier (flux = 1 mL/min), and inlet temperature of 250 C, the ion source and quadrupole were 310, 240 and 180 C, respectively. The oven temperature was first programed at 110 C and increased to 320 C within 11 min. To identify and quantify amino acids authentic standards were used for an internal calibration. Concentration values were presented in μmol/kg.

Statistical Analysis

The experiment was conducted as a completely randomized design with a 2 x 2 factorial arrangement of treatments, with main effects of aging type (wet and dry) and vitamin E inclusion (high and regular vitamin E), and their interaction. Data were analyzed using the generalized linear mixed model (GLIMMIX) procedure from statistical analysis software (SAS v 9.4; Cary, NC). Malondialdehyde determined by the thiobarbituric acid analysis (TBARS) was analyzed as a 2 x 2 factorial with repeated

measures over time (0, 42 and 42 + 8 days of retail display), using the Toeplitz covariance structure. Least square means of the significant effect treatment-by-aging ($P < 0.05$) was further determined on each day (analysis of variance by day). Similarly, objective color measurements (LAB) and subjective percent discoloration (as an average of 5 panelists) were assessed for 14 consecutive days and analyzed as repeated measures over time, using the Toeplitz covariance structure. Least square means of the effect of vitamin E inclusion (high vitamin E vs control)-by-aging type (wet vs dry) ($P < 0.05$) were determined by day. Lastly, the main effects of aging type, vitamin E inclusion, day, and their interactions on amino acid concentration in samples at day 0 and 42 were analyzed by the GLIMMIX procedure from SAS (v 9.4). The effect of day (zero vs 42) was significant ($P < 0.05$) in most amino acids, so least square means of amino acid concentration by aging type (wet vs dry) were obtained on day 42 with day 0 as a covariate. Least square means were considered significantly different at a $P < 0.05$. In each group of responses, multiple testing was adjusted by the Tukey-Kramer post-hoc test. Flavor data were analyzed as a 2 x 2 factorial using analysis of variance with the GLIMMIX procedure from SAS (v 9.4). To determine the relationship between trained sensory attributes and treatment characteristics principal component analysis was used and results were presented as a biplot. Trends were considered with *P*-values between 0.05 to 0.10.

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MANUSCRIPT

Running head: Vitamin E supplementation on dry-aged beef quality

Enhancement of Dry-Aged Beef Quality by Dietary Supplementation of High Levels of Vitamin E

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Abstract

The objective of this experiment was to determine if dietary supplementation of high levels of vitamin E could avoid the development of off-flavors and increase color stability in dry-aged beef strip loins. Crossbred cattle (n = 150; 10/pen) were grain-finished with the dietary addition of 2,200 international units of vitamin E (α-tocopherol) per head per day for 100 days. One low-Choice carcass (n = 12) was randomly selected from 12 of the 15 pens. Low Choice control carcasses (n = 12) were randomly selected from commercial, fed-cattle production to serve as controls. Strip loins were randomly assigned to wet or dry aging for 42 days. Lipid oxidation was measured using thiobarbituric acid reactive substances (TBARS) on day 0 (24 h after harvesting) and 42 of aging and after 8 d of retail display (post-aging). Free amino acids were measured on day 0 and day 42 of aging. Percentage discoloration was rated daily during 14 days of simulated retail display. A colorimeter measured L*, a*, and b* values. On day 42, control dry-aged loins had the highest TBARS values and vitamin E inclusion wet and dry-aged loins had lower TBARS values, wet-aged controls were not different from the other treatments (P = 0.043). After 8 days of retail display post-aging a trend was found. Control dry-aged and control wet-aged steaks had the highest TBARS values and vitamin E inclusion wet and dry-aged steaks tended to have lower TBARS values (P = 0.085). From the 30 amino acids analyzed, 27 of them increased after aging for 42 days and 14 amino acids increased after dry-aging when compared to wet-aging (P<.05). There was a three-way interaction for discoloration between vitamin E inclusion (control vs vitamin E), aging type (dry vs wet aging), and retail display day (P <0.0001). Wet-aged controls discolored fastest, followed by dry-aged controls and wet-aged vitamin E inclusion

samples. Dry-aged vitamin E inclusion samples had the lowest discoloration. There were aging type-by-day and aging-by-vitamin E inclusion interactions for a* values (P <.0001 and P = 0.0104, respectively). Generally, vitamin E inclusion samples sustained higher redness values for longer times. Principal component analysis of trained sensory data suggests that control dry-aged samples tend to have higher negative flavor notes. Samples from beef supplemented with vitamin E (wet and dry) and control wet aged samples, tended to cluster around positive flavor notes such as roasted, umami, and smokey/charcoal. These data indicate the feeding high doses of vitamin E reduces oxidation and improves flavor of dry-aged beef compared to the general population of fed cattle.

Introduction

Flavor, rather than tenderness, is the main reason for dry-aging, and it is the most important element that separates wet-aged from dry-aged beef (Kim et al., 2016). Dry aging acquires its unique flavor profile during prolonged exposure to oxygen. As a result, lipids and pigments oxidize more compared to traditionally wet-aged beef (Ribeiro et al., 2021). Research suggests positive (Campbell et al., 2001; Laster et al., 2008; Kim et al., 2016; Lee et al., 2019) and negative (Smith et al., 2014; Bernardo et al., 2020; Terjung et al., 2020) outcomes of dry aging. However, the results are specific to the parameters applied in the experiments. The contradictory results in the literature may arise because of the differences in the ratio between desired and undesired flavor development during dry aging. Dry-aged beef develops more free amino acids and reducing sugars related to positive flavors compared to wet-aged beef (Kim et al., 2016; Lee et al., 2019). These compounds are reactants in the Maillard reaction where amine compounds adhere to

reducing sugars under high heat conditions creating a wide variety of flavor compounds (Calkins and Hodgen, 2007). However, dry aging may result in greater lipid oxidation compared to wet-aged beef (Bernardo et al., 2020; Ribeiro et al., 2021). Oxidation of meat products can create the development of unpleasant odors and flavors leading to consumer rejection (Lima et al., 2013).

Antioxidants provide an opportunity to favor the positive outcomes of dry aging and reduce the negative effects produced by lipid oxidation. Early experiments on muscle-based products support the antioxidant effect of vitamin E produced by dietary supplementation of α tocopherol (Marusich et al., 1975; Tsai et al., 1978). Because of its non-polar nature vitamin E is incorporated in multiple tissues including skeletal muscle, pancreas, liver, and adipose tissue (Liu et al., 1995). An increase in intramuscular α tocopherol levels has been shown to reduce lipid oxidation in meat (Juarez et al., 2012). Additionally, vitamin E has been effective at delaying lipid oxidation during retail display when fed in relatively high amounts (Sanders et al.,1997). Dietary supplementation of a high amount of vitamin E (2,200 IU per head per day) could provide a pool of antioxidant capacity capable of enduring through the oxidative dry-aging process, producing a dry-aged product with reduced negative quality characteristics while maintaining the desired flavors of dry-aged beef. The objective of this experiment was to determine if dietary supplementation of a high level of α tocopherol could reduce development of off-flavors generated during dry aging, therefore, manipulating the ratio of positive and negative flavors and enhancing the quality of dry-aged beef.

Materials and Methods

Experimental Design

This experiment was conducted as a completely randomized design with a 2 x 2 factorial arrangement of treatments between vitamin E inclusion (control vs high vitamin E) samples and aging type (wet vs dry aging). The experimental unit was the beef strip loins (n = 24). Each treatment combination had 6 replicates.

Vitamin E Supplementation

Crossbred cattle were grain finished with the supplementation of 2,200 international units (IU) of α-tocopheryl-acetate (vitamin E) per head per day for 100 days. A total of 150 animals (10 animals/pen) were grain finished with vitamin E supplementation.

Sample Collection and Preparation

Longissimus lumborum (beef loins) from USDA low-Choice, A-maturity cattle were obtained from Greater Omaha Packing, LLC 24 h post-slaughter (day zero represents samples acquired 24 h after harvest). Twelve of the selected loins were from cattle fed a diet supplemented with high vitamin E content and 12 loins were obtained from the general population of fed cattle at the plant to serve as controls. Commercial cattle in the U. S. are typically fed ca. 50 IU per head per day. On arrival at University of Nebraska-Lincoln Loeffel meat lab, excess subcutaneous fat was removed, so all loins had about 2 ± 0.2 cm mm, and a specific UNL ID was randomly assigned to all 24 loins. From the 12 vitamin E inclusion loins, six were randomly assigned for dry aging and six were for wet aging. From the 12 control loins, six were randomly designated for dry

aging and six were for wet aging. From all loins, two 1.27 cm steaks were taken on day zero, one from the anterior end and one from the posterior end, for laboratory analysis. All steaks were vacuum packaged and frozen at -80 C. From the rest of all loins, a 2.40 cm steak was taken from the anterior end for Warner-Bratzler Shear force analysis (WBSF) to be performed on that same day. However, this data was not used in the experiment due to the need for more steaks from each loin to complete laboratory analyses. All loins chosen for wet aging were re-vacuum packaged with a nylon/polyethylene oxygen resistant vacuum bag (Bunzl Processor, Clarity 3-mil, Riverside, MO) packaged with a vacuum packager (Multivac, C 500, Morgan Hill, CA) and the loins chosen for dry aging were placed in the previously sanitized aging chambers.

