EXPLORING THE EFFECT OF LEAN SOURCE ON COLOR STABILITY AND FLAVOR OF GROUND BEEF PATTIES

A Thesis

by

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ABSTRACT

To explore the effect of lean source on the color and flavor stability of ground beef patties, two separate study were conducted. The first study focused on the impact of animal diet on the lean source composition to the color and flavor of ground beef by utilizing differing percentages of grass- and grain-fed lean. Although pH was not different among batches, grain-fed batches exhibited more redness and less discoloration at the end of a retail display period. Similarly, lipid oxidation values were smaller in grain-fed patties compared to grass-fed. Furthermore, grass-fed patties contained greater oleic acid (18:1n-9) and less stearic acid (18:0) compared to grain-fed patties. While volatile compound differences were expected be linearly affected by the percentage of grass-fed lean over the display period, the 33% and 67% grass-fed lean patties exhibited vast differences in volatile concentration over the display period. A second study was conducted focusing on the impact of lean source and fat percentage on flavor. To conduct this, lean sources were selected to vary in breed origin, quality grade, and diet to create a large variation. However, few volatile compounds were significantly impacted by lean source and even fewer were impacted by fat level. Furthermore, fatty acid composition was found to be impacted by both lean source, fat percentage, and the interaction of the two. Overall, it can be concluded that difference in both the lean source, fat level, and the interaction between these two variables can be attributed to flavor differences in ground beef patties. Additionally, it can be said that lean source has

a major impact on the color stability during retail display and flavor compounds produced in ground beef patties.

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1. INTRODUCTION

Ground beef is one of the most consumed beef products as it accounts for 40% of total beef sales (Suman, Hunt, Nair, & Rentfrow, 2014). Mancini & Hunt (2005) described visual color as the "gold standard" for consumer perception of meat products. Because consumers view discoloration as a sign of poor quality, meat color is the primary driving force for meat purchasing decisions. Beef discoloration is caused by the oxidation of the iron in myoglobin, which forms the brown colored pigment metmyoglobin (Mancini & Hunt, 2005). Preventing beef from oxidizing via the action of an antioxidant would delay myoglobin oxidation and increase the length of time of consumer acceptability in retail display. The lean portion of beef from grass-fed cattle may contain higher concentrations of natural antioxidant compounds such as vitamin E than beef from grain-fed cattle (Daley, Abbott, Doyle, Nader, & Larson, 2010a; De la Fuente et al., 2009; Fruet et al., 2018; Luciano, Moloney, et al., 2011; Yang, Lanari, Brewster, & Tume, 2002). Previous research has shown vitamin E is an antioxidant that improves beef color and color stability (Faustman, Chan, Schaefer, & Havens, 1998).

The autoxidation of polyunsaturated fatty acids in meat products is a major economic problem (Frankel, 1980). Lipid oxidation is associated with oxidized flavor, also known as warmed-over flavor (B. E. Greene & Cumuze, 1982). These flavors can be further described by the attributes cardboardy, rancid, stale, and metallic, and have been associated with the volatile compounds 2,3-octanedione and hexanal (St. Angelo et al., 1987). It has long been hypothesized that myoglobin oxidation and lipid oxidation are linked and that oxidation of both molecules would occur simultaneously (Alderton,

Faustman, Liebler, & Hill, 2003; Faustman et al., 1998; Lynch & Faustman, 2000). However, previous research in demonstrated that in grass-fed ground beef, color stability and flavor stability were inversely related (Sledge, 2008). This relationship needs to be further explored to understand the correlation between color and lipid stability in ground beef. We hypothesize that adding lean from grass-fed cattle to a ground beef blend in small quantities could naturally increase the vitamin E present in the blend, which would increase the retail shelf life without decreasing flavor acceptability of the product.

Although meat color drives consumer purchasing decisions, flavor is the most important attribute for consumer acceptance of beef as long as tenderness is acceptable (Kerth & Miller, 2015; Killinger, Calkins, Umberger, Feuz, & Eskridge, 2004; O'Quinn, Legako, Brooks, & Miller, 2018). "Fat is flavor" is a well-known adage among the beef industry. Due to this line of thinking, the USDA quality grade system, which evaluates carcass quality based on maturity and marbling, has been a long-used tool for consumers to predict quality and for processors and retails to set prices on products. Thus, numerous studies have been conducted examining marbling and fat deposition as they relate to consumer satisfaction and determining logical slaughter endpoints to increase profitability by producers. In turn, the degree of marbling has been shown to be a factor that impacts tenderness and consumer acceptability of beef products (Platter et al., 2003; Wheeler, Cundiff, & Koch, 1994).

In contrast, marbling has been only moderately correlated with flavor differences between breeds of beef cattle (Arshad et al., 2018). Additionally, research by Kerth & Miller (2015) reported that "beef identity" and "brown/roasted" flavor attributes as

defined by the beef lexicon (Adhikari et al., 2011) is highly correlated to Maillard reaction products and consumer liking. The Maillard reaction, in which reducing sugars and amino acids react during high dry heat conditions, produces the browning and caramelization present in beef steaks (Dashdorj, Amna, & Hwang, 2015). However, there remains a lack of information about how particular Maillard products are produced. Recent research by Dinh, Legako, Miller, & Brooks (2018) has even shown the role of sulfur containing amino acids, certain sugars, and nucleic compounds such as idenosine monophosphate to play a larger role in the generation of beef and brown flavor compounds than was initially thought.

Many pre-harvest factors have been shown to have an influence on metabolites which act as substrates in flavor-producing reactions. Specifically, Arshad et al. (2018) noted breed variations resulted in over 40 Maillard reaction products that differed because of genetic differences. Because the role of small sugar molecules, peptide chains, and free amino acids in the development of flavor is largely unknown outside of impacts on basic tastes, this leads to the hypothesis that genetic differences, and thus the regulation of different metabolites, may result in flavor differences across lean source.

In the beef industry, numerous branded programs exist based on differences in breed and meat quality. These claims have led to premium products which are based on high degrees of marbling and a guaranteed tender product. Consequently, consumers will pay premium prices for these products. These claims have not been confirmed by sensory and basic meat science research. However, novel instrumentation and research methods allow us to explore the water-soluble metabolites in the lean portion of meat

completely separate from the lipid portion. Defining differences in metabolites due to genetic or breed differences could provide evidence for superior flavor in certain lean sources due to up- or down-regulation of muscle metabolism. We hypothesize that differences in flavor compounds in the lean portion of ground beef will be the driving force for flavor difference across ground beef patty types.

2. REVIEW OF LITERATURE

2.1. Meat Color

Meat color is the largest factor that influences consumer purchasing decisions of fresh meat products (Govindarajan & Snyder, 1973; Mancini & Hunt, 2005; Seideman, Cross, Smith, & Durland, 1984). Consumers rely on color as an indicator of wholesomeness, freshness, and quality (Mancini & Hunt, 2005; Seideman et al., 1984; Suman & Joseph, 2013). Meat color is determined by a combination of proteins present in the muscle and how those proteins refract or absorb light (Walters, 1975). There are several proteins present in the muscle that contribute to color. The most significant are myoglobin, hemoglobin, and cytochrome (Suman & Joseph, 2013). Of these three proteins, myoglobin has the largest impact on meat color (Mancini & Hunt, 2005). Myoglobin is responsible for oxygen storage and transfer in the muscle (Berg, Tymoczko, Gatto Jr., Gregory, & Stryer, 2012). In living tissue, myoglobin only accounts for 10% of the total iron in the muscle (Clydesdale & Francis, 1971). However, after exsanguination, 95% of the total iron in muscle comes from myoglobin (Clydesdale & Francis, 1971). This change in the relative amount of myoglobin to hemoglobin in the muscle makes myoglobin the largest contributor to fresh meat color.

Although myoglobin is the most important protein, hemoglobin still plays a role in meat color. Hemoglobin is the protein in blood responsible for the transport of oxygen from the lungs to body tissues and transport of carbon dioxide from the tissues to the lungs (Berg et al., 2012). During the slaughter process, approximately 40 to 60% of blood is cleared from the animal through the process of exsanguination, with the

majority of the blood remaining in the viscera (Warriss, 2000). In muscle tissue, it is estimated only 2 to 9 mL/kg remains after exsanguination (Warriss, 2000). However, it has also been estimated that in well-bled animals, hemoglobin is responsible for 20 to 30% of the total color pigments (J. B. Fox, 1966).

Myoglobin and hemoglobin have similar structures, and as a result have similar functionalities (Berg et al., 2012). Due to their structural likeness, myoglobin and hemoglobin bind to the same ligands and produce nearly identical pigments in the muscle postmortem (Suman & Joseph, 2013). Although there are many commonalities between the two proteins, the largest difference is myoglobin is a monomer while hemoglobin is a tetramer of globular proteins (Berg et al., 2012). A single subunit of hemoglobin has a nearly identical amino acid sequence to myoglobin (Berg et al., 2012). However, because myoglobin is a monomer, the proteins have differences in properties such as binding affinity to oxygen.

Additionally, a small fraction of meat color is the result of a family of proteins called cytochromes (Suman & Joseph, 2013). Cytochrome proteins can be found in almost every organism besides a small number of bacteria (Heldt & Piechulla, 2011). The defining characteristics of cytochromes is that they have a prosthetic group that contains a heme iron (Cole & Eastoe, 1988). Cytochrome proteins are divided into three subdivisions (cytochrome-a, -b, or -c) based on the chemical structure of the heme group (Heldt & Piechulla, 2011). Their main physiological function is to transfer electrons by the oxidation and reduction of the heme iron (Cole & Eastoe, 1988). Cytochromes act as enzymes catalyzing cellular processes such as respiration and mitochondrial reactions

(Mowat & Chapman, 2013; Rousseau & Han, 2002). Cytochrome plays a larger role in the color of poultry and fish than in livestock species (Suman & Joseph, 2013).

Another factor that impacts meat color is the structure of muscle and its subsequent ability to scatter light (Hughes, Clarke, Purslow, & Warner, 2020). The variation in the lightness or darkness of meat is likely due to differences in the ultrastructure of a muscle cell (Hughes et al., 2020). Three basic parameters have been proposed to cause asymmetry in light refractivity of muscles: transverse shrinkage of muscle fibers, longitudinal shrinkage of muscle fibers, and the protein concentration of the sarcoplasm and extracellular media (Hughes et al., 2020). Transverse shrinkage of myofibrils results in the ability of light to scatter between diagonal elements within the cell (Hughes et al., 2020). Transverse shrinkage of myofibrils occurs postmortem and has been shown to be closely related to the concurrent decline in pH (Hughes et al., 2020). As the pH of the muscle decreases, the muscle fiber diameter shrinks and the extracellular space increases allowing the muscle to scatter more light (Hughes, Oiseth, Purslow, & Warner, 2014). Muscles that have a lesser decline in pH have an increased diameter, less extracellular space and lesser ability to scatter light compared to lower pH muscles (Hughes, Clarke, Purslow, & Warner, 2017, 2018).

Longitudinal shrinkage of the sarcomere within the muscle fiber changes the protein density of the A-band and I-band of the sarcomere, leading to differences in light scattering (Hughes et al., 2020). Research done by Hughes et al. (2018) showed darker muscles had shorter sarcomere lengths when compared to lighter muscles. However, it has been hypothesized that sarcomere length has little effect on total light scattering, and

that the effects of longitudinal shrinkage may be confounded by the transverse shrinkage of fibers (Hughes et al., 2020).

The protein composition of the sarcoplasm and extracellular fluid is hypothesized to affect the light scattering ability of the muscle by altering the optical protein density and the refractive index (Hughes et al., 2020). Refractive index is defined as "the ratio of the velocity of light of a specified wavelength in the air to its velocity in the examined substance" (Wypych, 2019). An increase of particles present in the sarcoplasm will slow the speed of light passing through it, which leads to a smaller refractive index (Hughes et al., 2020). The total protein concentration, solubility, and aggregation within the sarcoplasm is expected to affect the speed and angle of light as it passes through the muscle, thus affecting the overall refractive index (Hughes et al., 2020). However, research comparing drip-loss from light and dark beef muscles showed the concentration of proteins and the refractive index of the purge was similar, with differences resulting only in the quantity of purge from the muscle (Hughes, Clarke, Li, Purslow, & Warner, 2019). For this reason, researchers hypothesize protein concentrations has a minor role compared to the structure of the fibers (Hughes et al., 2020).

2.1.1. Chemical Structure of Myoglobin

Myoglobin is the primary protein responsible for the color of meat (Govindarajan & Snyder, 1973). In living tissue, myoglobin stores and delivers oxygen from the bloodstream to the muscle mitochondria for use during aerobic metabolism (Suman & Joseph, 2013). Myoglobin is a water-soluble protein found in the sarcoplasm of the muscle tissue (Govindarajan & Snyder, 1973). Myoglobin has two major structural

components: the globin protein and the prosthetic group located in the hydrophobic pocket of the protein (Mancini & Hunt, 2005). Myoglobin is a monomer comprised of 153 amino acids, and has a secondary structure that contains eight α-helices linked by nonhelical sections (Mancini & Hunt, 2005). The alpha helical regions of the globin portion contain polar amino acids on the outside of the helix while non-polar amino acids are arranged on the inside of the helix (Berg et al., 2012). The tertiary structure of myoglobin is a globular protein that contains a heme moiety in the hydrophobic pocket located in the middle of the protein (Berg et al., 2012). The globular protein protects the heme ring from oxidation and binding of unwanted molecules (Faustman & Cassens, 1990).

The heme moiety is comprised of an organic group known as the protoporphyrin ring, which is a large structure made of four combined pyrrole rings (Damodaran, Parkin, & Fennema, 2008). The center of the ring contains the heme iron (Fe) molecule which has six different coordination sites (Berg et al., 2012). Four of those binding sites are bound to nitrogen atoms in the tetrapyrrole ring of the porphyrin structure (Damodaran et al., 2008). The fifth binding site is bound to a histidine amino acid of the globin protein (Damodaran et al., 2008). This distal histidine is located at the sixty-fourth position of the globin amino acid sequence (Suman & Joseph, 2013). Binding of the heme ring to this amino acid residue induces a tilt into the three-dimensional structure of the molecule, which helps to further protect the heme iron from binding to larger, unwanted molecules (Suman & Joseph, 2013). This altered shape reduces the binding affinity of heme to carbon monoxide by one hundred-fold (Berg et al., 2012).

The distal histidine also stabilizes the binding of oxygen to the sixth coordination site of the heme iron (Faustman & Cassens, 1990). The sixth coordination site can bind numerous molecules. The iron can be present in either the reduced, (ferrous; Fe^{2+}) or the oxidized state (ferric; Fe^{3+}; Seideman et al., 1984). Oxidation is defined as the loss of an electron (Seideman et al., 1984). The oxidation of the iron in myoglobin results in decreased binding affinity of the molecule to oxygen (Faustman & Cassens, 1990). This results in the physiological inactivation of the metmyoglobin molecule, and ensures myoglobin only binds with oxygen in the ferrous form (Berg et al., 2012; Faustman & Cassens, 1990) The binding of different molecules in addition to the oxidation state of the heme iron creates the various colors associated in red meat.

Myoglobin has a greater affinity to oxygen than hemoglobin (Berg et al., 2012). If the partial pressure of oxygen is 2 mmHg, half of the myoglobin molecules are bound to oxygen (Berg et al., 2012). However, the partial pressure of oxygen must be 26 mmHg before half of the hemoglobin molecules will be bound (Berg et al., 2012). The difference is due to the cooperative binding exhibited by the hemoglobin molecule (Berg et al., 2012). Hemoglobin's cooperative binding causes the oxygen binding curve of the molecule to be sigmoidal, compared to myoglobin which exhibits a hyperbolic curve (Berg et al., 2012). Physiologically, hemoglobin's lower binding affinity for oxygen is the reason oxygen can be transferred from the hemoglobin in blood to the myoglobin in muscle tissue (Berg et al., 2012). In meat, this also means that more myoglobin molecules will bind to oxygen at lower partial pressures compared to residual hemoglobin in the muscle.

2.1.2. Myoglobin States

In red meat, myoglobin most commonly exists in one of three different states: deoxymyoglobin, oxymyoglobin, or metmyoglobin (Govindarajan & Snyder, 1973; Seideman et al., 1984). In the absence of oxygen, myoglobin exists in the deoxymyoglobin state. Deoxymyoglobin is also formed through deoxygenation, the reaction in which oxygen becomes dissociated from oxymyoglobin (Seideman et al., 1984). In this pigment, the sixth position of the heme iron is not bound to any ligand (Suman & Joseph, 2013). The iron of the heme ring is in its ferrous state (Fe^{2+}; Suman & Joseph, 2013), and the meat will appear a purplish-red color (Mancini & Hunt, 2005). This is commonly seen when meat is vacuumed-packaged (Renerre, 1990). However, this state is also present in the center of meat cuts, where oxygen cannot permeate (Govindarajan & Snyder, 1973).

Oxymyoglobin is formed when the sixth coordination site in the heme iron of myoglobin is bound to an oxygen molecule (O_2; Mancini & Hunt, 2005). The process of oxygen binding to the myoglobin is called oxygenation (Suman & Joseph, 2013). Oxygenation can be seen readily in meat products at atmospheric oxygen concentrations. The oxygenation process is commonly referred to as “blooming” in the industry (Suman & Joseph, 2013). Bloom time can vary depending on the cut but is oftentimes seen 30 to 60 min. after the surface is exposed to oxygen (Suman & Joseph, 2013). The iron of the heme ring is in the ferrous state (Suman & Joseph, 2013). The pigment produced is a red color and is seen in packaging where oxygen is plentiful such as high-oxygen modified atmosphere packaging (MAP) and overwrap packaging.

Metmyoglobin occurs when the iron molecule in the heme ring becomes oxidized to its ferric form (Mancini & Hunt, 2005). The iron becomes bound to a water molecule (Suman & Joseph, 2013), and the pigment produces a brown color (Mancini & Hunt, 2005). Metmyoglobin forms in low partial pressures of oxygen. Metmyoglobin is the predominant pigment visible over time when meat is stored in packaging with high oxygen permeability (Seideman et al., 1984). This color is unfavorable to consumers as they consider it to be lower quality or unsafe (Mancini & Hunt, 2005). Metmyoglobin often will first appear in spots across the surface of meat. Then, as time progresses, the entire surface will eventually become brown. Because of consumer bias against meat exhibiting metmyoglobin, retailers will drop the price after the pigment appears in order to persuade consumers to purchase the product quickly. In the United States, it has been estimated that 15% of retail sales of meat were discounted due to discoloration, leading to a loss of $1 billion of revenue annually (Troy & Kerry, 2010). If the surface of meat becomes discolored, the product can be minced or ground and marketed as such, which lowers the value of muscle significantly (Faustman & Cassens, 1990; USDA, 2019). However, if it still does not sell after that point, the product will be thrown away causing not only a loss of profit for the producer but waste of a high-quality protein source for the consumer.

In the early postmortem period, if the muscle is exposed to oxygen all three states of myoglobin will exist in a dynamic equilibrium (Govindarajan & Snyder, 1973). Prior to exposure to the atmosphere, the primary pigment present is deoxymyoglobin (Govindarajan & Snyder, 1973). However, once the meat is cut the myoglobin molecules

at the surface of the muscle are exposed to oxygen. Oxygenation occurs and the surface appears a bright red color as oxymyoglobin becomes the predominant pigment (Govindarajan & Snyder, 1973). Just below the surface, the partial pressure of oxygen is low as most oxygen is either bound to myoglobin or is being used by aerobic pathways in the muscle (Govindarajan & Snyder, 1973). This low partial pressure of oxygen leads to oxidation of the heme iron, and a layer of metmyoglobin forms (Govindarajan & Snyder, 1973). Below the layer of metmyoglobin, the pigments are not exposed to any oxygen and exist as deoxymyoglobin (Govindarajan & Snyder, 1973). For a period of time, reducing enzymes present in muscle will reduce the metmyoglobin to deoxymyoglobin, which can then be oxygenated to form oxymyoglobin (Mancini & Hunt, 2005). When meat is packaged with oxygen, as aerobic metabolism continues in the muscle it lowers the partial pressure of oxygen in the package (Suman & Joseph, 2013). Additionally, as time progresses, reducing enzymes become less able to reduce metmyoglobin back to deoxymyoglobin (Mancini & Hunt, 2005). These factors cause metmyoglobin pigments to thicken and move towards the surface of the meat (Mancini & Hunt, 2005). When 60% or more of the myoglobin molecules in an area become oxidized to metmyoglobin, the brown pigment visually predominates in that area (Seideman et al., 1984). However, consumer acceptance and sales begins to decline when as little as 20% metmyoglobin appears on the surface of meat (Warriss, 2000).

Another pigment that can be present in meat is carboxymyoglobin. Carboxymyoglobin forms when the heme iron binds a carbon monoxide atom (Gee & Brown, 1978). The iron is in its reduced, ferrous state. This pigment is a bright cherry

red and is more stable than oxymyoglobin because carbon monoxide can form a more stable bond to myoglobin compared to oxygen (Šuput et al., 2013). Visually, this color is indistinguishable from oxymyoglobin (Cornforth & Hunt, 2008). Carboxymyoglobin is seen when meat is packaged in a modified atmosphere packaging that contains a small amount of carbon monoxide. According to FDA regulations, carbon monoxide levels of 0.4% or less are generally recognized as safe and is the maximum amount usable in meat packaging (Šuput et al., 2013) .

There are other pigments that can be present but are less commonly seen in meat. These pigments include: sulfmyoglobin, choleglobin, and cyanmetmyoglobin (MacDougall, 1982; Nicol, Shaw, & Ledward, 1970; Schricker & Miller, 1983). Sulfmyoglobin typically exists when meat is contaminated with certain sulfur-reducing bacteria such as *Pseudomonas mephitica* which produces hydrogen sulfide (Nicol et al., 1970). The sulfur binds to the myoglobin molecule giving the meat a green color (Nicol et al., 1970). Choleglobin is also a green-colored pigment occurs when hydrogen peroxide (H_2O_2) is present as a bacterial by-product (Seideman et al., 1984). The heme iron can either be in the ferrous or ferric form (Seideman et al., 1984). Cyanmetmyoglobin produces a red colored pigment and is the result of myoglobin binding with cyanide (Savell, 2015).

2.2. Color Perception

Waves of light enter the eye through the pupil. The iris, which surrounds the pupil, controls the diameter of the pupil (R. S. Smith, John, Nishina, & Sundberg, 2002). The lens of the eye focuses the light onto the retina, which contains cells that are able to

translate the light into electrical impulses. The optic nerve then sends those impulses to the brain which interprets images. The retina contains two types of photoreceptor cells called cones and rods (Raviola & Gilula, 1973). Cones are located toward the center of the retina and interpret color (Curcio, Sloan, Packer, Hendrickson, & Kalina, 1987). Rods are located nearer to the periphery of the retina and assists in catching light during darker times (Farber, Flannery, Lolley, & Bok, 1985). Human retinas contain three different types of cone cells (L, M, and S) which are differentiated by the wavelengths at which the cells are most sensitive (Mustafi, Engel, & Palczewski, 2009). L-cones make up the majority of cells and are most responsive to low frequency photons, with their maximum sensitivity occurring at 555 to 565 nm (Mustafi et al., 2009). M-cones are sensitive to middle frequency photons, and are most sensitive from 530 to 537 nm (Mustafi et al., 2009). Human eyes have over twice as many L-cones than M-cones on average (Mustafi et al., 2009). S-cones account for only 5% of cone cells and are sensitive to supra-frequency photons, with maximum sensitivity occurring between 415 to 430 nm (Mustafi et al., 2009; Roorda, Metha, Lennie, & Williams, 2001).

When ligands are bound to the sixth coordination site on myoglobin, it causes a conformational shift in the myoglobin molecule. This conformational shift changes the reflectance of light from the protein along the visible color spectrum wavelengths (390 to 760 nm; Francis, 1995), causing the different chemical states of myoglobin to appear different colors. The relative amount of the different chemical states of myoglobin will ultimately determine the overall visual color of meat to consumers. The lighting source will also affect the perception of color (Francis, 1995). In the meat case, it is possible to

make cuts of meat appear more red to the consumer by either using a pink lighting source or having red reflective paint on or near the display case (Francis, 1995). Therefore, it is important to consider potential ways lighting differences could affect the color perception of your product if presenting it to a trained or untrained visual panel.

2.2.1. Methods of Identifying and Quantifying Color

2.2.1.1. Subjectively

Color can be quantified either instrumentally or visually. Instrumentally measuring color is an objective method to quantify color. Instrumental color is most often reported by the Commission Internationale de l'Eclairage (CIE) tristimulus color space values L*, a*, and b* (CIELAB). This color space was created in 1976 as an extension of the XYZ tristimulus color space (O'Farrell, 2011). The CIELAB color space values are most often used because it is the closest to a human's color perception (O'Farrell, 2011). These values define color on a three-dimensional sphere with three different axes. The L* axis defines the lightness of the sample. L* values range from 0 to 100 with 0 representing the absence of light (black) and 100 being complete saturation with light (white). The a* axis goes from green (-60) to red (+60) and is used to quantify the redness of meat. The b* axis goes from blue (-60) to yellow (+60) and is used to report the yellowness of meat.

Additionally, other values often reported include chroma and hue angle. Chroma can be defined as the vividness of the color present in the sample or the total saturation of color in the sample. Chroma is a value calculated from a* and b* and is equal to the square root of the sum of a* squared and b* squared (American Meat Science

Association, 2012). Hue angle is another value calculated from a* and b* and is equal to the arctangent of the ratio of b* to a* (American Meat Science Association, 2012) Hue angle defines the brownness in the sample, with a higher hue angle being indicative of a browner sample (Farouk & Wieliczko, 2003).

