THE EFFECTS OF EXERCISE ON BEEF CATTLE HEALTH, PERFORMANCE, AND CARCASS QUALITY; AND THE EFFECTS OF EXTENDED AGING, BLADE TENDERIZATION, AND DEGREE OF DONENESS ON BEEF AROMA VOLATILE FORMATION

by

BRYCE MARK GERLACH

B.S., University of Nebraska-Lincoln, 2008
M.S., Kansas State University, 2010

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Animal Sciences and Industry
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Abstract

Two experiments were conducted to evaluate the effects of exercise on feedlot cattle well-being, growth performance, and carcass characteristics (Experiments 1 and 2). Additionally, two experiments assessed the volatile compound profiles of beef in response to various postmortem processes (Experiments 3 and 4). Experiment 1 compared heifers (n=30) assigned to either a sedentary treatment or an exercise regimen 3 times/wk (20 min/d for the first 2 wk, 30 min/d for the next 2 wk, and 40 min/d for the final wk). Exercised heifers showed decreased blood insulin, daily gains, final body weights, and lower numerical yield grades in conjunction with decreased a* and b* color values of *longissimus* muscle lean. Experiment 2 exposed steers (n=419) to 1 of 4 treatments; not exercised (CON), or exercised 3 times/wk by animal handlers for 20-30 min for the first 10 wk (EARLY), the last 7 wk (LATE), or for the entire 116 d finishing period (ALL). EARLY treatment cattle exhibited a decrease in blood insulin while EARLY and ALL treatment cattle produced carcasses with decreased marbling scores in comparison to CON cattle. Experiment 3 assessed the volatile compounds generated by raw and cooked beef *gluteus medius* steaks (n=30) subjected to blade tenderization and aging times ranging from 5 to 61 d. Raw steaks aged longer than 19 d produced greater concentrations of heptanal, octanal, and nonanal than raw steaks aged 5 d, but cooked steaks showed no change in these compounds in response to aging. Additionally, blade tenderization reduced the concentrations of lipid oxidation and Maillard reaction products in cooked steaks. Experiment 4 investigated cross-sections, external, and internal locations of beef *longissimus lumborum* steaks (n=54) cooked to 63, 71, or 77 °C and aged for 5, 21, or 37 d. External locations generated aldehydes in greater amounts than internal locations while pyrazines are produced exclusively at external locations. Increases in degree of doneness increased all aldehydes, except nonanal, in steak cross-sections. Aldehydes, except hexanal and octanal, generated from external locations became similar to internal locations as aging times increased. Pyrazines from external locations were reduced by increased aging times.

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Preface

This dissertation is written in accordance with the style guidelines designated by Meat Science, the official scientific journal of the American Meat Science Association. This dissertation covers two distinct topics. **Chapters 1-4** are focused on the influence of exercise in feedlot cattle. **Chapter 1** is a review of literature that pertains to the areas of consumer perception of meat production, cattle health, cattle temperament, meat quality, and meat composition. **Chapter 2** details a pilot study that evaluated routine exercise of feedlot heifers. **Chapter 3** investigates routine exercise of feedlot steers in a commercial scenario. **Chapter 4** concludes the first section and discusses overall conclusions regarding the implementation of routine exercise in feedlot cattle. **Chapters 5-8** are concentrated on the evaluation of aroma volatile compounds generated by raw and cooked beef. **Chapter 5** is a review of literature that relates to the development of cooked beef aroma and alterations of flavor instigated by postmortem handling of beef. **Chapter 6** investigates the volatile compounds produced by raw and cooked *gluteus medius* steaks that were subjected to multiple aging times and blade tenderization. **Chapter 7** further evaluates cooked beef aroma volatiles from different areas of *longissimus lumborum* steaks cooked to different degrees of doneness and subjected to different aging times. **Chapter 8** draws conclusions from the studies that evaluated aroma volatiles produced by beef.

Chapter 1 - Review of literature: Exercise of feedlot cattle

1. Consumer perceptions of meat production

Over the past 15 years consumers have increased their concern regarding the way food products are produced (Grunert, Bredahl, and Brunso, 2004). Grunert and others (2004) reported that consumers are interested in: 1) organic production, 2) animal welfare, and 3) products manufactured in a "natural" way. The USDA recognizes many verified processes like "Non-Hormone Treated Cattle (NHTC)", "Natural", and "Organic" that are gaining traction as a marketing element. These processes are many times perceived to be animal-friendly even though they may not be directly related to animal welfare. Animal welfare is interpreted differently by different people and scientific research may not be an accurate gauge of how society views animal welfare issues. A majority of society assesses animal welfare using emotion and perception of rearing practices, but assessments of animal welfare in scientific literature are based on observed measures (Buller & Roe, 2012).

Progressive livestock operations in the U.S. may develop alternative production practices that appeal to consumers from an animal welfare perspective in an effort to garner monetary incentives from the retail sector. Buller and Roe (2012) detailed that 'naturality' along with confinement and density are the most common aspects of animal welfare that could be advanced to attract certain consumers. 'Naturality' was most commonly associated with a term like 'free-range' and confinement is readily linked with stocking densities. Lastly, an overriding theme related to animal welfare is the thought that it enhances the quality of a product or is somehow improving product taste and healthfulness or indicative of a local, independent producer. This study displays that consumers have instinctive beliefs in regards to the manner in which meat animals are produced.

Food production and manufacturing processes can influence the way consumers make purchasing decisions and may even influence their opinions on regulatory issues regarding food animals. California and Arizona, among other states, have voted in favor of laws that ban the use of cages for housing egg-laying chickens as well as stalls and crates for housing sows and veal calves (Richards, Allender, & Fang, 2013). These laws on livestock production substantiate the public interests and attention to welfare of livestock. The increase in governmental regulation

also signals a need for farms and ranches to evaluate their practices and develop methods that are designed to enhance animal well-being.

Understanding how consumers will react or accept production methods developed to improve animal well-being is of utmost importance. Frewer, Kole, Van De Kroon, and De Lauwere (2005) surveyed Dutch consumers on attitudes towards husbandry of swine and fish. Results demonstrated that consumers thought about animal welfare in two broad categories: 1) animal health, and 2) suitability of the environment in which animals live. Consumers also rated animal welfare orientated production as more important in pig production than fish production. Respondents were very adamant that food products produced with the use of animal friendly production systems be labeled as such. Farmers were labeled as more trustworthy and knowledgeable than government and supermarkets in matters associated with animal welfare. This study makes it readily apparent that consumers are concerned with animal production methods and request that those producers making distinct efforts to improve animal well-being have the opportunity to differentiate their products at the retail counter.

Animal welfare is important to consumers, but it is necessary to assess if this translates to their purchasing habits. A study conducted by Olynk, Tonsor, and Wolf (2010) evaluated willingness of consumers to purchase pork chops and milk produced from verified processes. The authors conducted a survey using direct and indirect questioning to evaluate acceptance of four production processes: individual crates/stalls, pasture access, antibiotic use, and certified trucking/transport. Results from this study indicated that consumers were more willing to pay for products that were raised by processes verified by the USDA compared to processes verified by a private party, consumer group, or by producers. Their attitude toward USDA certification was quantified by their willingness to pay an additional $1.50-$2/lb for pork produced using USDA verified production methods compared to other verification types. Additionally, respondents were most willing to pay for products verifying that animals had access to pasture followed by verification of antibiotic use. Many meat purchasers show an interest in regard to methods used to produce the meat they purchase and, with proper oversight, certain production claims can entice consumers to pay a premium for these products.

The creation and use of a verified production system that promotes animal welfare could provide additional revenue streams for beef producers. The industry must not lose sight of creating an ample supply of beef that is affordable for a majority of buyers that are not enticed by

different production claims. Additionally, it is crucial that palatability components of beef are not adversely impacted so that consumer acceptance of beef is maintained.

2. Insulin-resistance in feedlot cattle

Glucose is necessary for the function of many tissues. Insulin levels increase after a meal is consumed to stimulate glucose transport, metabolism, and storage by muscle and adipocytes (Shepherd & Kahn, 1999). An overabundance of glucose can be toxic (Rosetti, Giaccari, & DeFronzo, 1990) and chronic hyperglycemia can induce insulin-resistance. The resulting impaired glucose transport is associated with type 2 diabetes mellitus in humans (Shepherd & Kahn, 1999).

Cattle fed concentrate-based diets in feedlot settings have increased performance, efficiency, and ribeye area as well as increased quality grades compared to cattle fed forage-based diets (Bennett et al., 1995). As a result, most beef in the U.S. is produced from cattle fed high-concentrate diets primarily composed of corn (Vasconcelos & Galyean, 2007), which is high in starch content. Ruminant animals that are fed high-starch diets produce greater amounts of propionate which is readily converted to glucose (Huntington, 1997). The high levels of glucose produced via ruminal fermentation of high-starch diets could create hyperglycemic conditions that lead to increased insulin-resistance. Limiting the intake of high-starch feedstuffs is not a viable option as they are integral to producing high quality beef in an efficient and economically feasible manner. Therefore, it is important to evaluate complimentary production approaches that may alleviate concerns with insulin-resistance to improve health of feedlot cattle.

In humans, physical exercise has been labeled an important addition to treatment of both type 1 and type 2 diabetes mellitus (Goodyear & Kahn, 1998). A meta-analysis conducted by Boulé, Haddad, Kenny, Wells, and Sigal (2001) evaluated the effects of exercise on glycemic control to combat symptoms of type 2 diabetes mellitus. Studies that evaluated exercise interventions of at least 8 wk in duration and were devoid of drug cointerventions were included in the analysis. Exercised treatment groups had decreased levels of glycosylated hemoglobin in comparison to control groups. The observed levels of glycosylated hemoglobin were similar to those observed in treatment groups that combined diet and exercise. Contrarily, the body mass of subjects receiving exercise treatment were similar to body weights of the non-exercised control

groups. The reduction of glycosylated hemoglobin by exercise should reduce incidences of diabetic complications without altering body mass in humans and it is important to understand why this occurs.

Exercise induced changes in insulin sensitivity are due to an increase of glucose uptake in the body. Goodyear and Kahn (1998) reviewed the impact of exercise on glucose uptake via the GLUT 4 transporter. A single session of exercise increases glucose uptake in skeletal muscles which is mediated by the GLUT 4 transporter. Additionally, chronic exercise has shown the capability to increase GLUT 4 expression in skeletal muscle and enhance the responsiveness of muscle glucose uptake to insulin. These effects have also been seen in other mammals as exercise increases insulin sensitivity (Da Silva et al., 2010) and improves glucose metabolism in obese rats (Nara, Takahashi, Kanda, Shimomura, & Kobayashi, 1997).

Research evaluating the implementation of exercise to combat insulin-resistance in feedlot cattle is negligible; however, other animal models have been used. Exercise has shown potential to inhibit the development of type 2 diabetes mellitus in *Psammomys obesus*, rats that develop type 2 diabetes mellitus within 2 wk of exposure to high energy diets (Heled et al., 2005). *Psammomys obesus* were used in a 2 x 2 factorial experiment and assigned to low or high energy diets and exercise or non-exercised treatments. All animals in the high energy, non-exercised group became diabetic, but none of the animals in the high energy exercised group became diabetic. This study showed that exercise enhanced the expression of TNFα, its receptor R1 and the glucose transporter GLUT 1 in muscle tissue. This study outlines the potential for exercise to alleviate diabetic symptoms in animals produced in situations that promote the development of insulin-resistance.

Carter, McCutcheon, Valle, Meilahn, and Geor (2010) evaluated the effect of exercise training on overweight or obese, insulin-resistant geldings. Horses were either exercised at a high intensity for 4 wk, low intensity for 4 wk, or remained sedentary throughout the study. Although weight and fat mass were decreased, these researchers observed no change in the parameters of glucose and insulin dynamics in the exercised geldings. It is apparent that exercise may not improve animal health, in regards to diabetic symptoms, in similar manners across species and it is necessary to evaluate the species of interest to better understand their response to exercise.

Feedlot cattle live a very sedentary lifestyle as their only daily activities are associated with eating and drinking. The unhealthy lifestyle of a sedentary animal fed a high-starch diet

could contribute to diabetic-like symptoms indicative of poor health. The capability of exercise to inhibit the development of type 2 diabetes mellitus in other species suggests that exercise has potential to improve the health of cattle fed high-starch diets. Exercise could possibly decrease insulin-resistance in feedlot cattle by increasing expression of GLUT 1 and GLUT 4 transporters, ultimately increasing their glucose uptake in skeletal muscle and reducing diabetic symptoms.

3. Cattle temperament and animal performance

Livestock temperament is showing an increased importance as it can influence animal performance and carcass traits. Various methods have been used to assess cattle temperament and stress when processing cattle through a handling chute. Chute scores, that consider the animals' calmness and excitability while being restrained in a chute, and the velocity at which the cattle exit a processing chute are often used to assess temperament (Burdick, Randel, Carroll, & Welsh, 2011). These types of temperament scoring systems have related directly to basal concentrations of glucocorticoids and catecholamines (Burdick et al., 2011). In most mammals, including humans and cattle, cortisol is the primary glucocorticoid released during stress (Burdick et al., 2011). Additionally, during stress in cattle, epinephrine is the primary catecholamine produced by the adrenal medulla in conjunction with limited amounts of norepinephrine (Burdick et al., 2011). All of these factors can be used in classifying cattle into temperament groups as well as assessing the stress they incur during handling.

Managing cattle to have docile temperaments could be beneficial to producers and packers as it pertains to animal performance and carcass quality. Reinhardt, Busby, and Corah (2009) used feedlot cattle produced in southwest Iowa from 2002 to 2006 to correlate the relationship between various performance and carcass traits with phenotypic traits, such as disposition. Steers and heifers were assigned disposition scores (1 = calm, slowly walks out of chute and down exit alley; 6 = extremely excitable, agitated, jumps when exiting chute, runs away from chute) during initial on-test weighing, at re-implanting, and during final sorting prior to shipment to the abattoir. Disposition influenced multiple traits. Cattle with higher disposition scores showed decreased initial body weight, average daily gain, and final body weight. Moreover, temperamental cattle produced carcasses that displayed decreased fat thickness, hot carcass weights, *longissimus* muscle areas, marbling scores, and USDA quality grades. Additionally, cattle with higher disposition scores exhibited a decreased percentage of carcasses

that graded USDA Prime and Upper 2/3 Choice, but increased the percentage of carcasses that graded USDA Select and Standard. Lastly, cattle with higher disposition scores produced a greater percentage of Yield grade 1 and 2 carcasses along with a reduced percentage of Yield grade 3 and 4 carcasses. Animal disposition, in regards to handling, is not only important for ease of animal handling, but can provide performance and beef quality improvements which offer monetary incentive to producers to rear docile animals.

Animal temperament is usually assessed when humans are present, which is often only associated with times of processing and shipping. Many rearing systems have minimal amounts of human-animal interaction which could cause cattle to react disproportionally to stressful situations when humans are involved. There is evidence that an animal's fear of humans may change with improved and more frequent experiences with humans (Grandin, 1997; Jago, Krohn, & Matthews, 1999). In a 12-mo backgrounding scenario, Petherick, Doogan, Venus, Holroyd, & Olsson (2009) subjected steers to three human handling and yarding scenarios: Good handling/yarding, Poor handling/yarding, and Minimal handling/yarding. Steers were then lot-fed for 78 d with no handling treatments imposed prior to being transported for commercial slaughter. At the end of backgrounding, all three treatments produced steers with similar body weights; however, plasma cortisol levels were lowest for Good treatment steers compared to the Poor and Minimal treatment groups at the end of lot-feeding. Steers subjected to minimal handling/yarding seemed to experience more stress than either the Good and Poor handling groups, likely due to the novelty of being handling and confined. This study verifies that increased exposure to proper handling can positively alter the disposition of cattle.

Francisco, Cooke, Marques, Mills, and Bohnert (2012) evaluated the implementation of an acclimation system for steers entering a backgrounding program post-weaning. Steers were assessed for temperament score 35 d after weaning and then assigned to either an acclimation treatment that entailed processing through a handling facility twice weekly or a control treatment that remained undisturbed on pasture throughout the duration of the study. At feedlot receiving, the acclimated steers displayed decreased temperament scores and plasma cortisol suggesting they were calmer and incurring less stress; however, the acclimated steers exhibited lower average daily gains, gain-to-feed ratios, and tended to have lower dry matter intakes when compared to the control steers. To effectively improve cattle temperament it may be necessary to

explore various handling methods so that the improvements in temperament are not negated by decreases in performance.

Stress can occur during transportation to slaughter. Cattle shipped 200 km to a commercial abattoir exhibited greater blood cortisol concentrations at exsanguination than cattle shipped a short distance and harvested in a research abattoir (Tume and Shaw, 1992). Moreover, in a study evaluating transport of temperamental and calm bulls, temperamental bulls showed greater blood glucose and cortisol than calm bulls up to 48 h after 4 h of transport (Hulbert et al., 2011). Additionally, calm bulls showed a greater likelihood to resist microbial invasion 96 h after transportation when compared to temperamental bulls (Hulbert et al., 2011). Cattle are often shipped long distances to commercial slaughter facilities and cattle that are more docile should handle this stressful situation better than their temperamental counterparts.

Temperament has shown to be of vital importance to the beef industry. Not only can calm cattle be more productive, but they can yield carcasses with improved quality measures. Additionally, calm cattle should be more tolerable to stressful situations which could be beneficial to animal health.

4. Exercise and meat color

Fresh meat color is an important characteristic that consumers use to select meat cuts at the retail counter. Consumers perceive bright-pink to bright-red meat color as being desirable and any deviation from this is unacceptable (Kropf, 1980). There are many ante mortem and postmortem factors that contribute to the development of meat color. A vast majority of research on ante mortem factors and meat color has been directed towards nutrition. Research has detailed that diet can alter glycogen storage, chilling rate, or antioxidant accumulation, all of which can alter pH, oxygen consumption, and metmyoglobin reducing activity of a muscle which influence meat color (Mancini & Hunt, 2005). Animal stress and production methods have also received attention by scientific research to investigate their influence on meat color. Many authors assumed that livestock subjected to great amounts and frequency of physical activity, like that associated with grazing, should produce meat that is darker than normal (Muir, Smith, Wallace, Cruickshank, & Smith, 1998). Most available research regarding physical activity and beef color utilize scenarios where grazing is compared with grain-fed beef and observed results may be

confounded due to nutritional differences associated with the two systems (Dunne, Monahan, & Moloney, 2011).

Lean color differences have been seen between pasture-fed and grain-fed cattle. Dunne, O'Mara, Monahan, French, and Moloney (2005) set out to determine if this difference was due to nutrition or the difference in daily exercise that the cattle endured. In this study, 18-mo old steers were housed indoors and exercise treatment cattle were exposed to walking exercise (4.41km/d for 6 d/wk), while non-exercised steers remained in their pen over the duration of the study. The exercise treatment did not affect muscle lightness ('L'), redness ('a'), and yellowness ('b') of the *longissimus dorsi* and the *extensor carpi radialis*, but decreased 'a' values of the *semimembranosus*. Also, there was no observed treatment effect on ultimate pH in all three muscles. Significant differences were seen between muscles for 'L', 'a', and 'b' values along with pH. A muscle × exercise interaction was seen for 'a' values showing that redness is influenced in a muscle-dependent manner with the *semimembranosus* being altered the most and *longissimus dorsi* the least. Additionally, treatment did not influence the total myoglobin concentration of all muscles sampled. The results of this research contrast the common notion that exercise changes meat color.

Muscle tissues can adapt due to certain stimuli, such as prolonged physical activity. Vestergaard, Oksbjerg, and Henckel (2000) evaluated the impact of intensive (ad libitum feed and tie-stall housing) and extensive (restricted forage-based feeding and loose housing) production methods on meat color and muscle characteristics. Their research displayed that *longissimus dorsi* and *semitendinosus* muscles were darker in color and had higher pigmentation in the extensive production group compared with the intensive group. Additionally, the extensive treatment produced *longissimus dorsi* muscles with decreased redness and yellowness when compared with the intensive group, whereas, no difference was observed in the *semitendinosus* and *supraspinatus*. These results demonstrated changes in color which is commonly thought to occur in response to increased physical activity, but the results were compromised due to the diets used.

Physical activity or exercise could present issues in regards to producing beef with acceptable color. Still, there is an unnecessary assumption that exercise will produce dark beef when the body of literature is not present to support the claim. There is a need to investigate this

matter in a way where evaluation of exercise or physical activity treatment is the primary focus and limited dietary factors are included in the research design.

5. Exercise and meat tenderness

The eating experience associated with beef is one of the primary attributes that consumers rely on to make future meat purchases. Tenderness has been identified as one of the most important factors affecting consumer acceptance of cooked beef in the U.S. (Miller et al., 1995) and consumers change their purchasing habits after they encounter beef that is unacceptably tough (Boleman et al., 1997). Negatively affecting meat tenderness to promote new production methods could adversely affect the salability of that specific product.

The impact of physical activity on tenderness has received limited attention, especially in beef. Aalhus, Price, Shand, and Hawrysh (1991) evaluated meat characteristics from lambs that received treadmill exercise 5 d per wk throughout the finishing period. These authors showed that exercise increased tenderness as significant decreases in Warner-Bratzler shear force were observed in *semimembranosus* and *vastus lateralis* muscles from exercised lambs. Moreover, in a prior experiment where Aalhus and Price (1990) subjected lambs to resistance jumping exercise, a slight reduction in Warner-Bratzler shear force of the *vastus lateralis* was detected. The observed tenderness improvements detailed in these studies are indicative of muscles that are directly involved in movement, but support muscles that are of a greater monetary value must also be evaluated.

Enfalt et al. (1993) observed results that were contradictory to those of Aalhus et al. (1991) when evaluating exercise in pigs. Pigs are generally raised indoors with very limited space, ultimately restricting their ability to engage in physical exercise. To combat this, Enfalt et al. (1993) designated pigs to either a control treatment or an exercise treatment that partook in moderate exercise 5 d per wk throughout the fattening period. This study revealed no differences in Warner-Bratzler shear force values of *longissimus dorsi* and *biceps femoris* muscles between control and exercised groups. The *longissimus dorsi* is not directly involved in exercise as it is a support muscle but it could provide evidence of how other support muscles should respond to exercise.

Lopez-Bote et al. (2008) evaluated the effect of exercise on various meat quality traits in Iberian pigs. The exercised treatment was fed in a manner that required the pigs to travel 2 km

every day while the sedentary group was housed indoors. Rheological traits were assessed on the *psoas major* muscle at 0 and 8 d postmortem. Hardness was slightly reduced for the exercised group at both of the storage times. Sample chewiness was significantly reduced for the exercise treatment compared to the sedentary group on d 8 of storage suggesting that exercise samples were more tender.

The muscle connective tissues, endomysium, perimysium and epimysium, make up a network of collagen and elastin fibers that are embedded in a matrix of proteoglycans (Lepetit, 2008). The perimysium represents about 90% of total connective tissues in muscles (McCormick, 1999). The amount of connective tissue is highly variable between muscles and is generally present in greater amounts in muscles that are considered tough. Both raw muscle collagen and cooked meat collagen contents are highly correlated with tenderness (Dransfield et al., 2003). Exercise training is associated with increased prolyl-4-hydroxylase activity in skeletal muscle without an increase in collagen concentration (Kovanen & Suominen, 1989) which suggests that the rate of collagen turnover may be increased in exercised animals (Gosselin, Adams, Cotter, McCormick, & Thomas, 1998). Little research has evaluated the effect of exercise on skeletal muscle collagen in beef, but other livestock species have been evaluated.

Petersen, Berge, Henckel and Soerensen (1997) evaluated multiple rearing systems and their impact on collagen characteristics in pigs. Crossbred pigs were delegated to one of the following rearing treatments: confined, trained, or free. The confined and trained pigs were housed individually and the trained pigs were exercised 5 times per wk on a treadmill for 10 min at the beginning of the study and progressing to 20 min by the end of the experiment. The "free" treatment housed 8 pigs in a 36 m^2 pen. Total collagen, heat stable collagen, and collagen solubility were analyzed from *biceps femoris* and *longissimus dorsi* samples collected 24 h postmortem. Trained and free-raised female pigs exhibited greater amounts of heat-stable collagen and decreased collagen solubility in the *biceps femoris* when compared to the confined treatment group. *Biceps femoris* samples from male pigs presented no differences between treatments for total collagen and heat-stable collagen, but trained pigs demonstrated greater collagen solubility than both confined and free-raised treatments. Additionally, no differences were seen between treatment groups for total collagen, heat-stable collagen, and collagen solubility in the *longissimus dorsi*. This study further shows that muscles with different functions may have different responses to exercise as the *biceps femoris*, which is involved in movement,

demonstrated changes that should decrease tenderness in female pigs while the *longissimus dorsi*, a support muscle, remained unchanged.

An earlier study involving pigs and exercise was conducted by Hawrysh, Murray, and Bowland (1974). Control and exercised pigs were housed in similar individual pens. Pigs assigned to the exercise treatment were exercised on a treadmill 3 d per wk for 30 min at the beginning of the trial and up to 60 min at the end of the study. Collagen was assessed based on hydroxyproline analysis from *psoas major* and *biceps femoris* samples collected 3.5 d postmortem. Hydroxyproline was greater in the *biceps femoris* compared to the *psoas major*, but there were no discernible differences between the treatments for both the *biceps femoris* and *psoas major* muscles. The lack of change in the *biceps femoris* contrasts the findings of Petersen et al. (1997) and the varying results may be attributed to the duration of the exercise regimen employed.

There are multiple factors that may be causing the observed tenderness and collagen differences, or lack thereof. First there is a lack of consistency to the terms "physical activity" and "exercise". These are very open ended phrases that fail to distinguish the intensity or rigorousness of the activity endured by an animal. Physical activity is often associated with the act of grazing which is not necessarily an intense act, but is constantly occurring throughout the day. Another issue is that many studies fail to keep nutritional aspects the same; this is a readily apparent problem when evaluating the physical stress of grazing as the other treatment group may have a higher energy diet. Lastly, muscle location could be of serious importance when assessing tenderness and collagen composition as some muscles are used much more than others when the animal is subjected to exercise.

6. Exercise impact on fatty acid composition

Fatty acids influence many regulatory processes including gene expression and synthesis of lipid or lipid-derived messengers (Nikolaidis & Mougios, 2004). The fatty acid profile found in skeletal muscle and fat depots in a carcass may be altered when the physical activity of an individual is altered for extended periods of time. To date, limited research has evaluated how exercise affects the fatty acid profile of skeletal muscle in major livestock species; however, this subject has been evaluated extensively in other species commonly used for research predicting human applications.

The use of fatty acids as substrates during physical activity in rabbits was evaluated by Szabo, Romvari, Febel, Bogner, and Szendro (2002). Pannon White rabbits were assigned to a control group or an exercised treatment that subjected the rabbits to treadmill exercised until exhaustion twice daily for a duration of 4 wk. Homogenized samples from the *longissimus dorsi* and *vastus lateralis* were assessed for fatty acid composition at the completion of the trial. The proportion of oleic acid (C18:1 n-9) was significantly increased by exercise in both the *longissimus dorsi* and *vastus lateralis* muscles. Additionally, exercise treatment produced a decrease in the proportion of stearic acid (C18:0) in the *vastus lateralis*. These results demonstrate that certain fatty acids may be influenced by the implementation of an exercise regimen.

The influence of physical activity on the fatty acid composition of skeletal muscle has also been evaluated in rats. Quiles, Huertas, Manas, Battino, and Mataix (1999) investigated the implementation of physical activity on Wistar rats that were being supplemented with different dietary fats. Rats were either supplemented olive oil or sunflower oil and each dietary treatment was split into exercise and sedentary treatments. Rats assigned to exercise treatment underwent training on a treadmill for 40 min once a d, 5 d per wk for 8 wk. Fatty acid profiles were attained for skeletal muscle mitochondrial membranes collected from the *vastus lateralis*. Exercise treatment decreased total polyunsaturated fatty acids (PUFA) and total n-6 PUFA. Total n-3 PUFA was decreased by exercise training as was 22:5n-3 in animals of both diets while 22:6n-3 was decreased in the olive oil diet and increased in the sunflower oil diet. This study further shows that exercise influences fatty acid concentrations in a muscle related to movement.

Different animal species provide differing results in regard to the influence exercise has on fatty acid concentrations in skeletal muscle. Additionally, the various exercise regimens used produced different results as more intense exercise seems to change fatty acid concentrations in a more drastic fashion. It is quite possible that exercise could alter the fatty acid profile of skeletal muscle in feedlot cattle.

7. Summary

Animal welfare is continually receiving additional attention by producers and consumers alike. The high concentrate diets and sedentary lifestyles of cattle in confinement feeding operations could lead to poor animal health due to insulin-resistance. Exercise has demonstrated

the capability to reduce insulin-resistance in non-livestock species and could provide an avenue to improve the health of cattle produced in confinement. Animal performance, carcass characteristics, and meat quality measures must be evaluated to ensure high quality beef is produced efficiently if exercise is introduced in cattle production.

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Chapter 2 - Effects of exercise on feedlot heifer performance, animal well-being, and carcass quality

Abstract

Heifers (n=30) remained sedentary or were exercised 3 times/wk (20 min/d for the first 2 wk, 30 min/d for the next 2 wk, and 40 min/d for the final 4 wk) to assess the influence of exercise on animal health, performance, and carcass characteristics. Sedentary heifers had greater blood cortisol than exercised heifers at d 28 and 60 (treatment × day interaction, $P = 0.007$). Exercise decreased ($P = 0.009$) blood insulin, while no other blood constituents were affected ($P > 0.05$) by exercise. Exercise decreased ($P < 0.05$) final live weight, ADG, 12^{th} rib fat, and USDA yield grade, as well as a* and b* values of exposed *longissimus* muscle. Exercise treatment did not affect ($P > 0.05$) gain:feed, DMI, HCW, marbling score, or LM area. Additionally, Warner-Bratzler shear force, total fatty acids, SFA, MUFA, PUFA, and CLA concentrations of four muscles were unaffected ($P > 0.05$) by exercise.

Keywords: beef, exercise, performance, quality, welfare, yield.

1. Introduction

Cattle feeding in the U. S. has evolved into large, concentrated units whose primary focus is to produce large quantities of high quality beef for consumers at affordable prices. Current production facilities are designed to promote efficient growth and accelerate fat accumulation in cattle, but inadvertently limit the amount of physical activity in which cattle can partake. Cattle housed in feedlots incur rather limited amounts of interaction with humans throughout the finishing process which may result in cattle responding disproportionately to stressful situations associated with processing, shipping, and harvest. These issues can compromise animal well-being, animal handler safety, and may cause undesirable changes to meat quality.

Current production systems have come under increasing scrutiny from consumers. Consumers are placing more emphasis on how animals are raised, which is reflected in their purchasing decisions at the retail counter (Grunert, Bredahl, & Brunso, 2004). Animal welfare, health, and living environment of livestock are key concerns for consumers (Frewer, Kole, Van De Kroon, & De Lauwere, 2005). The increased willingness of consumers to pay more for products from livestock raised by verified processes has led to an increased availability of beef products marketed as “natural”, “organic”, “grass-fed”, and “certified humane” at the retail counter (Olynk, Tonsor, & Wolf, 2010). Beef producers could potentially benefit from altering their production methods by generating value through niche marketing in addition to improving performance of cattle by enhancing animal health and limiting animal stress.

The combination of a sedentary lifestyle and high energy diet is common in beef cattle production and may compromise some health related traits. Hyperglycemia is a toxic condition in the body caused by an overabundance of glucose (Rosetti, Giaccari, & DeFronzo, 1990). Glucose transport, metabolism, and storage are facilitated by insulin, which is increased after a meal is consumed (Shepherd & Kahn, 1999). Chronic hyperglycemia can lead to insulin-resistance, which prevents glucose transport and is a common symptom of type 2 diabetes mellitus in humans (Shepherd & Kahn, 1999). A majority of beef cattle finished in the U.S. are fed high starch diets that are primarily composed of corn (Vasconcelos & Galyean, 2007). Fermentation of high-starch feeds yields propionate in the rumen, which is further converted into glucose (Huntington, 1997). Insulin-resistance could occur in feedlot cattle, as the aforementioned lifestyle contributes to hyperglycemic conditions.

Physical exercise is commonly identified as a method to reduce insulin-resistance associated with type 2 diabetes mellitus in humans (Goodyear & Kahn, 1998). Additionally, exercise has the capability to inhibit the development of type 2 diabetes in the *Psammomys obesus* due to increased expression of TNFα and GLUT 1 glucose transporter in skeletal muscle (Heled et al., 2005). Additionally, the implementation of a routine exercise program for feedlot cattle would increase cattle's exposure to handling by humans. Increased interaction between cattle and humans has been attributed to lowering blood cortisol, which is a primary indicator of animal stress (Boandl, Wohlt, & Carsia, 1989). Animals with lower blood cortisol levels could be expected to be more docile when handled and docile cattle have improved performance and carcass traits in comparison to temperamental cattle (Reinhardt, Busby, & Corah, 2009).

Exercising beef cattle in a confinement setting has the potential to improve animal health and welfare but could have an impact on meat characteristics that influence consumer purchasing decisions. Fresh meat color is an important characteristic that consumers use to select meat cuts at the retail counter. Walking 4.41 km on a daily basis had no influence on instrumental color values of the *longissimus dorsi* in 18 mo old steers (Dunne, O'Mara, Monahan, French, and Moloney, 2005), but no research has reported how other types of exercise regimens can impact meat color in cattle. Additionally, tenderness is one of the most important factors affecting consumer acceptance of cooked beef in the United States (Miller et al., 1995) as the consumption of meat cuts that are unacceptably tough can alter purchasing decision of consumers (Boleman et al., 1997). Exercise of sheep decreased Warner-Bratzler shear force (WBSF) of the *semimembranosus* and *vastus lateralis* due to a dilution of collagen in relation to myofibrillar protein (Aalhus, Price, Shand, & Hawrysh, 1991), so meat from livestock receiving routine exercise may incur a tenderness improvement.

Producers and consumers alike will continue to place high priority to the issue of animal welfare. Improving animal health and reducing animal stress should be of high importance in addressing the issue of animal welfare. Routine exercise of feedlot cattle provides a potential avenue to improve cattle welfare and docility which could improve live animal efficiency and meat quality; however, there are possibilities that exercise may be detrimental to certain performance and carcass characteristics. The objectives of this study were to assess the impact of an exercise regimen on feedlot cattle with specific attention to animal welfare, animal performance, carcass composition, and meat quality.

2. Materials and methods

2.1 Animal background

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in this study. Crossbred heifers (n=30; 448 ± 27 kg initial body weight) were used in a randomized complete block experiment. Heifers were stratified by initial body weight (BW) and body condition into 15 strata prior to being randomly assigned, within strata, to Sedentary or Exercised treatment groups. Heifers were housed individually in pens (1.5 m × 6.5 m) and fed a finishing diet that consisted of 93% concentrate and 7% roughage. Daily feed allocations were recorded over the duration of the study and BW were measured on d 0, 28, and 60.

2.2 Exercise protocol

Sedentary treatment cattle remained in their pens throughout the duration of the study except for data collection days. Exercised treatment cattle were exercised three times per wk. Exercised cattle were removed from their pens and forced to move by an animal handler on foot at a pace of 5 to 6 km/h (20 min/d for the first 2 wk, 30 min/d for the next 2 wk, and 40 min/d for the next 4 wk resulting in a total of 8 weeks of exercise).

2.3 Blood constituents

Blood samples were collected via jugular vein puncture on d 28 and 60 to assess blood glucose and lactate while blood samples to assess insulin, IGF-1, and cortisol were collected on d 0, 28, and 60. Blood used to evaluate blood plasma concentrations of glucose, lactate, insulin, IGF-1, and cortisol were placed in tubes containing an anticoagulant and centrifuged for 15 min at 2,000 × *g* at 13 ºC. Blood plasma glucose and lactate were measured using a glucose-lactate autoanalyzer (2300 Stat Plus, YSI Inc., Yellow Springs, OH). Commercial radioimmunoassay kits were used to analyze both serum cortisol (Cortisol Coat-A-Count, #TKIN, Siemens Medical Solutions Diagnostics, Los Angeles, CA) and insulin (Insulin Coat-a-Count, #TKCO1, Siemens Medical Solutions Diagnostics, Los Angeles, CA) concentrations while plasma IGF-1 was analyzed using a commercial ELISA kit (# AC-27F1, Immunodiagnostic Systems Inc., Fountain Hills, AZ).

2.4 Carcass characteristics

Heifers (n=16) remained in strata and were randomly selected to be slaughtered at the Kansas State University Meat Laboratory on d 62. Hot carcass weights (HCW) were recorded at the completion of slaughter. Percentage of dress yield was determined as HCW divided by final BW. *Longissimus* muscle (LM) area, kidney, pelvic, and heart fat percentage (KPH%), subcutaneous fat thickness over the 12th rib, USDA yield grade, and marbling score was evaluated 24 h postmortem. Ultimate pH was assessed in the *longissimus thoracis* dorsal to the 12^{th} rib 24 h postmortem using a portable pH meter (HI 9025; Hanna Instruments, Wilmington, NC). Carcasses were fabricated and 2.54 cm thick steaks were taken from the *longissimus lumborum*, *triceps brachii*, s*emitendinosus*, and *semimembranosus* muscles for Warner-Bratzler shear force (WBSF) assessment. Additionally, fresh samples were taken from each of the four previously stated muscles for analysis of fatty acid methyl esters.

2.5 Instrumental color

The exposed *longissimus thoracis* between the 12^{th} and 13^{th} rib was assessed for objective lean color after 30 min of bloom time. International Commission on Illumination (CIE) instrumental color (L*, a*, and b*, Illuminant A) was measured with a HunterLab Miniscan XE Plus spectrophotometer (2.54-cm-diameter aperture, 10^{o} standard observer; Hunter Associates Laboratory, INC., Reston, VA.). Three readings were taken for each carcass and values were averaged prior to statistical analysis.