Wet and Dry-Aging

Wet-aged loins were stored in a vacuum package at a refrigerated temperature of 3-6 C, and they were placed besides each aging chamber. Loins assigned for dry aging were placed in the aging chambers created by Lau et al. (2019) with the following specifications: temperature 3-6 C, 50% relative humidity, and 2,200 revolutions per minute fan speed. The spatial orientation of all the control and all the vitamin E inclusion loins in the aging chambers were consistent. The posterior ends of the loins were facing the same sides of their respective aging chambers to ensure equal aging conditions. The lights in the refrigerator were off to prevent photooxidation. Both wet and dry-aged loins were aged for 42 days. Wights were recorded for all dry-aged loins every 5 min for 42 days.

Fabrication

On Day 42, wet-aged loins were taken out of the vacuum bags and purge and loin weights were recorded. Right afterwards, dry-aged loins were taken out from the aging chambers, and the hardened outer crust of the loin was removed. Weights of the trim and the loins were recorded. A slicer was used to cut all loins (controls first) into the following steaks anterior to posterior: one 1.27 cm steak to face-off the loin; three 2.40 cm steaks for lab analyses, trained sensory analysis, and retail display analysis; three 2.40 cm steaks for UNL sensory analysis; and one 1.27 cm steak for thiobarbituric acid reactive substances (TBARS) analysis. Subcutaneous fat from the TBARS steak was trimmed off and the longissimus muscle was split into two equal rectangular 2.40 cm by 5.08 cm halves. One of them was immediately vacuum packaged and the other one was placed on a tray covered with oxygen permeable polystyrene film with an oxygen transmission rate of 2.25 mL/cm^2/24 h at 23 (Prime Source, PSM 18 #75003815, Kansas City, MO) to analyze oxidation after retail display. The rest of the loins were vacuum packaged and frozen at -80 C.

Retail Display and Color Measurements

On day 42 of aging, a 2.40 cm steak from vitamin E inclusion and control loins was placed on Styrofoam trays (220 mm x 170 mm x 30 mm, Styro-Tech, Denver, CO) and subjected to a retail display simulation for 14 days. Originally the retail display was set to 8 d, however, because of the color stability results the retail display simulation was extended to 14 d. This explains the difference between TBARS retail display days and

color retail display d. All steaks were wrapped with an oxygen permeable polystyrene film with an oxygen transmission rate of 2.25 mL/cm^2/24 h at 23 C (Prime Source, PSM 18 #75003815, Kansas City, MO). The retail display simulation conditions were the same as described by Ribeiro (2021): refrigerated temperatures (3-6 C) and a constant 24 hour 32-W white fluorescent light exposure of 1,000 to 1,800 Lux of illuminance. During this period, an objective color measurement was taken from all steaks at 11 AM every day. The measurement was made with a Minolta Chromameter CR-400 (Minolta Camera Company, Osaka Japan) spectrophotometer taking the average of 6 readings across the steaks through the oxygen permeable film. The measurement diameter was 8 mm with an 11 mm illumination diameter. The assigned illuminant was D65 and the standard observer was 2 degrees. The Minolta Chromameter was calibrated before the daily readings using a blank calibration plate covered with the plastic film used to package the steaks. This objective color measurement obtained the following readings: L* = psychometric lightness; a* = positive values represent redness and negative values represent greenness; and b* = positive values represent yellowness and negative values represent blueness. Additionally, five panelists were asked to evaluate the subjective percent discoloration of the steaks daily. All panelists were asked to arrive at the same time every day and rank all steaks for discoloration from 0 to 100%. Images representing examples of the percent discoloration from 0% to 5% and 10% increments until 100% discoloration were available for students to serve as a baseline. Linear approximation was used to calculate when samples reached 20 percent discoloration and 14.5 a* values. Based on the assumption that discoloration and a* reduction was linear, the rate from the last day before reaching the thresholds was used, and using the rate proportion, the exact

time point in days was calculated. Total color change ΔE was calculated between d 1 of retail display and d 14. Using the square root of the sum of the squared differences between d 14 L* a* and b* and d 1 L*a*b*.

Oxidative Rancidity

On day zero (24 h after harvest), a 1.27 cm steak was sliced from the anterior end of all loins. The subcutaneous fat was trimmed off and longissimus muscle was isolated while the sample was still cold. Samples were then vacuum packaged with a nylon/polyethylene oxygen resistant vacuum bag (Bunzl Processor, Clarity 3-mil, Riverside, MO) and frozen at -80 C. The isolated longissimus samples were thawed in their respective bags for 8 h at 3-6 C. Once the samples were thawed, the longissimus was cut into cubes and quickly frozen in liquid nitrogen. Then, the samples were pulverized, with a heavy-duty food blender (Waring Commercial, 700S, Torrington, CT). All samples were bagged, tagged and frozen at -80 C. On day 42 (after wet or dry aging), 1.27 cm steaks were sliced from the anterior side from all loins to remove the outer surface. The other 6 steaks were sliced (as described above) and a 1.27 cm steak was sliced for TBARS. One half of the 1.27 cm steak was designated to analyze oxidation on day 42 after wet or dry aging and the other was for oxidation analysis after an additional 8 days of retail display. The longissimus muscle was isolated from the 1.27 cm steak and two rectangles of 2.40 x 5.08 cm were cut from them. For TBARS on day 42 the steak was frozen and pulverized. For the TBARS analysis following retail display, the samples were placed on a tray covered with oxygen permeable polystyrene film and exposed to light during retail display days (same retail display materials and conditions as samples shown above). After the retail display, all samples were pulverized. All samples were

analyzed using the TBARS protocol presented by Hart et al. (2019). The concentration of malonaldehyde was calculated using the absorbance of a standard curve with known concentrations of malonaldehyde.

Water Activity

Steaks from day zero (1.27 cm from the posterior side), and 2.40 cm steaks from day 42 were frozen at -80 C. Samples were thawed at 3-6 C for 8 h in their respective vacuum bags. Once all samples were thawed, the longissimus muscle was isolated, and hand cut into 0.5 by 0.5 cm squares. Thus, the dimensions were 0.5 cm x 0.5 cm x 1.27 cm. The resulting block of meat were cut on the 1.27 cm side , making pieces close to 0.5 cm x 0.5 cm x 0.63 cm. Particles were placed in water activity cups covering the bottom area and maintaining the volume to a specific level denoted on the disposable cups. All steaks were analyzed in triplicate (three different samples per steak) with an Aqualab water activity meter (Decagon Devices, Inc., Pullman, WA). Day 42, 2.40 cm steaks were thawed in their respective vacuum bags. Once thawed the samples were cut into squares of 0.5 by 0.5 cm, making a rectangle shape 0.5 cm x 0.5 cm by 2.40 cm. The longitudinal 2.40 cm side was hand cut in half twice (in 1/4ths) producing particles with a volume of about 0.5 cm x 0.5 cm x 0.63 cm. The average of the triplicates was used to estimate the water activity. Left over samples from day 0 and 42 were vacuum packaged and stored at -80 C for possible future laboratory analysis.

Sensory Analysis

Sensory analysis was conducted at Texas A&M University (College Station, Texas). A Group of 6 trained sensory panelists analyzed a 2.40 cm steak from all

treatment combinations. There were 6 control dry-aged steaks, 6 control wet-aged steaks, 6 vitamin E inclusion dry-aged steaks, and 6 vitamin E inclusion wet-aged steaks. An independent random number ID tag was placed to all steaks to avoid bias. Samples were cooked to an internal temperature of 30 C and then flipped to and cooked to a final temperature of 71 C on a stainless steel, electric stove top grill (Manufacturing International, StarMax, 536GF, St. Louis, MO). Raw and cooked weights and temperatures were recorded. Samples were wrapped in aluminum foil and placed in a Bain Marie warmer (APW, Wyott W-Vi, Alan, TX) at 63 C for no longer than 20 minutes before being served to the trained panel. Steaks were cut into 1.27 x 1.27 x 2.40 cm cubes (the 2.40 dimension was the natural thickness of the steak). Samples had no visible fat nor connective tissue.

