Post-harvest influences on beef flavor development and tenderness

by

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A Dissertation

In

Animal Science

Submitted to the Graduate Faculty
of Texas Tech University in
Partial Fulfillment of
the Requirements for
the Degree of

DOCTOR OF PHILOSOPHY

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May 2020

ACKNOWLEDGMENTS

My three years at Texas Tech have been a time of change and growth with many ups and downs. It is hard to believe that it is already coming to an end. There are so many people I would like to thank for their help during this time. First and foremost, I have to thank my major advisor, Dr. Jerrad Legako. You have truly taught me so much and helped shape me into an independent researcher. Thank you for showing and teaching me different laboratory techniques, challenging me to critically evaluate my own and other research, and teaching me to approach writing and research from multiple different angles. You have also taught me what it means to be a mentor and a leader by example with your three rules to success. I will be forever grateful for your guidance and leadership.

I would also like to thank my committee members: Dr. Brooks, Dr. Woerner, Dr. Miller, and Dr. Wheeler; for all of their assistance during my Ph.D. program. Thank you for answering all of my questions throughout research and interpretation, as well as your support, guidance, and encouragement during my program. I sincerely appreciate your time to serve on my committee.

I have had the opportunity to work with some amazing individuals during my tenure at Texas Tech. Thank you to all of my fellow graduate students who have helped with product collections, consumer panels, trained panels, and who served as trained panelists through the adventures that are shelf-life service projects. A special thank you to: Savannah Forgey, Taylor Horton, April Englishbey, Andrea Krieg, Loni Lucherk, Meghan Clancy, Sam Barker, and Hope Hall for all of their help, support, and encouragement. Additionally, thank you so much to the Legako lab undergrads, Colton

Smith, Nathan Johnson, and Jacie Henefey, for all of their help. I would probably still be weighing samples for Westerns if it wasn't for them.

Finally, I would like to thank my parents, Steven and Dawn, and my grandparents, Dennis and Joan, for all their love and support during this time. I'm so glad they went the extra mile to get me involved with agriculture through 4-H and FFA to drive this crazy dream of mine. They have never questioned my ability to truly succeed and are always there to catch me or light a fire under me if need be. Thank you for being the best substitute shepherds for the Rose Hill flock and continuing our vision of success while I'm completing my education.

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ABSTRACT

The objective of these studies was to determine: the influence of dry-heat cookery method on beef flavor development in two USDA quality grades following sous vide preparation; the influence of dry heat cookery on beef flavor development of multiple beef muscles; the influence of package and muscle type on postmortem proteolysis and subsequent release of flavor contributing free-amino acids during storage and distribution.

In study 1, there were no cooking method × quality grade interactions ($P \geq 0.076$) for all consumer traits evaluated. Overall, salamander cooked (SALA) steaks were preferred ($P < 0.05$) by consumers over clamshell (CLAM) steaks for all palatability traits. Oven steaks had greater liking scores ($P < 0.05$) than CLAM steaks for juiciness, tenderness, and overall liking but were similar to CLAM steaks ($P > 0.05$) for flavor. Charbroiler (CHAR) steaks were similar ($P > 0.05$) to CLAM steaks for flavor but were preferred ($P < 0.05$) for tenderness, juiciness, and overall liking. Steaks cooked using the OVEN method produced a greater concentration of lipid derived volatiles, such as alcohols, aldehydes, and carboxylic acids. In direct contrast, CHAR steaks produced a higher concentration of pyrazines and Strecker aldehydes, which are derived from the Maillard reaction.

In study 2, no interactions were observed between cooking method and muscle ($P \geq 0.344$) for any palatability traits evaluated. Consumers preferred CHAR steaks ($P < 0.05$) to CLAM steaks for flavor, tenderness, juiciness, and overall liking. Additionally, CLAM steaks were rated lower ($P < 0.05$) than all other methods for tenderness and juiciness. Oven (OVEN) and SALA steaks were rated higher ($P < 0.05$) than CLAM steaks by consumers for tenderness and juiciness but were similar ($P > 0.05$) to CLAM

steaks for overall liking. Charbroiler steaks produced a greater concentration of Maillard compounds, including Strecker aldehydes, pyrazines, and sulfur-containing compounds compared to the other cooking methods. Steaks cooked using OVEN and SALA ($P < 0.05$) produced more lipid oxidation products, including carboxylic acids and esters. Additionally, CHAR steaks produced the greatest ($P < 0.05$) total volatiles compared to all other treatments, which may be a result of the combination of Maillard reaction products and the lipid degradation products.

In study 3, high oxygen (HIOX) steaks exhibited ($P < 0.05$) the highest Warner-Bratzler shear force values, lowest desmin degradation rate ($P < 0.05$), and the highest ratings for fishy, bitter, sour, and oxidized flavors, the lowest overall tenderness scores ($P < 0.05$), and, in general, produced the lowest amount of free amino acids ($P < 0.05$) compared to all other treatments. Contrastingly, rollstock (ROLL) packaging produced the highest ratings for beef flavor identity, brown/roasted, bloody/serumy, and umami flavors ($P < 0.05$). Additionally, ROLL packaging exhibited ($P < 0.05$) greater desmin degradation in comparison to HIOX steaks.

Beef flavor development and tenderness are readily impacted by dry heat cookery method and packaging types. Sous vide cooking could minimize the effects between USDA quality grades. Steaks should not be cooked using a clamshell grill or packaged in a high oxygen environment to provide the optimum combination of flavor and tenderness.

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CHAPTER 1
REVIEW OF LITERATURE

Palatability

In relation to meat products, palatability is defined as the overall eating experience and includes flavor, tenderness, and juiciness (Smith and Carpenter, 1974; Platter et al., 2003; O'Quinn et al., 2018). When estimating the contributions of each of these three major tenants to the consumer eating experience, O'Quinn et al. (2018) reported that flavor contributed 49.4%, tenderness contributed 43.4%, and juiciness contributed 7.4%. Palatability is an important driver for consumer purchasing decisions. Without a consistent, high quality product, consumers are less likely to continue to purchase beef (Claborn et al., 2011; Wilfong et al., 2016). Flavor has been well established as a main contributor to beef palatability. Recent studies on beef quality factors have indicated an increased focus on flavor from the consumer (Corbin et al., 2014; Lucherk et al., 2016; Wilfong et al., 2016; Nyquist et al., 2018; Vierck et al., 2018). During consumer evaluation, these studies indicated that flavor was the most important palatability trait when consuming beef with over 50 percent of the respondents selecting flavor. This has drastically increased from approximately 30 percent in older studies (Huffman et al., 1996). Additionally, flavor has been strongly correlated ($r = 0.88$, 0.85) to consumer overall liking (O'Quinn et al., 2012; Legako et al., 2015a). Beef flavor can be evaluated in several different ways, including trained panels, consumer panels, and volatile flavor analysis. The gold standard for flavor analysis is with human subjects in a panel environment. However, to understand the true mechanisms behind flavor development and formation, volatile compound analysis via gas chromatography-mass spectrometry is used. Volatile flavor compounds contribute substantially to flavor

through the aroma fraction (Mottram, 1998; Legako et al., 2015a). These compounds are formed through various pathways of flavor development, including the Maillard reaction and thermal lipid degradation (Mottram, 1993, 1998).

Flavor perception

Flavor is a complex sensory trait, as it is the combination of odors, aromas, and basic tastes, and how those factors react with the human olfactory, somatosensory, and gustatory systems (Frank and Byram, 1988; Smith and Margolskee, 2001; Small and Prescott, 2005; Viana, 2010). Additionally, the tangible sensation of food as it is being consumed, often termed texture or mouthfeel, can readily impact the perception of flavors (Smith and Margolskee, 2001; Small and Prescott, 2005). Furthermore, when describing flavor to consumers, it often is described as taste, despite flavor and taste being two separate components to the sensory experience (Prescott, 1999).

The first part of flavor can be described as the five basic tastes: sweet, salty, sour, bitter, and umami. Each one of these tastes are detected on the tongue via taste receptors. It was once believed that each taste had a certain region of the tongue where it was best detected, such as bitter on the back of the tongue and salty on the sides of the tongue (Jung et al., 2003). However, more current research has indicated that these receptors are spread across the entirety of the tongue, through taste buds, which are bulb-like receptors which contain multiple types of detectors (umami, salty, sweet, sour, and bitter) within one receptor (Smith and Margolskee, 2001; Jung et al., 2003). Of the four types of papillae on the tongue, only three have the ability to detect tastes, as they contain the necessary taste buds and receptors: fungiform, foliate, and circumvallate (Jung et al., 2003). The fungiform papillae are the most numerous and smallest of the three gustatory

papillae, as they cover the greatest amount of surface area on the tongue, particularly the oral part of the tongue toward the front of the mouth (Jung et al., 2003). The circumvallate and foliate papillate are located on the back of the tongue in the pharyngeal area, near the back of the throat (Jung et al., 2003). These types of papillae are much larger and contain more receptors than the fungiform papillae (Jung et al., 2003). As food products are being masticated, small molecules called tastants are released and enter the taste pores at the top of the receptor. These tastants interact with the microvilli of the taste receptor, signaling electrochemical changes to stimulate signals to the brain (Viana, 2010).

These signals, along with those perpetuated by odors and aromas in the olfactory pathways, are interpreted as flavors by the brain (Smith and Margolskee, 2001). The brain uses multiple neural areas, including the anterior insula, frontal operculum, orbitofrontal cortex, and anterior cingulate cortex and their interactions to interpret and detect flavors once sensed by the trigeminal system (Small and Prescott, 2005). To make flavor more complex, not only are multiple areas of the brain being used to detect and analyze flavors, multimodal neurons are attempting to concurrently decode signals from multiple types of sensory attributes: odors, aromas, and basic tastes, then unite those attributes to give the full flavor experience (Small and Prescott, 2005). Studies evaluating the brain's response to eating meat are few in number. However, when people are fed high quality steaks, such as USDA Prime, greater connectivity with the lateral orbitofrontal cortex, insular cortex, and striatum is observed through functional magnetic resonance imaging (Tapp et al., 2017). Greater connectivity through these particular areas indicate increased positive hedonic experiences and are linked to increased positive

reward stimuli (Tapp et al., 2017). Similarly, greater functional connectivity in the insula and orbitofrontal cortex was observed with panelists consuming grain-fed beef compared to chicken and grass-fed beef (Beyer et al., 2019).

These odors, aromas, and tastes are detected via the trigeminal somatosensory system (Viana, 2010). The trigeminal nerves have endings in the mucous membranes of the oral and nasal cavities, as well as the facial skin, cornea, and conjunctiva of the eyes (Viana, 2010). These nerves are responsible for reactions to foods, especially the chemesthesis reaction of spicy foods, which is the spicy pungency or irritation of food that can cause eyes to water and sneezing (Viana, 2010). These tastes can be enhanced or detracted with certain odors (Frank and Byram, 1988). Tastes and odors that are associated with one another (ex. strawberry and sucrose) are able to enhance one another in comparison to ones not typically associated together (ex. lemon and salt) (Frank and Byram, 1988; Small and Prescott, 2005).

In addition to the complexities observed with the perception of flavor from a sensory standpoint, flavor can also be readily affected by psychological impacts, such as memory, attention, and feelings (Small and Prescott, 2005; Viana, 2010). Additionally, taste-odor integration, as compared to audio-visual integration, occurs much earlier in development and therefore is more likely to be affected by memories, experience, and emotional factors (Prescott, 1999; Small and Prescott, 2005). This initial integration is further supported by the almost unanimous sensory impact of taste perception, oral sensations, and olfaction at one time when consuming food products, as it is hard for humans to separate fractions unless trained to do so (Prescott, 1999; Small and Prescott, 2005).

Mechanisms of flavor development

Flavor is elucidated from meat products via three major pathways: the Maillard reaction, thermal degradation, and lipid oxidation. Each one of these pathways contribute unique flavor compounds to meat flavor. The Maillard reaction at its most basic form is a non-enzymatic browning reaction between a reducing sugar (carbonyl component) and an amino acid (amine component) at high temperatures (Mottram, 1993). The Maillard reaction can result in a large variety of different compounds, including furans, carbonyls, aldehydes, sulfur and nitrogen compounds, ketones, pyrroles, pyrazines, thiazoles, thiophenes, and furanthiols. The complete reaction was first described in 1953 by the Hodge Scheme and is comprised of three different stages: initial condensation and rearrangement to form a glycosylamine, dehydration, and finally, the conversion of these Maillard intermediates, which include reductones, dehydroreductones, carbonyl groups, and other compounds, into aroma compounds via heterocyclization and further reactions with amine groups and amino acids (Hodge, 1953; Mottram, 1993). Maillard intermediates, specifically reductones and dehydroreductones, can interact with various compounds through three major avenues: Strecker degradation, retro-aldolization, and interactions with hydrogen sulfide (H_2S) and ammonia (NH_3). For Strecker degradation and retro-aldolization, these compounds can continue to interact and undergo heterocyclization (Hodge, 1953; Mottram, 1993, 1998).

Strecker degradation is the degradation of α-amino acids that is initiated with carbonyl compounds to form Strecker aldehydes, α –aminoketones, methional, and free NH_3 and H_2S from cysteine (Hodge, 1953; Mottram, 1993, 1998). During Strecker degradation, these amino acids are decarboxylated and deaminated, which results in two

major compounds: Strecker aldehydes and α –aminoketones. α –aminoketones are important precursors for the formation of thiazoles and pyrazines (Mottram, 1993). The Strecker aldehydes produced are specific to the amino acids being degraded. Each α-amino acid, with the exception of proline and hydroxyproline, can be degraded into a certain Strecker aldehyde with unique flavors and aromas produced. For example, leucine is degraded into 3-methylbutanal, which is characteristic of malty, fruity, and toasted bread odors (Farmer et al., 1989; Mottram, 1993, 1998). Proline and hydroxyproline are unable to participate in this reaction because they do not contain an additional amino group in a pyrroline ring; instead, these amino acids produce nitrogen based heterocyclic compounds (Mottram, 1993). The sulfur groups removed from cysteine and methionine during Strecker degradation are used to react and form sulfur containing compounds, such as carbon disulfide. These compounds are important to meat flavor but have a low odor threshold, which indicates that at high concentrations, they may have negative impacts on flavor (Farmer et al., 1989; Mottram, 1993, 1998).

Lipid oxidation

Lipid oxidation is a causative reaction of many deterioration issues in meat quality (Love and Pearson, 1971; Min and Ahn, 2005; Bekhit et al., 2013). Meat products that are higher in unsaturated fatty acids, such as poultry and fish, are more susceptible to lipid oxidation due to one or more double bonds present, which makes it more labile to oxidation (Bekhit et al., 2013). Additionally, phospholipids, which make up the cell membrane, are especially susceptible to lipid oxidation (Min and Ahn, 2005; Bekhit et al., 2013). Lower USDA quality grades, such as Select and Standard possess a greater proportion of the polar lipid fraction, and therefore are more sensitive to lipid oxidation

(Wood et al., 2008; Legako et al., 2015b). The lipid oxidation reaction can occur during a different segments of the meat processing chain, including type of product, processing, freezing, storage, retail display, and cooking, as well as being intrinsically pro-oxidative, which makes it extremely hard to mitigate all factors initiating the reaction (Bekhit et al., 2013).

Lipid oxidation occurs primarily in three stages: initiation, propagation, and termination as a free radical chain reaction (Min and Ahn, 2005; Bekhit et al., 2013). Free radicals in meat systems include reactive oxygen species (ROS), super oxide anion radicals (O_2), hydroperoxyl radicals (HO_2), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), iron-oxygen complexes, and free iron (Min and Ahn, 2005; Bekhit et al., 2013). Initiation begins when a free radical abstracts a hydrogen atom from the lipid chain, leaving unpaired electrons on the carbon chain. To stabilize the chain after the hydrogen abstraction, a molecular rearrangement to form a conjugated diene occurs (Min and Ahn, 2005). This process will continue within the lipid through propagation. Lipid peroxides can abstract hydrogens from adjacent or nearby lipid molecules or fatty acids to continue to form lipid hydroperoxides or conjugated dienes, which are considered primary products of lipid oxidation (Min and Ahn, 2005; Ross and Smith, 2006). Propagation will continue to wreak havoc on the lipid component of meat until there is no more substrate. Lipid peroxides will then begin to interact with each other or begin to instead self-destruct (termination), resulting in non-radical products (Min and Ahn, 2005).

Through this process, there are many secondary products that can be produced during propagation and termination, including carbonyls, alcohols, hydrocarbons, and furans which produce negative, rancid off-flavors in meat products (Shahidi and Pegg,

1994; Min and Ahn, 2005; Bekhit et al., 2013). Carbonyls, especially aldehydes, are produced in the greatest concentration during this reaction, possibly due to their ability to act as secondary messengers to disseminate initial free radical reactions (Min and Ahn, 2005). Additionally, aldehydes, such as hexanal, promote oxymyoglobin oxidation, metmyoglobin's pro-oxidant activity, and reduce metmyoglobin reducing activity (Shahidi and Pegg, 1994; Min and Ahn, 2005). It has been proposed by Lynch and Faustman (2000) that aldehydes induce this oxidation because it is present within all stages of oxidation and are escalated with the production of secondary products. Lynch and Faustman (2000) also indicate that of the aldehydes produced, hexanal is of the most consequence because it is one of the strongest volatiles produced during oxidation.

Protein oxidation

In addition to lipid oxidation, proteins can also be impacted by oxidation reactions. However, instead of the more generalized effect observed in lipid oxidation, protein oxidation has a more targeted approach, depending on the specific radical (Davies, 2005). Generally speaking, the most reactive species, such as hydroxyl radicals, are less selective, as the majority of the backbone and side chains will be oxidized to some extent. However, less reactive oxidants such as diatomic oxygen, has a very targeted approach and primarily impacts proteins with a greater electron count on side chains, such as proteins high in tryptophan, tyrosine, histidine, methionine, and cysteine (Davies, 2005). These reactive radicals are electron deficient and are most reactive with these particular amino acid side chains because of their greater electron count. Protein oxidation can occur in two major places of the protein: the peptide backbone and in the side chains of individual amino acids (Davies, 2005). In free amino acids, the primary

site of oxidation is the side chain, rather than the α-carbon (Davies, 2016). However, the method of attack by free radicals is highly dependent on the individual amino acid's side chain. In cases of aromatic amino acids, such as tyrosine or phenylalanine, an addition is made to the ring structure (Davies, 2005, 2016). In comparison, amino acids with sulfur attached, such as methionine or cysteine, hydrogen abstraction occurs and can give way to thiyl radicals to perpetuate and produce carbon radicals at both side chains and the α-carbon backbone (Davies, 2005, 2016).

In the protein backbone, little damage is observed with non-radical oxidants as the rate of reaction is slow (Davies, 2005). Additionally, due to the complex secondary structure, such as beta sheets and alpha helices, it makes more challenging for free radicals to interact with the protein backbone (Davies, 2005, 2016). Comparatively, radical species, such as hydroxyls, react primarily with the backbones of proteins through hydrogen atom abstraction at the alpha-carbon of proteins (Davies, 2005).

Postmortem aging: impact on palatability

Postmortem aging is one of the beef industry's most readily used applications to improve tenderness. Through several biochemical mechanisms, including the calpain and caspase system and the multi-catalytic proteinases complex, meat is intrinsically tenderized via protein degradation (Aberle et al., 2001; Lonergan et al., 2010). Aging begins through degradation of the z-disks via postmortem proteolysis, which leads to weakening and possible fragmentation of myofibrils (Huff-Lonergan et al., 1996; Lonergan et al., 2010). The weakening of the protein structure allows for reduction of Warner-Bratzler shear force values and increased tenderness ratings by both consumer and trained panelists, regardless of age or animal gender and has been well described in

the literature (Goll et al., 1964; Huff and Parrish Jr, 1993; Jeremiah and Gibson, 2003b; Lepper-Blilie et al., 2016). Following z-disk degradation, multiple proteins, including desmin, troponin-T, titin, and nebulin, are degraded and used to produce new polypeptides (Goll et al., 1983; Huff-Lonergan et al., 1995; Koohmaraie, 1996; Lonergan et al., 2010). In addition to the positive tenderness attributes observed during aging, aging also impacts flavor. When aging beef to 2, 9, 13, 23, or 30 d, Watanabe et al. (2015) observed substantial increases in volatile flavor compound production, especially in Maillard products, such as pyrazines and pyrroles, as well as lipid degradation products, such as aldehydes, alcohols, and ketones. Additionally, aging increases the availability of important flavor precursors, especially free amino acids, which are released from proteins during degradation (Ba et al., 2014). Free amino acids and small peptides are integral to the development of flavor though the Maillard reaction and are specific taste active compounds (Dashdorj et al., 2015). Amino acids and small peptides contribute sweet, sour, bitter, umami, and characteristic meat-like flavors to cooked meat products (Dashdorj et al., 2015). Previous literature has indicated an increase in free amino acids after aging in both the Longissimus lumborum and Biceps femoris (Yang et al., 2018, 2019)

One major mechanism of aging is the calpain system. Calpains are a large group of cysteine-based proteases (Goll et al., 1983; Koohmaraie, 1996; Kemp et al., 2010). The calpain system requires calcium to function and in skeletal muscle, contains three principal components: μ-calpain (calpain-1), m-calpain (calpain-2), and calpastatin, an endogenous inhibitor of calpains (Koohmaraie and Geesink, 2006; Kemp et al., 2010; Lonergan et al., 2010). Calpains degrade very specific targets, degrading both

myofibrillar and cytoskeletal proteins, including titin, nebulin, filamin, desmin, and troponin-T (Huff-Lonergan et al., 1996; Kemp et al., 2010; Lonergan et al., 2010). Calpains have no effect on the major myofibrillar proteins, myosin and actin; additionally, because of their specificity, calpains do not degrade proteins to individual amino acids (Lonergan et al., 2010). Calpains are also self-limiting in terms of degradation (Huff-Lonergan, 2014). Unlike plant-based enzymes, such as bromelin or papain, calpains are self-regulated, and therefore do not over tenderize the product to the point of being mushy or grainy (Huff-Lonergan, 2014). The calpain system can be inhibited through several types of environments and conditions, including beta-agonist usage, breed type, and genetic mutations such as callipyge. As the pH is reduced in postmortem conditions, the effectiveness of these two enzymes is reduced (Lonergan et al., 2010). In fact, an ultra-accelerated pH decline, such as that observed with pale, soft, and exudative pork, has been implicated as almost completely inhibitory to calpain degradation, which completely arrests the aging process (Lonergan et al., 2010). Additionally, oxidative environments, such as those found in irradiated steaks or high oxygen packaging, have been found to inhibit calpain activity, leading to substantial toughening of meat products (Rowe et al., 2004; Lindahl et al., 2010).

To analyze postmortem proteolysis, various proteins are used as biomarkers to observe degradation over a certain time period or with certain treatment conditions. Troponin, along with desmin, are used as protein markers for postmortem proteolysis due to their sensitivity to the aging process. Due to the location of troponin and desmin on the outer part of the myofibril, they are more easily degraded during protein turnover and are more likely to be degraded by calpains postmortem.

Desmin

Desmin is a type-III cytoskeletal protein that is located on the edges of the z-disk as a part of the intermediate filament (Paulin and Li, 2004; Hnia et al., 2015). It is only located in muscles with striation, such as skeletal and cardiac muscle and has a molecular mass of 53 kDA (Paulin and Li, 2004). The main function of desmin is to form a 3-dimensional scaffold to surround the myofibrillar z-disk to connect the contractile unit to the subsarcolemmal cytoskeleton, nuclei, and other important organelles (Paulin and Li, 2004; Hnia et al., 2015). In addition to serving as a scaffold, desmin also composes lengthwise connections between consecutive z-disks and the plasma membrane of the myofiber to aid in holding adjacent myofibrils in place (Paulin and Li, 2004; Hnia et al., 2015). Because of desmin's location on the periphery of the z-disk, it is particularly abundant at the neuromuscular junction and the myotendinous junction of skeletal muscle.