2.6 Warner-Bratzler shear force

Steaks were vacuum packaged in oxygen impermeable film (Prime Source Vacuum Pouches, Kansas City, MO; 76.2 μm, STP Barrier, Nylon/PE Vacuum Pouch; oxygen transmission rate 0.04 g/254 cm^2/24 h at 0 ºC; water vapor transmission rate 0.2 cc/254 cm^2/24 h at 0 ºC at 0% relative humidity) and aged for 14 d at 0-2 °C before being frozen at -20 °C. Steaks were thawed for approximately 12 h at 0-2 °C and cooked to 40 °C, turned, and cooked to a final internal temperature of 70 °C in a dual flow, forced-air convection gas oven (Blodgett, model DFC-102 CH3; G.S. Blodgett Co., Burlington, VT) preheated to 163 °C. Steak temperatures were monitored with copper-constantan thermocouples (Omega® Engineering, Stamford, CT) inserted into the approximate geometric center of each steak and attached to a Doric temperature

recorder (model 205; Vas Engineering, San Francisco, CA). The steaks were chilled overnight at 0-2 °C before 8 round cores (1.27-cm diameter) were obtained from each steak parallel to the long axis of the muscle fibers using a 1.27-cm corer (G-R Manufacturing Co., Manhattan, KS). Each core was sheared once perpendicular to the direction of the muscle fibers using a Warner-Bratzler V-shaped blunt blade (G-R Manufacturing Co., Manhattan, KS) attached to an Instron Universal Testing Machine (model 4201, Instron Corp., Canton, MA) with a 50-kg compression load cell and a crosshead speed of 250 mm/min. Peak shear force values were recorded in kg and values from the 8 cores were averaged for statistical analysis.

2.7 Fatty acid composition

Tissue samples were stored in a sterilized plastic container (Whirl Pack, Nasco, Modesta, CA) at -20 ºC. A modified gas chromatography procedure of Sukhija and Palmquist (1988) was used for analysis of long chain fatty acids. Each sample was frozen in liquid nitrogen, pulverized using a tabletop blender (model 33BL79; Waring Products, New Hartford, CT), and analyzed for fatty acid methyl esters. Muscle (50 μg) samples were combined with 2 mL of methanolic-HCl and 3 mL of internal standard (2 mg/mL of methyl heptadecanoic acid (C17:0) in benzene) and heated in a water bath for 120 min at 70 °C for transmethylation. After cooling, addition of 2 mL of benzene and 3 mL of K_2CO_3 allowed methyl esters to be extracted and transferred to a vial for subsequent quantification of the methylated fatty acids by gas chromatography. Injection port and detector temperatures were set at 250 °C, with a helium flow rate of 1mL/min and a split ratio of 100:1. Oven temperature began at 140 °C and was increased at 2 °C/min to 200 °C and then increased at 4 °C /min to 245 °C where it was held for 17 min. Fatty acids from each of the muscle samples were expressed as a proportion of the total sample.

2.8 Statistical analysis

Data were analyzed as a randomized complete block design with weight strata serving as the block. Statistical analysis was performed using the Proc Mixed procedure of SAS (SAS Institute, Inc., Cary, NC) with the fixed effect of exercise treatment and random effects of strata and exercise regime × strata. Time and time × exercise regimen were included as fixed effects for BW and blood parameters. Post-hoc mean separation was carried out using Fisher's least significant difference. All data were analyzed at a 5% level of significance.

3. Results and discussion

3.1 Animal performance

A treatment × day interaction ($P = 0.013$) was observed for body weight (Figure 2.1). Body weights were similar ($P > 0.05$) between treatments on d 0 and 28, but exercised heifers had decreased ($P < 0.05$) body weights in comparison to their sedentary counterparts on d 60. Treatment means for other live animal performance measures are shown in Table 2.1. Heifers subjected to exercise had decreased average daily gains (ADG; $P = 0.048$), but similar dry matter intakes (DMI; $P = 0.057$) and gain:feed (G:F; $P = 0.084$) when compared to their sedentary counterparts. Daily walking exercise (4.41 km daily for six days per week for 8 weeks) of 18 mo old steers reduced final BW and ADG in comparison to non-exercised steers (Dunne et al., 2005), which supports findings of the current study. Conversely, treadmill exercise produced similar live weights and daily gains in pigs (Enfalt et al., 1993). Research of exercise in livestock is rather limited, but exercise decreased body weights (Bell, Spencer, & Sheriff, 1997) and reduced DMI, ADG, and feed efficiency (Mirand et al., 2004) in rodent models.

Exercise commonly is performed to achieve weight loss in humans, but the regulation of body weight is complex as it is simultaneously affected by genetic architecture, the environment, and their interactions (Kelly, Nehrenberg, Hua, Garland Jr., & Pomp, 2011). To effectively gain weight, energy intake must exceed energy expenditure (Donnelly & Smith, 2005). In the present study, cattle in different treatments displayed similar DMI, but produced different BW. Exercise could possibly suppress weight gain due to the increased energy requirements of the exercised cattle. Cattle in the current study were progressed through multiple exercise times to adapt to a 40 min/d exercise regimen. Reducing the intensity or duration of the exercise regimen could possibly achieve a state where BW remains unaffected due to decreased energy expenditure.

3.4 Blood constituents

There were no treatment × day interactions ($P > 0.05$) for both blood glucose or lactate (Table 2.2). Additionally, glucose and lactate were unaffected ($P > 0.05$) by exercise treatment (Table 2.2). Sheep had increased blood glucose and lactate during exercise (Apple, Minton, Parsons, Dikeman, & Leith, 1994), but blood glucose has been shown to decrease while lactate increases in exercised horses (Ferrante & Kronfeld, 1994). Glucose is a critical energy source for

working muscle (Apple et al., 1994) and it is logical that it may be affected during bouts of exercise; however, this may depend on when sampling for glucose occurs. The current study evaluated blood glucose at predetermined weigh periods and were not conducted immediately after exercise, which may explain the lack of differences between treatment groups for both constituents. Glucose increases as metabolizable energy intake is increased (Ellenberger et al., 1989) but did not change in heifers over the duration of a finishing period (Yambayamba, Price, & Foxcroft, 1996). The DMI for both treatments were similar in the present study, so it is rational that blood glucose was also similar between treatments.

Blood lactate is evaluated often in cattle as abnormal increases of blood lactate are indicative of acidosis (Owens, Secrist, Hill, & Gill, 1998). Lactate can be utilized by specific bacteria in the rumen and these bacteria are present in greater numbers when cattle are properly adapted to a high concentrate diet (Huber, Cooley, Goetsch, & Das, 1976). Lactate can enter the blood of ruminants by absorption from the rumen or metabolism of propionate in the rumen epithelium (Dziuk, 1984). The current study shows that exercise should not alter the ability of cattle to properly dispose of lactate.

No treatment × day interaction ($P = 0.062$) was observed for blood insulin, but blood insulin decreased ($P = 0.009$) with exercise (Table 2.2). Previous research has demonstrated that blood insulin can increase (Schwaiger, Beauchemin, & Penner, 2013) or remain unchanged (Yambayamba et al., 1996) during finishing. The decreases in blood insulin levels observed in exercised cattle suggest that they may have decreased insulin in comparison to sedentary cattle. Insulin-resistance occurs when an individual endures chronic hyperglycemia, which is a major contributor to type 2 diabetes mellitus in humans (Shepherd & Kahn, 1999). Carter, McCutcheon, Valle, Meilahn, and Geor (2010) observed that exercise failed to decrease insulin-resistance in obese horses; however, exercise effectively inhibits the development of insulin-resistance in diabetes-prone rats (Heled et al., 2005).

There was no treatment × day interaction ($P = 0.368$) or treatment effect ($P = 0.344$) on blood IGF-1 levels, but IGF- 1 decreased ($P = 0.012$) over time (Table 2.2). Previously, blood IGF-1 in beef heifers did not change throughout the finishing period (Yambayamba et al., 1996). Other research has shown that IGF-1 changes in direct proportion to dietary intake (Ellenberger et al., 1989). The role of IGF-1 is similar to insulin and has various effects during exercise (Giesel et al., 2009). The effect of exercise on IGF-1 has been evaluated in rats. Exercise evoked

greater increases in IGF-1 for rats that had no prior physical training compared to rats that had been trained (Giesel et al., 2009). A similar comparison could be made between the current study and the research of Giesel et al. (2009), except IGF-1 assessment was conducted during an instance of handling. This study shows that routine exercise does not alter the amount of IGF-1 produced during instances of handling.

A treatment × day interaction ($P = 0.007$) was observed for blood cortisol (Figure 2.2). Exercise cattle showed greater ($P < 0.05$) blood cortisol concentrations than sedentary cattle at d 0 but sedentary cattle displayed greater ($P < 0.05$) blood cortisol concentrations at d 28 and 60. Low blood cortisol levels are generally associated with calm cattle (Hulbert et al., 2011; King et al., 2006). A possible method for conditioning animals to better cope with stress is to increase human-animal interactions (Grandin, 1997; Jago, Krohn, & Matthews, 1999), which could be achieved by using an animal handler to administer exercise. Contrary to the current study, increased animal handling was shown to be ineffective in reducing cortisol levels in a feeder calf backgrounding situation (Petherick, Doogan, Venus, Holroyd, & Olsson, 2009). Heifers in the current study were housed in individual pens and exercised cattle were exercised simultaneously in one large group before returning to their individual pens. The decreased cortisol levels of exercised cattle in the current study could be attributed to increased interaction between animals as group housed cattle have decreased blood cortisol in comparison to individually housed animals (Titto et al., 2010).

3.3 Carcass characteristics

Carcass characteristics are summarized in Table 2.3. No differences were observed between exercise and sedentary treatments for HCW ($P = 0.152$) or percentage of dressed yield ($P = 0.417$). Conflicting results have been reported with regard to the influence of exercise on muscling characteristics in livestock. The current study found no differences ($P = 0.169$) between treatments for LM area. Carcass weight and dressed yield were unchanged but overall carcass conformation decreased when cattle were exposed to daily walking exercise (Dunne et al., 2005). Conversely, treadmill exercise of sheep failed to increase muscle mass of the *vastus lateralis* and *semimembranosus* (Aalhus & Price, 1991). The previously described exercise methods are associated with endurance exercise, but implementation of resistance jumping increased mass of the *biceps brachii* and *brachialis* in sheep (Aalhus & Price, 1990). Resistance

exercise may provide more musculature change than endurance exercise if the primary focus of implementing exercise is to increase muscle mass, but this could present logistical issues in species such as cattle.

Exercised and sedentary heifers had similar (P = 0.849) KPH%, but exercised heifers produced leaner carcasses with less 12th rib fat (P = 0.006) and decreased USDA yield grades (P = 0.002) in comparison to sedentary heifers. Steers subjected to walking exercise elicited similar results to those observed in this study, as carcass fat scores were decreased (Dunne et al., 2005). Additionally, multiple rodent models display a propensity for exercise to result in decreased body fat (Bell et al., 1997; Mirand et al., 2004). Body fat can be reduced with exercise of sufficient intensity (Pacy, Webster, & Garrow, 1986), so the results observed in this study are expected.

Marbling score was similar (P = 0.245) between treatments. Scientific literature is limited with respect to intramuscular fat deposition in response to exercise in livestock and other animal models. Intramuscular fat is deposited when excess energy is available to the animal (Robelin, 1986). In the current study, exercise may have increased the energy requirements to a point where subcutaneous fat was not deposited to the same extent in exercised carcasses in comparison to carcasses from sedentary cattle; however, required energy did not influence deposition of marbling. The current study was conducted for 8 wk, which is significantly shorter than normal finishing periods. Differences in marbling score may be observed if the finishing period is lengthened.

3.4 Instrumental color

Instrumental color least squares means are detailed in Table 2.4. Exercised heifers produced carcasses that had decreased a* (P = 0.007) and b* (P = 0.009) values at the exposed *longissimus thoracis* between the 12th and 13th ribs. Contrarily, L* values did not differ (P = 0.467) between treatments. Steers exercised by walking produced *longissimus dorsi* muscles with color attributes similar to those from control steers; however, exercise decreased redness (‘a’ values) of the *semimembranosus* (Dunne et al., 2005). Animals with increased activity have greater amounts of myoglobin than their sedentary or inactive counterparts (Essen-Gustavsson, 1996). The exercised animals in the current study exhibited decreased redness in the *longissimus thoracis*, which is contradictory to what should occur if myoglobin is increased. Exercise did not

change the amount of myoglobin present in the *semimembranosus*, a muscle that had decreased redness in response to exercise (Dunne et al., 2005). Consequently, muscle myoglobin content may not be responsible for the observed color change in the current study. Carcasses from exercised heifers displayed less subcutaneous fat at the 12th rib. Decreased amounts of subcutaneous fat present on carcasses can make *longissimus* muscle appear darker and increase the presence of heat ring (May, Dolezal, Gill, Ray, & Buchanan, 1992), which could contribute to some of the observed differences between treatments for instrumental color values.

Muscle pH has a great influence on meat color development as it affects structural and physiochemical properties which cause light scattering at the meat surface (MacDougall, 1982). Carcass ultimate pH (P = 0.669) did not differ between exercise and sedentary treatments in the present study (Table 2.4), which agrees with finding of Dunne et al. (2005) who also noted similar ultimate pH from exercised and control steer carcasses. Apple et al. (2006) subjected cattle to various exercise treatments immediately prior to slaughter in an attempt to alter postmortem pH and ultimately create dark cutting beef, which is characterized by a pH > 6.0. These authors observed no differences between control and exercised treatments for pH decline or tristimulus color values. The single bout of exercise employed by Apple et al. (2006) was used to simulate stress that cattle may incur prior to slaughter, which is different than the exercise used in the current study. Nonetheless, the lack of change in pH of the aforementioned research and the current study provide no evidence that ultimate pH of carcasses from exercised cattle should be affected.

3.5 Warner-Bratzler shear force

Least squares means for instrumental tenderness are presented in Table 2.4. There was no treatment effect on WBSF of steaks derived from the *longissimus dorsi* (P = 0.135), *triceps brachii* (P = 0.859), *semitendinosus* (P = 0.243), or *semimembranosus* (P = 0.742). Prior research involving exercise of livestock has shown varying effects on meat tenderness. Treadmill exercise of sheep decreased WBSF of the *semimembranosus* and *vastus lateralis* (Aalhus et al., 1991); however, resistance jumping exercise of sheep had no effect on WBSF of the *vastus lateralis* (Aalhus & Price, 1990). Decreases in WBSF noted by Aalhus et al. (1991) were attributed to dilution of collagen, as myofibrillar protein increased on a per gram of muscle basis while collagen decreased (Aalhus et al., 1991). Exercise can influence activity of enzymes that

are integral to collagen biosynthesis (Kovanen & Suominen, 1989) so evaluating the development of collagen crosslinks may deserve attention to further explain changes or lack thereof in muscles of exercised cattle. Additionally, the current study evaluated multiple muscles of varying function and involvement with the employed exercise activity. Evaluation of intensified or muscle specific exercise may be integral to achieve alterations in tenderness as seen in past research (Aalhus et al. 1991).

3.6 Fatty acids

Individual SFA and MUFA are shown in Table 2.5. Carcasses from exercised cattle contained less ($P < 0.05$) margaric acid (17:0) in the *longissimus lumborum* in comparison to the sedentary treatment group, while all other SFA across all evaluated muscles remained unchanged ($P > 0.05$) by exercise treatment. Additionally, exercise treatment reduced ($P < 0.05$) nervonic acid (24:1) in the *longissimus lumborum* and vaccenic acid (18:1n-7c) in the *triceps brachii*. No distinguishable differences ($P > 0.05$) were present between treatments for total fatty acids, SFA, MUFA, PUFA, and CLA for all four muscles assessed (Table 2.6). Previous research has provided inconsistent results of the influence of exercise on fatty acid composition of skeletal muscles in other species (Nikolaidis & Mougios, 2004). Exercise in rabbits has been reported to increase oleic acid (C18:1 n-9) in the *longissimus lumborum* and *vastus lateralis*, but decrease stearic (C18:0) and arachidonic (C20:4 n-6) acids in the *vastus lateralis* (Szabo, Romvari, Febel, Bogner, & Szendro, 2002). Moreover, the exercise of rats has yielded lower percentages of arachidonic acid (C20:4 n-6) and polyunsaturated omega-3 fatty acids in the *vastus lateralis* (Quiles, Huertas, Manas, Battino, & Mataix, 1999). Multiple muscles were evaluated in the current study and none exhibited vast changes in fatty acid composition. A variety of muscles have been evaluated from other species to assess changes in fatty acid composition in response to exercise and results show that any changes are independent of muscle and fiber type (Nikolaidis & Mougios, 2004). Species, other than cattle, that showed changes in fatty acid profile were subjected to more intense exercise regimens. Increasing the intensity to levels necessary to achieve consistent changes in fatty acid profile may be illogical to implement in a beef feedlot setting.

4. Conclusions

Routine exercise could potentially improve the health and reduce the stress response of feedlot cattle by decreasing blood insulin and cortisol concentrations. Exercise can hinder live animal performance, but these disadvantages are not transferred to carcass composition in terms of carcass weight, LM area, and marbling scores. Additionally, exercised cattle yield leaner carcasses with lower numerical yield grades than their sedentary counterparts. Exercise of feedlot cattle can decrease redness and yellowness of LM muscle; however, exercise should not compromise other quality attributes, such as tenderness. Routine exercise regimens resembling the one described in this study can alter some blood constituents, but exercise intensity must be carefully monitored so live animal performance traits and meat color are not adversely affected.

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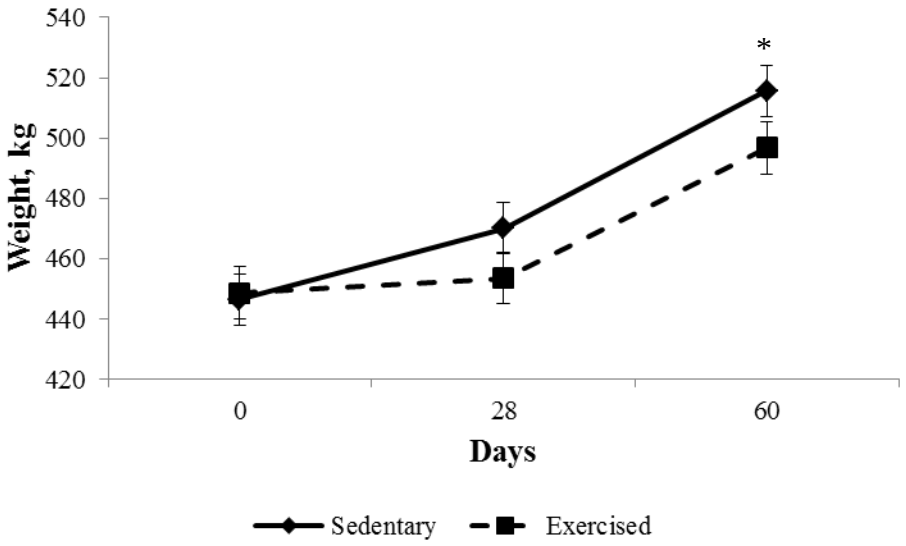

Figure 2.1 Body weights of sedentary and exercised heifers.
Body weights of sedentary heifers (n=15) and heifers (n=15) subjected to exercise (5 to 6 km/h for 20 min/d for the first 2 wk, 30 min/d for the next 2 wk, and 40 min/d for the remainder of the finishing period) 3 times per wk throughout the 8 wk finishing period. Error bars represent the mean ± SEM. *$P < 0.05$. Exercise treatment × day interaction $P = 0.013$.

Table 2.1 Growth performance of sedentary heifers and heifers subjected to exercise 3 times per wk throughout the finishing period.

	Treatment[1]			
	Sedentary	Exercise	SEM	*P*-Value
DMI, kg/d	10.03	8.97	0.362	0.057
ADG, kg	1.12	0.78	0.113	0.048
G:F	0.049	0.038	0.0043	0.084

[1]Sedentary = No exercise throughout the finishing period; Exercise = Heifers exercised at 5 to 6 km/h 3 times per wk (20 min/d for the first 2 wk, 30 min/d for the next 2 wk, and 40 min/d for the remaining 4 wk of the finishing period).

Table 2.2 Blood constituents[1] of sedentary heifers and heifers subjected to exercise 3 times per wk throughout the finishing period.

	Treatment[2]			*P*-Values[3]		
	Sedentary	Exercise	SEM	E	D	E × D
Glucose, mM				0.398	0.833	0.856
Day 28	5.0	5.5	0.54			
Day 60	4.7	5.5	0.55			
Lactate, mM				0.771	0.653	0.932
Day 28	4.4	4.7	0.83			
Day 60	3.8	4.3	0.86			
Insulin, ng/mL				0.009	0.812	0.062
Day 0	0.96	0.96	0.203			
Day 28	1.76	0.42	0.203			
Day 60	1.46	0.81	0.203			
IGF-1, ng/mL				0.791	< 0.001	0.154
Day 0	239.9	250.8	11.48			
Day 28	264.6	233.8	11.48			
Day 60	187.0	192.0	11.61			

[1]Glucose, lactate, and IGF-1 analyzed using blood plasma. Insulin analyzed using blood serum.
[2]Sedentary = No exercise throughout the finishing period; Exercise = Heifers exercised at 5 to 6 km/h 3 times per wk (20 min/d for the first 2 wk, 30 min/d for the next 2 wk, and 40 min/d for the remaining 4 wk of the finishing period).
[3]E = effect of exercise treatment; D = effect of days on feed; E × D = interaction between exercise treatment and days on feed.

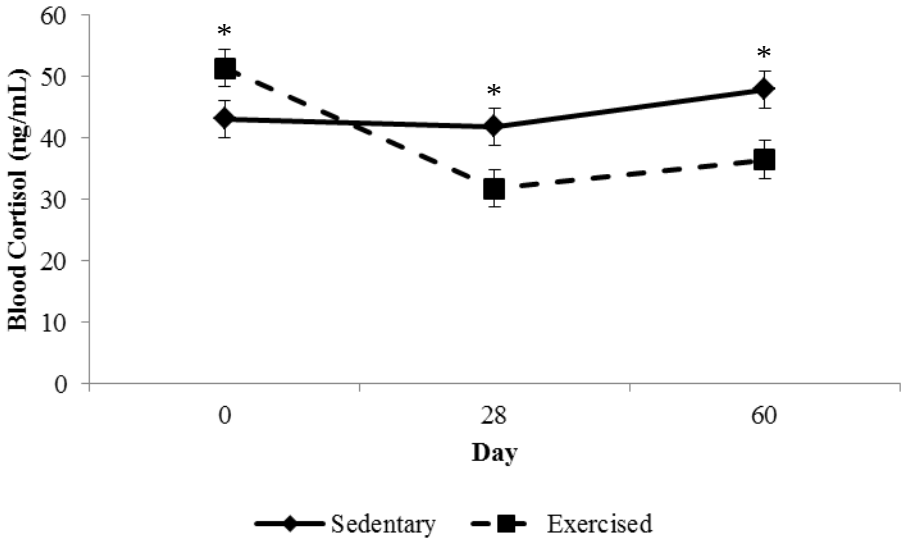

Figure 2.2 Blood serum cortisol concentrations from sedentary and exercised heifers.
Blood serum cortisol concentrations from sedentary heifers (n=15) and heifers (n=15) subjected to exercise (5 to 6 km/h for 20 min/d for the first 2 wk, 30 min/d for the next 2 wk, and 40 min/d for the remainder of the finishing period) 3 times per wk throughout 8 wk finishing period. Error bars represent the mean ± SEM. *$P < 0.05$. Exercise treatment × day interaction $P = 0.007$.

Table 2.3 Carcass characteristics of sedentary heifers and heifers subjected to exercise 3 times per wk throughout the finishing period.

	Treatment[1]			
	Sedentary	Exercise	SEM	*P*-Value
HCW, kg	321	312	4.34	0.152
Dress yield, %	64.1	64.8	0.563	0.417
LM area, cm^2	84.4	88.7	2.07	0.169
KPH, %	2.1	2.1	0.12	0.849
12th-rib fat, cm	1.45	1.09	0.0791	0.006
USDA yield grade	2.9	2.2	0.12	0.002
Marbling score[2]	401	374	15.9	0.245

[1]Sedentary = No exercise throughout the finishing period; Exercise = Heifers exercised at 5 to 6 km/h 3 times per wk (20 min/d for the first 2 wk, 30 min/d for the next 2 wk, and 40 min/d for the remaining 4 wk of the finishing period).
[2]Marbling score 300 to 399 = Slight; 400 to 499 = Small.

Table 2.4 Meat quality traits from carcasses of sedentary heifers and heifers subjected to exercise 3 times per wk throughout the finishing period.

	Treatment[1]			
	Sedentary	Exercise	SEM	*P*-Value
Instrumental Color[2]				
L*	46.2	44.6	1.40	0.467
a*	33.9	31.5	0.450	0.007
b*	26.5	23.7	0.563	0.009
Ultimate pH	5.56	5.51	0.114	0.669
WBSF, kg				
Longissimus lumborum	3.5	4.1	0.26	0.135
Triceps brachii	3.7	3.8	0.13	0.859
Semitendinosus	4.3	4.7	0.22	0.243
Semimembranosus	4.4	4.5	0.19	0.742

[1]Sedentary = No exercise throughout the finishing period; Exercise = Heifers exercised at 5 to 6 km/h 3 times per wk (20 min/d for the first 2 wk, 30 min/d for the next 2 wk, and 40 min/d for the remaining 4 wk of the finishing period).

[2]Instrumental color evaluated on the exposed *longissimus thoracis* between the 12th and 13th ribs approximately 24 h postmortem.

Table 2.5 Proportions of saturated and monounsaturated fatty acids (mg/g) in raw muscle samples from carcasses of sedentary heifers and heifers subjected to exercise 3 times per wk throughout the finishing period.

	Longissimus Lumborum			*Triceps Brachii*			*Semitendinosus*			*Semimembranosus*		
	Treatment[1]			Treatment[1]			Treatment[1]			Treatment[1]		
	Sedentary	Exercise	SEM	Sedentary	Exercise	SEM	Sedentary	Exercise	SEM	Sedentary	Exercise	SEM
SFA[2]												
14:0	2.09	1.66	0.292	1.16	1.16	0.159	1.50	1.47	0.234	1.21	1.23	0.211
15:0	0.316	0.236	0.0354	0.185	0.173	0.0215	0.259	0.232	0.0366	0.199	0.200	0.0338
16:0	18.2	14.4	2.08	11.0	10.5	1.35	14.1	13.4	2.02	11.8	11.5	1.69
17:0	1.14[a]	0.801[b]	0.118	6.64	5.75	0.0842	0.845	0.736	0.119	0.702	0.671	0.114
18:0	10.0	7.88	1.03	5.82	5.61	0.593	6.57	6.53	0.905	6.16	6.36	0.819
20:0	0.070	0.064	0.0055	0.040	0.048	0.0045	0.051	0.049	0.0078	0.054	0.051	0.0080
21:0	0.189	0.143	0.0259	0.125	0.115	0.0202	0.187	0.165	0.0264	0.137	0.124	0.0183
22:0	0.030	0.027	0.0017	0.027	0.029	0.0020	0.031	0.029	0.0021	0.031	0.026	0.0024
24:0	0.042	0.033	0.0044	0.038	0.040	0.0025	0.035	0.033	0.0048	0.037	0.036	0.0024
MUFA[2]												
14:1	0.489	0.414	0.0907	0.289	0.319	0.0490	0.436	0.449	0.0789	0.306	0.323	0.0659
16:1	2.28	1.91	0.317	1.54	1.59	0.229	2.11	2.12	0.333	1.59	1.62	0.276
17:1	0.612	0.442	0.0615	0.419	0.389	0.0499	0.549	0.487	0.0731	0.431	0.408	0.0659
18:1n-7c	1.31	1.09	0.128	0.995[a]	0.465[b]	0.152	1.06	1.17	0.160	0.557	0.729	0.202
18:1n-9c	25.1	19.5	2.92	15.8	15.1	2.00	19.7	19.2	2.86	17.0	16.3	2.41
18:1n-9t	1.58	1.26	0.180	0.831	0.822	0.0813	1.18	1.18	0.156	0.979	1.06	0.170
18:1n-11t	0.182	0.155	0.0199	0.090	0.102	0.0107	0.128	0.144	0.0177	0.108	0.130	0.0194
20:1	0.124	0.100	0.0153	0.080	0.081	0.011	0.106	0.103	0.0197	0.090	0.090	0.010
24:1	0.036[a]	0.028[b]	0.0023	0.030	0.028	0.0025	0.032	0.031	0.0031	0.028	0.030	0.0020

[1]Sedentary = No exercise throughout the finishing period; Exercise = Heifers exercised at 5 to 6 km/h 3 times per wk (20 min/d for the first 2 wk, 30 min/d for the next 2 wk, and 40 min/d for the remaining 4 wk of the finishing period).

[2]Fatty acids are represented as number of carbon atoms:number of carbon-carbon double bonds.

[a-b]Means with different superscripts within a certain muscle group and row differ ($P < 0.05$).

Table 2.6 Proportions of conjugated linoleic acids, total fatty acids (% of sample) and total fatty acid concentrations (mg/g) in raw muscle samples from carcasses of sedentary heifers and heifers subjected to exercise 3 times per wk throughout the finishing period.

	Longissimus Lumborum			*Triceps Brachii*			*Semitendinosus*			*Semimembranosus*		
	Treatment[1]			Treatment[1]			Treatment[1]			Treatment[1]		
	Sedentary	Exercise	SEM	Sedentary	Exercise	SEM	Sedentary	Exercise	SEM	Sedentary	Exercise	SEM
n-6 fatty acids[2]												
18:2n-6c	2.72	2.48	0.123	2.65	2.76	0.0886	2.50	2.65	0.108	2.73	2.92	0.113
18:3n-6c	0.072	0.062	0.0065	0.041	0.053	0.0079	0.076	0.057	0.011	0.052	0.046	0.0072
20:3n-6	0.195	0.184	0.0103	0.219	0.236	0.0107	0.208	0.223	0.0997	0.236	0.250	0.0953
20:4n-6	0.038	0.030	0.0052	0.030	0.031	0.0032	0.028	0.032	0.0046	0.035	0.043	0.0047
n-3												
18:3n-3	0.142	0.118	0.0106	0.117	0.118	0.00788	0.140	0.127	0.0122	0.119	0.121	0.0103
20:5n-3	0.062[a]	0.031[b]	0.010	0.049	0.040	0.015	0.116[a]	0.064[b]	0.017	0.090	0.058	0.016
22:5n-3	0.125	0.124	0.00835	0.136	0.139	0.0211	0.131	0.134	0.00687	0.127[b]	0.147[a]	0.00656
22:6n-3	0.044	0.036	0.0045	0.046	0.048	0.0063	0.055[a]	0.042[b]	0.0039	0.039	0.045	0.0063
CLA												
18:2, cis-9, trans-11	0.024	0.029	0.0038	0.021	0.020	0.0039	0.031	0.025	0.0057	0.022	0.019	0.0032
18:2, trans-10, cis-12	0.006	0.010	0.003	0.006	0.007	0.002	0.015	0.013	0.0035	0.009	0.005	0.002
18:2, cis-9, cis-11	0.006	0.002	0.002	0.002	0.005	0.002	0.012	0.005	0.004	0.003	0.002	0.001
18:2, trans-9, trans-11	0.089	0.067	0.095	0.058	0.057	0.0081	0.091	0.081	0.011	0.065	0.063	0.011
SFA[3]	32.2	25.3	3.54	19.1	18.3	2.20	23.7	22.7	3.32	20.4	20.3	2.87
MUFA[4]	3.65	3.03	0.476	2.49	2.50	0.325	3.39	3.33	0.484	2.60	2.60	0.414
PUFA[5]	31.8	25.2	3.37	21.1	20.0	2.28	25.5	25.1	3.31	22.2	22.0	2.82
CLA[6]	0.123	0.108	0.0153	0.090	0.090	0.015	0.149	0.124	0.0192	0.101	0.090	0.013
Total fatty acids, %	6.76	5.36	0.735	4.28	4.08	0.479	5.26	5.11	0.708	4.52	4.48	0.607

[1]Sedentary = No exercise throughout the finishing period; Exercise = Heifers exercised at 5 to 6 km/h 3 times per wk (20 min/d for the first 2 wk, 30 min/d for the next 2 wk, and 40 min/d for the remaining 4 wk of the finishing period).
[2]Fatty acids are represented as number of carbon atoms:number of carbon-carbon double bonds.
[3]SFA = sum of fatty acids that contain no double bonds.
[4]MUFA = sum of fatty acids that contain 1 double bond.
[5]PUFA = sum of fatty acids that contain 2 or more double bonds.
[6]CLA = sum of conjugated linoleic acids.
[a-b]Means with different superscripts within a certain muscle group and row differ ($P < 0.05$).

Chapter 3 - The effects of routine exercise on feedlot steer well-being, performance, and carcass characteristics.

Abstract

This research evaluated the effects of routine exercise of feedlot steers on various live animal and meat traits. Steers (n=419) were assigned to 1 of 4 treatments: not exercised during the finishing period or exercised 20-30 min, 3 times/wk by animal handlers for the first 10 wk; for the last 7 wk; or for the entire 17-wk finishing period. Live animal performance traits were similar ($P > 0.05$) between treatments. No treatment × day interactions were observed ($P > 0.05$) for blood constituents and temperament score. Cattle that began exercise at the beginning of the study had decreased ($P = 0.022$) USDA marbling scores in contrast to non-exercised animals. Additionally, there were no differences ($P > 0.05$) between treatments for instrumental color, WBSF, total fatty acids, and collagen content of beef *longissimus lumborum* muscle. Routine exercise as implemented in this study has minimal impact on live animal and meat characteristics.

Keywords: animal welfare, beef, exercise, meat quality, performance, stress

1. Introduction

Cattle feeding operations in the United States are designed to maximize growth and accelerate fat accumulation to efficiently produce high quality beef. Beef producers in the U.S. have optimized efficiency by placing cattle in concentrated animal feeding operations. These operations minimize the space needed to produce beef which inherently limits the physical activity of cattle. Additionally, feedlot cattle encounter limited amounts of human-animal interaction aside from processing upon entry to the feedlot and before shipping cattle for slaughter, which may result in the cattle overreacting to potentially stressful situations involving animal handlers.

Recent trends indicate that consumers are becoming more conscious of how and where their food is produced. In terms of meat production, the general public has shown specific interest in animal welfare and production practices used in the livestock industry. Consumers feel that sensory characteristics, healthiness, convenience and process characteristics (how animals were raised) are of high importance when making their purchasing decisions at the retail counter (Grunert, Bredahl, & Brunso, 2004). Buyers have also shown a willingness to pay more for certain products that were raised by verified processes which has led to an increased availability of "natural", "organic", and "grass-fed" beef products in the market-place (Olynk, Tonsor, & Wolf, 2010). Incorporation of practices to improve cattle well-being may provide niche marketing opportunities; however, these practices must be examined to determine how they affect live animal efficiency as well as carcass traits.

Rearing cattle in large feedlots may present adverse effects to animal health due to their sedentary lifestyle and high energy diet. Glucose is necessary for the function of many tissues, but an overabundance of glucose in the body can be toxic and is known as hyperglycemia (Rosetti, Giaccari, & DeFronzo, 1990). Insulin levels increase after a meal is consumed to stimulate glucose transport, metabolism, and storage by muscle and hepatocytes (Shepherd & Kahn, 1999). Chronic hyperglycemia can impair the function of insulin in the body creating insulin-resistance, which prevents glucose transport and is attributed to type 2 diabetes mellitus in humans (Shepherd & Kahn, 1999). Most beef in the U.S. is produced from cattle fed high-concentrate diets primarily composed of corn (Vasconcelos & Galyean, 2007) which is high in starch content. Ruminant animals that are fed on high-starch diets produce greater amounts of

propionate which is readily converted to glucose (Huntington, 1997). It is quite possible that cattle produced in these settings could be developing insulin-resistance due to hyperglycemic conditions.

Consumers' concerns for animal welfare are centered on animal health and living environment (Frewer, Kole, Van De Kroon, & De Lauwere, 2005). Reconstructing current cattle production systems would be rather costly, however, there is potential to incorporate practices that can enhance the well-being of livestock produced in current feedlot systems. Exercise is a vital component to a healthy lifestyle and reduces type 2 diabetes mellitus in humans (Goodyear & Kahn, 1998). A pilot study conducted at Kansas State University investigated this hypothesis and showed that moderate exercise reduced blood insulin levels in feedlot heifers (Chapter 2, p. 39), which suggests that exercised cattle may have improved insulin sensitivity. The implementation of exercise using animal handlers would increase the amount of human-animal interaction that feedlot cattle encounter which decreases blood cortisol (Boandl, Wohlt, & Carsia, 1989). Increased handling may decrease the stress inherent with handling and loading of cattle prior to slaughter (Grandin, 1997). Additionally, performance and carcass traits can be improved if handling can improve cattle docility (Reinhardt, Busby, & Corah, 2009).

Moderate exercise could alter meat quality components that are crucial in consumer purchasing decisions and product acceptability. Fresh meat color is the most important characteristic that consumers use to select meat cuts at the retail counter. Consumers perceive bright-pink to bright-red meat color as being desirable and any deviation from this is unacceptable (Kropf, 1980). Additionally, tenderness is one of the most important factors affecting consumer acceptance of cooked beef in the United States (Miller et al., 1995). The consumption of meat cuts that are unacceptably tough can drastically impact the future purchasing decisions of consumers (Boleman et al., 1997). The value of an alternative production system would be diminished if it yielded products of unacceptable quality. The exercise of feedlot heifers showed no influence on the tenderness of four muscles but negatively influenced meat color (Chapter 2, p. 42).

Exercise could lead to a change in physical and structural composition of an animal. Compositional changes could influence the development of quality traits and influence the nutritional profile of a product. Collagen is highly correlated with meat tenderness (Dransfield et al., 2003) and the amount and types of collagen in a muscle can be very influential on the degree

of tenderness that is perceived by a consumer. Research evaluating the effects of exercise on collagen composition in livestock has produced mixed results. Exercise has shown decreases in collagen amount in *vastus lateralis* from exercised sheep (Aalhus, Price, Shand, & Hawrysh, 1991) whereas exercise did not alter collagen content in the *longissimus dorsi* of swine (Petersen, Berge, Henckel, and Soerensen, 1997). Endurance exercise may require that animals utilize stored adipose tissue as an energy source. Evidence, from species other than cattle, indicate that chronic exercise can alter portions of the fatty acid profile in skeletal muscle (Szabo, Romvari, Febel, Bogner, & Szendro, 2002; Quiles, Huertas, Manas, Battino, & Mataix, 1999) suggesting that exercise could potentially yield beef with an altered fatty acid profile. It is still unclear if exercise influences the composition of collagen and fatty acids in skeletal muscle of exercised beef cattle.

The combination of animal welfare and effective labeling of animal products can help increase the demand for perceived animal welfare-friendly products (Napolitano, Girolami, & Braghieri, 2010). To date there has been limited research investigating the application of exercise regimens of cattle in a commercial feedlot setting. Therefore, the objective of this study was to examine the effects of a feedlot steer exercise regimen on animal live performance, behavior, physiology, meat quality, and meat composition.