Instrumental color can be measured with either a tristimulus colorimeter or a spectrophotometer. A tristimulus colorimeter functions by emitting radiant power from a light source in the machine onto the surface of an object (Choudhury, 2014). The light reflected from the surface will pass through one of three tristimulus filters and onto a photo-detector (Choudhury, 2014). This data then goes to the microprocessor of the machine, which will compute the corresponding CIE-value (Choudhury, 2014). Some tristimulus colorimeters are photoelectric colorimeters, which means they use a phototube or photocell and reflected light passes through a rotating, filter-containing disc to measure the color space values separately by a photoelectric photon detector (Choudhury, 2014). The Minolta (KONICA MINOLTA, INC. Marunouchi, Chiyoda-ku, Tokyo, Japan), a photoelectric tristimulus colorimeter, is the most common instrument used to objectively measure color, with 60% journal articles during 1998 to 2007 reporting using the Minolta to collect color data (Tapp, Yancey, & Apple, 2011).

A reflectance spectrophotometer measures the intensity of reflected light compared to a reference (Hollinger, Siegemund, Cueni, & Steiner, 2018). A spectrophotometer can measure both the color and the intensity of reflected light quantitatively (Wallace, Wax, Roberts, & Graf, 2009). The spectrophotometer emits a beam of visible light through a prism or other diffraction mechanism (Vo & Shim,

2020). In a single beam spectrophotometer, this light then passes through the sample and the reflected light passes onto a detector (Whetzel, 2015). However, in double beam spectrophotometers, this light is split into two different beams (Whetzel, 2015). The first beam will pass through a reference, while the second passes through the sample (Whetzel, 2015). Both reflected light beams will pass through a monochromator detector (Whetzel, 2015). Because the double beam spectrophotometer measures the reference and sample simultaneously, it ensures that each sample is independent from variation that may be present in the light source (Nilapwar, Nardelli, Westerhoff, & Verma, 2011). The most common spectrophotometer used is an instrument by HunterLab (Hunter Associates Laboratory, Reston, VA, USA) such as the Miniscan. However, only 31.6% of articles reported using a Hunter instrument to measure color (Tapp et al., 2011).

No matter which instrument is used to measure color, differences in the illuminant, aperture size, observation angle, and number of readings per sample can lead to variation in CIE values. The illuminant is the light source that is used in the instrument. Some instruments allow you to select the illuminant, while other instruments only have one option. The different illuminants represent different lighting sources and intensity. Illuminant A represents average, incandescent lighting at an intensity of 2857 K, while Illuminant D_{65} represents noon daylight and has an intensity of 6500 K (American Meat Science Association, 2012). Illuminant A has been shown to have a greater proportion of wavelengths in the red portion of the spectrum and is therefore more highly correlated with visual color (Tapp et al., 2011). Although the American Meat Science Association recommends using Illuminant A to measure meat color,

Illuminant D65 was the most reportedly used illuminant (American Meat Science Association, 2012; Tapp et al., 2011). In addition, nearly half of color papers surveyed neglected to report which illuminant was used during the experiment (Tapp et al., 2011).

The aperture of the instrument is the window used to view the sample (J. W. S. Yancey & Kropf, 2008). The aperture size has been shown to affect the reflectance of samples. As the aperture decreases, L*, a*, and b* values also decrease, indicating a darker, less red, less yellow sample (Sterrenburg, 1989). Additionally, as the aperture size decreases, the reflectance percentage decreases, especially for the wavelengths between 600 to 700 nm which primarily causes contrasts in a* values (J. W. S. Yancey & Kropf, 2008). Some instruments come with a preset aperture size, while others have the option to adjust the aperture diameter. The American Meat Science Association recommends using the largest aperture size without compromising the ability to take multiple measurements of the sample (American Meat Science Association, 2012).

The observation angle of the instrument is another variable that can lead to discrepancies in color values. The observer is defined by the American Meat Science Association's Color Measurement Guidelines as "a human or instrument used to detect color differences" (American Meat Science Association, 2012). The angle at which the sample is viewed by the observer is the observation angle. Some instruments contain multiple degrees of observers, with the most common being 2^{o} and 10^{o} (American Meat Science Association, 2012). The 10° observer is the most reported in literature and is the recommended size by the American Meat Science Association (American Meat Science Association, 2012; Tapp et al., 2011). Because there is such a large number of

instrumental factors that can cause variances in CIE values, they should not be numerically compared from experiment to experiment. Rather, trends in values (in example, increases or decreases in a* values) should be compared when discussing results.

2.2.1.2. Objectively

Meat color can also be quantified using either trained or untrained visual color panels. Trained panels must be screened and undergo training, but are able to provide quantified data that can be compared to anchored, known references (American Meat Science Association, 2012). Trained panelists must be able to discriminate between samples of different colors. Panelists acuity should be tested using the Farnsworth Munsell Hue test before training (American Meat Science Association, 2012). Panelists should then undergo training using standard references and be statistically validated before data collection begins (American Meat Science Association, 2012). Using a trained visual panel provides objective, quantitative data on visual color (American Meat Science Association, 2012). On the other hand, an untrained color panel is used to collect subjective data on consumer preferences of meat color (American Meat Science Association, 2012). Consumer panelists can be asked to rate samples on color liking/disliking, overall acceptability, or other questions to best address the research objective (American Meat Science Association, 2012).

2.3. Factors Affecting Color

2.3.1. Antemortem Factors

The species of animal impacts meat color largely due to differing concentrations of myoglobin present in the muscle. Species known for paler color of meat contain less myoglobin than species with a more vivid red color of meat. For example white meat chicken cuts like the breast, only possess on average 0.31 mg of myoglobin/g of muscle compared to the dark meat cuts like the thigh which contains 1.17 mg myoglobin/g of muscle (Kranen et al., 1999). Lamb, which is dark red in color, contains on average 6.6 mg of myoglobin/kg of muscle (Calnan, Jacob, Pethick, & Gardner, 2016). Young cattle can have on average 4 to 10 mg myoglobin/g of meat while meat from mature cattle can have 16 to 20 mg myoglobin/g of meat (Seideman et al., 1984). Myoglobin's affinity for oxygen can also differ between myoglobin molecules of different species. Nerimetla et al., (2017), found that when bovine and porcine myoglobin molecules are at the same pH, porcine myoglobin has a greater binding affinity to oxygen than bovine myoglobin. Species also impacts the ability for the muscle to reduce metmyoglobin back to deoxymyoglobin. Elroy et al., (2015), found that bovine myoglobin had greater non-enzymatic metmyoglobin reduction than equine and porcine myoglobin molecules *in vitro*. However, in an earlier study, it was reported that the redox stability of equine oxymyoglobin was higher than bovine, while bovine was equal to porcine and ovine (Yin et al., 2011).

Sex of the animal also affects both color and color stability. Gagaoua, Picard, & Monteils, (2018), showed that meat from cows was redder than meat from steers which

was redder than meat from young bulls. Contradictory to those results, a separate study showed that young bulls had greater a* values when compared to cull cows (Imazaki, Elansary, Scippo, Daube, & Clinquart, 2019). Additionally, this study showed that at initial retail display time, cull cows had a greater percentage of metmyoglobin compared to young bulls. However, after 7 d of retail display, young bulls had a greater percentage of metmyoglobin, suggesting cull cows had greater oxidative stability compared to young bulls (Imazaki et al., 2019). Other studies have shown when age is the same, meat from male animal is usually darker than meat from female animals, as male animals have been shown to have greater concentrations of myoglobin (Seideman et al., 1984).

As animals increase in age, the concentration of myoglobin also increases either to ensure the muscle has enough to oxygen to function efficiently or to meet an increasing demand for aerobic metabolism in the muscle (Cho, Kang, Seong, Park, & Kang, 2015; Gagaoua et al., 2018; Lawrie, 1950; Moon, Yang, Park, & Joo, 2006). This affects both the color and color stability of meat. Moon et al., (2006), demonstrated older animals had significantly greater a* and b* values compared to animals in younger age groups. More recent research has shown meat from older animals had decreased L* values and hue angles in addition to greater a*, b*, and chroma values (Gagaoua et al., 2018). This indicates that meat from older animals is darker, more red, more yellow and has a more vivid color, while exhibiting less discoloration than meat from younger animals. Past research has shown that meat from older animals had lower oxidative stability due to increased lipid oxidation, not increased myoglobin oxidation (Xiong et al., 2007).

The effect of breed of cattle on color has been researched, with several studies focusing on breed differences (Neethling, Suman, Sigge, Hoffman, & Hunt, 2017). Holsteins have been shown to have greater metmyoglobin and thiobarbituric acid reactive substances during postmortem aging compared to crossbreed steers (Faustman & Cassens, 1991). Similarly, Holstein meat was found to be less red when compared to meat from Hanwoo cattle (Hur, Park, & Joo, 2008). When comparing *Longissimus dorsi* muscles from Limousin, Charolais, and Hereford cattle, Limousin was the lightest, most red, most yellow, and most vivid (Pogorzelska et al., 2013). Charolais had greater L*, a*, and b* values compared to Hereford (Pogorzelska et al., 2013). When comparing the *Longissimus dorsi* from Podolian, Friesian, and crossbred cattle over a 14 d aging period, initially the Friesian and crossbreed loins were the most red (Marino et al., 2014). However, at the end of the aging period, the Podolian loins had the most red color of any treatment (Marino et al., 2014). Other studies have not found a difference in color due solely to breed (Vieira, Cerdeno, Serrano, Lavin, & Mantecon, 2007). Ultimately, due to the large number of cattle breeds, one study could not possibly compare color and color stability of muscles from all breeds. Additionally, cross-breeding leads to an even large set of possible variables. It has been proposed that differences in color and color stability can be attributed to genetic differences in total myoglobin, reducing capacity, and oxygen consumption rate of the muscles (Neethling et al., 2017).

The impact of diet on color and color stability of beef has been a topic of interest for a long time, with published reports on the subject dating back as far as 1936 (Longwell, 1936). Studies have looked at a variety of factors including the impact of

different feedstuffs and additives. Grass-fed cattle have a darker lean color compared to grain-fed cattle (Crouse, Cross, & Seideman, 1984; Realini, Duckett, Brito, Dalla Rizza, & De Mattos, 2004; Yang, Lanari, et al., 2002). It is suggested that the darker lean color could be due to differences in chilling rates of carcasses (Yang, Lanari, et al., 2002). Previous research has shown that both subcutaneous fat thickness and carcass weight will affect the chilling rate and subsequently the color of meat (P. Mallikarjunan & G. S. Mittal, 1994). Research has also shown grass-fed cattle tend to be smaller and leaner than their grain-fed counterparts when cattle are harvested at the same age (Crouse et al., 1984; Alessandro Priolo, Micol, & Agabriel, 2001; Realini et al., 2004; Yang, Lanari, et al., 2002). Therefore, when placed in the same cooler conditions, the grass-fed carcass will chill at a quicker rate resulting in a darker color. In studies in which the carcass weights did not significantly differ, grass-fed carcasses were not always darker (Alessandro Priolo et al., 2001). Vestergaard, Oksbjerg, & Henckel, (2000), suggest that the darker color of cattle fed grass-finishing diets is due to greater amounts of aerobic metabolism in the muscle antemortem, which limits the glycogen available in the muscle to be metabolized during rigor. While grass-finished cattle have a noticeably more yellow fat, several studies have reported no differences in the muscle color of grass-finished cattle compared to grain-finished (French et al., 2001, 2000; Muramoto, Shibata, & Nakanishi, 2003). Contrarily, Realini et al., (2004), reported that pasture raised cattle have greater L*, a*, and b* values when compared to cattle finished on a concentrate diet.

Color stability is also affected by finishing diet, and may be greater in grass-finished cattle than grain-fed cattle. Lanari, Brewster, Yang, & Tume, (2002) reported that in certain muscles, the shelf life of both fresh and aged muscles was greater in pasture-finished animals than in grain-finished cattle. O'Sullivan et al., (2003), reported that diet did not impact color stability of steaks packaged in overwrap packaging, but when packaged in a high-oxygen MAP, color stability of forage-finished cattle was greater than that of concentrate-finished cattle. Additionally, although Vitamin E concentration was not significantly different, pasture-finished cattle contained higher levels of the antioxidant enzyme superoxide dismutase compared to cattle finished on a mixed diet (Mercier, Gatellier, & Renerre, 2004). Consequently, thiobarbituric acid reactive substances (TBARS) values were lower in meat from the grass-finished animals (Mercier et al., 2004). Differences in muscle metabolism exist between grain-fed and grass-fed cattle (Apaoblaza et al., 2020). Compounds that are known to act as biological antioxidants such as alpha-tocopherol (Vitamin E) and beta-carotene can be present in greater quantities in tissues from grass-fed cattle (Yang, Lanari, et al., 2002).

Finally, antemortem stress affects the final color of beef products. Animals that undergo stress immediately prior to slaughter can have a condition called pale, soft, and exudative (PSE) which is caused by a combination of rapid glycolysis postmortem and elevated temperatures of the muscle (Adzitey & Nurul, 2011; Fox, Wolfram, Kemp, & Langlois, 1980). In pork, PSE can also be caused by genetic factors (Joo, Kauffman, Kim, & Kim, 1994). This meat is pale in color, soft in texture, and contains exudate on the surface (Fox et al., 1980). Additionally, it has poor ability to hold onto water and

exudes liquid more rapidly than normal meat (Aalhus, Best, Murray, & Jones, 1998). PSE meat also has less pigment stability than normal meat (Livingston & Brown, 1981; Sørheim, Erlandsen, Nissen, Lea, & Høyem, 1997). PSE is more likely to occur in pork than in beef due to differences in muscle type as porcine muscles contain a greater proportion of glycolytic muscle fibers (Aalhus et al., 1998).

On the other hand, animals that endure long term stress prior to slaughter can have the condition called dark, firm, and dry (DFD; Adzitey & Nurul, 2011). In this condition, the muscle has little glycogen available to metabolize postmortem, resulting in a high ultimate pH (Adzitey & Nurul, 2011). In cattle, this condition is commonly referred to as dark cutting beef or "dark cutters" (Egbert & Cornforth, 1986). DFD meat is dark in color, firm in texture, and appears dry on the surface. This meat has an increased ability to hold onto water (Adzitey & Nurul, 2011). Additionally, DFD meat differs in color stability compared normal meat. Hughes et al., (2017), reported that meat with a high ultimate pH promoted greater myoglobin oxidation than normal meat. Contrarily, other studies have suggested that DFD meat has less metmyoglobin production over retail display (Zhu & Brewer, 1998). These differing results could be explained by the presence of spoilage bacteria, as DFD meat is more subject to bacterial spoilage due to the high ultimate pH of the meat (Gill & Newton, 1981).

2.3.2. Postmortem Endogenous Factors

The pH decline and ultimate pH of muscle largely influence the color of fresh meat products. Abril et al., (2001), reported that all color variables (L*, a*, b*, chroma, and hue angle) are affected by ultimate pH. The ultimate pH of normal meat should range between

5.4 and 5.8 (Faustman & Cassens, 1990). A lower pH is more favorable for myoglobin oxidation as the lower pH increases the release of superoxide molecules (Faustman & Cassens, 1990; Livingston & Brown, 1981). The pH decline of postmortem muscle is determined by a number of factors including stress, glycolytic potential, and muscle type. As previously discussed, if the pH declines too rapidly or is ultimately too low, the meat appears too light in color as seen in PSE meat (J. D. Fox et al., 1980). However, if the pH does not drop low enough, the color will appear too dark (Adzitey & Nurul, 2011). The glycolytic potential of the muscle is the amount of substrates in the muscle that can be used for anaerobic metabolism to produce lactic acid (Mancini & Hunt, 2005). Several studies have shown that an increase in glycolytic potential in muscle is correlated to a lighter color (Hamilton, Miller, Ellis, McKeith, & Wilson, 2003; Meadus & MacInnis, 2000; Moeller, Bass, Leeds, Emnett, & Irvin, 2003). The muscle type and location can also impact pH. Baublits, Pohlman, Brown, Yancey, & Johnson, (2006), reported differences in pH of *Infraspinatus* and *Biceps femoris* muscles in the same animals. Large, thick muscles such as the *Semimembranosus* can vary in pH across areas of the same muscle due to differences in chilling rate and glycolytic metabolism within the muscle (M. S. Lee, Yancey, Apple, Sawyer, & Baublits, 2007). It has been shown in other studies that muscles with higher concentration of fast-twitch fibers have a lower pH compared to muscles with higher concentrations of slow-twitch fibers (Chikuni et al., 2010). Other factors that have been shown to impact the pH of muscle include fat thickness, sex of the animal, and breed (Page, Wulf, & Schwotzer, 2001). Ultimately, pH is a dynamic system

that is affected by many factors pre- and post-mortem. This makes pH a difficult variable to predict and control in the product.

Muscle fiber type can also affect color and color stability. Muscles were originally sorted as red or white muscles depending on their visual appearance (Zierath & Hawley, 2004). These thoughts led to muscle fiber types being classified as red and white muscle fibers, which differ in myoglobin content (Zierath & Hawley, 2004). Fibers then became designated into slow- and fast-twitch fibers based on staining differences (Zierath & Hawley, 2004). Fast-twitch fibers can now be divided into glycolytic or oxidative fast-twitch fibers (Zierath & Hawley, 2004). Currently, four isoforms of muscle fibers have been defined in skeletal muscle: Type I, IIA, IIX, and IIB (Picard, Gagaoua, & Gagaoua, 2020). Type I fibers are red in color, more oxidative, and have a slow contraction speed (Picard et al., 2020). Type IIA, IIX, and IIB fibers have a faster contraction speed (Picard et al., 2020). While Type IIA fibers are red and have an equal affinity for both oxidative and glycolytic metabolism, Type IIX and IIB fibers are paler in color and use more glycolytic metabolism (Picard et al., 2020). Muscle fiber composition can vary by sex, breed, and sire (Komiya et al., 2020; Seideman & Crouse, 1986). Within an animal, muscles differ in the proportion of fiber types based on muscle functionality (Van Bibber-Krueger et al., 2020). For example, the *Infraspinatus* which is classified as a red muscle has 46.6% β-red (Type I) fibers, 28.5% α-red fibers (Type IIA), and 24.9% α-white (Type IIB) fibers while a white muscle such as the *Longissimus dorsi* is comprised of 35% β-red fibers, 21.8% α-red fibers, and 43.2% α-white fibers (Kirchofer, Calkins, & Gwartney, 2002). Muscle fibers can also be classified as

intermediate such as the *Subscapularis* which is 39.5% β-red fibers, 33.1% α-red fibers, and 27.5% α-white fibers (Kirchofer et al., 2002). Muscles with a greater concentration of red fibers are more color-labile than muscles with more white fibers (Canto et al., 2015).

Oxygen consumption rate determines oxygen penetration depth into the muscle and subsequently the depth of the oxymyoglobin layer in meat (Bendall, 1972). The oxygen consumption rate of the muscle also affects the color stability of meat by determining the depth of metmyoglobin formation (Madhavi & Carpenter, 1993). If metmyoglobin initially forms close to the surface of the muscle, the color deteriorates quicker (Madhavi & Carpenter, 1993). This occurs when the oxygen consumption rate of the muscle is high (Madhavi & Carpenter, 1993). Oxygen consumption rate is greatest initially in postmortem muscle, and declines during aging (Madhavi & Carpenter, 1993). Oxygen consumption rate varies by species and muscle (Faustman & Cassens, 1990). Of meat animal species, lamb has the greatest oxygen consumption rate, followed by pork, and then by beef (Faustman & Cassens, 1990). Lanari & Cassens, (1991), reported that regardless of species, the *Gluteus Medius* had greater oxygen consumption rate compared to the *Longissimus dorsi* throughout the postmortem aging period. Additionally, O'Keeffe & Hood, (1982) reported that the *Psoas major* had a greater oxygen consumption rate than the *Longissimus dorsi.* Oxygen consumption rate in muscle is also affected by endogenous and exogenous factors such as the pH and temperature of the muscle postmortem. Increases in both the temperature and pH lead to an increase in oxygen consumption (Faustman & Cassens, 1990; O'Keeffe & Hood,

1982). The amount of substrate available for mitochondrial activity also impacts the oxygen consumption rate of muscle. Oxygen consumption rate decreases as the amount of substrate decreases (O'Keeffe & Hood, 1982). Additionally, the way the muscle is processed can influence the oxygen consumption rate of muscle. Compared to whole muscle cuts, grinding or mincing of muscle pre-rigor increases the oxygen consumption rate of the muscle (Faustman & Cassens, 1990). This could be another reason that ground meat has a decreased shelf-life compared to whole muscle cuts.

The reducing capacity of the muscle plays a large role in the color stability of meat products. The anaerobic metmyoglobin reducing activity (MRA) was the first system reported to convert metmyoglobin back to deoxymyoglobin in meat (Stewart, Hutchins, Zipser, & Watts, 1965). Metmyoglobin reducing activity in ground meats was reported to be affected by pH and temperature, with greater temperatures and higher pH resulting in increased metmyoglobin reduction (Stewart et al., 1965). Similarly, another system known as the aerobic metmyoglobin reduction (ARA) was reported soon after (Ledward, 1970). It has also been shown that metmyoglobin reduction is accomplished by mitochondria and mitochondrial enzymes involved in glycolysis, the TCA cycle, and the electron transport chain (Giddings & Hultin, 1974; Govindarajan & Snyder, 1973; O'Keeffe & Hood, 1982). Additionally, it has been reported that reduction is accomplished through enzymes called metmyoglobin reductases, which rely on NADH as a cofactor (Faustman & Cassens, 1990). Metmyoglobin reducing activity in meat decreases post rigor due to the decline in muscle pH, decrease of substrates and cofactors for reduction, and less mitochondrial integrity (Giddings & Hultin, 1974; O'Keeffe &

Hood, 1982). Elroy et al., (2015), stated there are many different mechanisms for metmyoglobin reduction, but the most extensively studied are the systems relying on NADH. Non-enzymatic systems for reducing metmyoglobin have also been reported in meat (Brown & Snyder, 1969; Elroy et al., 2015). Non-enzymatic metmyoglobin reduction is greater at the average postmortem muscle pH of 5.6 compared to the physiological pH of 7.4 (Elroy et al., 2015). Several early studies reported no correlation in metmyoglobin reducing activity and color stability (B. E. Greene, 1969; Ledward, 1970). Govindarajan & Snyder, (1973) hypothesized that the disparity in the results could be due to errors in the protocol to measure metmyoglobin reduction. More recent studies have shown that increased MRA leads to an increased color stability in the product (Bekhit & Faustman, 2005).

Finally, fat will affect the color of the product, with lightness most commonly influenced. Troutt et al., (1992), reported that as the fat percentage of ground beef patties increased, the L* values increased, while a* and b* values did not differ. Berry, (1998), also reported greater L* values for patties with higher fat content. Additionally, Suman et al., (2004), reported that patties made from the *Psoas major* (13.21% fat) were lighter and less red than patties made from the *Longissimus lumborum* (9.32% fat). It is hypothesized the color differences were driven by the differences in fat content (Suman et al., 2004). However, a more recent study by Ismail, Lee, Ko, & Ahn, (2009), reported no differences in lightness in patties containing 10%, 15%, or 20% fat. Although fat percentage has not been shown to affect color in all studies, it is likely that patties that have greater fat percentages will be lighter in color. Furthermore, the fatty acid

composition present in fat can affect color stability (Wood et al., 2008). Beef that contains greater polyunsaturated fatty acids are more susceptible to lipid oxidation, which can lead to greater discoloration (Faustman, Sun, Mancini, & Suman, 2010; Suman et al., 2014). This can be an issue for grass-finished cattle, as they contain higher concentrations of polyunsaturated fatty acids such as 18:3 (linolenic acid; Mancini & Hunt, 2005).

2.3.3. Postmortem Exogenous Factors

Processing whole muscles impacts the color and color stability of the product. Madhavi & Carpenter, (1993), reported that grinding led to decreased color stability compared to whole muscles. It was hypothesized that these differences were due to an increased oxygen consumption rate of the product after tissue disruption (Madhavi & Carpenter, 1993). Although little is known about the role of mitochondrial matrix enzymes on meat discoloration, by grinding muscle, these enzymes are released from the matrix which could impact the state of myoglobin (Ramanathan, Nair, Hunt, & Suman, 2019). In addition, lipid oxidation increases due to grinding (Faustman et al., 2010; Gray, Gomaa, & Buckley, 1996). Other factors such as the temperatures of the muscle during processing will affect the color. Many studies across various species increased temperatures during processing decrease the color stability of meat (Neethling et al., 2017; O'Keeffe & Hood, 1982; Rosenvold & Wiklund, 2011).

The aging time of muscles can have a positive or negative effect on meat color depending on the length of aging. Wet aging muscles for up to 21 days has been shown to increase the redness and total color of the meat (Oliete et al., 2006, 2005; Suman et

al., 2014). However, wet-aging for extended periods of time prior to retail display reduces the color stability of muscle (King, Shackelford, Kalchayanand, & Wheeler, 2012; Suman et al., 2014). Therefore, it is important to assure that aging times are similar between treatments, in order to minimize variation in color stability caused by aging time.

Packaging has a large effect on the color and color stability of fresh meats. Ground beef is predominantly packaged in either overwrap or high-oxygen modified atmosphere packaging (MAP) in the United States (Suman et al., 2014). Other packaging systems used in the industry include: vacuum-packaging, low-oxygen MAP, and carbon monoxide MAP. Modified atmosphere packaging has been a large advancement to meat color and color stability. High-oxygen atmospheres (80% oxygen) promote the formation of oxymyoglobin and increases the display life of products (Mancini & Hunt, 2005). However, the display life of products can be longer than the flavor life of products (Jayasingh, Cornforth, Carpenter, & Whittier, 2001; Mancini & Hunt, 2005). Low-oxygen MAP can minimize aerobic bacterial growth and lipid oxidation, however, achieving meat bloom is difficult during prolonged storage (Mancini & Hunt, 2005). The addition of carbon monoxide to MAP improves color of meat while in the package (Hunt et al., 2004; Mancini & Hunt, 2005; Suman et al., 2014). However, exposure to air and light can lead to discoloration of the product when removed from packaging (Hunt et al., 2004; Mancini & Hunt, 2005; Suman et al., 2014). The largest issue with using carbon monoxide in MAP is federal regulations. Carbon monoxide MAP was outlawed by the European Economic Area in 2004 (Suman et al., 2014). Conversely, the United States

approved use of up to 0.4% carbon monoxide in packages in 2004 (Suman et al., 2014). Meat packaged in vacuum packages appear to be a purple-red color as opposed to the traditional bright red (Suman et al., 2014). Vacuum packaged ground beef has been shown to maintain its color for seven days in retail display (Jeong & Claus, 2011; Suman et al., 2014). However, consumers prefer the cherry-red color of beef as seen in a PVC overwrap (Carpenter, Cornforth, & Whittier, 2001; Mancini & Hunt, 2005). Carpenter et al., (2001), hypothesized that this preference would slow the progress of MAP and vacuum packaging.