The Institutional Review Board for Use of Humans in Research at Texas A&M approved the research. Panelists (with more than 400 hours of experience) were asked to rank a variety of flavors from 0 to 15 (0 = none and 15 = extremely intense). These flavors include beef flavor, brown/roasted, bloody, fat-like, bitter, salty, sweet, umami, metallic, burnt, buttery, cardboardy, animal hair, green, fishy, panty, rancid, stale, soapy, putrid, and juiciness. References were provided to panelists continually during training and testing.

Free Amino Acid Analysis

Amino acids were extracted from 1 g of sample in 5 mL of distilled water, followed by a purification through a 0.2 μm and a 3 kDa membrane filter. Amino acids were combined with 200 nmol/mL of norvaline to serve as an internal standard and derivatized by propyl chloroformate (Kaspar et al., 2008). Isooctane was used to extract

the derivatives and the solution was transferred to a 2 mL glass vial with a fixed insert (Agilent Technologies, Santa Carla, CA) for gas chromatography-mass spectrometry determination. The 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). Using helium as a gas carrier (flux = 1.1 mL/min), and inlet temperature of 250 C, the ion source and quadrupole were 310, 240 and 180 C, respectively. The oven temperature was first programed at 110 C and increased to 320 C within 11 min. To identify and quantify amino acids authentic standards were used for an internal calibration. Concentration values were presented in μmol/kg.

Statistical Analysis

The experiment was conducted as a completely randomized design with a 2 x 2 factorial treatment design, with main effects of aging method (wet and dry) and vitamin E inclusion (high vitamin E and control), and their interaction. Data were analyzed using the Generalized Linear Mixed model (GLIMMIX) procedure from statistical analysis software (SAS v 9.4; Cary, NC). Malondialdehyde determined by the TBARS procedure was analyzed as a 2 x 2 factorial with repeated measures over time (0, 42 and 42 + 8 days of retail display), using the Toeplitz covariance structure. Least square means of the significant effect treatment-by-aging ($P < 0.05$) was further determined on each day (analysis of variance by day). Similarly, objective color measurements (LAB) and subjective percent discoloration (as an average of 5 panelists) were assessed for 14 consecutive days and analyzed as repeated measures over time, using the Toeplitz

covariance structure. Least square means of the effect of vitamin E inclusion by aging type ($P < 0.05$) were determined by day. Lastly, the main effects of aging type, vitamin E inclusion, day, and their interactions on amino acid concentration in samples at day 0 and 42 were analyzed by the GLIMMIX procedure from SAS (v 9.4). The effect of day (0 vs 42) was significant ($P < 0.05$) in most amino acids, so least square means of amino acid concentration by aging type (wet vs dry) were obtained on day 42 with day 0 as a covariate. Least square means were considered significantly different at a $P < 0.05$. In each group of responses, multiple testing was adjusted by the Tukey-Kramer post-hoc test. Flavor data were analyzed as a 2 x 2 factorial using analysis of variance with the GLIMMIX procedure from SAS (v 9.4). To understand the relationship between trained sensory attributes and treatment characteristics principal component analysis was used and results were presented as a biplot. On all analyses, trends were considered with *P*-values between 0.05 to 0.10.

Results and Discussion

Lipid Oxidation

Lipid oxidation was measured using TBARS on day 0 (24 h after harvest), day 42 of wet or dry aging, and day 8 of retail display following aging. On day 0, control and vitamin E inclusion loins did not differ (Figure 1) in lipid oxidation ($P = 0.936$). On day 42, control dry-aged loins had the highest TBARS values and vitamin E inclusion wet and dry-aged loins had lower TBARS values, control wet aged samples were not different from the other treatments ($P = 0.043$). A trend was found ($P = 0.085$) after 8 days of retail display post-aging, control dry-aged had the highest TBARS values and vitamin E inclusion wet and dry-aged steaks tended to have lower, control wet aged samples were

not different from the other treatments (Figure1) TBARS values. These results concur with Kim et al. (2016), Passetti et al. (2020) and Ribeiro et al. (2021). Faustman et al. (1989 b), Sherbeck et al. (1995) and Sanders et al. (1997) also found less lipid oxidation during retail display from beef fed diets supplemented with vitamin E after 6 and 7 days of retail display, respectively. Retail display simulation was done for 8 days (after wet or dry aging) and the average malondialdehyde content of vitamin E wet-aged and vitamin E dry-aged were 4.94 and 2.56 mg/Kg, respectively. The results from this experiment suggest that dietary supplementation of 2,200 IU per head per day of vitamin E provided enough antioxidant capacity to reduce lipid oxidation after 42 days of wet or dry extended aging and after simulated retail display conditions. Reduction in lipid oxidation by dietary supplementation of vitamin E represents an opportunity for dry-aged beef, increasing its shelf life and potentially reducing the off-flavors generated by lipid oxidation during and after dry aging.

Free Amino Acids

Free amino acids were measured on day 0 (24 h after harvest) and day 42 (after wet or dry aging). From the 30 amino acids analyzed, 27 of them increased (Table 1) after aging for 42 days ($P < .05$). Amino acids such as 3-hydroxyproline, 4-hydroxyproline, α-aminobutyric acid, alanine, glutamine, glycine, histidine, isoleucine, biphenyl, proline, serine, sarcosine, valine and beta-aminoisobutyric acid had greater concentrations in samples dry-aged for 42 days compared to samples wet-aged for 42 days (Table 2). Likewise, Lee et al. (2019) found more free amino acids in samples from dry-aged beef compared to wet-aged samples. Amino acids play an important role in flavor because they can react with reducing sugars to create multiple flavor compounds

(Calkins and Hodgen, 2007). Additionally, free amino acids can react with non-polar compounds (fats and oils), changing the profile of volatiles formed under high heat conditions (Whitfield et al., 1988). Differences in free amino acids between wet-aged and dry-aged beef affect the sensory attributes of the product. Therefore, a greater amount of free amino acids may be responsible for the unique positive flavor development of dry-aged beef. Because no differences were detected in free amino acids between samples from cattle fed high vitamin E and control samples, it appears that dietary supplementation of vitamin E does not decrease the quality or intensity of the flavors generated during prolonged dry aging.

Sensory Analysis

Trained sensory panelists found similar individual flavor attributes between control and vitamin E inclusion samples. Vitamin E inclusion effects were found for fishy flavor (P = 0.0181), with control dry-aged samples and control wet-aged samples presenting the highest values. Both vitamin E inclusion dry-aged and wet-aged produced the lowest fishy flavor scores. An aging type-by-vitamin E inclusion effect was detected on the spoiled/putrid flavor (P = 0.0369), primarily because this flavor note was only detected for the control dry-aged samples. This suggests that off-flavors can be generated during prolonged dry aging and that vitamin E is capable of reducing the development of these particular flavor notes making it an exceptional practice to avoid off-flavor in dry aged beef.

Because flavor is a sum of multiple flavors acting at a given time, it is necessary to analyze their combined effect on consumer perception. Principal component analysis was performed with the sensory analysis data (Figure 5). A relationship was established

between vitamin E inclusion (high vitamin E vs Control) and aging type (dry vs. wet) combination effects on specific flavor characteristics. Two major factors were determined and responsible for 34.97 percent of the variation. Factor 1 represented the “beefy” flavors such as beef ID, roasted, smokey and salty (Table 4). Factor 2 was related to the flavors that can be created by oxidation such as cardboardy, fishy, warmed over, green, liver-like, rancid, and refrigerator stale (Table 4). Control dry-aged samples tended to have the highest factor 2 scores (Figure 5), meaning they had negative flavor notes associated with lipid oxidation. As stated above, vitamin E was able to reduce lipid oxidation in dry aged beef which likely explains the reduced association to oxidized flavors. Because the free amino acids between vitamin E inclusion and control samples were not different, the vitamin E inclusion dry-aged samples were expected to be associated with flavors similar to the ones of wet-aged control and wet-aged vitamin E inclusion. The reduction of the potent negative flavors of dry-aged beef was achieved by dietary supplementation of vitamin E. The absence of these negative flavors appears to allow dry-aged beef from cattle supplemented with high levels of vitamin E to possess positive flavor attributes very similar to traditionally wet-aged beef. These results suggest that it is possible to manipulate the ratio of negative and positive flavor notes during dry aging to produce a product with fewer negative flavor attributes, allowing the positive flavors that are created during extended dry aging to appear.

Objective Color

Values for L* had vitamin E inclusion by aging type effects on 3 of the 14 days of retail display ($P<.0001$). Collectively, all treatments decreased in lightness as the retail display progressed. Thus, the longer the time the product spends under retail display

conditions, the darker it becomes. An aging type (wet vs. dry)-by-vitamin E inclusion (control vs. high vitamin E) effect was observed (P = 0.0017) on b* values for the last 7 days of retail display. Vitamin E inclusion wet-aged samples decreased more on average for b* values compared to the rest of the treatments. No aging type -by-vitamin E inclusion effects were observed for a* values. A vitamin E inclusion-by-days of retail display effect was significant (P<.0001) with vitamin E inclusion samples exhibiting more red color than controls for 10 of the 14 days of retail display (Figure 4). Total color change (Δ E) values were evaluated between day 0 and 14 of retail display (Table 5). The vitamin E inclusion effect (P = 0.0002) was significant, suggesting that control samples changed more in color during 14 days of retail display compared to vitamin E inclusion samples. Faustman et al. (1989 a) also reported higher a* values and lower total color change during retail display on steaks from cattle supplemented with vitamin E. These results indicate that dietary supplementation of 2,200 IU of vitamin E per head per day improved redness and color stability (after 42 days of wet and dry aging) during prolonged retail display conditions.