2. Materials and methods

2.1 Animal background

The Kansas State University Institutional Animal Care and Use Committee approved the protocols (#3014) used in this study. Steers (n = 420; 377 ± 25 kg initial body weight) were transported to Kansas State University's Beef Cattle Research Center (BCRC) where they were stratified by weight and randomly assigned within strata to one of four treatments: 1) no exercise for the 116-d finishing period (CON); 2) exercised three times per wk for the first 10 wk of the 116-d finishing period (EARLY); 3) exercised three times per wk for the final 7 wk of the 116-d finishing period (LATE); and 4) exercised three times per wk throughout the 116-d finishing period (ALL). There were 28 pens used (7 pens per treatment and 15 animals per pen). One steer did not complete the study for reasons unrelated to the treatment. As a result, 1 pen contained only 14 steers at the end of the study. Steers were placed on a 21-d step-up diet prior to the application of exercise treatments, where they were fed a diet that consisted of 93% concentrate

and 7% roughage. Steers were housed in dirt-surfaced pens (9.9 × 24 m) throughout the study. Body weights (BW) were obtained on d 0, 72, and 116. Daily feed additions were recorded over the duration of the study.

2.2 Exercise protocol

Exercise was administered to exercise treatment groups between the times of 5:00 am and 7:00 am. Animal handlers exercised the cattle by moving them at a trotting pace (4.8 – 6.4 km/h) for 20 to 30 min on a 0.79 km long path surrounding a portion of the BCRC. Exercise time began at the moment the cattle exited their pen and concluded at the moment they reentered their pen. Animal handlers utilized noise paddles when necessary to accelerate the pace of the cattle's trot. Cattle were not subjected to exercise on days where the National Weather Service (Silver Spring, MD) predicted a heat index above 43 ºC or recorded that the nightly low temperature did not go below 26 ºC to avoid heat stress. Exercise was not conducted on days when severe storms or lightning were present in the vicinity of the BCRC to ensure the safety of the steers and animal handlers.

2.4 Temperament

Temperament scores were obtained on d 0, 72, and 116. Temperament scores were determined while cattle were held in a handling chute (Model HSC-10; Daniels Manufacturing Company, Ainsworth, NE) and assigned a score by the same person each day of evaluation using the following rubric: 1) calm and no movement, 2) restless shifting, 3) squirming and occasional shaking of handling chute, 4) continuous vigorous movement and shaking of handling chute.

2.5 Blood constituents

Three steers from each pen (n=84) were randomly selected to monitor blood constituents throughout the study. Blood samples were collected on d 0, 72, and 116 via jugular vein puncture a minimum of 24 h after the most recent bout of exercise. An additional blood sample was taken at the harvest facility immediately after exsanguination. Blood used to assess concentrations of glucose, lactate, insulin, cortisol, epinephrine, and norepinephrine were placed in tubes containing an anticoagulant and centrifuged for 15 min at 2,000 × *g* at 13 ºC. Blood plasma glucose and lactate were measured using a glucose-lactate autoanalyzer (2300 Stat Plus; YSI Inc., Yellow Springs, OH). Commercial radioimmunoassay kits were used to analyze both blood

serum insulin (Insulin Coat-a-Count, #TKCO1; Siemens Medical Solutions Diagnostics, Los Angeles, CA) and serum cortisol (Cortisol Coat-A-Count, #TKIN; Siemens Medical Solutions Diagnostics, Los Angeles, CA) concentrations.

Plasma epinephrine and norepinephrine were isolated using activated alumina and 0.1 M $HClO_4$ and quantified in duplicate using HPLC as described by Holladay and Edens (1987). A plasma sample of 0.5 ml was combined with 250 ng of the internal standard; 3,4-dihydroxybenzylamine hydrobromide (DHBA). Catecholamine:DHBA peak height ratios for samples and standards were determined and sample catecholamine concentrations were calculated using the regression equation generated from each catecholamine standard. Duplicate samples with CV greater than 5% were re-analyzed until variation was within the acceptable limits.

2.6 Carcass data collection

Steers were slaughtered on d 117 at a commercial abattoir located 451 km away from the BCRC. Hot carcass weights (HCW) were recorded on the day of slaughter and percentage of dressed yield was determined as HCW divided by final BW. Additionally, livers were assessed for incidence and severity of abscesses on the day of slaughter. Abscesses were evaluated and scored based on the Elanco scoring system: 0 = no abscesses, A- = 1 or 2 small abscesses or abscess scars, A 0 = 2 to 4 small, well-organized abscesses, and A + = 1 or more large or active abscesses with or without adhesions (Brink, Lowry, Stock, & Parrott, 1990). *Longissimus* muscle (LM) area, subcutaneous fat at the 12th rib, kidney, pelvic, and heart fat percentage (KPH%), USDA yield grade, and USDA marbling score were determined after a 30-h chill.

2.7 Meat sample collection and portioning

Approximately 48 h postmortem, 84 strip loins (IMPS 180) were randomly selected from the right side of the carcasses so that each live pen was represented by three loins. The strip loins were then transported to the Kansas State University Meat Laboratory where they were portioned to perform multiple analyses. Strip loins were removed from their oxygen impermeable vacuum package and the *longissimus lumborum* (LL) was trimmed free of fat. The anterior end of each LL muscle was removed for collagen and fatty acid analysis. A 2.54 cm thick steak was taken from the anterior portion of the remaining muscle to measure Warner-Bratzler shear force and

the remaining portion was used to determine International Commission on Illumination (CIE) L* (lightness), a* (redness), and b* (yellowness) color values for lean color assessment.

2.8 Instrumental color

The portions of the LL used for lean color assessment were vacuum packaged in oxygen impermeable film (Prime Source Vacuum Pouches, Kansas City, MO; 76.2 μm, STP Barrier, Nylon/PE Vacuum Pouch; oxygen transmission rate 0.04 g/254 cm^2/24 h at 0 ºC; water vapor transmission rate 0.2 cc/254 cm^2/24 h at 0 ºC at 0% relative humidity) and stored at 0-2 ºC until 14 d postmortem. On d 14, the sections were removed from their packaging and two 2.54 cm steaks were removed from the anterior end with the second steak that had not been exposed to oxygen being used for lean color analysis. The steaks were placed on a styrofoam tray (2S; Cryovac Sealed Air, Duncan, SC) with a moisture absorbent pad (Dri-Loc Pad; Sealed Air Corporation, Elmwood Park, NJ) and wrapped in oxygen permeable polyvinyl chloride film (Prime Source, oxygen transmission rate 0.6 g/254 cm^2/24 h at 0 ºC; water vapor transmission rate 0.6 cc/254 cm^2/24 h at 0 ºC and 0% relative humidity). After packaging, the steaks were allowed 30 min to bloom before color measurements were taken. Instrumental color of the lean surface (CIE L*, a*, and b*, Illuminant A) was measured with a HunterLab Miniscan XE Plus spectrophotometer (2.54-cm-diameter aperture, 10º standard observer; Hunter Associates Laboratory, INC., Reston, Va., U.S.A.). Three readings were taken for each steak and values were averaged prior to statistical analysis.

2.9 Warner-Bratzler shear force

Steaks were vacuum packaged in oxygen impermeable film (Prime Source Vacuum Pouches, Kansas City, MO; 76.2 μm, STP Barrier, Nylon/PE Vacuum Pouch; oxygen transmission rate 0.04 g/254 cm^2/24 h at 0 ºC; water vapor transmission rate 0.2 cc/254 cm^2/24 h at 0 ºC at 0% relative humidity) and aged for 14 d at 0-2 °C before being frozen at -20 °C. Steaks were thawed for approximately 12 h at 0-2 °C and cooked to 40 °C, turned, and cooked to a final internal temperature of 70 °C in a dual flow, forced-air convection gas oven (Blodgett, model DFC-102 CH3; G.S. Blodgett Co., Burlington, VT) preheated to 163 °C. Steak temperatures were monitored with copper-constantan thermocouples (Omega® Engineering, Stamford, CT) inserted into the approximate geometric center of each steak and attached to a Doric temperature recorder (model 205; Vas Engineering, San Francisco, CA). The steaks were chilled overnight at

0-2 °C before 8 round cores (1.27-cm diameter) were obtained from each steak parallel to the long axis of the muscle fibers using a 1.27-cm corer (G-R Manufacturing Co., Manhattan, KS). Each core was sheared once perpendicular to the direction of the muscle fibers using a Warner-Bratzler V-shaped blunt blade (G-R Manufacturing Co., Manhattan, KS) attached to an Instron Universal Testing Machine (model 4201, Instron Corp., Canton, MA) with a 50-kg compression load cell and a crosshead speed of 250 mm/min. Peak shear force values were recorded in kg and the values from the cores were averaged for statistical analysis.

2.10 Collagen extraction

Samples used for collagen assays were stored at -80 ºC in a sterilized plastic container (Whirl Pack, Nasco, Modesta, CA) and thawed at 0-2 ºC prior to assessment. A modified version of Hwang, Mizuta, Yokoyama, and Yoshinaka (2007) was used to purify collagen from the LL samples. Samples (5 g) were homogenized (Power Gen 1000; Fisher Scientific, Waltham, MA) in 5 mL of 0.1 M NaOH. After rocking for 24 h at 4 ºC, samples were centrifuged (Model 5810 R; Eppendorf North America, Hauppauge, NY) at 12,000 × *g* for 45 min at 4 ºC and the supernatant was removed. The remaining pellet was then dried and mixed with 5 mL of 0.5 M acetic acid before rocking overnight at 4 ºC. The samples were again centrifuged at 12,000 × *g* for 45 min at 4 ºC and the supernatant was removed. The pellet was dried and weighed prior to enzymatic extraction (0.05 mL/mg tissue) in a pepsin solution (1 mg/mL in 0.5 M acetic acid) for 24 h. The samples were again centrifuged at 12,000 × *g* for 45 min at 4 ºC.

2.11 SDS-PAGE analysis

Protein from the pepsin/acetic acid supernatant was resuspended in 2 M Tris Base and sample protein concentrations were quantified using a BCA protein assay kit (Pierce, Rockford, IL). Protein (5 μg) was loaded into 7.5% separating polyacrylamide gels with 3.5% stacking gels and separated at 25 mA. Gels were stained with SYPRO Ruby protein gel stain (Lonza, Rockland, ME). Proteins were fixed in the gel using 40% methanol/10% acetic acid (v/v) for 20 min. The gel was then incubated in the SYPRO Ruby protein gel stain solution overnight and then washed twice in 10% methanol/7% acetic acid (v/v) solution for 30 min. Gels were then visualized by excitation at 300 nm using a UV transilluminator and photographed using a GelDoc-It 415 Imaging System (UVP LLC, Upland, CA) with a 605 nm ethidium bromide emission filter. Band intensities were quantified using VisionWorks LS Image Acquisition and

Analysis Software (UVP LLC, Upland, CA) and values for EARLY, LATE, and ALL treatments were normalized to the average of 100% of CON samples on each gel. Values are reported relative to 100% CON.

2.12 Fatty acid composition

Fatty acid samples were stored in a sterilized bag (Whirl Pack, Nasco, Modesta, CA) at -20 ºC. A modified gas chromatography procedure of Sukhija and Palmquist (1988) was used for fatty acid analysis of LL samples. Each LL sample was frozen in liquid nitrogen, pulverized using a tabletop blender (model 33BL79; Waring Products, New Hartford, CT), and analyzed for fatty acids. Muscle (50µg) samples were combined with 2 mL of methanolic-HCl and 3 mL of internal standard (2 mg/mL of methyl Heptadecanoic acid (C17:0) in benzene) and heated in a water bath for 120 min at 70 °C for transmethylation. After cooling, the addition of 2 mL of benzene and 3 mL of K_2CO_3 allowed the methyl esters to be extracted and transferred to a vial for subsequent quantification of the methylated fatty acids by gas chromatography for fatty acid analysis. Injection port and detector temperatures were set at 250 °C, with a helium flow rate of 1mL/min and a split ratio of 100:1. Oven temperature began at 140 °C and was increased at 2 °C/min to 200 °C and then increased at 4 °C /min to 245 °C where it was held for 17 min. Fatty acids from each of the LL samples were expressed as a proportion of the total sample.

2.13 Statistical analysis

Data were analyzed as a randomized complete block design with weight strata serving as the block. Statistical analysis was performed using the Proc Mixed procedure of SAS (SAS Institute, Inc., Cary, NC). Exercise treatment was a fixed effect and strata served as a random effect. Time and time × exercise treatment were included as fixed effects for BW, temperament, and blood constituents. Treatment means were computed with the LSMEANS option. Post-hoc mean separation was carried out using Fisher's least significant difference. All treatment means were considered significantly different at the 5% level of significance.

3. Results and discussion

Exercise groups did not attain the maximum possible days of exercise due to weather-related cancellations. Cattle were exercised a total of 26, 18, and 44 d for the EARLY, LATE, and ALL treatments, respectively. All cattle did not exert the same willingness to exercise as

some would begin running without being provoked, while others were very resistant to begin moving.

3.1 Live animal performance

Body weight displayed a treatment × day interaction ($P = 0.015$; Table 3.1). All treatments were similar ($P > 0.05$) on d 0 and increased ($P < 0.05$) at d 72 and 116. LATE and CON cattle were heavier ($P < 0.05$) than EARLY cattle on d 72, while LATE cattle were also heavier ($P < 0.05$) than ALL cattle. At d 116, CON cattle were heavier ($P < 0.05$) than both LATE and ALL cattle. Main effect treatment means for feedlot growth performance traits are presented in Table 3.2. Cattle from all treatments showed similar average daily gains (ADG; $P = 0.178$), dry matter intakes (DMI; $P = 0.100$), and gain:feed ratios (G:F; $P = 0.116$). Results of prior research contradict those seen in the current study. Heifers exposed to exercise for 60 d (Chapter 2, pp. 37-38) and 18 mo old steers subjected to daily waking exercise (Dunne, O'Mara, Monahan, French, & Moloney, 2005) both showed lighter live weights and decreased ADG in comparison to their sedentary counterparts. Conversely, DMI and G:F was similar between exercised and sedentary heifers (Chapter 2, p. 38), which agrees with the current study. The exercise treatments implemented in the current study could be considered less intense than the previously mentioned research, as it was either for a shorter time period (Chapter 2, p. 22) or covered a shorter distance (Dunne et al., 2005) which may explain the lack of differences seen between treatment groups. It is quite possible that the exercise employed in the current study was not of sufficient intensity to evoke enough change in required energy for maintenance to interrupt weight gain and feed efficiency.

Exercise of cattle has received little attention in scientific literature; however, other animal models evaluating exercise have been researched frequently. Rats with access to activity wheels displayed lighter body weights than those without access to activity wheels (Bell, Spencer, & Sherriff, 1997). Moreover, exercised (1 h/d, 6 d/wk) rats exhibited decreased DMI, ADG, and feed efficiency when compared to their sedentary counterparts (Mirand et al., 2004).

Exercise is a subjective term as it is defined in numerous ways throughout the scientific literature. The current study defined exercise as moving animals for 20-30 min at a trotting pace, while others have exercised for longer periods of time, longer distances, and at several intensities. It is important to take these differences into consideration when evaluating an

exercise regimen as the various definitions of exercise could produce different live animal performance measures.

3.2 Temperament

Animals that have frequent contact with people are generally less stressed due to restraint and handling than those that are rarely in contact with people (Grandin, 1997). In the current study, no treatment × day interaction ($P = 0.213$) was observed for temperament scores (Table 3.3). Individually, both treatment and day had no effect on temperament scores ($P > 0.05$). The lack of change over the duration of the current study contradicts prior findings that showed cattle subjected to a handling acclimation scenario twice per wk had lower temperament scores than non-acclimated cattle (Francisco, Cooke, Marques, Mills, Bohnert, 2012). The increase in human-animal interaction that exercised steers received in this study did not alter the cattle's demeanor during blood and weight collection times.

3.3 Blood constituents

No treatment × day interactions were observed ($P > 0.05$) for any of the blood constituents assessed in this study (Table 3.4). Blood glucose and lactate were not influenced ($P > 0.05$) by exercise treatments, but glucose increased ($P < 0.001$) and lactate decreased ($P = 0.005$) as time progressed. Glucose is a significant fuel for working muscle and exercised sheep exhibited an increase in both blood glucose and lactate in contrast to their non-exercised counterparts (Apple, Minton, Parsons, Dikeman, & Leith, 1994). Additionally, blood glucose levels were decreased and blood lactate levels were increased due to a session of exercise in horses (Ferrante & Kronfeld, 1994). Evaluation of blood characteristics by Apple et al. (1994) as well as Ferrante and Kronfeld (1994) were done during or immediately after exercise while the current study evaluated blood constituents after cattle had an opportunity to recover from a session of exercise. The changes of blood glucose and lactate, which were time related, are most likely a consequence of feeding and adaptation to feeding. The cattle in this study were fed a high concentrate diet which readily produces glucose via fermentation in the rumen (Huntington, 1997). The current study agrees with the findings of Ellenberger et al. (1989) who showed that glucose increased in response to greater intake of metabolizable energy.

A vast portion of research investigating blood lactate in feedlot cattle is focused on the early portion of the feeding period as abnormally high levels of blood lactate are associated with

acidosis (Owens, Secrist, Hill, & Gill, 1998). In advanced cases of acidosis, lactate can enter the blood of ruminants due to absorption from the rumen or metabolism of propionate in the rumen epithelium (Dziuk, 1984). The decreases of blood lactate in this study agrees with the findings of Schwaiger, Beauchemin, and Penner (2013) who observed a decrease of blood lactate during recovery after an acidosis challenge. Decreased blood lactate during finishing, as seen in this study, is most likely attributed to an increase of lactate-utilizing bacteria in the rumen which is indicative of a proper adaptation to the diet at the onset of the finishing phase (Huber, Cooley, Goetsch, & Das, 1976).

Only blood serum insulin was affected by treatment ($P = 0.022$) as EARLY treatment cattle had reduced ($P < 0.05$) blood insulin in comparison to CON and ALL cattle. Insulin increased ($P < 0.001$) dramatically over the feeding period. Prior research showed that exercise effectively decreased blood insulin in finishing heifers (Chapter 2, p. 39). Moreover, insulin has been shown to increase (Schwaiger et al., 2013) or remain unchanged during finishing (Yambayamba, Price, & Foxcroft, 1996). Insulin is a key component of glucose disposal in muscle. When insulin sensitivity is reduced, more insulin is released in the body to facilitate glucose disposal, which is why elevated blood insulin is a primary symptom of type 2 diabetes mellitus in humans (Heled et al., 2005). The dramatic increase in insulin over time may signify that steers in the current study became more insulin-resistant as time progressed. Throughout the study, the LATE and ALL treatment cattle had blood insulin levels similar ($P > 0.05$) to CON cattle, which agree with the lack of change in blood insulin of obese horses subjected to low and high intensity exercise (Carter, McCutcheon, Valle, Meilahn, & Raymond, 2010).

None of the stress-related blood constituents evaluated in this study were affected ($P > 0.05$) by treatment. Blood cortisol was affected ($P < 0.001$) by day as it was lowest at d 72. Additionally, epinephrine and norepinephrine increased ($P < 0.001$) after d 0. Generally, calmer and less excitable cattle have lower blood cortisol levels than excitable cattle when they are handled (Hulbert et al., 2011; King et al., 2006). Furthermore, epinephrine and norepinephrine have been associated with animals exposed to stressful situations (Burdick, Randel, Carroll, & Welsh, 2011). The lack of differences between treatments for temperament scores in this study align with the lack of treatment differences for cortisol, epinephrine, and norepinephrine, which suggests that human-animal interactions associated with the employed exercise regimens did not change cattle temperament. The current research agrees with findings of Petherick, Doogan,

Venus, Holroyd, and Olsson (2009) who demonstrated that various intensities and methods of handling produced no differences in blood cortisol of feeder calves during backgrounding. Previously, Titto et al. (2010) showed that blood cortisol concentrations of steers housed in group pens decreased over a 112 d finishing period which contrasts results of the current study showing blood cortisol was the same at the beginning and end of the study. It is quite apparent that the human-animal interaction associated with the exercise treatments used in the current study was unable to condition animals to better cope with stress incurred during handling.

Assessment of blood constituents taken during exsanguination are presented in Table 3.5. There was no apparent influence of treatment ($P > 0.05$) on any of the blood constituents assessed in this study. Stress prior to slaughter can cause not only discomfort for the animal, but can contribute to various carcass defects, such as dark-cutting beef (Warren, Mandell, & Bateman, 2010). Cattle often have to be transported long distances prior to slaughter and cortisol's association with transportation has been investigated frequently. A 4 h transport resulted in an increase in cortisol concentrations of cattle (Murata, Takahashi, & Matsumoto, 1987) and cattle transported 200 km had heightened cortisol levels in comparison to cattle slaughtered in a research abattoir where no extensive transportation was needed (Tume & Shaw, 1992). Prior research has also evaluated how cattle of differing temperaments handle the stress associated with transportation. Hulbert et al. (2011) investigated how bulls of different temperaments coped with the stress of transportation and showed no difference between calm and temperamental bulls up to 24 h after transport. The lack of differences observed in the current study suggests that exercise did not alter how cattle cope with transportation-related stress prior to slaughter.

3.5 Carcass characteristics

Exercise is commonly known to change body composition. Exercised did not alter ($P > 0.05$) HCW and percentage of dressed yield (Table 3.6). Additionally, the occurrence of liver abscesses, LM area, 12^{th} rib fat, KPH%, and USDA yield grade were similar ($P > 0.05$) across treatments (Table 3.5). The only measurement of muscularity evaluated in this study was LM area. Dunne and others (2005) noted decreased conformation scores in carcasses from exercised steers which contrasts the lack of change in the current study. The LM is not heavily involved in animal movement so it is expected that it is minimally affected; however, muscles of various

function from lamb carcasses of treadmill exercised sheep also displayed a lack of change in mass (Aalhus & Price, 1991). Musculature could be influenced differently when resistance training is employed instead of endurance training, which was used in the current study. Sheep subjected to resistance jumping yielded larger *biceps brachii* and *brachialis* in comparison to non-exercised sheep (Aalhus & Price, 1990) demonstrating the relationship between muscle function and exercise type. The current study only evaluated the LM which may not portray the true musculature change that occurred due to exercise.

Exercise of sufficient intensity is generally regarded to reduce body fat (Pacy, Webster, & Garrow, 1986), so it is expected that carcass fat should be reduced from animals subjected to routine exercise. The results of the current study disagree with prior research that showed reductions in 12^{th} rib fat (Chapter 2, p. 41) and fat score (Dunne et al., 2005) in carcasses from exercised cattle. Multiple rodent models have further verified that exercise can result in a reduction of body fat (Bell et al., 1997; Mirand et al., 2004) so the lack of change in carcass fat detected in the current study could be due to insufficient exercise intensity that failed to change the energy requirements of the cattle.

Feed restriction decreases energy intake that induces a temporary reduction of fatness, which is not totally restored during the recovery period (Robelin, 1986). It is possible that exercise early in the finishing phase creates a similar effect because exercised animals require more energy for maintenance (Donnelly & Smith, 2005). Exercised animals may have less excess energy available to deposit as fat, specifically marbling. If suppressed early on, adipose tissues will undergo hypertrophy at a reduced rate during the remainder of finishing (Robelin, 1986). This scenario may explain why carcasses from EARLY and ALL treatment cattle produced decreased ($P = 0.022$) marbling scores in comparison to CON carcasses which maintained similar ($P < 0.05$) marbling scores to the LATE exercise treatment. Heifers exercised up to 40 min per d maintained similar marbling scores in comparison to sedentary heifers (Chapter 2, p. 41); however, these heifers were only exercised for an 8 wk period whereas the present study subjected exercise for as long as 17 wk.

3.6 Instrumental color

Treatment had no effect ($P > 0.05$) on the instrumentally-measured CIE L*, a*, and b* values as well as saturation index and hue angle of LL steaks (Table 3.7). Steers walked 4.41 km

on a daily basis produced no change in 'L', 'a', and 'b' values of the *longissimus dorsi* (Dunne et al., 2005) which aligns with the present study; however, implementation of routine exercise in feedlot heifers produced carcasses with decreased a* and b* values from the *longissimus thoracis* in comparison to their sedentary counterparts (Chapter 2, p. 42). Physical activity associated with grazing is thought to produce darker meat (Muir, Smith, Wallace, Cruickshank, & Smith, 1998), but the lack of change observed in L* values in the present study and others (Chapter 2, p. 42; Dunne et al., 2005) refute this idea. Attention may need to be directed towards changes in redness (a*, 'a' values) which have been observed in the *longissimus thoracis* (Chapter 2, p. 42) and *semimembranosus* muscles (Dunne et al., 2005) from carcasses of exercised cattle. Active animals contain more myoglobin in comparison to their inactive counterparts, but differences between muscles are also present (Essen-Gustavsson, 1996). Muscles more heavily associated with locomotion need to be evaluated to properly assess the role that exercise contributes in the development of meat color.

3.7 Warner-Bratzler shear force

No differences ($P = 0.860$) in LL WBSF were detected between treatment groups as WBSF were 4.46, 4.71, 4.62, and 4.55 kg for CON, EARLY, LATE, and ALL respectively. The reported WBSF values in this research would be considered to be "intermediate" or "tough" in terms of tenderness according to confidence intervals reported by Shackelford, Morgan, Savell, and Cross (1991). Previous research demonstrated that exercise does not alter WBSF of the *longissimus lumborum*, *triceps brachii*, *semitendinosus*, and *semimembranosus* muscles in feedlot heifers (Chapter 2, p. 42), which agrees with the current study; however, research in sheep demonstrated decreases in WBSF of the *semimembranosus* and *vastus lateralis* muscles in carcasses of exercised lambs (Aalhus et al., 1991). The reduction in shear force of the *vastus lateralis* was attributed to a dilution of collagen as collagen decreased and myofibrillar protein increased on a per gram of muscle basis (Aalhus et al., 1991).

Meat tenderness is affected by multiple factors including proteolysis, connective tissue, and muscle contractile state (Belew, Brooks, McKenna, & Savell, 2003) which can vary between muscles depending on their function. The LL has limited involvement in animal movement in comparison to muscles located in the chuck and round so it's logical that the LL would demonstrate minimal change in response to exercise. Once more, variations of exercise intensity

exist between the previously mentioned research and the present study. The current study contained less intense exercise than the 90 min bouts of exercise that Aalhus and others (1991) subjected to sheep 5 times/wk which yielded tenderness improvements. It appears that muscle function and exercise intensity are likely factors contributing to the lack of tenderness change observed in this study.

3.8 Type I collagen crosslink states

Separated collagen type I crosslink states of LL muscle are detailed in Table 3.8. Collagen analysis indicated no treatment effect ($P > 0.05$) for Beta 11, Beta 12 and Gamma collagen type I crosslink states. These findings coincide with the results of Petersen et al. (1997) who showed that treadmill training of pigs had no effect on the amount of total and heat-soluble collagen as well as the solubility of collagen in the *longissimus dorsi*. Prolyl-4-hydroxylase (PH) and galactosylhydroxylysylyl glucosyltransferase (GGT) both participate in the post-translational modifications of the collagen biosynthesis (Kivirikko & Myllyla, 1982). Exercise appears to affect PH and GGT activity differently in young and old rats. As animals age, both PH and GGT activity is decreased greatly (Kovanen & Suominen, 1989). Exercise had no influence on PH and GGT activity in rats as old as 4-mo, but exercise increased both PH and GGT activity in rats older than 4-mo (Kovanen & Suominen, 1989) suggesting that exercise may influence collagen biosynthesis in an age-dependent manner. Additional research revealed that exercise exhibited no effect on collagen concentration of young (3 mo old) and old (23 mo old) rats. Additionally, exercise did not change the proportion of the non-reducible collagen cross-link hydroxylsylpyridinoline (HP) in young rats, but greatly reduced the amount of HP in old rats (Gosselin, Adams, Cotter, McCormick, & Thomas, 1998). Cattle finished in feedlot systems reach market weights at a relatively young age so exercise may not influence their collagen composition; however, exercise use in finishing systems that take longer to finish animals, such as grass-feeding, may see alterations in certain collagen cross-links due to increased collagen biosynthesis.

3.9 Fatty acid composition

Proportions of individual saturated and monounsaturated fatty acids are displayed in Table 3.9 and proportions of conjugated linoleic acids (CLA), polyunsaturated fatty acids, and total fatty acid are available in Table 3.10. Treatment had no effect on concentrations of

individual fatty acids aside from the CLA 18:2, trans-10, cis-12 which was decreased ($P = 0.011$) in all exercise treatments when compared to the control treatment. Total fatty acid percentages were similar ($P = 0.663$) between treatments. Additionally, there were no distinguishable differences between treatments for total saturated fatty acids ($P = 0.671$), total monounsaturated fatty acids ($P = 0.679$), total polyunsaturated fatty acids ($P = 0.269$), and total CLA ($P = 0.151$). Prior research shows exercise of cattle has a minimal effect on the fatty acid profile of multiple muscles (Chapter 2, pp. 43-44). Exercise has been evaluated in multiple species aside from cattle, but results are inconsistent when evaluating the effect of exercise on fatty acid composition (Nikolaidis & Mougios, 2004). The findings of the current study contrast those of Szabo et al. (2002) who showed that exercise in rabbits produced an increase of oleic acid (C18:1 n-9) in the *longissimus dorsi* and *vastus lateralis* but decreased stearic (C18:0) and arachidonic (C20:4 n-6) acids in the *vastus lateralis*. Moreover, Quiles et al. (1999) showed that exercise of rats yielded lower percentages of arachidonic acid (C20:4 n-6) and polyunsaturated omega-3 fatty acids in the *vastus lateralis*. Both of the prior studies utilized exercise regimens that were more frequent, longer in duration, and higher in intensity than the current study. Also, numerous muscles from multiple animal sources have been evaluated to investigate the influence of exercise on fatty acid profiles, but results appear to show that these alterations are independent of muscle fiber type (Nikolaidis & Mougios, 2004). Exercise regimens of great intensity may need evaluation to determine the capabilities of exercise to change the fatty acid profile, but intensities greater than that of the current study may be difficult to administer in a feedlot scenario.

4. Conclusions

Feedlot cattle could be subjected to routine exercise to combat the lack of physical activity they incur on a daily basis. The EARLY exercise treatment evaluated in this study demonstrated a potential to decrease blood insulin, but additional findings suggest no other health improvement or stress reductions can be produced using the discussed exercise regimens. The exercise regimens used in this study provided no performance or carcass yield advantages. Additionally, EARLY and ALL treatment cattle had decreased marbling scores in comparison to CON cattle. If exercise is implemented, additional investigation is needed to evaluate how long cattle should be on feed prior to the initiation of exercise to ensure that marbling is not reduced.

Due to the lack of significant research in this subject area, further research needs to be conducted to evaluate varying intensities of exercise regimens and their effect on a wider range of beef carcass composition and beef quality traits from muscles located in the chuck and round.

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Table 3.1 Body weights of feedlot steers subjected to routine exercise treatments.

	Treatment[1]					*P*-Values[2]		
	CON	EARLY	LATE	ALL	SEM	E	D	E × D
Body weight						0.226	< 0.001	0.013
Day 0	376[f]	376[f]	378[f]	376[f]	3.36			
Day 72	548[cd]	540[e]	549[c]	542[de]	3.36			
Day 116	620[a]	618[ab]	613[b]	611[b]	3.36			

[1]CON = no exercise for the 116-d finishing period; EARLY = exercised three times per wk for the first 10 wk of the 116-d finishing period; LATE = exercised three times per wk for the final 7 wk of the 116-d finishing period; ALL = exercised three times per wk throughout the 116-day finishing period. Exercise was conducted by animal handlers who moved the cattle at a pace of 4.8-6.4 km/h for 20-30 min.

[2]E = effect of exercise treatment; D = effect of days on feed; E × D = interaction between exercise treatment and days on feed.

[a-f]Means with different superscripts differ ($P < 0.05$).

Table 3.2 Growth performance traits of steers subjected to routine exercise treatments.

	Treatment[1]					
	CON	EARLY	LATE	ALL	SEM	*P*-Value
ADG, kg	2.10	2.09	2.03	2.03	0.0657	0.178
DMI, kg	10.58	10.28	10.43	10.27	0.2876	0.101
G:F	0.196	0.201	0.191	0.194	0.00319	0.116

[1]CON = no exercise for the 116-d finishing period; EARLY = exercised three times per wk for the first 10 wk of the 116-d finishing period; LATE = exercised three times per wk for the final 7 wk of the 116-d finishing period; ALL = exercised three times per wk throughout the 116-day finishing period. Exercise was conducted by animal handlers who moved the cattle at a pace of 4.8-6.4 km/h for 20-30 min.

Table 3.3 Temperament scores[1] of steers subjected to routine exercise treatments.

	Treatment[2]					*P*-Values[3]		
	CON	EARLY	LATE	ALL	SEM	E	D	E × D
Temperament score						0.236	0.244	0.213
Day 0	1.9	1.9	2.0	2.0	0.17			
Day 72	1.4	2.0	2.0	1.8	0.17			
Day 116	1.8	1.6	1.8	1.9	0.17			

[1]Where 1 = calm and no movement, 2 = restless shifting, 3 = squirming and occasional shaking of handling chute, 4 = continuous vigorous movement and shaking of handling chute.
[2]CON = no exercise for the 116-d finishing period; EARLY = exercised three times per wk for the first 10 wk of the 116-d finishing period; LATE = exercised three times per wk for the final 7 wk of the 116-d finishing period; ALL = exercised three times per wk throughout the 116-day finishing period. Exercise was conducted by animal handlers who moved the cattle at a pace of 4.8-6.4 km/h for 20-30 min.
[3]E = effect of exercise treatment; D = effect of days on feed; E × D = interaction between exercise treatment and days on feed.

Table 3.4 Blood constituents[1] during feedlot finishing from steers subjected to routine exercise treatments.

	Treatment[2]					*P*-Values[3]		
	CON	EARLY	LATE	ALL	SEM	E	D	E × D
Glucose, mM						0.751	<0.001	0.372
Day 0	3.1	3.0	2.8	2.9	0.14			
Day 72	2.9	3.0	3.5	3.3	0.14			
Day 116	3.7	3.9	4.0	3.8	0.14			
Lactate, mM						0.145	0.005	0.812
Day 0	4.2	4.5	4.8	4.6	0.29			
Day 72	3.1	4.7	4.2	4.3	0.29			
Day 116	2.9	3.7	3.3	3.9	0.30			
Insulin, ng/mL						0.022	<0.001	0.237
Day 0	0.19	0.11	0.17	0.15	0.060			
Day 72	0.82	0.44	0.60	0.69	0.060			
Day 116	1.33	0.85	1.02	1.40	0.060			
Cortisol, ng/mL						0.911	<0.001	0.317
Day 0	35.3	37.2	43.4	40.3	1.48			
Day 72	24.8	24.6	19.4	20.7	1.48			
Day 116	32.9	33.4	35.7	34.6	1.51			
Epinephrine, pg/mL						0.553	<0.001	0.788
Day 0	67.7	77.3	72.7	73.7	2.70			
Day 72	101.8	107.8	100.5	109.0	2.70			
Day 116	94.7	92.8	91.8	98.9	2.72			
Norepinephrine,						0.184	<0.001	0.296
Day 0	42.4	43.6	42.1	37.8	2.36			
Day 72	71.1	63.9	72.7	71.0	2.37			
Day 116	68.5	63.5	80.1	66.0	2.38			

[1]Glucose, lactate, epinephrine, and norepinephrine analyzed using blood plasma. Insulin and cortisol analyzed using blood serum.

[2]CON = no exercise for the 116-d finishing period; EARLY = exercised three times per wk for the first 10 wk of the 116-d finishing period; LATE = exercised three times per wk for the final 7 wk of the 116-d finishing period; ALL = exercised three times per wk throughout the 116-day finishing period. Exercise was conducted by animal handlers who moved the cattle at a pace of 4.8-6.4 km/h for 20-30 min.

[3]E = effect of exercise treatment; D = effect of days on feed; E × D = interaction between exercise treatment and days on feed.

Table 3.5 Blood constituents[1] at exsanguination[2] from feedlot steers subjected to routine exercise treatments.

	Treatment[3]					
	CON	EARLY	LATE	ALL	SEM	*P*-Value
Glucose, mM	10.1	9.2	10.8	9.2	1.4	0.721
Lactate, mM	14.5	12.0	13.4	12.5	0.964	0.161
Insulin, ng/mL	0.49	0.55	0.47	0.47	0.090	0.910
Cortisol, ng/mL	34.8	27.9	35.7	29.5	6.26	0.759
Epinephrine, pg/mL	170.4	190.4	179.6	188.6	1.848	0.859
Norepinephrine, pg/mL	186.5	182.5	180.6	190.5	4.536	0.384

[1]Glucose, lactate, epinephrine, and norepinephrine analyzed using blood plasma. Insulin and cortisol analyzed using blood serum.
[2]Blood samples collected from blood flow initiated at exsanguination.
[4]CON = no exercise for the 116-d finishing period; EARLY = exercised three times per wk for the first 10 wk of the 116-d finishing period; LATE = exercised three times per wk for the final 7 wk of the 116-d finishing period; ALL = exercised three times per wk throughout the 116-day finishing period. Exercise was conducted by animal handlers who moved the cattle at a pace of 4.8-6.4 km/h for 20-30 min.

Table 3.6 Carcass characteristics from steers subjected to routine exercise treatments.

	Treatment[1]					
	CON	EARLY	LATE	ALL	SEM	*P*-Value
Hot carcass weight, kg	388	395	396	390	5.94	0.706
Dressed yield, %	60.9	61.2	60.3	61.2	0.88	0.611
Liver abscess, %	8.7	11. 5	14.3	14.5	3.4	0.381
A+[2]	1.0	1.0	0	3.9	1.2	0.101
A[3]	1.9	1.0	4.8	2.9	1.6	0.353
A-[4]	3.8	9.5	9.5	6.7	2.8	0.327
LM area, cm^2	97.8	102.2	101.8	95.4	2.43	0.188
12th-rib fat, cm	1.18	1.27	1.19	1.27	0.0862	0.826
KPH, %	2.1	2.3	2.3	2.3	0.093	0.225
USDA yield grade	2.5	2.4	2.4	2.7	0.18	0.526
USDA marbling score[5]	471^{a}	400^{c}	461^{ab}	419^{bc}	19.4	0.022

[1]CON = no exercise for the 116-d finishing period; EARLY = exercised three times per wk for the first 10 wk of the 116-d finishing period; LATE = exercised three times per wk for the final 7 wk of the 116-d finishing period; ALL = exercised three times per wk throughout the 116-day finishing period. Exercise was conducted by animal handlers who moved the cattle at a pace of 4.8-6.4 km/h for 20-30 min.