Desmin makes up approximately 0.35% of the entire skeletal muscle fraction and is encoded by a single gene (Paulin and Li, 2004). During myogenesis, desmin is one of the earliest muscle proteins to appear, being activated as early as d 7.5 of embryonic development in cardiac tissue and d 9 in skeletal muscle cells in mice (Paulin and Li, 2004). As muscle structure begins formation in myoblasts and satellite cells, desmin is present at a low concentration and increases in concentration in differentiated myotubes (Paulin and Li, 2004; Hnia et al., 2015). As such, desmin is used as a myogenic marker for somites, as desmin expression prefaces myogenic transcription factors such as MyoD, myogenin, and MRF-4. Only Myf-5 is expressed prior to desmin, which proposes desmin may play a role in myogenic commitment and differentiation (Hnia et al., 2015). It is

thought that because of desmin's scaffolding nature that it may be directly involved with the process of myoblast fusion, as recent research has indicated that fusion can only occur between desmin-positive cells (Hnia et al., 2015).

Desmin and troponin-T have been used as a marker for postmortem proteolysis in meat products because of their proximity to the z-disk (Huff-Lonergan et al., 1996; Koohmaraie, 1996; Melody et al., 2004; Lonergan et al., 2010; Maoa et al., 2012; Phelps et al., 2015). However, literature describing the correlation values or relationships between desmin degradation and tenderness are limited. Rhee et al. (2004) evaluated desmin degradation across 11 different muscles and found desmin degradation to be significantly correlated (r = -0.13) to overall tenderness ratings by trained panelists. However, this effect is varied across muscles, with the Longissimus possessing the strongest relationship (r = 0.56) to overall tenderness and (r = -0.62) Warner-Bratzler shear force (Rhee et al., 2004). In comparison, the Psoas major does not possess a significant relationship with desmin degradation, as its tenderness rating (r = 0.09) and Warner-Bratzler shear force (r = 0.14) are weakly related to desmin degradation.

Troponin

Troponin is a regulatory protein found in the myofibril. It makes up approximately 5 percent of the myofibrillar protein. Troponin's main function as a regulatory protein is to regulate muscle contraction, as it acts like a ratchet to protect the actin active sites (Filatov et al., 1999). Without troponin, there would be no reversible binding of calcium during muscle contraction. To achieve that goal, troponin is present in the grooves of the actin filament (Filatov et al., 1999). It is a calcium-ion dependent protein that requires calcium to function. Troponin is made up of an alpha-helix

surrounded by two globular domains (Filatov et al., 1999). Troponin has a molecular weight of 69,000 Daltons and has three subunits: T, I, and C.

Each one of these subunits have individual functions: C is responsible for binding calcium; I inhibits ATPase activity of actomyosin; and T allows tropomyosin to bind to troponin (Filatov et al., 1999). Troponin-C (**TnC**) binds calcium through conformational changes of the N-terminal domain, which are induced in the presence of calcium (Filatov et al., 1999). Once TnC has bound calcium, troponin-I (**TnI**) can inhibit actomyosin ATPase activity, allowing for more improved contact with tropomyosin for muscle contraction (Filatov et al., 1999). Troponin-I has three separate isoforms: two for skeletal muscle (one for fast and one for slow contraction speed) and one for cardiac tissue (Filatov et al., 1999). Furthermore, troponin-T (**TnT**) interacts with tropomyosin and is the true regulatory subunit of troponin (Filatov et al., 1999). Like TnI's multiple isoforms, TnT has approximately 128 isoforms, depending on the type of tissue it resides in and the species. Troponin-T has three binding sites for tropomyosin: the N-terminus, one within amino acid residues 156-227, and the third site is present close to cysteine-190 and is located closely to the C-terminus (Filatov et al., 1999). These binding sites allow for TnT to be connected to the actin-tropomyosin filament during contraction and also regulates actomyosin ATPase activity through binding with both TnC and TnI (Filatov et al., 1999).

Cooking method

Cooking method is one of the primary factors that consumers have control over in producing a highly palatable beef product for consumption. Cooking method has primarily been investigated as a factor in tenderness (Moody et al., 1978; Berry, 1993;

Dugan and Aalhus, 1998; Wheeler et al., 1998; Powell et al., 2000; Lawrence et al., 2001; Boles and Swan, 2002; Herring and Rogers, 2003b; Jeremiah and Gibson, 2003a; Obuz et al., 2003; McKenna et al., 2004; Yancey et al., 2011; Bowers et al., 2012; Yancey et al., 2016; Fabre et al., 2018). However, flavor and aroma in meat products is produced principally through cooking (Mottram, 1998). Consumers will use a wide variety of cooking methods to cook their meat to provide the optimum combination of tenderness, juiciness, and flavor (Savell et al., 1999; Bagley et al., 2010). Generally speaking, cookery methods fall into one of two categories: dry or moist heat. Dry heat cookery methods are those that use direct application of high temperature heat, whether through application of hot air (convection), a hot pan (conduction), or a radiant heat (such as a flame) (Bagg, 2003). Moist heat cookery instead uses liquid as a vector for heat at a substantially lower temperature, resulting in improved tenderness through breakdown of connective tissue via gelatinization (Bagg, 2003). Dry heat cookery methods, such as grilling, broiling, and pan-frying are more popular than moist heat cookery, such as braising or stewing (Savell et al., 1999). To date, much work regarding cookery methods have been focused on tenderness and have not evaluated the difference between dry heat cookery methods for flavor development (Berry, 1993; Savell et al., 1999; Powell et al., 2000; Lawrence et al., 2001; Obuz et al., 2003). However, flavor and aroma in meat products is produced principally through cooking (Mottram, 1998).

Cooking and therefore flavor development, is impacted by heat transfer rate, which can be impacted by product composition and muscle type. Differences in quality grades are attributed to differences in intramuscular fat, which can influence the way steaks conduct heat and therefore impact flavor development (O'Quinn et al., 2012;

Legako et al., 2015a). In addition to quality grade, muscle type has a direct impact on palatability ratings from consumers, which may be in part due to differing fiber types, fiber direction, or a combination of those factors (Hunt et al., 2014b; Legako et al., 2015a). Muscles from locomotive parts of the carcass, such as the round or chuck typically perform better with consumers with a moist form of cookery, in which collagen is allowed to gelatinize and improve tenderness (Jeremiah and Gibson, 2003a). Supportive muscles, such as the Longissimus or the Psoas major, are able to produce high quality eating experience via moist or dry heat cookery method (Yancey et al., 2011). Because of the major focus on tenderness, no research has been conducted on volatile compound analysis of different cookery methods.

Packaging method

Packaging method of meat products is an important factor in the meat industry, as it serves to protect product, improve shelf life and quality, as well as factors into the consumer's purchasing decision (McMillin, 2017; Polkinghorne et al., 2018). Different packaging types can result in different eating experiences. Multiple consumer studies in both the United States and Australia have consistently shown MAP packaging to be lower than both polyvinyl overwrap and vacuum packaging for tenderness, juiciness, flavor liking and overall liking when fed to consumers (Polkinghorne et al., 2019; Ponce et al., 2019). Additionally, high oxygen modified atmosphere packaging (**MAP**) has been implicated with increased toughness when compared to vacuum packaged or polyvinyl chloride overwrapped steak (Geesink et al., 2015; Moczkowska et al., 2017). The mechanism of the toughness observed has not been fully understood. Conflicting results have been observed, as Geesink et al. (2015) did not observe any differences in desmin

degradation in high oxygen MAP, however, Moczkowska et al. (2017) and Fu et al. (2017) both observed increased desmin degradation in vacuum packaging in comparison to high oxygen MAP. Increased desmin degradation would indicate a greater amount of postmortem proteolysis occurring, therefore resulting in a more tender product. As previously discussed, if the oxidative environment is inhibiting the calpain system, it would inhibit postmortem proteolysis, resulting in a much tougher product. From a flavor standpoint, high oxygen MAP has been shown to produce lower beef flavor identity, umami, and tenderness ratings, as well as increased oxidized, cardboardy, and sour flavors when analyzed by trained descriptive panels (Ponce et al., 2019). This is likely due to induced lipid oxidation from the high oxygen MAP's oxidative environment, which contributes greatly to production of off flavors (Min and Ahn, 2005; Bekhit et al., 2013).

Previous research indicates that packaging type has an impact on aging and flavor development of meat products. From an aging standpoint, Fu et al. (2017) reported increased desmin degradation in vacuum packaging in comparison to both polyvinyl overwrap and MAP. Similarly, Moczkowska et al. (2017) reported vacuum packaging had increased degradation of both desmin and troponin-T in both the Longissimus lumborum and Biceps femoris in comparison to MAP steaks. Moreover, Moczkowska et al. (2017) reported reduced Warner-Bratzler shear force values in vacuum packaged steaks for both muscles, which indicates an increased rate of proteolysis occurred in vacuum packages rather than MAP steaks.

Additionally, MAP and polyvinyl overwrap produced a greater amount of carbonyl products from oxidation in both the Psoas major and Semimembranosus in

comparison to vacuum packaging (Fu et al., 2017). Packaging type has a direct impact on cooked steak volatile flavor production, where high oxygen modified atmosphere packaging and polyvinyl overwrap produced a greater amount of lipid derived compounds from lipid oxidation (Ponce, 2018). In contrast, rollstock packaging produced greater amounts of dimethyl sulfide, which contributes to the "meaty aroma" possessed by meat products when cooked (Ponce, 2018). These volatile flavor compound differences translated to influence consumer ratings of beef flavor (Ponce et al., 2019). Steaks packaged in rollstock packages were rated higher for flavor liking than steaks in polyvinyl overwrap and high oxygen modified atmosphere packaging (Ponce et al., 2019). These results indicate that packaging scheme, in addition to postmortem aging, directly impacts flavor compounds produced during cooking. However, there is no previous research that has evaluated the impact of packaging type on amino acids produced from postmortem aging.

Quality grade

According to the USDA Agricultural Marketing Service, the USDA quality grade has been the U.S. industry standard of palatability since 1926 (Aberle et al., 2001). For similar maturity levels, different degrees of marbling indicate a different quality grade (Aberle et al., 2001). As marbling levels increase, a concurrent increase in proximate fat percentage is observed. As the lowest quality grade for A maturity cattle, Standard possesses the lowest percentage of fat (1.3-2.5%), followed by Select (2.5-4.5%), Low Choice (4.5-5.8%), Top Choice (the upper 2/3rds of the Choice grade) (6.0-9.0%), and Prime, which possesses the highest percentage of fat (10.4-14.8%) (Gilpin et al., 1965; Parrish et al., 1973; Savell et al., 1986; Luchak et al., 1998; Dow et al., 2011; Smith et al.,

2011; O'Quinn et al., 2012; Emerson et al., 2013; Hunt et al., 2014b; Legako et al., 2015a; Lucherk et al., 2016). Because of the large variation in fat percentage and its influence on eating quality, this results in a wide range of palatability.

Marbling, and therefore quality grade, has been well established as a driver and indication of the palatability of beef (Smith et al., 1985; Luchak et al., 1998; O'Quinn et al., 2012; Corbin et al., 2014; Lucherk et al., 2016; Drey et al., 2018; Nyquist et al., 2018). From a tenderness and juiciness standpoint, marbling can improve palatability through multiple theories: lubrication, bite, and strain theory. First, the lubrication theory works through marbling present in and around the muscle fibers and perimysial connective tissue layer is released through mastication and therefore resulting in a more tender and juicier product (Smith and Carpenter, 1974; Savell and Cross, 1988). The bite theory suggests that intramuscular fat lessens the effort needed to bite through cooked meat, as fat is less dense than denatured protein (Smith and Carpenter, 1974; Savell and Cross, 1988). As a result, increased quality grade has resulted in reduced Warner-Bratzler shear force values (WBSF) and increased tenderness ratings (Ueda et al., 2007; Dubost et al., 2013; Wilfong et al., 2016). Similarly, the strain theory proposes that as marbling is deposited within both the perimysium and endomysium, the added tension from the adipocytes reduces the strength through splintering of the connective tissue within beef, resulting in a more tender product (Smith and Carpenter, 1974; Savell and Cross, 1988). Histologically speaking, the strain theory has been confirmed in very high marbled Japanese Black cattle, which was reflected as more tender by trained panelists (Nishimura et al., 1999).

Previously, marbling and flavor have had a complicated relationship. Marbling has a significant impact on flavor, but it is not fully clear how it is impacted. Marbling is responsible in part for the species-specific flavors within a meat product, especially within red meat species (Smith and Carpenter, 1974; Savell and Cross, 1988). However, increased marbling levels have not been repeatedly correlated with an increase in volatile flavor compounds (Cross et al., 1980; Mottram et al., 1982; Mottram and Edwards, 1983; Mottram, 1998; Legako et al., 2015a). Furthermore, in the Longissimus muscle, consumer flavor liking scores have been moderately correlated (r = 0.25, 0.37, 0.27) with intramuscular fat percentage (O'Quinn et al., 2012; Hunt et al., 2014b; Legako et al., 2015a). More recent research with different sampling methodology, however, has indicated an increase in certain volatile compounds of steaks with higher quality grades (Gardner and Legako, 2018). Additionally, with a trained sensory panel, strong correlations (r = 0.84) have been observed between increased marbling scores and buttery/beef fat flavor (Emerson et al., 2013).

Muscle type

Meat quality and palatability is inherently related to muscle type and function. Within an animal, skeletal muscles are placed into one of two distinct functional categories: support and locomotion (Lee et al., 2010). These two functional roles result in distinctly different muscle fiber type profiles (Kirchofer et al., 2002; Joo et al., 2013). Muscle fiber type is responsible for contractile and metabolic properties of muscle, in addition to fiber cross-sectional area, glycogen and lipid content, and color via myoglobin content (Schiaffino et al., 1989; Kirchofer et al., 2002; Lee et al., 2010). These distinct changes between muscle can result in various flavor changes, due to fatty acid

composition, protein concentration, and oxidative stability of the muscle (Hunt et al., 2014b; Legako et al., 2015; Ponce, 2018).

Connective tissue is one of the major factors implicated in meat tenderness and is highly variable depending on muscle type (Whipple et al., 1990; Purslow, 2005; Purslow, 2014). Connective tissue is a supportive mechanism for the muscle structure and can have major impact on meat texture (Purslow, 2005). Muscles used for locomotion, especially those present in the chuck and round, have a high amount of connective tissue and supportive muscles, such as the Longissimus or the Psoas major, have much less due to their function as supportive muscles. Those locomotive muscles require moist cookery methods to break down the collagen present in the perimysium, endomysium, and epimysium through a process called gelatinization (Aberle et al., 2001). Elastin, unlike collagen, cannot be broken down through gelatinization, and therefore contributes to background toughness. Moreover, connective tissue is further developed with age. Older animals, due to greater muscle use, will develop greater crosslinking of collagen with age and therefore be tougher (Purslow, 2005; Purslow, 2014).

Rhee et al. (2004) conducted an evaluation of the variation in tenderness traits among eleven muscles within a beef carcass, including the Longissimus dorsi (LD), Psoas major (PM), Biceps femoris (BF), Triceps brachii (TB), and Infraspinatus (IF). Starting with the PM within this group of muscles, this muscle had the longest sarcomere length (> 2.9 μm), and lower collagen content and connective tissue amount during trained sensory panels, but the PM has the lowest percentage of desmin degradation. Similarly, another tender muscle, the IF, has a long sarcomere length (> 2.1 μm), greater connective tissue content than the PM, and an increased percentage of desmin

degradation compared to the PM. Following the IF, the TB has longer sarcomeres than the IF, but shorter than the PM, with a greater percentage of desmin degradation than both the IF and PM, as well as a greater amount of connective tissue according to trained panelists. The BF and the LD in this study were similar, with the shortest sarcomere lengths (1.8 μm) and the greatest percentages of desmin degradation (60.7%). However, the LD possessed substantially less connective tissue compared to the BF and was greater than both the TB and the IF. More tender muscles, such as the PM, possess longer sarcomere lengths, less connective tissue, and lower percentage of desmin degradation. Within the same study, correlation coefficients were used to measure the contribution of connective tissue, sarcomere length and desmin degradation to overall tenderness ratings. All three traits have strong correlations with tenderness. However, it is evident that various muscles are impacted differently by these traits.

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CHAPTER 2

EFFECTS OF DRY HEAT COOKERY METHOD ON BEEF STRIP LOIN STEAKS OF TWO QUALITY GRADES FOLLOWING SOUS VIDE PREPARATION

ABSTRACT

The objective of this study was to determine the influence of dry-heat cookery method on beef flavor development in two USDA quality grades following sous vide preparation. Beef strip loins were selected from two USDA quality grades: upper 2/3rds of Choice (Modest^{00}-Moderate^{100}) and Select (Slight^{00}-Slight^{100} marbling, n = 20/grade). Following 21 d of wet aging, strip loins were fabricated into 2.54 cm steaks and randomly assigned to one of four dry heat cookery methods: charbroiler grill (**CHAR**), clamshell grill (**CLAM**), convection oven (**OVEN**), and salamander broiler (**SALA**). Prior to untrained consumer panel and volatile compound analysis via gas chromatography-mass spectrometry, steaks were cooked under sous vide conditions for 1.5 h, then finished on the assigned cookery method. There were no cooking method × quality grade interactions ($P \geq 0.076$) for all consumer traits evaluated. Overall, SALA steaks were preferred ($P < 0.05$) by consumers than CLAM steaks for all palatability traits. Oven steaks had greater liking scores ($P < 0.05$) than CLAM steaks for juiciness, tenderness, and overall liking but were similar to CLAM steaks ($P > 0.05$) for flavor. Charbroiler steaks were similar ($P > 0.05$) to CLAM steaks for flavor but were preferred ($P < 0.05$) for tenderness, juiciness, and overall liking. Steaks cooked using the OVEN method produced a greater concentration of lipid derived volatiles, such as alcohols, aldehydes, and carboxylic acids. In direct contrast, CHAR steaks produced a higher concentration of pyrazines and Strecker aldehydes, which are derived from the Maillard reaction. This data indicates that cookery method, and therefore heat transfer method, has

a substantially stronger influence on consumer ratings and flavor development than USDA quality grade in this study when prepared using sous vide methods.

Key words: Consumers, cooking method, quality grade, sous vide, volatile compounds

INTRODUCTION

Consumers can effectively engage in many beef cookery methods, which may influence the final palatability of a steak. Generational changes in cooking styles due to increased time commitments, social influences, and others, consumers are looking for easier alternatives to cooking, especially meat products, as many have little to no cooking experience. Sous vide, which is French for "under vacuum", has recently gained popularity in both restaurants and homes as a method that provides a more evenly cooked product. Sous vide, at its core, is long temperature, long time cooking in a vacuum sealed environment in a circulating water bath (Baldwin, 2012; Dominguez-Hernandez et al., 2018). This method allows for a more evenly cooked product both internally and externally under precise temperature control (Baldwin, 2012). Typically, following sous vide preparation, steaks will be finished using a dry heat cookery method, such as a grill or cast iron or will be finished cooking through a reverse sear process.

Consumers may employ a variety of methods to cook their meat to provide their optimum combination of flavor, tenderness, and juiciness (Savell et al., 1999; Bagley et al., 2010). Generally speaking, cookery methods fall into one of two categories: dry or moist heat. Dry heat cookery methods are those that use direct application of high temperature heat, whether through application of hot air (convection), a hot pan (conduction), or a radiant heat (such as a flame). Moist heat cookery instead uses liquid as a vector for heat at a substantially lower temperature, resulting in improved tenderness

through breakdown of connective tissue via gelatinization. Dry heat cookery methods, such as grilling, broiling, and pan-frying are more popular than moist heat cookery, such as braising or stewing (Savell et al., 1999). To date, much work regarding cookery methods have been focused on tenderness and have not evaluated the difference between dry heat cookery methods for flavor development (Berry, 1993; Savell et al., 1999; Powell et al., 2000; Lawrence et al., 2001; Obuz et al., 2003). However, flavor and aroma in meat products is produced principally through dry-heat cooking (Mottram, 1998). Flavor is elucidated from meat products via two major pathways: the Maillard reaction and degradation of lipids. Each of these pathways contribute unique flavor compounds to meat flavor and are impacted by cooking. The Maillard reaction at its most basic form is a non-enzymatic browning reaction between a reducing sugar (carbonyl component) and an amino acid (amine component) at high temperatures (Mottram, 1993). The Maillard reaction can result in a large variety of different compounds, including furans, carbonyls, aldehydes, sulfur and nitrogen compounds, ketones, pyrroles, pyrazines, thiazoles, thiophenes, and furanthiols (Mottram et al., 1982; Mottram, 1993, 1998). These compounds are all influenced by temperature, heat application, and therefore, cooking method.

Previous studies have also indicated that differences in intramuscular fat can impact how steaks conduct heat and therefore, what volatile and flavor compounds are produced during cooking (O'Quinn et al., 2012; Legako et al., 2015; Gardner and Legako, 2018). Prior studies are conflicted on the true influence of intramuscular fat on volatile compound development. Marbling is responsible in part for the species-specific flavors within a meat product, especially within red meat species (Smith and Carpenter,

1974; Savell and Cross, 1988). However, despite increased marbling levels, it has not been repeatedly correlated with an increase in volatile flavor compounds (Cross et al., 1980; Mottram et al., 1982; Mottram and Edwards, 1983; Mottram, 1998; Legako et al., 2015a). However, Gardner and Legako (2018) observed a more linear response to volatile compound production with steaks of increasing USDA quality grade. Therefore, the objective of this study was to determine the influence of dry-heat cookery method on beef flavor development in two USDA quality grades following sous vide preparation.

MATERIALS AND METHODS

Carcass selection and steak fabrication

Beef strip loins (IMPS #180, NAMP 2010) were selected from one side from carcasses of two USDA quality grades: upper 2/3rds of Choice (Modest^{00}-Moderate^{100}) and Select (Slight^{00}-Slight^{100} marbling, n = 20/grade). Trained Texas Tech University (**TTU**) research personnel collected carcass data for yield and quality grade information, including preliminary yield grade, ribeye area, kidney, pelvic, and heart fat, as well as lean and skeletal maturity and marbling score. Following selection, all subprimals were transported under refrigeration (0 - 4°C) to the Gordon W. Davis Meat Laboratory at TTU. Subprimals were wet aged in the absence of light for 21 d at 0 - 4°C. Strip loins were fabricated anterior to posterior into 2.54 cm steaks using a slicer (Berkel X13A-Plus, Berkel, Inc, Houston, TX). Steaks were then randomly assigned within subprimals to one of randomly four cooking methods, vacuum packaged, and frozen at -20°C until further analysis.

Proximate analysis & pH

The percentage of moisture, fat, protein, and collagen was determined for raw steaks using an AOAC approved method (Anderson, 2007). Samples were thawed for 12 h at 4°C. Prior to analysis, all accessory muscles and heavy connective tissue were removed and then samples were cubed into approximately 3 cm^3 pieces. Sample pieces were then ground twice through a 4 mm plate on a tabletop grinder (#12 2/3 HP Electric Meat Grinder, Model MG-204182-13, Gander Mountain, St. Paul, MN). Proximate analysis was conducted using near-infared spectrophotometry (FoodScan, FOSS NIRsystems, Inc., Laurel, MD).

pH was measured using a slurry method, where 10 g of ground sample after proximate analysis was added to 90 mL of distilled water and were stirred with a stir bar until thoroughly mixed. To prevent the pH electrode (Jenway Model-3510, 120 VAC, Cole Parmer, Vernon Hills, IL) from being blocked with sample, all pH measurements were taken through a filter paper cone (Qualitative P8 Fisherbrand Filter Paper, Fisher Scientific, Pittsburgh, PA). Between each sample, the pH electrode was rinsed using distilled water and dried using low lint Kimwipes (Kimberly-Clark; 34120, Uline, Pleasant Prairie, WI).