The conditions in the cooler during retail display have a large effect on the color stability of products. Increased cooler temperatures result in decreased color stability, as higher temperatures provide a more favorable condition for myoglobin oxidation (Brown & Mebine, 1969; Faustman et al., 2010; Neethling et al., 2017). The lighting type used in the cooler will also affect the color stability of the product. Using LED lights in the retail display case results in lower cooler temperatures, while L*, a*, and b* values of ground beef do not differ compared to using fluorescent lights (Steele et al., 2016). In addition, the ground beef stored under LED lighting has a longer shelf life than ground beef stored under standard fluorescent lights (Steele et al., 2016). The display case or cooler may also have inherent variation in color, lighting intensity, and temperature within the system. To mitigate these potential differences, products are to be rotated throughout the cooler side to side and front to back regularly throughout the display period (American Meat Science Association, 2012).

2.4. Oxidation

Oxidation of lipids and proteins is one of the biggest problems currently faced in the meat industry (Falowo, Fayemi, & Muchenje, 2014; Sampels, 2013). The International Union of Pure and Applied Chemistry (IUPAC) defines oxidation as either a process where an atom loses electrons, an atom increases in oxidation state, or the loss of a hydrogen or gain of oxygen by an organic substrate (Silverstein, 2011). In meat products, oxidation is commonly thought of the as the loss of an electron (Seideman et al., 1984). Meat products contain many constituents that are subject to deterioration via oxidation including lipid, proteins, vitamins, and pigments (Domínguez et al., 2019). Oxidation can cause the product to lose nutritional value and produce compounds that can be potentially toxic (Domínguez et al., 2019). Oxidation is also a major cause of quality declines in meat products (Ladikos & Lougovois, 1990). In the United States alone, it was estimated that oxidation of myoglobin, which causes product discoloration, was visible on 15% of products at the retail level and responsible for a loss of one billion dollars of revenue annually (Troy & Kerry, 2010). Lipid oxidation products cause off flavors and odors in meat and meat products (Baron & Andersen, 2002). The rancidity caused by lipid oxidation begins to develop as soon as exsanguination and continues to increase in intensity until consumers will no longer accept the product (Gray et al., 1996). Additionally, oxidation of myofibrillar proteins can also cause detrimental effects to meat quality attributes such as tenderness (Bao, Boeren, & Ertbjerg, 2018). Overall, oxidation is a limiting factor in the consumer acceptability of meat as it affects all three

consumer quality attributes: appearance, texture, and flavor (Gray et al., 1996; Q. Liu, Lanari, & Schaefer, 1995).

2.4.1. Protein Oxidation

Protein oxidation in meat has increased in attention in recent years as consumers have noted the negative effects of protein oxidation (Jongberg, Lund, & Skibsted, 2017). While protein oxidation most typically refers to myofibrillar protein oxidation, myoglobin is a protein and thus the two will be discussed together. Myoglobin, the pigment producing protein in meat, is oxidized to the biologically inactive form, metmyoglobin, via autoxidation (Brown & Mebine, 1969). In the autoxidation process, deoxymyoglobin or oxymyoglobin can be oxidized to form metmyoglobin (Suman & Joseph, 2013). When myoglobin becomes oxidized, the iron in the heme ring of the molecule loses an electron (Suman & Joseph, 2013). The iron is then in the ferric (Fe^{3+}) form and is bound to a water molecule (Suman & Joseph, 2013). Myoglobin of ruminants, including cattle, is more susceptible to oxidation than porcine myoglobin (Gutzke & Trout, 2002; Rousseaux, Dautrevaux, & Han, 1976; Suman & Joseph, 2013). It has been hypothesized that the primary structure of myoglobin, which differs from species to species determines the rate of autoxidation of myoglobin (Suman & Joseph, 2013). Myoglobin proteins with a greater amount of oxidizable residues such as cysteine, oxidize more rapidly (Suman & Joseph, 2013). Differences in the primary amino acid sequence of myoglobin also causes differences in the ability of myoglobin to remain bound to the heme iron (Suman & Joseph, 2013). Myoglobin molecules that are not able to remain bound to the heme iron exhibit discoloration faster (Suman & Joseph,

2013). Myoglobin oxidation is affected by many endogenous and exogenous postmortem factors as thoroughly reviewed in Section III.

Several pre- and post-harvest techniques have been proposed to improve color stability of beef. Daniel, Dikeman, Arnett, & Hunt (2009) reported that restricting Vitamin A in cattle finishing diets could improve beef color over retail display. Grass-finished beef increases the redness of meat during retail display in aerobic packaging (Insani et al., 2008). It has been hypothesized that the increased consumption of antioxidant compounds from forages, specifically α-tocopherol and β-carotene, decreases oxidation of myoglobin in forage-finished beef (Insani et al., 2008; Suman et al., 2014). In ground beef, combining color-labile and color-stable muscles could be utilized to increase the color stability of the batch (Suman et al., 2014). Raines, Hunt, & Unruh (2010) reported that for 80% lean patties, creating ground beef batches that contain either 50% or greater color-stable muscles or limiting color-labile muscles to less than 25% of the batch can increase the color stability of the batch. Other research has examined changes in packaging, addition of exogenous antioxidant and/or reducing compounds, and antimicrobials to decrease myoglobin oxidation in meat products (Suman et al., 2014).

Oxidation of myofibrillar proteins in meat is a more complex process than oxidation of heme proteins or lipids and produces a wider variety of reaction byproducts (Jongberg et al., 2017). Oxidation generally affects the side chains of amino acids (Stadtman, 1990; Zhang, Xiao, & Ahn, 2013). Possible modifications from protein oxidation include thiol oxidation, aromatic hydroxylation, and formation of carbonyl

groups (Stadtman, 1990). Cysteine and methionine are the most susceptible amino acids to oxidation because they possess a reactive sulfur atom, which is a powerful nucleophile and contains many electrons that are easily removed (Shacter, 2000; Zhang et al., 2013). Oxidants, such as reactive oxygen species, aldehydes, and ketones are able to directly attack the backbone of proteins (Zhang et al., 2013). This can cause protein fragmentation or conformational changes that effect the tertiary or secondary structure of the protein, which can lead to aggregation and loss of functional properties (Martinaud et al., 1997; Morzel, Gatellier, Sayd, Renerre, & Laville, 2006; Zhang et al., 2013). Lund, Heinonen, Baron, & Estévez (2011) illustrated the basic causes and byproducts of protein oxidation in meat. These byproducts of protein oxidation change physical and chemical properties of proteins including their solubility, hydrophobicity, water-holding capacity, and tenderness (Liu & Xiong, 2000; Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004a, 2004b; Srinivasan & Hultin, 1997; Srinivasan & Xiong, 1996; Zhang et al., 2013).

Myofibrillar protein oxidation proceeds by a free radical chain reaction similar to lipid oxidation (Lund et al., 2011). Reactive oxygen species (ROS) can be produced by mitochondrial metabolism processes such as the electron transport chain, fatty acid metabolism, and cytochrome reactions (Beckman & Ames, 1998; Shacter, 2000; Zhang et al., 2013). Reactive oxygen species produced include free radical such as $^{\bullet}OH$, $O_2^{\bullet-}$, $RS^{\bullet}$, and $ROO^{\bullet}$ or nonradicals such as H_2O_2 and ROOH (Zhang et al., 2013).

Finishing animals on a grass-fed diet and/or supplementing the diets with antioxidants such as tocopherols has been shown to decrease the protein oxidation in

meat (Lund et al., 2011; Ruiz, Muriel, & Ventanas, 2002). Protein carbonyl concentration has a negative correlation to Vitamin E concentration in the muscle (Lund et al., 2011). It was proposed that decreasing the ratio of polyunsaturated fatty acids (PUFAs) to total fatty acids would decrease the incidence of protein oxidation (Lund et al., 2011). However, Lund, Lametsch, Hviid, Jensen, & Skibsted (2007) found no correlation of unsaturated fatty acid amount and protein oxidation in pork. Similarly, the addition of a hydrophilic antioxidant, reduced protein oxidation in meat (Baron, Berner, Skibsted, & Refsgaard, 2005; Lund et al., 2011). However, adding a lipophilic antioxidant to the product has no effect on protein oxidation (Baron et al., 2005). Another product that is being looked at to prevent protein oxidation is plant phenolic compounds (Lund et al., 2011). Phenolic compounds can be both hydrophilic and hydrophobic antioxidants and thus could act in both the aqueous and lipid phases of meat (Lund et al., 2011; Fereidoon Shahidi, Janitha, & Wanasundara, 1992). Whether a plant phenolic compound can have antioxidant properties is dependent on its chemical structure as well as extrinsic factors of the product (Edwin N. Frankel & Meyer, 2000; Lund et al., 2011). More research is necessary to definitively determine if phenolic compounds can be used as effective antioxidants in meat products.

2.4.2. Lipid Oxidation

Lipids are an important constituent in the flavor, aroma, tenderness, and juiciness of meat (Amaral, Solva, & Lannes, 2018). However, lipids are considered one of the most chemically unstable food components (Amaral et al., 2018; Min & Ahn, 2005). Lipid oxidation leads to deterioration of fatty tissues in meat and thus affects the quality,

color, texture, aroma, taste, and shelf-life of meat (Amaral et al., 2018; Min & Ahn, 2005). Lipid oxidation can be measured in meat by assessing the peroxide value or by measuring thiobarbituric acid-reactive substances (TBARS; Falowo et al., 2014). The oxidative stability of lipids in meat are impacted by the type of lipid structure and the environment as well as the degree of unsaturation, exposure to light and heat, the presence of oxygen, and the presence and concentration of pro-oxidant and antioxidant compounds (Amaral et al., 2018). Meat products naturally contain iron from myoglobin and hemoglobin, hydrogen peroxide, and ascorbic acid which can promote oxidation by catalyzing oxidizing reactions or generating reactive oxygen species (Amaral et al., 2018).

Lipids in meat products can be oxidized via autoxidation, photo-oxidation, or enzymatic oxidation (Domínguez et al., 2019). Autoxidation is a complex process that occurs from self-programming radical reactions (Amaral et al., 2018). These reactions are dependent on the temperature and pH of the product as well as the presence of metal ions and free radicals (Amaral et al., 2018). There are three steps to autoxidation: initiation, propagation, and termination (Cheng, 2016; Domínguez et al., 2019). During initiation, a radical compound removes a hydrogen from the carbon adjacent to a double bound of an unsaturated fatty acid forming an alkyl radical (Domínguez et al., 2019). The compound involved in the reaction can be a singlet oxygen, reactive oxygen species such as hydrogen peroxide, a superoxide anion, or a hydroxyl radical (Domínguez et al., 2019; Erickson, 2002). These compounds arise by the activation of oxygen by an energy source such as light or by the presence of pro-oxidant compounds such as transition

metals (Domínguez et al., 2019; Min & Ahn, 2005). During propagation, the radical lipids react with other lipids, exacerbating the formation of new radicals (Domínguez et al., 2019). The alkyl radical reacts with oxygen to form peroxy radicals (Min & Ahn, 2005). The peroxy radical can then remove a hydrogen from a second lipid, forming a hydroperoxide and a new alkyl radical (Domínguez et al., 2019). This cycle continues until termination (Domínguez et al., 2019). Hydroperoxides formed during propagation can also decompose and form new hydroxyl, peroxy, and alkyl radical compounds that can then initiate autoxidation in other lipids (Chaijan & Panpipat, 2017; Domínguez et al., 2019; Králová, 2015). Finally, termination occurs when the radical reacts with other radical or an antioxidant compound to produce non-radical products (Domínguez et al., 2019).

Photo-oxidation occurs by radiant energy mainly in the form of ultraviolet radiation in the presence of pro-oxidant compounds such as myoglobin (Amaral et al., 2018). Photo-oxidation occurs faster than autoxidation in meat products as they are generally directly exposed to light during retail display (Domínguez et al., 2019; J.M. Lorenzo, Dominguez, & Carballo, 2017). In this process, radical reactions occur producing unique hydroperoxides during initiation (Amaral et al., 2018). Initiation of photo-oxidation occurs when a singlet sensitizer, such as myoglobin or hemoglobin, absorbs light energy and becomes an excited triplet sensitizer (Domínguez et al., 2019). This excited sensitizer can either react with molecular oxygen to produce a singlet oxygen, react with a triplet oxygen to produce a superoxide radical anion, or abstract a

hydrogen from an unsaturated fatty acid and form an alkyl radical (Choe & Min, 2006; Domínguez et al., 2019; Erickson, 2002; Wasowicz et al., 2004).

Enzymatic oxidation is catalyzed by the enzyme lipoxygenase, which adds oxygen to the hydrocarbon fatty acid chain (Amaral et al., 2018). The reaction forms peroxides and hydroperoxides with conjugated double bounds, which then degenerate to form several products (Amaral et al., 2018; José M. Lorenzo & Gómez, 2012). The main difference between enzymatic oxidation and the free radical mechanisms previously discussed is the formation of hydroperoxides during initiation (Domínguez et al., 2019). During enzymatic oxidation, the active site of lipoxygenase contains a ferrous iron that abstracts a hydrogen atom from the methylene group of a polyunsaturated fatty acid (Domínguez et al., 2019; Ghnimi, Budilarto, & Kamal-Eldin, 2017). This forms a conjugated diene that will react with molecular oxygen to form a peroxy radical (Domínguez et al., 2019). The peroxy radical will then remove hydrogen from another unsaturated fatty acid molecule, generating a conjugated hydroperoxyl diene and alkyl radical (Chaijan & Panpipat, 2017; Domínguez et al., 2019; Wasowicz et al., 2004).

Diet has a major impact on lipid content and oxidation in meat products. Beef finished on a grass-based diet has an increased concentration of polyunsaturated fatty acids, and thus are more susceptible to lipid oxidation (Gatellier, Mercier, Juin, & Renerre, 2005; Larick & Turner, 1989; S. L. Melton, Black, Davis, & Backus, 1982; Yang, Lanari, et al., 2002). However, grass-fed cattle have a greater concentration of α-tocopherol (Vitamin E) which could delay lipid oxidation (Lanari et al., 2002; Luciano, Moloney, et al., 2011; Realini et al., 2004). Additionally, meat from grass-fed cattle has

lower TBARS values compared to meat from grain-finished cattle (Gatellier et al., 2005; Nuernberg et al., 2005; Realini et al., 2004). Therefore, grass-fed beef has been shown to decrease the lipid oxidation of beef while also providing higher concentrations of polyunsaturated fatty acids. This is likely due to grass-fed cattle having a higher ratio of antioxidant compounds to PUFA (Luciano, Moloney, et al., 2011).

2.4.3. Interrelationship Between Myoglobin, Lipid, and Myofibrillar Protein Oxidation

The interaction between myoglobin and lipid oxidation has been a topic of research for many years (Alderton et al., 2003; Faustman, Liebler, McClure, & Sun, 1999; Lynch & Faustman, 2000; Mancini & Hunt, 2005). It has been reported that oxidation of the heme iron in myoglobin catalyzed the oxidation of unsaturated fatty acids in meat (Baron & Andersen, 2002). However, Alderton et al., (2003) reported that a by-product of lipid oxidation (4-hydroxy-2-nonenal) decreases the stability of oxymyoglobin by binding to the histidine that stabilizes the compound. Although it has not clearly been defined which occurs first, the oxidation of both compounds was shown to be positively correlated (Gray et al., 1996; Q. Liu et al., 1995). Reactive aldehydes such as malondialdehyde produced during lipid peroxidation can also cause protein oxidation (Requena et al., 1997; Uchida & Stadtman, 1994; Zhang et al., 2013). It has been proposed that decreasing lipid oxidation in products would decrease the secondary byproducts that can oxidize proteins and thereby lower the protein oxidation in products (Lund et al., 2011). Additionally, myoglobin and hemoglobin can initiate myofibrillar protein oxidation, as the heme iron can act as a pro-oxidant (Baron & Andersen, 2002; Zhang et al., 2013). Furthermore, the secondary products of lipid oxidation can interact

with amino acid residues resulting in the oxidation of myofibrillar proteins (Zhang et al., 2013). Faustman et al., (2010) reported that protein oxidation increases lipid oxidation and vice versa. Myoglobin can also act as pro-oxidant to initiate and accelerate lipid and protein oxidation (Zhang et al., 2013). Protein oxidation products are thought to be active at the interface of water and lipids in meat products (Jongberg et al., 2017). This finding has caused researchers to hypothesize that this could be the connection between lipid, myofibrillar, and heme protein oxidation (Jongberg et al., 2017).

2.5. Antioxidants

Antioxidants are the principle compounds in food products that prevent the oxidative deterioration of lipids, proteins, carbohydrates, and other small molecules, thus preserving quality (Halliwell, Aeschbach, Löliger, & Aruoma, 1995; Fereidoon Shahidi et al., 1992). In addition, antioxidants in antemortem muscles also protect cells against damage by reactive oxygen species (ROS; Halliwell, Aeschbach, Löliger, & Aruoma, 1995). Biological tissues contain several endogenous antioxidants systems, however, food processing operations are capable of removing or overwhelming these antioxidants naturally present (Damodaran et al., 2008). Many studies have been conducted examining the use of natural and synthetic antioxidants to reduce oxidation and increase the shelf life of meat products (Falowo et al., 2014; Lanari et al., 2002; Liu et al., 1995; Yang, Brewster, Lanari, & Tume, 2002; Yang, Lanari, et al., 2002). Natural antioxidants can be added to muscle either through the diet of the animal or added directly to the meat product (Falowo et al., 2014). Synthetic antioxidants can be added to the product, however, their addition and use level is governed by federal regulations (Fereidoon

Shahidi et al., 1992). Synthetic antioxidants that can be added to foods are butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG), dodecyl gallate (DG), and tertiary-butylhydroquinone (TBHQ; Shahidi et al., 1992).

2.5.1. Antioxidant Mechanisms and Classifications

Antioxidant compounds can be classified as either natural or synthetic depending on their source. After the antioxidant source is defined, they can be further be classified based on their mechanism to prevent oxidation (Damodaran et al., 2008). Antioxidants used in food products have three potential mechanisms to increase the oxidative stability of foods (Damodaran et al., 2008). The first potential mechanism is controlling free radicals (Damodaran et al., 2008). By scavenging free radicals in products, these antioxidants can inhibit initiation, propagation, and β-scission reactions (Damodaran et al., 2008). These compounds are able to interact with peroxyl or alkoxyl radicals, but are thought to mostly react with peroxyl radicals due to their lower energy state (Damodaran et al., 2008). Free radical scavengers are able to donate a hydrogen atom to a free radical as long as the antioxidant has a lower reduction potential than that of the free radical and the reaction is kinetically feasible (Damodaran et al., 2008). Effective free radical scavengers are able to form lower energy radicals that will not react rapidly to oxygen (Damodaran et al., 2008). Phenolic compounds possess many of the aforementioned qualities of efficient free radical scavengers (Damodaran et al., 2008). The most common free radical scavengers in food are tocopherols including α-tocopherol (Vitamin E), plant phenolics such as anthocyanins and flavonoids, ascorbic acid, and thiols

(Damodaran et al., 2008). Synthetic phenolic compounds such as BHT, BHA,TBHQ, and propyl gallate can be added to foods during processing (Damodaran et al., 2008).

Since lipid oxidation is highly dependent on the concentration and activity of prooxidant compounds, controlling these compounds can increase the oxidative stability of foods (Damodaran et al., 2008). Prooxidants in foods include transition metals, singlet oxygen, and some enzymes (Damodaran et al., 2008). Some antioxidants are able to control prooxidant metals present in food by either chelating or sequestering the transition metals (Damodaran et al., 2008). Metal chelators inhibit the activity of metals by preventing metal redox cycling, occupying the metal coordination site, forming insoluble metal complexes, or using steric hindrance to prevent the interaction of metals and oxidation intermediates (Damodaran et al., 2008). Antioxidants that utilize this mechanism include ethylenediamine tetraacetic acid (EDTA), citric acid, phytate and other polyphosphates (Damodaran et al., 2008). Antioxidant such as transferrin, ferritin, and casein are proteins that are able to sequester metals to prevent oxidation (Damodaran et al., 2008). Singlet oxygen is an oxygen molecule in the excited state which will promote lipid oxidation in meat products (Damodaran et al., 2008). These molecules can be controlled by carotenoid compounds such as β-carotene, which have the ability to quench the singlet oxygen or lower the energy of the singlet oxygen to the ground-state triplet oxygen (Damodaran et al., 2008).

Finally, *in vivo* enzymes can prevent oxidation (Halliwell et al., 1995). Superoxide dismutase (SOD) catalyzes the breakdown of the superoxide anion radical, which is capable of forming perhydroxyl radicals and catalyzing lipid oxidation

(Damodaran et al., 2008). This reaction forms oxygen and hydrogen peroxide (Damodaran et al., 2008). Catalase is then able to inactive the hydrogen peroxide produced by SOD and produce water and molecular oxygen (Damodaran et al., 2008). Additionally, glutathione peroxidase is an enzyme that can decompose lipid hydroperoxides or hydrogen peroxide to form a fatty acid alcohol or water, respectively (Damodaran et al., 2008).

Antioxidants can also be considered as primary or secondary antioxidants, based on the products they interact with. Primary antioxidants will react directly with high-energy radicals and convert them to thermodynamically stable products (Damodaran et al., 2008; Fereidoon Shahidi et al., 1992). Secondary or preventative antioxidants will break down hydroperoxides capable of initiating the oxidation of other compounds, thus slowing the rate of oxidation in products (Fereidoon Shahidi et al., 1992). Free radical scavengers can be either primary or secondary antioxidants (Damodaran et al., 2008). Antioxidants can also be capable of interacting with each other to increase the total oxidative stability of foods (Damodaran et al., 2008). For example, a primary free radical scavenger such as α-tocopherol can have enhanced activity when it can be regenerated by a secondary free radical scavenger such as ascorbic acid (Damodaran et al., 2008). Metal chelators can also enhance the ability of some free radical scavengers (Damodaran et al., 2008). The metal chelator will reduce the number of free radicals formed via metal-catalyzed oxidation, thus slowing the rate of inactivation of free radical scavengers present (Damodaran et al., 2008).

2.5.2. Naturally Occurring Antioxidants

Natural antioxidants are mostly the products of fruits, vegetables, herbs, and spices (Falowo et al., 2014). Plant materials are composed of many phenolic compounds (Falowo et al., 2014). Phenolic compounds have the ability to become oxidized themselves, which delays oxidation of lipids and proteins in meats (Falowo et al., 2014). Plant phenolic compounds are classified into several different groups based on the chemical structures of their aromatic rings: phenolic acids, flavonoids, diterpenes, tannins, stilbenes, curcuminoids, coumarins, lignans, or quinones (Falowo et al., 2014; Fresco, Borges, Diniz, & Marques, 2006; Huang, Cai, & Zhang, 2009). Phenolic compounds can inhibit oxidation by two different mechanisms. First, the phenolic compound is able to donate electrons which results in termination of free radicals (Allen & Cornforth, 2010; Dangles & Dufour, 2006; Falowo et al., 2014). Second, the phenolic compounds can chelate metals and remove ROS initiators to prevent oxidation from occurring (Allen & Cornforth, 2010; Dai & Mumper, 2010; Falowo et al., 2014).

Vitamin E, also known as α-tocopherol, is a major lipid-soluble antioxidant compound located in muscle (Wood & Enser, 1997). It is present in muscle from animals on a grass-based diet including cattle, sheep and goats, and can also be supplemented in diets (Wood & Enser, 1997). Vitamin E is considered to be the most powerful propagation disruptor present in muscle (Descalzo & Sancho, 2008). Additionally, β-carotene is a carotenoid found in plants which is deposited in the inner part of lipid membranes and can cooperate with tocopherols to scavenge radical compounds (Descalzo & Sancho, 2008). Furthermore, β-carotene is active at low partial

pressures of oxygen to quench lipid oxidation byproducts (Descalzo & Sancho, 2008; Kennedy & Liebler, 1992). Vitamin C and polyphenols are hydrophilic compounds that can reduce peroxyradicals, but are unable to make contact reactions with lipid radicals (Descalzo & Sancho, 2008). Skeletal muscle also contains natural antioxidant enzymes including catalase, superoxide dismutase, and glutathione peroxidase (Descalzo & Sancho, 2008). Catalase and superoxide dismustase function as coupled enzymes (Descalzo & Sancho, 2008). Superoxide dismutase scavenges superoxide anions present, creating hydrogen peroxide as the product while catalase removes hydrogen peroxide and creates water and oxygen (Descalzo & Sancho, 2008). Glutathione can be oxidized by peroxides via catalysis by glutathione peroxidase (Descalzo & Sancho, 2008). The oxidized glutathione can then be reduced by glutathione reductase (Descalzo & Sancho, 2008). The concentration and activity of these enzymes in muscle is variable and depends on factors such as cell injury, stress, and inflammation (Descalzo & Sancho, 2008). In addition to this, after exsanguination, the muscle cells begin to die and become depleted of nutrients, meaning the enzyme activity is the only remnant of enzymes that were present prior to the onset of cell death (Descalzo & Sancho, 2008).