Holman et al. (2017) suggested that the threshold for 95% consumer acceptability was an a* value equal or above 14.5. Days to reach an a* value of 14.5 were calculated with a mathematical linear approximation based on the assumption that the rate of oxidation was constant. Vitamin E inclusion effects for days to reach a* below 14.5 during retail display were nearly significant (P = 0.0580), with vitamin E inclusion samples taking 5.05 days and controls samples 3.78 days, on average. Dietary supplementation of vitamin E can increase the retail display time of beef products

representing a possible economical revenue opportunity and a potential reduction in waste of meat products.

Subjective Percent Discoloration

Hood and Riordan (1973) described that the ratio of sales (between consumers that consider whether to buy a product or not) between bright red beef and beef with 20% metmyoglobin formation is 2:1, respectively, when discolored beef are present in the batch. Using a mathematical linear approximation, days to reach 20 percent discoloration was calculated based on the assumption that the rate of discoloration was constant. Days to reach 20 percent of discoloration had aging type ($P<.0001$) and vitamin E inclusion ($P<.0001$) effects. Dry-aged samples delayed discoloring on average 7.08 days and wet aged samples discolored in 4.99 days to surpass 20% discoloration. Vitamin E inclusion samples took on average 7.06 days to reach 20 percent discoloration and controls discolored in 5.01 days of simulated retail display. This may represent that both dry-aging and dietary supplementation of vitamin E are effective at increasing the value of beef by adding days of retail display and reducing metmyoglobin formation, providing a more attractive product for a longer time.

There was a three-way interaction (Figure 3) for discoloration between vitamin E inclusion (control vs high vitamin E), aging type (dry vs wet aging) and retail display day ($P< 0.0001$). Wet-aged controls discolored fastest, followed by dry-aged controls and wet-aged vitamin E inclusion samples. Dry-aged vitamin E inclusion samples had the lowest discoloration. Contradictory to Ribeiro et al. (2021), in this experiment dry-aged samples took longer to discolor compared to wet-aged. The observable differences in

discoloration between wet and dry-aged beef could be due, in part, to a concentration effect of vitamin E because of moisture loss.

Conclusion

Dietary supplementation with 2,200 international units (IU) of vitamin E (α-tocopheryl-acetate) per head per day provides enough antioxidant capacity to reduce lipid oxidation in dry-aged beef strip loins aged for 42 days. Additionally, samples from cattle supplemented high levels of vitamin E had improved color stability during retail display and developed less negative flavor attributes when compared to dry-aged samples from a general population of fed cattle in the U.S.

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TABLES

Table 1. Effects of aging time (0 vs 42 d of aging) by amino acid content(mmol/Kg) of beef strip loins.

	Day zero	Day 42	*P*-value	SEM
3-hydroxyproline	0.287	1.615	<.0001	0.076
4-hydroxyproline	0.072	0.463	<.0001	0.031
Lysine	0.020	0.023	0.239	0.001
α -aminobutyric acid	0.013	0.015	0.004	0.001
Alanine	1.062	3.577	<.0001	0.172
Polyamine antiporter	0.018	0.027	0.0109	0.003
Asparagine	0.028	0.115	<.0001	0.008
Aspartate	0.019	0.061	<.0001	0.004
Cysteine	0.012	0.035	<.0001	0.005
Glutamine	0.416	1.846	<.0001	0.116
Glutamic acid	0.228	0.780	<.0001	0.034
Glycine	0.327	1.623	<.0001	0.067
G-protein regulatory	0.023	0.033	<.0001	0.001
Histidine	0.171	0.775	<.0001	0.028
Tyrosine	0.022	0.025	0.1473	0.002
Isoleucine	0.152	0.973	<.0001	0.046
Leucine	0.383	1.945	<.0001	0.088
Lysine	0.224	0.536	<.0001	0.025
Methionine	0.026	0.440	<.0001	0.026
Ornithine	0.091	0.091	0.9534	0.007
Phenylalanine	0.217	1.148	<.0001	0.050
Biphenyl	0.033	0.046	<.0001	0.002
Proline	0.118	0.430	<.0001	0.021
Sarcosine	0.253	0.392	<.0001	0.011
Serine	0.044	0.611	<.0001	0.036
Threonine	0.042	0.509	<.0001	0.030
Tryptophan	0.071	0.234	<.0001	0.011
Tyrosine	0.230	1.164	<.0001	0.054
Valine	0.279	1.794	<.0001	0.085
Beta-aminoisobutyric acid	0.621	1.491	<.0001	0.193

[a-b] Means with different superscripts differ by row (*P*< .05).

Table 2. Comparison of free amino acids (mmol/Kg) of dry-aged vs. wet-aged boneless loins after 42 days of wet or dry aging.

Amino Acid	Wet	SEM	Dry	SEM	*P* - value
3-hydroxyproline	1.269 [b]	0.110	1.961 [a]	0.116	0.0004
4-hydroxyproline	0.355 [b]	0.045	0.570 [a]	0.047	0.0041
Lysine	0.020	0.002	0.023	0.002	0.2585
α -aminobutyric acid	0.010 [b]	0.001	0.017 [a]	0.001	<.0001
Alanine	3.034 [b]	0.248	4.121 [a]	0.261	0.0086
Polyamine antiporter	0.025	0.004	0.032	0.005	0.3470
Asparagine	0.105	0.011	0.125	0.011	0.2180
Aspartate	0.061	0.007	0.058	0.007	0.7564
Cysteine	0.036	0.007	0.034	0.007	0.8632
Glutamine	1.382 [b]	0.172	2.312 [a]	0.180	0.0017
Glutamic acid	0.753	0.049	0.806	0.052	0.4780
Glycine	1.301 [b]	0.094	1.948 [a]	0.099	0.0002
G-protein regulatory	0.033	0.002	0.034	0.002	0.9446
Histidine	0.633 [b]	0.041	0.915 [a]	0.043	0.0002
Tyrosine	0.020	0.003	0.026	0.003	0.1656
Isoleucine	0.865 [b]	0.069	1.083 [a]	0.072	0.0461
Leucine	1.772	0.128	2.119	0.134	0.0798
Lysine	0.499	0.036	0.574	0.038	0.1627
Methionine	0.435	0.037	0.445	0.039	0.8486
Ornithine	0.090	0.006	0.092	0.006	0.8061
Phenylalanine	1.069	0.073	1.226	0.077	0.1621
Biphenyl	0.039 [b]	0.003	0.051 [a]	0.003	0.0178
Proline	0.369 [b]	0.030	0.491 [a]	0.032	0.0138
Sarcosine	0.366 [b]	0.009	0.416 [a]	0.010	0.0018
Serine	0.526 [b]	0.052	0.698 [a]	0.055	0.0376
Threonine	0.439	0.045	0.578	0.048	0.0563
Tryptophan	0.229	0.016	0.236	0.016	0.7907
Tyrosine	1.136	0.078	1.193	0.082	0.6242
Valine	1.600 [b]	0.124	1.988 [a]	0.130	0.0471
Beta-aminoisobutyric acid	1.054 [b]	0.262	1.925 [a]	0.275	0.0343

[a-b] Means with different superscripts differ by row ($P < .05$).

Table 3. Percent weight loss comparison of vitamin E inclusion (high vitamin E vs control) by aging type (wet vs dry aging) effects after fabrication of boneless strip loins.

	Total loss	Trim loss	Water loss	Purge loss
Control Dry-aged	54.91 [a]	30.83 [a]	24.07 [a]	0 [b]
Vitamin E Dry-aged	51.62 [a]	27.55 [a]	24.06 [a]	0 [b]
Control Wet-aged	1.16 [b]	0 [b]	0 [b]	1.16 [a]
Vitamin E Wet-aged	0.60[b]	0 [b]	0 [b]	0.60 [a]
SEM	0.9701	0.9296	0.4531	0.253
Aging effect	<.0001	<.0001	<.0001	0.0022

[a-b] Means with different superscripts differ within column ($P < .05$).

For the dry-aged treatments the outer crust was removed from dry-aged samples. Wet-aged sample weight loss is due to purge.

Table 4. Trained sensory analysis results for vitamin E inclusion (control vs high vitamin E) by aging type (dry-aged vs wet-aged) effects.