Consumer sensory analysis

Prior to panels, steaks were thawed for 24 h at 2 – 4°C. Steaks were then cooked sous vide for approximately 1.5 h to a medium-rare degree of doneness (63°C) under vacuum in a circulating water bath (Immersion Circulator SmartVide 6, Sammic, Gipuzkoa, Spain) set at 63.5°C. Immediately prior to serving to panels, steaks were finished to a medium degree of doneness (71°C) on one of four randomly assigned

cooking methods: charbroiler grill (Cecilware Pro CCP24 Gas Charbroiler, Grindmaster-Cecilware Corp., Louisville, KY [**CHAR**]), clamshell grill (Cuisinart Griddler Deluxe GR-250, Cuisinart, Stamford, CT [**CLAM**]), convection oven (Mark V, Blodgett Corp., Burlington, VT [**OVEN**]), or salamander broiler (36-RB-N Salamander Broiler, Vulcan, Baltimore, MD [**SALA**]). Cooking surfaces were heated to 200°C ± 10 °C and monitored during cooking using surface thermocouples and dataloggers (Magnetic K thermocouple 88402K; RDXL4SD Datalogger Omega; Stamford, CT, USA). After cooking, steaks were cut into 2.54 × 1 × 1 cm cubes and two cubes were served to each panelist. Samples were then immediately served to panelists.

Consumer panels were conducted using the methods previously administered at TTU (O'Quinn et al., 2012; Legako et al., 2015a). Untrained consumer panelists (n = 100) were recruited from the Lubbock, Texas area in groups of 20. Panelists evaluated eight samples, one of each treatment, for flavor, tenderness, juiciness, and overall liking on unstructured 100-point line scales using a digital ballot (Qualtrics, Provo, UT) on an electronic tablet (iPad, Apple, Inc., Cupertino, CA). Each scale was verbally anchored at each endpoint and midpoint (0 = extremely dislike/extremely tough/extremely dry; 50 = neither dislike nor like/neither tough nor tender/neither dry nor juicy; 100 = extremely like/extremely tender/extremely juicy). Additionally, each panelist was also asked to rate each trait as acceptable or unacceptable and designate each sample as unsatisfactory, everyday, better than everyday, or premium quality. Each ballot consisted of a demographics sheet, a purchasing motivators sheet, and eight sample ballots. During the panel, panelists were provided with water, apple juice, and unsalted crackers to serve as palate cleansers.

Volatile compound analysis

The methods of Gardner and Legako (2018) were used to determine volatile compound composition of steaks. Steaks designated for volatile compound analysis were prepared as previously described for consumer sensory analysis. Immediately following cooking, steaks were bagged, then directly submerged into ice, vacuum packaged, and frozen at -20°C until volatile compound analysis. Prior to analysis, steaks were heated to 63.5°C using a circulating water bath for approximately 1 h. Following heating, six 1.27 cm cores were removed from the center of the steak perpendicular to the steak cut surface. The cores were then minced for 10 sec using a coffee grinder (4-12 cup Mr. Coffee grinder; Sunbeam Corporation, Boca Raton, FL). Five grams of sample was weighed into 20 mL glass vials (Gerstel Inc, Linthicum, MD). Ten microliters of internal standard (1, 2-dichlorobenzene, 2.5 mg/µL) was pipetted into the vial and then sealed using a polytetrafluoroethylene septa screw cap (#093640-040-00, 1.3 mm polytetrafluoroethylene septa and metal screw cap; Gerstel Inc, Linthicum, MD). The samples were then loaded using a Gerstel automatic sampler (MPS; Gerstel, Inc) for a 5 min incubation time at 65°C in the Gerstel agitator prior to a 20 min extraction time. Solid-phase microextraction (**SPME**) was used to collect the volatile compounds from the headspace of the sample with an 85 µm film thickness carboxen polydimethylsiloxane fiber (Supelco Inc., Bellefonte, PA). Volatile compounds extracted from the headspace were placed onto a VF-5 MS capillary column (30 m × 0.25 mm × 1.0 µm; Agilent J&W GC Column; Agilent Technologies, Inc., Santa Clara, CA). Authentic standards (Sigma-Aldrich, St. Louis, MO) were used to confirm compound identities through retention time.

Statistical analysis

Data was analyzed as a split plot arrangement using the PROC GLIMMIX procedure of SAS. (SAS, version 9.4; SAS, Inc., Cary, NC). For analysis, individual steak served as the experimental unit. Strip loin served as the whole plot factor and cooking method served as the subplot factor. Peak temperature was included in the model as a covariate. For consumer liking data, panel session and round served as a random effect. Consumer acceptance data was analyzed using a binomial distribution. The Kenward-Rogers adjustment was used to estimate denominator degrees of freedom. Differences were determined using $\alpha \leq 0.050$.

RESULTS & DISCUSSION

Carcass traits

Carcass characteristics are displayed in Table 2.1. Quality grade had no effect on lean, skeletal, or overall maturity, as well as ribeye area, preliminary, and adjusted fat thickness ($P > 0.05$). As expected, carcasses from the upper 2/3^{rds} of USDA Choice had greater marbling scores and a greater percentage of kidney, pelvic, and heart fat ($P < 0.05$) than Select carcasses.

Proximate analysis & pH

Results from proximate analysis are detailed in Table 2.2. Steaks from the upper 2/3rds of Choice possessed a greater ($P < 0.05$) percentage of fat and a correspondingly lower ($P < 0.05$) moisture percentages compared to Select steaks. As expected, no differences were observed ($P > 0.05$) between USDA quality grades for protein, collagen, and pH.

Consumer panel demographic characteristics and purchasing motivators

The demographic characteristics of the 100 consumers who participated in sensory evaluation are presented in Table 2.3. The majority of participants were Caucasian/White (54.0%) from four-person households (31.0%). Moreover, 54.0% of participants were male and 46.0% of participants were female. Additionally, 39.0% of participants were single and 61.0% were married. Most of the consumers were 30 to 39 years old (37.0%) with an annual income of $50,000-$74,999 (23.0%) or more than $100,000 (29.0%) and were college graduates (37.0%). When consuming beef, most consumers considered flavor the most important palatability trait (47.0%), followed by tenderness (39.0%), and preferred steaks cooked to medium-rare (29.0%) or medium (31.0%) and consumed beef 1 to 3 times per week (45.0%).

In addition to a demographics survey, participants were also asked to rate 15 different purchasing motivators for beef products (Table 2.4). Price, color, USDA grade, size, and eating satisfaction claims were ranked as the most important ($P < 0.05$) traits. Additionally, familiarity of cut, marbling levels, antibiotic use, nutrient content, growth promotant use, animal welfare, packaging types and natural/organic claims were more important ($P < 0.05$) than brand, grass-fed, or grain fed.

Consumer sensory analysis

Cooking method

There were no cooking method × quality grade interactions ($P \geq 0.076$) for all consumer traits evaluated (Table 2.5). Overall, SALA steaks were preferred ($P < 0.05$) by consumers than CLAM steaks for all palatability traits. Oven steaks had greater rating scores ($P < 0.05$) than CLAM steaks for juiciness, tenderness, and overall liking but were similar to CLAM steaks ($P > 0.05$) for flavor liking. Charbroiler steaks were similar ($P >$

0.05) to CLAM steaks for flavor liking but were preferred ($P < 0.05$) for tenderness, juiciness, and overall liking. When asked if samples were acceptable for each palatability trait, a greater percentage ($P < 0.05$) of SALA steaks were designated as acceptable for flavor, tenderness, juiciness, and overall acceptability than CLAM steaks (Table 2.6). Salamander steaks had the greatest percentage ($P < 0.05$) of steaks rated as acceptable for juiciness in comparison to all other treatments, which were similar ($P > 0.05$). For flavor acceptability, a similar percentage of OVEN and CHAR steaks were denoted as acceptable ($P > 0.05$). However, a greater percentage of OVEN steaks were designated as acceptable in comparison to CLAM steaks ($P < 0.05$). Clamshell steaks had the lowest percentage of steaks rated as acceptable ($P < 0.05$) for tenderness in comparison to all other treatments, which were similar ($P > 0.05$). Overall, SALA steaks had a higher percentage of steaks rated as acceptable for overall liking ($P < 0.05$) compared to CLAM steaks, however, CHAR and OVEN steaks were intermediate and all methods ($P > 0.05$). However, when asked to designate each sample as unsatisfactory, everyday, better than everyday, or premium quality, no differences were observed between cooking methods ($P > 0.05$) for the percentages of steaks rated as everyday, better than everyday, or premium quality (Table 2.7). In contrast, a higher percentage of CLAM steaks were rated as unsatisfactory quality ($P < 0.05$) in comparison to CHAR and SALA steaks, but were similar to OVEN steaks ($P > 0.05$).

No prior literature has discussed the impact of sous vide cooking followed by finishing the cooking process on a dry heat cookery method. Primarily, the majority of discussion on cooking method has revolved around its impact on tenderness, specifically Warner-Bratzler shear force (Wheeler et al., 1998; Powell et al., 2000; Lawrence et al.,

2001; Herring and Rogers, 2003a; Obuz et al., 2003; McKenna et al., 2004; Obuz et al., 2004; Bowers et al., 2012). Additionally, previous literature has focused on the tenderness of LL steaks in comparison to other lower quality muscles, such as the semimembranosus as attempts to reduce the impact of greater concentrations of connective tissue and large fiber size to improve tenderness ratings by consumers. However, when directly comparing cooking methods, clamshell grills have been found to be more consistent, rapid, and repeatable for research applications in comparison to electric broilers (McKenna et al., 2004). The results from the current study, however, indicate that clamshell grills may be detrimental to flavor research and actually may reduce consumer ratings of grilled beef strip loin steaks, especially for the palatability traits of tenderness, juiciness, and overall liking, as it was ranked the lowest for each of those traits.

Quality grade

Quality grade did not influence ($P \geq 0.07$) flavor, juiciness, overall liking or acceptability, as consumers rated both Top Choice and Select steaks similar for flavor, juiciness, and overall liking (Table 2.5). However, consumers preferred Select steaks for tenderness over Top Choice steaks ($P = 0.04$). There was no difference ($P = 0.210$) in acceptability of any trait (Table 2.6). When asked to rate each sample as unsatisfactory, everyday quality, better than everyday quality, or premium quality, quality grade did not impact ($P \geq 0.080$) the percentage of steaks rated as unsatisfactory, everyday quality, or better than everyday quality (Table 2.7). However, a greater percentage of Select steaks were rated as premium quality ($P < 0.05$) than Top Choice steaks. Increased levels of marbling and therefore higher quality grades have typically been associated with higher

consumer ratings of tenderness, juiciness and flavor (O'Quinn et al., 2012; Corbin et al., 2014; Lucherk et al., 2016). These studies had a much wider range of quality grades (Prime to Standard) rather than the smaller window in the present study (upper 2/3rds of Choice and Select). However, due to variation within quality grades and the reduced marbling score spread, consumers may have rated the two grades similarly. Other studies have reported similar results from similar quality grades (Savell et al., 1987; Legako et al., 2015a; Wilfong et al., 2016; Vierck et al., 2018). Additionally, sous vide preparation has been implicated with reducing tenderness variation within steaks (Baldwin, 2012). Sous vide allows for the degradation of proteins, including myofibillar, sarcoplasmic, and connective tissue proteins (Baldwin, 2012; Dominguez-Hernandez et al., 2018). Connective tissue proteins specifically are impacted by the low temperature, long time method of sous vide cooking. By exposing these proteins to gelatinization through sous vide cooking, this may have contributed to the reduced tenderness variation observed between quality grades (Baldwin, 2012; Dominguez-Hernandez et al., 2018).

Volatile compound analysis

Fifty-three volatile flavor compounds were evaluated from various flavor development pathways, including the Maillard reaction and lipid degradation. Primarily, these compounds were impacted by the main effect of cooking method (n = 28) and the interaction between cooking method and USDA quality grade (n = 4). No compound evaluated was solely impacted ($P \geq 0.06$) by USDA quality grade.

Four compounds, hexanoic acid, methyl ester, 1-octen-3-ol, pentanal, and 2-pentylfuran, were all impacted ($P \leq 0.044$; Table 2.8) by the interaction of cooking

method and USDA quality grade. These lipid derived products were present ($P < 0.05$) in the greatest concentration in Select OVEN steaks compared to all other treatments.

A similar trend existed for lipid degradation products affected by the cooking method main effect. Oven steaks produced ($P < 0.05$) the greatest concentration of lipid-derived alcohols (1-hexanol, 1-octanol, and 1-pentanol) compared to all other treatments. Similarly, OVEN steaks also produced ($P < 0.05$) the greatest concentration of 2-heptanone and d-limonene compared to all other treatments. However, when examining the group of lipid derived aldehydes, CHAR and OVEN steaks produced ($P < 0.05$) the highest concentration of decanal, dodecanal, nonanal, and octanal. This may be due to the re-volatilization of lipids as they strike the heat source (the radiant flame of the charbroiler grill or the hot air of the convection oven) and are aerosolized back on to the exposed surface of the steak. Previous work has indicated that lipids can be lost in meat products through evaporative and drip losses during the cooking process (Sigler et al., 1978). Lipids lost through the evaporative portion of cook loss have the opportunity to be re-circulated on to the steak, especially in a closed environment, such as a convection oven. The evaporative, volatile nature of flavor compounds are used to an advantage during the smoking process, in which volatile compounds, such as aldehydes, alcohols, carbonyls, and esters, are circulated through the smoke and absorbed by the meat product (Maga, 1987). This process could be emulated with the lipids reacting with the flames and being reabsorbed by the steak during the cooking process.

Contrastingly, CLAM steaks produced ($P < 0.05$) the highest concentration of butanal. The direct application of heat likely rapidly decomposed the lipid fraction of the steak, resulting in rapid breakdown into these lipid oxidation products. These two

aldehydes are noted for their contribution to oxidized and off-flavors, which likely reduced the consumer scores for flavor liking in CLAM steaks. Similar to the aldehydes, the carboxylic acids were present in the highest amounts in both CHAR and OVEN steaks, with the notable exception of benzoic acid. Benzoic acid was produced ($P < 0.05$) in the greatest concentration in CHAR steaks compared to all other treatments. Carboxylic acids, such as butanoic and hexanoic acid, contribute to sour, sweaty, and rancid off-flavors observed in meat products (Spanier et al., 1992; Stetzer et al., 2008; Kerth and Miller, 2015). Additionally, carboxylic acids are formed during oxidation of aldehydes or alcohols, which are considered secondary products of lipid oxidation (Min and Ahn, 2005; Bekhit et al., 2013). This indicates that CHAR steaks are producing end products of lipid oxidation, possibly produced through a longer thermal oxidation of lipids. In comparison, CLAM steaks produced a greater concentration of hexanal, which is a secondary product of lipid oxidation. This indicates that CLAM steaks have less of an opportunity to be oxidized further into carboxylic acids, whereas CHAR steaks continued to be oxidized during that cooking process.

A similar trend existed for decane, toluene, and p-xylene, lipid derived hydrocarbons. Charbroiler steaks produced ($P < 0.05$) the highest concentration of these lipid compounds compared to all other treatments.

When evaluating volatile compounds produced as a result of the Maillard reaction, CHAR steaks dominated the landscape. Charbroiler steaks produced ($P < 0.05$) the highest concentration of all pyrazines compared to all other treatments. Additionally, CHAR steaks produced the greatest concentration of methional and phenylacetaldehyde, two Strecker aldehydes. However, four notable exceptions occurred to this trend in

Maillard ketones and two Strecker aldehydes. Steaks cooked using CLAM produced ($P < 0.05$) the greatest concentration of 3-methylbutanal, isobutyraldehyde, 2,3-butanedione, and 3-hydroxy-2-butanone compared to all other treatments. This is likely due to the direct conduction of the heat source off the clamshell grill. The extremely rapid, continual application of heat from both sides of the steak would result in a more rapid cooking process and reduce the completion of the Maillard reaction and therefore, produce a greater concentration of intermediary products, such as Strecker aldehydes and Maillard ketones, such as 3-hydroxy-2-butanone (Cook times: CHAR 227.3 sec ± 103.6; CLAM: 126.7 sec ± 58.3; OVEN: 397.2 sec ± 75.4; SALA: 180.0 sec ± 45.2). These compounds can undergo further reactions, such as heterocyclization, resulting in the pyrazines observed in the CHAR steaks. It is likely that because the CHAR method is not as rapid as the CLAM method that steaks had more time to produce a greater concentration of final products, such as pyrazines. It appears that the CLAM method halts the Maillard reaction before heterocyclization can occur, but CHAR allows the Maillard reaction to further proceed. Overall, CHAR and OVEN steaks produced ($P < 0.05$) the greatest total concentration of volatile compounds compared to SALA steaks when all compounds were taken into account.

It is interesting to note that only one sulfur containing compound, methional, was impacted by cooking method. This depression in sulfur containing volatile flavor compound production is likely due to the sous vide cooking process prior to cooking. Moist heat cookery, such as a sous vide or boiling environment, has been linked to a significant detriment in volatile compounds characteristic of meat cooked in a high temperature environment (Utama et al., 2018). Since steaks were only finished from a

medium-rare degree of doneness (63℃) to medium (71℃), this may have severely restricted the flavor development possible and reduced the appearance of sulfur-containing compounds in the final product. Despite the possible influence from sous-vide preparation, it is clear that cooking method has a much stronger influence on consumer ratings and volatile flavor production in comparison to USDA quality grade when prepared using sous vide.

Table 2.1. Least squares means of beef carcass measurements of carcasses of two quality grade treatments.

Treatment	Lean Maturity[1]	Skeletal Maturity[1]	Overall Maturity[1]	USDA Marbling Score[2]	Preliminary Fat Thickness, cm	Adjusted Fat Thickness, cm	Ribeye Area, cm^2	Kidney, Pelvic, Heart Fat, %
Quality Grade								
Top Choice[3]	147	129	136	528[a]	1.36	1.44	91.0	3.6[a]
Select	138	137	139	344[b]	1.03	1.13	95.1	3.3[b]
SEM[4]	8.1	4.0	4.2	4.6	0.03	0.32	0.3	0.1
P-value	0.440	0.140	0.560	< 0.001	0.060	0.070	0.120	0.001

[1]100 = A; 200 = B.

[2]200 = Traces; 300 = Slight; 400 = Small; 500 = Modest; 600 = Moderate.

[3]USDA marbling score of Modest[00]-Moderate[100].

[4]SE (largest) of the least squares means in the same main effect (marbling texture or quality grade).

[ab]Least squares means in the same main effect (quality grade or marbling texture) without a common superscript differ ($P < 0.05$).

Table 2.2. Least square means for proximate analysis and pH for beef steaks (*n* = 160) from two USDA quality grades[1].

	%				
Quality grade	Fat	Moisture	Protein	Collagen	pH
Top Choice	5.9[a]	69.7[b]	22.0	1.7	5.5
Select	2.7[b]	71.5[a]	21.9	1.6	5.5
SEM[2]	0.24	0.85	0.26	0.05	0.03
P-value	< 0.001	< 0.001	0.731	0.589	0.914

[1]Top Choice: USDA marbling score of Modest[00]-Moderate[100]; Select: USDA marbling score of Slight[00]-Slight[100]

[2]SE (largest) of the least squares means.

[abcd]Least squares means without a common superscript differ ($P < 0.05$).

Table 2.3. Demographic characteristics of consumers (n = 100) who participated in consumer sensory panels.

Characteristic	Response	Percentage of Consumers
Gender	Male	54.0
	Female	46.0
Household size	1 person	11.0
	2 people	17.0
	3 people	19.0
	4 people	31.0
	5 people	17.0
	6 people	3.0
	>6 people	2.0
Marital Status	Single	39.0
	Married	61.0
Age	Under 20	4.0
	20-29	17.0
	30-39	37.0
	40-49	24.0
	50-59	10.0
	Over 60	8.0
Ethnic Origin	African-American	1.0
	Asian	0.0
	Caucasian/White	54.0
	Hispanic	42.0
	Native American	1.0
	Other	1.0
Annual household income	Under $25,000	11.0
	$25,000 - $34,999	9.0
	$35,000 - $49,999	8.0
	$50,000 - $74,999	23.0
	$75,000 - $100,000	20.0
	More than $100,000	29.0
Education level	Non-high school graduate	5.0
	High school graduate	15.0
	Some college/ technical school	30.0
	College graduate	37.0
	Post graduate	13.0
Beef consumption per week	None	0.0
	1-3 times	45.0
	4-6 times	35.0
	7 or more	20.0
Most important palatability trait	Flavor	47.0
	Juiciness	14.0
	Tenderness	39.0
Degree of doneness preference	Very rare	1.0
	Rare	8.0
	Medium-rare	29.0
	Medium	31.0
	Medium-well	23.0
	Well-done	7.0
	Very well-done	1.0

Table 2.4. Beef strip loin steak purchasing motivators[1] of consumers (*n* = 100) participating in consumer sensory panels.

Trait	Importance
Price	71.8[a]
Color	71.2[a]
USDA Grade	71.2[a]
Size, weight, thickness	67.4[ab]
Eating Satisfaction Claims	64.6[abc]
Familiarity of cut	64.4[bcd]
Marbling levels	61.7[bcd]
Antibiotic use in animal	57.0[cde]
Nutrient Content	56.2[cdef]
Growth promotant use in animals	55.8[def]
Animal welfare	52.4[efg]
Packaging type	50.2[efg]
Natural or organic claims	47.8[fgh]
Brand	47.4[gh]
Grass-fed	46.5[gh]
Grain-fed	39.5[h]
SEM	3.0
P-value	< 0.001

[1]Purchasing motivators: 0 = extremely unimportant, 100 = extremely unimportant.
[2]SE (largest) of the least squares means in the same main effect.
[abcdefgh]Least squares means without a common superscript differ ($P < 0.05$).

Table 2.5. Least squares means for consumer panel ratings[1] of beef strip loin steaks of two USDA quality grades (n = 160) cooked on four different dry cookery methods.

Treatment	Flavor Liking	Tenderness	Juiciness	Overall Liking
Cooking method				
Charbroiler	60.7[ab]	63.0[a]	53.8[a]	59.5[a]
Clamshell	55.9[b]	55.1[b]	45.7[b]	52.5[b]
Oven	62.0[ab]	65.7[a]	61.4[a]	63.5[a]
Salamander	63.9[a]	65.4[a]	57.4[a]	63.0[a]
SEM[2]	3.3	3.5	3.6	3.4
P-value	0.031	0.008	0.002	0.006
Quality grade				
Top Choice[3]	58.8	60.2[b]	52.8	57.8
Select[4]	62.4	64.4[a]	56.3	61.5
SEM	1.7	1.7	1.7	1.7
P-value	0.054	0.039	0.100	0.066
Method × Quality grade				
P-value	0.076	0.970	0.967	0.645

[1]Sensory scores: 0 = extremely tough/dry/dislike flavor/dislike overall, 50 = neither dry nor juicy/neither tough nor tender, 100 = extremely juicy/tender/like flavor/like overall.

[2]SE (largest) of the least squares means in the same main effect (cooking method or quality grade).

[3]USDA marbling score of Modest[00]-Moderate[100].

[4]USDA marbling score of Slight[00]-Slight[100]

[ab]Least squares means in the same main effect (cooking method or quality grade) without a common superscript differ ($P < 0.05$).

Table 2.6**.** Percentage of beef strip loin steaks of two quality grades cooked on four dry cookery methods rated as acceptable for flavor, tenderness, juiciness, and overall liking (*n* = 160).