Natural antioxidants can be added to meat products either by inclusion in animal diets or through technical strategies including direct inclusion of the antioxidant to the meat or by altering the packaging material to include the antioxidant extracts (Falowo et al., 2014). Meat from animals on a grass-fed diet possess high concentration of the natural antioxidants Vitamin E and β-carotene from plants (Lanari et al., 2002; Luciano, Moloney, et al., 2011; Luciano, Vasta, et al., 2011; Realini et al., 2004). Pasture-finished

animals also have greater superoxide dismutase activity compared to grain-fed animals (Descalzo & Sancho, 2008; Misra & Fridovich, 1972). However, catalase activity is not different between grain- and grass-fed cattle (Descalzo, Insani, Eyherabide, Guidi, & Pensel, 2000; Descalzo et al., 2007; Descalzo & Sancho, 2008). Conversely, glutathione peroxidase activity, which is negatively correlated with oxidation, is greater in grain-fed animals compared to grass-fed animals (Descalzo et al., 2000; Descalzo & Sancho, 2008; Gatellier, Mercier, & Renerre, 2004). However, due to the greater concentration of natural antioxidants present in muscle, meat from grass-fed animals should have greater antioxidant capacity when compared to grain-fed animals.

2.6. Flavor

Flavor is the most important attribute for consumer acceptance of beef, as long as tenderness is acceptable (Corbin et al., 2015; Kerth & Miller, 2015; Killinger, Calkins, Umberger, Feuz, & Eskridge, 2004; Miller, 2020; O'Quinn et al., 2012; O'Quinn, Legako, Brooks, & Miller, 2018). Additionally, the most recent National Beef Tenderness Survey reported that nearly 95% of all steaks fall into the very tender or tender category, with the exceptions being the top and bottom round steaks (Martinez et al., 2017). This shows that flavor is the driving factor influencing consumer acceptability of beef. Beef flavor is determined the interaction of extrinsic and intrinsic attributes and thus is a complex attribute of beef (Glascock, 2014; Kerth & Miller, 2015). Flavor in beef is driven by the Maillard reaction, lipid thermal degradation, and the interaction between the two processes (Kerth & Miller, 2015).

2.6.1. How does tasting occur?

Flavor can be defined as "the sum of perceptions resulting from stimulation of the sense ends that are grouped together at the entrance of the alimentary and respiratory tracts" (Meilgaard, Civille, & Carr, 1991). The more practically accepted definition is "the impressions perceived via the chemical senses from a product in the mouth" (Meilgaard et al., 1991). This includes a combination of volatile aromatic compounds which are perceived by the olfactory bulb, the gustatory perceptions, also known as the basic tastes, and the chemical feeling factors perceived by nerve ends in the buccanal and nasal cavities (Meilgaard et al., 1991).

2.6.1.1. Aroma

The perception of volatile aroma compounds, known as olfaction, occurs as airborne compounds are sensed by the olfactory bulb, found in the roof of the nasal cavity known as the posterior nares (Meilgaard et al., 1991). The olfactory bulb consists of millions of cilia covering the epithelium (Meilgaard et al., 1991). These cilia sense the odorant by an unknown mechanism (Meilgaard et al., 1991). Due to the location of the olfactory bulb, only a fraction of the air carrying these compounds can reach the cilia either through the nose or from the mouth during mastication and swallowing (Meilgaard et al., 1991). The greatest amount of compounds can reach the olfactory bulb by sniffing for one to two seconds (Meilgaard et al., 1991). However, after only two seconds of exposure to the stimulus, the receptors will have adapted to the aroma and must "de-adapt" for a period of five to twenty seconds before it can respond to a new sensation (Meilgaard et al., 1991). The sensitivity of the olfactory bulb to stimuli can

vary over a trillion fold range, making some compounds easier or more difficult to identify than others (Meilgaard et al., 1991). In fact, the olfactory bulb can be ten to one hundred times more sensitive to certain compounds than a gas chromatograph (Meilgaard et al., 1991). Nevertheless, it is still unknown how aroma signals are sent to the brain, how the brain processes the quality and strength of the odor, or how it processes different qualities of aromas simultaneously (Meilgaard et al., 1991). It has also been shown that human sensitivity to odors can vary depending on hunger or satiety, mood, concentration, respiratory infection, menstrual cycle, and pregnancy (Meilgaard et al., 1991). This makes the human response to odors and aromas an even more difficult subject to study.

2.6.1.2. Gustation

Gustation is the perception of stimuli dissolved in water, oil, or saliva by the taste buds (Meilgaard et al., 1991). The taste buds are located mainly on papillae on the surface of the tongue, although they are also found in the mucosa of the palate in addition to areas in the throat (Meilgaard et al., 1991). The tongue contains three types of papillae: circumvallate, foliate, and fungiform (Arvidson & Friberg, 1980; Bloom & Fawcett, 1975). Filiform papillae are the most numerous on the tongue, however, they do not contain any taste buds and are only involved in sensing mouthfeels (Smith & Margolskee, 2001). The fungiform papillae on the tongue are the most noticeable and contain one or more taste buds (Smith & Margolskee, 2001). Circumvallate papillae are located in an inverted V shaped on the back of the tongue and also contain taste buds (Smith & Margolskee, 2001).

Taste buds can contain between 50 and 100 taste cells, which each contain microvilli known as the taste pore (Smith & Margolskee, 2001). Chemicals from food are dissolved in the saliva and either interact with taste receptor proteins or with ion channels in the taste bud, which causes the chemical signal to the brain to identify basic tastes (Smith & Margolskee, 2001). There are five basic tastes: sweet, sour, salty, bitter, and umami (Smith & Margolskee, 2001). The sweet taste occurs when sugars or artificial sweeteners bind to G-protein receptors called gustducin on the surface of the taste cell (Smith & Margolskee, 2001). This activates an enzyme within the cell, which causes an action potential within the taste cell (Smith & Margolskee, 2001). The action potential leads to the release of neurotransmitters from the taste cell neuron to the brain to signal a sweet taste (Smith & Margolskee, 2001). Bitter compounds, such as quinine, follow a similar pathway as sweet compounds do (Smith & Margolskee, 2001). The compound interacts with the receptor on the taste cell, and through a secondary messenger system, an action potential occurs leading to the release of bitter neurotransmitters (Smith & Margolskee, 2001).

The sour taste is caused by hydrogen ions (H^+) from acids in solution (Smith & Margolskee, 2001). The hydrogen ions will enter the taste cell and/or interfere with the sodium-potassium channels on the taste cell causing an accumulation of positive charges within the cell (Smith & Margolskee, 2001). The accumulation of positive charges causes an action potential leading to the release of the sour neurotransmitters (Smith & Margolskee, 2001). The salty taste is signaled when sodium ions (Na^+) enter the taste cell and begin to accumulate (Smith & Margolskee, 2001). Similar to the sour taste, this

causes the action potential which leads to the release of salty neurotransmitters to the brain (Smith & Margolskee, 2001).

Umami is the most recently discovered and therefore the least researched basic taste (Smith & Margolskee, 2001). This taste is defined as savory or meaty and is the result of the amino acid glutamate (Smith & Margolskee, 2001). Similar to both the bitter and sweet tastes, glutamate interacts with the protein receptors on the taste cell, which causes the release of an unknown secondary messenger ultimately resulting in the release of a neurotransmitter signaling the umami taste (Smith & Margolskee, 2001). Although it was once hypothesized that different taste cells on the taste buds were responsible for tasting one specific basic taste, it is now known that each taste cell in the tongue is capable of responding to all stimuli, and therefore is capable of tasting all five basic tastes (Smith & Margolskee, 2001)

Since the stimuli are present in a solution, more regular contact is made between the receptor and the stimuli compared to aroma compounds (Meilgaard et al., 1991). However, it is possible for basic taste compounds to bind to the receptor proteins causing a lasting sensation (Meilgaard et al., 1991). The gustatory compounds are carried through the mouth via saliva which contains water, amino acids, proteins, sugar, salts, and organic acids. (Meilgaard et al., 1991). Additionally, the receptors are fed and maintained by blood, which contains a more complex mixture of similar substances (Meilgaard et al., 1991). Therefore, we can only perceive differences in substances due to our sensitivity level, which is defined by the concentration of those compounds in our saliva (Meilgaard et al., 1991). Additionally, the difference between the weakest and

strongest compound detected is a mere thousand-fold difference which is a much smaller range compared to aroma compounds (Meilgaard et al., 1991). Perception of basic tastes can be affected by the temperature and viscosity of the food in addition to the rate, duration, and application area of the product (Meilgaard et al., 1991). Furthermore, the chemical state of the saliva and the absence or present of other compounds in the product will also affect taste perception (Meilgaard et al., 1991).

2.7. Flavor Perception

2.7.1. Methods of Identifying and Quantifying Flavor

Flavors of beef can be identified and quantified by subjective or objective methods. Subjectively, consumers can be used to identify flavors they like and dislike about products. Consumer testing is key to determining if consumers can detect differences in products or to discover the degree to which consumers like or dislike products (American Meat Science Association, 2016). The American Meat Science Association (2016) outlines the methodology and uses for consumer testing. Consumers can be used for either qualitative or quantitative tests. During qualitative tests, consumers are able to provide a subjective response to the sample by talking about their feelings about the product (Meilgaard, Civille, & Carr, 1991). Quantitative consumer tests can help determine how well a product is liked, and the degree of consumer acceptability, using a numeric hedonic, just about right, or intensity scale. These tests can be conducted using previously recruited consumers with a central location test (CLT) or a home use test (HUT). Random consumers can also be tested using a mail intercept test to probe consumer opinions.

Objectively, beef flavors can be quantified using instrumentally using a gas chromatograph/mass spectrometer (GC/MS) or by using descriptive analysis. Measuring volatile aroma compounds utilizing headspace analysis is a straightforward and reliable research method presently used that was extensively outlined by Kerth & Miller (2015). Samples of cooked beef, typically the same sample tested by descriptive analysis or a consumer panel, is placed in a closed system with a solid phase microextraction (SPME) fiber which can absorb volatile compounds present in the headspace. The SPME is then inserted into a GC/MS, which can desorb, elute, identify, and quantify volatile aroma compounds present in the sample. The GC/MS may also contain an olfactory port, which gives researchers the ability to smell specific aromas from individual volatile compounds as they exit the GC. Identified aromas can then be matched to chemical compounds identified by the MS. Utilizing both GC/MS technology and descriptive or consumer tests on the same samples allows researchers to correlate specific compounds with consumer liking or disliking.

Furthermore, descriptive analysis uses a group of trained sensory panelists to identify and quantify the intensity of flavors present in beef. The Spectrum descriptive attribute analysis allows researchers to collect information of the intensities of aromas, flavors, and textures using an universal scale (American Meat Science Association, 2016). For specific products, a lexicon or a dictionary of attributes and references is used to define the attributes within the product (American Meat Science Association, 2016). The Beef Flavor Lexicon is the established lexicon for identifying major aroma and flavor attributes for whole-muscle beef products (Adhikari et al., 2011; American Meat

Science Association, 2016). Using this lexicon has provided a consistent research method to evaluate and quantify beef flavor (Kerth & Miller, 2015). Panelists are extensively trained using the lexicon to understand and scale attributes in products using a line or 16-point intensity scale (American Meat Science Association, 2016). Conducting a descriptive sensory test provides numerical differences of specific flavor attributes tested for amongst different treatments.

2.7.2. Positive Flavors Identified in Beef

Volatile aromas are the most important contributor to beef flavor (Kerth & Miller, 2015). The positive flavors present in beef were defined through the beef lexicon as beefy, brown/roasted, bloody/serumy, fat-like, sweet, salty, and umami (Kerth & Miller, 2015; Miller & Kerth, 2012). Beefy, brown/roasted, bloody/serumy, sweet, salty, and umami are derived from compounds from the lean component of meat (Kerth & Miller, 2015; Miller & Kerth, 2012). However, fat-like flavors arise from the lipid portion of meat products (Kerth & Miller, 2015; Miller & Kerth, 2012). Overall consumer liking of beef is positively correlated with the presence of beef flavor, brown/roasted, and fat-like (Luckemeyer, 2015). Furthermore, overall flavor liking is positively correlated with beef identity, brown/roasted, fat-like, umami, sweet, salty, overall sweet, and burnt (Luckemeyer, 2015).

Sugar compounds in beef are responsible for the sweet flavor, while sodium containing salts and free sodium ions cause the salty taste, and the amino acid glutamate is responsible for the umami taste (Smith & Margolskee, 2001). Because there are hundreds of compounds present in meat that contribute to the flavor and aroma of the

product, it is difficult to define exactly which compounds cause individual flavors (Calkins & Hodgen, 2007). It has been identified that sulfur-containing heterocyclic compounds are important to produce savory, meaty, roasted flavors (Donald S. Mottram, 1998). Additionally, Luckemeyer, (2015) reported that 32 volatile aroma compounds account for 67% of the variation in beef identity, 47 compounds account for 81% of the variation in brown/roasted, 30 compounds can account for 51% of the variation in bloody/serumy, and 53 compounds account for 77% of the variation in fat-like. However, one single compound did not account for a large variation for any specific flavors (Luckemeyer, 2015). This indicates that not only the concentration of a single compound but the interaction between these compounds may be more responsible for specific flavor attributes in beef.

2.7.3. Negative Flavors Identified in Beef

Negative flavors present in beef were also defined through the beef lexicon as metallic, liver-like, sour, barnyard, musty-earthy/humus, and bitter (Kerth & Miller, 2015; Miller & Kerth, 2012). Liver-like, metallic, and bitter compounds are derived from substrates originating from the lipid portion of beef (Kerth & Miller, 2015; Miller & Kerth, 2012). Liver-like and metallic are also related to the total myoglobin, higher pH values, and beef with oxidized lipids (Luckemeyer, 2015; Rhonda K. Miller & Kerth, 2012). Metallic and bitter flavors can result from compounds produced from the Maillard reaction (Luckemeyer, 2015). Overall consumer flavor liking of beef is negatively correlated with the flavor attributes liver-like, musty-earthy/humus, and carboard (Luckemeyer, 2015).

Sour flavors are the result of acids and other free protons present in the meat, such as aspartic, glutamic, or lactic acid (Luckemeyer, 2015; Smith & Margolskee, 2001). Bitter tastes in beef can be the result of bitter compounds such as hypoxanthine, anserine, carnosine, or other peptides (Luckemeyer, 2015). As with positive flavor compounds, negative flavor compounds are also a the result of a combination of multiple volatile compounds (Calkins & Hodgen, 2007). However, Luckemeyer (2015) identified 39 volatile aroma compounds responsible for 61% of the variation in metallic and 43 compounds responsible for 61% of the variation in liver-like. As with the positive flavor compounds, not one single volatile compound could account for a large proportion of the variation of any individual flavor (Luckemeyer, 2015).

2.8. Factors Affecting Flavor

2.8.1. Maillard Reaction

The flavor of cooked meats is the ultimately the result of non-volatile constituents of fresh meat including proteins, lipid and carbohydrates, the most important of which are fats and small water-soluble compounds (Chen & Ho, 1998; Khan, Jo, & Tariq, 2015; Luckemeyer, 2015; Resconi, Escudero, & Campo, 2013). Water-soluble compounds present in meat include amino acids, carbohydrates, nucleotides, peptides, and other nitrogenous compounds (Luckemeyer, 2015). Cysteine and methionine are considered the largest contributors to the development of meat flavor (Khan et al., 2015; Werkhoff et al., 1990). Cysteine, a sulfur containing amino acid, reacts with ribose, glucose, or xylose during heating to product meat-like flavors

(Luckemeyer, 2015; Morton, 1960). Ribose is the main reducing sugar present in muscle and is associated with ribonucleotides found in RNA and ATP (Luckemeyer, 2015).

The principal pathway for cooked meat aroma compounds is the Maillard reaction (Farmer & Mottram, 1990; Khan et al., 2015; D. S. Mottram & Nobrega, 2002). The Maillard reaction, also referred to as nonenzymatic browning, is the reaction between reducing sugars and amino acids during cooking (Hurrell, 1982; Luckemeyer, 2015; D. S. Mottram & Edwards, 1983; F. Shahidi, 1994). This process was first discovered by Louis Maillard in 1912 as the mechanism to explain reactions between amines and carbonyl compounds (Luckemeyer, 2015). The first step of the Maillard reaction is the condensation of an amino compound with the carbonyl group of a reducing sugar to form a glycoslyamine (Figure X(1); Luckemeyer, 2015). The glycoslyamine will be converted to a ketosamine through Amadori rearrangement (Figure X(2); Damodaran et al., 2008). Next, the intermediary products are dehydrated, the saccharidic moiety of the molecule is fragmented, and Strecker degradation occurs (Damodaran et al., 2008). Strecker degradation is the reaction of sugar degradation products such as glyoxal or methylglyoxal with amino acids formed aldehydes, ammonia, and carbon dioxide (Damodaran et al., 2008). Finally, the intermediary products from the first two steps of the Maillard reaction will form high molecular weight, brown pigments that are also heterocyclic flavor compounds responsible for the typical flavor of browned products (Damodaran et al., 2008). Heterocyclic flavor compounds that are produced from the Maillard reaction can be classified as pyrazines,

pyridines, pyrroles, furans, furanones, furfurals, thiazoles, thiophenes, and oxazoles (Damodaran et al., 2008; Donald S. Mottram, 1998).

2.9. Lipid Thermal Degradation

Lipid thermal degradation gives rise to the fatty aromas and flavors of cooked meat as well as possess compounds that determine species specific flavors (Mottram, 1998). Mottram (1998) thoroughly reviewed the process of lipid thermal degradation. During the cooking process, fatty acids in lipids are oxidized in reactions similar to those that cause rancid off-flavors. However, these reactions occur much faster during cooking which results in the production of different volatile compounds which produce desirable flavors. Additionally, unsaturated fatty acids will oxidize quicker compared to saturated fatty acids. The phospholipid portion of muscle cells contains a higher concentration of unsaturated fatty acids such as arachidonic acid compared to triglycerides. Therefore, it has been considered a more important source of volatile aroma compounds. Mottram & Edwards (1983) found that removal of phospholipids and triglycerides from lean muscle using a polar and non-polar solvent, respectively (methanol-chloroform) resulted in the absence of the meaty aroma and the presence of a new roast, biscuit-like aroma in the cooked product. When to compounds were analyzed, data showed there was an absence in lipid oxidation products but an increase of alkyl pyrazines (Mottram & Edwards, 1983). This showed that in normal meat products, lipid degradation products control the Maillard reaction steps that form heterocyclic aroma compounds (Mottram, 1998). Lipid degradation of triacylglycerides or phospholipids will produce flavor compounds

including alkanals, alkanones, alkanoic acids, alkanols, lactones, and alkylfurans (Mottram, 1998).

Furthermore, volatile aroma compounds can be the result of the interaction of lipid ad Maillard reaction compounds (Mottram, 1994; Mottram, 1998; Whitfield & Mottram, 1992). Compounds produced from these reactions include lipid-derived aldehydes, which are formed by the reaction between saturated or unsaturated aldehydes from lipid oxidation and carbonyl and amino compounds from the Maillard reaction (Mottram, 1998). Alkylthiazoles, alkylpyridines, and alkyl-substituted heterocyclic compounds can all arise from these reactions and contribute to the desirable fatty, fried flavors found in meat (Buttery, Ling, Teranishi, & Mon, 1977; Fogerty, Whitfield, Svoronos, & Ford, 1991; Ho, Carlin, Huang, Hawng, & Hau, 1987; Mottram, 1998). Additionally, some of the products formed from the interaction between phospholipids and Maillard reaction products include hydrocarbons, alkylfurans, saturated and unsaturated alcohols, aldehydes, and ketones (Mottram, 1998).

2.9.1. Intrinsic Factors

Several intrinsic and extrinsic factors have been shown to impact the flavor of beef. One intrinsic factor known to have an effect on flavor is breed of the animal. Breed affects total fat, intramuscular fat, and fatty acid composition of the animal (Khan et al., 2015). The intramuscular fat can vary between breeds as much as 2% (Khan et al., 2015; Piedrafita et al., 2003). Additionally, total fat content can differ by nearly 5% between species (Cepin, Zgur, & Cepon, 1998; Khan et al., 2015). Furthermore, breeds can differ in the amount of sulfur producing compounds present in cooked meats, which thus

affects the flavor (Insausti, Goñi, Petri, Gorraiz, & Beriain, 2005; Khan et al., 2015). Breed has also been shown to affect the rate of sensory changes, such as the rate of aging which affects the tenderness of the product (Campo, Sañudo, Panea, Alberti, & Santolaria, 1999; Khan et al., 2015). Volatile compounds also differ depending on the breed of animal. In cattle, Friesian cattle were found to have a stronger fatty flavor and aftertaste in additional to differences in volatile compounds produced compared to Pirenaica cattle (Gorraiz, Beriain, Chasco, & Insausti, 2002; Khan et al., 2015). Similarly, Van Ba, Ryu, Lan, & Hwang (2013) found differences in volatile flavor compounds and sensory characteristics of Hanwoo and Angus.

Marbling or total fat content effects the flavor and consumer liking of beef. Increases in marbling score linearly increases the flavor desirability of beef (Luckemeyer, 2015; Mcbee & Wiles, 1965; G. C. Smith, Savell, Cross, & Carpenter, 1983). Additionally, USDA Choice steaks have higher flavor intensity rating compared to USDA Select steaks (Luckemeyer, 2015; Miller et al., 1997). Not surprisingly, increasing marbling also increases the fat flavor of products (Luckemeyer, 2015; R.K. Miller, 2001). In beef steaks, less than 3% fat in products leads to unacceptable palatability scores (Luckemeyer, 2015). Cross, Berry, & Wells (1980) reported that ground beef patties formulated with 28% fat had greater tenderness and juiciness than leaner patties. Additionally, beef flavor intensity did not differ between fat levels of ground beef (Cross et al., 1980). Similarly, Berry & Leddy (1984) reported patties that were 24% fat were more tender and juicier than lower fat patties. Furthermore, when patties were cooked using the same cooking methods, flavor intensity did not differ

between fat level (Berry & Leddy, 1984). Furthermore, Troutt et al. (1992) reported similar results with patties ranging from 5% to 30% fat.

Diet is a well-known and heavily researched intrinsic factor affecting the flavor of beef. The animal diet will affect not only sensory characteristics, but also effects the proximate composition, fatty acid profile, tenderness, and color of the meat as previously discussed (Franco, Crecente, Vázquez, Gómez, & Lorenzo, 2013; Li et al., 2014; Ramírez-Retamal & Morales, 2014). Meat from grass-fed animals in leaner and thus is perceived as healthier compared to grain-finished beef (Khan et al., 2015; Sañudo et al., 2000; Wood et al., 2008). Sivadier, Ratel, & Engel (2010) suggested that the presence of 2,3-octanedione in the muscle could be used as a suitable biomarker to authenticate animals finished on a pasture-exclusive diet (Khan et al., 2015). Other compounds that have been noted in pasture-fed meat have been terpenes and diterpenoids (Khan et al., 2015; A. Priolo et al., 2004; O. A. Young, Berdagué, Viallon, Rousset-Akrim, & Theriez, 1997). As grain-based diets have greater carbohydrate availability, animals fed grain during finishing have higher concentrations of branched-chain fatty acids in addition to γ-lactones (Khan et al., 2015; O.A. Young & Braggins, 1998; Owen A. Young, Lane, Priolo, & Fraser, 2003). Conversely, pasture-finished animals have greater δ-lactones (Bailey, Suzuki, Fernando, Swartz, & Purchas, 1994; Khan et al., 2015).

2.9.2. Extrinsic Factors

Several extrinsic factors can affect the flavor of beef. Among these are postmortem aging, packaging, cooking method, and degree of doneness (Lorenzen, Davuluri, Adhikari, & Grün, 2005; Melton, 1999; Montgomery, Parrish, Olson, Dickson,

& Niebuhr, 2003; Prestat, Jensen, McKeith, & Brewer, 2002; Savell et al., 1999; Spanier, Flores, McMillin, & Bidner, 1997). These factors have been well reviewed in several papers (Dashdorj et al., 2015; Guerrero, Valero, Campo, & Sañudo, 2013). However, since the extrinsic factors were controlled and remained consistent throughout this study, these parameters should not have a significant effect on potential flavor differences amongst treatments and therefore will not be discussed at length.

2.10. Impact of Oxidation on Flavor

Off-flavor development as a result of oxidation has been a long-recognized problem during storage of meat (Gray et al., 1996). These oxidative off-flavors, also called rancidity, start to develop in the muscle soon after death of the animals, and increase as the product is held until the rancidity will make the product unacceptable to consumers (Gray et al., 1996). This is mainly due to the shifting balance of prooxidant and antioxidant compounds present in the muscle to control lipid oxidation postmortem (Gray et al., 1996).

2.10.1. Myofibrillar Proteins and Myoglobin Oxidation

Although it has often been suggested myoglobin is closely related to lipid oxidation, little evidence has been presented to support this theory (Renerre and Labadie, 1993; Calkins and Hodgen, 2007). However, a small correlation has been seen between myoglobin concentration and liver-like flavors in beef (Calkins & Hodgen, 2007; Yancey et al., 2006). It has also been proposed that lipid oxidation products catalyze the oxidation of myoglobin (Gray et al., 1996). If this mechanism was proven true, then metmyoglobin formation could be an accurate predictor of lipid oxidation in fresh meat

products. Contrarily, Sledge (2008) found that in grass-fed ground beef, color and lipid oxidative stability was inversely related to flavor stability. This data suggests that in grass-fed beef, either lipid oxidation products are not leading to myoglobin oxidation, or that a potential hydrophilic antioxidant is present that can control the rate of myoglobin and lipid oxidation.

As myofibrillar protein oxidation is a relatively new area of interest in meat science, little research has focused on the impact of myofibrillar protein oxidation and its effect on meat flavor (Lund et al., 2011). However, it is known that oxidation of myofibrillar proteins during storage can be attributed to an increase in the negative attributes bitter and sour as well as a decrease of the positive flavor attributes beefy and brothy (Spanier, Miller, & Bland, 1992). Additionally, oxidation of enzymatic proteins in the muscle, especially of μ-calpain, can affect the tenderness and water-holding capacity of meat, thus affecting tenderness and juiciness of the product (Xiong, 2000; Zhang et al., 2013). More research is necessary to determine the effect of myofibrillar protein oxidation on lipid oxidation and flavor of meat products (Zhang et al., 2013).