[2]A+ = 1 or more large, or multiple small, active abscesses, with or without adhesions.

[3]A = 2 to 4 small, well-organized abscesses.

[4]A- = 1 or 2 small abscesses or scars.

[5]Marbling score 400 to 499 = Small.

[abc]Means with different superscripts within the same row differ ($P < 0.05$).

Table 3.7 Instrumental color values of oxygenated *longissimus lumborum* steaks from carcasses of steers subjected to routine exercise treatments after 14 d of refrigerated storage in anaerobic packaging.

	Treatment[1]					
	CON	EARLY	LATE	ALL	SEM	*P*-Value
L* (lightness)	44.3	44.6	44.0	44.5	1.20	0.985
a* (redness)	32.7	32.3	33.0	33.4	0.527	0.563
b* (yellowness)	25.4	24.9	25.7	26.1	0.548	0.436
Hue angle[2]	37.8	37.4	37.8	38.0	0.236	0.316
Saturation index[3]	41.4	40.8	41.8	42.4	0.746	0.497

[1]CON = no exercise for the 116-d finishing period; EARLY = exercised three times per wk for the first 10 wk of the 116-d finishing period; LATE = exercised three times per wk for the final 7 wk of the 116-d finishing period; ALL = exercised three times per wk throughout the 116-day finishing period. Exercise was conducted by animal handlers who moved the cattle at a pace of 4.8-6.4 km/h for 20-30 min.

[2]Hue angle: $(b^*/a^*)^{tan-1}$.

[3]Saturation index: $(a^{*2} + b^{*2})^{1/2}$.

Table 3.8 Type 1 collagen crosslinks of beef *longissimus lumborum* from carcasses of steers subjected to routine exercise treatments[1].

	Treatment[2]					
	CON	EARLY	LATE	ALL	SEM	*P*-Value
Type 1 collagen crosslink state						
Beta 11	0.994	0.929	1.011	0.952	0.0931	0.856
Beta 12	0.986	0.973	1.002	0.950	0.0750	0.935
Gamma	1.011	1.053	1.108	1.043	0.0868	0.835

[1]Values calculated as a ratio of band density to average band density of CON within each SDS-PAGE gel.

[2]CON = no exercise for the 116-d finishing period; EARLY = exercised three times per wk for the first 10 wk of the 116-d finishing period; LATE = exercised three times per wk for the final 7 wk of the 116-d finishing period; ALL = exercised three times per wk throughout the 116-day finishing period. Exercise was conducted by animal handlers who moved the cattle at a pace of 4.8-6.4 km/h for 20-30 min.

Table 3.9 Proportions of saturated and monounsaturated fatty acids (mg/g) in raw *longissimus lumborum* steaks from steers subjected to routine exercise treatments.

	Treatment[1]					
	CON	EARLY	LATE	ALL	SEM	*P*-Value
Saturated fatty acids[2]						
14:0	1.23	1.03	1.17	1.26	0.110	0.481
15:0	0.243	0.219	0.238	0.250	0.0236	0.818
16:0	11.6	9.77	11.0	11.1	0.993	0.628
17:0	0.877	0.767	0.797	0.818	0.108	0.905
18:0	6.33	5.43	5.71	6.27	0.634	0.703
20:0	0.056	0.051	0.051	0.055	0.0063	0.897
21:0	0.127	0.105	0.989	0.111	0.0103	0.295
22:0	0.030	0.030	0.036	0.037	0.0038	0.415
24:0	0.028	0.029	0.034	0.029	0.0021	0.204
Monounsaturated fatty acids						
14:1	0.363	0.281	0.351	0.338	0.0281	0.183
16:1	1.65	1.34	1.56	1.61	0.135	0.377
17:1	0.501	0.428	0.465	0.453	0.0570	0.835
18:1n-7	0.979	0.853	0.919	0.926	0.0750	0.700
18:1n-9c	17.5	14.7	16.1	16.3	1.62	0.686
18:1n-9t	0.886	0.869	0.826	0.858	0.0925	0.972
18:1n-11	0.119	0.112	0.105	0.095	0.011	0.333
20:1	0.112	0.094	0.091	0.098	0.010	0.424
24:1	0.019	0.023	0.014	0.020	0.0030	0.293

[1]CON = no exercise for the 116-d finishing period; EARLY = exercised three times per wk for the first 10 wk of the 116-d finishing period; LATE = exercised three times per wk for the final 7 wk of the 116-d finishing period; ALL = exercised three times per wk throughout the 116-day finishing period. Exercise was conducted by animal handlers who moved the cattle at a pace of 4.8-6.4 km/h for 20-30 min.

[2]Fatty acids are represented as number of carbon atoms:number of carbon-carbon double bonds.

Table 3.10 Proportions of conjugated linoleic acids, total fatty acids (% of sample) and total fatty acid concentrations (mg/g) in raw *longissimus lumborum* steaks from steers subjected to routine exercise treatments.

	Treatment[1]					
	CON	EARLY	LATE	ALL	SEM	*P*-Value
n-6 fatty acids[2]						
18:2n-6c	1.89	1.81	1.75	1.85	0.0649	0.527
18:3n-6c	0.102	0.063	0.062	0.095	0.024	0.512
20:3n-6	0.130	0.116	0.129	0.125	0.00475	0.170
20:4n-6	0.017[b]	0.016[b]	0.019[ab]	0.022[a]	0.0014	0.035
n-3						
18:3n-3	0.015	0.013	0.013	0.014	0.00069	0.103
20:5n-3	0.010	0.008	0.009	0.009	0.001	0.239
22:5n-3	0.066	0.063	0.065	0.068	0.0039	0.797
22:6n-3	0.036	0.031	0.035	0.033	0.0025	0.497
Conjugated linoleic acids						
18:2, cis-9, trans-11	0.022	0.018	0.016	0.019	0.0025	0.355
18:2, trans-10, cis-12	0.018[a]	0.011[b]	0.013[b]	0.015[b]	0.0017	0.011
18:2, cis-9,cis-11	0.010	0.005	0.004	0.005	0.002	0.087
18:2, trans-9, trans-11	0.061	0.053	0.048	0.053	0.0057	0.456
SFA[3]	20.6	17.5	19.2	20.0	1.86	0.671
MUFA[4]	22.2	18.8	20.5	20.9	1.97	0.679
PUFA[5]	2.71	2.50	2.48	2.62	0.0903	0.269
CLA[6]	0.111	0.087	0.081	0.093	0.0095	0.151
Total fatty acids (%)	4.55	3.88	4.22	4.35	0.385	0.663

[1]CON = no exercise for the 116-d finishing period; EARLY = exercised three times per wk for the first 10 wk of the 116-d finishing period; LATE = exercised three times per wk for the final 7 wk of the 116-d finishing period; ALL = exercised three times per wk throughout the 116-day finishing period. Exercise was conducted by animal handlers who moved the cattle at a pace of 4.8-6.4 km/h for 20-30 min.

[2]Fatty acids are represented as number of carbon atoms:number of carbon-carbon double bonds.

[3]SFA = sum of fatty acids that contain no double bonds.

[4]MUFA = sum of fatty acids that contain 1 double bond.

[5]PUFA = sum of fatty acids that contain 2 or more double bonds.

[6]CLA = sum of conjugated linoleic acids.

[ab]Means with different superscripts within the same row are significantly different ($P < 0.05$).

Chapter 4 - General conclusions and implications: Exercise of feedlot cattle

Consumers are becoming more concerned with the health and welfare of meat producing animals. Feedlot cattle live a sedentary lifestyle while being fed a high concentrate diet which could prompt the development of diabetic-like symptoms due to hyperglycemia. The first study evaluated routine exercise of heifers in a pilot study and the second study implemented exercise in a commercial scenario using multiple pens of cattle. A primary objective of this research was to evaluate the influence that routine exercise would have on animal health. Blood insulin seemed to be the lone blood constituent that was affected by exercise although it was only influenced by 2 of the 4 exercise treatments used in these studies. A reduction in blood insulin could signify that those particular animals have enhanced insulin sensitivity in comparison to their sedentary counterparts. Exercise did not show any influence on other health related blood parameters that were assessed in either study.

Feedlot cattle have minimal interactions with humans on a daily basis. The lack of interaction with humans may result in cattle reacting disproportionately to stressful situations associated with processing and shipping. The increase of human-animal interaction through increased handling is thought to improve animal temperament and reduce the anxiety animals incur when subjected to stressful situations. Heifers that were individually stalled and exercised as a group had decreased blood cortisol levels at the midpoint of the pilot study, but cattle housed in group pens and exercised as a pen showed no difference in blood cortisol. The different results, in regard to cortisol, between studies suggest that the observed decrease in cortisol during the pilot study may not be due to exercise, but due to an increased amount of social interaction between animals. All other measurements of stress-related blood constituents were similar between treatment groups. Additionally, temperament scores assigned when cattle were held in a handling chute did not differ between treatments. It is fairly apparent the increase in handling associated with routine exercise has limited potential to improve cattle temperament.

Body weight and average daily gains were decreased by exercise in the pilot study but not in the commercial study; however, both studies showed similar feed consumption across treatments. The pilot study employed an exercise regimen that was twice as long during the last half of the study than what was used throughout the commercial study. The results of these

studies suggest that the exercise regimen in the pilot study was of great enough intensity to influence body weight while the exercise regimens in the commercial study were not. Differences in intensity could also explain the contrasting results observed in carcass cutability measures. The pilot study showed that exercise decreased 12th rib fat suggesting that exercised cattle maintained less excess energy available to store as fat in comparison to sedentary cattle. Conversely, 12th rib fat thickness was unaffected in the commercial study signifying that exercise treatments were not of great enough intensity to disrupt the energy balance necessary for subcutaneous fat deposition.

Marbling differences observed in the commercial study seem to be related with the time at which exercise is introduced during the finishing period. This was displayed as cattle that were exercised during the final seven wk of finishing had greater marbling scores than cattle exercised the first 10 wk. Cattle that are exercised earlier in the finishing period may suppress the amount of excess energy available for storage as intramuscular fat. The suppression of adipocyte growth early in the finishing period may translate to slower growth of those fat cells when exercise is stopped and more energy is available for storage as fat. It may be necessary to delay exercise in a manner similar to common implant strategies to maximize marbling development.

These studies showed that exercise did not influence tenderness in multiple muscles which contrasts prior research that shows exercise of sheep can improve tenderness. Another quality parameter that is vital to consumer purchasing decisions is meat color. Carcasses from exercised heifers in the pilot study had *longissimus* muscles with decreased redness and yellowness, while no instrumental color values were altered by exercise treatments in the larger commercial study. The contrasts between the pilot and commercial studies could be attributed to exercise intensity.

It appears that exercise, depending on regimen, has the potential for use in a feedlot setting as a means to reduce blood insulin of cattle to promote animal health. There seems to be little evidence that suggests that exercise could be implemented to improve performance or carcass quality as growth, marbling, and meat color can be negatively influenced by certain exercise regimens. If a marketing opportunity was present where exercising feedlot cattle could provide a premium for producers, additional research would be warranted to identify what exercise intensity and initiation time should be used to produce the greatest benefits for animal welfare, performance, and meat quality.

Chapter 5 - Review of literature: Beef aroma volatiles

1. Introduction

1.1 Flavor's role in beef palatability

Providing consumers with consistent and desirable beef products is paramount to the success of the beef industry. Consumer preferences and demands may change overtime, but palatability will continue to serve as a main driver to consumer acceptance of beef. Beef palatability is a multifaceted entity that combines various traits such as flavor, tenderness, and juiciness which all contribute to the beef eating experience. Numerous studies evaluating beef palatability and the correlation of these three traits with overall like of beef have been conducted.

Historically, tenderness has been identified as the main driver of beef palatability. Tenderness was more highly correlated than flavor and juiciness with consumers' overall like ratings of muscles from the beef chuck (*complexus, infraspinatus, serratus ventralis, supraspinatus and triceps brachii*) and rib (*longissimus thoracis*) (Kukowski, Maddock, & Wulf, 2004). Nevertheless, flavor was labeled the most important factor affecting consumers' meat buying habits when there are no differences in product tenderness (Sitz, Calkins, Feuz, Umberger, & Eskridge, 2005). Additionally, consumer panelists' flavor ratings were more highly correlated than their juiciness ratings to overall acceptability ratings of USDA Select to High Choice beef strip loin steaks when Warner-Bratzler shear forces were similar across all samples (Killinger, Calkins, Umberger, Feuz, & Eskridge, 2004). Flavor and tenderness are routinely identified as the most important components affecting beef palatability and have been shown to contribute equally to consumer overall like ratings of beef steaks in some studies (Lorenzen et al., 1999; Neely et al., 1998). More recently, research has suggested that flavor may be more integral to product acceptability than tenderness. In a study evaluating the effect of fat level on sensory traits of beef strip steaks, consumer overall liking was most highly correlated with flavor like rankings in comparison to tenderness and juiciness ratings (O'Quinn et al., 2012). These studies all suggest that flavor, juiciness, and tenderness interact with each other and that care must be taken to properly monitor each of these traits.

Currently, there are numerous marketing and labeling schemes that provide consumers with a wide range of information to make their purchasing decisions when buying beef.

Consumers are now presented products with natural and organic claims as well as nutritional value statistics on product labels at the retail counter. When asked to place a score on purchasing motivators (tenderness, juiciness, flavor, price, product consistency, ease of preparation, nutritional value, natural, and organic) of beef steaks and roasts, consumers still placed the most emphasis on product flavor, tenderness, and juiciness as their primary motivators when buying beef (Reicks et al., 2011). The importance of flavor to consumers can also be seen by the willingness of a consumer to pay more for a product that has a flavor profile that they prefer. One such instance is the vastly different flavor profile that grain-fed and grass-fed beef produce. Consumers that preferred the flavor of grain-fed beef over grass-fed beef showed a willingness to pay up to $2.00/0.45 kg more for that product (Sitz et al., 2005). Even though consumers are presented with numerous trends and marketing claims, palatability still drives their purchasing decisions and certain flavors can entice them to select specific products that are positively associated with past eating experiences.

Beef flavor is very complex as it contains numerous odors and flavor notes that combine to create one flavor. Properly describing flavors associated with beef becomes more complicated due to various cookery methods combined with live animal genetics, gender, age, and diet amongst other variables. In an effort to develop a comprehensive and effective lexicon to describe beef flavors, Adhikari and others (2011) used highly trained sensory panelists to evaluate different beef cuts of different quality grades cooked to multiple end-point cooking temperatures using various cooking methods. All samples (n=176) that were evaluated contained the following flavor notes: beef identity, brown/roasted, bloody/serumy, fat-like, metallic, sour aromatics, overall sweet flavor, and all five tastes. Additionally, liver-like, green-hay, green, chemical, burned, rancid, spoiled, warmed-over, animal hair, cocoa, leather, dairy, sour dairy, and cooked milk flavor notes and aromas were detected and had an important role in select sample groups. This lexicon shows the overwhelming complexity of beef flavor and the amount of individual flavors that may be present and contribute to the overall flavor of beef.

Beef palatability is a very important topic and must continually be evaluated to ensure that acceptable products are provided to consumers. Flavor seems to play a very important role in palatability. Flavor itself is complex and demands further investigation to fully understand how it should be analyzed.

1.2 Aroma's role in flavor

Flavor is complex, but is highly important in defining the sensorial acceptance of food as it is the food quality which entices humans to eat (Carden & Baird, 2000). The Society of Flavor Chemistry defines flavor as "a substance which may be a single chemical entity, or a blend of chemicals of natural or synthetic origin whose primary purpose is to provide all or part of the particular flavor effect to any food or other product taken in the mouth." (Carden & Baird, 2000). Flavor is mainly comprised of the two sensations of taste and smell, although the sensations of astringency, mouthfeel, and juiciness may play a role (Farmer, 1994).

Receptors in the mouth can distinguish the five main tastes (sweet, salty, sour, bitter, and umami). Foods contain water-soluble compounds that produce taste sensations recognized by the tongue which are due to the presence of inorganic salts (salty), hypoxanthine (bitter), sugars (sweet), and organic acids (sour) (Moody, 1983). The aromatic portion of flavor is significantly more diverse than taste as the human nose can identify hundreds or even thousands of different odors (Farmer, 1994). The volatile compounds responsible for aroma stimulate the nasal epithelium after they enter the nose (by smelling) or through the posterior nares at the back of the nose and throat while food is being chewed (Farmer, 1994). Aroma is such an important fraction to flavor that anosmics (people who lack the ability to smell) complain their food no longer tastes good even though simple tests affirm their capabilities to accurately detect sweet, sour, salty, and bitter tastes (Carden & Baird, 2000).

Aroma sensations can be attributed to thousands of low-molecular-weight compounds. Aromatic and aliphatic compounds generally contain a heteroatom (O, N, S) which creates a precise electronic configuration that can be recognized by the nasal receptors (Farmer, 1994). The compounds responsible for aromas are most often a result of reactions that occur during cooking and processing (Carden & Baird, 2000) and it is of great importance to recognize how they are formed in beef.

2. Aroma volatile formation

2.1 Meat aroma precursors

Raw beef has little flavor and a blood-like taste, but it contains numerous volatile precursors that are developed during cooking and contribute to the complex and bold flavors

associated with beef (Shahidi, 1989). Free amino acids, peptides, reducing sugars, vitamins, nucleotides and fatty acids are all present in meat and are converted into volatile compounds that develop during cooking (Madruga, Elmore, Oruna-Concha, Balagiannis, & Mottram, 2010).

Hornstein and Crowe (1960) researched the water-extractable flavor precursors in raw beef and pork. Beef and pork lean portions with fat completely removed were extracted with cold water and then heated to assess aroma. The aromas from the lean portions of each species were indistinguishable from the other. Conversely, fat portions of each species were evaluated in a similar manner to the lean portions and showed a stark contrast of aromas that were indicative of each of the species. This study ultimately concluded that flavor precursors of meat are low molecular weight compounds and that species flavor and aroma are mostly dependent upon the fat portion of the sample.

Macy, Naumann, and Bailey (1964a) further evaluated the water-soluble flavor precursors associated with beef, pork, and lamb. The amino acid profiles were relatively similar between all species as cysteine, histidine, hydroxyproline, isoleucine, carnosine, glutamine, glycerophosphoethanolamine, phosphoethanolamine, and urea were identified in lean samples of each species. Moreover, ribose, fructose, and glucose were the primary carbohydrates that were identified in the samples. Additional quantitative analysis of non-amino acid nitrogen compounds revealed the presence of ionosine, creatinine, and hypoxanthine. Macy, Naumann, and Bailey (1964b) then investigated the effects of heating on all of the previously mentioned compounds. Heating produced a dramatic decrease in the amounts of taurine, anserine-carnosine, and alanine. Moreover, cysteine, glutamic acid, glycine, lysine, serine, methionine, leucine, isoleucine, and methyl histidine were also degraded during heating. Ribose was recognized as the most heat labile sugar while fructose was the most stable. Overall amounts of carbohydrates were decreased by 72% in beef, 55% in lamb, and 64% in pork. It is readily apparent that the heating of amino acids and sugars that are contained in meat causes a significant change in the makeup of meat and lead to products that may contribute to cooked meat flavor.

Numerous studies have demonstrated that carbohydrates and amino acids are depleted during heating and that cysteine and ribose exhibited the greatest decrease (Mottram, 1998). The decrease of these two precursors directly correlates with thermal reactions responsible for a vast amount of flavor and aroma components that are crucial to meat flavor.

2.2 The Maillard reaction

Thermal processing is essential to the formation of volatiles that are responsible for a variety of aromas and flavors associated with meat. One of the primary reactions occurring during cooking is the Maillard reaction which is named after the French chemist Louis-Camille Maillard who observed the formation of brown pigments when heating a mixture of glucose and lysine (Maillard, 1912). The Maillard reaction is used to describe the complex series of chemical reactions between carbonyl and amino components derived from biological systems (Mottram, 1994) and is more commonly described as non-enzymatic browning (Fay & Brevard, 2005). This reaction normally occurs on the meat surface where the meat product becomes dehydrated (van den Ouweland, Peer, & Tjan, 1978).

The outline and descriptions of the Maillard reaction are detailed in the most depth by Hodge (1953). A comprehensive outline of the reaction is present in Figure 5.1. The initial stage involves sugar-amine condensation and an Amadori rearrangement. First, an addition reaction occurs between the amino group of an amino acid and the carbonyl group of an aldose sugar to form an N-substituted glycosylamine and water. The following step eliminates water and the N-glycosylamine undergoes an Amadori rearrangement to form an N-substituted 1-amino-1-deoxy-2-ketose. The Amadori products do not contribute directly to flavor but are important precursors to flavor compounds (Mottram, 1994).

The intermediate stage is composed of three reactions: 1) sugar dehydration, 2) sugar fragmentation, and 3) amino acid degradation (Hodge, 1953). The dehydration of Amadori products is dependent on the conditions of the reaction system. In acidic systems, furfurals are commonly produced after a 1,2 enolization. At a higher pH the favored reaction is a 2,3 enolization that precedes the formation of hydroxyl ketones, dicarbonyl compounds, and other reductones. The resultant sugars are fragmented which lead to multiple different products that have different potentials for browning.

Amino acids are broken down during Strecker degradation which is one of the most vital happenings during the Maillard reaction (Hodge, 1953). This degradation occurs when α-amino acids undergo oxidative deamination and decarboxylation in the presence of a dicarbonyl compound. The resultant products are aldehydes containing one less carbon atom and an α-aminoketone. The α-aminoketones formed during this process are intermediates that lead to the formation of many aroma-producing volatiles indicative of cooked meat such as furans,

pyrazines, pyrroles, oxazoles, thiazoles and other heterocyclic compounds (Fay & Brevard, 2005).

The final stage of the Maillard reaction yields high-molecular weight melanoidin polymers that create the characteristic brown color associated with the reaction (Hodge, 1953). The first portion of the final stage is the condensation of cyclic subunits. Pyrroles and pyrrole derivatives are examples of the types of compounds that are condensed to form aldols, N-free polymers, aldimines, and ketimines. These products are then polymerized to form the brown nitrogenous polymers and copolymers known as melanoidins. These reactions lead to a variety of aromatic compounds which have become characteristic of flavors associated with the cooking of beef.

2.3 Lipid oxidation

Lipids are vital components to meat flavor development. Lipids are precursors to both desirable and undesirable odors and a solvent for many lipophilic odor compounds (Farmer, 1996). Additionally, the presence of lipids affects the rate of flavor release in the mouth and their texture contributes to the mouthfeel and juiciness of a product which impacts flavor perception (Farmer, 1996). Lipid degradation during the cooking of meat contributes several hundred volatile compounds including aliphatic hydrocarbons, aldehydes, ketones, alcohols, carboxylic acids and esters (Mottram, 1998). Lipid-derived volatiles are the result of oxidation reactions of unsaturated fatty acids. Long term storage allows oxidation to produce off-flavors and can be attributed to the development of rancidity (Ladikos & Lougovois, 1990). The same oxidation reactions can occur during cooking, however, they occur more rapidly and form different compounds that produce desirable flavors (Calkins & Hodgen, 2007).

The process of lipid oxidation is generally described in three major steps: 1) initiation, 2) propagation, and 3) termination (Figure 5.2). A hydrogen atom (H) is removed from a methylene carbon in a fatty acid (RH) by binding to oxygen (O_2) during the initiation step. The propagation step entails that the fatty acyl radical (R•) reacts with O_2 to form a peroxyl radical (ROO•) which will then oxidize other unsaturated fatty acids and will initiate a chain reaction. The termination step occurs when there is no available oxygen for the fatty acyl radical to bind (Gray, 1978). Lipid oxidation forms hydroperoxides as its primary product. Hydroperoxides do not contribute to aroma, but they decompose into hydrocarbons, alcohols, ketones, and aldehydes which are

very influential in the aroma of meat (Shahidi, 1989). Lipid-derived volatiles have a high odor threshold and are much more influential in aroma development than the volatiles derived from water-soluble precursors (Mottram, 1998). Aldehydes are a major secondary product of lipid oxidation and malonaldehyde is one such aldehyde that is an oxidation product of polyunsaturated fatty acids that is often used to assess the degree of lipid oxidation in meat products (Shahidi, 1989). Even though malonaldehyde is a major lipid oxidation product, it has little effect on aroma (Shahidi, 1989). Aldehydes other than malonaldehyde, such as hexanal, can have a significant impact on meat aroma. Hexanal is formed from the breakdown of ω6 fatty acids and can contribute positively to beef flavor, but may produce undesirable flavors at higher concentrations (Melton, 1983). Lipids are readily broken down before, during, and after cooking so the products of lipid oxidation inherently play a vital role in beef aroma.

2.4 Interactions between lipid oxidation and the Maillard reaction

Lipid oxidation and the Maillard reaction are both important contributors to the presence of meat aroma and flavor volatile compounds. Both of these reactions are occurring simultaneously during the cooking of meat, so it is likely that primary and secondary products, as well as intermediates, of the two reactions may impact the other. One such example of lipid and Maillard products reacting is the formation of butyl and pentyl pyrazines. Butyl and pentyl pyrazines form when aldehydes, such as hexanal and pentanal, react with dihydropyrazines, which are formed from the condensation of two aminoketones (Mottram, 1998). Mottram and Whitfield (1995) added phospholipid to a cysteine and ribose mixture and heated it to investigate the impact that phospholipid has on the production of Maillard volatiles. Their results showed that the addition of a phospholipid, in this case phosphatidylcholine, had minimal impact on the amounts of Maillard/lipid interaction products, including 2-pentylthiophene, 2-hexylthiophene, 2-pentylthiapyran, and 2-pentylpryidine. This result contrasted the previous work of Farmer and Mottram (1990) that saw significant differences between volatile production in a similar scenario except the reactions were carried out in an aqueous system. Lipid oxidation and Maillard reaction products will interact during cooking and it appears that the type of cookery method used (wet or dry) can also be influential in the resultant volatile products created during cooking.

2.5 Phospholipids in meat flavor

Phospholipids are vital components of cells and contain a much higher proportion of unsaturated fatty acids than the triglycerides associated with the major fat depots. The differences in composition between these lipid sources could lead to differing aromas and flavors when heated. Mottram and Edwards (1983) discovered that if all triglyceride was extracted from a beef sample and the remaining portion was cooked that only minor changes occurred in the volatile compounds produced and the aroma still contained its "meat-like" characteristics. Upon the removal of both phospholipid and triglyceride, the resultant cooked meat sample had an aroma characterized by toasted and biscuit-like notes. Moreover, volatiles from samples devoid of triglyceride were similar to the unextracted control and the major components were aliphatic alcohols and aldehydes. When both triglycerides and phospholipids were removed, the volatile profile changed dramatically with aldehyde content greatly reduced and aliphatic alcohols present in only trace amounts. Additionally, the samples lacking triglycerides and phospholipids showed increased amounts of benzaldehyde and pyrazines. This study suggests that meaty aromas associated with meat can be attributed to structural phospholipids while the roasted aromas and the corresponding volatiles are decreased by the presence of lipid.

Farmer and Mottram (1990) further investigated the importance of triglycerides and phospholipids on production of aroma and aroma volatiles by incorporating them in a Maillard reaction between cysteine and ribose. Cysteine and ribose were heated in the presence of beef triglyceride, beef phospholipid, L-α-phosphatidylcholine (PC), or L-α-phosphatidylethanolamine (PE). The reaction mixture that contained beef triglyceride had a strong sulphurous aroma with some meaty notes and had a similar intensity to a mixture containing no phospholipid. All three phospholipid mixtures contained more intense meaty notes than what was observed with the beef triglyceride. The mixture containing beef phospholipid contained a distinctly meaty aroma, the PC mixture contained predominantly sulfurous aroma, and the PE mixture resembled the beef phospholipid mixture as it presented a very distinct meaty aroma. Mixtures with inclusions of phospholipids also produced greater amounts of lipid-derived volatile compounds including 2-pentylpyridine, 2-alkylthiophenes, alkenylthiophenes, a pentylthiapyran, and alkanethiols. The meaty aromas associated with beef appear attributable to phospholipids that are integrated into muscle structure rather than triglycerides stored in the major fat depots of a carcass.

3. Flavor notes contributed by aroma volatiles

The previously described reactions yield a multitude of volatile compounds. Many of these compounds contribute to flavor notes that are very important to the flavor of meat while others may play less of a role. It is important to identify specific volatiles and classes of compounds that contribute to certain flavor notes experienced when consuming beef.

There are numerous flavor notes present in meat flavor and each of these flavor notes are products of certain classes of compounds and possibly a singular compound. The most recognizable aromas are fatty, species-related, roasted, boiled-meat, and the "meaty" aroma characteristic of all meat products (Mottram, 1998). Table 5.1 compiled by Calkins and Hodgen (2007) outlines numerous volatiles that have been detected in meat and aromas that have been associated with them.

Sulfurous compounds are an important part of meaty aroma. Many of the sulfur compounds have low odor thresholds with sulfurous, onion-like, and meat aromas (Fors, 1983). Additionally, simulated meat flavorings used in processed savory food products have used furans and thiophenes with a thiol group in the 3-position, as well as related disulfides, that possess strong meat-like aromas (Mottram, 1998). The precursors for these compounds are most likely pentose sugars and cysteine. Mottram and Madruga (1994) evaluated one of the main sources of pentoses in meat which is inosine-5′-monophosphate (IMP) and its effect on the production of meat aroma volatiles during heating. The heated meat systems produced several thiols as well as di- and trisulfides containing 2-methyl-3-furyl and/or 2-furylmethyl groups. Many of the reported volatiles were in very low amounts and some were only present when IMP was added to the system. Additionally, all observed compounds were present in greater amounts with IMP added to the system. Beef contains IMP so it can be expected that sulfurous compounds play an integral role in beef flavor.

Farmer and Patterson (1991) evaluated volatile compounds produced from cooked beef heart, *semimembranosus*, and *psoas major* muscles and identified five structurally related late-eluting disulphides present from heart muscle. Bis(2-methyl-3-furyl) disulfide and 2-furfuryl-2-methyl-3-furyl disulfide were present in the greatest quantities, which is significant as the heart has a relatively strong flavor. Only one of the five identified disulphides, bis(2-methyl-3-furyl) disulphide, was present in significant amounts in the *semimembranosus* and *psoas major* samples, although it was in lower quantities in comparison to the heart. The remaining four

disulphides were found in trace amounts in either the *semimembranosus* or *psoas major*. Additionally, after conducting gas chromatography-odor assessments, the authors determined that bis(2-methyl-3-furyl) disulfide and 2-furfuryl-2-methyl-3-furyl disulphide elicited a meaty, roasted, burnt aroma while bis(2-furfuryl) disulfide lacked meaty notes but still had a roasted and burnt aroma. This study also displays how sulfurous compounds are associated with strong meaty flavors.

Lipid degradation products are very important to meat aroma as fatty flavor is generated from the lipid portion of meat. Fatty acids such as linoleic and arachidonic acid start to oxidize to 9-hydroperoxide and 11-hydroperoxide, respectively, which can form 2,4-decadienal, 2-nonenal, 1-octen-3-one, 2,4-nonadioenal, and 2-octenal with 2-nonenal and 2,4-decadienal providing similar intensities of meaty aromas as those provided by sulfur compounds (Calkins & Hodgen, 2007). Aldehydes, ketones and lactones, which are major secondary products of lipid oxidation, will contribute to the fatty aromas in cooked meats (Mottram, 1998). Additionally, aldehydes and ketones can create burnt, sweet, fatty, metallic, and rancid aromas (Shahidi, 1989).

Hexanal can have a significant impact on meat aroma as it is the most prominent volatile compound in cooked meat (Calkins & Hodgen, 2007). Hexanal is formed from the breakdown of ω6 fatty acids and can contribute to unpleasant, rancid, strong green, and unripe fruit notes (Calkins & Hodgen 2007, Madruga et al., 2010) Additionally, hexanal is directly proportional to thiobarbituric acid reactive substances (TBARS) which is a measure of lipid oxidation and is often negatively associated with flavor (Shahidi & Pegg, 1994; Ullrich & Grosch, 1987). Lipid degradation products produce many desirable flavors in beef, especially fatty flavor notes, but they can also be associated with off-flavors and rancidity that could be detrimental to beef flavor.

Lipids are generally regarded as the primary source of species flavors. Different livestock species have varying levels of unsaturated fatty acids in the triglycerides and this gives rise to differing amounts of unsaturated aldehydes that may be influential into identifying species flavor (Noleau & Toulemonde, 1987). Lamb is unique in the quantity of methyl-branched saturated fatty acids it contains, which is vastly different from other species, and this is directly associated with the characteristic flavor of mutton which the Chinese describe as “soo” flavor (Wong, Nixon, & Johnson, 1975). Beef-like aroma has been attributed to 12-methyltridecanal which was

observed in stewed beef at much higher levels than lamb, veal, deer, pork, chicken, and turkey (Guth & Grosch, 1993, 1995).

Roasted flavors appear to be associated with heterocyclic compounds formed in the later stages of the Maillard reaction (Mottram, 1998). Pyrazines, thiazoles, and oxazoles are compounds that are prevalent in cooked meat. These classes of compounds show dramatic increases when heat treatment is intensified, and pyrazines are the dominant class of volatiles associated with well-done grilled meat (Mottram, 1985). Oxygenated furans and pyrans contribute caramel-like odors, as do pyrroles. Oxazoles and oxazolines have been found in meat and contribute woody, musty, and green flavors (Mottram, 1994).

It is readily apparent why beef flavor is such a complex trait due to the multitudes of compounds contributing to its formation. The various classes of compounds that combine to produce beef aroma are derived from different types of meat components which can vary in availability between breeds, animals, and even muscles. These factors are further complicated by the various practices employed by meat processors, retailers, and consumers.

4. Instrumental analysis of volatile aroma compounds

4.1 Headspace sampling techniques

Separation of flavor compounds is commonly conducted using gas chromatography (GC), but there are various ways to prepare and collect analytes that are injected into a GC. Multiple sample preparation methods, including porous polymer trapping and distillation-extraction, were compared by Jennings and Filsoof (1977) and they concluded that no isolation technique produced results that duplicated an original neat sample. Solvent extraction and distillation can be used to isolate compounds from food; however, headspace analysis is frequently preferred to take advantage of the volatility of aroma compounds which leave a food matrix and travel through the air to be perceived (Wampler, 2002). Headspace analysis is often divided into two categories: static headspace and dynamic headspace/purge and trap. The fundamental principal of both techniques is that volatile analytes from a solid or liquid material are sampled by evaluating the atmosphere around the sample, leaving the actual sample material behind (Wampler, 2002).

Static headspace sampling is the most readily automated and validated of all headspace sampling techniques (Snow, 2002). Wampler (2002) described the basic mechanics of static headspace sampling. A food sample is placed into a headspace vial, sealed and warmed to enhance vaporization of volatiles and then allowed to stand for a period of time to establish equilibrium. A syringe is then used to withdraw an aliquot of the headspace gas to inject into a GC injection port. Static headspace-GC has been used for the analysis of natural aromas and odors in several industries, but its main limitation is limited sensitivity (Snow, 2002).

Dynamic headspace involves moving the analytes away from the sample matrix by use of a carrier gas that constantly sweeps the atmosphere around the sample away to a trap that is later extracted and injected into a GC (Wampler, 2002). "Dynamic headspace" is generally used to describe sampling of a solid material while "purge and trap" is used to describe analysis by bubbling a carrier gas through a liquid (Wampler, 2002). Dynamic headspace or purge and trap procedures increase the size of the sample headspace beyond that of a vial and do not allow the establishment of an equilibration state (Wampler, 2002). Dynamic headspace methods are preferred for low concentrations of volatile organic compounds in aqueous matrices and have been implemented in a wide array of industry scenarios including the analysis of food and aromas (Snow, 2002).

4.2 Solid-phase microextraction

Isolation of volatile compounds using steam distillation, solvent extraction, trapping volatiles on adsorbents, or combinations of these methods are time consuming and may introduce impurities (Harmon, 2002). Solid-phase microextraction (SPME), which was first described by Berlardi and Pawliszyn (1989), is a quick extraction method that uses a fused-silica fiber which is coated on the outside with an appropriate stationary phase (Kataoka, Lord, & Pawliszyn, 2000). The SPME fibers are coated with polymers ranging from the nonpolar polydimethylsiloxane (PDMS) to the more polar Carbowax or can be coated with a combination of numerous polymers (Harmon, 2002). During sampling, the fiber is immersed in the sample (usually the gas phase above the sample) where it absorbs analytes present in the sample. The fiber is removed from the sample and anlaytes are thermally desorbed in the injector of a GC (Holt, 2001). Elmore, Papantoniou, and Mottram (2001) compared the use of SPME to headspace entrainment on Tenax to evaluate aroma volatiles of cooked beef. This study showed

that Tenax extracted greater amounts of most volatiles, but SPME extracted greater amounts of polar volatiles, such as hydroxyfuranones and hydroxyketones. Additionally, SPME extracts contained multiple Maillard reaction intermediates that were not observed when Tenax was used. The quick use and ability to preferentially extract polar volatiles make SPME a viable option for analyzing a large number of samples for volatile aroma compounds.

5. Postmortem factors influencing flavor development

There are hundreds of volatile compounds that contribute to beef flavor and aroma. Many of these compounds can be altered through storage and cooking (Calkins & Hodgen, 2007). The vast number of volatiles that impact flavor make evaluating meat flavor extremely difficult. Many volatiles have been attributed to the presence of specific aromas, so it is important to evaluate how certain practices manipulate their presence in beef.

5.1 Aging

Beef is commonly subjected to "wet aging" which is a process that entails anaerobically packaged cuts being stored at refrigerated temperatures for an extended period of time. Practically all beef in the U.S. is anaerobically packaged at the packer level (Smith et al., 2008), so most U.S. beef undergoes wet aging to some extent. Postmortem aging improves tenderness of beef through the naturally occurring proteolytic degradation of cytoskeletal proteins (Koohmaraie, 1996). Use of aging will inherently alter the flavor profile of beef which can be both beneficial and detrimental to its palatability. To effectively utilize aging, it is necessary to examine the length needed to provide acceptable flavor and tenderness.