Treatment	Flavor Acceptability	Tenderness Acceptability	Juiciness Acceptability	Overall Acceptability
Cooking method				
Charbroiler	84.2[ab]	87.6[a]	72.1[b]	83.7[ab]
Clamshell	79.0[b]	76.6[b]	67.1[b]	76.2[b]
Oven	87.3[a]	86.6[a]	74.1[b]	81.7[ab]
Salamander	88.3[a]	91.4[a]	82.9[a]	88.3[a]
SEM[1]	0.3	0.3	0.2	0.3
P-value	0.050	< 0.001	0.006	0.020
Quality grade				
Top Choice[2]	84.4	84.7	73.1	81.7
Select[3]	85.6	87.8	75.9	84.1
SEM	0.3	0.2	0.1	0.2
P-value	0.666	0.213	0.384	0.381
Method × Quality grade				
P-value	0.056	0.963	0.692	0.855

[1]SE (largest) of the least squares means in the same main effect (cooking method or quality grade).

[2]USDA marbling score of Modest[00]-Moderate[100].

[ab]Least squares means in the same main effect (cooking method or quality grade) without a common superscript differ ($P < 0.05$).

Table 2.7. Percentage of beef strip loin steaks (*n* = 160) of two quality grades cooked on four dry cookery methods rated identified as different perceived quality levels by consumer panelists (*n* = 100).

Treatment	Unsatisfactory Quality	Everyday Quality	Better than Everyday Quality	Premium Quality
Cooking method				
Charbroiler	15.5[b]	52.5	25.0	6.4
Clamshell	26.5[a]	46.0	22.4	3.7
Oven	18.3[ab]	51.0	21.4	7.7
Salamander	12.4[b]	53.0	23.0	9.2
SEM[1]	0.3	0.1	0.2	0.4
P-value	0.004	0.485	0.855	0.244
Quality grade				
Top Choice[2]	18.4	53.8	24.1	4.5[b]
Select[3]	16.9	47.5	21.8	9.1[a]
SEM	0.2	0.1	0.1	0.3
P-value	0.594	0.078	0.441	0.016
Method × Quality grade				
P-value	0.216	0.141	0.232	0.360

[1]SE (largest) of the least squares means in the same main effect (cooking method or quality grade).

[2]USDA marbling score of Modest[00]-Moderate[100].

[3]USDA marbling score of Slight[00]-Slight[100]

[ab]Least squares means in the same main effect (cooking method or quality grade) without a common superscript differ ($P < 0.05$).

Table 2.8. Interaction of dry heat cookery method[1] and USDA quality grade[2] on production of volatile compounds produced by beef strip loin steaks.

	Hexanoic acid, methyl ester	1-octen-3-ol	2-pentylfuran	Pentanal
Top Choice				
Charbroiler	0.31^{b}	5.79^{b}	1.52^{b}	1.95^{bc}
Clamshell	0.42^{b}	4.85^{b}	1.07^{b}	2.71^{bc}
Oven	0.49^{b}	5.30^{b}	1.40^{b}	2.19^{bc}
Salamander	0.38^{b}	5.82^{b}	1.49^{b}	3.63^{abc}
Select				
Charbroiler	0.49^{b}	3.56^{b}	1.15^{b}	1.31^{c}
Clamshell	0.34^{b}	6.20^{b}	2.50^{b}	4.00^{b}
Oven	0.93^{a}	13.50^{a}	5.63^{a}	6.17^{a}
Salamander	0.33^{b}	4.54^{b}	1.33^{b}	2.09^{bc}
SEM[3]	0.11	2.21	0.83	0.99
P-value	0.007	0.044	0.016	0.021

[1]Cooking methods included charbroiler grill, clamshell grill, convection oven, and salamander broiler.

[2]Top Choice: USDA marbling score of Modest^{00}-Moderate^{100}, Select: USDA marbling score of Slight^{00}-Slight^{100}.

[3]SE (largest) of the least squares means in the same main effect (cooking method or quality grade).

[ab]Least squares means in the same column without a common superscript differ ($P < 0.05$).

Table 2.9. Least squares means of volatile compounds produced from beef strip loin steaks prepared using four dry heat cookery methods.

	Cooking method[1]					
Compound, ng/g sample	CHAR	CLAM	OVEN	SALA	SEM[2]	*P*-value
Maillard reaction products						
Pyrazines						
Methylpyrazine	1.90[a]	0.40[b]	0.15[b]	0.19[b]	0.60	< 0.001
2,5-dimethylpyrazine	3.48[a]	0.81[b]	0.21[c]	0.33[bc]	0.19	< 0.001
2-ethyl-3,5-dimethylpyrazine	2.79[a]	0.74[b]	0.27[b]	0.28[b]	0.22	< 0.001
3-ethyl-2,5-dimethylpyrazine	3.02[a]	0.80[b]	0.28[b]	0.29[b]	0.24	< 0.001
Trimethylpyrazine	3.48[a]	0.58[b]	0.20[b]	0.21[b]	0.27	< 0.001
Strecker aldehydes						
3-methylbutanal	0.83[b]	1.52[a]	0.65[b]	1.13[ab]	0.18	0.007
Isobutyraldehyde	5.22[b]	9.31[a]	4.50[b]	6.01[b]	0.81	< 0.001
Methional	4.33[a]	2.67[b]	2.70[b]	2.28[b]	0.33	< 0.001
Phenylacetaldehyde	1.26[a]	0.97[b]	0.95[b]	0.76[b]	0.10	0.006
Maillard ketones						
2,3-butanedione	20.01[b]	56.75[a]	29.48[b]	36.27[b]	6.69	0.001
3-hydroxy-2-butanone	38.17[c]	93.94[a]	69.29[b]	73.96[ab]	8.44	< 0.001
Lipid degradation products						
Aldehydes						
Butanal	0.25[bc]	0.49[a]	0.22[c]	0.38[ab]	0.06	0.003
Decanal	437.83[a]	194.54[b]	397.18[a]	138.64[b]	34.70	< 0.001
Dodecanal	6.07[a]	3.30[b]	4.71[a]	2.82[b]	0.51	< 0.001
Hexanal	31.08[b]	62.44[a]	79.23[a]	49.41[ab]	11.16	0.020
Nonanal	9.14[ab]	6.95[bc]	11.50[a]	5.42[c]	1.33	0.008
Octanal	3.00[ab]	2.52[b]	1.29[a]	1.97[b]	0.48	0.005
Alcohols						
1-hexanol	0.49[b]	0.74[b]	1.45[a]	0.58[b]	0.26	0.015
1-octanol	6.90[b]	4.58[bc]	9.92[a]	3.40[c]	1.06	< 0.001
1-pentanol	3.27[b]	8.96[a]	9.44[a]	7.02[ab]	1.89	0.036
Carboxylic acids						
Benzoic acid	7.28[a]	3.84[b]	2.23[b]	2.76[b]	1.25	0.022
Heptanoic acid	2.50[ab]	1.82[bc]	2.97[a]	1.37[c]	0.28	< 0.001
Octanoic acid	57.64[a]	28.59[b]	60.59[a]	25.72[b]	4.55	< 0.001
Hydrocarbons						
2-heptanone	1.27[b]	1.26[b]	1.91[a]	1.12[b]	0.20	0.022
D-limonene	0.030[b]	0.072[a]	0.066[a]	0.060[a]	0.001	0.001
Decane	2.10[a]	1.32[b]	1.50[b]	1.18[b]	0.13	< 0.001
Toluene	13.33[a]	9.43[b]	8.38[b]	7.67[b]	1.25	0.006
p-Xylene	708.77[a]	264.32[b]	247.26[b]	232.99[b]	55.11	< 0.001
Total volatile production	1611.29[a]	965.59[bc]	1308.73[ab]	791.15[c]	131.61	< 0.001

[1]Cooking methods included charbroiler grill (CHAR), clamshell grill (CLAM), convection oven (OVEN), and salamander broiler (SALA).

[2]SE (largest) of the least squares means in the same row.

[abc]Least squares means in the same row without a common superscript differ ($P < 0.05$)

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CHAPTER 3
EVALUATION OF DRY HEAT COOKERY METHOD ON VOLATILE FLAVOR COMPOUND DEVELOPMENT AND CONSUMER EVALUATION OF SIX BEEF MUSCLES

ABSTRACT

The objective of this study was to determine the influence of dry heat cookery on beef flavor development of multiple beef muscles. Beef strip loins, top sirloin butts, paired tenderloins, paired shoulder clods, and chuck rolls were collected from USDA Low Choice Carcasses (Small^{00}-Small^{100} marbling; n = 20). Subprimals were wet aged in the absence of light for 21 d at 0 - 4°C. Subprimals were fabricated into 2.54-cm thick steaks representative of the following muscles: Gluteus medius (**GM**), Infraspinatus (**IF**), Longissimus lumborum (**LL**), Psoas major (**PM**), Serratus ventralis (**SV**), and Triceps brachii (**TB**) and randomly assigned to one of four dry heat cookery methods: charbroiler grill (**CHAR**), clamshell grill (**CLAM**), convection oven (**OVEN**), and salamander broiler (**SALA**). Steaks were finished to a medium degree of doneness (71°C) on the randomly assigned cooking method. Untrained consumer panelists (n = 300) evaluated each sample for flavor, tenderness, juiciness, and overall liking. No interactions were observed between cooking method and muscle ($P \geq 0.344$) for any palatability traits evaluated. Consumers preferred CHAR steaks ($P < 0.05$) than CLAM steaks for flavor, tenderness, juiciness, and overall liking. Additionally, CLAM steaks were rated lower ($P < 0.05$) than all other methods for tenderness and juiciness. Oven and SALA steaks were rated higher ($P < 0.05$) than CLAM steaks by consumers for tenderness and juiciness but were similar ($P > 0.05$) to CLAM steaks for overall liking. Charbroiler steaks produced a greater concentration of Maillard compounds, including Strecker aldehydes, pyrazines, and sulfur-containing compounds compared to the other cooking methods. Steaks cooked

using OVEN and SALA ($P < 0.05$) produced more lipid oxidation products, including carboxylic acids and esters. Additionally, CHAR steaks produced the greatest ($P < 0.05$) total volatile production compared to all other treatments, which may be a result of the combination of Maillard reaction products and the lipid degradation products.

INTRODUCTION

Flavor and aroma in meat products is produced principally through cooking (Mottram, 1998). Flavor development occurs through the Maillard reaction and thermal degradation of lipids and thiamin, which produces the characteristic brown color and roasted, brown flavors associated with cooked meat products (Mottram, 1993, 1998). Cooking and therefore flavor development, is impacted by heat transfer rate, which can be impacted by product composition and muscle type. Differences in quality grades are attributed to differences in intramuscular fat, which can influence the way steaks conduct heat and therefore impact flavor development (O'Quinn et al., 2012; Legako et al., 2015a). In addition to quality grade, muscle type has a direct impact on palatability ratings from consumers, which may be in part due to differing fiber types, fiber direction, or a combination of those factors (Hunt et al., 2014a; Legako et al., 2015a).

Cooking method is one of the primary factors that consumers have control over in producing a highly palatable beef product for consumption. However, the majority of literature surrounding cooking method's impact on palatability has focused primarily on tenderness, rather than all attributes of palatability (Berry, 1993; Savell et al., 1999; Powell et al., 2000; Lawrence et al., 2001; Obuz et al., 2003). Consumers will use a wide variety of cooking methods to cook their meat to provide the optimum combination of tenderness, juiciness, and flavor (Savell et al., 1999; Bagley et al., 2010). Previously,

individual muscle types have been evaluated by cooking methods for tenderness evaluation, but differentiation between dry heat cookery methods for flavor analysis is non-existent in the literature. By matching individual muscles to dry heat cookery methods that improve flavor, beef marketing can be improved, therefore resulting in a better eating experience for the consumer. Additionally, restaurants can better improve the consumer's eating experience by using a variety of cooking methods to better match muscles being served. Therefore, the objective of this study was to determine the influence of dry heat cookery on beef flavor development of multiple beef muscles.

MATERIALS & METHODS

Product selection & subprimal fabrication

Beef strip loins (IMPS #180), top sirloin butts (IMPS #184), paired tenderloins (IMPS #189), paired shoulder clods (IMPS #114), and chuck rolls (IMPS #116) were collected from USDA Low Choice Carcasses ($Small^{00}$-$Small^{100}$ marbling; n = 20) from a large commercial beef processing facility. Trained Texas Tech University (**TTU**) research personnel collected carcass data for yield and quality grade information, including preliminary yield grade, ribeye area, kidney, pelvic, and heart fat, as well as lean and skeletal maturity and marbling score. Following selection, all subprimals were transported under refrigeration (0 - 4°C) to the Gordon W. Davis Meat Laboratory at TTU. Subprimals were wet aged in the absence of light for 21 d at 0 - 4°C.

During fabrication, subprimals were fabricated into the following muscles: Gluteus medius (**GM**), Infraspinatus (**IF**), Longissimus lumborum (**LL**), Psoas major (**PM**), Serratus ventralis (**SV**), and Triceps brachii (**TB**). Subprimals were then fabricated into 2.54 cm steaks using a slicer (Berkel X13E, Berkel Equipment, Louisville, KY).

Steaks were then randomly assigned within paired subprimals to one of the four cooking methods, vacuum packaged, and frozen at -20°C until further analysis.

Proximate analysis & pH

The percentage of moisture, fat, protein, and collagen was determined using an AOAC approved method. Samples were thawed for 12 h at 4°C. Prior to analysis, all accessory muscles and heavy connective tissue were removed and then samples were cubed into approximately 3 cm^3 pieces. Sample pieces were then ground twice through a 4 mm plate on a tabletop grinder (#12 2/3 HP Electric Meat Grinder, Model MG-204182-13, Gander Mountain, St. Paul, MN). Proximate analysis was conducted using near-infared spectrophotometry (FoodScan, FOSS NIRsystems, Inc., Laurel, MD).

pH was measured using a slurry method, where 10 g of ground sample after proximate analysis was added to 90 mL of distilled water and were stirred with a stir bar until thoroughly mixed. To prevent the pH electrode (Jenway Model-3510, 120 VAC, Cole Parmer, Vernon Hills, IL) from being blocked with sample, all pH measurements were taken through a filter paper cone (Qualitative P8 Fisherbrand Filter Paper, Fisher Scientific, Pittsburgh, PA). Between each sample, the pH electrode was rinsed using distilled water and dried using low lint Kimwipes (Kimberly-Clark; 34120, Uline, Pleasant Prairie, WI).

Consumer sensory analysis

Prior to panels, steaks were thawed for 24 h at 2 – 4°C. Prior to panel evaluation, steaks were finished to a medium degree of doneness (71°C) on one of four randomly assigned cooking methods: charbroiler grill (Cecilware Pro CCP24 Gas Charbroiler, Grindmaster-Cecilware Corp., Louisville, KY [**CHAR**]), clamshell grill (Cuisinart

Griddler Deluxe GR-250, Cuisinart, Stamford, CT [**CLAM**]), convection oven (Mark V, Blodgett Corp., Burlington, VT [**OVEN**]), or salamander broiler (36-RB-N Salamander Broiler, Vulcan, Baltimore, MD [**SALA**]). Cooking surfaces were heated to 200℃ ± 10 ℃ and monitored during cooking using surface thermocouples and dataloggers (Magnetic K thermocouple 88402K; RDXL4SD Datalogger Omega; Stamford, CT, USA). Approximately every three minutes, steaks were flipped on the charbroiler, oven, and salamander to avoid burning on either side and to evenly distribute the heat source. Steaks were cooked to a medium degree of doneness (71°C) using hand-held thermometers (Thermapen Mk4, ThermoWorks, Inc, Salt Lake City, UT), then immediately placed into in a vacuum bag, then ice. Steaks were vacuum packaged and chilled for approximately 6 h until panel sessions. One hour prior to panel sessions, vacuum packaged steaks were placed into a circulating water bath (Immersion Circulator SmartVide 6, Sammic, Gipuzkoa, Spain) set at 63.5℃ until serving. After reheating, steaks were cut into steak thickness × 1 × 1 cm cubes and two cubes were served to each panelist. Samples were then immediately served to panelists.

Consumer panels were conducted using the methods previously administered at TTU (O'Quinn et al., 2012; Legako et al., 2015a). Untrained consumer panelists (n = 300) were recruited from the Lubbock, Texas area in groups of 20. An incomplete block design was used to evaluate the samples due to the number of treatments (n = 24). Panelists evaluated each sample for flavor, tenderness, juiciness, and overall liking on unstructured 100-point line scales using a digital ballot (Qualtrics, Provo, UT) on an electronic tablet (iPad, Apple, Inc., Cupertino, CA). Each scale was verbally anchored at each endpoint and midpoint (0 = extremely dislike/extremely tough/extremely dry; 50 = neither dislike nor

like/neither tough nor tender/neither dry nor juicy; 100 = extremely like/extremely tender/extremely juicy). Additionally, each panelist was also asked to rate each trait as acceptable or unacceptable and designate each sample as unsatisfactory, everyday, better than everyday, or premium quality. Each ballot consisted of a demographics sheet, a purchasing motivators sheet, and eight sample ballots. During the panel, panelists were provided with water, apple juice, and unsalted crackers to serve as palate cleansers.

Volatile compound analysis

The methods of Gardner and Legako (2018) were used to determine volatile compound composition of steaks. Steaks designated for volatile compound analysis were prepared as previously described for consumer sensory analysis. Immediately following cooking, steaks were placed in an unsealed bag, then directly submerged into ice, vacuum packaged, and frozen at -20°C until volatile compound analysis. Prior to analysis, steaks were heated to 63.5°C using a circulating water bath for approximately 1 h. Following heating, six 1.27 cm cores were removed from the center of the steak perpendicular to the steak cut surface. The cores were then minced for 10 sec using a coffee grinder (4-12 cup Mr. Coffee grinder; Sunbeam Corporation, Boca Raton, FL). Five grams of sample was weighed into 20 mL glass vials (Gerstel Inc, Linthicum, MD). Ten microliters of internal standard (1, 2-dichlorobenzene, 2.5 mg/µL) was pipetted into the vial and then sealed using a polytetrafluoroethylene septa screw cap (#093640-040-00, 1.3 mm polytetrafluoroethylene septa and metal screw cap; Gerstel Inc, Linthicum, MD). The samples were then loaded using a Gerstel automatic sampler (MPS; Gerstel, Inc) for a 5 min incubation time at 65°C in the Gerstel agitator prior to a 20 min extraction time. Solid-phase microextraction (**SPME**) was used to collect the volatile compounds from

the headspace of the sample with an 85 µm film thickness carboxen polydimethylsiloxane fiber (Supelco Inc., Bellefonte, PA). Volatile compounds extracted from the headspace were placed onto a VF-5 MS capillary column (30 m × 0.25 mm × 1.0 µm; Agilent J&W GC Column; Agilent Technologies, Inc., Santa Clara, CA). Authentic standards (Sigma-Aldrich, St. Louis, MO) were used to confirm compound identities through retention time.

Statistical analysis

Data was analyzed as a split plot arrangement using the PROC GLIMMIX procedure of SAS. (SAS, version 9.4; SAS, Inc., Cary, NC). For analysis, individual steak served as the experimental unit. Subprimal served as the whole plot factor and cooking method served as the subplot factor. Peak temperature was included in the model as a covariate For consumer data, panel session and round also served as a random effect. Consumer acceptance data was analyzed using a binomial distribution. For all analyses, differences were considered significant at $\alpha < 0.05$. The Kenward-Rogers adjustment was used to estimate denominator degrees of freedom.

RESULTS & DISCUSSION

Carcass characteristics

Carcass characteristics are represented in Table 3.1. Carcasses used in this objective were A maturity and had a marbling score of Small. Additionally, carcasses exhibited an average of 1.2 cm of preliminary and adjusted fat thickness and 98.5 cm^2 of ribeye area. Additionally, they possessed 3.3% kidney, pelvic, and heart fat.

Proximate analysis & pH

Proximate analysis and pH results are present in Table 3.2. Raw steaks from the SV ventralis and IF had higher ($P < 0.05$) percentages of fat compared to all other muscles. Contrastingly, steaks from the TB and the PM possessed the greatest ($P < 0.05$) percent of moisture while the IF contained the lowest ($P < 0.05$) percentage of moisture. For protein percentage, the GM and LL contained the greatest ($P < 0.05$) percentage compared to all other treatments, while the SV had the lowest ($P < 0.05$) percentage of protein. Serratus ventralis steaks possessed the greatest ($P < 0.05$) percentage of collagen compared to all other treatments, while the TB possessed the lowest ($P < 0.05$) percentage of collagen. For pH, PM and IF steaks were the highest ($P < 0.05$) in pH compared to all other treatments. Additionally, the SV was higher ($P < 0.05$) in pH compared to the GM, which was the lowest ($P < 0.05$) in pH values.

Consumer panel demographics characteristics and purchasing motivators

The demographic characteristics of the 300 consumers who participated in the sensory evaluation are presented in Table 3.3. The majority of participants were Caucasian/White (54.7%) from households of four people (27.3%). Male participants were present at 46.3% and there were 53.7% female participants. The consumers were predominately married (54.0%), 30 to 39 years of age (31.0%) with an annual income of more than $100,000 (22.9%) and some college or technical school education (35.0%). When consuming beef, 50.0% of consumers considered flavor the most important palatability trait, followed by tenderness (38.6%). Additionally, consumers primarily ate beef 1 to 3 times per week (39.3%) or 4 to 6 times per week (37.0%) and preferred their beef cooked to medium rare (34.7%) or medium (32.3%).

Consumers were also asked to rank 15 beef product purchasing motivators (Table 3.4). Price, USDA grade, color, and size, weight, and thickness were the most important ($P < 0.05$), followed by marbling levels, eating satisfaction claims, familiarity of cut, and nutrient content. Moreover, animal welfare, antibiotic use, and growth promotant use were more important ($P < 0.05$) than natural/organic claims, grass-fed diet, packaging type, brand, and grain-fed diet, which were considered the least important ($P < 0.05$).

Consumer sensory analysis

Cooking method

No interactions were observed between cooking method and muscle ($P \geq 0.344$) for any palatability traits evaluated. Consumers preferred CHAR steaks ($P < 0.05$) to CLAM steaks for flavor, tenderness, juiciness, and overall liking (Table 3.5). Additionally, CLAM steaks were rated lower ($P < 0.05$) than all other methods for tenderness and juiciness. Moreover, OVEN steaks were rated similar ($P > 0.05$) for flavor to both CHAR and CLAM steaks ($P > 0.05$). Oven and SALA steaks were rated higher ($P < 0.05$) than CLAM steaks by consumers for tenderness and juiciness but were similar ($P > 0.05$) to CLAM steaks for overall liking. Salamander steaks were rated similar ($P > 0.05$) to CLAM steaks for flavor. When asked to rate steaks as acceptable for tenderness or juiciness, CLAM steaks had a lower ($P < 0.05$; Table 3.6) percentage of steaks rated as acceptable in comparison to all other treatments. No differences were observed ($P = 0.06$) between cooking methods for overall liking, as well as the percentage of steaks rated as acceptable for flavor and overall ($P = 0.44$, 0.26). When asked to designate each sample as unsatisfactory, everyday, better than everyday, or premium quality, CLAM steaks produced a greater ($P < 0.05$) percentage of unsatisfactory steaks than OVEN or CHAR

steaks but was similar to SALA ($P > 0.05$; Table 3.7). Clamshell steaks also produced a greater ($P < 0.05$) percentage of steaks as everyday quality than SALA or CHAR steaks but was similar ($P > 0.05$) to OVEN. No differences were observed ($P = 0.08$) between cooking methods for the percentage of steaks rated as better than everyday quality. Charbroiler and SALA steaks had the greatest ($P < 0.05$) percentage of steaks rated as premium quality in comparison to CLAM steaks, which produced the lowest ($P < 0.05$) percentage.