2.10.2. Lipid Oxidation

Lipid oxidation in fresh meats during storage can lead to a decrease in the meaty flavor of the product soon after it has been cooked and cooled (Milton E. Bailey & Um, 1992). The degree of lipid oxidation in meat is determined by a number of factors including the concentration of phospholipids, polyunsaturated fatty acids, and pro-oxidants in the meat (Calkins & Hodgen, 2007). Lipid oxidation produces undesirable flavor compounds such as aldehydes, lactones, hydrocarbons, furans and ketones which

are responsible for the distinct rancid off-flavors in products (Calkins & Hodgen, 2007; Ladikos & Lougovois, 1990). Similar to other flavors found in beef, oxidation of lipids produces many volatile compounds that result in the oxidized flavors. Some of the specific volatile compounds that have been associated with oxidation of unsaturated fatty acids include hexanal, 2-octenal, 2-nonenal, 1-octen-3-ol, 1-octen-3-one, methyl 5-oxopentanoate, pentane, 2,3-decadienal, *trans*-4,5-epoxy-*(E)*-2-decenal, and 2,4,7-tridecatrienal (Artz, Perkins, & Salvador-Henson, 1993; Blank, Lin, Vera, Welti, & Fay, 2001; Calkins & Hodgen, 2007; Ullrich & Grosch, 1987). When measuring the levels of lipid oxidation in meat, thiobarbituric acid reactive substances (TBARS) values above 0.5 have been shown to indicate a level of rancidity that can be perceived by consumers in odor and flavor as either oxidized or warmed-over (St. Angelo et al., 1987; Wood et al., 2008).

In cooked meats during refrigerated storage, unsaturated fatty acids are continually oxidized producing an undesirable odor and flavor described as “old, stale, rancid, and metallic” (Milton E. Bailey & Um, 1992). This characteristic flavor is called warmed-over flavor (WOF), and is more prominent when refrigerated meat is reheated (Milton E. Bailey & Um, 1992). Oxidized flavors in cooked meats can become apparent after 48 hours (Spanier, Miller, et al., 1992). Additionally, grinding raw meat disrupts the muscle structure and enhances the development of WOF in the product when it’s cooked (Spanier, Miller, et al., 1992; Spanier, Vercellotti, & James, 1992). Lipid oxidation of phospholipids in muscle is the primary source of the off-flavor notes produced (Spanier, Miller, et al., 1992). The volatile compound hexanal in addition to

the products of TBARS can be related to off-flavor descriptors such as painty (Larick & Turner, 1990; Lillard, 1987; Spanier, Miller, et al., 1992; Spanier, Vercellotti, et al., 1992).

2.11. Objective 1 – Determining the impact of lean source on color and color stability of ground beef patties

The objectives of the first chapter of this thesis was to determine differences in color, protein, and lipid oxidation in grass-fed and grain fed beef and to further explain the relationship between color stability and flavor stability in grass-fed and grain-fed beef. Based on the results of the previous study by Sledge (2008), the hypothesis was that adding a small percentage of grass-fed lean to a commercial ground beef blend could help improve the color stability in an overwrap packaging system while not having detrimental effects on the flavor. The possibility of grass-fed lean as the antioxidant source would allow the processors to improve color stability of the product by adding a natural ingredient containing antioxidants that would not have to be added to an ingredient statement on the label.

2.12. Objective 2 – Exploring how the biological type of the lean source utilized drives flavor development of ground beef patties

The objectives of the second chapter of this thesis were to determine the beef flavor potential of lean with a novel HPLC-qTOF technology in raw and cooked meat from cattle differing in lean source and to define sugar and amino acid differences amongst beef types as they pertain to the Maillard reaction and serve as potential for positive beef flavor. The hypothesis of this study is that genetic variation leans to

differences in protein expression. Differences in the amino acids of proteins expressed combined with different reducing sugars present is the driving force for flavor differences. If these sugars and amino acids were identified, there is the potential to predict beef flavor potential in raw samples.

2.13. References

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3. UTILIZING A MIXTURE OF GRAIN-FED AND GRASS-FED LEAN IN A GROUND BEEF BLEND IMPACTS COLOR AND FLAVOR STABILITY OF GROUND BEEF PATTIES

3.1. Abstract

To test the hypothesis that adding a small percentage of grass-fed lean to a commercial ground beef blend could help improve the color stability in an overwrap packaging system while not having detrimental effects on the flavor, rounds were sourced from commercially-available grain- and grass-fed cattle, coarse ground, and combined to make the following treatment combinations of grass-fed lean/grain-fed lean: 0%/100%, 33%/67%, 67%/33%, 100%/0%. All treatment batches were formulated to 15% fat using standard fat sourced from grain-fed cattle less than 30 months of age, then fine-ground and formed into patties. Initial color and pH were measured, then patties were placed in overwrap packaging and stored in retail display for 3 or 5 d then frozen for analyses. Instrumental color was measured every 24 h. Thiobarbituric acid reactive substances (TBARS) and volatile aroma compounds were measured on patties held in retail display for 0, 72, and 120 h. Fatty acid composition was measured on each individual batch. Patties formulated with 100% grass-fed lean contained higher percentages of oleic acid than 0% grass-fed lean patties ($P < 0.05$). Furthermore, the percentage of grass-fed lean linearly increased hue angle ($P < 0.05$) and quadratically decreased a* to 33% then increased to 100% ($P < 0.05$). Patties containing 100% grass-fed lean had higher TBARS values than other treatments after 3 d of retail display ($P <$

0.05). Therefore, patties containing 100% grass-fed lean exhibited greater amounts of lipid and myoglobin oxidation compared to formulations containing grain-fed lean. We concluded that grass-fed lean did not improve the color stability or prevent lipid oxidation in ground beef patties.

3.2. Introduction

Ground beef is one of the most consumed beef products, as it accounts for 40% of total beef sales (Suman et al., 2014). Mancini and Hunt (2005) described visual color as the "gold standard" for consumer perception of meat products. Because consumers view discoloration as a sign of poor quality, meat color is the primary driving force for meat purchasing decisions. Beef discoloration is caused by the oxidation of the iron in myoglobin, which forms the brown-colored pigment metmyoglobin (Mancini & Hunt, 2005). Preventing beef from oxidizing via the action of an antioxidant would delay myoglobin oxidation and increase the length of time of consumer acceptability in retail display. Additionally, the "clean label" trend currently seen in consumers in industrialized countries has driven the perception of artificial ingredients as "unhealthy and unfamiliar" (Asioli et al., 2017). Therefore, addition of synthetic antioxidant to a product could be perceived as less healthy, as it violates the 'free from' artificial ingredients/additives program (Asioli et al., 2017). This could cause the product to be perceived as less healthy by the consumer. However, the lean portion of beef from grass-fed cattle contains higher concentrations of natural antioxidant compounds such as vitamin E than beef from grain-fed cattle (Daley et al., 2010a; De la Fuente et al., 2009; Fruet et al., 2018; Luciano, Moloney, et al., 2011; Yang, Lanari, et al., 2002). Previous research has shown vitamin E is an antioxidant that improves beef color and color stability (Faustman et al., 1998).

Oxidation is the limiting factors for consumer acceptability of meat products as it affects all three quality attributes: appearance, texture, and flavor (Gray et al., 1996; Q.

Liu et al., 1995). The autoxidation of polyunsaturated fatty acids in meat products is a major economic problem (E.N. Frankel, 1980). Lipid oxidation is associated with oxidized flavor, also known as warmed-over flavor (B. E. Greene & Cumuze, 1982). These flavors can be further described by the attributes cardboardy, rancid, stale, and metallic, and have been associated with the volatile compounds 2,3-octanedione and hexanal (St. Angelo et al., 1987). Beef from grass-fed cattle contains a greater concentration of polyunsaturated fatty acids compared to beef from grain-fed cattle, making meat from grass-fed animals more susceptible to lipid oxidation (Gatellier et al., 2005; Lanari et al., 2002; Larick & Turner, 1989; S. L. Melton et al., 1982). However, grass-fed beef also has greater concentrations of α-tocopherol (Vitamin E) which has been shown to delay lipid oxidation (Gatellier et al., 2005; Lanari et al., 2002; Luciano, Moloney, et al., 2011; Nuernberg et al., 2005; Realini et al., 2004).

It has been hypothesized that myoglobin and lipid oxidation were linked and would occur simultaneously (Alderton et al., 2003; Faustman et al., 1998; Lynch & Faustman, 2000). However, previous research in our laboratory demonstrated that in grass-fed ground beef, color stability and flavor stability were inversely related (Sledge, 2008). This relationship needs to be further explored to understand the correlation between color and lipid stability in ground beef. It is hypothesized that adding lean from grass-fed cattle to a ground beef blend in small quantities could naturally increase the vitamin E present in the blend, which would increase the retail shelf life without decreasing flavor acceptability of the product.

3.3. Materials and Methods

3.3.1. Product Procurement and Patty Formation

Beef inside rounds were purchased from a commercial meat purveyor. Rounds were purchased commercially and sourced from processors advertising the beef product as either finished on a grain diet or forage/grass/hay diet. Rounds were ground through a 12.7-mm plate before mixing lean types at varying levels to create 4 treatment groups: 1) 100% grain-finished lean/0% forage-finished lean; 2) 67% grain-finished lean/33% forage-finished lean; 3) 33% grain-finished lean/67% forage-finished lean; 4) 0% grain-finished lean/100% forage-finished lean. Treatment groups are henceforth referred to by the percentage of grass-fed lean utilized as a percentage of the entire batch (0% grass-fed, 33% grass, 67% grass-fed, and 100% grass-fed, respectively). Once lean sources were mixed, treatment batches were formulated to a target 85% lean/15% fat percentage using one homogenous fat source collected from grain-fed cattle less than 30 months of age in order to mitigate differences in inherent fatty acid composition. Fat percentage was validated using the Foss FoodScan2 Meat (FOSS Global, Hilleroed, Denmark). Batches were then fine ground through a 9.5-mm plate and then formed into 150-g patties that were using a handheld hamburger patty press (Oneida Hospitality Group, Lincolnshire, IL, USA). Patties were packaged with four patties to a package in an overwrap tray, ensuring the patties were not touching each other. Patties were randomly assigned to retail display times of 0, 72, or 120 h. After initial analyses were measured, 0-d patties were crust frozen at -10°C, vacuum-packaged, and held in frozen storage at -20°C until further analyses were completed. Patties assigned to retail display for 72 or

120 h were packaged in foam trays using an oxygen oxygen-permeable polyvinyl chloride overwrap film. At the end of the assigned retail display period, patties were crust frozen at -10°C, vacuum-packaged, and then kept in frozen storage at -20°C until further analyses. Each replication of the study contained 18 patties/treatment batch. Three replications of each treatment batch were created, for a total of 216 patties for the experiment.

3.3.2. Retail Display

The temperature of the cooler used for retail display was monitored daily and maintained at less than 4.5°C. Patties were placed under lights containing 40-watt mercury bulb (Philips F40T12/CW Plus Alto collection; Philips, Amsterdam, Netherlands) with an intensity of 1,612.5 to 2,152 lux. Intensity of light on the patties was measured at the height of the surface of the patty with a lux meter. Patties were rotated throughout the cooler front to back and side to side every 24 h to mitigate potential differences in temperature and light intensities throughout the cooler.

3.3.3. Instrumental Color and pH

Instrumental color (CIE-values L*, a*, b*) were measured in triplicate on each patty every 24 h using a HunterLab MiniScan 4500L (Hunter Labs, Reston, VA, USA) using Illuminant A with a 2.54-cm aperture and a 10° observation angle. Before initial color was measured, patties were allowed to bloom for at least 30 min at atmospheric oxygen before packaging. Initial color measurements were taken prior to packaging, with the overwrap film placed over the instrument while measurements were taken to mitigate differences in color readings. The instrument was standardized using black and

white tiles (Hunter Labs, Reston, VA, USA) at the beginning of each session and as prompted by the instrument during use. Overwrap film was placed over the tiles during standardization to prevent potential differences caused by the packaging. For all color readings after the 0 h measurements, color on each individual patty was measured in the unopened package in triplicate with samples averaged across each patty for analyses. Hue angle and chroma was calculated using CIE-a* and -b* values (American Meat Science Association, 2012). Reflectance values were measured at wavelengths every 10 nm from 400 to 700 nm. Percentages of deoxymyoglobin (DMb), oxymyoglobin (OMb), and metmyoglobin (MMb) were calculated from the isosbestic wavelengths (American Meat Science Association, 2012) using the following equations:

(1) $A = \log\frac{1}{R}$

(2) $\%\ MMb = \left\{1.395 - \left[\frac{(A572 - A730)}{(A525 - A730)}\right]\right\} \times 100$

(3) $\%\ DMb = \left\{2.375 - \left[1 - \frac{(A473 - A730)}{(A525 - A730)}\right]\right\} \times 100$

(4) $\%\ OMb = 100 - (\%MMb + \%DMb)$

Initial pH was measured on each treatment batch using a hand-held pH meter (Model: HI98163; Hanna Instruments, Carrolton, TX USA). Six reading were taken per treatment batch and averaged to determine the pH of the batch. The pH meter was standardized at the beginning of the day and as prompted by the instrument using pH 4.0, 7.0, and 10.0 standards (Hanna Instruments, Carrolton, TX USA).

3.3.4. Lipid Oxidation Analysis

Lipid oxidation in patties was measured using the thiobarbituric acid reactive substances (TBARS) for Oxidative Rancidity Rapid, Wet Method protocol (AMSA Meat Color Guidelines, 2012). Samples were powdered and duplicate 0.5-g samples were weighed out. Then 2.5 mL of TBA stock solution (0.375% thiobarbituric acid, 15% trichloroacetic acid, and 0.25 N HCl) was added. Samples were then heated in boiling water in loosely capped tubes. Tubes were then cooled in approximately 7.62 cm of tap water for 5 minutes and then centrifuged at 5,000 × *g* for 10 min at 4°C. The supernatant was pipetted into a 96-well plate (VWR, Radnot, PA, USA) and the absorbance was measured at 532 nm using an Epoch monochromator (Biotek Instruments, Inc., Winooski, VT, USA) and processed using the Gen5 Microplate Data Collection and Analysis software (Biotek Instruments Inc., Winooski, VT, USA) against a blank containing all reagents except the sample. TBARS numbers were calculated and presented as mg malonaldehyde (MDA)/kg meat in addition to the TBARS value (ppm) according to the standard formulas:

TBARS number (mg MDA/kg) = sample A_{532} × (1 M TBA chromagen)/156,000) × 1 mol/L/M × (0.003 L/0.5 g meat) × (72.07 g MDA/mol MDA) × 1,000 mg/g × 1,000 g/kg

TBARS value (ppm) = sample A_{532} × 2.77

3.3.5. Volatile Compound Analysis (GC/MS)

Patties were thawed in refrigerated storage at 4^{o}C for 12 to 24 h prior to cooking. Patties were cooked on a 2.54-cm thick flat top Star Max 536TGF 91.44cm Countertop Electric Griddle with Snap Action Thermostatic Controls (Star International Holdings

Inc. Company, St. Louis, MO, USA). Griddles were preheated to 204°C, then patties were placed on the griddle. Patties were flipped when the internal temperature reached 32°C and removed when the internal temperature reached 71°C. Internal temperature was monitored using an Omega HH501BT Type T handheld thermometer (Omega Engineering, Stanford, CT, USA) and measured in the geometric center of the patty. Immediately after cooking, patties were frozen in liquid nitrogen and stored in -80^{o}C until collection of volatile compounds. Frozen patties were first powdered, then 5 g of powdered sample was placed in a 20-mL glass vial with a Teflon lid and placed on a heating block (Block analog 2 120V with block modular 28M, VWR) and held for 20 min at 65°C. The volatile compounds present in the headspace were collected using a solid-phase micro-extraction (SPME) Portable Field Sampler (Supelco 504831, 75 µm carboxen/ polydimethylsiloxane, Sigma-Aldrich, St. Louis, MO) for 20 minutes. Volatile aroma compounds were eluted from the SPMEs and separated using gas chromatography (GC; Agilent Technologies 7920 series GC, Santa Clara, CA). The sample was desorbed at 280°C for three minutes. The sample was then loaded onto a gas chromatograph column (AgilentVF 5MS 30 m × 0.25 mm ID/1µ film thickness, SGE Analytical Sciences, Austin, TX). Through the column, the temperature started at 40°C (held for one minute) and increased at a rate of 20°C/min until reaching 250°C. Compounds were identified and quantified with a mass spectrometer (MS; Agilent Technologies 5975 series MSD, Santa Clara, CA) using the Wiley Chemical Library (Palisade, Ithaca, NY, USA).

3.3.6. Fatty Acid Analysis

Total lipids of raw patties were extracted by a modification of the method of Folch, Lees, &Stanley (1957). Five grams of homogenized beef was extracted in chloroform: methanol (2:1, v/v) and fatty acid methyl esters (FAME) were prepared as described by Archibeque, Lunt, Tume, & Smith (2005). The FAME were analyzed using a Varian gas chromatograph (model CP-3800 fixed with a CP-8200 auto sampler, Varian Inc., Walnut Creek, CA). Separation of FAME was accomplished on a fused silica capillary column CP-Sil88 [100 m × 0.25 mm (i.d.)] (Chrompack Inc., Middleburg, The Netherlands), with hydrogen as the carrier gas (flow rate = 35 mL/min; split ratio 20:1). Initial oven temperature was 150°C; oven temperature was increased at 5°C/min to 220°C and held for 22 min. Injector and detector temperatures were set at 270°C and 300°C, respectively. Individual fatty acids were identified using genuine external standard GLC-68D (Nu-Chek Prep, Inc., Elysian, MN).

3.3.7. Statistical Analysis

All data were analyzed as a completely randomized design, and the analysis of variance (ANOVA) was generated using the generalized linear mixed models procedure of SAS version 9.4 (SAS Inst., Inc., Cary, NC) with the percentage of grass-fed beef trimmings and batch as the fixed and random effects, respectively. Additionally, the instrumental color data were analyzed as repeated measures using the PROC MIXED function of SAS version 9.4 (SAS Institute, Inc., Cary, NC) with patty as the subject of the repeated display (0, 24, 48, 72, 96, and 120 h). When a significant ($P \leq 0.05$) F-test was observed, least squares means (LSMEANS option) were separated at $P < 0.05$ using

paired t-tests (PDIFF option of SAS). In addition, linear and quadratic polynomial contrasts were used to test the main effects of the percentage of grass-fed trimmings on ground beef quality attributes; however, because of unequal distribution of data across all display times (some instrumental data for patties in display for 24 h was lost due to equipment error), PROC IML of SAS was used to generate the appropriate coefficients for linear and quadratic polynomial contrasts for display time. Fatty acid composition was analyzed as a completely randomized design with percentage of grass-fed lean as the main effect and batch as a random effect.

3.4. Results

3.4.1. Instrumental Color and pH

The initial pH did not differ among batches containing 0%, 33%, 67%, or 100% grass-fed lean (Table 1; $P > 0.05$). For L*-values, there was no significant interactions between percentage of grass-fed lean utilized and day of retail display (Table 2; $P > 0.05$). However, there was a significant interaction between percentage of grass-fed lean and day of retail display for all other measured color parameters ($P < 0.05$). Patties that contained 100% grass-fed lean at 0 h of retail display were more red than any other day × treatment combination ($P < 0.05$). Initially (0 h), patties that contained 67% grass-fed lean were more red than patties containing 33% grass-fed lean, but were less red than 100% grass-fed patties ($P < 0.05$). However, at 24 and 48 h of retail display, 0% grass-fed lean were more red than all other treatments ($P < 0.05$) which were statistically equal ($P > 0.05$). Patties of all treatments had the lowest a*-values after 72 h of retail display compared to other display times ($P < 0.05$). At 72 h, 33% and 67% grass-fed lean patties

were less red than 0% grass-fed patties with 100% grass-fed patties being intermediate ($P < 0.05$). At 96 h, 67% grass-fed lean was more than 100% grass-fed lean patties with the other treatments being intermediate ($P < 0.05$). Furthermore, at 120 h of retail display, there was no difference in redness between the treatments ($P > 0.05$). Surprisingly, a* values for all treatments at 120 h were greater than a* values at 72 and 96 hours of retail display ($P < 0.05$).

At 0 h, 100% grass-fed lean patties were more yellow than all patties (Table 2; $P < 0.05$). After 24 h of display, 67% grass-fed lean patties were less yellow ($P < 0.05$) than all other treatments, which did not differ from each other ($P > 0.05$). Additionally, at 24 h, b* values were highest for all treatments compared to any other day of retail display. At 48 h of retail display, all patties had statistically equal b* values ($P > 0.05$). However, at 72 h, 100% grass-fed lean patties were more yellow than 0% and 67% grass-fed lean patties, while 33% grass-fed lean patties were intermediate ($P < 0.05$). Similarly, at 96 h, 100% grass-fed lean patties were more yellow than 0% grass-fed lean patties, while 33% and 66% grass-fed lean patties were intermediate ($P < 0.05$). After 120 h of retail display all patties did not differ in yellowness ($P > 0.05$). Although patties were more yellow after 24 h, with increased display time patties tended to have slightly lower b* scores and tended to be similar across all treatments at 48, 72, 96, and 120 h of display.

Initially (0 h), the total color saturation (chroma) of ground beef patties was greatest for patties made with 100% grass-fed lean ($P < 0.05$). However, after 24 h of retail display, total color was greater ($P < 0.05$) for 0% grass-fed lean patties than 33%

and 100% grass-fed lean patties, which were statistically equal ($P < 0.05$) and greater than 67% grass-fed lean patties. At 48 h of retail display, 0% grass-fed lean patties had greater chroma values than 67% grass-fed lean patties, while 33% and 100% grass-fed lean patties were intermediate ($P < 0.05$). Furthermore, within each hour of display time from 72 h through 120 h, chroma values did not differ among the treatments.

Initial hue angle values for all treatments were not different, and were lower than any other day × treatment combination ($P < 0.05$). However, after 24 h of retail display, 100% grass-fed lean patties had greater hue angles than 0% and 67% grass-fed lean patties, while 33% grass-fed lean patties were intermediate ($P < 0.05$). Furthermore, after 48 h, 0% grass-fed lean patties had smaller hue angles ($P < 0.05$) than all other treatments, which did not differ ($P > 0.05$). The largest hue values for all treatments occurred after 72 h of retail display, at which time 0% grass-fed patties were lower ($P < 0.05$) than all other treatments, which did not differ ($P > 0.05$). Interestingly, hue angles decreased between 72 h and 120 h of retail display ($P < 0.05$). At 96 h, 100% grass-fed lean had greater hue angles ($P < 0.05$) than all other treatments, which were statistically equal ($P > 0.05$). However, after 120 h, hue angles did not differ among any of the treatments ($P > 0.05$). Additionally, after 120 h of retail display, hue angles did not differ between day two and day three of retail display ($P < 0.05$).

A significant interaction existed among days of retail display and percentage of grass-fed lean for the percentages of oxymyoglobin, deoxymyoglobin, and metmyoglobin (Table 3; $P < 0.05$). Initially (0 h), the percentage of oxymyoglobin was greater for 100% grass-fed patties than for 0% or 33% grass-fed patties, with 67% grass-

fed patties being intermediate ($P < 0.05$). However, after 48 h of retail display, patties containing 0% grass-fed lean contained higher ($P < 0.05$) percentages of oxymyoglobin, with all other treatments being equal ($P > 0.05$). On 72 h of retail display, 0% grass-fed patties continued to contain greater percentages of oxymyoglobin compared to other treatments ($P < 0.05$). Conversely, on 96 h of retail display, 67% grass-fed patties had greater oxymyoglobin percentages than all other treatments ($P < 0.05$). After 120 h of retail display, all treatments contained the same percentages of oxymyoglobin ($P > 0.05$).

Deoxymyoglobin percentages were lowest ($P < 0.05$) during 0 h and 48 h days of retail display, with all treatments containing equal amounts of the pigment ($P > 0.05$). However, after 72 h of retail display, 0% grass-fed lean patties contained more ($P < 0.05$) deoxymyoglobin than the other treatments, which did not differ ($P > 0.05$) from each other. However, after 96 h of retail display, all patties increased ($P < 0.05$) in the percentage of deoxymyoglobin, with 100% grass-fed lean patties containing less deoxymyoglobin than all other treatments ($P < 0.05$). Additionally, at 120 h of retail display, all patties contained the greatest percentages of deoxymyoglobin compared to previous hours of retail display, with 100% grass-fed lean patties containing less deoxymyoglobin than all other treatments ($P < 0.05$).

Metmyoglobin percentages were lowest at 0 h of retail display and increased each day to 72 h of retail display (Table 3; $P < 0.05$). At 0 h, 100% grass-fed lean patties had greater percentages of metmyoglobin than 0% grass-fed lean patties with 33% and 67% grass-fed lean patties being intermediates ($P < 0.05$). However, at 48 h and 72 h of

retail display, 0% grass-fed lean patties had less myoglobin than all other treatments ($P < 0.05$) than all other treatments, which did not differ ($P > 0.05$). However, metmyoglobin percentages decreased for all treatments between 72 h and 96 h of retail display ($P < 0.05$). However, on 96 h patties with 100% grass-fed lean had greater metmyoglobin percentages than all other treatments ($P < 0.05$). Additionally, at 120 h, the percentage of metmyoglobin decreased for all treatments compared to 96 h ($P < 0.05$). At 120 h, patties with 100% grass-fed lean continued to have more ($P < 0.05$) metmyoglobin than all other treatments. Furthermore, at 120 h patties containing 67% grass-fed lean had less metmyoglobin than 0% grass-fed lean patties, with 33% grass-fed lean patties being intermediate ($P < 0.05$). The ratio of the reflectance of 630 nm/580 nm is another indicator of discoloration, with lower ratios indicating greater discoloration present. This data mirrors the metmyoglobin percentage, with the most discoloration occurring at day three of retail display (Table 3; $P < 0.05$).