Flavor	Control Dry-Aged	Control Wet-Aged	Vitamin E Dry-Aged	Vitamin E Wet-Aged	P-Value	SEM
Animal Hair	0.06	0.00	0.00	0.00	0.3293	0.0277
Barnyard	0.25	0.08	0.06	0.00	0.0484	0.0780
Beef Flavor ID	8.28	7.86	8.19	7.69	0.8924	0.342
Bitter	1.92	2.14	1.81	1.72	0.4069	0.1803
Bloody/Serumy	1.75	1.97	1.56	2.47	0.2278	0.2791
Brown/ Roasted	8.64	8.56	8.75	8.42	0.7351	0.3642
Burnt	0.47^{ab}	0.86 ^{a}	0.47 ^{ab}	0.28^{b}	0.0592	0.1458
Buttery	0.61	0.42	0.67	0.42	0.7711	0.0942
Cardboardy	0.94	0.61	0.50	0.67	0.3577	0.2656
Connective Tissue	11.25	11.36	11.56	11.19	0.3782	0.2620
Fat-Like	2.53	2.97	2.56	3.11	0.7447	0.1683
Fishy	0.47 ^{A}	0.10^{AB}	0.08^{AB}	0.00 ^{B}	0.3156	0.1079
Green	0.33	0.03	0.08	0.00	0.2270	0.0891
Heated Oil	0.06	0.17	0.00	0.06	0.6595	0.0621
Juiciness	8.36	9.06	8.86	9.19	0.5409	0.2902
Liver-Like	0.61	0.53	0.36	0.25	0.9459	0.2021
Metallic	2.31	2.39	2.39	2.36	0.7193	0.1524
Muscle Tenderness	9.78	10.39	10.33	10.33	0.4569	0.4027
Musty-Earthy/Humus	0.33	0.39	0.08	0.19	0.8337	0.1306
Painty	0.03	0.00	0.00	0.00	0.3293	0.0138
Rancid	0.06	0.00	0.00	0.00	0.1295	0.0175
Refrigerator Stale	0.06	0.08	0.06	0.00	0.4753	0.0572
Salty	1.86	1.67	1.78	1.56	0.9099	0.1212
Smokey Charcoal	0.06	0.08	0.11	0.00	0.2055	0.0530
Soapy	0.00	0.00	0.00	0.00		
Sour	2.06	1.67	1.78	1.97	0.1855	0.2127
Sour Milk/Dairy	0.08	0.08	0.03	0.19	0.1395	0.0541
Spoiled putrid	0.17 ^{a}	0.00 ^{b}	0.00 ^{b}	0.00 ^{b}	0.0369	0.0372
Sweet	0.50	0.47	0.42	0.47	0.6880	0.1023
Umami	4.36	4.78	4.89	4.61	0.0527	0.1686
Warmed over	0.14	0.14	0.00	0.03	0.8341	0.0654

Values based on the average of 6 trained panelists. Scale from 0-15, zero being the lowest flavor intensity and 15 representing the highest.

$^{a, b}$ Means with different superscripts differ for aging type by vitamin E inclusion interaction effects ($P < .05$).

$^{A, B}$ Means with different superscripts differ for vitamin E inclusion effects ($P < .05$).

Table 5. Loading factors of principal component analysis.

	F1	F2
Beef Identity	0.810	0.007
Brown/Roasted	0.800	-0.050
Bloody/Serumy	-0.592	-0.413
Fat-like	-0.561	-0.242
Bitter	0.735	-0.126
Salty	0.666	0.197
Sweet	-0.255	0.585
Sour	-0.341	0.034
Umami	0.338	-0.343
Metallic	0.071	0.066
Musty/Earthy/Humus	-0.051	0.352
Cardboardy	-0.292	0.691
Animal Hair	-0.268	0.005
Barnyard	-0.049	0.439
Burnt	0.611	-0.035
Buttery	-0.037	0.020
Sour Milk/Sour Dairy	-0.195	-0.049
Fishy	0.052	0.680
Green	0.094	0.510
Heated Oil	-0.208	0.560
Liver-like	-0.165	0.551
Painty	0.503	0.133
Rancid	0.001	0.523
Refrigerator Stale	0.063	0.584
Smokey/Charcoa/Woody	0.801	-0.169
Spoiled Putrid	0.390	0.419
Warmed Over	-0.207	0.816
Juiciness	-0.551	-0.478
Muscle Fiber Tenderness	-0.218	-0.397
Connective Tissue Amount	0.203	-0.231

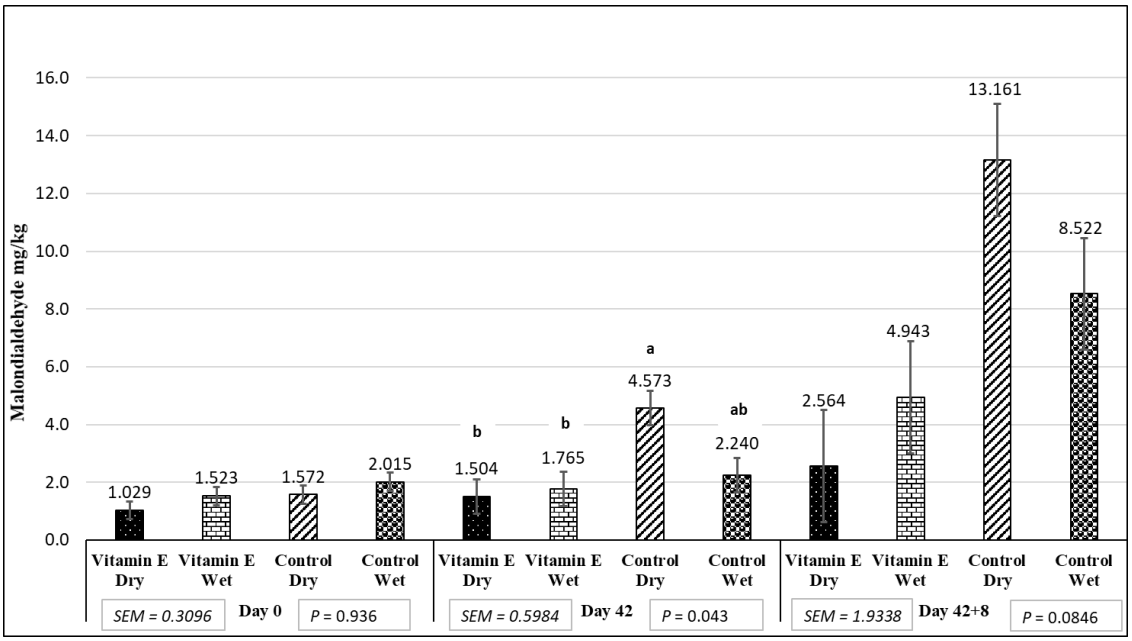
Table 6. Vitamin E inclusion effect (High vitamin E vs control) on objective and subjective linear approximation for days to specific color change during retail display.

Item	Vitamin E	Control	SEM	*P*-value
Days to 20% discoloration	7.06[a]	5.01[b]	0.208	<.0001
Days to <14.5 a*	5.05	3.79	0.444	0.0580
Δ E	10.81[b]	13.04[a]	0.350	0.0002

[a-b] Means with different superscripts differ within column ($P < .05$).

FIGURES

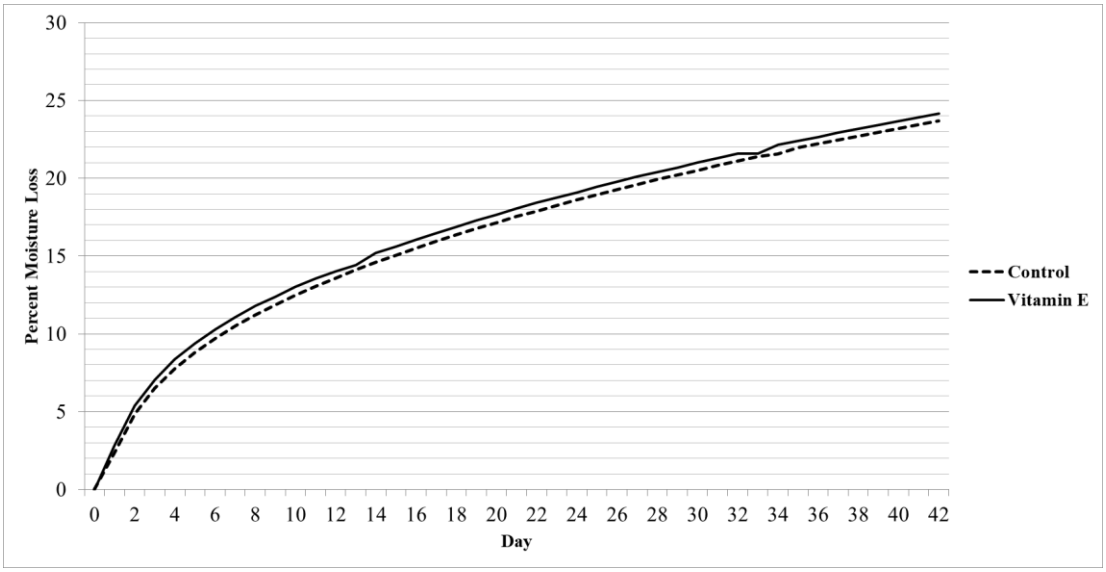
Figure 1. Oxidative rancidity measured with thiobarbituric acid reactive substances for all treatment combinations on day zero, 42 and 42 + 8 days of retail display.