Previously, when comparing multiple muscles over a variety of cooking methods, statistical differences have been observed between cooking methods, however, the magnitude of the differences are 0.01 to 0.5 on an eight-point scale (Herring and Rogers, 2003a). When fed to consumers, however, there have been distinct differences observed between cooking methods. Sepulveda et al. (2019) reported that beef strip loin steaks cooked on a flat top grill were rated lower by consumers than steaks cooked on a charbroiler grill, clamshell grill, and salamander broiler for tenderness, juiciness, flavor liking, and overall liking. This supports that cooking method can directly impact consumer ratings of beef steaks and consumers are able to differentiate between cooking method in terms of palatability.

Muscle

Psoas major steaks were rated higher ($P < 0.05$; Table 3.5) than all other muscles for flavor, tenderness, and overall liking. Additionally, PM steaks had the greatest ($P < 0.05$) percentage of steaks rated as acceptable for flavor and tenderness. Consumers rated IF steaks similar ($P > 0.05$) to PM steaks for juiciness and had a similar percentage of steaks rated as acceptable for juiciness and overall acceptability. For flavor, tenderness,

and overall liking, IF steaks were rated lower ($P < 0.05$) than PM steaks, but higher ($P < 0.05$) than all other muscles. Consumers rated SV steaks similar ($P > 0.05$) to IF, GM, LL, and TB steaks for flavor. Serratus ventralis steaks were also rated higher ($P < 0.05$) than GM, LL, and TB steaks for juiciness, but were similar ($P > 0.05$) to TB steaks for overall liking. Consumers rated GM, LL and TB steaks the lowest ($P < 0.05$) for flavor, tenderness, and overall liking.

When asked to rate steaks as acceptable for flavor, PM steaks had the greatest percentage of steaks rated as acceptable ($P < 0.05$), followed by IF steaks, which were similar ($P > 0.05$) to LL, SV, and TB steaks, but higher ($P < 0.05$) than GM steaks. A similar trend was observed for tenderness acceptability, however, IF steaks had a greater ($P < 0.05$) percentage of steaks rated as an acceptable for tenderness than all other muscles with the exception of the PM. Consumers rated a greater percentage of PM and IF steaks as acceptable ($P < 0.05$) for juiciness than all other muscles, followed by SV and TB steaks ($P < 0.05$), then LL and GM steaks had the lowest ($P < 0.05$) percentage of steaks rated as acceptable for juiciness. For overall acceptability, PM and IF steaks had the highest percentage of steaks rated as acceptable ($P < 0.05$) and were greater ($P < 0.05$) than all other muscles, which were lower and similar ($P > 0.05$). When asked to designate samples as unsatisfactory, everyday, better than everyday, or premium quality, consumers rated a greater percentage of GM, LL, SV, and TB steaks as unsatisfactory ($P < 0.05$) than IF or PM steaks. Psoas major steaks had the lowest ($P < 0.05$) percentage of steaks rated as unsatisfactory. A similar trend was observed for the percentage of steaks rated as everyday quality. Serratus ventralis, PM, and IF steaks had the lowest ($P < 0.05$) percentage of steaks rated as everyday quality, compared to the GM, LL, and TB, which

were similar and greater ($P < 0.05$). For better than everyday quality, IF steaks produced the greatest ($P < 0.05$) percentage of steaks, followed by PM and SV, which were greater ($P < 0.05$) than LL, GM, and TB steaks. The PM had the greatest percentage of steaks rated as premium quality ($P < 0.05$), followed by IF, which was greater ($P < 0.05$) than the SV, GM, and LL.

It is important to note the lack of interactive effect between cooking method and quality grade. This indicates that rather than selecting an optimum cooking for each individual muscle, instead, a variety of cooking applications can be used with equal success on high quality muscles. In the 2010 National Beef Tenderness Survey, IF (top blade) steaks were rated the highest out of LL (top loin) steaks and GM (top sirloin) steaks for overall liking, tenderness, and juiciness, but was similar to the LL for flavor like and flavor level (Guelker et al., 2013). Hunt et al. (2014b) reported similar consumer ratings for GM, SV, and LL steaks, which were similar for tenderness, juiciness, and flavor. Nyquist et al. (2018) reported similar results, as the IF outperformed the LL and TB for flavor liking, juiciness, tenderness, and overall liking. However, the SV was reported to be similar to the IF for juiciness, but was lower for all other traits evaluated (Nyquist et al., 2018). However, these results directly contrast the findings from Legako et al. (2015a), In this study, steaks from Low Choice PM, LL, and GM were rated similar for tenderness, juiciness, and flavor liking, and overall liking. Carmack et al. (1995) also reported no differences between the GM, IF, LL, PM, SV, and TB for beef-flavor intensity, tenderness, or juiciness. This may be due to the wide range of muscles used in these studies, which also included traditionally low-quality muscles such as the semimembranosus and semitendinosus, which have typically been drier and tougher than

the muscles used within the present study. Additionally for chuck muscles specifically, Kukowski et al. (2005) reported that LL and IF steaks were rated similar for tenderness, juiciness, and flavor intensity, but higher than both the SV and TB.

Volatile compound analysis

Seventy-two compounds were evaluated for their contribution to beef flavor development. Of these compounds, 19 compounds were impacted by the interaction of cookery method and muscle (Tables 3.8 and 3.9), 26 compounds were solely impacted by the cooking method main effect (Table 3.10), and 24 compounds were impacted by muscle type alone (Table 3.11).

Cooking method

When evaluating differences in compounds produced from various dry heat cookery methods, very different profiles emerged between methods. CHAR steaks produced a greater concentration of Maillard compounds, including Strecker aldehydes, pyrazines, and sulfur-containing compounds compared to the other cooking methods evaluated (Table 3.10). Specifically, for Strecker aldehydes, CHAR steaks produced the greatest ($P < 0.05$) concentration of 2-methylbutanal, benzaldehyde, and phenylacetaldehyde compared to OVEN and SALA steaks. However, an opposite trend existed for 3-methylbutanal, where OVEN steaks produced the lowest ($P < 0.05$) concentration compared to all other treatments. Charbroiler steaks produced the greatest ($P < 0.05$) concentration of methylpyrazine and trimethylpyrazine compared to all other treatments. Additionally, for trimethylpyrazine, CLAM steaks produced a greater ($P < 0.05$) concentration than OVEN or SALA steaks, but was still lower ($P < 0.05$) than CHAR steaks. Moreover, CLAM and CHAR steaks produced the greatest concentration

of sulfur-containing compounds. For methanethiol, CLAM steaks produced the greatest ($P < 0.05$) concentration compared to OVEN and SALA steaks, but were similar ($P > 0.05$) to CHAR steaks. Similarly, CLAM steaks produced a greater ($P < 0.05$) concentration of dimethyl disulfide compared to all other cooking methods. However, for carbon disulfide, SALA produced the greatest ($P < 0.05$) concentration compared to all other treatments. Additionally. CHAR steaks produced the greatest ($P < 0.05$) concentration of 2-methylthiophene compared to all other treatments. For Maillard products, CHAR and CHAR steaks followed a similar trend, indicating that more direct applications of heat increased Maillard product production.

Steaks cooked using OVEN and SALA ($P < 0.05$) produced more lipid oxidation products, including carboxylic acids and esters. Specifically, OVEN steaks produced the greatest ($P < 0.05$) concentrations of 1-octanol, octanoic acid, and heptanoic acid compared to all other treatments. Salamander steaks produced the greatest ($P < 0.05$) concentration of acetic acid and pentanal compared to all other treatments. For nonanoic acid, methyl ester, pentanal, pentane, OVEN and SALA steaks produced a greater ($P < 0.05$) concentration than CHAR or CLAM steaks. In direct contrast, however, CHAR steaks produced the greatest ($P < 0.05$) concentration of propanoic acid, methyl ester, 2-pentanone, and toluene compared to all other treatments. Additionally, CHAR steaks produced the greatest ($P < 0.05$) total volatile production compared to all other treatments, which may be a result of the combination of Maillard reaction products and the lipid degradation products. This increase in lipid-derived products may be produced by recirculation of lipid products throughout the cooking process, as lipids are dripped down into the flames during the cooking process, then aerosolized back on to the cooking

surface and steak of the oven, charbroiler grill, and the salamander broiler. Oven and SALA steaks also produced the lowest concentration ($P < 0.05$) of sulfur-containing compounds and pyrazines, which indicate radiant and convection heat transfer methods produce lower concentrations of Maillard products, due to their less direct heat application and transfer. However, as the CHAR grill is also a radiant heat transfer, it may explain the increase in lipid derived products produced by the cooking method.

Muscle

When evaluating the impact of muscle type on flavor development, the SV stood out as the muscle that produced the greatest ($P < 0.05$; Table 3.11) concentration of total volatile production compare to all other muscles with the exception of the GM (Table 3.11). Across the classes of compounds, the SV produced the greatest ($P < 0.05$) concentration of 2,3-butanediol, carbon disulfide, 1-octen-3-ol, octanoic acid, 2-propanone, 2-pentanone, octane, and pentane. This increase in total volatile compound production may be due to the plentiful flavor precursors present in the SV. The SV has been well-established as a muscle with a high fat percentage in comparison to other muscle types within a USDA quality grade (Hunt et al., 2016; Nyquist et al., 2018). Additionally, Hunt et al. (2016) reported that SV steaks possessed a higher concentrations of fatty acids, which can interact with products formed during the Maillard production and produce key compounds to flavor development.

Similarly, the GM produced a higher ($P < 0.05$) concentration of Maillard reaction products, including benzaldehyde and methylpyrazine. This contributed to an increased ($P < 0.05$) total concentration compared to IF and LL steaks. In direct contrast, the IF produced the lowest concentration of most compounds. The PM also produced a

wide range of compounds, however, it was not to the extremes possessed by the SV. This intermediate effect may contribute to the increased ratings by consumers for flavor liking (Table 3.5), rather than swinging the pendulum to one extreme (lipid degradation) to the other (Maillard reaction products). These major differences in muscle type were not observed in previous literature. Previously, Hunt et al. (2016) and Legako et al. (2015a) observed differences between muscles for Strecker aldehydes and carboxylic acids, as well as certain ketones, including 2,3-butanedione. In the study conducted by Legako et al. (2015a), the semimembranosus outperformed the SV for the Maillard derived compounds, likely due to its reduced fat percentage, whereas the SV produced greater concentrations of lipid derived carboxylic acids. No differences were observed between muscles for pyrazines or sulfur containing compounds in Legako et al. (2015a). These differences were further echoed in Hunt et al. (2016). This may be due to differing sampling methodologies, as the steaks in the current study were re-heated using the sous vide method. Reheating may have allowed for further development of certain compounds, such as those derived from lipid degradations.

Interaction of cooking method and muscle type

When evaluating the interactive effects of dry heat cookery and muscle type, much of the main effects from cooking method and muscle were further echoed. Charbroiler steaks from GM, IF, and SV subprimals produced the greatest ($P < 0.05$; Table 3.8) concentration of methional, a Strecker aldehyde. Similar trends existed across for Maillard reaction products, including isobutyraldehyde, 2,5-dimethypyrazine, 3-ethyl-2,5-dimethylpyrazine, and 2-3-ethyl-3,5-dimethylpyrazine. However, for 3-hydroxy-2-butanone, a Maillard ketone, CLAM SV and SALA GM steaks produced the highest ($P <$

0.05) concentration compared to all other treatments. Similar to the main effects, CHAR steaks of all muscles produced a higher ($P < 0.05$) concentration of p-xylene and tetradecane. However, for butanal, octanoic acid, methyl ester, 1-octene, hexanal, 2-heptanone, and decane, CLAM SV and OVEN SV steaks produced a greater ($P < 0.05$) concentration compared to all other treatments.

Table 3.1. Least square means (± SD[1]) of beef carcass (n = 20) measurements.

Carcass Characteristics	
Quality Attributes	
Lean maturity[2]	152 ± 35
Skeletal maturity[2]	125 ± 20
Overall maturity[2]	136 ± 20
Marbling score[3]	446 ± 30
Yield Attributes	
Preliminary fat thickness, cm	1.2 ± 0.6
Adjusted fat thickness, cm	1.2 ± 0.6
Ribeye area, cm^2	98.5 ± 1.3
Hot carcass weight, kg	
Kidney, pelvic, and heart fat, %	3.3 ± 0.3
Final yield grade	

[1]Standard deviation.
[2]100 = A; 200 = B.
[3]200 = Traces; 300 = Slight; 400 = Small.

Table 3.2. Least square means for proximate analysis and pH for beef steaks (n = 480) from six different muscles.

	%				
Muscle	Fat	Moisture	Protein	Collagen	pH
Gluteus medius	3.4^{b}	72.6^{bc}	23.0^{a}	1.8^{bc}	5.4^{d}
Infraspinatus	8.0^{a}	70.8^{d}	19.7^{c}	1.9^{b}	5.7^{a}
Longissimus lumborum	3.9^{b}	71.4^{cd}	22.7^{a}	1.8^{bc}	5.5^{cd}
Psoas major	3.3^{b}	73.4^{ab}	21.0^{b}	1.8^{bc}	5.8^{a}
Serratus ventralis	7.3^{a}	72.1^{c}	18.2^{d}	2.2^{a}	5.6^{b}
Triceps brachii	2.8^{b}	74.1^{a}	21.7^{b}	1.7^{c}	5.6^{bc}
SEM[1]	0.79	0.43	0.53	0.15	0.04
P-value	0.004	< 0.001	< 0.001	< 0.001	< 0.001

[1]SE (largest) of the least squares means.

[abcd]Least squares means without a common superscript differ (P < 0.05).

Table 3.3. Demographic characteristics of consumers (n = 300) who participated in consumer sensory panels.

Characteristic	Response	Percentage of Consumers
Gender	Male	46.3
	Female	53.7
Household size	1 person	11.0
	2 people	18.3
	3 people	17.0
	4 people	27.3
	5 people	15.6
	6 people	6.3
	>6 people	4.3
Marital Status	Single	46.0
	Married	54.0
Age	Under 20	12.0
	20-29	19.7
	30-39	31.0
	40-49	22.0
	50-59	6.0
	Over 60	9.3
Ethnic Origin	African-American	6.7
	Asian	0.3
	Caucasian/White	54.0
	Hispanic	35.7
	Native American	1.0
	Other	0.3
Annual household income	Under $25,000	11.0
	$25,000 - $34,999	11.0
	$35,000 - $49,999	15.7
	$50,000 - $74,999	16.3
	$75,000 - $100,000	20.0
	More than $100,000	22.9
Education level	Non-high school graduate	5.0
	High school graduate	23.3
	Some college/ technical school	35.0
	College graduate	25.0
	Post graduate	11.6
Beef consumption per week	None	0.0
	1-3 times	39.3
	4-6 times	37.0
	7 or more	23.7
Most important palatability trait	Flavor	50.0
	Juiciness	11.3
	Tenderness	38.6
Degree of doneness preference	Very rare	0.7
	Rare	4.3
	Medium-rare	34.7
	Medium	32.3
	Medium-well	15.7
	Well-done	9.7
	Very well-done	2.6

Table 3.4. Beef steak purchasing motivators[1] of consumers (n = 300) participating in consumer sensory panels.

Trait	Importance
Price	67.9[a]
USDA Grade	67.6[a]
Size, weight, thickness	66.9[a]
Color	66.8[a]
Marbling level	58.6[b]
Eating Satisfaction Claims	57.8[b]
Familiarity of cut	57.4[bc]
Nutrient Content	55.8[bc]
Animal welfare	50.9[cd]
Antibiotic use in animal	48.4[d]
Growth promotant use	48.2[d]
Natural or organic claims	43.1[e]
Grass-fed	41.0[ef]
Packaging type	40.8[ef]
Brand	40.8[ef]
Grain-fed	37.9[f]
SEM	1.8
P-value	< 0.001

[1]Purchasing motivators: 0 = extremely unimportant, 100 = extremely unimportant.
[2]SE (largest) of the least squares means in the same main effect.
[abcdef]Least squares means without a common superscript differ ($P < 0.05$).

Table 3.5. Least squares means of palatability ratings of beef steaks (n = 480) from six muscles and cooked on four different cooking methods.

Treatment	Flavor	Tenderness	Juiciness	Overall Liking
Cooking method				
Charbroiler	60.1[a]	64.3[a]	55.1[a]	59.8[a]
Clamshell	54.5[b]	55.7[b]	47.2[b]	54.0[b]
Oven	57.9[ab]	62.1[a]	52.0[a]	57.6[ab]
Salamander	56.1[b]	62.7[a]	54.8[a]	57.0[ab]
SEM[1]	1.9	1.5	1.6	1.7
P-value	0.023	< 0.001	< 0.001	0.033
Muscle				
Gluteus medius	53.1[c]	54.9[c]	43.6[d]	51.2[d]
Infraspinatus	58.9[b]	70.3[b]	64.1[a]	62.6[b]
Longissimus lumborum	53.5[c]	55.7[c]	42.0[d]	51.4[d]
Psoas major	64.7[a]	74.9[a]	59.9[a]	67.4[a]
Serratus ventralis	56.2[bc]	56.8[c]	55.2[b]	56.7[c]
Triceps brachii	55.5[c]	54.6[c]	48.8[c]	53.5[cd]
SEM	2.1	1.7	1.7	1.8
P-value	< 0.001	< 0.001	< 0.001	< 0.001
Method × Muscle				
P-value	0.344	0.902	0.487	0.518

[1]SE (largest) of the least squares means in the same main effect.
[abc]Least squares means in the same main effect (cooking method or muscle) without a common superscript differ (P < 0.05).

Table 3.6. Percentage of beef steaks of six muscles cooked on four dry cookery methods rated as acceptable for flavor, tenderness, juiciness, and overall liking (n = 480).

Treatment	Flavor Acceptability	Tenderness Acceptability	Juiciness Acceptability	Overall Acceptability
Cooking method				
Charbroiler	81.2	89.5[a]	79.3[a]	82.4
Clamshell	81.8	82.8[b]	69.3[b]	79.2
Oven	83.7	90.5[a]	78.5[a]	82.8
Salamander	80.1	89.8[a]	76.4[a]	79.3
SEM[1]	0.2	0.2	0.1	0.1
P-value	0.442	0.001	< 0.001	0.264
Muscle				
Gluteus medius	77.0[c]	80.5[c]	64.9[c]	74.6[b]
Infraspinatus	83.0[b]	92.2[b]	87.4[a]	85.9[a]
Longissimus lumborum	80.3[bc]	82.1[c]	61.0[c]	74.4[b]
Psoas major	89.0[a]	97.5[a]	84.3[a]	89.7[a]
Serratus ventralis	79.3[bc]	82.1[c]	78.0[b]	78.3[b]
Triceps brachii	79.6[bc]	83.3[c]	73.2[b]	78.6[b]
SEM	0.2	0.4	0.2	0.2
P-value	< 0.001	< 0.001	< 0.001	< 0.001
Method × Muscle				
P-value	0.231	0.909	0.316	0.599

[1]SE (largest) of the least squares means in the same main effect.
[abc]Least squares means in the same main effect (cooking method or muscle) without a common superscript differ (P < 0.05).

Table 3.7. Percentage of beef steaks (*n* = 480) from six muscles cooked on four dry cookery methods identified as different perceived quality levels by consumer panelists (*n* = 300).

Treatment	Unsatisfactory Quality	Everyday Quality	Better than Everyday Quality	Premium Quality
Cooking method				
Charbroiler	16.4[b]	41.8[c]	26.3	11.3[a]
Clamshell	22.0[a]	49.5[a]	20.8	4.1[c]
Oven	16.2[b]	48.3[ab]	26.9	5.3[bc]
Salamander	20.1[ab]	42.6[bc]	24.4	8.5[ab]
SEM[1]	0.1	0.09	0.1	0.3
P-value	0.030	0.014	0.077	< 0.001
Muscle				
Gluteus medius	25.0[a]	51.3[a]	19.2[c]	2.8[d]
Infraspinatus	15.3[b]	35.8[b]	36.0[a]	11.7[b]
Longissimus lumborum	24.6[a]	53.1[a]	17.1[c]	3.1[d]
Psoas major	9.6[c]	40.2[b]	28.7[b]	18.5[a]
Serratus ventralis	21.4[a]	41.0[b]	28.8[b]	8.1[bc]
Triceps brachii	19.7[ab]	52.4[a]	20.7[c]	5.9[cd]
SEM	0.2	0.1	0.1	0.3
P-value	< 0.001	< 0.001	< 0.001	< 0.001
Method × Muscle				
P-value	0.344	0.742	0.761	0.208

[1]SE (largest) of the least squares means in the same main effect.

[abc]Least squares means in the same main effect (cooking method or muscle) without a common superscript differ ($P < 0.05$).

Table 3.8. Interaction of dry heat cookery method and muscle[1] on Maillard reaction-derived volatile compound production of beef steaks (n = 480).

	Strecker aldehydes		*Sulfur compounds*		*Pyrazines*			*Maillard ketones*	
Compound, ng/g	Methional	Isobutyraldehyde	Dimethyl sulfide	Dimethyl sulfone	2,5-dimethylpyrazine	3-ethyl-2,5-dimethylpyrazine	2-ethyl-3,5-dimethylpyrazine	2,3-butanedione	3-hydroxy-2-butanone
Treatment									
Charbroiler									
GM	4.67^{a}	18.98^{abc}	7.36^{cde}	0.648^{bc}	9.95^{a}	8.36^{a}	7.79^{a}	90.60^{abcd}	148.18^{bcd}
IF	4.47^{a}	6.68^{ed}	4.69^{def}	0.378^{c}	5.33^{de}	4.03^{c}	3.68^{c}	18.60^{g}	31.21^{ij}
LL	2.87^{b}	11.29^{cde}	4.23^{def}	0.613^{bc}	8.02^{bc}	6.59^{b}	6.00^{b}	39.46^{efg}	67.03^{ghij}
PM	1.89^{cde}	11.44^{cde}	6.69^{cdef}	0.516^{bc}	4.04^{ef}	3.14^{cd}	2.93^{cd}	55.83^{defgh}	98.35^{cdefghi}
SV	4.04^{a}	19.03^{abc}	6.59^{cdef}	0.838^{bc}	6.69^{cd}	6.25^{b}	5.86^{b}	73.65^{bcde}	109.35^{bcdefg}
TB	2.24^{bc}	5.87^{ed}	5.91^{def}	0.245^{c}	9.07^{ab}	6.29^{b}	5.69^{b}	26.23^{fg}	46.85^{ghij}
Clamshell									
GM	1.46^{cde}	7.75^{ed}	5.70^{def}	0.203^{c}	3.99^{ef}	3.08^{cd}	2.79^{cd}	39.44^{efg}	63.89^{ghij}
IF	1.39^{cde}	3.42^{e}	4.07^{def}	0.283^{c}	2.14^{fgh}	1.60^{ef}	1.46^{ef}	14.51^{g}	23.38^{j}
LL	1.06^{e}	7.26^{ed}	4.37^{def}	0.310^{c}	1.30^{hi}	1.07^{ef}	0.96^{ef}	27.67^{fg}	41.20^{hij}
PM	1.35^{cde}	8.88^{ed}	6.64^{cdef}	0.650^{bc}	1.63^{ghi}	0.69^{f}	0.61^{f}	58.85^{cdef}	87.57^{defghij}
SV	1.76^{cde}	24.81^{a}	6.99^{cdef}	2.700^{a}	1.79^{ghi}	1.61^{ef}	1.47^{ef}	129.40^{a}	225.89^{a}
TB	1.32^{cde}	7.70^{ed}	4.03^{ef}	0.270^{c}	3.37^{fg}	2.29^{de}	2.10^{de}	44.63^{efg}	68.37^{fghij}
Oven									
GM	1.38^{cde}	8.05^{ed}	12.55^{ab}	0.467^{bc}	0.58^{hi}	0.46^{f}	0.41^{f}	92.52^{abcd}	154.30^{bc}
IF	1.56^{cde}	5.60^{ed}	4.84^{def}	0.691^{bc}	0.55^{hi}	0.41^{f}	0.33^{f}	18.44^{g}	47.05^{ghij}
LL	1.27^{ed}	7.77^{ed}	2.99^{f}	0.951^{bc}	0.26^{i}	0.25^{f}	0.24^{f}	44.34^{efg}	78.20^{efghij}
PM	1.56^{cde}	8.56^{de}	6.69^{cdef}	0.740^{bc}	0.68^{hi}	1.00^{ef}	0.76^{f}	70.31^{bcde}	105.46^{cdefgh}
SV	2.20^{bc}	10.29^{cde}	5.25^{def}	1.246^{bc}	0.79^{hi}	0.85^{ef}	0.72^{f}	88.43^{bcd}	140.26^{bcde}
TB	1.19^{ed}	7.34^{ed}	8.14^{cd}	0.440^{bc}	0.92^{hi}	1.00^{ef}	0.82^{ef}	42.83^{efg}	78.48^{efghij}
Salamander									
GM	1.85^{cde}	12.80^{bcd}	10.14^{bc}	0.755^{bc}	1.08^{hi}	0.91^{ef}	0.84^{ef}	104.46^{ab}	175.00^{ab}
IF	1.84^{cde}	7.16^{ed}	7.29^{cde}	0.449^{bc}	0.99^{hi}	0.80^{f}	0.75^{f}	43.21^{efg}	70.31^{fghij}
LL	1.80^{cde}	10.62^{cde}	5.87^{def}	0.634^{bc}	1.16^{hi}	0.95^{ef}	0.89^{ef}	53.02^{efgh}	92.19^{cdefghi}
PM	1.51^{cde}	13.78^{bcd}	5.94^{def}	0.886^{bc}	0.62^{hi}	0.43^{f}	0.40^{f}	77.01^{bcde}	136.19^{bcdef}
SV	2.09^{bcd}	13.58^{bcd}	7.77^{cde}	0.888^{bc}	1.20^{hi}	0.93^{ef}	0.87^{ef}	87.92^{bcd}	144.94^{bcd}
TB	2.98^{b}	21.06^{ab}	14.67^{a}	1.445^{b}	1.42^{hi}	1.27^{ef}	1.20^{ef}	97.63^{abc}	155.35^{bc}
SEM[2]	0.37	3.58	1.48	0.411	0.74	0.58	0.50	16.01	26.67
P-value	<0.001	0.030	0.002	0.018	<0.001	<0.001	<0.001	0.042	0.020

[1]Muscles included Gluteus medius (GM), Infraspinatus (IF), Longissimus lumborum (LL), Psoas major (PM), Serratus ventralis (SV), and Triceps brachii (TB).