Different muscles from a beef carcass serve different purposes and therefore have different properties. It is quite possible that different primal cuts may require different aging regimens to create an acceptable and consistent product. To evaluate this issue, Gruber and others (2006) investigated the impact of multiple postmortem aging times on 17 different beef muscles. They showed that across two different USDA quality grades, 16 of the 17 evaluated muscles showed steady decreases in Warner-Bratzler shear force (WBSF) up to 28 d of aging. This study also showed that aging times need to be tailored to specific muscles as some muscles required longer aging times to achieve a significant decrease in WBSF. These results suggest that subprimals including muscles like the *gluteus medius* may require longer aging periods than are

necessary with other muscles such as the *longissimus dorsi*. Additional research has shown that aging up to 40 d can still improve tenderness in beef (King et al., 2009). These authors also showed total proteolysis was increased by aging (40, 46, and 60% for 12, 26, and 40 d aging, respectively) in *gluteus medius* steaks. Since proteolysis is increased with longer aging periods it should be expected that tenderness can be increased in longer aging periods as well, which could entice purveyors and retailers to age product longer than normal.

With the proven tenderness benefits that aging provides it is still necessary to evaluate how it influences other factors in palatability, mainly flavor. Jeremiah and Gibson (2003) evaluated four different aging times (7, 14, 21, and 28 d) and their effects on various sensory properties of beef loins and ribs. Beef flavor intensity, flavor desirability, and overall palatability exhibited an increase as aging time increased with the most satisfactory scores assigned to the longest aging period (28 d). Longer aging times were also associated with increased intensity of "browned" and "livery" aromas along with a decreased incidence of "bloody" flavor. Moreover, salty and bitter flavors were more intense for beef aged longer periods of time. The observed improvement in mechanical tenderness measurements of longer aged beef was affirmed by sensory panel through higher initial tenderness and overall tenderness scores. Although longer aging times caused some off-flavors, such as livery, they also contributed to other flavors that are considered to be desirable.

Processors, distributors, and retailers across the industry are very inconsistent in the way they manage anaerobically packaged beef. On average, anaerobically packaged beef is held in refrigerated storage for 20.5 d to ensure tenderness, but storage times can range from 1 d to as long as 358 d before product reaches the retail counter (Guelker et al., 2013). The inconsistency of product handling and storage of beef could yield a great deal of inconsistency in product palatability. Juarez et al. (2010) evaluated the effects of subjecting beef to a wide range of aging times. Six different subprimals were evaluated in the study and were aged up to 56 d for instrumental tenderness and as long as 42 d for sensory characteristics. Beef strip loins showed decreased shear forces from 0 to 56 d of aging. Blade-eye, eye of round, and chuck tenders showed their lowest shear forces at 35 d of aging. Strip loins, outside rounds, inside rounds, and eye of rounds exhibited the highest flavor intensities at 0 and 14 d of age and flavor intensities decreased to their lowest levels on d 42. Off-flavors for all muscles assessed were lowest on d 0 and increased to their highest levels during the longest aging periods (28 or 42 d). It is yet to be

seen that if the observed differences of flavor and off-flavor intensities are due to a decrease in desirable flavor compounds, an increase in off-flavor compounds, or if off-flavors are masking the desirable beef flavors.

Positive and negative trends in flavor development have been observed in product subjected to aging times as long as 28 d. Although beef flavor may intensify in some cuts, the development of off-flavors could be detrimental to product palatability. Yancey, Dikeman, Hachmeister, Chambers IV, and Milliken (2005) showed that aging beef top sirloin steaks up to 21 and 35 d increased metallic, rancid, and sour flavors when compared to aging periods of 7 and 14 d. Extended aging periods of greater than 28 d can still provide tenderness improvement but they may also create unwanted and objectionable off-flavors.

Knowing that aging impacts flavor, it is important to see how aging influences aroma volatiles of beef. Beef *semimembranosus* muscles were aged for 0 h, 4 h, 2 d, 4 d, 7 d, and 14 d before being ground and cooked to medium doneness in patty form (Spanier, Flores, McMillin, & Bidner, 1997). The patties were then assessed by a sensory panel and were subjected to analysis for aroma volatiles. This study showed aging decreased beefy, brothy, browned-caramel, and sweet flavors and increased bitter and sour flavors. Additionally, there were no correlations between hexanal and total volatiles with any of the flavor traits assessed by sensory panel. This study showed a relatively short aging period and contrasted other studies which showed flavor improvement up to 28 d. No data were given for volatiles at each individual aging time.

Ma, Hamid, Bekhit, Robertson, and Law (2012) evaluated the impact of aging beef up to 21 days in addition to researching the impact of pre-rigor injection of proteases on cooked beef volatiles. Hot-boned *semimembranosus* muscles from 5-yr old cows were injected with a variety of proteases and cooked volatiles were assessed after 1 and 21 d of postmortem aging. Out of 56 volatiles that were detected, the control group, that did not receive injection, only had three volatile compounds that showed significant changes between 1 and 21 d of aging. Furfural and 2-nonanone increased from 1 to 21 d of aging while tetradecane decreased over the aging period. This study showed that volatile compounds produced during cooking may not endure significant change prior to 21 d of aging.

Aging is commonplace in the beef industry due to its tremendous impact on tenderness. Additionally, aging most definitely influences beef flavor. Beef aged for longer time periods

seems to have increased occurrences of off-flavors but has exhibited different results on flavor intensity and beefy flavor attributes. It is still unclear how aging times greater than 21 days influences the aroma volatile profile of beef.

5.2 Aging effects on volatile precursors

Beef is normally subjected to postmortem aging to enhance product palatability. Koutsidis and others (2008) researched the impact that postmortem aging of up to 21 d had on the precursors responsible for meat aroma and flavor in beef *longissimus lumborum*. Glycogen showed no significant change over the aging period. Glucose, fructose, mannose, and ribose showed a linear increase throughout the study while mannose-6-phosphate, fructose-6-phosphate, and glucose-6-phosphate all decreased as aging time increased. Ionisine 5′-monophosphate (IMP) was degraded linearly over the aging period as was its accumulation products ribose, ionisine, and hypoxanthine. Free amino acids increased during aging especially between d 7 and 14. Methionine showed a sevenfold increase, but phenylalanine, lysine, leucine, and isoleucine also showed significant increases during aging. Additionally, 21 d of aging yielded nearly a 3-fold increase in the amount of free cysteine available. The increased pool of free amino acids accompanying the observed increases in ribose, methionine, and cysteine as aging time was lengthened suggests that long aged meat could produce greater amounts of volatiles formed via the Maillard reaction.

5.3 Blade tenderization

Specific muscles and production practices can produce beef that is inherently tough to a point where postmortem aging fails to provide adequate tenderization. One of the most common processing methods used to alleviate this issue is blade tenderization. Blade tenderization can provide ample tenderness improvement by disrupting the structures of muscle and connective tissues (Bowker, 2007). King and others (2009) showed that blade tenderization could decrease slice shear force by nearly 4 kg in *longissimus lumborum* steaks and over 6 kg in *gluteus medius* steaks. Brooks and others (2010) demonstrated the effects of blade tenderization on beef strip loins from cattle fed zilpaterol hydrochloride on consumer sensory scores. Although blade tenderization increased tenderness scores, it decreased juiciness and had no influence on flavor when compared to the non-tenderized control. Overall, blade tenderization had a higher

percentage of samples that were considered to be acceptable by consumer panelists in comparison to control steaks.

Some research suggests that blade tenderization can alter the flavor intensity of beef. Bidner, Montgomery, Bagley, and McMillin (1985) contrasted the effects of blade tenderization, electrical stimulation, and aging on palatability. At 72 h postmortem, strip loins were assigned to 21 d of wet aging, blade tenderization, or a control treatment. Blade tenderization exhibited lower sensory panel flavor intensity scores than control and the 21 d aged treatment groups. The influence of blade tenderization may differ between muscles. Seideman, Smith, Carpenter, & Marshall (1977) evaluated blade tenderization on the palatability of beef *psoas major* and *semimembranosus* steaks. *Psoas major* steaks that were blade tenderized once demonstrated increased flavor desirability and overall palatability scores. Conversely, *semimembranosus* steaks tenderized 0, 1, 2, or 3 times showed similar flavor desirability scores, but overall palatability increased with number of times tenderized.

Most research has suggested that blade tenderization does not influence the flavor of beef. (George-Evins, Unruh, Waylan, and Marsden, (2004) investigated the cumulative effect of postmortem aging, blade tenderization, and endpoint cooking temperature on the tenderness and sensory attributes of top sirloins. Sirloins were aged for 7, 14, or 21 d and then blade tenderized 0, 1, or 2 times. A single round of blade tenderization did not decrease WBSF when compared to non-tenderized sirloins but two rounds of blade tenderization elicited a significant decrease in WBSF of sirloin steaks. Sensory panel results showed that any amount of blade tenderization improved myofibrillar and overall tenderness, but there were no effects of blade tenderization on sensory panel flavor scores. The effects of blade tenderization on palatability of beef strip loin and top sirloin steaks at 4 and 18 d of postmortem aging were investigated by Savell, McKeith, Murphey, Smith, & Carpenter (1982). Blade tenderization displayed no influence on flavor of both strip loin and top sirloin steaks at 4 and 18 d of aging. Overall palatability of strip loins steaks was also unaffected by blade tenderization, however, blade tenderization increased overall palatability of top sirloins steaks which is most likely due to changes in tenderness. Additional research has displayed a lack of change in flavor of beef *longissimus* (Medeiros, Field, Menkhaus, Riley, & Russell, 1989; Glover, Forrest, Johnson, Bramblett, & Judge, 1977) and *semimembranosus* muscles (Medeiros et al., 1989, Glover et al., 1977). These studies suggest

that flavor is not altered by blade tenderization; instead the increased palatability of blade tenderized steaks is based solely on tenderness.

Blade tenderization is commonly used to improve tenderness from cuts like the top sirloin butt (George-Evins et al., 2004). Blade tenderization has shown mixed results in terms of its effects on beef flavor even though most research suggests it does not influence flavor. Limited insight into the cumulative effects of blade tenderization and aging on flavor is available. Moreover, there has been minimal research on the impact blade tenderization has on the aroma volatile profiles of raw and cooked beef.

5.4 Degree of doneness

The endpoint temperature of cooked beef is known as the degree of doneness. Consumers have a distinct control over what degree of doneness their steak is cooked to. People have diverse preferences; therefore, one steak could have vastly different eating qualities depending on the consumer. The most commonly known degrees of doneness used in the U.S. are rare, medium rare, medium, medium well and well done (Reicks et al., 2011) which correlate with 60, 63, 71, 74, and 77 °C respectively (AMSA, 1995). It has been well-documented that end-point cooking temperature has a distinct impact on palatability as it alters tenderness and flavor (Parrish, Olson, Miner, & Rust, 1973). Altering tenderness and flavor could prove to be detrimental to product acceptability as both are equally responsible for the variation of overall product likeability in beef (Neely et al., 1999). Prior research has shown that beef cooked to well done (77 °C) has higher shear forces than beef cooked to medium (71 °C) in both steaks (Lorenzen, Davuluri, Adhikari, & Grün, 2005) and ground patties (Berry, 1994). Likewise, as degree of doneness increases the juiciness, as perceived by consumers, decreases (Lorenzen et al., 1999). During cooking, numerous chemical reactions, including protein denaturation, occur that likely influence the aforementioned palatability traits.

The extent of cooking impacts the perceived flavor of beef. The longer beef is cooked, the longer that the reactions responsible for flavor development can occur. Resultant flavors from meat cooked to differing degrees of doneness may be associated with positive and negative flavor perceptions. Lorenzen et al. (2005) evaluated multiple sensory characteristics as well as volatile compounds associated with six different endpoint temperatures. Beef *longissimus* steaks were cooked to 55, 60, 63, 71, 77, and 82 ºC before sensory panel and volatile analysis. Beef

flavor as determined by sensory panelists was similar between cooking temperatures. Additionally, as endpoint temperatures were increased, the prevalence of roasted and burnt flavors increased while the occurrence of bloody and fatty flavors decreased. It is logical that roasted and burnt flavors should increase as higher degrees of doneness are achieved, but the lack of bloody and fatty flavors could be attributed to a masking effect due to more potent roasted and burnt flavor notes. Consumer panelists showed no preference for liking of flavor between cooking temperatures. Steaks cooked to 55, 60, and 63 ºC had no specific volatiles associated with them. Steaks cooked to 71 ºC were characterized by 2-nonanone, 2-octanone, 2-hexanone, 3-methyl-1-butanol, and 3-methythiophene. Steaks cooked to 77 and 82 ºC were both associated with 3-penten-2-one, hexanal, and nonanal while the steaks cooked to 82 ºC were also strongly associated with 2-pentanone. It is readily apparent that degree of doneness influences beef flavor and that aroma may be a vital aspect of the observed changes.

Consumers are not alike in that they have different preferences to what is deemed acceptable and palatable when eating beef. Schmidt and others (2010) evaluated how consumer preferences aligned with beef cooked to different degrees of doneness. Consumers who stated that they preferred rare or medium rare steaks placed higher likeability ratings to rare and medium rare steaks in comparison to higher degrees of doneness. Conversely, consumers who stated that they preferred medium steaks showed no difference in acceptability of medium rare, medium, and medium well steaks, but preferred rare steaks over those cooked well done. Additionally, consumers preferring medium well steaks showed a preference for medium rare and medium USDA select steaks compared to well-done steaks, but likeability scores of USDA choice steaks did not favor one degree of doneness over another. Overall this study showed that if consumers say they prefer rare or medium rare steaks they prefer that their steak is cooked rare or medium rare, while those who say they prefer well done steaks prefer well done steaks over rare or medium rare steaks. Lastly, those who say they prefer medium or medium well steaks do not necessarily prefer steaks cooked to those specified degrees of doneness.

6. Summary

Flavor has a great influence on palatability of beef and aroma plays an integral role in the development of flavor. There are vast numbers of aroma volatiles produced in cooked beef that present various aromas that contribute to the complex flavor of beef. Wet aging and blade

tenderization are two practices utilized by the meat industry to improve beef palatability. Both of these applications involve disruption of the muscle structure which can possibly influence flavor and other palatability traits. Additionally, beef is cooked to varying degrees of doneness to fulfill the preferences of consumers and the wide range of endpoint cooking temperatures used can contribute to vastly different eating experiences. The aforementioned practices could all influence the aroma volatile profiles of beef that could greatly influence flavor and palatability.

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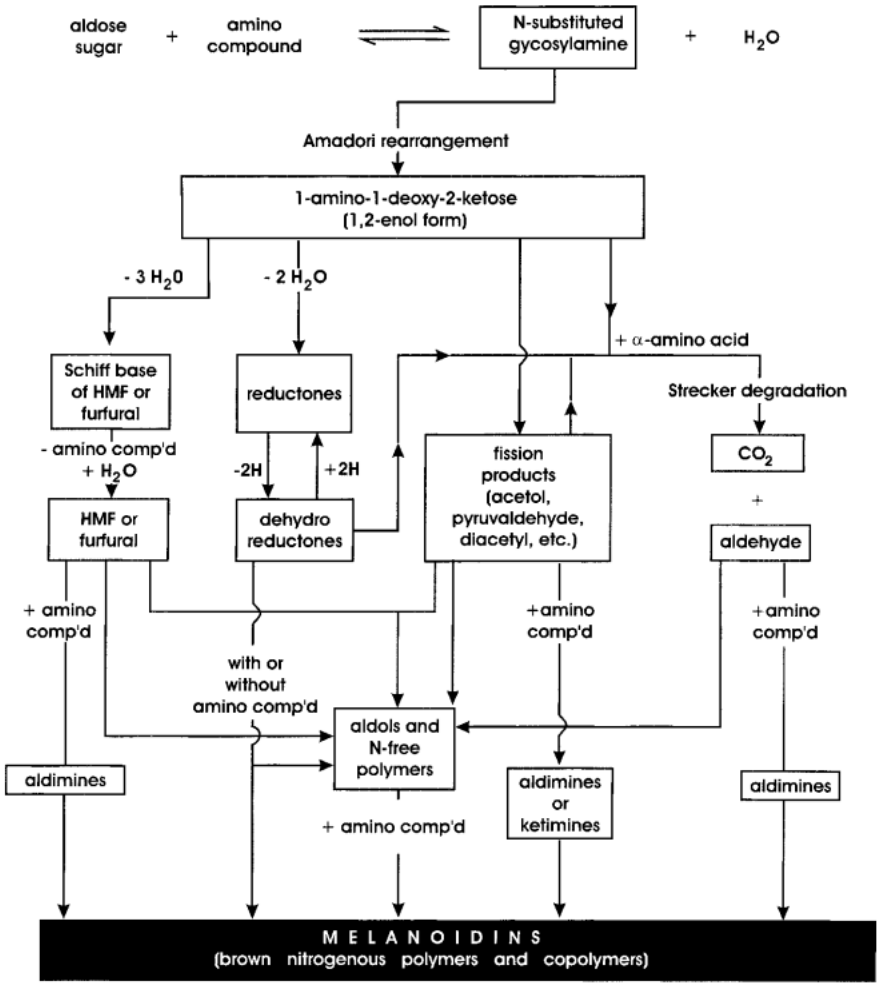

Figure 5.1 The Maillard reaction (Fay & Brevard, 2005).

Initiation

$RH + O_2 \rightarrow R\bullet + \bullet OH$

Propagation

$R\bullet + O_2 \rightarrow ROO\bullet$

$ROO\bullet + RH \rightarrow ROOH + R\bullet$

Termination

$R\bullet + R\bullet \rightarrow RR$

$R\bullet + ROO\bullet \rightarrow ROOR$

$ROO\bullet + ROO\bullet \rightarrow ROOR + O_2$

Figure 5.2 Mechanism for lipid oxidation (Gray, 1978).

Table 5.1 Compounds identified in beef and characteristic flavors and/or aromas associated with those compounds.[1]

Compound name	Characteristic flavors/aromas
Benzaldehyde	Volatile almond oil, bitter almond, burning aromatic taste
Benzene	Pleasant, distinct
sec-Butanamine	Seafood, green, onion
Butenal	Malty, green, roast
n-Caprioc acid	Goaty
3-Carene	Sweet and pungent odor but more agreeable than turpentine, orange
Cyclobutanol	Roasted
2,2,6-Trimethylcyclohexanone	Mint, acetone
2,4-Decadienal	Deep fat flavor, chicken flavor at 10 ppm, citrus/orange/grapefruit
Decanal	Powerful, waxy, aldehydic, orange, citrus peel
2-Decenal	Tallow, orange
1,3-Bis(1,1-dimethylethyl) benzene	Cooked beef
N,N'-Dimethyl 1,2-ethanediamine	Ammonia
5-Ethylcyclopent-1-enecarboxaldehyde	Fragrant, perfume
2-Pentylfuran	Green bean, butter
2,4-Heptadienal	Nut, fat
Heptanal	Oily, fatty, rancid, unpleasant, penetrating, fruity odor in liquid
1-Heptanol	Fragrant, woody, oily, green, fatty, winey, sap, herb
2-Heptanone	Fruity, spicy, cinnamon, penetrating fruity odor in liquid
6-Methyl 2-heptanone	Cloves, menthol, eugenol
2-Heptenal	Soapy, fatty, almond, fishy, unpleasant
Hexanal	Fatty-green, grassy, strong green, tallow, fat, unripe fruit when dilute
Hexane	Faint peculiar odor
Hexanol	Woody, cut grass, chemical-winey, fatty, fruity, weak metallic
2-Ethyl 1-hexanol	Resin, flower, green
2-Hexen-1-ol	Green, sharp, leafy, fruity, unripe banana
3-Methylbutanal	Pungent apple-like odor, malt
2,4-Nonadienal	Fat, was, green, watermelon, geranium, pungent
Nonanal	Floral, citrus, fatty, grassy, waxy, green
2-Nonanone	Hot milk, soap, gree, fruity, floral
2-Nonenal	Cardboardy, orris, fat, cucumber
Octanal	Harsh, fatty, orange peel, soapy, lemon, green, honey
1-Octanol	Penetrating aromatic odor, fatty, waxy, citrus, oily, walnut, moss,
2-Methyl 3-Octanone	Herb, butter, resin, gasoline
2-Octenal	Green, nut, fat
1-Octen-3-ol	Mushrooms, compound excreted by many insects
2-Octen-1-ol	Green citrus
3-Octen-2-one	Nut, crushed bug, earthy, spicy, herbal, sweet, mushroom, hay,
Pentanal	Almond, malt, pungent, acrid
Pentane	Very slight warmed-over flavor, oxidized
1-Pentanol	Mild odor, fusel oil, fruit, balsamic
5-Amino 1-pentanol	Mild
α-Pinene	Piney, fruity, citrus, turpentine
Piperazine	Salty
Propanol	Alcoholic
Tetradecane	Alkand
Tridecane	Alkane
2-Tridecenal	Sweet, stong, spicy

[1]Adapted from Calkins and Hodgen (2007).

Chapter 6 - The effects of extended postmortem aging and blade tenderization on the volatile compound profile of raw and cooked beef *gluteus medius* steaks.

Abstract

The aim of this study was to evaluate impact of 5, 19, 33, 47, or 61 d of postmortem aging and blade tenderization (BT) on aroma volatile compound generation from raw and cooked beef *gluteus medius* steaks. Aging time × BT treatment interactions ($P < 0.05$) were observed for 4-methylphenol from raw and cooked steaks. Heptanal, octanal, and nonanal concentrations were greater ($P < 0.05$) from raw steaks aged longer than 19 d in comparison to steaks aged 5 d, but exhibited no response ($P > 0.05$) to aging in cooked steaks. These results suggest aging-induced lipid oxidation may be best assessed using raw steaks. The only Maillard reaction product from cooked steaks that was affected ($P = 0.006$) by aging was 3-ethyl-2,5-dimethylpyrazine. Numerous Maillard reaction and lipid oxidation products from cooked steaks were decreased ($P < 0.05$) by use of BT, implying that BT may alter beef aroma.

Keywords: aging, aroma, beef, blade tenderization, GC/MS, SPME

1. Introduction

Beef flavor is complex and heavily influences the acceptability of beef. Flavor is mainly comprised of the two sensations of taste and smell (Farmer, 1994). The tongue can recognize water-soluble compounds responsible for the basic tastes (sweet, salty, sour, bitter, and umami) (Moody, 1983). The aroma portion of flavor is more diverse than the taste portion as the human nose can identify thousands of different odors (Farmer, 1994). Beef aroma is influenced by the presence of hundreds of different volatile compounds that form during cooking (Calkins & Hodgen, 2007).

Raw meat has little aroma and a blood-like taste (Stetzer, Cadwallader, Singh, Mckeith, & Brewer, 2008), but it contains numerous precursors, such as amino acids, peptides, reducing sugars, vitamins, nucleotides and fatty acids, that can generate aromas through the Maillard reaction and lipid oxidation during cooking (Madruga, Elmore, Oruna-Concha, Balagiannis, & Mottram, 2010). Hodge (1953) provides a thorough explanation of the Maillard reaction, which is characterized by non-enzymatic browning induced by reactions between amino acids and reducing sugars in the presence of heat. A diverse array of volatile compounds is generated during the Maillard reaction and includes aldehydes, sulfur compounds, furans, pyridines, pyrazines, and thiazoles among other classes of compounds that have strong aromatic properties (Mottram, 1994). Off-flavor development and formation of rancidity occur during fresh meat storage as a result of lipid oxidation (Ladikos & Lougovois, 1990). Cooking can initiate oxidation reactions similar to those that contribute to rancidity; however, they occur more rapidly and form different compounds that produce desirable flavors (Ladikos & Lougovois, 1990). Hydroperoxides are the primary products of lipid oxidation and do not contribute to aroma, but once these compounds decompose, they form hydrocarbons, alcohols, ketones, and aldehydes which strongly influence aroma (Shahidi, 1989).

Commonly, beef is subjected to postmortem aging under anaerobic conditions through a process called “wet aging.” Aging can increase product tenderness due to proteolytic degradation of cytoskeletal proteins (Koohmaraie, 1996) and aging periods up to 28 d improve beef palatability (Brewer & Novakofski, 2008; Jeremiah & Gibson, 2003). Storage times of anaerobically packaged beef in the U.S. are very inconsistent. On average, anaerobically packaged beef is held in refrigerated storage for 20.5 d, but storage times can range from 1 d to

as long as 358 d before product reaches the retail counter (Guelker et al., 2013). A wide range of storage times could increase the variability of beef palatability traits within the beef supply which could compromise consumer confidence in beef.

Wet aging for periods longer than 30 d is common when handling product that is inherently tough or when product is exported. Beef aged as long as 28 d exhibits tenderness improvement, but not all muscles demonstrate the same response to aging, as some require longer aging periods to achieve maximum tenderness (Gruber et al., 2006). Additionally, proteolysis, which improves tenderness, can still increase in the *gluteus medius* when aging times are extended to 40 d postmortem (King et al., 2009).

Although extended aging times can improve tenderness, they may adversely influence beef flavor. Beef aged up to 35 d has an increased perception of sour and rancid flavors in addition to increased off-flavor intensity when compared to beef aged for a shorter time period (Yancey, Dikeman, Hachmeister, Chambers IV, & Milliken, 2005). Moreover, flavor intensity decreases while off-flavor intensity increases when aging times are extended to 42 d in beef strip loins and cuts from the round (Juarez et al., 2010). The natural proteolysis responsible for increased tenderness also increases the pool of available aroma precursors which could alter the quantity of volatiles formed via the Maillard reaction during cooking and ultimately impact beef aroma (Koutsidis et al., 2008).

Beef top sirloin butts commonly undergo blade tenderization to improve tenderness (George-Evins, Unruh, Waylan, & Marsden, 2004). Blade tenderization decreases Warner-Bratzler shear force and increases perceived tenderness in top sirloin butts as assessed by sensory panel (George-Evins et al., 2004). Most research has determined that blade tenderization does not influence beef flavor (George-Evins et al., 2004; Savell, McKeith, Murphey, Smith, & Carpenter, 1982; Medeiros, Field, Menkhaus, Riley, & Russell, 1989), but other studies have shown that blade tenderization decreases flavor intensity scores of beef top sirloin steaks (Jeremiah, Gibson, & Cunningham, 1999) and beef strip loin steaks (Bidner, Montgomery, Bagley, & McMillin, 1985). Physical disruption of meat structure, as seen in blade tenderization, used to increase tenderness may alter the form of aroma precursors and ultimately cause an alteration to the volatile profile of raw and cooked beef.

Aroma volatile production has been frequently evaluated in beef that has undergone postmortem aging as long as 21 d, but research investigating the impact of longer aging periods

on the presence of aroma volatiles has been limited. Additionally, the amount of research evaluating the effects of blade tenderization on aroma volatile formation is negligible. Beef top sirloin butts, which contain the *gluteus medius*, are frequently blade tenderized and/or aged longer than 21 d postmortem to improve tenderness. Aging and blade tenderization can improve tenderness, but the effects of these processes on aroma compound formation are essentially unknown. Therefore, the objectives of this study were to evaluate the changes in the aroma volatile profiles from raw and cooked *gluteus medius* steaks caused by extended postmortem aging and the application of blade tenderization.

2. Materials and methods

2.1 Experimental design

Five top sirloin butts (IMPS 184) were purchased from three commercial beef processing facilities (n=15) and were transported to the Kansas State University Meat Laboratory (Manhattan, KS). The five top sirloin butts from each source were randomly assigned to 5, 19, 33, 47, or 61 d of postmortem aging. The top sirloins butts were stored in their original anaerobic packages at 2 ºC throughout the duration of each aging period. Upon the completion of their respective aging treatment, top sirloin butts were removed from their packaging and the *gluteus medius* (GM) was removed and trimmed free of fat. The GM was cut down the center, parallel with the muscle fibers, to yield two approximately equal sized pieces which were randomly assigned to either no tenderization (NT) or blade tenderization (BT) treatment. The BT-treated GM pieces were processed through a blade tenderizer (Ross model T7001 tenderizer, Midland, VA) twice, being turned over between cycles. Each GM piece yielded one 2.54-cm thick steak (n = 30) that was removed by cutting perpendicular to the muscle fibers. Steaks were vacuum packaged (Prime Source Vacuum Pouches; 76.2 μm, STP Barrier, Nylon/PE Vacuum Pouch; oxygen transmission rate 0.04 g/254 cm^2/24 h at 0 ºC; water vapor transmission rate 0.2 cc/254 cm^2/24 h at 0 ºC at 0% relative humidity) and stored at –20 ºC until analyses were conducted.

2.2 Aroma volatile extraction

Steaks were thawed for approximately 24 h at 0-2 ºC prior to sample preparation for volatile analysis. Two samples (0.5 g) were attained by cutting a thin strip across the center of the steak and then removing the center portion of the strip so the sample spanned from cooked

surface to cooked surface. Samples were minced with a knife while the remaining steak portion was re-packaged in a vacuum sealed bag (Prime Source Vacuum Pouches; 76.2 μm, STP Barrier, Nylon/PE Vacuum Pouch; oxygen transmission rate 0.04 g/254 cm^2/24 h at 0 ºC; water vapor transmission rate 0.2 cc/254 cm^2/24 h at 0 ºC at 0% relative humidity) and stored at 0-2 ºC until needed for volatile analysis of cooked steaks. Minced samples were placed in a 10 mL screw cap vial (Supelco, Bellefonte, PA) fitted with a polytetrafluoroethylene/silicone septum (Supelco, Bellefonte, PA). Each sample had 10 μL of internal standard (1 ppm 1, 3 dichlorobenzene) added to the vial prior to equilibration. Samples were analyzed using headspace-solid phase microextraction (HS-SPME) as described by Koppel, Adhikari, and Di Donfrancessco (2013) using the following modifications. Sample vials were equilibrated at 45 ºC and agitated at 250 rpm for 15 min using an autosampler (Pal system, model CombiPal; CTC Analytics, Zwingen, Switzerland). A 50/30 μm divinylbenzene/carboxen/polydimethyl-siloxane fiber (Supelco, Bellefonte, PA) was then exposed to the sample headspace for 30 min at 45 ºC. The analytes were desorbed from the fiber coating to the injection port of a gas chromatograph (GC) at 270 ºC for 3 min in splitless mode. The fiber was baked at 270 ºC for 20 min between samples to eliminate residual analytes.

Steaks used for cooked volatile analysis were cooked to an internal temperature of 60 ºC using a George Foreman Grill (Model GRP12, Miramar, FL). The grill was powered on and allowed to heat for 10 min and then the raw steak was placed on the grill and the top was closed. Steak temperatures were monitored using a traceable expanded-range thermometer (Fisher Scientific, Pittsburgh, PA) that was inserted into the approximate geometric center of the steak. Two samples (0.5 g) were taken to represent a cross-section from the center of each cooked steak. Samples were held for no longer than 45 min before being prepared and analyzed as previously described.

2.3 Volatile identification and quantification

Volatile separation, identification, and quantification were conducted on a GC (Varian GC CP3800; Varian Inc., Walnut Creek, CA) combined with a Varian mass spectrometer (MS) detector (Saturn 2000). An RTX-5MS (Crossbond 5% diphenyl/95% dimethyl polysiloxane) column (Restek U.S., Bellefonte, PA; 30 m × 0.25mm × 0.25 μm film thickness) was used to

separate the volatiles. The column was initially heated at 40 ºC for 4 min and temperature increased by 5 ºC per min to 260 ºC, where it was held for 7 min.

Volatile compounds were identified using mass spectra as well as linear retention indices (NIST/EPA/NIH Mass Spectral Library, Version, 2.0, 2005). The retention times for a C7-C40 saturated alkane mix (Supelco Analytical, Bellefonte, PA) were used in determining experimental linear retention indices for the volatile compounds that were identified.

2.4 Statistical analysis

Data were analyzed as a split-plot design with processing facility serving as the whole-plot and aging time being the whole-plot treatment. Each top sirloin served as the split plot with BT treatments functioning as the split-plot treatment. Treatment differences were determined using the Proc Mixed procedures of SAS (SAS Institute, Inc., Cary, NC). Post-hoc mean separation was carried out using Fisher's least significant differences and all data were analyzed at a 5% level of significance. Due to the numerous volatile compounds assessed, principal component analysis (PCA) of raw and cooked steaks was also conducted using Unscrambler® (Version 10.2; Camo Software, Oslo, Norway) to reduce dimensionality of the data set.

3. Results and discussion

A summary of all significant main and interaction affects that aging and BT had on the presence and concentration of aroma volatiles from raw and cooked GM steaks are presented in Table 6.1.

3.1 Effects of aging and blade tenderization on volatiles from raw GM steaks

A total of 1 alcohol, 4 aldehydes, 4 carboxylic acid methylesters, 1 furan, 1 hydrocarbon, 2 ketones, 2 phenols, and 1 pyrazine were present in quantifiable amounts in raw steak samples. The lone compound to display an aging × tenderization interaction ($P = 0.018$) was 4-methylphenol (Figure 6.1). The concentration of 4-methylphenol was similar ($P > 0.05$) between BT and NT steaks that were aged 5, 33, and 47 d. The NT steaks that were aged 19 and 61 d displayed greater ($P < 0.05$) concentrations of 4-methylphenol than BT steaks. The concentration of 4-methylphenol produced by NT steaks aged 5 d was less ($P < 0.05$) than NT steaks aged 19, 47, and 61 d but similar ($P > 0.05$) to NT steaks aged 33 d. The NT steaks aged 19, 33, 47, and 61 d maintained similar ($P > 0.05$) concentrations of 4-methylphenol. The BT steaks from all

aging treatments displayed similar ($P > 0.05$) concentrations of 4-methylphenol. 4-Methylphenol has previously demonstrated a relationship with animal odor in cooked lamb (Young, Berdague, Viallon, Rousset-Akrim, & Theriez, 1997) but has received limited attention in other research evaluating beef aroma volatiles.

The effects of aging time on volatile compound concentrations from raw GM steaks are displayed in Table 6.2. The only compounds that displayed a response to aging were the aldehydes heptanal ($P = 0.047$), octanal ($P = 0.002$), and nonanal ($P = 0.019$). Concentrations of heptanal, octanal, and nonanal from steaks aged 5 d were similar ($P > 0.05$) to steaks aged 19 d but less ($P < 0.05$) than steaks aged 33, 47, and 61 d. Additionally, steaks aged 19 d produced reduced ($P < 0.05$) concentrations of heptanal, octanal, and nonanal than steaks aged 61 d, while steaks aged 19, 33, and 47 d produced similar ($P > 0.05$) concentrations of all three compounds. Moreover, heptanal, octanal, and nonanal were found in similar ($P > 0.05$) concentrations in steaks aged 33, 47, and 61 d. The aroma of heptanal has been characterized as oily, rancid, and unpleasant (Calkins & Hodgen, 2007; Madruga et al., 2010), while soapy, lemon, and green have been used to characterize the aroma and flavors that are experienced when octanal is present (Calkins & Hodgen, 2007). Aromas and flavors associated with nonanal have been described as soapy and tallowy (Stetzer et al., 2008) as well as floral, citrus, and grassy (Calkins & Hodgen, 2007). Aldehydes form by free radical initiated lipid autoxidation reactions (Mottram, 1998). As aging time increases, it is expected that lipid oxidation will increase (Yancey et al., 2006) which should result in a greater accumulation of the aforementioned aldehydes and could lead to the formation of rancid off-flavors (Mottram, 1998).

All compounds, aside from 4-methylphenol, heptanal, octanal, and nonanal, identified in quantifiable amounts from raw samples remained similar ($P > 0.05$) across aging treatments. Hexanal was among the compounds that showed no change ($P = 0.066$) in response to the aging treatments. Hexanal is one of the most prevalent volatile compounds commonly generated by beef. Hexanal is known to be directly proportional to thiobarbituric acid reactive substances (TBARS), which is a measure of lipid oxidation (Shahidi & Pegg, 1994; Ullrich & Grosch, 1987). The lack of change in hexanal due to aging time contrasts prior research which demonstrates hexanal increases when aging time is increased in ground beef patties (Ismail, Lee, Ko, & Ahn, 2009); however, the current study used steaks which should exhibit less oxidation as

there is a decreased surface area that interacts with oxygen and no free radicals that are produced during grinding.

The impact of BT treatment on the generation of volatile compounds from raw GM steaks is depicted in Table 6.3. The implementation of BT decreased the concentrations of 2-pentylfuran ($P = 0.006$) and methyl butanoate ($P = 0.014$), while all other volatile compounds detected in raw steaks remained unaffected ($P > 0.05$) by BT treatment. The most likely route of formation of 2-pentylfuran is through the 9-hydroperoxide of linoleic acid (Mottram, 1991) while butanoate can be formed by lipid oxidation (Stetzer et al., 2008). The physical disruption of muscle structure suggests that BT would increase the amounts of free radicals available to initiate lipid oxidation; however, the BT treatment used in the current study was applied prior to cutting steaks at the completion of the designated aging time. It is quite possible that BT could initiate a greater amount of lipid oxidation in raw steaks if the BT treatment was applied prior to the designated aging time.

3.2 Principal component analysis (PCA) of volatile compounds generated by raw steaks

Figure 6.2 outlines PCA conducted on volatile compounds from raw GM steaks subjected to BT or NT at five aging times. This PCA describes 65% and 16% of the total variation associated with principal components (PC) 1 and 2, respectively. Heptanal, octanal, nonanal, 2,3-octanedione, and 1-octen-3-ol exhibited great positive scores along PC 1 while 3-hydroxy-2-butanone displayed the greatest negative scores along PC 1. The scores of the previously mentioned compounds suggests they contributed greatly to the variability associated with PC1 and minimally to the variation of PC 2, where they all displayed scores close to 0. Both BT and NT steaks aged 5 d as well as BT steaks aged 19 d showed the greatest negative scores along PC 1 and were the most closely associated with 3-hydroxy-2-butanone. The 47 BT, 33 BT, and 61 NT samples were separated from 5 BT, 5 NT, and 19 BT along PC 1 as they exhibited positive scores implying they contained greater concentrations of heptanal, octanal, nonanal, 2,3-octanedione, and 1-octen-3-ol.

3.3 Effects of aging and blade tenderization on volatiles from cooked GM steaks

A total of 1 alcohol, 5 aldehydes, 4 carboxylic acid methylesters, 1 furan, 1 hydrocarbon, 2 ketones, 2 phenols, and 2 pyrazines were produced in quantifiable amounts from cooked steaks. The only compound that displayed an aging × tenderization treatment interaction (P = 0.027) was 4-methylphenol (Figure 6.3). The 4-methylphenol concentrations from NT steaks were greater ($P < 0.05$) than concentrations from BT steaks at 19 and 61 d of age. The NT steaks generated 4-methylphenol at 5 d of aging in lesser ($P < 0.05$) concentrations than 19, 33, and 61 d but produced concentrations similar ($P = 0.410$) to those observed with steaks aged 47 d. Steaks that were BT maintained similar ($P > 0.05$) concentrations of 4-methylphenol across all aging treatments.