[a, b] Means within day 42 with different superscripts differ ($P<.05$).

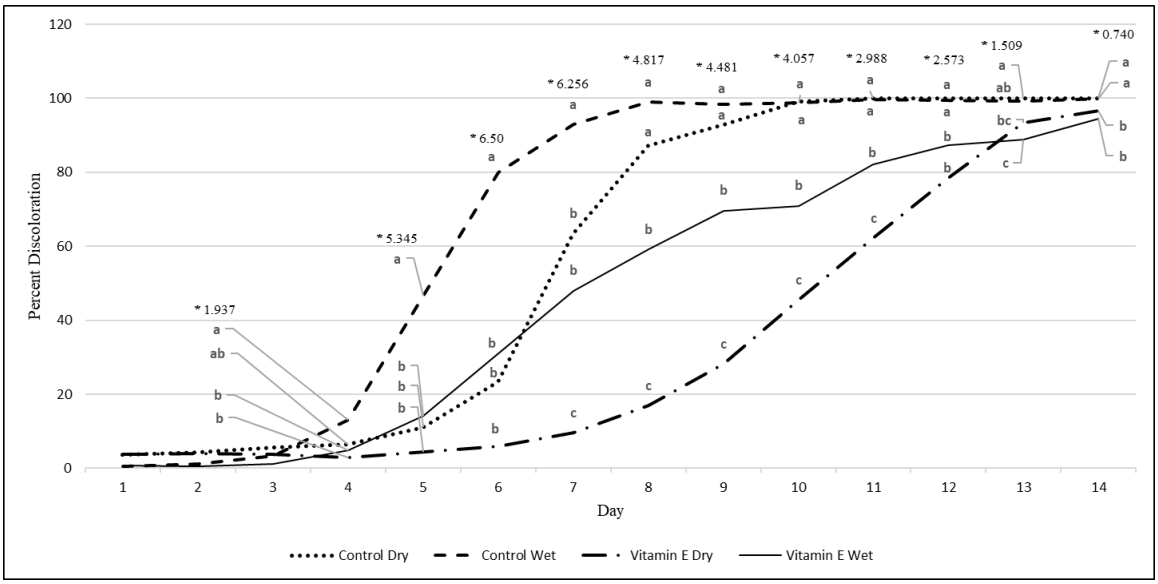
[A, B] Means within day 42 + 8 days of retail display differ ($P<.05$).

Figure 2. Effect of vitamin E inclusion (high vitamin E vs. control) on total moisture loss of boneless strip loins during 42 days of dry aging



P-value = 0.7080; SEM = 0.9142.

Figure 3. Subjective percent discoloration of all treatment combinations (control-dry-aged, control wet-aged, high vitamin E dry-aged and high vitamin E wet-aged) during 14 days of simulated retail display.

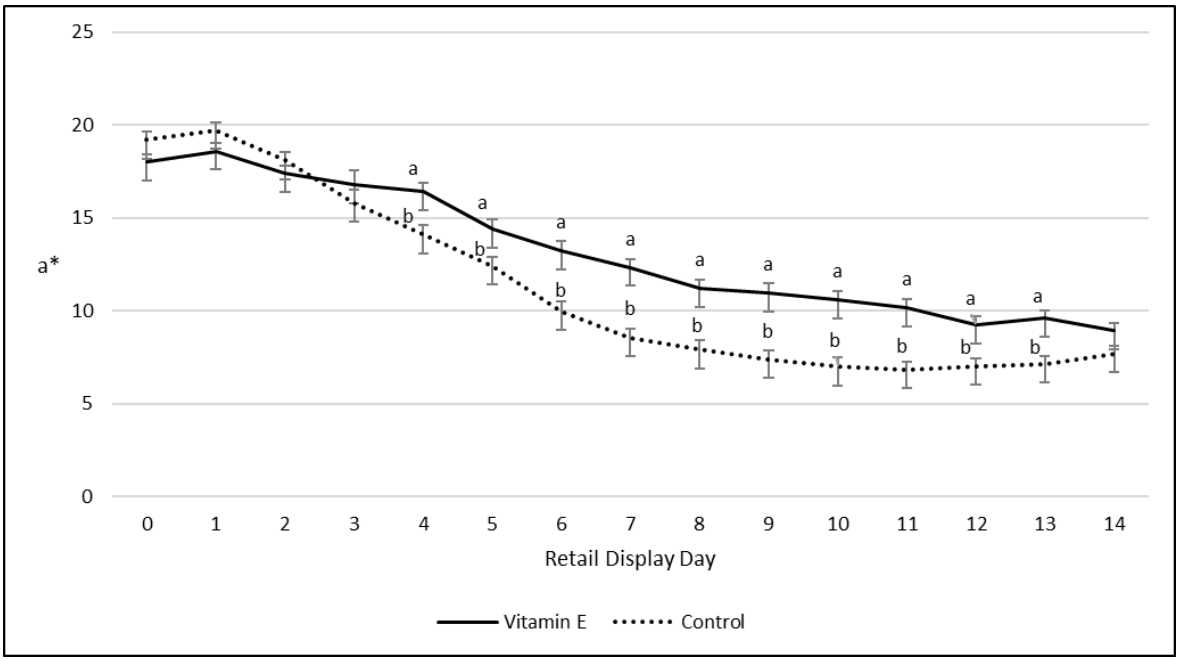


Aging type-by-vitamin E inclusion-by-day interaction ($P < .0001$).

[a,b,c] Means with different superscripts differ within day ($P < .05$).

* Superscript represents the standard error of the mean (SEM) of treatment comparisons by day.

Figure 4. Redness values (a*) of vitamin E inclusion (high vitamin E vs control) main effects during retail display for 14 days.



Vitamin E inclusion-by-day effects (P <.0001).

[a, b] Means within a day with different superscripts are different (P < 0.05). Error bars represent actual standard error of the mean (SEM) values.

Figure 5. Principal component analysis biplot.