[2]SE (largest) of the least squares means in the same main effect.

[abcdefghi]Least squares means in the same main effect (cooking method or muscle) without a common superscript differ ($P < 0.05$).

Table 3.9. Interaction of dry heat cookery method and muscle[1] on lipid-derived volatile compound production of beef steaks (n = 480).

	Carboxylic acids		*Ester*	*Alkenes*		*Aldehydes*		*Ketone*	*Alkanes*	
Compound, ng/g	Benzoic acid	Butanoic acid	Octanoic acid, methyl ester	1-octene	p-Xylene	Butanal	Hexanal	2-heptanone	Decane	Tetradecane
Charbroiler grill										
GM	0.238^{cd}	79.18^{ab}	0.364^{cd}	1.41^{bcd}	1740.48^{a}	1.04^{abc}	14.82^{c}	1.46^{bcdef}	1.338^{bc}	1.48^{f}
IF	0.438^{cd}	26.25^{ef}	0.306^{cd}	0.63^{de}	1122.14^{b}	0.34^{de}	8.89^{c}	1.56^{bcde}	0.850^{cde}	23.83^{a}
LL	0.524^{cd}	21.30^{ef}	0.410^{cd}	1.63^{bc}	1101.75^{b}	0.60^{cde}	14.43^{c}	1.30^{defg}	1.522^{ab}	4.69^{def}
PM	0.390^{cd}	55.53^{bcde}	0.390^{cd}	0.95^{cde}	635.62^{c}	0.61^{cde}	53.40^{bc}	1.64^{bcde}	1.326^{bcd}	3.95^{def}
SV	0.423^{cd}	54.24^{bcde}	0.467^{cd}	3.19^{a}	1227.44^{b}	1.05^{abc}	32.00^{bc}	1.89^{bc}	1.867^{a}	1.44^{f}
TB	0.344^{cd}	26.71^{def}	0.256^{c}	0.56^{de}	1408.94^{ab}	0.30^{de}	8.57^{c}	1.09^{efgh}	0.767^{de}	12.60^{bcd}
Clamshell grill										
GM	0.202^{d}	30.39^{def}	0.300^{cd}	0.66^{de}	223.62^{d}	0.41^{de}	13.60^{c}	0.81^{gh}	0.814^{de}	6.41^{bcdef}
IF	0.306^{cd}	22.25^{ef}	0.240^{d}	0.57^{de}	227.70^{d}	0.16^{e}	20.96^{c}	1.11^{efgh}	0.900^{cde}	9.18^{bcdef}
LL	0.174^{d}	16.41^{f}	0.314^{cd}	0.58^{de}	194.05^{d}	0.38^{de}	27.88^{c}	0.69^{h}	0.822^{de}	10.71^{bcde}
PM	0.176^{d}	50.24^{bcde}	0.352^{cd}	0.82^{de}	180.22^{d}	0.47^{de}	80.04^{bc}	1.27^{defg}	0.992^{cde}	7.47^{bcdef}
SV	1.887^{bc}	114.47^{a}	1.056^{a}	1.79^{b}	466.57^{cd}	1.42^{a}	39.59^{bc}	1.61^{bcde}	1.973^{a}	2.25^{ef}
TB	0.422^{cd}	30.72^{cdef}	0.321^{cd}	0.67^{de}	346.79^{cd}	0.40^{de}	35.82^{bc}	1.42^{bcdef}	0.693^{e}	13.19^{bc}
Convection oven										
GM	0.231^{d}	45.71^{bcdef}	0.400^{cd}	1.05^{bcde}	166.35^{d}	0.41^{de}	42.47^{bc}	1.63^{bcde}	0.894^{cde}	1.95^{ef}
IF	0.279^{cd}	35.87^{cdef}	0.391^{cd}	0.87^{de}	234.51^{d}	0.28^{de}	29.59^{c}	1.42^{defg}	0.844^{de}	3.53^{ef}
LL	0.344^{cd}	24.81^{ef}	0.506^{bcd}	1.13^{bcde}	131.95^{d}	0.41^{de}	50.71^{bc}	1.30^{defg}	0.917^{cde}	1.65
PM	0.295^{cd}	54.84^{bcde}	0.746^{b}	1.40^{bcd}	201.96^{d}	0.44^{de}	61.76^{bc}	1.99^{b}	1.101^{bcde}	2.17^{ef}
SV	0.556^{cd}	64.77^{bc}	0.561^{bc}	3.24^{a}	314.76^{cd}	0.56^{cde}	244.70^{a}	2.96^{a}	1.117^{bcde}	1.54^{ef}
TB	0.353^{cd}	38.04^{cdef}	0.397^{cd}	0.88^{cde}	285.93^{cd}	0.38^{de}	17.25^{c}	1.28^{defg}	0.965^{cde}	2.16^{ef}
Salamander broiler										
GM	0.367^{cd}	61.72^{bcd}	0.394^{cd}	1.39^{bcd}	265.29^{d}	0.67^{bcde}	66.51^{bc}	1.34^{cdef}	1.170^{bcde}	1.80^{ef}
IF	0.411^{cd}	51.20^{bcde}	0.378^{cd}	0.52^{e}	215.62^{d}	0.36^{de}	67.88^{bc}	1.26^{defgh}	1.527^{ab}	5.49^{cdef}
LL	0.412^{cd}	21.83^{ef}	0.382^{cd}	0.54^{de}	254.22^{d}	0.56^{cde}	67.71^{bc}	0.89^{fgh}	0.983^{cde}	2.67^{ef}
PM	0.383^{cd}	64.13^{bcd}	0.436^{cd}	0.77^{de}	193.53^{d}	0.74^{bcd}	33.65^{bc}	1.15^{efgh}	1.271^{bcd}	13.88^{b}
SV	3.800^{b}	62.58^{bcd}	0.451^{cd}	1.28^{bcde}	278.57^{cd}	0.73^{bcd}	106.72^{b}	1.74^{bcd}	1.138^{bcde}	1.87^{f}
TB	5.727^{a}	64.98^{bc}	0.490^{bcd}	1.28^{bcde}	326.79^{cd}	1.15^{ab}	39.34^{bc}	1.42^{bcdef}	1.11^{bcde}	5.68^{cdef}
SEM[2]	1.058	14.81	0.111	0.36	141.63	0.21	30.71	0.23	0.240	3.73
P-value	<0.001	0.024	0.008	0.004	0.011	0.034	0.005	0.038	0.003	0.001

[1]Muscles included Gluteus medius (GM), Infraspinatus (IF), Longissimus lumborum (LL), Psoas major (PM), Serratus ventralis (SV), and Triceps brachii (TB).

[2]SE (largest) of the least squares means in the same main effect.

[abcdef]Least squares means in the same column without a common superscript differ ($P < 0.05$).

Table 3.10. Least squares means of volatile compounds produced from beef steaks cooked under four different dry heat cookery methods[1] (n = 480).

	Cooking method					
Compound	CHAR	CLAM	OVEN	SALA	SEM[2]	*P*-value
Strecker aldehydes						
Acetaldehyde	15.34	20.40	14.10	15.15	2.57	0.709
3-methylbutanal	2.76[a]	2.07[a]	1.32[b]	2.20[a]	0.27	<0.001
2-methylbutanal	3.31[a]	1.80[bc]	1.07[c]	2.11[b]	0.34	<0.001
Benzaldehyde	34.48[a]	29.10[ab]	20.54[c]	26.17[bc]	2.56	0.002
Phenylacetaldehyde	1.123[a]	1.030[a]	0.557[c]	0.697[b]	0.045	<0.001
Maillard intermediate						
2,3-butanediol	38.69	56.90	53.68	64.44	10.37	0.206
Pyrazines						
Methyl-pyrazine	4.05[a]	1.23[b]	0.57[b]	0.75[b]	0.27	<0.001
Trimethylpyrazine	3.73[a]	1.51[b]	0.48[c]	0.69[c]	0.17	<0.001
Sulfur-containing compounds						
Methanethiol	3.27[ab]	4.50[a]	3.19[b]	2.79[b]	0.57	0.027
Dimethyl disulfide	0.026[b]	0.082[a]	0.036[b]	0.042[b]	0.016	0.040
Carbon disulfide	4.56[b]	4.10[b]	4.87[b]	7.61[a]	0.33	<0.001
2-methyl thiophene	0.801[a]	0.309[b]	0.239[b]	0.212[b]	0.056	<0.001
Lipid-derived alcohols						
Ethanol	6.94	10.01	7.20	8.94	1.29	0.245
1-octen-3-ol	2.48	3.03	4.27	4.15	0.58	0.081
1-octanol	4.81[b]	4.90[b]	7.29[a]	4.55[b]	0.49	<0.001
Carboxylic acids						
Acetic acid	3.37[b]	3.04[b]	3.19[b]	4.26[a]	0.15	<0.001
Heptanoic acid	1.88[b]	1.72[b]	2.68[a]	1.73[b]	0.11	<0.001
Nonanoic acid	0.559[bc]	0.636[ab]	0.434[c]	0.719[a]	0.057	<0.001
Octanoic acid	63.87[b]	62.86[b]	79.56[a]	57.86[b]	4.13	0.002
Esters						
Hexanoic acid, methyl ester	0.604[ab]	0.351[b]	1.032[a]	0.745[ab]	0.174	0.048
Nonanoic acid, methyl ester	0.250[bc]	0.232[c]	0.282[a]	0.274[ab]	0.010	0.001
Propanoic acid, methyl ester	0.877[a]	0.761[b]	0.724[b]	0.777[b]	0.035	0.007
Ketones						
2-propanone	47.6	48.4	53.0	64.4	5.40	0.092
2-pentanone	0.301[a]	0.193[b]	0.207[b]	0.237[b]	0.020	<0.001
Lipid-derived aldehydes						
Decanal	225.84[a]	168.50[b]	181.05[b]	198.05[ab]	13.38	0.021
Heptanal	12.43	14.08	16.76	16.22	1.70	0.234
Nonanal	7.74[b]	10.57[a]	7.42[b]	6.55[b]	0.87	0.007
Pentanal	0.99[c]	1.58[bc]	2.24[ab]	2.63[a]	0.38	0.011
Hydrocarbons						
Toluene	18.00[a]	7.08[bc]	5.70[c]	8.28[b]	0.91	<0.001
Octane	2.23	1.67	1.63	1.69	0.21	0.126
Pentane	4.11[b]	4.65[b]	5.91[ab]	7.12[a]	0.74	0.006
Total volatile production	1955.99[a]	966.34[b]	989.17[b]	1120.21[b]	92.80	<0.001

[1]Cooking methods included charbroiler grill (CHAR), clamshell grill (CLAM), convection oven (OVEN), and salamander broiler (SALA).

[2]SE (largest) of the least squares means in the same main effect.

[abc]Least squares means in the same column without a common superscript differ ($P < 0.05$

Table 3.11. Least squares means of volatile compounds produced from six different beef muscles (n = 480).

	Muscle							
Compound	GM	IF	LL	PM	SV	TB	SEM	*P*-value
Strecker aldehydes								
Acetaldehyde	15.4^{bc}	9.6^{c}	12.1^{bc}	16.8^{abc}	24.1^{a}	19.5^{ab}	3.20	0.018
3-methylbutanal	2.38^{ab}	1.35^{c}	2.21^{abc}	1.73^{bc}	2.97^{a}	1.88^{bc}	0.33	0.002
2-methylbutanal	3.18^{a}	0.88^{b}	1.88^{b}	1.35^{b}	3.39^{a}	1.75^{b}	0.44	<0.001
Benzaldehyde	30.05^{ab}	20.82^{c}	24.21^{bc}	25.35^{bc}	27.25^{bc}	37.70^{a}	3.14	0.003
Phenylacetaldehyde	1.048^{a}	0.698^{bc}	0.831^{b}	0.680^{c}	0.802^{bc}	1.05^{a}	0.053	<0.001
Maillard intermediate								
2,3-butanediol	36.60^{cd}	58.45^{bc}	21.45^{d}	78.65^{ab}	84.91^{a}	40.50^{cd}	15.75	<0.001
Pyrazines								
Methyl-pyrazine	2.15^{a}	1.46^{bc}	1.51^{abc}	0.95^{c}	1.92^{ab}	1.91^{ab}	0.30	0.026
Trimethylpyrazine	1.59	1.46	1.42	1.36	1.61	2.19	0.22	0.077
Sulfur-containing compounds								
Methanethiol	3.63^{ab}	2.15^{b}	2.81^{b}	3.30^{b}	5.41^{a}	3.34^{b}	0.79	0.050
Carbon disulfide	5.11^{bc}	4.38^{cd}	4.29^{cd}	5.61^{b}	8.45^{a}	3.87^{d}	0.41	<0.001
Dimethyl disulfide	0.040	0.016	0.044	0.033	0.092	0.053	0.021	0.124
2-methyl thiophene	0.354	0.368	0.314	0.324	0.505	0.478	0.065	0.062
Lipid derived alcohols								
Ethanol	9.50^{a}	3.88^{b}	7.30^{ab}	7.72^{ab}	10.22^{a}	11.04^{a}	1.79	0.029
1-octen-3-ol	2.73^{bc}	2.81^{bc}	1.95^{c}	3.81^{b}	6.48^{a}	3.11^{bc}	0.67	<0.001
1-octanol	4.69	5.19	4.63	5.71	6.75	5.37	0.66	0.117
Carboxylic acids								
Acetic acid	3.51^{b}	2.93^{c}	3.01^{c}	3.77^{ab}	4.03^{a}	3.53^{b}	0.17	<0.001
Heptanoic acid	1.74^{cd}	2.01^{bc}	1.62^{d}	2.18^{ab}	2.50^{a}	1.94^{bcd}	0.13	<0.001
Nonanoic acid	0.482	0.684	0.540	0.549	0.565	0.704	0.07	0.098
Octanoic acid	69.66^{b}	60.26^{b}	45.84^{c}	64.89^{b}	85.34^{a}	70.23^{b}	4.84	<0.001
Esters								
Hexanoic acid, methyl ester	0.532^{b}	0.468^{b}	0.476^{b}	1.327^{a}	0.848^{ab}	0.444^{b}	0.208	0.010
Nonanoic acid, methyl ester	0.256	0.240	0.261	0.270	0.274	0.257	0.012	0.414
Propanoic acid, methyl ester	0.695	0.769	0.827	0.826	0.795	0.799	0.042	0.165
Ketones								
2-propanone	62.5^{b}	32.2^{cd}	29.5^{d}	56.9^{b}	88.8^{a}	50.4^{bc}	6.7	<0.001
2-pentanone	0.215^{bc}	0.211^{bc}	0.178^{c}	0.246^{b}	0.339^{a}	0.218^{bc}	0.024	<0.001
Lipid-derived aldehydes								
Decanal	179.12	196.38	189.01	207.61	187.69	200.34	16.00	0.800
Heptanal	12.90^{b}	11.67^{b}	12.80^{b}	16.46^{ab}	20.69^{a}	14.73^{b}	2.11	0.024
Nonanal	7.93	7.85	6.76	7.83	8.35	9.65	1.08	0.541
Pentanal	1.25^{b}	1.36^{b}	1.25^{b}	2.30^{ab}	3.37^{a}	1.64^{b}	0.47	0.005
Hydrocarbons								
Toluene	11.99^{a}	7.25^{c}	8.67^{bc}	7.37^{c}	10.72^{ab}	12.58^{a}	1.07	<0.001
Octane	1.89^{b}	1.32^{bc}	1.44^{bc}	1.97^{b}	3.07^{a}	1.15^{c}	0.26	<0.001
Pentane	5.02^{bc}	3.74^{c}	3.34^{c}	6.04^{b}	9.41^{a}	5.10^{bc}	0.90	<0.001
Total volatile production	1402.58^{ab}	1038.64^{c}	1006.69^{c}	1136.19^{bc}	1627.38^{a}	1336.07^{b}	106.76	<0.001

[1]Muscles included Gluteus medius (GM), Infraspinatus (IF), Longissimus lumborum (LL), Psoas major (PM), Serratus ventralis (SV), and Triceps brachii (TB).
[2]SE (largest) of the least squares means in the same main effect.
[abc]Least squares means in the same main effect (cooking method or muscle) without a common superscript differ (P < 0.05).

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CHAPTER 4
DETERMINATION OF PACKAGE AND MUSCLE TYPE INFLUENCE ON PROTEOLYSIS, BEEF FLAVOR CONTRIBUTING FREE AMINO ACIDS, FINAL BEEF FLAVOR AND TENDERNESS

ABSTRACT

The objectives of this study were to determine: the influence of package and muscle type on postmortem proteolysis and subsequent release of flavor contributing free-amino acids during storage and distribution. Additionally, to determine the influence of packaging type on final beef flavor and tenderness through volatile flavor compounds, descriptive sensory analysis, and Warner-Bratzler shear force. Strip loins and top sirloin butts (n = 20/subprimal) from USDA Low Choice carcasses were fabricated into 2.54 cm steaks (M. Longissimus lumborum and M. Gluteus medius) at 7 d postmortem. Steaks were randomly assigned to packaging treatments: carbon monoxide motherbag (**CO**), high oxygen modified atmosphere packaging (**HIOX**), polyvinyl overwrap (**OW**) and rollstock (**ROLL**); and aged for 14 d in dark storage. Steaks intended for OW were initially vacuum packaged during dark storage, then overwrapped just prior to display. Steaks were placed in coffin-style retail display for 48-h under fluorescent lighting. High oxygen steaks exhibited ($P < 0.05$) the highest Warner-Bratzler shear force values, lowest desmin degradation rate ($P < 0.05$), and the highest ratings for fishy, bitter, sour, and oxidized flavors, the lowest overall tenderness scores ($P < 0.05$), and, in general, produced the lowest amount of free amino acids ($P < 0.05$) compared to all other treatments. Contrastingly, ROLL packaging produced the highest ratings for beef flavor identity, brown/roasted, bloody/serumy, and umami flavors ($P < 0.05$). Additionally, ROLL packaging exhibited ($P < 0.05$) greater desmin degradation in comparison to

HIOX steaks. This data indicates the optimum package for storage and aging is an anaerobic environment to maintain flavor, tenderness, and postmortem proteolysis.

Key words: aging, beef, packaging type, postmortem proteolysis, tenderness, volatile flavor compounds

INTRODUCTION

Packaging method of meat products is an important factor in the meat industry, as it serves to protect product, improve shelf life and quality, as well as factors into the consumer's purchasing decision (McMillin, 2017; Polkinghorne et al., 2018). Different packaging types can result in different eating experiences. Multiple consumer studies in both the United States and Australia have consistently shown modified atmosphere packaging (**MAP**) to be lower than both polyvinyl overwrap and vacuum packaging for tenderness, juiciness, flavor liking, and overall liking when fed to consumers (Polkinghorne et al., 2019; Ponce et al., 2019). Additionally, high oxygen MAP has been implicated with increased toughness when compared to vacuum packaged or polyvinyl chloride overwrapped steaks (Geesink et al., 2015; Moczkowska et al., 2017). Currently, toughening of beef steaks after exposure to high oxygen environments is not fully understood. Conflicting results are reported, as Geesink et al. (2015) did not observe any differences in desmin degradation in high oxygen MAP. However, Moczkowska et al. (2017) and Fu et al. (2017) both observed increased desmin degradation in vacuum packaging in comparison to high oxygen MAP. Increased desmin degradation would indicate a greater amount of postmortem proteolysis occurring, therefore resulting in a more tender product.

From a flavor standpoint, high oxygen MAP has been shown to produce lower beef flavor identity and umami ratings, as well as increased oxidized, cardboardy, and sour flavors when analyzed by trained descriptive panels (Ponce et al., 2019). This is likely due to induced lipid oxidation from the high oxygen MAP's oxidative environment, which contributes greatly to production of off flavors (Min and Ahn, 2005; Bekhit et al., 2013).

Previous research indicates that packaging type has an impact on tenderization and flavor development of meat products. With regard to tenderization, Fu et al. (2017) reported increased desmin degradation in vacuum packaging in comparison to both polyvinyl overwrap and modified atmosphere packaging. Similarly, Moczkowska et al. (2017) reported vacuum packaging had increased degradation of both desmin and troponin-T in both the M. Longissimus lumborum and M. Biceps femoris. Moreover, Moczkowska et al. (2017) reported reduced Warner-Bratzler shear force values in vacuum packaged steaks for both muscles, which indicates an increased rate of proteolysis occurred in vacuum packages rather than modified atmosphere packaged steaks. Additionally, modified atmosphere packaging and polyvinyl overwrap produced a greater amount of carbonyl products from oxidation in both the M. Psoas major and M. Semimembranosus in comparison to vacuum packaging (Fu et al., 2017). The impact of many postmortem production practices on beef flavor is unknown, or the scope has not been fully investigated. Therefore, the objective of this study was to determine the influence of package and muscle type on postmortem proteolysis and subsequent release of flavor contributing free-amino acids during storage and distribution and on final beef character.