Linear and quadratic effects were evaluated for the main effects of the days of retail display and the percentage of grass-fed lean. Day of retail display had a significant quadratic effect on L*, a*, b*, chroma, and hue angle ($P < 0.05$; Table 4). L* quadratically decreased to day one of retail display, then increased ($P < 0.05$). Redness quadratically decreased to day three of retail display, then increased ($P < 0.05$). Inversely, hue angle increased to day three of retail display, then decreased ($P < 0.05$). Both b* and chroma quadratically increased to day one of retail display, then decreased ($P < 0.05$). Additionally, the percentage of grass-fed lean had a significant quadratic effect on L*, a*, b*, and chroma ($P < 0.05$; Table 5). L* values quadratically increased

to 33% grass-fed lean, then decreased ($P < 0.05$). Conversely, a* values quadratically decreased to 33% grass-fed lean, then increased ($P < 0.05$). Both b* and hue angles quadratically decreased to 67% grass-fed lean, then increased ($P < 0.05$). Additionally, hue angle values linearly increased between 0% and 100% grass-fed lean ($P < 0.05$).

3.4.2. Lipid Oxidation

There was a significant interaction between day of retail display and percentage of grass-fed lean on the level of lipid oxidation ($P < 0.05$; Table 6). Both the initial amount of malonaldehyde (mg MDA/kg) and TBARS substances (ppm) in ground beef patties was greater in 33% grass-fed lean patties than all other treatments, which were equal ($P < 0.05$). Oxidative products increased on day three, with 100% grass-fed lean patties being greater than any other treatments ($P < 0.05$). Conversely, after five days of retail display, 0% grass-fed lean patties had less milligrams of malonaldehyde than 67% grass-fed lean patties, with 33% grass-fed lean patties being intermediate ($P < 0.05$). 100% grass-fed lean patties had greater malonaldehyde than 33% grass-fed lean patties, with 67% grass-fed lean patties being intermediate ($P < 0.05$). Furthermore, on day five 0% grass-fed lean patties had less TBARS (ppm) than any other treatment. 100% grass-fed lean patties had greater TBARS values than 67% grass-fed lean patties, with 33% grass-fed lean patties being intermediate ($P < 0.05$).

3.4.3. Volatile Compound Analysis

Of the 102 total compounds analyzed, only one compound was significantly affected by the percentage of grass-fed lean utilized (Table 7). 2,3-butanedione (strong, buttery odor) was statistically equal in 0% and 33% grass-fed lean patties and present in

a higher concentration compared to 66% and 100% grass-fed lean patties, which were statistically equal.

Day of retail display had a significant main effect on the concentration of twenty-two additional compounds (Table 8). Of those compounds, 12 were identified as having flavors and/or aromas that could contribute to overall beef flavor. Of the alcohols that were significantly affected by time of retail display, 1-pentanol (fusel-like sweet and pleasant odor; burning taste) was present in the greatest concentration at 72 h and 120 h of retail display ($P < 0.05$). 2,3-butanediol, 3-methyl-1-butanol (whiskey characteristic, pungent odor and taste), and ethanol (characteristic odor; burning taste) had the highest concentrations after 120 h of retail display ($P < 0.05$). Additionally, 2-ethyl-1-hexanol (mild, oily, sweet, floral odor; fatty-flavor taste) had a greater concentration on 120 h compared to 0 h, while 72 h was intermediate ($P < 0.05$). Of the statistically significant alkane compounds present, 2,3,3-trimethyl-pentane was present in a higher concentration after 0 h of retail display compared to 120 h of retail display, with 72 h being intermediate ($P < 0.05$). Similarly, 4-methyldecane was present in greater concentration after 0 h of display ($P < 0.05$) compared to 72 h and 120 h of display, which did not differ ($P > 0.05$). Conversely, butane was higher ($P < 0.05$) after 120 h of retail display compared to 0 h and 72 h of display, which did not differ ($P > 0.05$). Furthermore, pentane was present in higher concentrations at 72 h and 120 h of retail display compared to 0 h of display ($P < 0.05$).

Cycloalkanes and cycloalkenes were also identified to be affected by retail display time. 1-methyl-3-propylcyclohexane and 1,3/4-dimethylbenzene were present in

greater concentrations at 0 h of display compared to 72 h and 120 h ($P < 0.05$). Similarly, 1,2,4-trimethylcyclohexane and dl-limonene (pleasant, lemon-like odor) were present in greater concentrations at 0 h compared to 72 h of display ($P < 0.05$), however, 120 h of retail display was intermediate. Only one furan was found to be affected by retail display time. 2-pentylfuarn (fruity, green bean, metallic, vegetable) was present in greater concentrations at 72 h and 120 h of retail display compared to 0 h of display ($P < 0.05$). Several ketones were found to increase during retail display 2-heptanone (fruity, spicy, cinnamon, banana), 2-pentanone (ethereal, fruit odor), and 2,3-butadione (strong, buttery odor) were found in higher concentrations at 72 h and 120 h compared so 0 h of display. Interestingly, the only ketone that did not follow a similar pattern was 2,3-pentanedione (sweet, quinone odor; penetrating buttery taste) which was present in higher concentrations at 72 h compared to 120 h, with 0 h of display being intermediate. Furthermore, the organic acid acetic acid, ethyl ester was present in higher concentrations in 120 h of display compared to 72 h of display, which was greater than 0 h of display ($P < 0.05$). Interestingly, the isomerization of pyrazines was also affected by retail display time. While 2-ethyl-3,5-dimethylpyrazine (toasted nut, sweet woody, roasted cocoa odor) was greater in 0 h and 72 h of retail display, its isomer 3-ethyl-2,5-dimethylpyrazine was present in greater concentration at 72 h and 120 h of display ($P < 0.05$). Furthermore, the sulfur containing compound dimethyl disulfide (diffuse, intense onion odor) was present in higher concentrations at 72 h compared to 0 h, while 120 h was intermediate ($P < 0.05$).

Furthermore, of the 102 compounds there was a significant interaction between the percentage of grass-fed lean and the day of retail display seen in twenty of the compounds ($P < 0.05$; Table 9). Of those twenty compounds 6 have been identified as having specific flavors or aromas. Of the alcohols significantly impacted, 1-hexanol was not present in any of the lean treatments at 0 h of retail display. However, at 72 h of display, 67% grass-fed lean patties had greater hexanol (herbaceous, woody odor; sweet, green fruity flavor) than 0% and 33% grass-fed lean patties ($P < 0.05$), while 100% grass-fed lean patties were not different from 0% or 67% grass-fed lean batches ($P > 0.05$). At 120 h of retail display, 67% grass-fed lean patties had less ($P < 0.05$) 1-hexanol than all other treatments, which did not differ ($P > 0.05$). Additionally, 1-octen-3-ol (powerful, sweet earthy odor; sweet, herbaceous taste) at 0 h of display was higher in 33% grass-fed lean than all other treatments ($P < 0.05$). However, at 72 h 67% and 100% grass-fed lean patties had higher concentrations than 33% grass-fed lean patties, with 0% grass-fed lean patties being intermediate to 33% and 100% grass-fed lean ($P < 0.05$). After 120 h of retail display, 0% grass-fed lean patties had lower ($P < 0.05$) concentrations of 1-octen-3-ol than all other treatments, which did not differ ($P > 0.05$).

Of the aldehydes that had significant effects, both 3-methyl-thiopropanal () and benzeneacetaldehyde (powerful onion, meat-like odor; pleasant meat and soup like flavor) were not present in any of the grass-fed lean treatments at 0 h of retail display. However, after 72 h of display, 3-methyl-thiopropanal was greater in 67% grass-fed lean than all other treatments ($P < 0.05$). Additionally, 100% grass-fed lean contained more 3-methyl-thiopropanal compared to 33% grass-fed lean patties, with 0% grass-fed lean

being intermediate ($P < 0.05$). Similarly, at 72 h of display benzeneacetaldehyde had a greater ($P < 0.05$) concentration in 67% grass-fed lean patties, compared to all other treatments which did not differ ($P > 0.05$). Furthermore, at 120 h of display, 3-methyl-thiopropanal was not present in any of the treatments, while benzeneacetaldehyde was only present in 100% grass-fed lean patties ($P < 0.05$). At 0 h of display, heptanal (harsh, green odor; unpleasant, pungent bitter flavor that can be fruit like at low levels) was present in higher concentrations in 33% and 100% grass-fed lean patties compared to 0% grass-fed lean patties, while 67% grass-fed lean patties were intermediate ($P < 0.05$). However, after 72 h of display, heptanal was lower ($P < 0.05$) in 33% grass-fed lean patties compared to all other treatments, which did not differ ($P > 0.05$). After 120 h of display, all treatments had statistically equal amounts of heptanal ($P > 0.05$).

Interestingly, several alkanes were found to be significantly affected by both lean source and time of display. 2-methylundecane and 2,6-dimethylundecane were both only present in 100% grass-fed lean patties at 0 h of display. However, at 72 h of display, all of the treatments had statistically equal amounts of both compounds ($P > 0.05$). Furthermore, after 120 h of display, 2-methylundecane was present in higher ($P < 0.05$) concentration in 67% grass-fed lean patties compared to all other treatments, which did not differ ($P > 0.05$), while 2,6-dimethylundecane continued to remain statistically equal between all grass treatments ($P > 0.05$). At 0 h, 2/4-methylheptane was present in greater concentration in 0% and 100% grass-fed lean patties compared to 67% grass-fed lean patties, while 33% grass-fed lean patties were intermediate ($P < 0.05$). After 72 h of display. 67% patties contained lesser amounts ($P < 0.05$) than all other treatments, which

did not differ ($P > 0.05$). At the end of the display period (120 h), 67% grass-fed lean patties had a greater concentration of 2/4-methylheptane than 0% grass-fed lean patties, while 33% and 100% grass-fed lean patties were intermediate ($P < 0.05$). Similarly, at 0 h 4-methylnonane was present in a higher concentration in 0% and 100% grass-fed lean patties compared to 67% grass-fed lean patties, while 33% grass-fed lean patties were intermediate ($P < 0.05$). After 72 h of display, patties did not significantly differ in 4-methylnonane concentration ($P > 0.05$). However, after 120 h of display, 67% grass-fed lean patties contained a greater concentration ($P < 0.05$) than all other compounds, which did not differ ($P > 0.05$). Decane and octane concentrations both did not differ between grass-fed lean treatments at 0 h of display. However, at 72 h 100% grass-fed lean patties had less decane ($P < 0.05$) that all other treatments while octane concentrations did not differ between grass-fed lean treatments ($P > 0.05$). Furthermore, after 120 h of display, decane concentration did not differ between grass-fed lean treatments ($P > 0.05$) while octane concentrations were lower ($P < 0.05$) in 0% grass-fed lean patties than all other treatments, which did not differ ($P > 0.05$). Additionally, at 0 h of display, nonane concentrations were greater in 100% grass-fed lean patties compared to 67% grass-fed lean patties, while 0% and 33% grass-fed lean patties were intermediate ($P < 0.05$). At 72 h of display, lean treatments did not differ in nonane concentration ($P > 0.05$). After 120 h of retail display, 67% grass-fed lean patties had greater nonane concentration compared to 0% grass-fed lean, while 33% and 100% grass-fed lean patties were intermediate ($P < 0.05$).

Also, interestingly, cycloalkanes were also significantly affected by the interactions of time in retail display and percentage of grass-fed lean. At 0 h, butyl-cyclohexane was present in greater concentration in 0% grass-fed lean compared to 33% and 67% grass-fed lean patties, while 100% grass-fed lean patties were intermediate ($P < 0.05$). After 72 h of display, all grass-fed lean treatments were not different ($P > 0.05$). However, after 120 h, 67% grass-fed lean patties had greater ($P < 0.05$) concentrations of butyl-cyclohexane than all other treatments, which did not differ ($P > 0.05$). At 0 h, c*is*-1-ethyl-3-methylcyclohexane was present in greater concentrations ($P < 0.05$) in 0% and 100% grass-fed lean treatments compared to 33% and 67% grass-fed lean batches, which did not differ ($P < 0.05$). At 72 h of display, there were no significant differences among treatments ($P > 0.05$), however, after 120 h, 67% grass-fed lean patties had greater concentrations than all other treatments ($P < 0.05$). At 0 h, ethyl-cyclohexane concentrations did not differ between treatments ($P < 0.05$). However, at 72 h of display 33% grass-fed lean had a higher concentration than any other treatment ($P < 0.05$), while at 120 h, 67% grass-fed lean had a higher concentration than any other treatment ($P < 0.05$). At 0 h, 0% grass-fed lean had a greater concentration of methylcyclohexane ($P < 0.05$). However, at 72 h, 33% grass-fed lean had greater concentration compared to all other treatments ($P < 0.05$). After 120 h, methyl-cyclopentane was not present in any of the treatments. At 0 h, 0% grass-fed lean had a greater concentration of propyl-cyclohexane than 33% grass-fed lean, while 67% and 100% grass-fed lean patties were intermediate ($P < 0.05$). After 72 h, 33% grass-fed lean had a greater concentration compared to 67% and 100% grass-fed lean, while 0% grass-fed lean was intermediate (*P*

< 0.05). However, after 120 h of retail display 67% grass-fed lean had a greater concentration of propyl-cyclohexane than all other treatments ($P < 0.05$).

The ketone compound 2-hexanone was not present in any of the treatments at 0 h of retail display. However, at 72 h of retail display, 33% grass-fed lean had greater concentrations ($P < 0.05$) than any other treatments, which did not differ ($P > 0.05$). However, after 120 h of retail display, 100% grass-fed lean had greater concentrations ($P < 0.05$) of 2-hexanone than all other treatments, which were not different ($P > 0.05$). Initially (0 h) there was no difference in acetic acid methyl ester (pleasant fruity odor; slightly bitter flavor) among any grass-fed lean treatments ($P > 0.05$). However, at 72 h of retail display, 100% grass-fed lean a greater concentration of acetic acid methyl ester than 0% and 33% grass-fed lean, while 67% grass-fed lean was intermediate ($P < 0.05$). Furthermore, at 120 h of display, 0% grass-fed lean was greater ($P < 0.05$) than all other treatments, while all other treatments did not differ ($P > 0.05$).

3.4.4. Fatty Acid Composition

The percentage of myristic acid (14:0), myristoleic acid (14:1n-5), palmitic acid (16:0), palmitoleic acid (16:1n-7), *cis*-vaccenic (18:1n-7), linoleic (18:2n-6), arachidic (20:0), eicosenoic (20:1n-9), eicosandienoic (20:2), arachidonic (20:4n-6), behenic (22:0), erucic (22:1n-9), lignoceric (24:0), nervoic (24:1n-9), docosahexaenoic (22:6), total saturated fatty acid, total monounsaturated fatty acids, and total polyunsaturated fatty acids did not differ among batches containing differing percentages of grass-fed lean (Table 10; $P > 0.05$). The 100% grass-fed lean patties contained less stearic acid ($P < 0.05$; 18:0) than all other treatments, which were equal ($P > 0.05$). However, oleic acid

(18:1n-9) was greater ($P < 0.05$) in 100% grass-fed lean patties than 0% or 33% grass-fed lean patties, with 67% grass-fed lean patties being intermediate ($P > 0.05$). Furthermore, nervoic (24:1) was greater ($P < 0.05$) in 0% grass-fed lean patties than all other treatments, which were did not differ ($P > 0.05$).

3.5. Discussion

Since pH was not different among the treatments, differences in color of the treatments cannot be explained by differences in pH. However, in future research experiments, the pH of the patty should be measured each day during retail display to determine if pH changes during this time. Differences in pH over the course of the shelf life could help determine if metabolism is occurring in the muscle postmortem. It is possible that metabolic differences could affect the oxygen consumption and the production and/or use of reductive enzymes. Chikuni et al. (2010) reported that muscles with a higher concentration of fast-twitch fibers have a lower pH when compared to slow-twitch fibers. This is due to the postmortem metabolism occurring in the muscle fibers, as slow-twitch fibers are more glycolytic, while slow-twitch fibers are more oxidative in metabolism (Picard et al., 2020). An increase in the pH over time could also be an indication of microbial growth in the product (Rhee, Krahl, Lucia, & Acuff, 1997). Microbial growth occurring on the surface of meat could also be the reason for the surface discoloration of the ground beef patties, as aerobic bacteria can be utilizing the oxygen from the packaging which would reduce the oxygen partial pressure at the surface of the meat, leading to the formation of metmyoglobin (Rhee et al., 1997).

With increasing storage times, the redness of patties across treatments decreased in redness to 72 h. While 24 h patties were slightly less red in display compared to 0 h patties, patties containing no grass fed lean increased in a* values in the first 24 h. This indicated that the grass-fed lean was detrimental to color stability within 24 h of retail display. The largest decrease in redness was seen between 24 h and 48 h of retail display. Interestingly, for all treatments, the color improved (greater a*, lower hue angles) between the 72 and 120 h of retail display. During this time period, there was a significant decrease in metmyoglobin and increase in deoxymyoglobin. This is indicative of metmyoglobin being reduced to deoxymyoglobin in the muscle. However, there is little change in the amount of oxymyoglobin during this time. This could be the result of the metmyoglobin being reduced below the surface of the patty, where oxygen tensions are too low to regenerate oxymyoglobin (Govindarajan & Snyder, 1973). Additionally, Seideman, Cross, Smith, & Durland (1984) reported that if 60% or more of the pigments in one area are oxidized, the brown pigment is predominately seen by the human eye. Therefore, reduction of pigments could be improving, but not enough to see a complete visual change in color. Improvement in color after three days of retail display could be due to the increased activity of metmyoglobin reducing activity below the surface of the patty. Initially, if the mitochondria present in the muscle is metabolizing oxygen, the oxygen partial pressure would be reduced in the package creating metmyoglobin over time (Seideman et al., 1984). However, the mitochondria would increase the NADH and other reducing enzymes which could account for the increase is reduction over the course of the shelf life (Faustman & Cassens, 1990; Giddings & Hultin, 1974;

Govindarajan & Snyder, 1973; O'Keeffe & Hood, 1982). During retail display, the patties containing muscle with greater oxidative metabolism could be actively functioning, leading to the rapid decline in color between day 0 and 3 of retail display (Canto et al., 2015). However, as the oxygen is used up and the muscle is no longer able to metabolize, reducing agents involved in the TCA cycle and electron transport chain (such as NADH) could be available to reduce metmyoglobin back to deoxymyoglobin, as seen by the increase in redness and percentage of deoxymyoglobin present as well as the decrease in hue angle and metmyoglobin exhibited in patties of all treatments.

Although the 100% grass-fed lean patties initially appeared the most red in color, they did not maintain their superior redness through the 120 h of retail display. These results were unexpected as previous research has shown grass-fed beef has greater color stability and a* over the course of retail display (Lanari et al., 2002; Sledge, 2008). However, this study differed from previous research, as we utilized fat sourced from grain-finished cattle in addition to the small amount of inherent fat from the muscle of grass-fed animals. This suggests that the differences in this study and previous research could be due to the absence or smaller concentration of a lipophilic antioxidant, such as α-tocopherol or β-carotene, that is commonly found deposited in the lipid portions of grass-fed beef. The results of this study did align with the results of O'Sullivan et al. (2003) who reported that diet did not impact color stability of steaks when packaged in overwrap packaging. The study did report that if steaks were packaged in high-oxygen modified atmosphere packaging (MAP), beef from forage-finished cattle had greater color stability compared to that of concentrate-finished cattle. Future research

opportunities could examine the effects of these treatments under different packaging systems, especially high oxygen MAP, as this packaging system, in addition to overwrap packaging, is the most common packaging system for ground beef (Suman et al., 2014).

Moreover, the increased myoglobin oxidation of the 100% grass-fed lean could be influenced by the fatty acid composition of 100% grass-fed lean compared to the grain-fed lean. Batches that contained 100% grass-fed lean contained a greater percentage of oleic acid (18:1n-9) and a lesser percentage of stearic acid (18:0) compared to the 0% grass-fed lean batched. Because the 100% grass-fed lean patties contained greater myoglobin oxidation and there is a positive correlation between lipid oxidation and myoglobin oxidation, this could be why there was a greater amount of oxidation in the 100% grass-fed lean patties (Faustman et al., 2010; Fruet et al., 2018; Luciano, Vasta, et al., 2011; Min & Ahn, 2005; Spanier, Miller, et al., 1992).

As expected, lipid oxidation increased throughout the first 72 h of shelf life. It was expected that lipid oxidation would continue to increase between day three and five, but instead oxidation plateaued. This could be because oxidation occurred quickly in the product. Since the rounds were received vacuum packaged from a major producer, it is unknown how long the product was aged for before processing. It was also unexpected that 33% grass-fed lean patties would exhibit greater initial lipid oxidation compared to other treatments. Since these patties were formulated from the same grass- and grain-fed rounds to make the other treatment batches, and since patties were made and frozen and virtually the same time, there is no reason these patties should have higher levels of lipid oxidation.

Furthermore, it was unexpected that the 100% grass-fed lean patties exhibited the greatest amount of lipid oxidation as Sledge (2008) reported that 0% grass-fed lean patties exhibited the highest TBARS values within overwrap packaging. This could be the result of the 100% grass-fed lean patties containing greater concentrations of oleic acid, which is an unsaturated fatty acids and thus more susceptible to lipid oxidation (Cheng, 2016; Faustman et al., 2010). However, while the grass-fed beef is expected to have a greater concentration of unsaturated fatty acids, if is expected that it would have greater concentrations of the polyunsaturated fatty acids such as α-linolenic acid (18:3n-3; Mancini & Hunt, 2005). Additionally, several studies have reported grain-fed beef containing higher concentrations of oleic acid compared to grass-fed beef (Z. C. T. R. Daniel, Wynn, Salter, & Buttery, 2004; Hwang & Joo, 2017; S. B. Smith, Lunt, Smith, & Walzem, 2020). These results make us question the validity of the grass-fed beef utilized in the study. Additionally, this is further indication that a lipophilic antioxidant is present in the lipid portion of grass-fed beef that becomes overwhelmed by the addition of fat from grain-fed animals.

The interactive affects between the lean utilized and days in retail display are both interesting and difficult to interpret. Several compounds are present in only one of the batches on each tested day of retail display. Additionally, the batch in which this compound shows up often changes over the time of retail display. However, little research is present that has examined the volatile compounds produced as the result of retail display time. Although the study by Blackmon et al. (2015) reported several of the same volatile compounds derived from the Maillard reaction and lipid thermal

degradation being affected by fat percentage and lean source, specifically from different primal cuts of beef. Therefore, it is likely that the compounds identified in this study play a significant role in the formation of flavor compounds in beef. Conducting more research in this area, with specific regards to the volatile compounds produced, could help further identify the role of these products and strengthen our understanding of the impact of these factors on flavor.

The main information we can perceive from this is that the lean sources are changing throughout retail display and are affecting the Maillard reaction differently. However, the intermediate treatment batches (33% and 66% grass-fed lean) do not affect compounds in a linear manner as anticipated. Combining the two different lean sources could cause the nonvolatile compounds in the raw product to react with each other, thus forming different compounds during the Maillard reaction. In the future, looking at changes in the lean component over shelf life using HPLC technology could help to explain how the sources are interacting with each other and what is driving the differences in compounds created during cooking.

It was surprising that the lean source utilized only significantly affected one volatile compound produced. One possible explanation is the nonvolatile compounds of the lean source (amino acids and sugars) could be so similar that they do not drive the formation of different end products when cooked. This theory could be validated by examining both the raw and cooked nonvolatile compounds in the product. Another potential reason for the similarities could be the large amount of fat utilized in the ground beef batches could interfere with the Maillard reaction and thus be the driving

force for the volatile compounds created during cooking. However, in a similar study by Blackmon, Miller, Kerth, & Smith (2015), reported differences in several Maillard-derived volatile compounds. Several of the compounds reported in that study were found to be affected by the interaction of lean source and day of retail display. Because the previous study did not include a retail display period, it is most likely that lean source did not affect more compounds as a main effect due to the interactive effect of lean components and retail display time. This seems more likely, as the fatty acid composition for the two products were so similar. However, this theory would need to be validated with further testing. When looking at the main effect of retail display time, several volatile compounds that can be attributed to off-flavors from lipid oxidation were present in the treatments, such as pentanal, heptanal, and several alkane compounds. As expected, these compounds tended to increase between 0 h and 72 h of retail display. However, concentrations did not always increase when comparing samples from 72 h and 120 h. This could be the result of near complete lipid oxidation in the product.

Since all batches contained large amounts of fat from the standard fat source, it is not surprising that there were few differences in the non-polar fatty acid composition of the ground beef batches. It is possible that more differences would be present if the polar fatty acids had also been analyzed, as previous research has shown that grass-fed cattle had polar lipids with higher concentrations of linoleic, α-linolenic, eicosatrienoic, arachidonic, and docosapenaenoic acid compared to grain-finished cattle (Larick & Turner, 1989). However, the differences in oleic acid between the 0% grass-fed batch and the 100% grass-fed batch is inconsistent with results from previous research (Daley,

Abbott, Doyle, Nader, & Larson, 2010b; Leheska et al., 2008; Westerling & Hedrick, 1979). It was expected that if differences existed between the grain- and grass-fed lean batches, that the 0% grass-fed batch would have lower concentration of oleic acid. However, since grass-fed beef tends be leaner compared to grain-fed beef, it is possible that the batches containing greater percentages of grass-fed lean had greater amounts of the standard, grain-fed fat source added to the formulation in order to reach the targeted 15% fat level in each batch. Overall, further research should aim to explore differences in both polar and non-polar fatty acids. Additionally, research should be focused on examining the inherent fatty acid composition of lean from these two systems without overwhelming them with fatty acids from a single source.