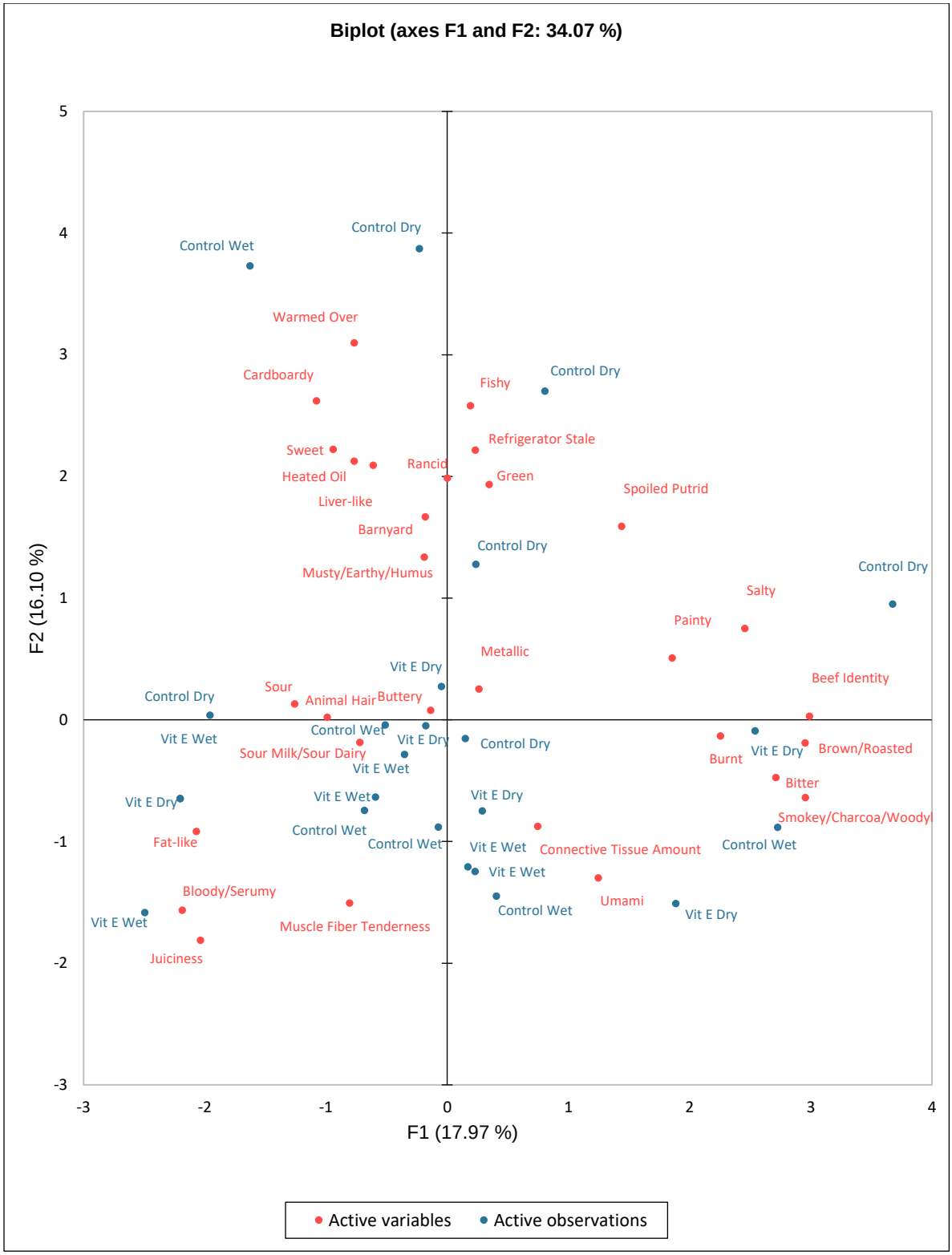
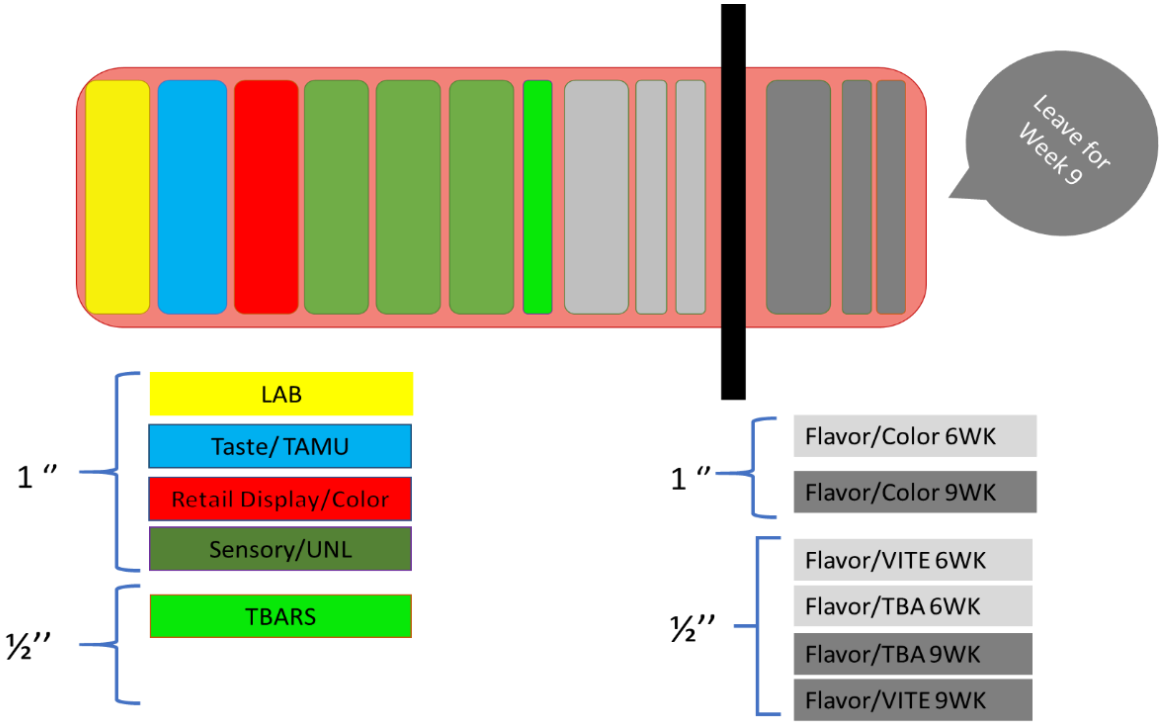


Figure 6. Fabrication map for all vitamin E inclusion and control loins after 42 d of wet or dry aging.



FUTURE RESEARCH RECOMMENDATION

Dry aging has proven to be a very complex process applied to a complex system. The main reason for producing dry-aged beef is flavor, however, a prominent quality defect of dry aged beef is high lipid oxidation, because most of the flavor in meat arises from lipids. It is necessary to understand the effects of lipid oxidation on dry-aged beef.

I state the hypothesis that oxidation of lipids is responsible for both the positive and negative aspects of dry-aged beef flavor. Controlling the extent of lipid oxidation may provide the opportunity to produce desired flavors while avoiding negative flavor profiles. Because of the close relationship between positive and negative effects of lipid oxidation on dry-aged beef an experiment is necessary to test the previews hypothesis.

Performing dry aging with a variety of times could potentially induce oxidation in a controlled manner. Dry aging beef loins for 10, 20, 30 and 40 days can be a means to control the extent of lipid oxidation. The same aging times could be done for wet-aged beef to compare both flavor and oxidation. Untrained sensory analysis would be done to detect the level of aging that the majority of consumers prefer. Oxidation analysis such as TBARS would be necessary to relate oxidation to flavor. Gas chromatography could be utilized to determine differences in volatiles with different aging times. Finally, a trained sensory panel can be done to detect at what level of oxidation positive flavors persist the most while maintaining low levels of negative off flavors.

Another experiment that could be done to understand dry-aged beef is to test the effect of exposure to dry-aged beef flavor on consumer acceptance. Because of the complexity of beef flavor, consumers may not enjoy the flavor notes the first time they try the product. However, tasting dry-aged beef multiple times could result in a higher

consumer acceptance that can be compared to traditional wet aged beef. For these experiments it would be necessary to understand the effect that the name “Dry-aged beef” has on the consumers first, followed by an evaluation of the effects of exposure to dry-aged beef flavor. If done properly this experiment could provide the data to prove that dry-aged beef flavor is a sophisticated, acquired taste and that consumer flavor perception is dependent on consumer exposure.

APPENDICES

Appendix 1. Project Design and Fabrication Maps

Total # of Cattle Loins: 24

Total # of treatments: 4 (6 loins per treatment)

Vitamin E wet aging

Vitamin E dry aging

Control wet aging

Control Dry aging

Cattle:

-High dosage Vitamin E (n = 12), Controls (n = 12)

-**Wet age: 6** Loins of treatment-vitamin E and **6** Controls

-**Dry age: 6** Loins of treatment-vitamin E and **6** Controls

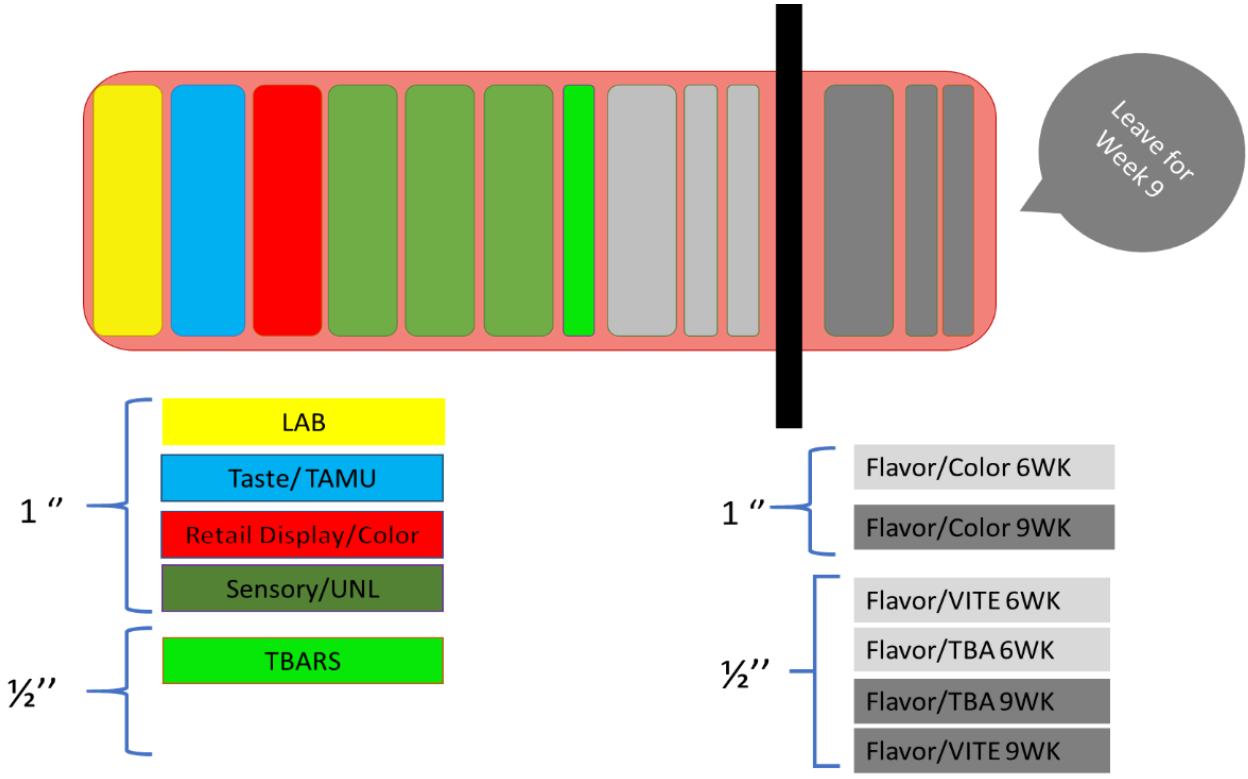
Fabrication Maps

June 18th and 19th:

Face both sides of the loins and weigh the faces, bag, and tag for lab analyses.