All detected aldehydes showed no change ($P > 0.05$) due to aging time (Table 6.4) which contrasts what was observed when raw samples were evaluated as heptanal, octanal, and nonanal were increased at longer aging periods. Aldehydes can be generated via autoxidation of unsaturated fatty acids during long-term storage and lead to rancid off-flavors if they are present in excessive amounts (Mottram, 1998). In cooked meat, lipid oxidation reactions occur quickly and lead to the production of volatile compounds associated with desirable flavors (Calkins & Hodgen, 2007). Saturated and unsaturated aldehydes with 6-10 carbons are major volatile components of all cooked meats and play an important role in meat flavor (Mottram, 1998). Aldehydes, especially hexanal, are often used to assess lipid oxidation in meat products as they are major products of lipid oxidation and have shown a strong correlation with TBARS (St. Angelo et al., 1987); however, hexanal and TBARS from cooked beef have previously shown a lack of correlation with each other (Spanier, Flores, McMillin, & Bidner, 1997). Aldehydes observed in the current study presented differences between aging treatments in raw samples; however, these differences may be equilibrated in cooked samples as those aldehydes can enter Maillard reaction pathways and are generally produced in greater and more variable quantities during the thermal degradation of lipid (Mottram, 1998).

Only methyl hexanoate (P = 0.029) and 3-ethyl-2,5-dimethylpyrazine (P = 0.006) were influenced by aging treatments (Table 6.4). Methyl hexanoate was present in greater ($P < 0.05$) concentrations in steaks aged 33 d in comparison to steaks aged 5 and 61 d. Additionally, steaks aged 19 and 47 d generated methyl hexanoate in concentrations that were similar ($P > 0.05$) to steaks from all other aging treatments. Methyl hexanoate has been identified in fruits and has

been associated with pineapple, sweet, and fruity aromas (Spanier et al., 1998). Hexanoate has a "sweaty" odor (Spanier, Vercellotti, & James, 1992) and has been shown to double in concentration as beef is aged from 7 d to 14 d (Stetzer et al., 2008).

3-Ethyl-2,5-dimethylpyrazine was the only Maillard reaction product that was altered by aging as it was present in greater ($P < 0.05$) concentrations from steaks aged 33 and 61 d in comparison to steaks aged 5, 19, and 47 d. The Maillard reaction occurs when reducing sugars and amino acids react during heating (Mottram, 1994). Meat contains numerous Maillard reaction precursors. Free sugars and amino acids are known to increase when beef is aged as long as 21 d, which suggests that the production of Maillard reaction compounds should increase as aging time is increased (Koutsidis et al., 2008). Numerous Maillard reaction products and intermediates, such as benzaldehyde, 3-hydroxy-2-butanone, and 2,5-dimethylpyrazine, were unaffected ($P > 0.05$) by changes in aging time which contrasts the thought that increased availability of volatile precursors results in an increase in Maillard reaction products.

Aging beef as long as 28 d can be used to improve beef palatability through tenderness improvement (Gruber et al., 2006). In the current beef supply, some anaerobically packaged subprimals are exposed to shorter or much longer aging times than the average 20.5 d (Guelker et al., 2013). Multiple studies show a decrease in beef flavor intensity and increase in off-flavor intensity as beef is aged for longer time periods (Juarez et al., 2010; Yancey et al., 2005). The current study provides little explanation to why a decrease in beef flavor intensity can occur during aging as none of the volatiles detected in this study demonstrated a decrease as aging time increased. Bitter, sour, and livery are a few off-flavors that reportedly increased when aging times are increased (Spanier, Flores, McMillin, & Bidner, 1997; Yancey et al., 2005). The current study only displayed two volatile compounds that were affected by aging time. 3-Ethyl-2,5-dimethylpyrazine should contribute to the perception of nutty aromas, while hexanoic acid methylester could contribute some negative flavors as it has been associated with fruity and sweaty odors (Spanier et al. 1992; Spanier et al., 1998). Bitter and sour are commonly known as basic tastes and livery off-flavor has been associated with increases of free iron (Yancey et al., 2006). It is quite possible that the development of off-flavors during aging are more associated with taste rather than aroma as neither 3-ethyl-2,5-dimethylpyrazine nor hexanoic acid methylester have been associated with bitter, sour, or livery flavors.

Main effects of blade tenderization treatment on cooked steak volatile compound concentrations are found in Table 6.5. Hexanal, which is formed from the breakdown of ω6 fatty acids and can present fatty, green, and unripe-fruit aromas (Calkins & Hodgen, 2007; Madruga et al., 2010), was decreased ($P = 0.037$) by BT as was heptanal ($P = 0.021$) which is associated with oily, rancid, and unpleasant aromas (Calkins & Hodgen, 2007; Madruga et al., 2010). Additionally, the Maillard reaction product benzaldehyde, which is associated with almond oil-like and bitter almond flavors in beef, was reduced ($P = 0.038$) by BT. Moreover, both octanal and nonanal were decreased ($P < 0.05$) by the implementation of BT. Octanal has been related to soapy, lemon, and green aromas (Calkins & Hodgen, 2007), while nonanal is associated with soapy and tallowy aromas (Stetzer et al., 2008). Many aldehydes identified in this study can be produced during the thermal degradation of fatty acids during cooking or through Strecker degradation during the Maillard reaction (Mottram, 1998).

The lone furan, 2-pentylfuran, was reduced ($P = 0.042$) by the use of BT. The aroma and flavor of 2-pentylfuran has been described as green, earthy, and beany (Stetzer et al., 2008). Moreover, both 2,5-dimethylpyrazine and 3-ethyl-2,5-dimethylpyrazine, which are Maillard reaction products, were both present in decreased ($P < 0.05$) concentrations in BT steaks. Cocoa and roasted nut have been used to describe aromas and flavors attributed to the presence of 2,5-dimethylpyrazine (Madruga et al., 2010).

Most research evaluating the influence of BT on flavor have reported no differences in regard to flavor intensity (George-Evins et al., 2004; Savell et al., 1982), but other research have demonstrated decreased flavor intensities from BT beef top sirloin steaks and ribeye steaks (Jeremiah et al., 1999) as well as BT beef striploin steaks (Bidner et al., 1985). The results of the current study suggest that flavor should be decreased by BT as many thermally generated compounds were reduced by the incorporation of BT. All cooked steaks assessed in this study were cooked to a specific internal temperature (60 °C). The structure and composition of muscle tissue serves as an insulatory barrier that restricts heat from entering the center of a steak (Hallstrom, Skjoldebrand, & Tragardh, 1988). Blade tenderization achieves tenderness improvement through physical disruption of muscle structure (Bowker et al., 2007). The muscle structure of a BT steak is much different than NT steaks and the alteration of muscle structure could reduce the cooking time needed to meet a specified degree of doneness as has been previously reported (Savell, Smith, & Carpenter, 1977; Schwartz & Mandigo, 1974). If cooking

time is reduced by BT, there is a reduced amount of time for both lipid oxidation and Maillard reactions to occur which may explain the observed decreases of aldehydes and pyrazines in the current study.

Another potential mechanism that may explain the suppressed volatile production associated with BT steaks could be the presence of free water. The Maillard reaction is dependent on water activity and occurs more readily when moisture levels are reduced (Jaeger, Janositz, & Knorr, 2010). The disruptions of muscle structure due to BT can cause an increase of purge during storage (Davis, Smith, & Carpenter, 1977) and disruption of myofibrillar protein can increase the amount of free water present in a muscle (Huff-Lonergan & Lonergan, 2005). The increased amounts of free water that may be present in BT steaks could potentially interact with the cooked meat surface and ultimately suppress the amount of Maillard reaction products generated during cooking.

3.4 Principal component analysis (PCA) of volatile compounds generated by cooked steaks

The PCA conducted on cooked steak volatiles is displayed in Figure 6.4. This PCA described 57% of the total variation for PC 1 and 32% of the total variation for PC 2. Many compounds routinely associated with cooked beef, such as aldehydes, showed high positive scores along PC 1, suggesting they contributed greatly to the variability associated with PC 1. Aside from the samples aged 47 d, BT samples had decreased values along PC1 in comparison to NT samples. The greater values along PC 1 exhibited by NT samples agree with the findings detailed in Table 6.5 that showed greater concentrations of aldehydes and pyrazines for NT samples in comparison to BT samples. Both NT and BT samples from 33 d of aging showed the greatest values with respect to PC 2 which suggests they are more associated with the observed carboxylic acid methylesters. Table 6.4 illustrated no differences ($P > 0.05$) between aging times for most compounds; however, methyl hexanoate concentrations were greater ($P < 0.05$) at 33 d of aging in comparison to 5 and 61 d of aging, which agrees with the PCA displayed in Figure 6.4.

4. Conclusions

Raw GM steaks aged as long as 61 d exhibited increased concentrations of heptanal, octanal, and nonanal which are associated with lipid oxidation; however, these changes were not seen in cooked GM steaks. The results of this study suggest that if the primary objective of volatile analysis is to assess lipid oxidation, it is best to assess raw beef as lipid oxidation products can be altered by thermal degradation or involvement in the Maillard reaction during cooking. The effects of aging time on cooked GM steaks was minimal as only two compounds, methyl hexanoate and 3-ethyl-2,5-dimethylpyrazine, were changed in response to aging which suggests that aging should have minimal influence on cooked beef aroma. Maillard reaction products were found in relatively low quantities with the sampling method used in this study which could explain the minimal changes observed due to aging treatment. Identifying specific areas, such as the cooked meat surface, may clarify the influence aging has on compounds associated with cooking. The implementation of BT had a significant influence on volatile compound production. The use of BT decreased the presence of multiple lipid oxidation and Maillard reaction products generated by cooked GM steaks. The inhibition of volatile production due to BT could affect flavor development in BT products and this matter warrants further investigation to understand the role that heat transfer and free water availability have in volatile production.

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Table 6.1 Summary of treatment effects on volatile compound concentrations from raw and cooked beef *gluteus medius* steaks.

	Linear Retention Index	Raw			Cooked		
		Aging Time	Tenderization Treatment	Aging × Tenderization	Aging Time	Tenderization Treatment	Aging × Tenderization
Alcohols							
1-Octen-3-ol	981	NS	NS	NS	NS	NS	NS
Aldehydes							
Hexanal	800	NS	NS	NS	NS	*	NS
Heptanal	902	**	NS	NS	NS	*	NS
Benzaldehyde	974	ND	ND	ND	NS	*	NS
Octanal	1004	**	NS	NS	NS	*	NS
Nonanal	1106	*	NS	NS	NS	*	NS
Carboxylic acid methylesters							
Methyl butanoate	726	NS	*	NS	NS	NS	NS
Methyl pentanoate	825	NS	NS	NS	NS	NS	NS
Methyl hexanoate	923	NS	NS	NS	*	NS	NS
Methyl octanoate	1122	NS	NS	NS	NS	NS	NS
Furans							
2-Pentylfuran	992	NS	*	NS	NS	*	NS
Hydrocarbons							
Toluene	774	NS	NS	NS	NS	NS	NS
Ketones							
3-Hydroxy-2-butanone		NS	NS	NS	NS	NS	NS
2,3-Octanedione	983	NS	NS	NS	NS	NS	NS
Phenols							
Phenol	978	NS	NS	NS	NS	NS	NS
4-Methylphenol	1075	NS	**	*	NS	*	*
Pyrazines							
2,5-Dimethylpyrazine	917	ND	ND	ND	NS	*	NS
3-Ethyl-2,5-dimethylpyrazine	1082	NS	NS	NS	*	*	NS

* Treatment means differ ($P < 0.05$).
**Treatment means differ ($P < 0.005$).
NS: No difference detected between treatment means ($P > 0.05$).
ND: Not detected in quantifiable amount and least squares mean not computed for treatment group.

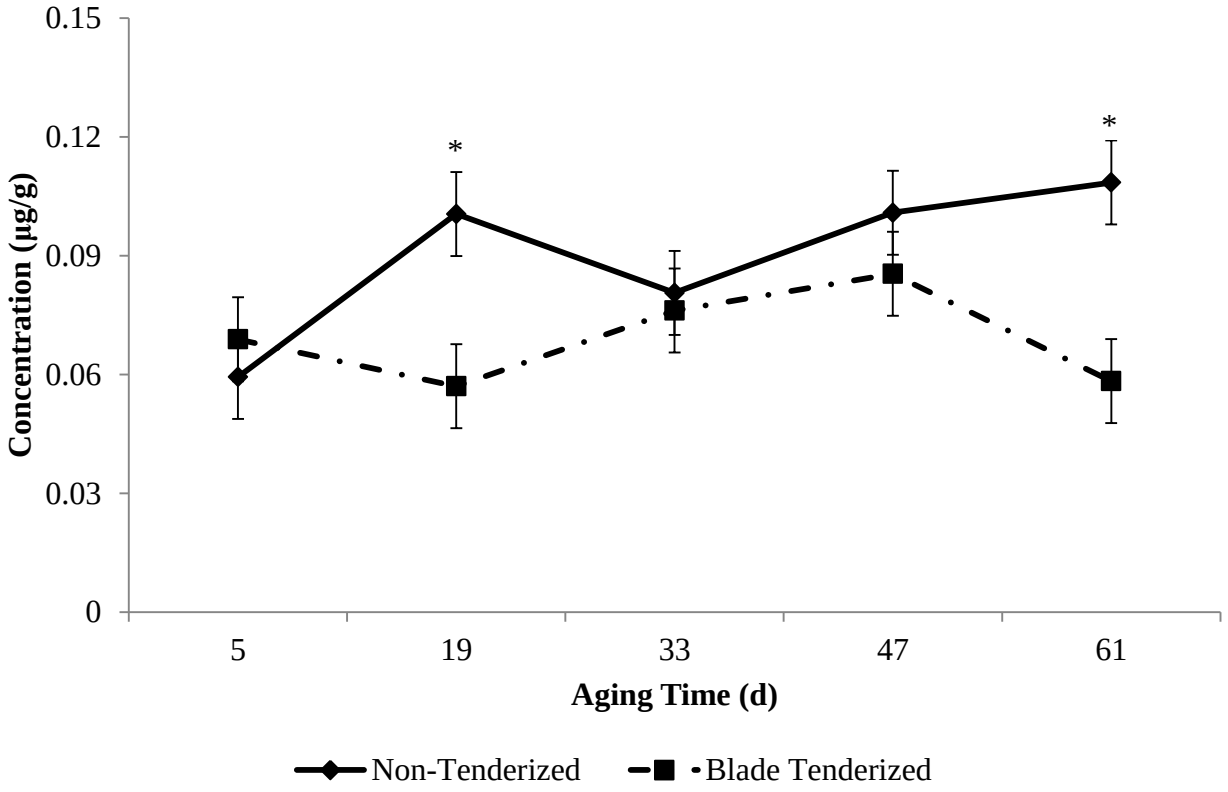

Figure 6.1 Concentrations of 4-methylphenol from raw *gluteus medius* steaks as affected by aging time and blade tenderization treatment.
Top sirloin butts were aged in anaerobic packaging at 2 °C for 5, 19, 33, 47, or 61 d. *Gluteus medius* pieces yielding blade tenderized steaks were processed twice through a blade tenderizer. Error bars represent the mean ± SEM. *$P < 0.05$. Aging time × tenderization treatment interaction $P = 0.019$.

Table 6.2 Effects of postmortem aging on volatile compound concentrations (μg/g) from raw *gluteus medius* steaks.

	Aging Time (d)						
	5	19	33	47	61	SEM	*P*-Value
Alcohols							
1-Octen-3-ol	0.015	0.023	0.057	0.051	0.062	0.015	0.113
Aldehydes							
Hexanal	0.044	0.067	0.141	0.175	0.156	0.042	0.066
Heptanal	0.007^{c}	0.013^{bc}	0.020^{ab}	0.020^{ab}	0.025^{a}	0.003	0.005
Octanal	0.008^{c}	0.014^{bc}	0.024^{ab}	0.024^{ab}	0.032^{a}	0.004	0.002
Nonanal	0.045^{c}	0.076^{bc}	0.108^{ab}	0.116^{ab}	0.155^{a}	0.021	0.019
Carboxylic acid methylesters							
Methyl butanoate	0.179	0.211	0.207	0.196	0.193	0.0530	0.989
Methyl pentanoate	0.027	0.034	0.038	0.040	0.031	0.0076	0.785
Methyl hexanoate	0.390	0.434	0.567	0.517	0.451	0.0871	0.643
Methyl octanoate	0.115	0.106	0.187	0.175	0.137	0.0474	0.552
Furans							
2-Pentylfuran	0.001	0.002	0.006	0.002	0.007	0.002	0.076
Hydrocarbons							
Toluene	0.077	0.087	0.085	0.086	0.078	0.0091	0.883
Ketones							
3-Hydroxy-2-butanone	0.072	0.048	0.041	0.025	0.038	0.021	0.587
2,3-Octanedione	0.008	0.012	0.028	0.025	0.027	0.009	0.238
Phenols							
Phenol	0.006	0.008	0.007	0.007	0.005	0.002	0.875
Pyrazines							
3-Ethyl-2,5-dimethylpyrazine	0.004	0.002	0.006	0.002	0.002	0.003	0.743

[abc]Means with different superscripts within the same row differ ($P < 0.05$).

Table 6.3 Effects of blade tenderization on volatile compound concentrations (μg/g) from raw *gluteus medius* steaks.

	Non-Tenderized	Blade Tenderized	SEM	*P*-Value
Alcohols				
1-Octen-3-ol	0.045	0.039	0.011	0.637
Aldehydes				
Hexanal	0.126	0.108	0.0334	0.522
Heptanal	0.020	0.015	0.0025	0.069
Octanal	0.024	0.017	0.0024	0.058
Nonanal	0.114	0.086	0.014	0.166
Carboxylic acid methylesters				
Methyl butanoate	0.215	0.180	0.0323	0.014
Methyl pentanoate	0.037	0.031	0.0038	0.072
Methyl hexanoate	0.499	0.445	0.0426	0.155
Methyl octanoate	0.144	0.144	0.0330	0.998
Furans				
2-Pentylfuran	0.005	0.002	0.0008	0.006
Hydrocarbons				
Toluene	0.085	0.081	0.0052	0.559
Ketones				
3-Hydroxy-2-butanone	0.041	0.048	0.011	0.467
2,3-Octanedione	0.022	0.018	0.0065	0.423
Phenols				
Phenol	0.008	0.005	0.001	0.057
Pyrazines				
3-Ethyl-2,5-dimethylpyrazine	0.002	0.004	0.002	0.437

[ab]Means with different superscripts within the same row differ ($P < 0.05$).

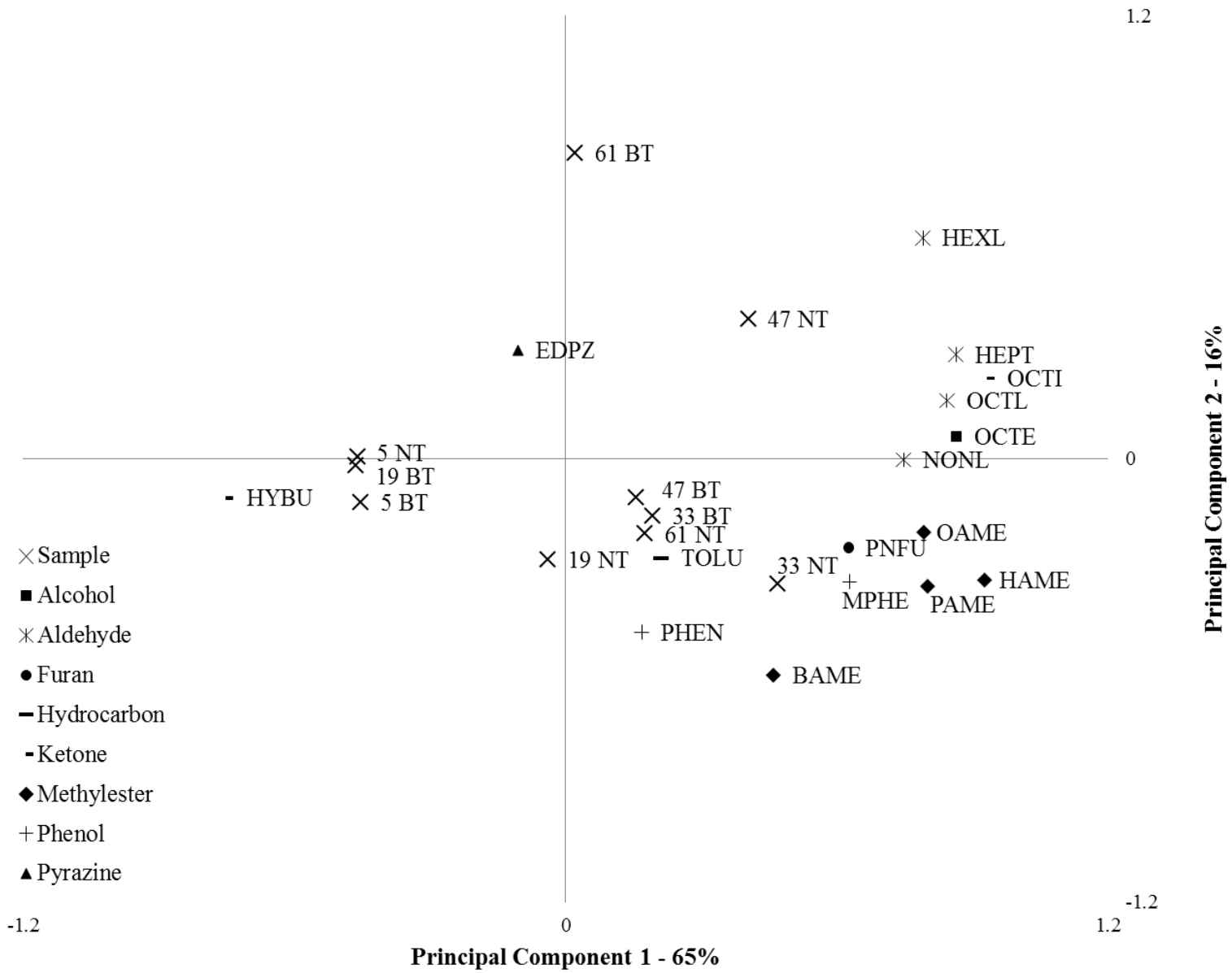
1.2
61 BT
HEXL
47 NT
EDPZ
HEPT
OCTI
OCTL
OCTE
5 NT
19 BT
NONL
0
HYBU
5 BT
47 BT
33 BT
61 NT
OAME
PNFU
19 NT
TOLU
33 NT
HAME
MPHE
PAME
PHEN
BAME
Sample
Alcohol
Aldehyde
Furan
Hydrocarbon
Ketone
Methylester
Phenol
Pyrazine
-1.2
0
1.2
-1.2
Principal Component 1 - 65%
Principal Component 2 - 16%

Figure 6.2 Bi-plots of principal component 1 and principal component 2 for volatile compounds from raw beef *gluteus medius* steaks. Top sirloin butts were aged in anaerobic packaging at 2 °C until the completion of the assigned aging time. *Gluteus medius* pieces yielding blade tenderized steaks were processed twice through a blade tenderizer. Legend – 5: 5 d postmortem aging, 19: 19 d postmortem aging, 33: 33 d postmortem aging, 47: 47 d postmortem aging, 61: 61 d postmortem aging. NT: non-tenderized, BT: blade tenderized. OCTE: 1-octen-3-ol, HEXL: hexanal, HEPT: heptanal, BENZ: benzaldehyde, OCTL: octanal, NONL: nonanal, BAME: methyl butanoate, PAME: methyl pentanoate, HAME: methyl hexanoate, OAME: methyl octanoate, PNFU: 2-pentylfuran, TOLU: toluene, HYBU: 3-hydroxy-2-butanone, OCTI: 2,3-octanedione, PHEN: phenol, MPHE: 4-methylphenol, and EDPZ: 3-ethyl-2,5-dimethylpyrazine.

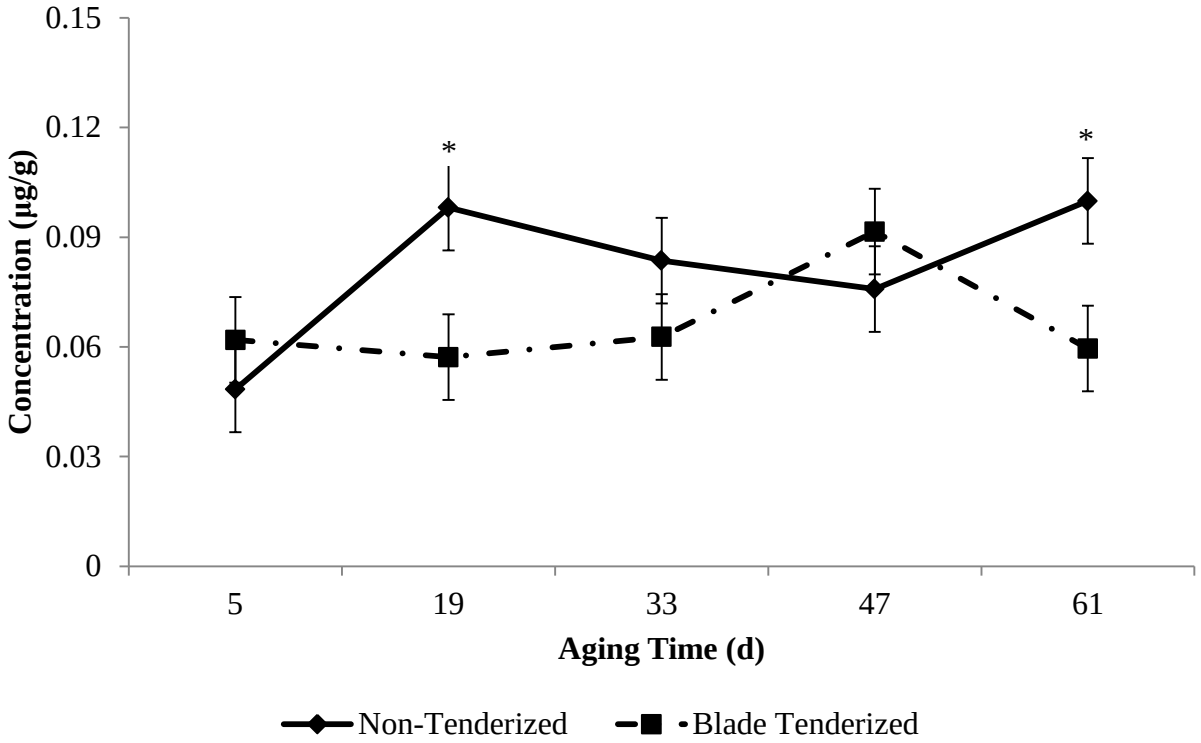

Figure 6.3 Concentrations of 4-methylphenol from *gluteus medius* steaks cooked to 60 °C as affected by aging time and blade tenderization treatment.
Top sirloin butts were aged in anaerobic packaging at 2 °C for 5, 19, 33, 47, or 61 d. *Gluteus medius* pieces yielding blade tenderized steaks were processed twice through a blade tenderizer. Error bars represent the mean ± SEM. *$P < 0.05$. Aging time × tenderization treatment interaction $P = 0.027$.

Table 6.4 Effects of postmortem aging on volatile compound concentrations (μg/g) from cooked *gluteus medius* steaks.

	Aging Time (d)						
	5	19	33	47	61	SEM	*P*-Value
Alcohols							
1-Octen-3-ol	0.011	0.020	0.019	0.025	0.024	0.0060	0.522
Aldehydes							
Hexanal	0.158	0.220	0.156	0.217	0.160	0.0524	0.842
Heptanal	0.044	0.059	0.050	0.051	0.057	0.013	0.902
Benzaldehyde	0.012	0.010	0.015	0.015	0.021	0.0038	0.373
Octanal	0.070	0.107	0.109	0.105	0.140	0.026	0.500
Nonanal	0.124	0.242	0.182	0.179	0.241	0.0635	0.661
Carboxylic acid methylesters							
Methyl butanoate	0.070	0.134	0.184	0.133	0.076	0.040	0.136
Methyl pentanoate	0.009	0.021	0.029	0.020	0.018	0.008	0.421
Methyl hexanoate	0.104[b]	0.209[ab]	0.339[a]	0.217[ab]	0.093[b]	0.065	0.029
Methyl octanoate	0.013	0.040	0.054	0.034	0.011	0.015	0.217
Furans							
2-Pentylfuran	0.001	0.003	0.004	0.004	0.006	0.002	0.505
Hydrocarbons							
Toluene	0.074	0.162	0.109	0.095	0.101	0.033	0.444
Ketones							
3-Hydroxy-2-butanone	0.059	0.073	0.069	0.037	0.076	0.019	0.260
2,3-Octanedione	0.005	0.010	0.009	0.012	0.006	0.004	0.591
Phenols							
Phenol	0.010	0.007	0.011	0.009	0.012	0.002	0.427
Pyrazines							
2,5-Dimethylpyrazine	0.020	0.023	0.048	0.029	0.048	0.0091	0.149
3-Ethyl-2,5-dimethylpyrazine	0.006[b]	0.013[b]	0.024[a]	0.015[b]	0.025[a]	0.004	0.006

[ab]Means with different superscripts within the same row differ ($P < 0.05$).

Table 6.5 Effects of blade tenderization on volatile compound concentrations (μg/g) from cooked *gluteus medius* steaks.

	Non-Tenderized	Blade Tenderized	SEM	*P*-Value
Alcohols				
1-Octen-3-ol	0.024	0.016	0.0040	0.129
Aldehydes				
Hexanal	0.213	0.147	0.0285	0.037
Heptanal	0.065	0.039	0.0073	0.021
Benzaldehyde	0.017	0.012	0.0020	0.038
Octanal	0.136	0.076	0.015	0.007
Nonanal	0.256	0.131	0.0402	0.041
Carboxylic acid methylesters				
Methyl butanoate	0.125	0.115	0.0314	0.661
Methyl pentanoate	0.023	0.016	0.0052	0.161
Methyl hexanoate	0.204	0.180	0.0504	0.622
Methyl octanoate	0.036	0.025	0.010	0.427
Furans				
2-Pentylfuran	0.005	0.002	0.001	0.042
Hydrocarbons				
Toluene	0.130	0.086	0.021	0.155
Ketones				
3-Hydroxy-2-butanone	0.061	0.065	0.016	0.727
2,3-Octanedione	0.010	0.006	0.003	0.100
Phenols				
Phenol	0.010	0.010	0.0012	0.660
Pyrazines				
2,5-Dimethylpyrazine	0.043	0.024	0.0051	0.005
3-Ethyl-2,5-dimethylpyrazine	0.021	0.012	0.0023	0.012

[ab]Means with different superscripts within the same row differ ($P < 0.05$).

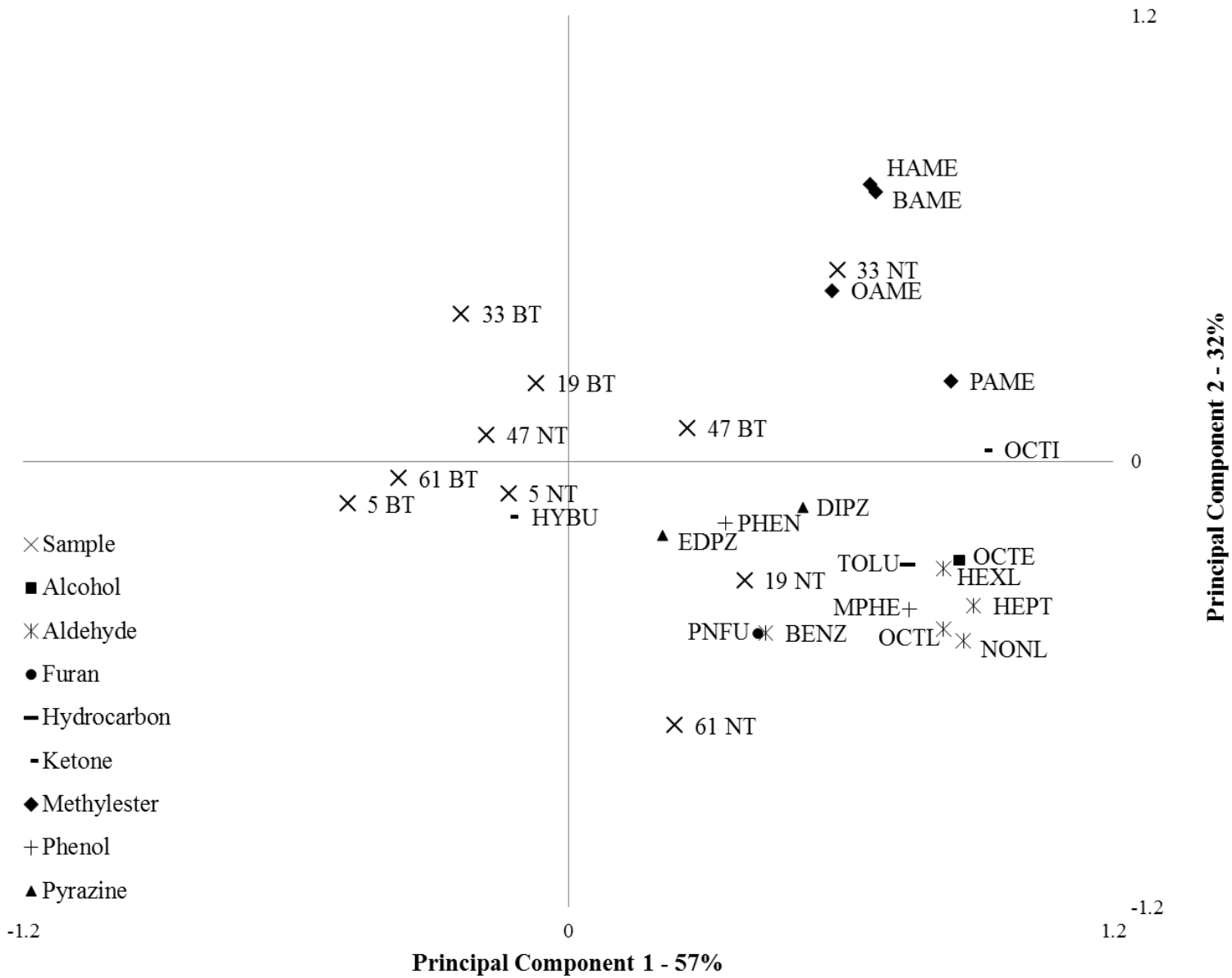
1.2
HAME
BAME
33 NT
OAME
33 BT
19 BT
PAME
47 NT
47 BT
OCTI
0
61 BT
5 BT
5 NT
HYBU
DIPZ
PHEN
EDPZ
TOLU
OCTE
HEXL
19 NT
MPHE
HEPT
PNFU
BENZ
OCTL
NONL
61 NT
Sample
Alcohol
Aldehyde
Furan
Hydrocarbon
Ketone
Methylester
Phenol
Pyrazine
-1.2
0
1.2
-1.2
Principal Component 2 - 32%
Principal Component 1 - 57%

Figure 6.4 Bi-plots of principal component 1 and principal component 2 for volatile compounds from beef *gluteus medius* steaks cooked to an internal temperature of 60 ºC.
Top sirloin butts were aged in anaerobic packaging at 2 °C until the completion of the assigned aging time. *Gluteus medius* pieces yielding blade tenderized steaks were processed twice through a blade tenderizer.
Legend – 5: 5 d postmortem aging, 19: 19 d postmortem aging, 33: 33 d postmortem aging, 47: 47 d postmortem aging, 61: 61 d postmortem aging. NT: non-tenderized, BT: blade tenderized. OCTE: 1-octen-3-ol, HEXL: hexanal, HEPT: heptanal, OCTL: octanal, NONL: nonanal, BAME: methyl butanoate, PAME: methyl pentanoate, HAME: methyl hexanoate, OAME: methyl octanoate, PNFU: 2-pentylfuran, TOLU: toluene, HYBU: 3-hydroxy-2 -butanone, OCTI: 2,3-octanedione, ,PHEN: phenol, MPHE: 4-methylphenol, DIPZ: 2,5-dimethylpyrazine, and EDPZ: 3-ethyl-2,5-dimethylpyrazine.

Chapter 7 - The effects of degree of doneness, postmortem aging, and sampling location on the volatile compound profile of beef *longissimus lumborum* steaks.

Abstract

Cooked steak cross-sections and external and internal steak locations were evaluated to determine effects of degrees of doneness (63, 71, or 77 °C) and aging times (5, 21, or 37 d) on volatile compounds generated by beef *longissimus lumborum* steaks. External locations produced aldehydes in greater amounts ($P < 0.001$) than internal locations, while pyrazines and 4-pyridinamine were produced exclusively at external locations. Increased degrees of doneness increased ($P < 0.05$) aldehydes, except nonanal, from cooked steak cross-sections. At external locations, only trimethylpyrazine was affected ($P = 0.038$) by degree of doneness. Aldehydes, except hexanal, and pyrazines from cross-sections remained similar ($P > 0.05$) as aging times increased. Aldehydes, aside from hexanal and octanal, generated from external locations were similar ($P < 0.05$) to internal locations at 37 d of aging. Pyrazines from external locations were reduced ($P < 0.05$) by increased aging times.

Keywords: aging, aroma, beef, degree of doneness, GC/MS, SPME

1. Introduction

Flavor is complex, but is of great importance when defining the sensorial acceptance of food. Flavor is comprised of the two sensations of taste and smell, although the sensations of astringency, mouthfeel, and juiciness may play a role (Farmer, 1994). Water-soluble compounds produce the basic tastes (sweet, salty, sour, bitter, and umami) that are recognized by the tongue (Moody, 1983). The aromatic portion of flavor is significantly more diverse than taste as the human nose can identify hundreds or even thousands of different odors (Farmer, 1994).

Aroma is a major contributor to the flavor of cooked beef. Beef flavor itself is complex; however, it is further complicated by the wide variety of handling and cooking procedures that are used prior to consumption. Beef aroma is influenced by the presence of hundreds of different volatile compounds (Calkins & Hodgen, 2007). If altered, aromatic volatile compounds may be present in differing quantities and proportions which could alter consumers' perception of some flavors, leading to altered eating experiences.