3.6. Conclusion

In conclusion, when creating ground beef batches utilizing grass- or grain-fed lean and a grain-fed fat source, the grass-fed lean had a negative impact on both the color and flavor stability of the ground beef patties. However, it is important to note that all patties had unfavorable (complete surface discoloration) after 72 h of retail display. Interestingly, lipid oxidation plateaued and a small amount of myoglobin was reduced between 72 h and 120 h of retail display. This was likely the result of reducing compounds such as NADH present in the muscle as a by-product of oxygen metabolism. This study showed that the increased color stability of grass-fed lean is likely not present in the lean portion of meat, and is not present in high enough concentrations when an additional grain-fed fat source is added. Volatile compounds produced were significantly impacted by the interaction of the lean source and retail display time. However, more

research is needed to determine the distinct differences in perceived flavors of the patties. Conducting a descriptive sensory analysis test could also help identify the role of the nondescript volatile compounds have in flavor perception of ground beef. This, in addition to HPLC technology, could also help determine the impact non-volatile compounds have the oxidative and flavor stability of ground beef patties. Further research examining the interactions that occur when mixing grain- and grass-fed lean should also be done to help determine the driving force of volatile aroma compound differences in grass- and grain-fed lean.

3.7. References

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4. LEAN SOURCE CREATES DIFFERENCES IN FLAVOR POTENTIAL OF GROUND BEEF PATTIES

4.1. Abstract

To test the hypothesis that differences in flavor compounds in the lean portion of ground beef are the driving force for flavor difference across ground beef patties, rounds were procured from each of the following treatment groups: premium upper two-thirds choice brand containing USDA Modest and higher marbling (CH), Japanese influenced heart-healthy beef (HRT), natural, grass-fed (NAT), and USDA Select (SE). Rounds were ground and supplemented with a commercial fat source to form treatment batches containing 10% or 20% fat. Batches were then fine ground and formed into 112-g patties. Patties were vacuum packaged and frozen until analyzed to determine fatty acid composition and volatile compound composition. Formulating the patties to 20% fat resulted in a higher percentage of saturated fatty acids across all treatments and a lower percentage of monounsaturated fatty acids amongst all lean sources except SE ($P < 0.05$). Additionally, NAT patties contained higher concentrations of (E)-2-heptanal (grassy, herbaceous; $P < 0.05$). Higher quality-grade patties contained higher concentrations of dimethyl disulfide (intense onion) compared to lower-grade patties ($P < 0.05$). Overall, lean source appeared to play a significant role in the volatile compound and fatty acid composition of patties.

4.2. Introduction

Flavor is the most important attribute for consumer acceptance of beef as long as tenderness is acceptable (Kerth & Miller, 2015; Killinger, Calkins, Umberger, Feuz, & Eskridge, 2004; O'Quinn, Legako, Brooks, & Miller, 2018). "Fat is flavor" is a well-known adage among the beef industry. Due to this line of thinking, the USDA quality grade system, which evaluates carcass quality based on maturity and marbling, has been a long-used tool for consumers to predict quality and for processors and retails to set prices on products. Thus, numerous studies have been conducted examining marbling and fat deposition as they relate to consumer satisfaction and determining logical slaughter endpoints to increase profitability by producers (Deering, 1994; B. B. Greene, Backus, & Riemann, 1989; Henchion, McCarthy, & Resconi, 2017; Jeremiah, 1982; Killinger et al., 2004; Lusk & Parker, 2009). In turn, the degree of marbling has been shown to be a factor that impacts tenderness and consumer acceptability of beef products (Jeremiah, 1996; Killinger et al., 2004).

Many pre-harvest factors have been shown to have an influence on metabolites which act as substrates in flavor-producing reactions. Specifically, Arshad et al. (2018) noted breed variations due to genetic differences in metabolites resulted in over 40 different Maillard reaction products. Because the role of small sugar molecules, peptide chains, and free amino acids in the development of flavor is largely unknown outside of impacts on basic tastes, this leads to the hypothesis that genetic differences, and thus the regulation of different metabolites, may result in flavor differences across lean source.

In the beef industry, numerous branded programs exist based on differences in breed and meat quality. These claims have led to premium products which are based on high degrees of marbling and a guaranteed tender product. Consequently, consumers will pay premium prices for these products (Banović, Grunert, Barreira, & Fontes, 2010; Font-i-Furnols & Guerrero, 2014; Killinger et al., 2004; Papanagiotou, Tzimitra-Kalogianni, & Melfou, 2013). These claims have not been confirmed by sensory and basic meat science research. However, novel instrumentation and research methods allow us to explore the water-soluble metabolites in the lean portion of meat completely separate from the lipid portion. Defining differences in metabolites due to genetic or breed differences could provide evidence for superior flavor in certain lean sources due to up- or down-regulation of muscle metabolism. We hypothesize that differences in flavor compounds in the lean portion of ground beef will be the driving force for flavor difference across ground beef patty types.

4.3. Materials and Methods

4.3.1. Product Procurement and Patty Formation

Beef inside rounds that qualify and represent four different lean source groups were purchased from a commercial distributor: premium branded program (upper 2/3 choice; CH); labeled heart-healthy (from Wagyu cattle containing a high degree of marbling; HRT); all-natural program (meeting specifications for all natural in addition to consuming a grass-based diet (Grass Run Farms, Greely, CO); NAT); USDA Select (control group; SE). Rounds were trimmed of any visible exterior fat and ground using a 12.7 mm plate. Patties were mixed to an assigned targeted fat percentage (10% fat or

20% fat) using a standard fat source purchased from a commercial meat purveyor. Actual fat percentages were validated using the Foss FoodScan2 Meat (FOSS Global, Hilleroed, Denmark). After patties were formulated at the appropriate fat percentage, the blend was reground using a 4.76 mm plate. Patties were weighed and hand-formed using a handheld hamburger patty press (Oneida Hospitality Group, Lincolnshire, IL, USA) to achieve a 113.4-gram patty with a 12.7 mm thickness and 11.43 cm diameter. Patties were randomly designated for GC/MS analysis (1 patty per round per treatment) or fatty acid analysis (1 patty per round per treatment). Patty paper was placed on either side of each patty, and patties were individually crust frozen at -10^{o}C, vacuum packaged, and stored at -20^{o}C until analysis.

4.3.2. Volatile Compound Analysis – GC/MS

Patties were thawed in refrigerated storage at 4^{o}C for 12 to 24 h prior to cooking. Patties were cooked on a 2.54-cm thick flat top Star Max 536TGF 91.44cm Countertop Electric Griddle with Snap Action Thermostatic Controls (Star International Holdings Inc. Company, St. Louis, MO, USA). Griddles were preheated to 204°C, then patties were placed on the griddle. Patties were flipped when the internal temperature reached 32°C and removed when the internal temperature reached 71°C. Internal temperature was monitored using an Omega HH501BT Type T handheld thermometer (Omega Engineering, Stanford, CT, USA) and measured in the geometric center of the patty. Immediately after cooking, patties were frozen in liquid nitrogen and stored in -80^{o}C until collection of volatile compounds. Frozen patties were powdered, and 5 g of powdered sample was placed in a 20-mL glass vial with a Teflon lid and placed on a

heating block (Block analog 2 120V with block modular 28M, VWR) held at 65°C. The volatile compounds present in the headspace were collected using a solid-phase micro-extraction (SPME) Portable Field Sampler (Supelco 504831, 75 µm carboxen/polydimethylsiloxane, Sigma-Aldrich, St. Louis, MO) for 20 minutes. Volatile aroma compounds were eluted from the SPMEs and separated using gas chromatography (GC; Agilent Technologies 7920 series GC, Santa Clara, CA). The sample was desorbed at 280°C for three minutes. The sample was then loaded onto the gas chromatograph column (AgilentVF 5MS 30 m × 0.25 mm ID/1µ film thickness, SGE Analytical Sciences, Austin, TX). Through the column, the temperature started at 40°C (held for one minute) and increased at a rate of 20°C/min until reaching 250°C. Compounds were identified and quantified with a mass spectrometer (MS; Agilent Technologies 5975 series MSD, Santa Clara, CA) using Wiley Chemical Library (Palisade, Ithaca, NY, USA).

4.3.3. Fatty Acid Analysis

Total lipids of raw patties were extracted by a modification of the method of Folch, Lees, & Sloane Stanley (1957). Five grams of homogenized beef were extracted in chloroform: methanol (2:1, v/v) and fatty acid methyl esters (FAME) were prepared as described by Archibeque, Lunt, Tume, & Smith, (2005). The FAME were analyzed using a Varian gas chromatograph (model CP-3800 fixed with a CP-8200 auto sampler, Varian Inc., Walnut Creek, CA). Separation of FAME was accomplished on a fused silica capillary column CP-Sil88 ([100 m × 0.25 mm (i.d.)]; Chrompack Inc., Middleburg, The Netherlands), with hydrogen as the carrier gas (flow rate = 35 mL/min;

split ratio 20:1). Initial oven temperature was 150°C; oven temperature increased at 5°C/min to 220°C and was held for 22 min. Injector and detector temperatures was set at 270°C and 300°C, respectively. Individual fatty acids were identified using genuine external standard GLC-68D (Nu-Chek Prep, Inc., Elysian, MN, USA).

4.3.4. Statistical Analysis

Data were analyzed as a completely randomized design with lean source and fat percentage as the main effects for each analysis with the alpha value set at 5% (0.05) using JMP version 15 (SAS Institute, Inc., Cary, NC, USA). Interactions were included in the model for analysis. When the F-test was determined to be significant, student's t-test was utilized for mean separation of treatment.

4.4. Results

4.4.1. Volatile Compound Analysis

A total of 70 volatile compounds present across treatments were analyzed. Of those compounds, there were no interactions between the lean source and percentage of fat ($P > 0.05$). Six compounds were significantly impacted by the lean source (Table 11; $P < 0.05$). Dimethyl disulfide, a compound described as having an intense onion odor (Burdock, 2009), was present in greater concentrations in CH and HRT patties than NAT patties ($P < 0.05$). SE were intermediate ($P > 0.05$). Additionally, 2,3,3-trimethylpentane was present in greater ($P < 0.05$) concentrations in NAT patties than HRT and CH patties, while SE patties were intermediate ($P > 0.05$). Furthermore, (E)-2-heptenal which has a brassy, herbaceous, lemon-like odor and a fruity, green somewhat bitter taste (Burdock, 2009), was greater ($P < 0.05$) in NAT patties than SE and CH patties,

with HRT patties being intermediate ($P > 0.05$). NAT patties also had greater ($P < 0.05$) concentrations of δ-3-carene, described as sweet, pungent turpentine like taste (Burdock, 2009), compared to CH and SE patties, while HRT patties were intermediate ($P > 0.05$). Similarly, NAT patties had greater ($P < 0.05$) concentrations of 1,4-dimethyl-2-ethyl-benzene, compared to CH and SE, while HRT patties were intermediate ($P > 0.05$). Finally, NAT patties had greater concentrations of 4-octene, than any other treatment ($P < 0.05$).

An additional three compounds were present in different concentrations depending on the amount of fat used in the formulation. Patties formulated with 10% fat contained greater ($P < 0.05$; Table 12) concentrations of 1-octen-3-ol and pentanal (powerful, acrid odor with a slightly-fruity, nut-like flavor; Burdock, 2009) compared to 20% fat patties ($P < 0.05$). Conversely, patties formulated with 20% fat had greater ($P < 0.05$) concentrations of 2-ethyl-1,4-dimethyl-benzene than 10% fat patties.

4.4.2. Fatty Acid Composition

Of the fatty acids measured, eicosenoic acid (20:1), eicosadienoic acid (20:2), eicosopentaenoic acid (20:5), and docosahexaenoic acid (22:6) were not significantly impacted by lean source or fat percentage ($P > 0.05$). However, the lean source impacted the percentage of five of the fatty acids measured (Table 13. There was a higher ($P < 0.05$) percentage of myristic acid (14:0) in SE patties compared to NAT patties, while CH patties were intermediate ($P > 0.05$). HRT patties contained the lowest percentage of myristic acid ($P < 0.05$). Arachidic acid (20:0) was similar in NAT and SE patties and greater than CH and HRT patties, which were also similar ($P > 0.05$). Furthermore,

arachidonic acid (20:4) was greater in HRT-heathy patties than SE patties, while NAT patties were intermediate ($P < 0.05$). NAT patties contained higher ($P < 0.05$) percentage of arachidonic acid than CH patties, while SE were intermediate ($P < 0.05$). HRT patties also contained a greater percentage of lignoceric acid (24:0) compared to USDA and CH patties, while NAT patties were intermediate ($P < 0.05$). CH and SE patties contained a statistical equal percentage of 22:1 and were greater than HRT and NAT patties ($P < 0.05$).

Additionally, nine of the fatty acids were impacted by the percentage of fat ($P < 0.05$; Table 13). Patties containing 10% fat had a greater percentage of myristoleic acid, *cis*-vaccenic acid, linoleic acid, arachidonic acid, lignoceric acid, and total PUFA compared to 20% fat patties (Table 14; $P < 0.05$). Conversely, 20% fat patties had higher percentages of myristic acid, palmitic acid, arachidic and 22:0 compared to 10% fat patties ($P < 0.05$).

Of the fatty acids analyzed, there was an interaction between lean source and fat percentage exhibited for five fatty acids (Table 15). Palmitoleic was similar ($P > 0.05$) for CH and HRT healthy patties at 10% fat, and was present at a higher percentage than any other treatments ($P < 0.05$). NAT patties formulated with 10% fat had a higher ($P < 0.05$) percentage than SE patties containing 20% fat, while HRT patties containing 20% fat were intermediate ($P > 0.05$). HRT patties containing 20% fat were equal ($P > 0.05$) to CH patties containing 20% fat, and greater than all of treatments, which were equal ($P > 0.05$). Stearic acid (18:0) was present in a greater percentage for SE 20% patties than all other treatments ($P < 0.05$). NAT 20% fat, CH 20%, and SE 10% were all similar (P

> 0.05) and had a higher percentage than HRT healthy 20% patties ($P < 0.05$). HRT 20% fat patties had more stearic acid that NAT and CH 10% patties ($P < 0.05$). HRT patties containing 10% fat had the smallest percentage of stearic acid ($P < 0.05$). Oleic acid percentage was greatest in the HRT 10% fat patties ($P < 0.05$). NAT 10% fat contained more ($P < 0.05$) oleic acid than NAT 20% fat patties, while HRT healthy 20% fat and CH 10% fat patties were intermediate ($P > 0.05$). All other treatments had similar amounts of oleic acid ($P > 0.05$). SE 10% fat and CH 20% fat patties had higher ($P < 0.05$) concentrations of 18:3 compared to CH 10% fat patties, while SE 20% fat patties were intermediate ($P > 0.05$). CH 10% fat patties had more ($P < 0.05$) 18:3 than HRT 10% fat and NAT 20% fat patties, while HRT 20% fat patties were intermediate ($P > 0.05$). Additionally, HRT 20% fat patties had a higher ($P < 0.05$) percentage of 18:3 than HRT 10% fat patties, while all other treatments were intermediate ($P > 0.05$). Furthermore, 24:1 concentration was similar ($P > 0.05$) in CH 10% and NAT 10% fat present at a higher percentage than all other treatment combinations, which were similar ($P > 0.05$). The total percentage of saturated fatty acids was highest in the SE 20 % fat patties and lowest in the HRT 10% fat patties ($P < 0.05$). Additionally, the 10% fat HRT patties had the greatest percentage of total monounsaturated fatty acids while the SE 10% fat, SE 20% fat, CH 20% fat, and NAT 20% fat all had the statistically lowest percentage of MUFAs ($P < 0.05$).

4.5. Discussion

It is not surprising that the NAT patties exhibited more differences in volatile aroma compounds, as this was the only treatment finished on a grass-based diet. It is

assumed that cattle for all other treatments were finished on a concentrate diet. Furthermore, δ-3-carene is a terpenoid derived compound common in woody, herbaceous plants, and therefore, could contribute to the distinct grassy-flavor found in grass-fed beef (Abdelgaleil, Saad, & Hassan, 2014; Obiloma et al., 2019). Additionally, 2-heptenal could also be a contributing factor to the grassy-flavor as it has been described as herbaceous (Burdock, 2009). Dimethyl disulfide was found in greater concentrations in patties made with lean from higher USDA quality grades. Gardner & Legako (2018) reported the highest concentration of dimethyl disulfide in USDA Prime steaks, and decreasing concentration with decreasing quality grades. Therefore, dimethyl disulfide could be partially responsible for increase in beefy, brown/roasted flavors that drive consumer liking of premium branded projects. Elmore et al. (2004) reported greater concentrations of 2-octene, an isomer of 4-octene in cattle finished on a silage diet. However, dimethyl disulfide was not reported to be significantly different amongst diet treatments (Elmore et al., 2004). Additionally, Elmore et al. (2004) reported cattle fed on concentrate diets had greater concentrations of 1-octen-3-ol and pentanal compared to silage-finished cattle. Pentanal, which was found in higher concentrations in patties formulated with 10% fat, could be partially responsible for a nut-like, fat-like flavor driving consumer preference of ground beef patties.

From the interaction between lean source and fat percentage, that adding fat decreased the concentration of palmitoic acid (16:1) in all treatments except SE which did not change. Additionally, adding fat increased the concentration of stearic acid in all lean sources except SE. Furthermore, the percentage of oleic acid decreased with added

fat for all treatments except SE. Interestingly, adding the fat source decreased the amount of α-linolenic acid (18:3) in CH and HRT batches, but had no effect on the NAT and SE batches. Conversely, the additional fat decreased the nervonic acid (24:1) in CH and SE but did not affect the HRT or NAT patties. Overall, the addition of fat from the grain-fed source resulted in an increase in the total saturated fatty acids for all lean sources and decrease of the monounsaturated fatty acids for all lean sources except SE. This is unfavorable from a human health perspective as unsaturated fatty acids in food products have been shown to have more beneficial aspects compared to saturated fatty acids (Lee & Park, 2014; Lunn & Theobald, 2006).

Since the ground beef batches were formulated with such high percentages of fat compared to the fat inherently present from the lean source in the round, it was expected that the percentages of fatty acids would not be impacted by the lean source. There are two different theories for why differences were found. The first is that the batches formulated from CH and HRT lean contained a high enough concentration of intramuscular fat that they were not impacted as heavily by the additional fat, especially in the lower fat formulation (Lunt, Riley, & Smith, 1993). Conversely, the additional fat source was purchased in a large amount and thus contained fat from several cattle. Therefore, the fat source itself could have been so diverse that it still led to differences between the treatment batches. Analyzing the lean sources prior to the addition of the fat and analyzing the fat separately could also help prove or disprove that theory. In future research, creating a baseline fatty acid composition from the lean source prior to

formulating could help explain the differences in final composition and could help form a better theory for how fat is impacting flavor.

4.6. Conclusion

In summary, differences in lean source alone did not seem to be a major driver for volatile aroma compound differences in ground beef patty, as few Maillard reaction and lipid thermal derived compounds were impacted by the lean source utilized Conversely, the fat composition also did not appear to be a major driver, as even fewer volatiles were affected by the differences in fat levels. Furthermore, the non-polar fatty acid composition was found to be affected by lean source, fat level, and the interaction of the two. Future research including descriptive sensory analysis, HPLC analysis, and polar fatty acid composition will be conducted. Understanding the perceived attribute differences in addition to the non-volatile composition of both raw and cooked patties and the polar fatty acid composition will help to strengthen our knowledge of the factors impacting flavor of ground beef patties.

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5. CONCLUSIONS

5.1. Utilizing a mixture of grain-fed and grass-fed lean in a ground beef blend impacts color and color stability of ground beef patties

Overall, in this study we found when creating ground beef batches utilizing grass- or grain-fed lean and including additional fat from a grain-fed fat source, the grass-fed lean had a negative impact on both the color and flavor stability of the ground beef patties. However, surprisingly, lipid oxidation plateaued and a small amount of myoglobin was reduced between 72 h and 120 h of retail display. This pigment reduction and lag phase of lipid oxidation can likely be attributed to the action of reducing compounds such as NADH present in the muscle as a by-product of oxygen metabolism in the muscle. From this study, we can conclude that the increased color stability of grass-fed beef reported in other studies is not the result of a compound in the lean compound of muscle, but is rather a compound present in the lipid portion. Additionally, this reducing compound is not present in high enough concentrations when an additional grain-fed fat source is added. Volatile compounds produced were significantly impacted by the interaction of the lean source and retail display time. However, further research will be done to determine the distinct aroma and flavor differences of the patties as perceived by human senses by conducting a descriptive sensory analysis test. This data, in addition to HPLC technology, could also help determine the impact non-volatile compounds have the oxidative and flavor stability of ground beef patties.

5.2. Lean source creates differences in flavor potential of ground beef patties

To conclude the second study, we found few significant differences in volatile aroma compound concentration between both the lean source treatment and the fat level. Interestingly, none of the compounds identified were affected by the interactive effect of the lean source and the fat percentage. We hypothesize this is due to the large amount of total fat interfering with the Maillard reaction and driving the formation of similar concentration of volatile compounds among treatments. Overall, differences in lean source alone did not appear to be a major driver for volatile aroma compound differences in ground beef patty, as few Maillard reaction and lipid thermal derived compounds were impacted by the lean source utilized Conversely, the fat composition also did not appear to be a major driver, as even fewer volatiles were affected by the differences in fat levels. Furthermore, the non-polar fatty acid composition was found to be affected by lean source, fat level, and the interaction of the two. Future research including descriptive sensory analysis, HPLC analysis, and polar fatty acid composition will be conducted. Understanding the perceived attribute differences in addition to the non-volatile composition of both raw and cooked patties and the polar fatty acid composition will help to strengthen our knowledge of the factors impacting flavor of ground beef patties.

5.3. Overall Conclusions

In conclusion, both the color and flavor of ground beef patties are determined by a vast number of dynamic chemical reactions and can be impacted by several intrinsic and extrinsic factors. However, from both studies we learned that the lean component of

the meat is a significant factor creating differences in color and flavor of ground beef patties. Future research goals to continue these studies are to examine the non-volatile compounds of the lean in raw and cooked patties to determine the impact these compounds have on flavor, identify the polar fatty acid composition and determine its impact on color and flavor stability, and to conduct descriptive sensory analysis to define the differences in the intensity of specific flavor attributes between treatments. By completing all of these analyses, we hope this will strengthen our knowledge and understanding of the driving forces determining color and flavor of ground beef.

APPENDIX A

Table 1. Least squares means of the initial pH of batches of ground beef containing 0%, 33%, 67%, or 100% grass-fed lean

	0%	33%	67%	100%	SEM	*P*-value
Initial pH	5.52	5.55	5.56	5.57	0.035[1]	0.26

[1]Standard error of the mean

Table 2. Least squares means of the interactive effect of day of retail display and percentage of grass-fed lean on instrumental color characteristics of fresh ground beef patties.

	L*[1]	a*[1]	b*[1]	Chroma[2]	Hue Angle[2]
0 h display					
0% grass-fed	47.25	31.27^{BC}	25.00^{D}	40.05^{C}	38.44^{G}
33% grass-fed	47.70	30.66^{C}	24.91^{D}	39.52^{C}	38.93^{G}
67% grass-fed	47.60	31.58^{B}	25.26^{D}	40.46^{C}	38.56^{G}
100% grass-fed	46.69	33.07^{A}	26.72^{C}	42.54^{AB}	38.81^{G}
24 h display					
0% grass-fed	36.01	30.03^{C}	31.08^{A}	43.22^{A}	45.99^{F}
33% grass-fed	36.33	28.52^{D}	30.42^{A}	41.70^{B}	46.87^{EF}
67% grass-fed	36.93	27.68^{D}	29.45^{B}	40.42^{C}	46.82^{F}
100% grass-fed	34.13	28.12^{D}	31.17^{A}	42.00^{B}	47.97^{E}
48 h display					
0% grass-fed	43.20	17.95^{E}	19.77^{F}	26.73^{D}	47.82^{E}
33% grass-fed	43.94	16.63^{FGH}	19.69^{F}	25.81^{DE}	49.93^{D}
67% grass-fed	44.25	16.19^{H}	19.35^{F}	25.26^{E}	50.15^{D}
100% grass-fed	42.40	16.56^{GH}	20.44^{F}	25.95^{DE}	50.34^{D}
72 h display					
0% grass-fed	43.71	14.07^{J}	20.44^{F}	24.84^{E}	55.56^{B}
33% grass-fed	45.02	13.00^{K}	20.58^{EF}	24.35^{E}	57.71^{A}
67% grass-fed	44.58	12.90^{K}	20.36^{F}	24.11^{E}	57.62^{A}
100% grass-fed	42.95	13.27^{JK}	20.94^{E}	24.80^{E}	57.61^{A}
96 h display					
0% grass-fed	41.21	15.63^{HI}	20.36^{F}	25.71^{DE}	52.56^{C}
33% grass-fed	41.86	15.49^{HI}	20.51^{EF}	25.76^{DE}	52.97^{C}
67% grass-fed	41.62	16.48^{GH}	20.71^{EF}	26.59^{DE}	51.78^{C}
100% grass-fed	40.94	14.59^{IJ}	21.20^{E}	25.79^{DE}	55.52^{B}
120 h display					
0% grass-fed	40.39	17.71^{EF}	19.36^{F}	26.30^{DE}	47.66^{E}
33% grass-fed	41.04	17.39^{EFG}	19.37^{F}	26.13^{DE}	48.23^{DE}
67% grass-fed	40.30	17.68^{EFG}	19.31^{F}	26.31^{DE}	47.95^{E}
100% grass-fed	38.93	17.46^{EFG}	20.28^{F}	26.84^{D}	49.43^{D}
SEM[3]	0.819	0.686	0.743	1.018	0.836
P-value	0.23	<0.001	0.048	<0.001	< 0.001

[1]L* is a measure of darkness to lightness (greater L* values indicate a lighter color); a* is a measure of redness (greater a* values indicate a redder color); and b* is a measure of yellowness (greater b* values indicate a more yellow color).