July 30th and 31st:

One half inch face was fist taken from the anterior side of all samples before this manufacturing step.



Grey color represents another project.

Appendix 2. Water Activity for day 0 and 42

Treatment Combination	Day 0	aw	Day 42	aw
Dry-Control	1	0.9926	1	0.9905
Dry-Control	2	0.9923	2	0.9905
Dry-Control	3	0.9950	3	0.9880
Dry-Control	4	0.9931	4	0.9884
Dry-Control	5	0.9940	5	0.9862
Dry-Control	6	0.9948	6	0.9824
Wet-Control	7	0.9928	7	0.9934
Wet-Control	8	0.9935	8	0.9953
Wet-Control	9	0.9928	9	0.9938
Wet-Control	10	0.9934	10	0.9954
Wet-Control	11	0.9930	11	0.9965
Wet-Control	12	0.9923	12	0.9923
Dry-Vitamin E	13	0.9923	13	0.9881
Dry-Vitamin E	14	0.9924	14	0.9863
Dry-Vitamin E	15	0.9917	15	0.9815
Dry-Vitamin E	16	0.9928	16	0.9885
Dry-Vitamin E	17	0.9943	17	0.9869
Dry-Vitamin E	18	0.9927	18	0.9869
Wet-Vitamin E	19	0.9954	19	0.9900
Wet-Vitamin E	20	0.9956	20	0.9958
Wet-Vitamin E	21	0.9937	21	0.9954
Wet-Vitamin E	22	0.9918	22	0.9954
Wet-Vitamin E	23	0.9948	23	0.9960
Wet-Vitamin E	24	0.9974	24	0.9941

Appendix 3. Example of Retail Display

Retail simulation samples at day 8

Control Dry-Age

Control Wet-Age

Vit E Dry-Age

Vit E Wet-Age

Appendix 4. Wet vs Dry Aging Set Up

Appendix 5. Thiobarbituric Acid Reactive Substances Protocol

Buege and Aust (1978) Modifications made by Ahn et al. (1998).

Reagents:

- 1,1,3,3-Tetraethoxypropane-Dilute 99 microliters of TEP (97%) bring to a volume of 100mL with double distilled water (1×10^{-3}M).
- 2-Thiobarbituric Acid (TCA)/Trichloroacetic Acid Solution (TBA) (1L)- Mix 15% TCA (w/v) and 20 mM TBA (MW 144.5) in with double distilled water. First dissolve 2.88 g of TBA in warm double distilled water, and then add TCA (150 g) and bring volume to 1L with with double distilled water.
- ButylatedhydroxyAnisole (BHA)-Add 10% stock solution in a volume of 90% ethanol. Ten grams of BHA in 90 mL of ethanol (90%). Then add 5 mL of with double distilled water.

Standards: In duplicate

Blank:	1 ml ddH20	Moles of TEP
Standard 5:	100 µl working TEP + 1.90 mL ddH20	(5x10-5M)
Standard 4:	1 mL Std. 5 + 1 mL ddH20	(2.5x10-5M)
Standard 3:	1 mL Std. 4 + 1 mL ddH20	(1.25x10-5M)
Standard 2:	1 mL Std. 3 + 1 mL ddH20	(.625x10-5M)
Standard 1:	1 mL Std. 2 + 1 ml ddH20	(.3125x10-5M)
	Remove 1 mL of Standard 1 and discard it, leaving 1 mL behind.	

Procedure

- Mix all reagents and standards before beginning.
- Transfer 5 g of powdered sample into a 50 ml conical tube, add 14 ml of ddH2O 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 □l from each tube into wells on a 96 well plate.
- Read absorbance at 540nm.

Calculations: mgs of malonaldehyde/kg of tissue

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

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

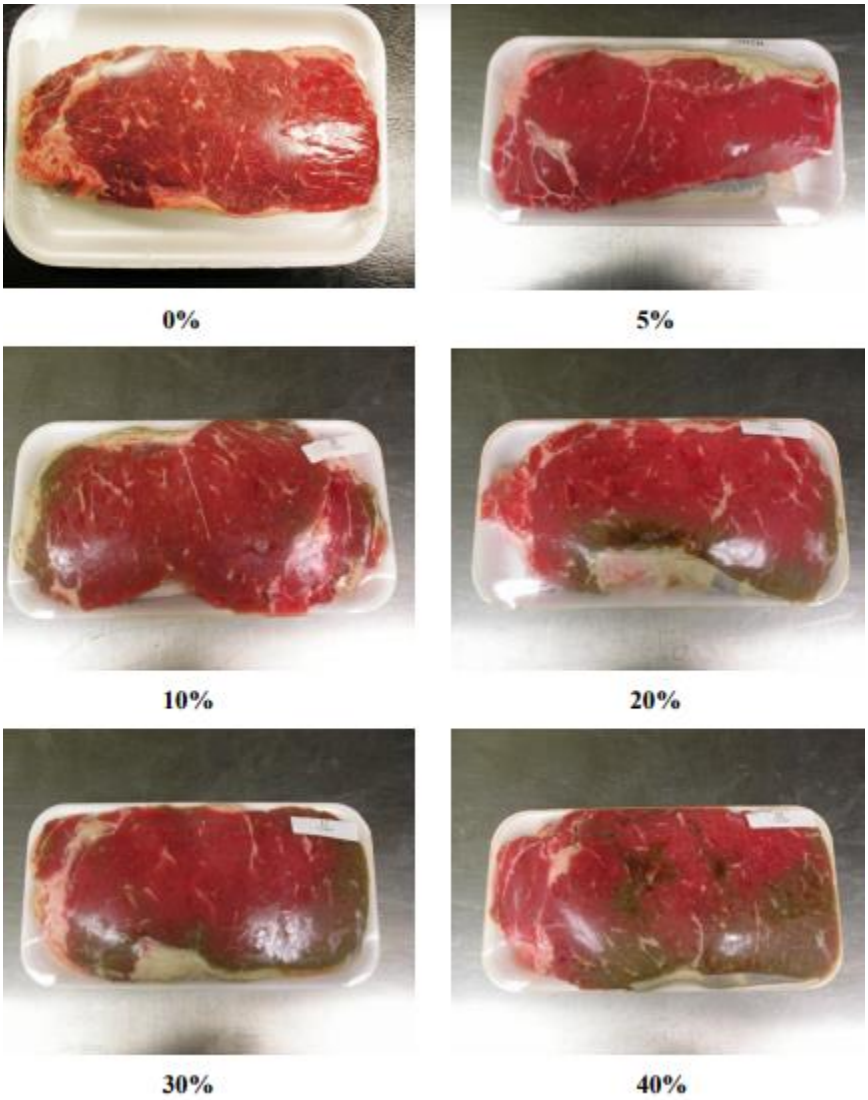
A=Absorbance of standard MW=MW of malonaldehyde (72.063 g/mole)

E= sample equivalent (1) P=Percent recovery

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

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

Appendix 6. Visual Guide for Subjective Percent Discoloration



50%

60%

70%

80%

90%

100%

Appendix 7. Proximate analysis results

	Day 0					Day 42				
	Water	**Protein**	**Ash**	**Fat**	**Total**	**Water**	**Protein**	**Ash**	**Fat**	**Total**
Control Dry Aged	68.39	21.03	1.27	9.31	100.00	66.62	24.66	1.46	7.39	100.13
Vitamin E Dry Aged	68.82	21.57	1.22	8.39	100.00	66.36	25.41	1.37	6.86	99.99
Control Wet Aged	69.22	20.01	1.35	9.43	100.00	69.42	21.07	1.27	8.24	100.00
Vitamin E Wet Aged	69.90	20.22	1.24	8.65	100.00	70.17	21.60	1.13	7.09	100.00