MATERIALS & METHODS

Product selection & subprimal fabrication

Beef strip loins (IMPS #180, NAMP 2010) and top sirloin butts (IMPS #184, NAMP 2010) were selected from USDA Low Choice A maturity carcasses (n = 20) free of quality defects for this study. Trained Texas Tech University (**TTU**) personnel collected data for yield and quality grading, including preliminary yield grade; ribeye area, kidney, pelvic, and heart fat; skeletal and lean maturity; and marbling score. Subprimals were collected in three separate collections. Following each collection, subprimals were transported under refrigeration (0 to 4°C) to the Gordon W. Davis Meat Laboratory in Lubbock, Texas. Subprimals were wet aged in the dark for 7 d postmortem, then fabricated into representative steaks of the M. Gluteus medius (**GM**) and the M. Longissimus lumborum (**LL**). A steak from the most anterior portion of each subprimal was saved (not subjected to retail display) and immediately frozen at -20°C to be used as a negative control for raw steak analyses. Steaks were then randomly assigned into one of four packaging schemes: carbon monoxide motherbag (0.4% CO/ 30% CO_2/ 69.6% N_2; **CO**), high oxygen modified atmosphere lidded trays (80% O_2/20% CO_2; **HIOX**), polyvinyl overwrap (**OW**), and rollstock (forming and non-forming films (T6035B and T6235B, Sealed Air, Cryovac, Charlotte, NC, **ROLL**). High oxygen modified atmosphere packages were created using a Mondini Tray Sealer, CV/VG-S (Cologne, Italy). The trays used for MAP packages had an oxygen transmission rate (OTR) of 0.1 cc/d at 73°C at 0% relative humidity (RH), and a moisture vapor transmission rate (MVTR) of 2 g/d. The tray film used for the MAP packages had an OTR of 7 cc/m^2/d at 40°C at 0% RH, and a MVTR of 9 g/m^2/d at 38°C at 100% RH. Steaks placed in ROLL packaging were produced using a Multivac Baseline F100 (Kansas City, MO) using a

forming film (OTR: 2 cc/m^2/d at 23°C at 0% RH; MVTR of 7 g/m^2/d at 38°C at 100% RH) and non-forming film (OTR of 3 cc/m^2/d at 23°C at 0% RH, and a MVTR of9 g/m^2/d at 38°C at 100% RH). A Minipack-torre, Minispenser (Dalmine, Italy) was used to produce the OW packaging. Prior to retail display, OW steaks were stored in ROLL packaging. Steaks were held in their respective packaging type for an additional 14 d of aging in the absence of light. Following the aging period, steaks were subjected to a 48-h retail display under continuous fluorescent lighting in coffin-style cases with a temperature range of 2 - 4℃. Cases were rotated every 12 h with light intensity measurements taken concurrently. Immediately following retail display, steaks were removed from their respective packaging and vacuum packaged, then frozen at -20℃ until further analysis.

Trained descriptive panel analysis

Trained descriptive panel analysis was conducted according to the AMSA Sensory Guidelines (AMSA, 2015). Seven panelists were trained according to the AMSA Sensory guidelines for thirteen traits: beef flavor identity, brown/roasted, bloody/serumy, fat-like, liver-like, oxidized, fishy, buttery, umami, bitter, sour, overall juiciness, and overall tenderness, described in Table 4.1. Panelists evaluated two cubed samples on continuous 100 point line scales using digital surveys on tablets (Qualtrics Surveys, Provo, UT; iPad, Apple, Inc., Cupertino, CA). Each scale was anchored at each endpoint and had a neutral midpoint (0 = extremely bland/dry/tough; 50 = neither tough/dry nor tender/juicy, 100 = extremely tender/juicy/intense). Panels consisted one steak of each treatment (n = 8) in a randomly assigned order. Prior to panel analysis, steaks were thawed for 24 h at 2 to 4℃. Using clamshell grills (Cuisinart Griddler Deluxe GR-250,

Cuisinart, Stamford, CT), steaks were cooked to a medium degree of doneness (71°C). After cooking, steaks were cut into steak thickness × 1 × 1 cm cubes and two cubes were served to each panelist.

Western blot analysis

Western blot analysis was conducted using the methods of Knobel (2014) and Phelps et al. (2015). Samples for both Western blot and free amino acid analysis were prepared for analysis through liquid nitrogen homogenization. Accessory muscles, external fat, and connective tissue were removed, then steaks were diced. The cubes were placed into liquid nitrogen and frozen, then homogenized using a food processor (Robot Coupe Blixer 3, Robot Coupe USA, Jacksonville, MS). Following homogenization, samples were stored at -80°C for approximately 1 month until further analysis.

Desmin and troponin-T (**TnT**) were the proteins of interest. Proteins were isolated from muscles using whole muscle extraction buffer. Following the addition of the buffer, samples were mixed on a vortex mixer at 2000 RPM for 2 min, then centrifuged for 15 min at 15,000 × *g.* Protein concentration was determined using the Pierce BCA protein assay (Thermo Fisher Scientific, Fairlawn, NJ). To confirm protein concentration, a NanoDrop 1000 spectophotometer was used to analyze protein concentration at 562 nm. Following concentration analysis, all samples were diluted to a similar concentration using phosphate buffered saline (**PBS**) and Modified Wang's tracking solution was added with β-mercaptoethanol, then incubated for 10 min at 100°C. Proteins were loaded on to a Novex 4 to 12% Bis Tris Gel (Invitrogen, Grand Island, NY) and were separated via electrophoresis. Gels ran for 35 min at 165 V and 30 mA. Following running, gels were transferred to nitrocellulose membranes for 7 min. Membranes were then incubated with

non-fat dry milk (Bio-Rad, Hercules, CA) and 10% 10 × tris buffered saline (**TBS**) for 1 h at 25℃ to block for non-specific antibody binding. Primary antibody solution consisting of antibodies for desmin (1:10 dilution, ab6322, Anti-desmin cytoskeleton marker, Abcam, Cambridge, UK) and TnT (1:10 dilution, ab83907, Anti-Troponin/TNT antibody, Abcam), and 1% TBS-Tween was then added, and samples were incubated and gently rocked overnight at 4℃. Membranes were then rinsed with 1% TBS-Tween solution 3 times each for 5 min, then secondary antibodies were added to the membranes and allowed to incubate in the absence of light on a rocker for 1 h at 25℃. Secondary antibody solution consisted of 1% TBS-Tween solution and 1:2 dilution of antibodies (desmin: A21126, AlexaFluor 633 Goat Anti-Mouse; TnT: A21070, AlexaFluor 633 Goat Anti-Rabbit; Thermofisher Scientific). Following secondary antibody incubation, membranes were again rinsed 3 times for 5 min with 1% TBS-Tween solution. Following incubation, membranes were dried and imaged using a VersaDoc Imaging System (Bio-Rad); bands were detected and measured using the Quantity One Band Analysis software (Bio-Rad). Degraded and intact forms of desmin were measured with bands located at approximately 55 kDA and TnT was measured at approximately 30 kDA. Band intensity was equalized to a pooled sample on each blot. Average intensity of each band was measured in relation to the internal standard and reported as measurements of relative degradation.

Free amino acid analysis

Free amino acid analysis was conducted using the modified methods of Koutsidis et al. (2008). For analysis, 3 g of sample was weighed into a 50 mL conical tube. Ten mL of autoclaved, cold, double-distilled water was added to each sample, then shaken for 10

min. Following shaking, samples were centrifuged at 29,900 × g for 33 min. All supernatant was decanted, then an additional 5 mL of water was added. Samples were re-extracted as described previously, then the two extracts were combined together. The combined supernatant was filtered through a 0.2 µm disc filter. Free amino acids (n = 23) were derivatized using 100 µL of the aqueous extract from the combined supernatant and an EZ-Faast amino acids kit (Phenomenex, Torrance, CA). Free amino acid content was determined using a gas chromatography-mass spectrometry in electron impact mode with a 3:1 split ratio (6890A; 5975B, Agilent, Santa, Clara, CA). Derivatives were separated using a Zebron ZB-AAA capillary column (10 m × 0.25 mm; 0.25 µm film thickness, Phenomenex). A three-level calibration curve based on response and concentration ratios between an internal standard (norvaline) and authentic standards for each amino acid was used for quantitation (mmol/ kg initial wet sample).

Warner-Bratzler shear force

Warner-Bratzler shear force (**WBSF**) was conducted according to the AMSA Sensory Guidelines (AMSA, 2015). Steaks were cooked as previously described for both trained panel and volatile compound analysis. Following the removal of volatile compound analysis cores, steaks were chilled for 12 h at 2 to 4°C. Six 1.27 cm cores were removed parallel to the muscle fiber orientation. Cores were then sheared perpendicular to the muscle fiber using a WBSF instrument equipped with a v-shaped blade with a 200 mm/min crosshead speed (GR-151, Tall Grass Solutions, Manhattan, KS). Measurements were recorded as peak force (kg) and averaged across the six cores for each steak.

Volatile compound analysis

The methods of Gardner and Legako (2018) were used to determine volatile compound composition of steaks. Steaks designated for volatile compound analysis were prepared as previously described for trained descriptive panel analysis. Immediately following cooking, six 1.27 cm cores were removed from the center of the steak perpendicular to the steak cut surface. The cores were then minced for 10 sec using a coffee grinder (4 to 12 cup Mr. Coffee grinder; Sunbeam Corporation, Boca Raton, FL). Five grams of sample was weighed into 20 mL glass vials (Gerstel Inc, Linthicum, MD). Ten microliters of internal standard (1, 2-dichlorobenzene, 2.5 mg/µL) was pipetted into the vial and then sealed using a polytetrafluoroethylene septa screw cap (#093640-040-00, 1.3 mm polytetrafluoroethylene septa and metal screw cap; Gerstel Inc, Linthicum, MD). The samples were then loaded using a Gerstel automatic sampler (MPS; Gerstel, Inc) for a 5 min incubation time at 65°C in the Gerstel agitator prior to a 25 min extraction time. Solid phase microextraction (**SPME**) was used to collect the volatile compounds from the headspace of the sample with an 85 µm film thickness carboxen polydimethylsiloxane fiber (Supelco Inc., Bellefonte, PA). Volatile compounds extracted from the headspace were placed onto a VF-5 MS capillary column (30 m × 0.25 mm × 1.0 µm; Agilent J&W GC Column; Agilent Technologies, Inc., Santa Clara, CA). Authentic standards (Sigma-Aldrich, St. Louis, MO) were used to confirm compound identities through retention time. Furthermore, authentic standards were utilized to quantitate individual volatile compounds relative to sample weight (ng/g).

Statistical analysis

Data was analyzed as a 2 × 4 factorial design with muscle, packaging type, and their interaction serving as fixed effects. Individual package served as the experimental unit. Collection and carcass ID were incorporated into the model as a random effect for all analyses. For cooked analyses, peak temperature was included as a covariate. Probability values (p-values) less than or equal to $\alpha = 0.05$ were considered significant. The Kenward-Rogers adjustment was also used to estimate denominator degrees of freedom.

RESULTS & DISCUSSION

Carcass characteristics

Carcass characteristics are present in Table 4.2. Carcasses used in this study were from the USDA Choice quality grade (Small^{00}-Small^{100} marbling score) and A maturity. Additionally, carcasses possessed approximately 1.0 cm preliminary and 1.2 cm adjusted fat thickness with 100.6 cm^2 ribeye area. Moreover, carcasses also possessed approximately 3.5% kidney, pelvic, and heart fat, with an average final yield grade of 2.9.

Trained descriptive panels

No interactions ($P \geq 0.233$) between packaging scheme and muscle type were observed for trained panel attributes (Table 4.3). Additionally, no differences ($P \geq 0.056$) in any sensory traits were observed between the GM and the LL. However, packaging type had a substantial impact on flavor and tenderness when evaluated by the trained panel. No differences were observed ($P \geq 0.357$) between packaging schemes for fat-like, liver-like, buttery, or salty flavors. When evaluating positive flavor traits, steaks in OW and ROLL packaging produced greater beef flavor identity ($P < 0.05$) in comparison to

both CO and HIOX packaging. Moreover, HIOX steaks were the lowest for beef flavor identity ($P < 0.05$) when compared to all other treatments. Overwrap and ROLL steaks produced more brown roasted flavors ($P < 0.05$) in comparison to HIOX steaks; however, CO steaks were similar to both treatment groups ($P > 0.05$). For bloody/serumy, HIOX steaks produced ($P < 0.05$) the least bloody/serumy flavor compared to all other treatments. However, ROLL steaks produced ($P < 0.05$) the highest ratings for bloody/serumy compared to OW steaks but was similar ($P > 0.05$) to CO steaks. When evaluating negative flavor traits, HIOX packaging produced the most intense oxidized and fishy flavors ($P < 0.05$) in comparison to all other treatments, followed by CO packaging ($P < 0.05$). For the basic tastes, ROLL and OW produced the most intense umami flavor ($P < 0.05$) compared to all other treatments. For bitter, HIOX steaks were most intense ($P < 0.05$) compared to all other treatments, followed by CO and ROLL packaging ($P < 0.05$), with OW steaks producing the least intense bitter flavor ($P < 0.05$). Additionally, HIOX packaging produced the most intense sour flavor ($P < 0.05$) compared to all other treatments. Furthermore, CO packaging produced juicier steaks ($P < 0.05$) than HIOX steaks, but ROLL and OW were similar to both treatments ($P > 0.05$). High oxygen packaging also produced the least tender steaks ($P < 0.05$) in comparison to all other treatments.

From a flavor standpoint, HIOX MAP has been shown to produce lower beef flavor identity, umami, and tenderness ratings, as well as increased oxidized, cardboardy, and sour flavors when analyzed by trained descriptive panels (Ponce et al., 2019). This is likely due to induced lipid oxidation from the oxidative environment of HIOX MAP, which contributes greatly to production of off flavors (Min and Ahn, 2005; Bekhit et al.,

2013). Additionally, this is likely due to the increased lipid oxidation products observed in the volatile compound analysis.

Rollstock and OW packages produced a greater umami flavor compared to all other treatments. This is likely due to the increased concentration of aspartic and glutamic acid (Table 4.7), two free amino acids linked to increased umami flavors (Dashdorj et al., 2015).

Western blot analysis

Results from Western blot analysis are present in Table 4.4. No differences were observed in TnT degradation for packaging type ($P = 0.442$), muscle ($P = 0.074$) or their interaction ($P = 0.093$). However, desmin was readily impacted by both packaging type ($P < 0.001$) and muscle ($P < 0.001$) with no interactive effects ($P = 0.153$). Initial samples pulled 7 d postmortem and HIOX samples possessed ($P < 0.05$) the greatest relative intensity of desmin compared to all other packaging types, which indicates a higher concentration of desmin and less degradation during postmortem proteolysis. Additionally, GM samples exhibited ($P < 0.05$) greater relative intensity of desmin when compared to LL samples, which indicates that LL steaks had a greater amount of desmin degradation.

These results are partially in agreement with previous work. Moczkowska et al. (2017) and Fu et al. (2017) both reported increased desmin degradation in LL steaks stored in vacuum packaging, as observed in the current study. However, in the current study, TnT degradation was similar across treatments, whereas Moczkowska et al. (2017) observed increased TnT degradation in LL steaks. Additional research with oxidative environments has also indicated similar rates of TnT degradation throughout packaging

types (Kim et al., 2010; Xue et al., 2012). However, both of these studies have reduced aging periods (14 d and 9 d) compared to the current study (21 d). Additionally, because of TnT's location in the thin filament of the sarcomere, it is less likely to be impacted by postmortem aging than desmin, a cytoskeletal protein located on the z-disks of the sarcomere, which is one of the first sites of the sarcomere impacted through postmortem proteolysis (Filatov et al., 1999; Lonergan et al., 2010).

Free amino acid analysis

Only one amino acid, beta-alanine, ($P \geq 0.629$) was not significant for muscle, packaging type, or their interaction. Three amino acids- aspartic acid, cysteine, and orthinine were impacted by the interaction of packaging type and muscle ($P < 0.011$; Table 4.5). For aspartic acid and cysteine, LL ROLL and OW steaks produced ($P < 0.05$) the greatest amount of each respective amino acid. Aspartic acid and cysteine contribute umami, savory, meat-like, and sulfurous flavors to meat products (Dashdorj et al., 2015). These flavors were present in increased intensity in ROLL and OW steaks, according to the trained panelists in the current study (Table 4.5). The increased concentration of aspartic acid and cysteine as free amino acids in LL ROLL and OW steaks indicate an increased reservoir of positive amino acids to contribute to beefy, savory flavors in steaks through the Maillard reaction. In comparison, LL and GM HIOX steaks produced ($P < 0.05$) the lowest concentration of aspartic acid and cysteine, as LL ROLL steaks exhibited almost seven times more cysteine than LL HIOX steaks. Similarly, GM ROLL steaks possessed 5.25 times more cysteine than GM HIOX steaks. For ornithine, LL ROLL steaks possessed ($P < 0.05$) the most ornithine compared to all other treatments, possessing 1.5 times more ornithine than GM ROLL steaks.

Six amino acids (alanine, cystine, glycine, proline, tyrosine, and valine) were impacted ($P \leq 0.01$) by both the main effects of packaging (Table 4.6) and muscle (Table 4.7). For all six amino acids, LL steaks possessed a greater ($P < 0.05$) concentration of each respective amino acid in comparison to GM steaks. Additionally, initial, d 0, unaged samples possessed the lowest ($P < 0.05$) concentration of all free amino acids, as they were not able to be freed through the postmortem aging process. Valine and glycine were present in the greatest ($P < 0.05$) concentration in OW and ROLL steaks, followed by CO which was greater than HIOX ($P < 0.05$). High oxygen steaks only contained greater ($P < 0.05$) concentrations of valine and glycine than the initial unaged subprimal samples. Similarly, ROLL and OW steaks possessed ($P < 0.05$) a greater concentration of proline than HIOX steaks; however, CO steaks were intermediate and similar ($P > 0.05$) to both treatment groups in proline concentration. Cystine and alanine were present ($P < 0.05$) in greater concentrations in OW steaks compared to HIOX steaks. Both ROLL and CO steaks were intermediate ($P > 0.05$) and similar to both treatments for cystine and alanine concentration. Cystine is known for contributing meat-like, sweet, and sulfurous flavor to meat products due to its sulfurous side chain (Dashdorj et al., 2015). Alanine also contributes both sweet and sour flavors to meat products (Dashdorj et al., 2015). These free amino acids may have contributed to the increase in beef flavor identity ratings for OW steaks compared to HIOX steaks when fed to trained panelists in the current study. Previously, no work has evaluated the impact of packaging types on flavor precursors, such as free amino acids.

Contrastingly, tyrosine was present ($P < 0.05$) in the highest concentration in HIOX steaks compared to ROLL steaks. The increase in tyrosine in HIOX steaks likely

influenced the bitterness ratings observed by trained panelists, as tyrosine is a water-soluble taste-active compound that contributes to bitter flavors (Dashdorj et al., 2015). Additionally, proteins with large amounts of tyrosine residues are more susceptible to oxidation via singlet oxygen (Papuc et al., 2017). Moreover, tyrosine also has a hydroxyl group present on the aromatic ring of its side chain which renders it especially labile to oxidation (Papuc et al., 2017). By forcing tyrosine's abstraction from peptide chains through protein oxidation, it would be present in greater amounts in HIOX environments compared to the anaerobic ROLL environment.

The majority of free amino acids (n = 12; Table 4.6) were impacted solely by the packaging main effect ($P < 0.04$). Initial samples from the beginning of the aging period exhibited the lowest concentration of amino acids compared to all other treatments, with the exception of histidine ($P < 0.05$). With the exception of histidine, ROLL and OW steaks possessed ($P < 0.05$) the greatest concentration of the remaining free amino acids, followed by CO steaks ($P < 0.05$), then HIOX steaks ($P < 0.05$). Histidine was present ($P < 0.05$) in greater concentrations in HIOX steaks in comparison to ROLL, CO, and initial steaks. Overwrap steaks were similar to all other treatments ($P > 0.05$). Proteins with amino acid residues with high electron density, such as histidine or tyrosine, are very labile to oxidation by singlet oxygen (Papuc et al., 2017). Examples of these proteins would be myoglobin, which uses histidine to play key structural roles in maintaining myoglobin structure and function (Mancini and Hunt, 2005). In an oxidative environment, it could contribute to increased release of histidine from HIOX steaks. This increase in histidine concentration, similar to tyrosine, likely contributed to the increased

bitter intensity of HIOX steaks reported by the trained panelists in the current study, as it has been linked to bitter flavors (Dashdorj et al., 2015).

Glutamine was the lone amino acid impacted by a muscle main effect ($P = 0.001$; Table 4.7). Similar to other amino acids, LL steaks possessed ($P < 0.05$) a greater concentration of glutamine compared to GM steaks. Glutamine has been observed to be a precursor to α-ketoglutarate, an important component to the Krebs cycle (Tapiero et al., 2002). The LL has consistently been rated higher by trained panelists than the GM for beef flavor, and it is likely that glutamine's contribution to those beefy flavors have aided with that advantage (Calkins and Hodgen, 2007).

Warner-Bratzler shear force

No interactions or muscle effects were observed ($P > 0.05$) for WBSF (Table 4.8). However, HIOX packaging produced the greatest ($P < 0.05$) WBSF values compared to all other treatments, followed by CO, ROLL, and OW. These packaging results are in agreement with the previous literature. Moczkowska et al. (2017), Zakrys-Waliwander et al. (2012), and Lagerstedt et al. (2011) observed substantial differentiation between HIOX MAP and vacuum packaged LL steaks, as HIOX steaks were substantially higher for WBSF. Additionally, Kim et al. (2010) observed increased star probe values (another instrumental determination for tenderness) for HIOX steaks compared to vacuum packaged steaks. Although an instrumental measurement of tenderness, the packaging differences also translated to a difference observed by the trained panelists in the current study (Table 4.3), as they rated HIOX steaks lower for tenderness compared to all other treatments. This is likely due to reduced protein degradation occurring postmortem, as observed by the Western blot results of this study. Previous literature indicates that

oxidative environments can arrest the aging process through the inactivation of calpains (Rowe et al., 2004; Kemp et al., 2010; Lonergan et al., 2010; Xue et al., 2012). If calpains are being inactivated during the aging period because of the HIOX environment, it would explain the increased WBSF, reduced desmin degradation, and reduced tenderness scores observed by trained panelists in HIOX steaks.

Volatile compound analysis

Seven compounds- benzaldehyde, 2,3-butanediol, hexanal, hexanoic acid, 2-pentylfuran, nonanal, and ethanol, elicited a packaging type × muscle interaction ($P \leq 0.034$; Table 4.9). For all interactions except 2,3-butanediol and ethanol, HIOX GM steaks produced the greatest concentration ($P < 0.05$) compared to all other treatments. High oxygen GM steaks produced ($P < 0.05$) 2.6 times the amount of hexanal than the next closest mean. This trend was apparent throughout these compounds, however for hexanoic acid, HIOX LL steaks were similar ($P > 0.05$) to HIOX GM steaks, indicating that regardless of muscle, hexanoic acid was produced in exorbitant amounts in HIOX packaging. These lipid derived compounds are primarily products of lipid oxidation (Min and Ahn, 2005). The combination of the HIOX packaging with the oxidation labile GM escalated the lipid oxidation process and forced oxidation products to be produced in inflated concentrations. For 2,3-butanediol and ethanol, CO GM steaks produced these intermediate compounds in the highest ($P < 0.05$) concentrations compared to all other treatments. 2,3-butanediol is a C4 sugar fragment Maillard reaction intermediate that originates from the retro-aldol reactions of reducing sugars and is a metabolite of acetaldehyde (Yaylayan and Keyhani, 1999; Martins et al., 2000). Additionally, Enterobacteriaceae have been implicated with the production of 2,3-butanediol through

fermentation and the metabolism of acetaldehyde. Previous work has indicated that the GM possesses increased concentrations of these compounds (Legako et al., 2015a; Hunt et al., 2016), however, this effect was limited to only CO GM steaks. This indicates that CO may have interfered with the cooking process, hence increasing the concentration of 2,3-butanedione. Since 2,3-butanedione is a Maillard intermediate, these results imply that CO is halting the Maillard reaction prior to the retro-aldol reaction, Strecker degradation, and production of sulfur containing compounds, resulting in a build-up of 2,3-butanedione during cooking (Mottram et al., 1982; Mottram, 1993, 1998).