[2]Chroma is a measure of the total color of the sample (greater chroma values indicate a more vivid color); Hue angle is a measure of discoloration of the sample (greater hue angle values indicate more discoloration).

[3]Standard error of the mean

[abcdefghijk] Least squares means in a column with different superscripts are statistically different ($P < 0.05$).

Table 3. Least squares means of the change in the percentages of oxymyoglobin, deoxymyoglobin, metmyoglobin, and the ratio of reflectance of 630 nm/580 nm throughout the retail display period as effected by the percentage of grass-fed lean.

	Oxymyoglobin%	Deoxymyoglobin%	Metmyoglobin%	Ratio[1]
0 h display				
0% grass-fed	71.23^{B}	2.44^{E}	26.33^{I}	5.69^{B}
33% grass-fed	71.85^{B}	2.61^{E}	25.54^{IJ}	5.45^{B}
67% grass-fed	72.32^{AB}	2.36^{E}	25.32^{IJ}	5.49^{B}
100% grass-fed	73.11^{A}	2.15^{E}	27.74^{J}	6.28^{A}
48 h display				
0% grass-fed	47.53^{C}	3.09^{E}	49.39^{FG}	1.99^{C}
33% grass-fed	43.71^{D}	2.79^{E}	53.50^{DE}	1.72^{CD}
67% grass-fed	43.21^{D}	2.79^{E}	54.00^{D}	1.66^{CD}
100% grass-fed	42.40^{D}	2.50^{E}	55.10^{D}	1.66^{CD}
72 h display				
0% grass-fed	32.62^{EF}	4.52^{D}	62.86^{B}	1.22^{EF}
33% grass-fed	30.82^{GH}	2.87^{E}	66.30^{A}	1.08^{F}
67% grass-fed	30.82^{GH}	2.92^{E}	66.25^{A}	1.08^{F}
100% grass-fed	30.89^{GH}	2.01^{E}	67.09^{A}	1.08^{F}
96 h display				
0% grass-fed	29.73^{H}	12.83^{C}	57.44^{C}	1.43^{DEF}
33% grass-fed	31.25^{FGH}	10.98^{C}	57.76^{C}	1.43^{DEF}
67% grass-fed	33.47^{E}	11.15^{C}	55.37^{CD}	1.60^{CDE}
100% grass-fed	30.63^{GH}	6.22^{D}	63.15^{B}	1.25^{EF}
120 h display				
0% grass-fed	31.12^{FGH}	21.41^{A}	47.48^{FG}	1.91^{C}
33% grass-fed	32.40^{EFG}	19.47^{A}	48.13^{GH}	1.89^{CD}
67% grass-fed	32.90^{EF}	20.02^{A}	47.08^{H}	2.02^{C}
100% grass-fed	32.32^{EFG}	16.02^{B}	51.66^{EF}	1.81^{CD}
SEM[2]	1.421	0.974	1.556	0.198
P-value	<0.001	<0.001	<0.001	<0.001

[1]Ratio of reflectance of 630 nm/580 nm; lower values indicate more discoloration present

[2]Standard error of the mean

[abcdefghij]Least squares means in a column with different superscripts are statistically different ($P < 0.05$).

Table 4. Linear and quadratic effects of retail display time on color characteristics of fresh ground beef patties made with different percentages of grass-fed lean.

	L*	a*	b*	Chroma	Hue Angle
0 h display	47.31	31.65	25.47	40.64	38.69
24 h display	35.85	28.59	30.53	41.4	46.91
48 h display	43.45	16.83	19.69	25.94	49.56
72 h display	44.06	13.31	20.58	24.53	57.13
96 h display	41.41	15.55	20.69	25.96	53.21
120 h display	40.17	17.56	19.58	26.40	48.32
SEM[1]	0.707	0.676	0.631	0.868	0.719
Linear *P*-value	<0.001	<0.001	<0.001	<0.001	<0.001
Quadratic *P*-value	<0.001	<0.001	<0.001	<0.001	<0.001

[1]Standard error of the mean

Table 5. Linear and quadratic effects of percentage of grass-fed lean included in the ground beef blend on color characteristics of fresh ground beef patties during retail display.

	L*	a*	b*	Chroma	Hue Angle
0% grass-fed lean	41.96	21.11	22.67	31.14	48.01
33% grass-fed lean	42.65	20.28	22.58	30.55	49.11
67% grass-fed lean	42.55	20.42	22.40	30.53	48.81
100% grass-fed lean	41.01	20.51	23.38	31.32	49.95
SEM[1]	0.685	0.651	0.309	0.839	0.679
Linear *P*-value	<0.001	0.018	0.003	0.56	<0.001
Quadratic *P*-value	<0.001	0.004	0.001	0.001	0.92

[1]Standard error of the mean

Table 6. Least squares means of the amount of lipid oxidation in ground beef blends containing different percentages of grass-fed lean over a retail display period.

	MDA[1]	TBARS[2]
0 h display		
0% grass-fed	0.26^{f}	0.26^{f}
33% grass-fed	0.56^{e}	0.58^{e}
67% grass-fed	0.33^{f}	0.35^{f}
100% grass-fed	0.23^{f}	0.23^{f}
72 h display		
0% grass-fed	0.65^{de}	0.68^{cde}
33% grass-fed	0.79^{cd}	0.80^{cd}
67% grass-fed	0.81^{cd}	0.84^{c}
100% grass-fed	1.06^{a}	1.10^{a}
120 h display		
0% grass-fed	0.60^{e}	0.61^{de}
33% grass-fed	0.82^{cd}	0.86^{bc}
67% grass-fed	0.86^{bc}	0.84^{c}
100% grass-fed	1.02^{ab}	1.05^{ab}
SEM[3]	0.063	0.067
P-value	0.003	0.003

[1]Milligrams of malonaldehyde per kilogram of meat

[2]Thiobarbituric acid reactive substances measured in parts per million

[3]Standard Error of the Mean

[abcdef]Least squares means in a column with differing superscripts are significantly different ($P < 0.05$)

Table 7. The main effect of the percentage of grass-fed lean on the concentration of volatile compounds[1] present in cooked ground beef patties.

	0% Grass[2]	33% Grass	66% Grass	100% Grass	SEM[3]	*P*-value
Ketones						
2,3-butanedione[4]	11.45[a]	11.43[a]	5.46[b]	5.57[b]	1.723	0.021

[1]Least square means reported for the logn+1 concentration of volatile compounds
[2]Percentage of grass-fed lean utilized in the ground beef batch
[3]Standard error of the mean
[4]Strong, buttery odor

Table 8. The main effect of the total time in retail display on the concentration of volatile compounds[1] present in cooked ground beef patties.

Volatile compound	0 h display	72 h display	120 h display	SEM[2]	*P*-value
Alcohols					
1-pentanol[3]	5.79[b]	10.75[a]	9.58[a]	1.202	0.02
2,3-butanediol	0.00[b]	0.00[b]	6.80[a]	1.129	0.001
2-ethyl-1-hexanol[4]	0.00[b]	2.95[ab]	6.37[a]	1.277	0.007
3-methyl-1-butanol[5]	0.00[b]	0.00[b]	9.14[a]	0.704	<0.001
Ethanol[6]	0.00[b]	0.86[b]	9.61[a]	0.785	<0.001
Alkanes					
2,3,3-trimethyl-pentane	4.29[a]	1.51[ab]	0.00[b]	1.054	0.026
4-methyldecane	3.87[a]	0.00[b]	0.00[b]	0.974	0.013
Butane	0.00[b]	1.09[b]	5.08[a]	1.205	0.016
Pentane	0.00[b]	8.16[a]	4.90[a]	1.498	0.003
Cycloalkanes					
1-methyl-3-propyl-cyclohexane	4.43[a]	0.00[b]	0.00[b]	0.889	0.002
1,2,4-trimethyl-cyclohexane	5.67[a]	0.96[b]	1.82[ab]	1.33	0.044
Cycloalkenes					
1,3/4-dimethyl-benzene	9.50[a]	4.59[b]	3.01[b]	1.464	0.013
dl-Limonene[7]	6.02[a]	3.19[ab]	0.88[b]	1.214	0.022
Furans					
2-pentylfuran[8]	0.00[b]	10.42[a]	10.43[a]	0.761	<0.001
Ketones					
2-heptanone[9]	0.85[b]	11.12[a]	12.52[a]	0.786	<0.001
2-pentanone[10]	5.79[b]	9.83[a]	10.35[a]	1.131	0.017
2,3-butanedione[11]	4.94[b]	10.67[a]	9.82[a]	1.493	0.026
2,3-pentanedione[12]	1.49[ab]	4.08[a]	0.00[b]	1.022	0.03
Organic acids					
Acetic acid, ethyl ester	0.00[c]	9.11[b]	15.03[a]	0.923	<0.001
Pyrazines					
2-ethyl-3,5-dimethyl-pyrazine[13]	3.91[a]	4.55[a]	0.00[b]	1.184	0.025
3-ethyl-2,5-dimethyl-pyrazine	2.00[b]	8.18[a]	9.13[a]	0.996	<0.001
Sulfur Containing					
Dimethyl disulfide[14]	0.84[b]	7.28[a]	3.33[ab]	1.369	0.01

[1]Least square means reported for the logn+1 concentration of volatile compounds; [2]Standard error of the mean; [3]Fusel-like sweet and pleasant odor; burning taste; [4]Mild, oily, sweet, floral odor; fatty-flavor; [5]Whiskey characteristic; pungent odor and taste; [6]Characteristic odor; burning taste; [7]Pleasant, lemon-like odor; [8]Fruity, green bean, metallic, vegetable odor; [9]Fruity, spicy, cinnamon, banana odor; [10]Ethereal, fruity odor; [11]Strong, buttery odor; [12]Sweet, quinone odor; penetrating buttery taste; [13]Toasted nut, sweet woody, roasted cocoa odor;[14]Diffuse, intense onion odor

Table 9. The interactive effect of the total time in retail display and the percentage of grass-fed lean on the concentration of volatile compounds[1] present in cooked ground beef patties.

	0 h display				72 h display				120 h display					
	0%	33%	67%	100%	0%	33%	67%	100%	0%	33%	67%	100%	SEM[2]	*P*-value
Alcohols														
1-hexanol[3]	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	3.48^{bc}	0.00^{c}	11.12^{a}	7.28^{ab}	7.54^{ab}	11.97^{a}	0.00^{c}	7.86^{ab}	2.148	0.002
1-Octen-3-ol[4]	0.00^{c}	3.67^{bc}	0.00^{c}	0.00^{c}	3.64^{bc}	0.00^{c}	11.50^{a}	7.70^{ab}	3.69^{bc}	11.73^{a}	7.23^{ab}	11.91^{a}	2.401	0.029
Aldehydes														
3-methyl-thiopropanal[5]	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	2.73^{bc}	0.00^{c}	9.15^{a}	5.64^{b}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	1.175	0.01
Benzene-acet-aldehyde[6]	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	10.14^{a}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	3.71^{b}	1.091	<0.001
Heptanal[7]	3.73^{b}	11.51^{a}	7.97^{ab}	11.86^{a}	11.27^{a}	3.85^{b}	12.18^{a}	11.80^{a}	11.61^{a}	11.66^{a}	7.67^{ab}	11.62^{a}	2.236	0.031
Alkanes														
2-methyl-undecane	0.00^{b}	0.00^{b}	0.00^{b}	7.61^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	7.26^{a}	0.00^{b}	1.528	0.003
2/4-methyl-heptane	10.41^{a}	6.12^{abcd}	3.50^{cd}	10.03^{ab}	6.94^{abc}	10.51^{a}	2.89^{d}	9.63^{abc}	0.00^{d}	6.42^{abcd}	9.98^{ab}	6.28^{abcd}	2.307	0.022
2,6-dimethyl-undecane	0.00^{b}	0.00^{b}	0.00^{b}	7.44^{a}	0.00^{b}	3.57^{ab}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	3.80^{ab}	0.00^{b}	1.853	0.047
4-methyl-nonane	7.51^{ab}	3.79^{abc}	0.00^{c}	10.57^{a}	0.00^{c}	3.73^{abc}	0.00^{c}	3.21^{bc}	0.00^{c}	0.00^{c}	7.24^{ab}	0.00^{c}	2.352	0.021
Decane	12.15^{a}	7.83^{ab}	7.21^{ab}	12.15^{a}	10.67^{a}	11.92^{a}	10.75^{a}	3.83^{b}	6.95^{ab}	10.82^{a}	12.46^{a}	10.33^{a}	2.200	0.047
Nonane	12.55^{ab}	7.85^{abcd}	4.39^{bcd}	12.67^{a}	10.35^{abc}	9.01^{abc}	10.63^{abc}	3.68^{cd}	0.00^{d}	7.24^{abcd}	13.21^{a}	6.83^{abcd}	2.834	0.016
Octane	12.00^{a}	11.92^{a}	11.53^{a}	12.38^{a}	11.72^{a}	12.25^{a}	12.40^{a}	12.21^{a}	4.23^{b}	12.42^{a}	12.23^{a}	12.41^{a}	1.254	0.016

[1]Least square means reported for the logn+1 concentration of volatile compounds; [2]Standard error of the mean; [3]Herbaceous, woody odor; sweet, green fruity flavor; [4]Powerful, sweet earthy odor; sweet, herbaceous taste; [5]Powerful onion, meat-like odor; pleasant meat and soup like flavor; [6]Harsh, green odor; unpleasant, pungent bitter flavor that can be fruit like at low levels; [7]Strong, fatty, harsh pungent odor; unpleasant fruity taste; [8]Pleasant fruity odor; slightly bitter flavor

Table 9 cont. The interactive effect of the total time in retail display and the percentage of grass-fed lean on the concentration of volatile compounds[1] present in cooked ground beef patties.

	0 h display				72 h display				120 h display					
	0%	33%	67%	100%	0%	33%	67%	100%	0%	33%	67%	100%	SEM[2]	*P*-value
Cycloalkanes														
Butyl-cyclohexane	9.66^{a}	3.54^{bc}	3.33^{bc}	6.97^{ab}	0.00^{c}	3.51^{bc}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	9.83^{a}	0.00^{c}	2.018	0.006
Cis-1-ethyl-3-methyl-cyclohexane	6.93^{a}	0.00^{b}	0.00^{b}	7.65^{a}	0.00^{b}	4.00^{ab}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	7.51^{a}	0.00^{b}	2.192	0.011
Ethyl-cyclohexane	7.87^{a}	3.69^{ab}	3.83^{ab}	7.60^{a}	0.00^{b}	7.99^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	10.92^{a}	0.00^{b}	2.489	0.009
Methyl-cyclopentane	11.53^{a}	3.50^{b}	4.37^{b}	4.15^{b}	3.65^{b}	12.04^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	2.309	0.023
Propyl-cyclohexane	7.86^{ab}	0.00^{c}	3.83^{bc}	3.81^{bc}	3.00^{bc}	7.98^{ab}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	11.31^{a}	0.00^{c}	2.408	0.005
Ketones														
2-hexanone	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	6.91^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	3.54^{ab}	1.429	0.034
Organic Acids														
Acetic acid, methyl ester[8]	3.39^{bc}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	6.13^{abc}	6.75^{ab}	11.05^{a}	3.68^{bc}	0.00^{c}	3.12^{bc}	2.158	0.015

[1]Least square means reported for the logn+1 concentration of volatile compounds; [2]Standard error of the mean; [3]Herbaceous, woody odor; sweet, green fruity flavor; [4]Powerful, sweet earthy odor; sweet, herbaceous taste; [5]Powerful onion, meat-like odor; pleasant meat and soup like flavor; [6]Harsh, green odor; unpleasant, pungent bitter flavor that can be fruit like at low levels; [7]Strong, fatty, harsh pungent odor; unpleasant fruity taste; [8]Pleasant fruity odor; slightly bitter flavor

Table 10. Least squares means of the percentages of fatty acids present in ground beef batches formulated with differing amounts of grass-fed lean.

Fatty acid	0% Grass[1]	33% Grass	67% Grass	100% Grass	SEM	*P*-value
Myristic, 14:0	3.01	2.90	2.89	2.76	0.063	0.12
Myristoleic, 14:1	0.82	0.83	0.83	0.59	0.106	0.37
Palmitic, 16:0	24.95	24.65	24.70	24.49	0.369	0.85
Palmitoleic, 16:1n-7	3.15	3.16	3.14	3.13	0.045	0.97
Stearic, 18:0	15.93[a]	15.63[a]	15.58[a]	14.82[b]	0.230	0.046
Oleic, 18:1n-9	40.63[b]	40.90[b]	41.50[ab]	42.51[a]	0.391	0.039
cis-Vaccenic, 18:1n-7	0.28	0.73	0.32	0.33	0.241	0.55
Linoleic, 18:2n-6	3.33	3.41	3.38	3.45	0.248	0.99
α-Linolenic, 18:3n-3	0.21	0.41	0.25	0.24	0.099	0.52
Arachidic, 20:0	0.31	0.30	0.35	0.13	0.165	0.79
Eicosaenoic, 20:1	0.29	0.24	0.26	0.24	0.022	0.38
Eicosadienoate, 20:2	0.05	0.05	0.05	0.04	0.004	0.40
Eicosatrienoic, 20:3	0.00	0.00	0.01	0.01	0.004	0.42
Arachidonic, 20:4	0.39	0.37	0.31	0.42	0.035	0.25
Eicosapentaenoic, 20:5	0.00	0.00	0.01	0.00	0.002	0.052
Behenoic, 22:0	0.01	0.00	0.01	0.00	0.005	0.21
Erucoic, 22:1	0.04	0.00	0.04	0.03	0.012	0.17
Lignoceric, 24:0	0.03	0.04	0.01	0.02	0.012	0.41
Nervonoic, 24:1	0.11[a]	0.08[b]	0.07[b]	0.08[b]	0.006	0.008
Docosahexaenoic, 22:6	0.01	0.00	0.02	0.01	0.004	0.07
Other	6.46	6.31	6.27	6.71	0.255	0.63
Total SFA[2]	44.26	43.52	43.54	42.22	0.710	0.31
Total MUFA[2]	45.30	45.94	46.16	46.91	0.350	0.07
Total PUFA[2]	3.98	4.23	4.03	4.17	0.328	0.94

[1]Percentage of grass-fed lean utilized in the ground beef batch

[2]Total SFA (saturated fatty acids), sum of all saturated fatty acids analyzed. Total MUFA (monounsaturated fatty acids), sum of all monounsaturated fatty acids analyzed. Total PUFA (polyunsaturated fatty acids), sum of all polyunsaturated fatty acids analyzed.

Table 11. The effect of lean source on the concentration[1] of volatile aroma compounds present in cooked ground beef patties.

	CH[2]	HRT[3]	NAT[4]	SE[5]	SEM[6]	*P*-value
1,4-dimethyl-2-ethyl-benzene	0.00[b]	1.72[ab]	2.07[a]	0.00[b]	0.670	0.046
2,3,3-trimethyl-pentane	1.81[b]	0.65[b]	5.76[a]	3.13[ab]	1.152	0.014
4-octene	0.00[b]	0.00[b]	2.16[a]	0.50[b]	0.572	0.024
Delta 3-carene	3.45[b]	6.83[ab]	8.02[a]	3.73[b]	1.289	0.0278
Dimethyl disulfide	3.82[a]	3.36[a]	0.00[b]	2.00[ab]	1.039	0.043
(E)-2-heptenal	0.00[b]	1.69[ab]	3.05[a]	0.00[b]	0.802	0.02

[1]Least squares means of logn+1 volatile aroma compound concentration
[2]Premium branded program (upper 2/3 choice)
[3]Labeled heart-healthy (from Wagyu cattle containing a high degree of marbling)
[4] All-natural program (meeting specifications for all natural in addition to consuming a grass-based diet)
[5]USDA Select (control group)
[6]Standard error of the mean

Table 12. The effect of fat percentage on the concentration[1] of volatile aroma compounds present in cooked ground beef patties.

	10%	20%	SEM[2]	*P*-value
1-Octen-3-ol	3.54	0.00	0.653	0.001
2-ethyl-1,4-dimethyl-benzene	0.29	1.61	0.467	0.048
Pentanal	7.66	2.81	1.098	0.003

[1]Least squares means of logn+1 volatile aroma compound concentration
[2]SEM

Table 13. Least squares means of the percentages of fatty acids present in ground beef batches formulated with differing amounts of grass-fed lean.

Fatty acid	CH[1]	HRT[2]	NAT[3]	SE[4]	SEM[5]	*P*-value
Myristic, 14:0	2.88^{ab}	2.69^{c}	2.82^{b}	2.93^{a}	0.035	<0.001
Myristoleic, 14:1	0.51	0.53	0.47	0.49	0.028	0.46
Palmitic, 16:0	24.84	24.65	24.50	24.60	0.097	0.095
cis-Vaccenic, 18:1n-7	0.79	0.47	0.74	0.69	0.112	0.19
Linoleic, 18:2n-6	3.23	3.37	3.54	3.30	0.090	0.083
Arachidic, 20:0	0.18^{b}	0.18^{b}	0.22^{a}	0.23^{a}	0.011	0.003
Eicosenoic, 20:1	0.19	0.17	0.19	0.19	0.010	0.44
Eicosadienoic, 20:2	0.04	0.08	0.03	0.04	0.016	0.21
Arachidonic, 20:4	0.31^{c}	0.43^{a}	0.41^{ab}	0.34^{bc}	0.030	0.018
Eicosapentaenoic, 20:5	0.00	0.01	0.00	0.00	0.003	0.37
Behenic, 22:0	0.03	0.03	0.02	0.03	0.004	0.17
Erucic, 22:1	0.03^{a}	0.00^{b}	0.00^{b}	0.03^{a}	0.004	<0.001
Lignoceric, 24:0	0.04^{b}	0.07^{a}	0.05^{ab}	0.04^{b}	0.006	0.002
Docosapentaenoic, 22:6	0.03	0.45	0.02	0.03	0.211	0.38
Other	6.22	6.56	6.16	6.08	0.286	0.65
Total PUFA[6]	3.87	4.55	4.22	3.98	0.249	0.22

[1]Premium branded program (upper 2/3 choice)
[2]Labeled heart-healthy (from Wagyu cattle containing a high degree of marbling)
[3]All-natural program (meeting specifications for all natural in addition to consuming a grass-based diet)
[4]USDA Select (control group)
[5]Standard error of the mean
[6]Total PUFA (polyunsaturated fatty acids), sum of all polyunsaturated acids.

Table 14. Least squares means of the percentages of fatty acids present in ground beef batches formulated with differing amounts of grass-fed lean.

Fatty acid	10% Fat	20% Fat	SEM[1]	*P*-value
Myristic, 14:0	2.77	2.89	0.024	0.002
Myristoleic, 14:1	0.54	0.46	0.019	0.005
Palmitic, 16:0	24.54	24.75	0.068	0.031
cis-Vaccenic, 18:1n-7	0.80	0.55	0.079	0.022
Linoleic, 18:2n-6	3.63	3.08	0.063	<0.001
Arachidic, 20:0	0.20	0.21	0.008	0.30
Eicosenoic, 20:1	0.19	0.18	0.007	0.42
Eicosadienoic, 20:2	0.06	0.03	0.011	0.091
Arachidonic, 20:4	0.48	0.27	0.021	<0.001
Eicosapentaenoic, 20:5	0.00	0.00	0.003	0.31
Behenic, 22:0	0.02	0.03	0.003	<0.001
Erucic, 22:1	0.02	0.01	0.003	0.18
Lignoceric, 24:0	0.07	0.04	0.004	<0.001
Docosapentaenoic, 22:6	0.23	0.03	0.150	0.35
Other	6.47	6.04	0.202	0.14
Total PUFA[2]	4.64	3.67	0.175	0.001

[1]Standard error of the mean

[2]Total PUFA (polyunsaturated fatty acids), sum of all polyunsaturated acids.

Table 15. Least squares means of the percentage of fatty acids present in ground beef patties formulated with different lean sources and different fat levels.

	10% Fat				20% Fat					
	CH[1]	HRT[2]	NAT[3]	SE[4]	CH[1]	HRT[2]	NAT[3]	SE[4]	SEM[5]	*P*-value
Palmitoic,16:1	2.30^{a}	2.38^{a}	2.06^{b}	1.69^{de}	1.85^{cd}	1.96^{bc}	1.72^{de}	1.57^{e}	0.063	0.036
Stearic, 18:0	20.36^{d}	18.20^{e}	20.45^{d}	23.55^{b}	23.55^{b}	22.08^{c}	23.50^{b}	24.96^{a}	0.359	0.005
Oleic, 18:1n-9	37.20^{bc}	39.49^{a}	37.56^{b}	34.30^{e}	35.45^{de}	36.83^{bcd}	35.86^{cde}	35.21^{de}	0.640	0.026
α-Linoleic, 18:3	0.25^{bc}	0.20^{e}	0.22^{de}	0.27^{a}	0.27^{a}	0.23^{cd}	0.22^{d}	0.27^{ab}	0.008	0.039
Nervonic, 24:1	0.09^{a}	0.05^{b}	0.04^{b}	0.09^{a}	0.05^{b}	0.04^{b}	0.04^{b}	0.05^{b}	0.008	0.038
Total SFA[6]	48.27^{d}	45.66^{e}	47.89^{d}	51.36^{b}	51.57^{b}	50.00^{c}	51.35^{b}	52.88^{a}	0.398	0.004
Total MUFA[6]	41.31^{b}	43.30^{a}	41.19^{b}	37.66^{d}	38.67^{cd}	39.80^{bc}	38.89^{cd}	38.06^{d}	0.601	0.007

[1]Premium branded program (upper 2/3 choice)

[2]Labeled heart-healthy (from Wagyu cattle containing a high degree of marbling)

[3]All-natural program (meeting specifications for all natural in addition to consuming a grass-based diet)

[4]USDA Select (control group)

[5]Standard error of the mean

[6]Total SFA (saturated fatty acids), sum all saturated fatty acids. Total MUFA (monounsaturated fatty acids), sum of all unsaturated fatty acids.