Raw meat has little aroma and a blood-like taste (Stetzer, Cadwallader, Singh, Mckeith, & Brewer, 2008) but it contains numerous precursors, such as amino acids, peptides, reducing sugars, vitamins, nucleotides and fatty acids, that can generate aromas during cooking (Madruga, Elmore, Oruna-Concha, Balagiannis, & Mottram, 2010). The primary pathways for flavor development during cooking are the Maillard reaction and lipid oxidation. The Maillard reaction is detailed at length by Hodge (1953) and is characterized as non-enzymatic browning that is derived through reactions between amino compounds and reducing sugars in the presence of heat. The Maillard reaction produces aldehydes, sulfur compounds, furans, pyridines, pyrazines, and thiazoles amongst other classes of compounds that have strong aromatic properties (Mottram, 1994). Lipid oxidation that occurs during storage of fresh meat can lead to formation of off-flavors and rancidity (Ladikos & Lougovois, 1990). Thermally initiated lipid oxidation reactions occur during cooking; however, they occur more rapidly than oxidation reactions that occur during storage and form different compounds which produce desirable flavors (Calkins & Hodgen, 2007). The primary products of lipid oxidation are hydroperoxides which do not contribute to aroma, but once these compounds decompose, they form hydrocarbons, alcohols, ketones, and aldehydes which strongly influence aroma (Shahidi, 1989).

Consumers have different preferences in regard to the endpoint cooking temperatures, which are known as degrees of doneness, used to prepare their beef products. The most common degrees of doneness used in the U.S. are rare, medium rare, medium, medium well, and well done (Reicks et al., 2011), which correlate with endpoint cooking temperatures of 60, 63, 71, 74, and 77 °C, respectively (AMSA, 1995). Consumer preference for a specific degree of doneness is not based solely on palatability, as consumers who said they preferred medium or medium well steaks did not necessarily prefer the sensory characteristics of steaks cooked to those specified degrees of doneness (Schmidt et al., 2010). Steaks from an individual subprimal could have different sensory properties based solely on the degree of doneness used to prepare it. Beef steaks cooked to high degrees of doneness are associated with roasted and burnt flavors, while steaks cooked to low degrees of doneness are associated with bloody and fatty flavors in (Lorenzen, Davuluri, Adhikari, & Grün, 2005). These results should be expected as there is a longer time for reactions to occur when steaks are prepared to higher degrees of doneness. It is yet to be seen if there are volatile compounds associated with bloody and fatty flavors that are decreased during cooking or if these flavors are simply masked by the potency of other aromas.

Beef subprimals are commonly stored in anaerobic packaging at refrigerated temperatures for an extended period of time during a process called "wet aging." Practically all beef in the U.S. is packaged anaerobically at the packer level (Smith et al., 2008), so most U.S. beef undergoes wet aging to some extent. During aging, naturally occurring proteolytic degradation of cytoskeletal proteins increases product tenderness (Koohmaraie, 1996). The degradation of muscle structure during aging also increases the pool of available aroma precursors which could alter the quantity of volatiles formed via the Maillard reaction during cooking (Koutsidis et al., 2008).

On average, anaerobically packaged beef is held in refrigerated storage for 20.5 d, but storage times can range from 1 d to as long as 358 d before product reaches the retail counter (Guelker et al., 2013). The wide range of storage times could contribute to a beef supply with highly variable palatability characteristics which could compromise consumer confidence in beef. Wet aging beef for periods longer than 30 d is common when product is exported, but aging for 30 d or longer can contribute to the development of off-flavors in beef. Research has shown that beef aged up to 35 d has an increased perception of sour and rancid flavors when compared to beef aged for shorter time periods (Yancey, Dikeman, Hachmeister, Chambers IV,

& Milliken, 2005). Additionally, these authors reported that off-flavor intensity was higher in beef with longer aging time periods. Moreover, flavor intensity has been shown to decrease while off-flavor intensity increases when aging times of beef strip loins and cuts from the round are extended to 42 d (Juarez et al., 2010).

Both endpoint cooking temperature and aging have the capability to impact beef flavor. Currently, most aroma and flavor volatile compound research has been concentrated on beef products aged for less than 30 d and minimal research has focused on volatile compounds associated with different degrees of doneness. A majority of volatile compounds detected in meat can be related to the Maillard reaction, which occurs on the external surface where meat becomes dehydrated (van den Ouweland, Peer, & Tjan, 1978). Little is known about the specific volatile profiles of the center and external portions of a cooked steak which could provide insight in to the primary reactions responsible for flavor changes due to cooking or aging. Therefore, the aim of this research was three-fold. First, determine how degree of doneness influences volatile compounds formed during cooking of beef *longissimus lumborum* (LL) steaks. Second, observe the impact of multiple aging times on the volatile compound profile of cooked beef LL steaks. Third, determine how volatile compound profiles of beef differ when taking samples from external and internal layers.

2. Materials and methods

2.1 Experimental design

USDA Low Choice beef strip loins (IMPS 180) (n=9) were procured from a commercial beef processing facility and transported to the Kansas State University Meat Laboratory (Manhattan, KS) where they were stored at 2 ºC. Loins were removed from their industrial packaging at 5 d postmortem. Each loin served as a block and the portion of the loin containing the *gluteus medius* was not used so the only muscle assessed was the LL. Each loin was split into thirds which were randomly assigned to postmortem aging treatments of 5, 21, and 37 d for use in two experiments.

2.2 Experiment 1 - Effects of degree of doneness on volatile compound concentrations assessed using cooked steak cross-sections or layered sections

Strip loin sections assigned to 5 d of aging were used in Experiment 1. Each section had the anterior portion removed for proximate analysis and the remaining section was cut into three 2.54 cm thick steaks. The three steaks from each loin were randomly assigned to three degrees of doneness (medium rare: 63 ºC; medium: 71 ºC; and well done: 77 ºC), vacuum packaged (Prime Source Vacuum Pouches; 76.2 µm, STP Barrier, Nylon/PE Vacuum Pouch; oxygen transmission rate 0.04 g/254 cm^2/24 h at 0 ºC; water vapor transmission rate 0.2 cc/254 cm^2/24 h at 0 ºC at 0% relative humidity), and frozen at -40 ºC until volatile analysis was conducted.

2.2.1 Aroma volatile analysis

Steaks were thawed for approximately 24 h at 0-2 ºC prior to sample preparation for volatile analysis. Prior to cooking, each steak was split in half. One half of the steak used to determine the volatile profile using cross-sections and the second half used to analyze volatile profiles at external and internal locations. Steaks were cooked using a George Foreman Grill (Model GRP12; Miramar, FL) which was powered on and allowed to heat for 10 min after which the raw steak was placed on the grill and the top was closed. Steaks were cooked to their assigned internal endpoint temperature which was monitored using a traceable expanded-range thermometer (Fisher Scientific, Pittsburgh, PA) placed in to the approximate geometric center of the steak. Cross-sections were attained by cutting a thin strip across the center of the steak and then removing the center portion of the strip so the sample spanned from cooked surface to cooked surface. External locations were obtained by removing the edges of the steak and then slicing off the outer 6.35 mm of the steak that was in direct contact with the upper cooking surface of the grill to produce a sample representative of the external surface. A subsequent 6.35 mm thick slice from the remaining portion was taken to yield a sample representative of the internal section. Samples from cross-sections, external, and internal locations were then minced using a knife and 0.5 g were weighed from each and placed into their own 10 mL screw cap vial (Supelco, Bellefonte, PA) fitted with a polytetrafluoroethylene/silicone septum (Supelco, Bellefonte, PA).

Each sample had 10 µL of an internal standard (0.1 ppm 1, 3 dichlorobenzene) added prior to equilibration. Samples were analyzed using headspace-solid phase microextraction (HS-

SPME) as described by Koppel, Adhikari, and Di Donfrancessco (2013) using the following modifications. Sample vials were equilibrated at 45 ºC and agitated at 250 rpm for 15 min using an autosampler (Pal system, model CombiPal; CTC Analytics, Zwingen, Switzerland). A 50/30 μm divinylbenzene/carboxen/polydimethyl-siloxane fiber (Supelco, Bellefonte, PA) was then exposed to the sample headspace for 30 min at 45 ºC. The analytes were desorbed from the fiber coating to the injection port of a gas chromatograph (GC) at 270 ºC for 3 min in splitless mode. The SPME fiber was baked at 270 ºC for 20 min between samples to eliminate residual volatiles.

2.2.2 Volatile identification and quantification

Volatile separation, identification, and quantification were conducted on a GC (Varian GC CP3800; Varian Inc., Walnut Creek, CA) combined with a mass spectrometer (MS) detector (Saturn 2000; Varian Inc., Walnut Creek, CA). An RTX-5 MS (Crossbond 5% diphenyl/95% dimethyl polysiloxane) column (Restek U.S., Bellefonte, PA; 30 m × 0.25mm × 0.25 μm film thickness) was used to separate the volatiles. The column was initially heated at 40 ºC for 4 min and temperature increased by 5 ºC per min to 260 ºC where it was held for 7 min.

Volatile compounds were identified using mass spectra as well as linear retention indices (NIST/EPA/NIH Mass Spectral Library, Version, 2.0, 2005). The retention times for a C7-C40 saturated alkane mix (Supelco Analytical, Bellefonte, PA) were used in determining experimental linear retention indices for the volatile compounds that were identified. Volatile compound concentrations were calculated as a proportion of the peak area of the target volatile to the area of the peak of the internal standard.

2.3 Experiment 2 - Effects of postmortem aging on volatile compound concentrations assessed using cooked steak cross-sections or layered sections

Steaks assigned to medium degree of doneness from Experiment 1 and sections aged 21 and 37 d were included in Experiment 2. Sections assigned to 21 d and 37 d of aging were vacuum packaged (Prime Source Vacuum Pouches; 76.2 μm, STP Barrier, Nylon/PE Vacuum Pouch; oxygen transmission rate 0.04 g/254 cm^2/24 h at 0 ºC; water vapor transmission rate 0.2 cc/254 cm^2/24 h at 0 ºC at 0% relative humidity) and held at 2 ºC until completion of their designated aging time. Upon the completion of the aging period, sections had their anterior portion removed for proximate analysis and a 2.54 cm thick steak was removed for volatile

analysis. Steaks were vacuum packaged (Prime Source Vacuum Pouches; 76.2 μm, STP Barrier, Nylon/PE Vacuum Pouch; oxygen transmission rate 0.04 g/254 cm^2/24 h at 0 ºC; water vapor transmission rate 0.2 cc/254 cm^2/24 h at 0 ºC at 0% relative humidity) and frozen at -40 ºC until volatile analysis was conducted. Steaks were prepared, cooked, and analyzed using the same methods described in Experiment 1 except that all steaks were cooked to an internal temperature of 71 ºC.

2.4 Proximate analysis

Raw LL samples were frozen in liquid nitrogen and pulverized in a table-top blender (Waring, Model #51BL32; Torrington, CT) and stored in a sterilized bag (Whirl Pack, Nasco, Modesta, CA) at -80 ºC until analyzed for protein (AOAC, 1994), moisture, and fat (AOAC, 2003).

2.5 Statistical analysis

All data collected in this study was analyzed using the Proc Mixed procedure of SAS (SAS Institute, Inc., Cary, NC) and each experiment was analyzed separately. Data collected from cross-section samples were analyzed as a randomized complete block design where degree of doneness or aging treatment served as a fixed effect and block was included as a random effect. Data collected from external and internal locations were analyzed as a split-plot design with the whole strip loin serving as the whole-plot and degree of doneness or aging being the whole-plot treatment. Each steak served as the split-plot with location functioning as the split-plot treatment. Compounds identified from external locations but not internal locations were assessed in a manner similar to cross-sections. All treatment means were computed with the LSMEANS option. Post-hoc mean separation was carried out using Fisher's least significant difference and all data were analyzed at a 5% level of significance. Due to the numerous volatile compounds assessed, principal component analysis (PCA) of cooked steaks was also conducted to reduce dimensionality of the data set using Unscrambler® (Version 10.2; Camo Software, Oslo, Norway).

3. Results and discussion

3.1 Experiment 1

A summary of all observed significant main and interaction effects that degree of doneness and location had on the presence and concentration of aroma volatile compounds in Experiment 1 are presented in Table 7.1.

3.1.1 Effects of degree of doneness on cooked steak cross-sections

Least squares means of volatile compound concentrations from cross-sections of steaks cooked at different degrees of doneness are presented in Table 7.2. A total of 6 aldehydes, 3 carboxylic acid methylesters (CAM), 2 hydrocarbons, 4 ketones, 3 pyrazines, and 1 pyridinamine were detected from steak cross-sections.

Well done steaks had a greater ($P < 0.05$) amount of pentanal, hexanal, and benzaldehyde when compared to medium rare and medium steaks. In agreement with the present study, prior research has identified hexanal as a compound that is highly related to well done and very well done LL steaks (Lorenzen, et al., 2005). The result of the current study should be expected as pentanal and hexanal are lipid oxidation products and the increased end-point temperature should allow a longer period for thermally induced lipid oxidation reactions to occur. Likewise, the increase in benzaldehyde, a product of the Maillard reaction, could also be attributed to longer cooking times allowing Maillard browning reactions to occur for a longer period of time. Pentanal can contribute almond or malty aromas while hexanal is associated with fatty and green aromas (Calkins & Hodgen, 2007). Benzaldehyde has been attributed to roasted pepper and nutty aroma in goat meat (Madruga et al., 2010) as well as almond oil-like and bitter almond flavor in beef (Calkins & Hodgen, 2007). Heptanal and octanal, also products of lipid oxidation, were produced in greater ($P < 0.05$) concentrations by well done steaks in comparison to medium rare steaks. The aroma of heptanal has been characterized as oily, rancid, and unpleasant (Calkins & Hodgen, 2007; Madruga et al., 2010), while octanal has been associated with soapy, lemon, and green flavors (Calkins & Hodgen, 2007).

All 3 CAM reported in this study showed similar responses due to changes in degree of doneness. Methyl butanoate ($P = 0.047$), methyl pentanoate ($P = 0.006$), and methyl hexanoate ($P = 0.010$) showed a distinct decrease when degree of doneness was increased above medium

rare. Methyl butanoate and methyl hexanoate can contribute pineapple, sweet, and fruity aromas in fruit (Spanier et al., 1998). The aroma of methyl pentanoate has been described as candy-like or resembling aromas indicative of alcohols, ketones, and aldehydes (Spanier et al., 1998). Butanoate and hexanoate have been identified in cooked beef and have aromas described as rancid and sweaty (Stetzer et al., 2008). The aforementioned compounds are products of lipid oxidation (Stetzer et al., 2008) and are most likely being further degraded by thermally initiated lipid oxidation reactions.

Toluene concentration was greater ($P = 0.014$) for well done steaks than both medium rare and medium steaks. Toluene has been associated with thermal degradation aromas that produce roast beef flavor (Min, Ina, Peterson, & Chang, 1977). Moreover, 2-butanone was generated in the greatest ($P < 0.05$) concentrations by well done steaks. Both hydrocarbons and ketones are generated by lipid oxidation (Mottram, 1998) and should be generated in greater amounts from steaks cooked to advanced degrees of doneness.

All identified pyrazines and the lone pyridinamine exhibited no change ($P > 0.05$) in response to changes in degree of doneness. These compounds are all direct products of the Maillard reaction and should be expected to increase as degree of doneness increases. Previous research has shown that pyrazines are highly associated with roasted and burnt flavors found in well done and very well done steaks (Lorenzen et al., 2005) which is contrary to results produced using cross-sections in this study. Steak cross-sections provide a small surface area of the cooked surface. The pyrazines and 4-pyridinamine were quantified in relatively low concentrations from cross-sections as they should form primarily on the cooked surface.

3.1.2 Effects of degree of doneness on layered sections of cooked steaks

External and internal locations generated different volatile profiles. The external samples contained 6 aldehydes, 3 CAM, 1 furan, 2 hydrocarbons, 4 ketones, 1 pyridinamine, and 3 pyrazines. Contrarily, the internal locations presented a volatile profile that contained quantifiable amounts of 6 aldehydes, 3 CAM, 2 hydrocarbons, and 4 ketones.

A degree of doneness × location interaction was observed ($P < 0.05$) for methyl pentanoate and methyl hexanoate (Figure 7.1). External locations produced lesser ($P < 0.05$) concentrations of methyl pentanoate than internal locations when steaks were cooked to medium rare. External locations did not generate methyl pentanoate when steaks were cooked to medium

and well done, while methyl pentanoate from internal locations was found in the greatest ($P < 0.05$) amount from medium rare steaks and the least ($P < 0.05$) from well done steaks. External and internal locations produced similar ($P > 0.05$) concentrations of methyl hexanoate from medium rare steaks, but external locations generated greater ($P < 0.05$) amounts of this compound than internal locations at both medium and well done degrees of doneness. External locations produced similar ($P > 0.05$) amounts of methyl hexanoate across all degrees of doneness, while medium rare steaks generated the greatest ($P < 0.05$) amount of methyl hexanoate at internal locations.

Main effects of degree of doneness on volatile compounds generated by both external and internal locations are detailed in Table 7.3. Benzaldehyde was generated in the least ($P < 0.05$) amount by steaks cooked to medium rare and remained at similar ($P > 0.05$) concentrations between medium and well done steaks while concentrations of other detected aldehydes remained similar ($P > 0.05$) across all degrees of doneness. Benzaldehyde is not present in raw meat while all other aldehydes detected in this study are known to be present in both raw and cooked meat (Chapter 6, p. 127). Benzaldehyde is a known Maillard reaction product so it is plausible that it would display a much greater change than lipid oxidation products due to increases of endpoint cooking temperatures.

Other compounds influenced by degree of doneness were butanoic acid methylester and 2-butanone. Methyl butanoate was generated in greater ($P < 0.05$) concentrations by steaks cooked to medium rare in comparison to steaks cooked to well done. Additionally, methyl butanoate generated from steaks cooked to medium were similar ($P > 0.05$) to both medium rare and well done steaks. The only ketone that displayed a response to different degrees of doneness was 2-butanone, which was generated in greater ($P < 0.05$) concentrations by medium steaks in comparison to medium rare steaks. When the degree of doneness of a beef steak is increased, there are also structural changes that occur such as collagen sollubilization and fibrillar protein changes (Parrish, Olson, Miner, & Rust, 1973). A change of the lipid portion due to thermally initiated lipid oxidation should be expected when there is an increase in degree of doneness. As the time and temperature increases during cooking, increased amounts of fatty acids should be degraded and form lipid oxidation products such as carboxylic acids and ketones.

No matter the degree of doneness, 2-pentylfuran, 2,5-dimethylpyrazine, trimethylpyrazine, 3-ethyl-2,5-dimethylpyrazine, and 4-pyridinamine were only detected at

external locations and their responses to varying degrees of doneness are detailed in Table 7.4. These five compounds can be formed during the Maillard reaction (van Boekel, 2006). Direct products of the Maillard reaction should be expected to be generated exclusively on the cooked surface of beef as that portion of the cut becomes dehydrated and presents a water activity that is more favorable for the Maillard reaction to occur (van den Ouweland et al., 1978). Trimethylpyrazine was the only compound found exclusively from external locations that was affected by degree of doneness as it was generated in greater ($P < 0.05$) concentrations by medium steaks in comparison to medium rare steaks. The lack of change ($P > 0.05$) in other pyrazines and 4-pyridinamine in response to changes in degree of doneness contradicts prior research showing that these types of compounds are more highly associated with flavors found in well done and very well done steaks (Lorenzen et al., 2005). The heat present at the products surface can directly affect the yield of pyrazines. Koehler and Odell (1970) demonstrated that pyrazine formation begins at 100 °C and when temperatures rise above 150 °C pyrazine formation becomes highly variable. Additionally, maximum pyrazine formation occurs at approximately 120 °C (Shibamoto & Bernhard, 1976). The cooking surface should progressively increase in temperature when medium and well-done degrees of doneness are being attained which may produce variable amounts of pyrazines explaining why no differences were observed for most of the pyrazines observed in this study.

Volatile concentrations produced by external and internal locations are detailed in Table 7.5. Every detected aldehyde was generated in greater ($P < 0.001$) concentrations by external locations in comparison to internal portions. Saturated and unsaturated aldehydes are major volatile components of all cooked meats (Mottram, 1998). External portions of beef have previously shown to produce greater amounts of the aldehydes malonaldehyde and hexanal in comparison to internal portions due to their proximity to oxygen (Spanier, Vercellotti, & James, 1992). External locations should produce aldehydes by lipid oxidation at an increased rate due to the proximity to oxygen and the cooking heat source. Additionally, aldehydes can be produced through Strecker degradation during the Maillard reaction which occurs primarily on the cooked meat surface (van Boekel, 2006).

Methyl butanoate was detected in greater ($P = 0.033$) concentrations from external locations in comparison to internal locations. Cross-section samples demonstrated a decrease of methyl butanoate when degree of doneness was increased suggesting it is thermally degraded.

Knowing that methyl butanoate is thermally degraded, it would be expected that it is present in lesser amounts in external samples where the heat source is much greater, which contradicts what was observed in this study. Butanoate has been labeled as a lipid oxidation product (Stetzer et al., 2008) and it is possible that thermal degradation of other carboxylic acids on the cooked surface is yielding methyl butanoate, which would explain its greater presence at external locations.

External locations generated greater ($P < 0.001$) concentrations of toluene than internal locations. Toluene, a hydrocarbon, has been associated with thermal degradation aromas that produce roast beef flavor (Min et al., 1977). Hydrocarbons can be produced by lipid oxidation reactions (Mottram, 1998) and toluene has been shown to increase due to irradiation treatment which is known to increase lipid oxidation (Ismail, Lee, Ko, & Ahn, 2009). All ketones identified in this study were present in greater ($P < 0.001$) concentrations at external locations when compared to internal portions. Ketones are known products of lipid oxidation (Mottram, 1998). The proximity to both oxygen and the source of heat during cooking implies that the external steak surface should generate a greater amount of lipid oxidation products in comparison to the internal portion of a cooked steak.

3.1.3 Principal component analysis (PCA) of experiment 1

The PCA of cross-sections and layered sections of steaks cooked to various endpoint temperatures is presented in Figure 7.2. This PCA described 59% of the total variation for principal component (PC) 1 and 7% of the total variation of PC 2. Aside from 2-pentanone, volatile compounds commonly associated with Maillard and lipid oxidation reactions showed high positive values in regard to PC 1, suggesting they had a great influence on the variation seen within PC 1. External locations cooked to well done and medium were the most highly associated with Maillard and lipid oxidation products which agrees with the findings demonstrated in Table 7.5. Internal and cross-section samples showed little association with lipid and Maillard reaction products in comparison to external samples. Internal locations and cross-sections were similar in regard to PC 1 but were different in regard to PC 2 with cross-sections having much lower values and being more associated with methyl butanoate, while internal locations were more closely associated with methyl pentanoate. External locations of steaks cooked to medium rare were more related to internal locations of medium steaks than external

locations of medium and well done steaks which showed negative values in regard to PC 2 and were aligned more closely with the identified pyrazines and 4-pyridinamine.

3.2 Experiment 2

A summary of all observed significant main and interaction effects that aging and location had on the presence and concentration of aroma volatile compounds in Experiment 2 are presented in Table 7.6.

3.2.1 Effect of postmortem aging on cooked steak cross-sections

Least squares means of volatile compound concentrations from cross-sections of cooked steaks subjected to various postmortem aging times are presented in Table 7.7. A total of 6 aldehydes, 3 CAM, 1 furan, 2 hydrocarbons, 4 ketones, 3 pyrazines, and 1 pyridinamine were detected in cross-section samples.

Hexanal was the lone aldehyde that was changed by aging time as it decreased ($P = 0.05$) when aging time was extended to 37 d. Hexanal is known to be directly proportional to thiobarbituric acid reactive substances (TBARS), which is a measure of lipid oxidation (Shahidi & Pegg, 1994; Ullrich & Grosch, 1987). The concentration of lipid oxidation products observed in the current study contrast previous research demonstrating that lipid oxidation increases when storage time increases (Yancey et al., 2006); but other research has shown that hexanal and TBARS from cooked beef have no correlation with each other (Spanier, Flores, McMillin, & Bidner, 1997). Prior research showed that hexanal demonstrated no change in cooked *gluteus medius* steaks subjected to various aging times (Chapter 6, pp. 127). The lack of hexanal in longer aged steaks could be a result of hexanal being degraded and forming other compounds or it is plausible that hexanal is participating in the Maillard reaction (Mottram, 1998).

The lone furan identified in this study, 2-pentylfuran, was not detected in steaks aged 5 or 21 d, but was detected in steaks aged 37 d. Furans are known to be Maillard reaction products (van Boekel, 2006) and the most likely route of formation of 2-pentylfuran is through the 9-hydroperoxide of linoleic acid (Mottram, 1991). The aroma of 2-pentylfuran has been described as green, earthy, and beany (Stetzer et al., 2008) but it has also been associated with buttery notes (Calkins & Hodgen, 2007).

The only ketone affected by aging was 3-hydroxy-2-butanone, which decreased ($P = 0.046$) as aging time increased. The current study contrasts prior research that demonstrated no

change in 3-hydroxy-2-butanone when top sirloins were exposed to increasing aging times (Chapter 6, p. 127). Additionally, 3-hydroxy-2-butanone has been correlated with livery off-flavor (Stetzer et al., 2008), while other research has described its aroma as pungent and grassy (Mottram 1998; Shahidi, 1989) as well as buttery, fatty, and sweaty (Madruga et al., 2010). 3-Hydroxy-2-butanone is an intermediate product of the Maillard reaction (Mottram, 1997) and other Maillard products, such as the detected pyrazines and 4-pyridinamine, all remained similar ($P > 0.05$) across all aging times. The use of steak cross-sections insists that the Maillard reaction is not increased when aging times are increased.

3.2.2 Effect of postmortem aging on layered sections of cooked steaks

External locations contained 6 aldehydes, 3 CAM, 1 furan, 2 hydrocarbons, 4 ketones, 3 pyrazines, and 1 pyridinamine. Internal locations presented a volatile profile that contained 6 aldehydes, 3 CAM, 1 furan, 2 hydrocarbons, and 4 ketones. Aging time × location interaction means are displayed in Table 7.8.

Pentanal displayed an aging time × location interaction ($P = 0.002$) as external locations from steaks aged 5 and 21 d produced greater amounts ($P < 0.05$) of pentanal than internal locations, but steaks aged 37 d produced similar ($P > 0.05$) concentrations of pentanal at both external and internal locations. Additionally, external locations produced decreased ($P < 0.05$) levels of pentanal as aging time increased while internal locations generated pentanal at similar ($P > 0.05$) concentrations across all aging times. Likewise, heptanal also demonstrated an aging time × location interaction ($P < 0.001$). External locations produced heptanal in greater ($P < 0.05$) concentrations than internal locations at 5 and 21 d of aging, while external and internal locations generated similar ($P > 0.05$) heptanal concentrations at 37 d of aging. Heptanal concentrations from external locations decreased ($P < 0.05$) as aging time was increased. Internal locations produced greater ($P < 0.05$) amounts of heptanal at 5 d of aging in comparison to 37 d, while heptanal concentrations from internal locations at 21 d of aging were similar ($P > 0.05$) to both 5 and 37 d.

An aging time × location interaction ($P = 0.011$) was present for benzaldehyde. Benzaldehyde was generated in greater ($P < 0.05$) concentrations by external locations in comparison to internal locations at 5 and 21 d of aging, but the different sampling locations produced benzaldehyde in similar ($P > 0.05$) concentrations at 37 d of aging. Benzaldehyde from

external locations was greatest ($P < 0.05$) at 5 and 21 d of aging while internal locations produced benzaldehyde in similar ($P > 0.05$) amounts for every aging treatment.

Nonanal, which is associated with fatty and grassy aromas (Calkins & Hodgen, 2007), demonstrated an aging time × location interaction ($P = 0.001$). Nonanal was generated by external locations in greater ($P < 0.05$) concentrations than internal locations at 5 d of aging; however, external and internal locations produced similar ($P > 0.05$) nonanal concentrations at 21 and 37 d of aging. Nonanal concentrations from external locations decreased ($P < 0.05$) as aging time was increased. At internal locations, nonanal concentrations were similar ($P > 0.05$) at 5 and 21 d of aging but decreased ($P < 0.05$) when aging was extended to 37 d.

Methyl hexanoate was the only CAM that exhibited an aging time × location interaction ($P = 0.028$). External locations generated methyl hexanoate in similar ($P > 0.05$) concentrations across all aging treatments and in greater amounts than internal locations at 5 d of aging. Concentrations of methyl hexanoate from internal locations were greater ($P < 0.05$) at d 37 in comparison to 5 d of aging. Additionally, an aging time × location interaction ($P = 0.005$) was observed for 2-pentylfuran. External locations produced greater ($P < 0.05$) amounts of 2-pentylfuran than internal locations at 5 and 21 d of aging, but similar ($P > 0.05$) concentrations were produced by the different locations within the 37 d aging treatment. The concentrations of 2-pentylfuran from external locations decreased ($P < 0.05$) at 37 d of aging while internal locations did not produce 2-pentylfuran in detectable quantities at 5 d of aging and were similar ($P > 0.05$) between 21 and 37 d of aging.

Aging time × location interactions were demonstrated for the ketones 2-butanone ($P = 0.004$) and 2,3-octanedione ($P = 0.010$). External locations generated both 2-butanone and 2,3-octanedione in greater ($P < 0.05$) concentrations than internal locations at 5 and 21 d of aging, but concentrations were similar ($P > 0.05$) between locations at 37 d of aging. 2-Butanone from external locations decreased ($P < 0.05$) as aging time was increased while internal locations produced similar ($P > 0.05$) concentrations of 2-butanone across all aging times. External locations showed a decrease ($P < 0.05$) of 2,3-octanedione at 21 d of age, but internal locations showed no alteration ($P > 0.05$) of 2,3-octanedione concentrations as aging time increased.

Most of the volatile compounds that displayed an aging time × location interaction are associated with lipid oxidation as aldehydes, ketones, and 2-pentylfuran can be produced by lipid oxidation. Aside from methyl hexanoate, external locations generated volatile compounds in

concentrations that were similar to that produced by internal locations as aging time was increased. A majority of lipid oxidation reactions that take place during cooking most likely occur on the product surface where there is an increased amount of heat and close proximity to oxygen.

Main effects of aging time on volatile compounds assessed from external and internal locations are displayed in Table 7.9. Hexanal decreased ($P < 0.001$) as aging time was increased while octanal was greater ($P < 0.05$) at 5 d of aging in comparison to 37 d of aging. Conversely, pentanoic acid methylester increased ($P = 0.048$) as aging time was increased while an aging time of 5 d produced the greatest ($P = 0.003$) amounts of 3-hydroxy-2-butanone.

All pyrazines and one pyridinamine observed in this study were located exclusively from external locations and aging effects on generation of these compounds are outlined in Table 7.10. Aging times of 5 and 21 d generated 2,5-dimethylpyrazine in similar ($P > 0.05$) concentrations, but both aging times produced 2,5-dimethylpyrazine in greater ($P < 0.05$) concentrations than what was produced at 37 d of aging. Trimethylpyrazine was produced in greater ($P < 0.05$) concentrations at 5 d of aging than at 21 and 37 d, while aging treatments of 5 and 21 d produced greater ($P < 0.05$) concentrations of 3-ethyl-2,5-dimethylpyrazine than 37 d of aging. 4-Pyridinamine was produced in greater ($P < 0.05$) concentrations from steaks aged 21 and 37 d in comparison to steaks aged 5 d. The aforementioned heterocyclic compounds are direct products of the Maillard reaction and are often associated with nutty and roasted aromas that are indicative of cooked meat (Mottram, 1998).

The primary variables that affect the extent of the Maillard reaction are the available reactants, pH, water activity, and the combination of temperature and time at which the reaction is allowed to occur (Jaeger, Janositz, & Knorr, 2010). In beef, the pool of free sugars and amino acids available to undergo the Maillard reaction increases as aging time increases, which suggests that Maillard browning should be increased in longer aged beef (Koutsidis et al., 2008). Aside from 4-pyridinamine, the current study saw a decrease of Maillard products as aging time was increased which disagrees with the theory of Koutsidis et al. (2008). The Maillard reaction is also highly influenced by the pH of a food system as melanoidin production via the Maillard reaction increases as pH increases (Martins & Van Boekel, 2005). The pH of beef has little variation once a beef carcass reaches an ultimate pH (Polak, Andrensek, Zlender, & Gasperlin,

2009). It is rather unlikely that the availability of Maillard precursors and pH are the root cause of the decrease in Maillard products observed in this study.

Maillard browning products are increased as water activity and moisture levels of the food source are reduced (Jaeger et al., 2010) which explains why Maillard products are primarily produced on the external surface where meat becomes dehydrated during cooking (van den Ouweland et al., 1978). Water-holding capacity of meat decreases over time due to the denaturation of myofibrillar proteins which increases the amount of immobilized and free water present in a cut of meat (Huff-Lonergan & Lonergan, 2005). Meat that has a reduced water-holding capacity appears exudative or moist on the surface as is seen with pale, soft, and exudative pork. Increased amounts of exudate on a meat product surface are most-likely attributed to the migration of free water to the product surface. Knowing that the Maillard reaction requires a reduced water activity to occur, an increase of free water at the external meat surface could potentially interrupt the degree of Maillard browning that occurs during the cooking of beef.

Steaks used to assess aging effects in this study were all cooked to an internal temperature of 71 °C, which is defined as "medium" degree of doneness (AMSA, 1995). The time needed to achieve a specified endpoint temperature could be a variable contributing to the differences of various volatile compounds observed in this study. The structure and composition of muscle tissue serves as an insulatory barrier that restricts heat from entering the center of a steak (Hallstrom, et al., 1988). As product is aged, the cytoskeletal proteins that comprise muscle structure are degraded (Koohmaraie, 1996). The disruption of muscle structure could possibly allow heat to transfer more freely through a steak during cooking, which could ultimately decrease the amount of time needed to cook a steak to a specified degree of doneness. Jeremiah and Gibson (2003) observed a 5 min/kg reduction in cooking time for beef aged 1 wk in contrast to beef that was not aged. If beef with longer aging periods is less resistant to heat, it could require a shorter period of time to cook and ultimately reduce the available time for the Maillard browning and lipid oxidation reactions to occur. Shortening the time available for both the Maillard and lipid oxidation reactions may explain why the current study exhibited numerous compounds that were reduced as aging time increased.

Numerous studies have reported that increased aging times of beef subprimals generated decreased beef flavor intensity and increased the presence of off-flavors (Juarez et al., 2010;

Yancey et al., 2006). The current study demonstrated a decrease in numerous compounds at external locations as aging time was increased. Decreases of flavor intensity may be explained by the decreased concentrations of volatiles generated during cooking, especially on the product surface. Conversely, there were no compounds that increased which could be associated with off-flavors seen in previous research. Bitter and sour are common off-flavors that are present when aging time is increased (Spanier et al., 1997; Yancey et al., 2005). Both bitter and sour are basic tastes, so it is plausible that aroma volatiles have a minimal contribution to their presence.

Mean volatile compound concentrations from external and internal locations are presented in Table 7.11. The lipid oxidation products hexanal and octanal were both generated in greater ($P < 0.001$) concentrations from external samples in contrast to internal samples. Pentanoic acid methylester was the only compound that was produced in the greatest ($P = 0.011$) concentrations by internal samples. Additionally, toluene was generated in the greatest ($P < 0.001$) amounts from external samples. Most compounds detailed in this study have their production accelerated by heat or are directly generated by heating so it is expected that the external portion of cooked steaks should produce greater amounts of the detected volatiles presented in this study. Pentanoic acid methylester displayed a reduction as degree of doneness increased in Experiment 1 suggesting that it is thermally degraded, which provides some explanation to why it would be generated in greater ($P < 0.011$) concentrations at the internal locations in comparison to external locations.

3.2.3 Principal component analysis of experiment 2

Figure 7.3 outlines PCA conducted on volatile compound production from multiple locations of cooked steaks subjected to 5, 21, or 37 d of postmortem aging. This PCA describes 55% and 8% of the total variation associated with PC 1 and PC 2, respectively. The majority of Maillard and lipid oxidation products showed high positive values in regard to PC 1. External locations from steaks aged 5 d demonstrated the greatest association with the majority of compounds identified in this study. As aging time increased, external locations became less associated with lipid oxidation and Maillard reaction compounds and became more similar to internal locations, which agrees with the findings detailed in Table 7.8. The cross-sections were separated along PC 2 with samples from 21 d of aging being the most highly associated with

nonanal while steaks from 5 and 37 d of aging were relatively similar to that of internal locations.

3.7 Proximate analysis

Means from proximate analyses are displayed in Table 7.12. Protein percentage did not change as aging times were lengthened ($P = 0.805$). Fat percentage increased from 5 d to 21 d of aging ($P < 0.05$) while moisture percentage decreased ($P < 0.05$) from 5 d to 21 d of aging. The implementation of aging has previously been associated with increased moisture loss in the form of purge (Geesink, Bekhit, & Bikerstaffe, 2000; Moore & Young, 1991). Raw beef has a very high water activity and reductions of moisture should promote the development of Maillard products. Moisture lost as purge in beef is very proteinaceous in nature (Savage, Warriss, & Jolley, 1990) and may contribute to the suppressed formation of Maillard volatiles observed in longer aged beef from this study.

4. Conclusions

The external surfaces of cooked steaks generate a greater amount of lipid oxidation and Maillard reaction products than internal portions. Additionally, pyrazines and pyridinamines are produced exclusively by the external surface of cooked steaks. All aldehydes, except nonanal, from steak cross-sections demonstrated increases when degree of doneness was increased, but these differences were not observed at external and internal locations. The effects of postmortem aging times, as long as 37 d, on steak cross-sections were minimal; however, multiple thermally produced compounds were reduced at external locations as aging times were increased. The reduction of browning and lipid oxidation reactions at the cooked meat surface, induced by extended postmortem aging, could result in decreased flavor intensities of long-aged beef products. Retailers and processors that market long-aged beef may need to consider further-processing techniques, such as product marination and injection, to alleviate potential flavor changes due to the lack of browning and oxidation reactions occurring at the cooked product surface.

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Table 7.1 Effects of degree of doneness on volatile compound concentrations from cross-sections and layered sections of cooked *longissimus lumborum* steaks.