Three compounds, 2-heptanone, 2-propanone, and octanoic acid, were impacted by both a packaging main effect ($P \leq 0.002$) and muscle main effect ($P \leq 0.013$; Table 4.10). For all three compounds, HIOX steaks produced ($P < 0.05$) a greater concentration than all other treatments. Additionally, GM steaks produced ($P < 0.05$) a greater concentration of all three compounds compared to LL steaks. Lipid derived ketones are primarily produced via lipid oxidation and have negative impacts on flavor (Min and Ahn, 2005). The GM is more labile to oxidation than the LL, which is more stable, which likely contributed to the increased concentration of 2-heptanone and 2-propanone produced (Lanari and Cassens, 1991; Colle et al., 2015). Similarly, octanoic acid, a straight chain saturated fatty acid, has an unpleasant, rancid odor and taste (Bekhit et al., 2013). It is developed through lipid oxidation, so it is logical for it to be present in the greatest amount in HIOX GM steaks (Bekhit et al., 2013)

Seventeen compounds, primarily lipid derived, were impacted by a packaging main effect ($P \leq 0.048$; Table 4.11). Not surprisingly, for all of the lipid derived alcohols, aldehydes, and the lone carboxylic acid, HIOX steaks produced ($P < 0.05$) the greatest

concentration compared to all other treatments. However, HIOX steaks also produced ($P < 0.05$) the highest concentration of 2,3-pentanedione, a Maillard reaction intermediate and methanethiol, a sulfur-containing compound developed during the Maillard reaction through cysteine, methionine, and methional degradation, compared to all other treatments (Resconi et al., 2013). These results indicate that oxidation can arrest the Maillard reaction, as these compounds typically undergo further reactions, such as the retro-aldol reaction and heterocyclization (Bekhit et al., 2013). Moreover, previous work has illustrated the antagonistic effect of lipid derived reactive carbonyls and phenolic compounds on production of Strecker aldehydes (Delgado et al., 2016). When added together with phenylalanine, phenylacetaldehyde production was substantially reduced (Delgado et al., 2016). This indicates that oxidation products halt the further production of different compounds.

Two compounds were impacted by the muscle main effect ($P \leq 0.003$; Table 4.12). The GM steaks produced a greater concentration of 2,3-butanedione ($P = 0.003$) and 3-hydroxy-2-butanone ($P = 0.002$) than the LL steaks. In previous studies, the GM has produced greater concentrations of 2,3-butanedione compared to the LL (Legako et al., 2015). Additionally, 3-hydroxy-2-butanone is a Maillard reaction-produced ketone, which is associated with buttery flavors and has previously been observed in increased levels in GM steaks over LL steaks (Legako et al., 2015)

CONCLUSIONS

This work clearly indicates environment and muscle type influence beef flavor and tenderness. Results from this study contribute to the growing understanding of beef flavor development and help to validate the impediment of proteolysis and tenderness

development by high oxygen environments. These results distinctly illustrate high oxygen packaging is detrimental to quality.

Table 4.1. Descriptive attributes and references.

Flavor attribute	Anchor	Location on scale (0 – 100)
Beef Flavor Identity	Beef broth (heated to 74°C, served warm)	30
	80% ground chuck (71°C)	50
	Brisket (71°C)	75
Bloody/Serumy	USDA Choice strip steak (60°C)	40
Brown/Roasted	80% ground chuck (71°C)	40
	Well done strip steak (77°C)	65
Fat-like	90/10 ground beef (71°C)	30
	70/30 ground beef (71°C)	60
Liver-like	Flat iron steak (71°C)	20
	Calf liver	90
Oxidized	Microwaved vegetable oil	30
	Cooked, stored (12 h at 4°C) and microwaved ground beef (71°C)	60
Buttery	Unsalted butter, 0.1 cm thick slice	65
Fishy	Cod liver oil	30
	Canned tuna	60
Umami	Beef broth, sodium free (heated to 74°C, served warm)	30
Sour	0.015% Citric acid	10
	0.050% Citric acid	25
Salty	0.15% NaCl	10
	0.25% NaCl	45
Bitter	0.01% Caffeine	15
	0.02% Caffeine	25
Overall tenderness	Eye of round (77°C)	30
	Strip steak (71°C)	55
	Tenderloin (65°C)	90
Overall juiciness	Strip steak (85°C)	25
	Strip steak (71°C)	50
	Strip steak (60°C)	75

Table 4.2. Least square means (± SEM[1]) of beef carcass (*n* = 20) measurements

Carcass Characteristics	
Quality Attributes	
Lean maturity[2]	139 ± 21
Skeletal maturity[2]	124 ± 28
Overall maturity[2]	130 ± 21
Marbling score[3]	443 ± 24
Yield Attributes	
Preliminary fat thickness, cm	1.0 ± 0.4
Adjusted fat thickness, cm	1.2 ± 0.4
Ribeye area, cm^2	100.6 ± 10.6
Hot carcass weight, kg	412.4 ± 39.4
Kidney, pelvic, and heart fat, %	3.5 ± 0.5
Final yield grade	2.9 ± 0.4

[1]SE of the mean.
[2]100 = A; 200 = B.
[3]200 = Traces; 300 = Slight; 400 = Small.

Table 4.3. Least squares means of trained descriptive panel evaluation[1] of beef steaks (n = 160) from two different muscles[2] and four different packaging schemes

	Beef Flavor Identity	Brown/ Roasted	Bloody/ Serumy	Fat-Like	Liver -Like	Oxidized	Fishy	Buttery	Umami	Salty	Bitter	Sour	Overall Juiciness	Overall Tenderness
Packaging type														
Carbon monoxide[3]	34.9^{b}	31.2^{a}	14.4^{ab}	14.2	8.2	29.3^{b}	23.6^{b}	13.5	22.6^{b}	7.1	10.4^{b}	11.7^{b}	47.1^{a}	52.3^{a}
High oxygen[4]	28.6^{c}	28.1^{b}	10.5^{c}	13.6	8.4	43.0^{a}	35.4^{a}	12.7	19.5^{c}	6.4	12.8^{a}	14.5^{a}	42.6^{b}	47.9^{b}
PVC overwrap[5]	39.3^{a}	33.8^{a}	13.6^{b}	13.5	8.6	23.8^{c}	17.9^{c}	13.0	25.2^{a}	6.8	8.8^{c}	10.1^{b}	44.6^{ab}	52.3^{a}
Rollstock[6]	41.7^{a}	32.6^{a}	17.3^{a}	15.1	8.4	18.8^{d}	14.3^{c}	14.9	26.2^{a}	7.3	10.2^{b}	11.1^{b}	44.7^{ab}	53.8^{a}
SEM[7]	1.5	1.5	1.4	0.9	0.9	2.0	2.2	1.3	0.9	0.4	0.7	0.9	1.3	1.6
P-value	< 0.001	0.003	< 0.001	0.390	0.971	< 0.001	< 0.001	0.497	< 0.001	0.357	< 0.001	< 0.001	0.048	0.011
Muscle														
GM	35.5	31.1	13.8	14.0	8.7	30.2	24.0	13.8	22.8	6.6	11.0	12.5	44.8	51.7
LL	36.7	31.8	14.0	14.2	8.1	27.2	21.5	13.3	23.9	7.2	10.1	11.2	44.7	51.3
SEM	1.3	1.3	1.2	0.8	0.8	1.7	1.7	1.1	0.8	0.3	0.6	0.8	1.0	1.3
P-value	0.250	0.536	0.834	0.787	0.389	0.058	0.174	0.639	0.143	0.063	0.065	0.056	0.995	0.747
Packaging × Muscle														
P-value	0.812	0.103	0.901	0.551	0.564	0.310	0.920	0.546	0.606	0.619	0.361	0.368	0.233	0.918

[1]Sensory scores: 0 = absence of specific flavor/extremely tough/dry, 50 = neither tough nor tender/neither dry nor juicy, 100 = extremely intense specific flavor/extremely tender/juicy.

[2]Muscles included the Gluteus medius (GM) and Longissimus lumborum (LL).

[3]Carbon monoxide motherbag (0.4% CO/ 30% CO_2/ 69.6% N_2)

[4]High oxygen modified atmosphere packaging (80% O_2/20% CO_2)

[5]Polyvinyl overwrap; prior to retail display, OW steaks were stored in ROLL packaging.

[6]Rollstock.

[7]SE (largest) of the least squares means in the same main effect (packaging type or muscle).

[abc]Least squares means in the same main effect (packaging type or muscle) without a common superscript differ (P < 0.05).

Table 4.4. Least squares means of relative intensity of desmin and troponin-T from beef steaks (n = 160) from two muscles and four packaging schemes

	Desmin	Troponin-T
Treatment		
Packaging type		
No packaging[1]	1.28[a]	0.99
Carbon monoxide[2]	0.97[b]	0.95
High oxygen[3]	1.03[a]	0.98
Overwrap[4]	0.98[b]	0.95
Rollstock[5]	0.97[b]	0.98
SEM[6]	0.06	0.03
P-value	< 0.001	0.442
Muscle		
Gluteus medius	1.09[a]	0.98
Longissimus lumborum	1.00[b]	0.96
SEM	0.05	0.01
P-value	< 0.001	0.074
Packaging × Muscle		
P-value	0.263	0.093

[1]Initial sample taken at the beginning of the aging period; no packaging treatment or aging applied.

[2]Carbon monoxide motherbag (0.4% CO/ 30% CO_2/ 69.6% N_2)

[3]High oxygen modified atmosphere packaging (80% O_2/20% CO_2)

[4]Polyvinyl overwrap; prior to retail display, overwrap steaks were stored in rollstock packaging.

[5]Rollstock.

[6]SE (largest) of the least squares means in the same main effect (packaging type or muscle).

[ab]Least squares means in the same main effect (packaging type or muscle) without a common superscript differ ($P < 0.05$).

Table 4.5. Interaction of packaging type and muscle on free amino acid content of beef steaks (n = 160) from two different muscles and four different packaging schemes

Free amino acid, mmol/kg	Aspartic acid	Cysteine	Orthinine
Gluteus medius			
No packaging[1]	0.019^{d}	0.389^{c}	0.068^{e}
Carbon monoxide[2]	0.048^{bcd}	0.186^{d}	0.101^{bcd}
High oxygen[3]	0.027^{cd}	0.130^{d}	0.085^{cde}
Overwrap[4]	0.051^{bc}	0.542^{bc}	0.106^{bc}
Rollstock[5]	0.043^{bcd}	0.682^{b}	0.117^{b}
Longissimus lumborum			
No packaging	0.022^{cd}	0.459^{c}	0.077^{e}
Carbon monoxide	0.066^{b}	0.420^{c}	0.087^{cde}
High oxygen	0.032^{cd}	0.158^{d}	0.077^{de}
Overwrap	0.099^{a}	0.942^{a}	0.112^{bc}
Rollstock	0.112^{a}	1.110^{a}	0.171^{a}
SEM[6]	0.011	0.081	0.012
P-value	0.011	0.004	0.009

[1]Initial sample taken at the beginning of the aging period; no packaging treatment or aging applied.

[2]Carbon monoxide motherbag (0.4% CO/ 30% CO_2/ 69.6% N_2)

[3]High oxygen modified atmosphere packaging (80% O_2/20% CO_2)

[4]Polyvinyl overwrap; prior to retail display, overwrap steaks were stored in rollstock packaging.

[5]Rollstock.

[6]SE (largest) of the least squares means in the same main effect (packaging type or muscle).

[abcde]Least squares means in the same main effect (packaging type or muscle) without a common superscript differ ($P < 0.05$).

Table 4.6. Least squares means of free amino acid content of beef steaks (n = 160) from four different packaging schemes.

	Packaging Type						
	No packaging[1]	Carbon monoxide[2]	High oxygen[3]	Overwrap[4]	Rollstock[5]	SEM[6]	*P*-value
Free amino acid, mmol/kg							
Alanine	3.797^{c}	5.240^{ab}	5.058^{b}	5.510^{a}	5.332^{ab}	0.284	0.008
Asparagine	0.167^{d}	0.298^{b}	0.257^{c}	0.360^{a}	0.342^{a}	0.014	< 0.001
Cystine	3.797^{c}	5.240^{ab}	5.058^{b}	5.511^{a}	5.332^{ab}	0.284	< 0.001
Glycine	1.094^{d}	1.478^{b}	1.356^{c}	1.673^{a}	1.610^{a}	0.090	0.003
Glutamic acid	0.720^{c}	1.520^{b}	1.293^{b}	1.781^{a}	1.863^{a}	0.091	< 0.001
Histidine	4.791^{b}	5.232^{b}	6.664^{a}	5.628^{ab}	5.395^{b}	0.431	0.036
Hydroxyproline	0.027^{c}	0.042^{ab}	0.038^{b}	0.047^{a}	0.041^{ab}	0.004	< 0.001
Isoleucine	0.428^{d}	0.928^{b}	0.792^{c}	1.047^{a}	1.055^{a}	0.471	< 0.001
Leucine	0.662^{c}	1.490^{b}	1.336^{b}	1.662^{a}	1.664^{a}	0.081	< 0.001
Lysine	0.309^{c}	0.650^{b}	0.650^{b}	0.762^{a}	0.708^{ab}	0.038	< 0.001
Methionine	0.128^{d}	0.362^{b}	0.309^{c}	0.430^{a}	0.420^{a}	0.020	< 0.001
Phenylalanine	0.255^{c}	0.626^{b}	0.562^{b}	0.700^{a}	0.704^{a}	0.035	< 0.001
Proline	0.332^{c}	0.437^{ab}	0.427^{b}	0.470^{a}	0.470^{a}	0.025	0.006
Serine	0.579^{b}	1.255^{a}	1.182^{a}	1.374^{a}	1.281^{a}	0.095	< 0.001
Threonine	0.394^{c}	0.731^{ab}	0.633^{b}	0.808^{a}	0.831^{a}	0.056	< 0.001
Tryptophan	0.028^{c}	0.060^{ab}	0.055^{b}	0.067^{a}	0.057ab	0.005	< 0.001
Tyrosine	0.245^{c}	0.508^{ab}	0.533^{a}	0.514^{ab}	0.461^{b}	0.043	< 0.001
Valine	0.786^{d}	1.613^{b}	1.412^{c}	1.809^{a}	1.816^{a}	0.088	0.01
Total free amino acids	15.227	22.735	22.773	25.334	24.987	1.043	< 0.001

[1]Initial sample taken at the beginning of the aging period; no packaging treatment or aging applied.

[2]Carbon monoxide motherbag (0.4% CO/ 30% CO_2/ 69.6% N_2)

[3]High oxygen modified atmosphere packaging (80% O_2/20% CO_2)

[4]Polyvinyl overwrap; prior to retail display, overwrap steaks were stored in rollstock packaging.

[5]Rollstock.

[6]SE (largest) of the least squares means in the same main effect (packaging type).

[abcd]Least squares means in the same main effect (packaging type) without a common superscript differ ($P < 0.05$).

Table 4.7. Least squares means of free amino acids from beef steaks (n = 160) from two different muscles

	Muscle Type			
	Gluteus medius	Longissimus lumborum	SEM[6]	*P*-value
Free amino acid, mmol/kg				
Alanine	4.825[b]	5.150[a]	0.263	< 0.001
Cystine	4.825[b]	5.150[a]	0.265	0.008
Glutamine	0.014[b]	0.017[a]	0.003	0.001
Glycine	1.387[b]	1.498[a]	0.084	< 0.001
Proline	0.408[b]	0.446[a]	0.022	< 0.001
Tyrosine	0.431[b]	0.474[a]	0.039	0.050
Valine	1.410[b]	1.564[a]	0.071	< 0.001

[1]SE (largest) of the least squares means in the same main effect (muscle type).
[ab]Least squares means in the same main effect (packaging type) without a common superscript differ ($P < 0.05$).

Table 4.8. Least squares means of Warner-Bratzler shear force values of beef steaks (*n* = 160) from two muscles and four packaging schemes

	Warner-Bratzler shear force, kgf
Packaging type	
Carbon monoxide[1]	2.5[b]
High oxygen[2]	3.1[a]
Overwrap[3]	2.2[c]
Rollstock[4]	2.4[bc]
SEM[5]	0.1
P-value	< 0.001
Muscle	
Gluteus medius	2.5
Longissimus lumborum	2.6
SEM	0.1
P-value	0.355
Packaging type × muscle	
P-value	0.275

[1]Carbon monoxide motherbag (0.4% CO/ 30% CO_2/ 69.6% N_2)

[2]High oxygen modified atmosphere packaging (80% O_2/20% CO_2)

[3]Polyvinyl overwrap; prior to retail display, overwrap steaks were stored in rollstock packaging.

[4]Rollstock.

[5]SE (largest) of the least squares means in the same main effect (packaging type or muscle).

[abc]Least squares means in the same main effect (packaging type or muscle) without a common superscript differ ($P < 0.05$).

Table 4.9. Interaction of packaging type and muscle on the production of volatile flavor compounds from beef steaks (n = 160)

Volatile compound, ng/g	Benzaldehyde	2,3-butanediol	Hexanal	Hexanoic acid	2-pentylfuran	Nonanal	Ethanol
Gluteus medius							
Carbon monoxide[1]	15.54^{b}	189.06^{a}	76.87^{b}	119.59^{bc}	4.72^{b}	2.37^{b}	90.46^{a}
High oxygen[2]	29.74^{a}	62.59^{b}	667.76^{a}	479.12^{a}	31.94^{a}	8.18^{a}	19.65^{b}
Overwrap[3]	14.64^{b}	119.43^{b}	156.46^{b}	157.73^{bc}	4.03^{b}	3.28^{b}	19.82^{b}
Rollstock[4]	14.06^{b}	89.79^{b}	109.39^{b}	116.66^{bc}	5.18^{b}	2.18^{b}	21.10^{b}
Longissimus lumborum							
Carbon monoxide[1]	12.35^{b}	77.49^{b}	86.98^{b}	113.79^{bc}	4.99^{b}	2.14^{b}	18.29^{b}
High oxygen[2]	16.19^{b}	76.43^{b}	254.40^{b}	220.08^{a}	7.11^{b}	3.75^{b}	10.69^{b}
Overwrap[3]	14.41^{b}	77.40^{b}	136.20^{b}	99.14^{c}	7.27^{b}	2.95^{b}	26.64^{b}
Rollstock[4]	13.33^{b}	81.74^{b}	118.23^{b}	108.12^{bc}	5.19^{b}	1.85^{b}	32.67^{b}
SEM[5]	2.61	28.83	74.00	42.02	4.19	0.84	13.68
P-value	0.034	0.019	0.008	0.003	0.002	0.020	0.005

[1]Carbon monoxide motherbag (0.4% CO/ 30% CO_2/ 69.6% N_2)

[2]High oxygen modified atmosphere packaging (80% O_2/20% CO_2)

[3]Polyvinyl overwrap; prior to retail display, overwrap steaks were stored in rollstock packaging.

[4]Rollstock.

[5]SE (largest) of the least squares means in the same main effect (packaging type or muscle).

[ab]Least squares means in the same main effect (packaging type or muscle) without a common superscript differ (P < 0.05).

Table 4.10. Least squares means of volatile compounds produced from beef steaks (*n* = 160) of four packaging schemes and two muscles

	2-heptanone	2-propanone	Octanoic acid
Packaging type			
Carbon monoxide[1]	2.86[b]	105.52[b]	26.55[b]
High oxygen[2]	5.68[a]	150.66[a]	32.95[a]
Overwrap[3]	2.06[b]	112.67[b]	25.72[b]
Rollstock[4]	2.04[b]	82.38[b]	24.80[b]
SEM[5]	0.53	12.27	2.37
P-value	< 0.0001	0.001	0.039
Muscle			
Gluteus medius	3.79[a]	130.53[a]	31.68[a]
Longissimus lumborum	2.53[b]	95.09[b]	23.33[b]
SEM	0.38	8.55	1.72
P-value	0.014	0.003	< 0.0001
Muscle × Packaging			
P-value	0.129	0.561	0.944

[1]Carbon monoxide motherbag (0.4% CO/ 30% CO_2/ 69.6% N_2)

[2]High oxygen modified atmosphere packaging (80% O_2/20% CO_2)

[3]Polyvinyl overwrap; prior to retail display, overwrap steaks were stored in rollstock packaging.

[4]Rollstock.

[5]SE (largest) of the least squares means in the same main effect (packaging type or muscle).

[ab]Least squares means in the same main effect (packaging type or muscle) without a common superscript differ ($P < 0.05$).

Table 4.11. Least squares means of volatile compounds produced from beef steaks (*n* = 160) of four packaging types

	Packaging type					
Volatile compound, ng/g	Carbon monoxide[1]	High oxygen[2]	Overwrap[3]	Rollstock[4]	SEM[2]	*P*-value
Maillard reaction products						
Strecker aldehydes						
Isobutyraldehyde	7.94[ab]	7.11[b]	10.38[a]	7.19	0.96	0.049
Methional	12.29[a]	9.68[b]	10.41[b]	10.18[b]	0.71	0.004
Ketone						
2,3-pentanedione	0.21[b]	0.82[a]	0.23[b]	0.19[b]	0.11	< 0.001
Sulfur-containing compound						
Methanethiol	3.37[ab]	4.10[a]	2.87[b]	2.63[b]	0.36	0.020
Lipid degradation products						
Alcohols						
1-hexanol	11.42[b]	70.18[a]	10.56[b]	12.68[b]	16.88	0.001
1-octanol	6.02[b]	9.97[a]	4.95[b]	4.22[b]	0.84	< 0.001
1-octen-3-ol	12.33[b]	29.89[a]	12.30[b]	11.67[b]	3.36	< 0.001
1-pentanol	11.66[b]	27.11[a]	12.44[b]	15.05[b]	2.78	< 0.001
Aldehydes						
Decanal	104.92[a]	83.67[ab]	67.86[b]	62.54[b]	9.99	0.008
Heptanal	9.58[b]	30.23[a]	13.32[b]	8.91[b]	3.11	< 0.001
Octanal	0.99[b]	2.18[a]	1.22[b]	0.85[b]	0.19	< 0.001
Pentanal	1.65[b]	4.80[a]	2.66[b]	2.06[b]	0.47	< 0.001
Alkanes						
Pentane	11.66[b]	17.74[a]	8.97[b]	7.43[b]	1.59	< 0.001
Tetradecane	0.71[a]	1.27[b]	0.83[b]	0.52[ab]	0.22	0.035
Carboxylic acid						
Benzoic acid	0.31[ab]	0.37[a]	0.40[ab]	0.25[b]	0.03	0.014
Esters						
Butanoic acid, methyl ester	0.82[b]	0.67[b]	1.33[a]	1.12[ab]	0.21	0.022
Hexanoic acid, methyl ester	9.97	24.34	9.28	13.13	4.94	0.038

[1]Carbon monoxide motherbag (0.4% CO/ 30% CO_2/ 69.6% N_2).

[2]High oxygen modified atmosphere packaging (80% O_2/20% CO_2).

[3]Polyvinyl overwrap; prior to retail display, overwrap steaks were stored in rollstock packaging.

[4]Rollstock.

[5]SE (largest) of the least squares means in the same main effect (packaging type).

[ab]Least squares means in the same main effect (packaging type) without a common superscript differ ($P < 0.05$).

Table 4.12. Least squares means of volatile compounds produced from beef steaks (*n* = 160) of two muscles

	Muscle type			
	GM	LL	SEM	*P*-value
2,3-butanedione	154.83[a]	113.16[b]	12.23	0.003
3-hydroxy-2-butanone	184.41[a]	125.33[b]	17.00	0.002

[1]Muscles in the study included the Gluteus medius (GM) and Longissimus lumborum (LL).

[2]SE (largest) of the least squares means in the same main effect.

[ab]Least squares means in the same main effect without a common superscript differ ($P < 0.05$).

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