	Cross-Section[1]	Layered Sections[2]			External Section[3]
	D[4]	D	L	D × L	D
Aldehydes					
Pentanal	**	NS	***	NS	-
Hexanal	*	NS	***	NS	-
Heptanal	**	NS	***	NS	-
Benzaldehyde	***	*	***	NS	-
Octanal	**	NS	***	NS	-
Nonanal	NS	NS	***	NS	-
Carboxylic acid methylesters					
Methyl butanoate	*	**	*	NS	-
Methyl pentanoate	*	**	***	*	-
Methyl hexanoate	*	*	**	*	-
Furans					
2-Pentylfuran	ND	-	-	-	NS
Hydrocarbons					
Toluene	*	NS	***	NS	-
3-Dodecene	NS	NS	NS	NS	-
Ketones					
2-Butanone	*	*	***	NS	-
2-Pentanone	NS	NS	***	NS	-
3-Hydroxy-2-butanone	NS	NS	**	NS	-
2,3-Octanedione	NS	NS	***	NS	-
Pyrazines					
2,5-Dimethylpyrazine	NS	-	-	-	NS
Trimethylpyrazine	NS	-	-	-	*
3-Ethyl-2,5-dimethylpyrazine	NS	-	-	-	NS
Pyridinamines					
4-Pyridinamine	NS	-	-	-	NS

*Treatment means differ ($P < 0.05$).
** Treatment means differ ($P < 0.005$).
***Treatment means differ ($P < 0.0001$).
NS: Treatment means are similar ($P > 0.05$).
ND: Compound not detected in any treatment.
[1]Cross-section: Samples prepared from a cross-section (contact surface to contact surface) in the middle of the steak.
[2]Layered sections: Compounds assessed at two locations (external and internal). External: samples prepared from outer 6.35 mm of steak; Internal: samples prepared from portion 6.35 – 12.7 mm from outside surface of steak.
[3]External section: Compounds not detected from internal locations so statistical analysis conducted on external locations only.
[4]D = effect of degree of doneness; L = effect of sample location; D × L = interaction between degree of doneness and sample location.

Table 7.2 Effects of degree of doneness on volatile compound concentrations (μg/g) from cross-sections[1] of cooked *longissimus lumborum* steaks.

	Degree of Doneness[2]				
	MR	MD	WD	SEM	*P*-Value
Aldehydes					
Pentanal	0.019[b]	0.019[b]	0.030[a]	0.0022	0.002
Hexanal	0.059[b]	0.074[b]	0.180[a]	0.032	0.016
Heptanal	0.012[b]	0.016[ab]	0.021[a]	0.0024	0.004
Benzaldehyde	0.006[b]	0.007[b]	0.011[a]	0.0008	<0.001
Octanal	0.021[b]	0.029[ab]	0.038[a]	0.0035	0.003
Nonanal	0.076	0.093	0.072	0.018	0.299
Carboxylic acid methylesters					
Methyl butanoate	0.064[a]	0.031[b]	0.034[b]	0.011	0.047
Methyl pentanoate	0.008[a]	0.002[b]	0.001[b]	0.001	0.006
Methyl hexanoate	0.054[a]	0.021[b]	0.013[b]	0.0089	0.010
Hydrocarbons					
Toluene	0.037[b]	0.037[b]	0.046[a]	0.0057	0.014
3-Dodecene	0.014	0.014	0.017	0.0012	0.074
Ketones					
2-Butanone	0.045[b]	0.045[b]	0.064[a]	0.0078	0.021
2-Pentanone	0.020	0.019	0.047	0.016	0.380
3-Hydroxy-2-butanone	0.028	0.025	0.028	0.0063	0.662
2,3-Octanedione	0.002	0.001	0.005	0.002	0.221
Pyrazines					
2,5-Dimethylpyrazine	0.013	0.015	0.074	0.030	0.287
Trimethylpyrazine	0.030	0.033	0.047	0.0060	0.113
3-Ethyl-2,5-dimethylpyrazine	0.004	0.005	0.008	0.003	0.220
Pyridinamines					
4-Pyridinamine	0.001	0.001	0.002	0.0008	0.538

[1]Cross-section: Samples prepared from a cross-section (contact surface to contact surface) in the middle of the steak.
[2]Steaks cooked to different internal temperatures as follows: Medium rare (MR): 63 ºC, medium (MD): 71 ºC, well done (WD): 77 ºC.
[ab]Means with different superscripts within the same row differ ($P < 0.05$).

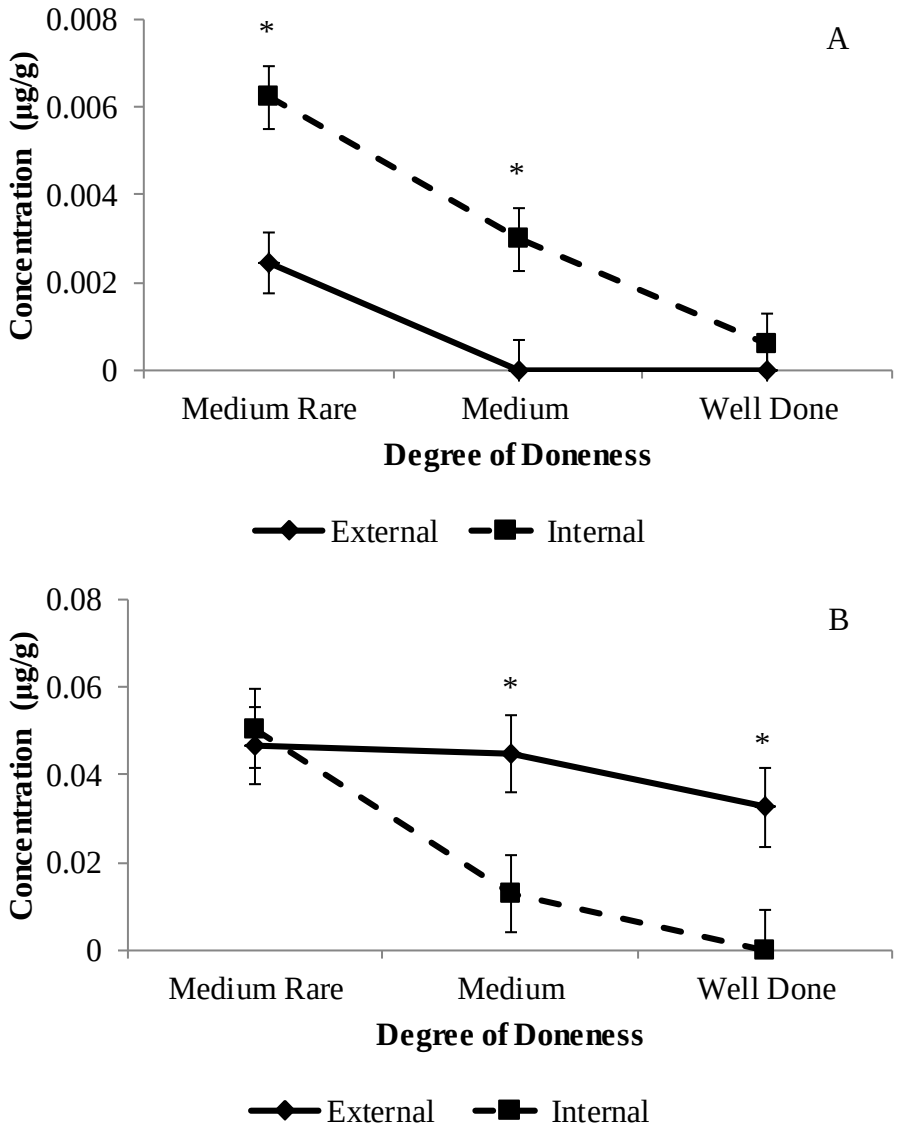

Figure 7.1 Volatile compound concentrations from external and internal locations of *longissimus lumborum* steaks cooked to different degrees of doneness.
Steaks cooked to different internal temperatures as follows: Medium rare: 63 ºC, medium : 71 ºC, well done: 77 ºC. External samples prepared from outer 6.35 mm of steak. Internal samples prepared from portion 6.35 – 12.7 mm from outside surface of steak. * $P < 0.05$. A) Concentrations of methyl pentanoate. Degree of doneness × location interaction $P = 0.047$. B) Concentrations of methyl hexanoate. Degree of doneness × location interaction $P = 0.023$.

Table 7.3 Effects of degree of doneness on volatile compound concentrations (μg/g) from external[1] and internal[2] locations of cooked *longissimus lumborum* steaks.

	Degree of Doneness[3]				
	MR	MD	WD	SEM	*P*-Value
Aldehydes					
Pentanal	0.031	0.045	0.038	0.0059	0.183
Hexanal	0.438	0.658	0.442	0.117	0.256
Heptanal	0.040	0.059	0.047	0.0086	0.121
Benzaldehyde	0.010[b]	0.020[a]	0.019[a]	0.0029	0.009
Octanal	0.052	0.095	0.090	0.020	0.079
Nonanal	0.100	0.124	0.108	0.0172	0.226
Carboxylic acid methylesters					
Methyl butanoate	0.041[a]	0.034[ab]	0.026[b]	0.0038	0.003
Hydrocarbons					
Toluene	0.045	0.055	0.049	0.0068	0.234
3-Dodecene	0.015	0.018	0.016	0.0016	0.207
Ketones					
2-Butanone	0.051[b]	0.071[a]	0.066[ab]	0.0098	0.034
2-Pentanone	0.018	0.024	0.022	0.0020	0.084
3-Hydroxy-2-butanone	0.028	0.032	0.031	0.0076	0.815
2,3-Octanedione	0.032	0.048	0.027	0.011	0.310

[1]External: samples prepared from outer 6.35 mm of steak.
[2]Internal: samples prepared from portion 6.35 – 12.7 mm from outside surface of steak.
[3]Steaks cooked to different internal temperatures as follows: Medium rare (MR): 63 ºC, medium (MD): 71 ºC, well done (WD): 77 ºC.
[ab]Means with different superscripts within the same row differ ($P < 0.05$).

Table 7.4 Effects of degree of doneness on concentrations (μg/g) of volatile compounds detected from external[1] locations but not from internal[2] locations of *longissimus lumborum* steaks.

	Degree of Doneness[3]				
	MR	MD	WD	SEM	*P*-Value
Furans					
2-Pentylfuran	0.010	0.014	0.013	0.0028	0.228
Pyrazines					
2,5-Dimethylpyrazine	0.045	0.139	0.093	0.026	0.053
Trimethylpyrazine	0.098[b]	0.227[a]	0.175[ab]	0.039	0.038
3-Ethyl-2,5-dimethylpyrazine	0.023	0.045	0.040	0.0077	0.096
Pyridinamines					
4-Pyridinamine	0.009	0.032	0.021	0.006	0.051

[1]External: samples prepared from outer 6.35 mm of steak.
[2]Internal: samples prepared from portion 6.35 – 12.7 mm from outside surface of steak.
[3]Steaks cooked to different internal temperatures as follows: Medium rare (MR): 63 ºC, medium (MD): 71 ºC, well done (WD): 77 ºC.
[ab]Means with different superscripts within the same row differ ($P < 0.05$).

Table 7.5 Effects of location on volatile compound concentrations (μg/g) from external[1] and internal[2] locations of *longissimus lumborum* steaks cooked to varying degrees of doneness[3].

	External	Internal	SEM	*P*-Value
Aldehydes				
Pentanal	0.055	0.021	0.0051	<0.001
Hexanal	0.801	0.224	0.0991	<0.001
Heptanal	0.075	0.022	0.0077	<0.001
Benzaldehyde	0.028	0.005	0.003	<0.001
Octanal	0.133	0.025	0.018	<0.001
Nonanal	0.144	0.077	0.016	<0.001
Carboxylic acid methylesters				
Methyl butanoate	0.037	0.030	0.0034	0.033
Hydrocarbons				
Toluene	0.064	0.036	0.0063	<0.001
3-Dodecene	0.017	0.016	0.0014	0.432
Ketones				
2-Butanone	0.083	0.043	0.0093	<0.001
2-Pentanone	0.026	0.017	0.0016	<0.001
3-Hydroxy-2-butanone	0.040	0.020	0.0072	<0.001
2,3-Octanedione	0.064	0.007	0.009	<0.001

[1]External: samples prepared from outer 6.35 mm of steak.
[2]Internal: samples prepared from portion 6.35 – 12.7 mm from outside surface of steak.
[3]Steaks cooked to different internal temperatures as follows: Medium rare: 63 ºC, medium: 71 ºC, well done: 77 ºC.
[ab]Means with different superscripts within the same row differ ($P < 0.05$).

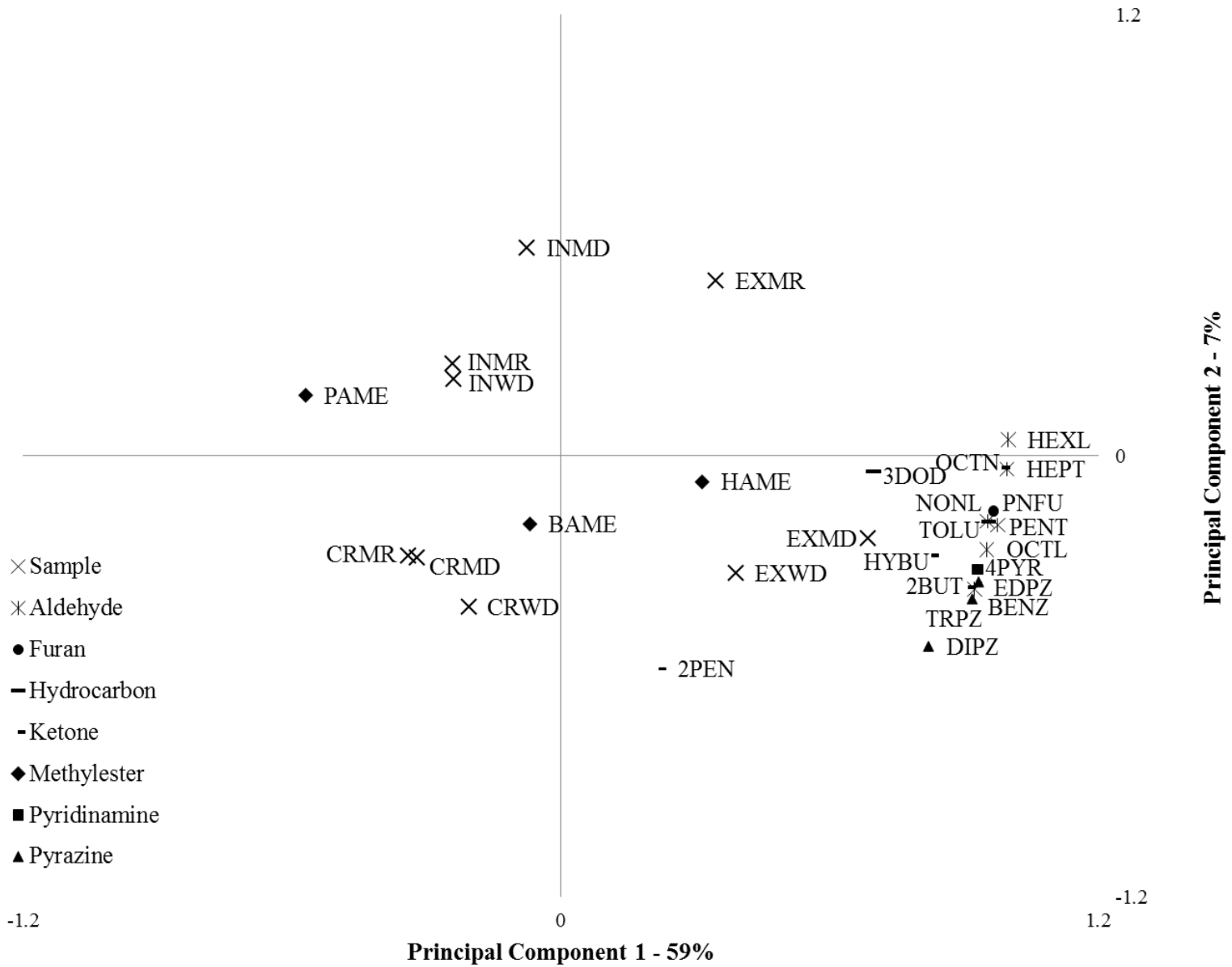
1.2
INMD
EXMR
INMR
INWD
PAME
HEXL
OCTN
HEPT
0
3DOD
HAME
NONL
PNFU
BAME
TOLU
PENT
EXMD
OCTL
CRMR
HYBU
CRMD
4PYR
EXWD
2BUT
EDPZ
CRWD
BENZ
TRPZ
DIPZ
2PEN
Sample
Aldehyde
Furan
Hydrocarbon
Ketone
Methylester
Pyridinamine
Pyrazine
-1.2
0
1.2
-1.2
Principal Component 2 - 7%
Principal Component 1 - 59%

Figure 7.2 Bi-plots of principal component 1 and principal component 2 for the volatile compounds from different locations of beef *longissimus lumborum* steaks cooked to different internal endpoint temperatures.
Legend – CR: samples prepared form a cross-section (contact surface to contact surface) in the middle of the steak. EX: samples prepared from outer 6.35 mm of steak. IN: samples prepared from portion 6.35 – 12.7 mm from outside surface of steak. MR: steaks cooked to an internal endpoint temperature of 63 ºC, MD: steaks cooked to an internal endpoint temperature of 71 ºC, WD: steaks cooked to an internal endpoint temperature of 77 ºC. PENT: pentanal, HEXL: hexanal, HEPT: heptanal, BENZ: benzaldehyde, OCTL: octanal, NONL: nonanal, BAME: methyl butanoate, PAME: methyl pentanoate, HAME: methyl hexanoate, PNFU: 2-pentylfuran, 3DOD: 3-dodecene, TOLU: toluene, 2BUT: 2-butanone, 2PEN: 2-pentanone, HYBU: 3-hydroxy-2-butanone, OCTN: 2,3 octanedione, DIPZ: 2,5-dimethylpyrazine, TRPZ: trimethylpyrazine, EDPZ: 3-ethyl-2,5-dimethylpyrazine, and 4PYR: 4-pyridinamine.

Table 7.6 Effects of 5, 21, and 37 d of postmortem aging on volatile compound concentrations from cross-sections and layered sections of cooked *longissimus lumborum* steaks.

	Cross-Section[1]	Layered Sections[2]			External Sections[3]
	A[4]	A	L	A × L	A
Aldehydes					
Pentanal	NS	***	***	**	-
Hexanal	*	***	***	NS	-
Heptanal	NS	***	***	**	-
Benzaldehyde	NS	***	***	*	-
Octanal	NS	**	***	NS	-
Nonanal	NS	***	***	**	-
Carboxylic acid methylesters					
Methyl butanoate	NS	NS	NS	NS	-
Methyl pentanoate	NS	*	*	NS	-
Methyl hexanoate	NS	NS	NS	*	-
Furans					
2-Pentylfuran	**	*	***	**	-
Hydrocarbons					
Toluene	NS	***	***	NS	-
3-Dodecene	NS	***	NS	NS	-
Ketones					
2-Butanone	NS	***	***	**	-
2-Pentanone	NS	NS	NS	NS	-
3-Hydroxy-2-butanone	*	**	NS	NS	-
2,3-Octanedione	NS	NS	***	*	-
Pyrazines					
2,5-Dimethylpyrazine	NS	-	-	-	*
Trimethylpyrazine	NS	-	-	-	**
3-Ethyl-2,5-dimethylpyrazine	NS	-	-	-	**
Pyridinamines					
4-Pyridinamine	NS	-	-	-	*

*Treatment means differ ($P < 0.05$).
** Treatment means differ ($P < 0.005$).
***Treatment means differ ($P < 0.0001$).
NS: Treatment means do not differ ($P > 0.05$)
[1]Cross-section: Samples prepared form a cross-section (contact surface to contact surface) in the middle of the steak.
[2]Layered sections: Compounds assessed at two locations (external and internal). External: samples prepared from outer 6.35 mm of steak; Internal: samples prepared from portion 6.35 – 12.7 mm from outside surface of steak.
[3]External sections: Compounds not detected from internal locations so statistical analysis conducted on external locations.
[4]A = effect of aging treatment; L = effect of sample location; A × L = interaction between aging treatment and sample location.

Table 7.7 Effects of postmortem aging time on volatile concentrations (μg/g) from cross-sections[1] of *longissimus lumborum* steaks cooked to 71 ºC.

	Aging Time[2] (d)				
	5	21	37	SEM	*P*-Value
Aldehydes					
Pentanal	0.019	0.037	0.011	0.011	0.244
Hexanal	0.074[a]	0.070[a]	0.034[b]	0.013	0.050
Heptanal	0.016	0.027	0.004	0.008	0.130
Benzaldehyde	0.007	0.011	0.006	0.002	0.192
Octanal	0.029	0.057	0.020	0.016	0.237
Nonanal	0.093	0.251	0.009	0.09	0.214
Carboxylic acid methylesters					
Methyl butanoate	0.031	0.039	0.020	0.0098	0.404
Methyl pentanoate	0.002	0.004	0.003	0.002	0.571
Methyl hexanoate	0.021	0.033	0.023	0.0084	0.568
Furans					
2-Pentylfuran	0.000[b]	0.000[b]	0.001[a]	0.0003	0.003
Hydrocarbons					
Toluene	0.037	0.021	0.027	0.0054	0.112
3-Dodecene	0.014	0.017	0.003	0.005	0.105
Ketones					
2-Butanone	0.045	0.049	0.023	0.0083	0.074
2-Pentanone	0.019	0.023	0.010	0.0056	0.223
3-Hydroxy-2-butanone	0.025[a]	0.016[ab]	0.010[b]	0.0044	0.046
2,3-Octanedione	0.001	0.002	0.002	0.0009	0.658
Pyrazines					
2,5-Dimethylpyrazine	0.015	0.027	0.018	0.0047	0.174
Trimethylpyrazine	0.033	0.056	0.020	0.012	0.133
3-Ethyl-2,5-dimethylpyrazine	0.005	0.020	0.015	0.006	0.249
Pyridinamines					
4-Pyridinamine	0.001	0.003	0.004	0.001	0.091

[1]Cross-section: Samples prepared form a cross-section (contact surface to contact surface) in the middle of the steak.
[2]Loin sections aged in anaerobic packaging at 2 °C until the designated aging time.
[abc]Means with different superscripts within the same row differ ($P < 0.05$).

Table 7.8 Volatile concentrations (μg/g) from external[1] and internal[2] locations of *longissimus lumborum* steaks subjected to various postmortem aging treatments cooked to 71 °C.

	Aging Time[3] (d)									
	5		21		37			*P*-Values[4]		
	External	Internal	External	Internal	External	Internal	SEM	A	L	A × L
Aldehydes										
Pentanal	0.065^{a}	0.025^{bc}	0.035^{b}	0.015^{c}	0.017^{c}	0.016^{c}	0.0059	0.001	<0.001	0.002
Heptanal	0.089^{a}	0.029^{bc}	0.042^{b}	0.017^{cd}	0.017^{cd}	0.006^{d}	0.006	<0.001	<0.001	<0.001
Benzaldehyde	0.034^{a}	0.005^{c}	0.028^{a}	0.003^{c}	0.013^{bc}	0.001^{c}	0.003	<0.001	0.001	0.011
Nonanal	0.167^{a}	0.080^{b}	0.107^{b}	0.082^{b}	0.022^{c}	0.016^{c}	0.013	<0.001	<0.001	0.001
Carboxylic acid methylesters										
Methyl hexanoate	0.045^{a}	0.013^{b}	0.032^{ab}	0.013^{b}	0.031^{ab}	0.047^{a}	0.0091	0.172	0.116	0.028
Furans										
2-Pentylfuran	0.014^{a}	0.000^{d}	0.012^{a}	0.002^{c}	0.005^{c}	0.001^{c}	0.002	0.030	<0.001	0.005
Ketones										
2-Butanone	0.095^{a}	0.048^{bc}	0.052^{b}	0.030^{c}	0.030^{c}	0.027^{c}	0.0082	< 0.001	< 0.001	0.004
2,3-Octanedione	0.086^{a}	0.010^{bc}	0.038^{b}	0.007^{c}	0.017^{bc}	0.005^{c}	0.01	0.006	<0.001	0.010

[1]External samples prepared from outer 6.35 mm of steak.
[2]Internal samples prepared from portion 6.35 – 12.7 mm from outside surface of steak.
[3]Loin sections aged in anaerobic packaging at 2 °C until the designated aging time.
[4]A = effect of aging treatment; L = effect of sample location; A × L = interaction between aging treatment and sample location.
[abc]Means with different superscripts within the same row differ ($P < 0.05$).

Table 7.9 Effects of postmortem aging on volatile concentrations (μg/g) from external[1] and internal[2] locations of *longissimus lumborum* steaks cooked to 71 ºC.

	Aging Time[3] (d)				
	5	21	37	SEM	*P*-Value
Aldehydes					
Hexanal	0.658[a]	0.377[b]	0.135[c]	0.0862	<0.001
Octanal	0.095[a]	0.061[ab]	0.026[b]	0.013	0.003
Carboxylic acid methylesters					
Methyl butanoate	0.034	0.022	0.030	0.0049	0.230
Methyl pentanoate	0.001[c]	0.002[b]	0.005[a]	0.001	0.048
Hydrocarbons					
Toluene	0.055[a]	0.023[b]	0.034[b]	0.0049	<0.001
3-Dodecene	0.018[a]	0.007[b]	0.005[b]	0.002	<0.001
Ketones					
2-Pentanone	0.024	0.014	0.015	0.0033	0.074
3-Hydroxy-2-butanone	0.032[a]	0.016[b]	0.013[b]	0.0050	0.003

[1]External: samples prepared from outer 6.35 mm of steak.
[2]Internal: samples prepared from portion 6.35 – 12.7 mm from outside surface of steak.
[3]Loin sections aged in anaerobic packaging at 2 °C until the designated aging time.
[abc]Means with different superscripts within the same row differ ($P < 0.05$).

Table 7.10 Effects of postmortem aging on volatile concentrations (μg/g) of compounds detected from external[1] locations but not from internal[2] locations of *longissimus lumborum* steaks cooked to 71 ºC.

	Aging Time[3] (d)				
	5	21	37	SEM	*P*-Value
Pyrazines					
2,5-Dimethylpyrazine	0.139[a]	0.109[a]	0.044[b]	0.026	0.021
Trimethylpyrazine	0.227[a]	0.136[b]	0.051[b]	0.033	0.002
3-Ethyl-2,5-dimethylpyrazine	0.045[a]	0.040[a]	0.015[b]	0.0084	0.005
Pyridinamines					
4-Pyridinamine	0.003[b]	0.021[a]	0.018[a]	0.008	0.038

[1]External: samples prepared from outer 6.35 mm of steak.
[2]Internal: samples prepared from portion 6.35 – 12.7 mm from outside surface of steak.
[3]Loin sections aged in anaerobic packaging at 2 °C until the designated aging time.
[ab]Means with different superscripts within the same row differ ($P < 0.05$).

Table 7.11 Effects of location on volatile concentrations (μg/g) from external[1] and internal[2] locations of *longissimus lumborum* steaks subjected to different postmortem aging treatments[3] and cooked to 71 ºC.

	External	Internal	SEM	*P*-Value
Aldehydes				
Hexanal	0.604	0.177	0.0727	<0.001
Octanal	0.103	0.019	0.011	<0.001
Carboxylic acid methylesters				
Methyl butanoate	0.028	0.030	0.0040	0.746
Methyl pentanoate	0.001	0.004	0.0008	0.011
Hydrocarbons				
Toluene	0.047	0.028	0.0035	<0.001
3-Dodecene	0.010	0.009	0.001	0.582
Ketones				
2-Pentanone	0.018	0.017	0.0027	0.678
3-Hydroxy-2-butanone	0.023	0.017	0.0045	0.060

[1]External: samples prepared from outer 6.35 mm of steak.
[2]Internal: samples prepared from portion 6.35 – 12.7 mm from outside surface of steak.
[3]Loin sections aged in anaerobic packaging at 2 °C for 5, 21, or 37 d.
[ab]Means with different superscripts within the same row differ ($P < 0.05$).

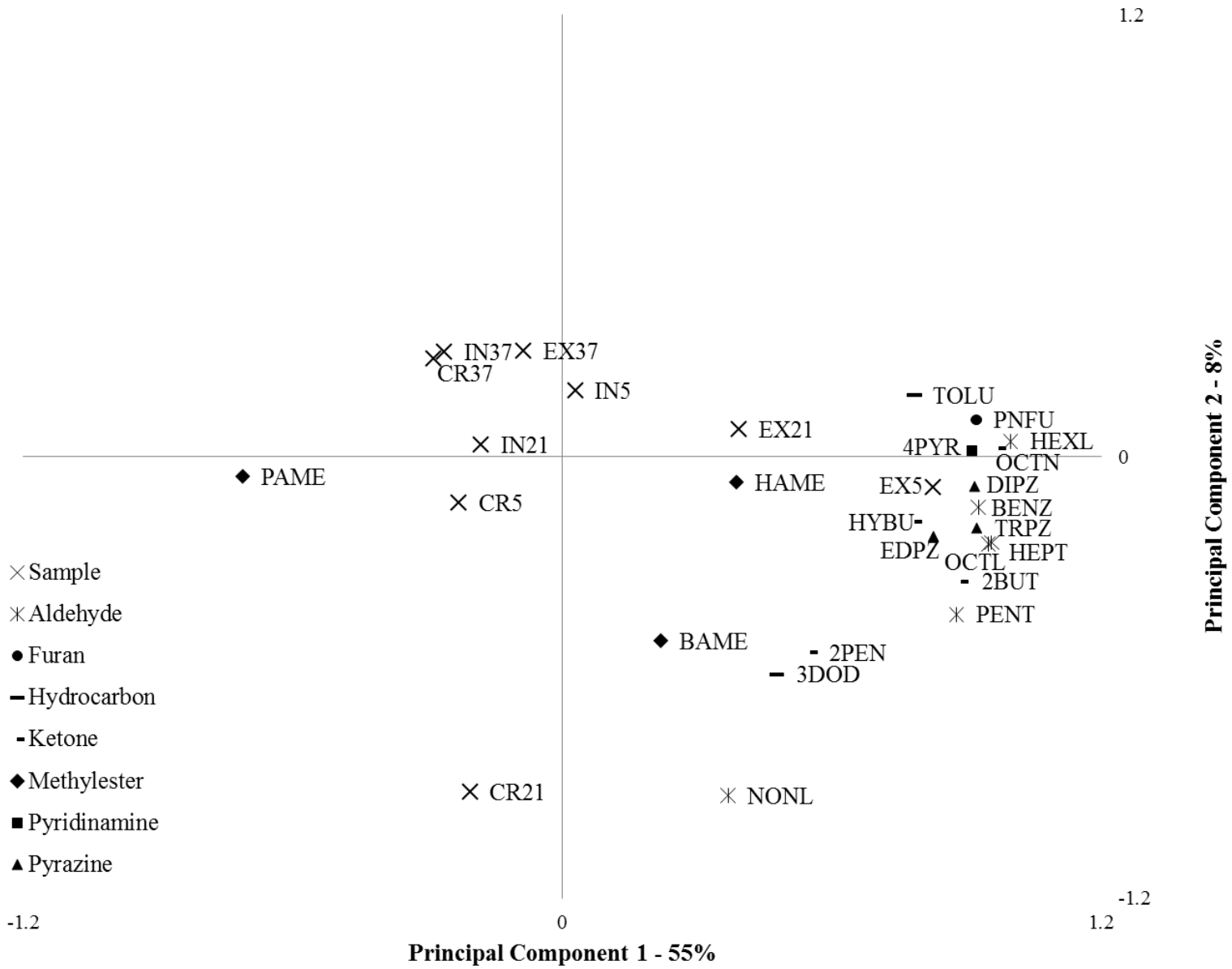
1.2
0
-1.2
-1.2
0
1.2
Principal Component 2 - 8%
Principal Component 1 - 55%
IN37
EX37
CR37
IN5
TOLU
PNFU
EX21
IN21
4PYR
HEXL
OCTN
PAME
HAME
EX5
DIPZ
CR5
BENZ
HYBU
TRPZ
EDPZ
HEPT
OCTL
2BUT
PENT
BAME
2PEN
3DOD
CR21
NONL
Sample
Aldehyde
Furan
Hydrocarbon
Ketone
Methylester
Pyridinamine
Pyrazine

Figure 7.3 Bi-plots of principal component 1 and principal component 2 for the volatile compounds from different locations of beef *longissimus lumborum* steaks subjected to different aging times and cooked to an internal endpoint temperature of 71 ºC. Strip loins were aged in anaerobic packaging at 2 °C until their designated aging time was achieved. Legend – CR: samples prepared form a cross-section (contact surface to contact surface) in the middle of the steak. EX: samples prepared from outer 6.35 mm of steak. IN: samples prepared from portion 6.35 – 12.7 mm from outside surface of steak. 5: 5 d of postmortem aging, 21: 21 d of postmortem aging, 35: 35 d of postmortem aging. PENT: pentanal, HEXL: hexanal, HEPT: heptanal, BENZ: benzaldehyde, OCTL: octanal, NONL: nonanal, BAME: methyl butanoate, PAME: methyl pentanoate, HAME: methyl hexanoate, PNFU: 2-pentylfuran, 3DOD: 3-dodecene, TOLU: toluene, 2BUT: 2-butanone, 2PEN: 2-pentanone, HYBU: 3-hydroxy-2-butanone, OCTN: 2,3 octanedione, DIPZ: 2,5-dimethylpyrazine, TRPZ: trimethylpyrazine, EDPZ: 3-ethyl-2,5-dimethylpyrazine, and 4PYR: 4-pyridinamine.

Table 7.12 Proximate analysis of *longissimus lumborum* steaks subjected to different postmortem aging treatments.

	Aging Time[1] (d)				
	5	21	37	SEM	*P*-Value
Protein, %	16.4	16.3	15.8	0.651	0.805
Fat, %	3.49[b]	4.72[a]	4.48[a]	0.194	<0.001
Moisture, %	72.1[a]	70.7[b]	70.5[b]	0.206	<0.001

[1]Loin sections aged in anaerobic packaging at 2 °C until the designated aging time.
[ab]Means with different superscripts within the same row differ ($P < 0.05$).

Chapter 8 - General conclusions and implications: Beef aroma volatiles

Aroma is a crucial contributor to the flavor and overall acceptance of beef. These studies investigated volatile aroma compounds that have been attributed to specific aromas and some that have received minimal attention in prior research. Aging, blade tenderization, and degree of doneness are variables that can affect the palatability of beef, but are not employed uniformly across the beef industry. The objectives of this research were to evaluate the effects of aging, blade tenderization, and degree of doneness on the volatile compound profile of beef and to investigate aroma volatile formation at different locations within a steak.

Postmortem aging and blade tenderization are common industry practices used to improve beef tenderness. Muscle structure degradation during aging and muscle structure disruption incurred during blade tenderization can improve tenderness, but both processes produce free radicals capable of initiating lipid oxidation. Aging increased lipid oxidation of raw *gluteus medius* (GM) steaks as increased amounts of aldehydes associated with lipid oxidation were produced when aging times were increased. The disruption of muscle structure associated with blade tenderization did not increase aldehydes associated with lipid oxidation in raw beef. Blade tenderization was applied at the completion of aging, so it is likely that free radicals produced by blade tenderization were unable to induce lipid oxidation to the extent achieved by free radicals produced during aging.

Raw beef has a relatively bland flavor but it contains volatile precursors that generate numerous compounds during cooking through lipid oxidation and the Maillard reaction. By evaluating external and internal samples of cooked LL steaks, it is readily apparent that compounds produced during cooking are primarily generated on the external steak surface where oxygen and the heat source have direct contact with the product. External locations of cooked LL steaks displayed greater amounts of lipid oxidation and Maillard browning products in comparison to internal locations. The meat surface becomes dehydrated during heating, which is beneficial for the production of Maillard browning products, and the proximity to heat and oxygen should catalyze lipid oxidation to progress at an increased rate.

Aging of fresh beef has been shown to increase the pool of aroma precursors available to undergo various reactions during cooking. The evaluation of cross-sections of both cooked GM

and LL steaks demonstrated that aging had an influence on a limited number of compounds. Conversely, the concentration of multiple lipid oxidation and Maillard reaction products from the external surface of cooked LL steaks became more similar to that of the internal steak portions as aging times increased. The structure of meat creates an insulatory barrier that impedes heat transfer, but as aging increases, the muscle structure is degraded which may allow less restriction of heat to the interior of a steak and could supply an increase of free water in the product. Decreased inhibition of heat transfer through a steak during cooking could mean that cooking times are reduced, which reduces the time that Maillard and lipid oxidation reactions are allowed to occur. Additionally, the increased availability of free water could interact with the product surface and create a water activity that reduces the amount of Maillard browning that can occur. Similar effects of heat transfer and free water may be present in tenderized GM steaks as lipid oxidation and Maillard products were drastically reduced in tenderized steaks.

Consumers have different preferences in regard to how their beef is prepared. My research evaluated how cooking LL steaks to medium rare, medium, and well done degrees of doneness influences the volatile compounds generated during cooking. When evaluating steak cross-sections, aldehydes are increased and carboxylic acid methylesters are decreased as degree of doneness is increased. Interestingly, trimethylpyrazine is the only compound found primarily at external locations that increased when degree of doneness was increased. Increased endpoint cooking temperatures allow the Maillard and lipid oxidation reactions to occur for a longer period of time than low endpoint cooking temperatures, which suggests that numerous compounds should increase in concentration when degree of doneness is increased. The lack of change at external locations due to degree of doneness implies that most compounds produced at external locations are generated early during the cooking process.

Volatile compounds can be affected by many processes used in the beef industry. Currently, cross-section samples are the most common sampling method used to evaluate volatile compounds produced by cooked beef. This research shows that evaluating external and internal steak portions may provide a better understanding of how volatile compounds respond to various processes. The current research provides the first evaluation of aroma volatile compounds produced by beef that is aged longer than 21 d and blade tenderized. The cumulative results from these studies can provide some explanation to observed flavor changes cited in prior research but cannot identify specific compounds contributing to off-flavors associated with

extended aging times. Additionally, this research may lead to future work that focuses on flavor formation at different locations within a steak in response to different cooking, packaging, and processing methods.

Appendix A - Supplementary data

Table 8.1 Mean volatile compound concentrations (μg/g) from raw *gluteus medius* steaks subjected to aging and tenderization[1] treatments.

	Aging Time (d)										
	5		19		33		47		61		
	NT	BT	NT	BT	NT	BT	NT	BT	NT	BT	SEM
Alcohols											
1-Octen-3-ol	0.016	0.014	0.027	0.018	0.059	0.056	0.049	0.054	0.072	0.053	0.020
Aldehydes											
Hexanal	0.051	0.039	0.084	0.049	0.158	0.124	0.231	0.120	0.103	0.208	0.051
Heptanal	0.009	0.006	0.017	0.009	0.024	0.016	0.024	0.017	0.026	0.025	0.005
Octanal	0.010	0.006	0.019	0.009	0.028	0.020	0.026	0.022	0.036	0.028	0.005
Nonanal	0.046	0.044	0.102	0.050	0.123	0.094	0.109	0.124	0.189	0.121	0.030
Carboxylic acid methylesters											
Methyl butanoate	0.183	0.174	0.227	0.196	0.234	0.180	0.206	0.186	0.224	0.162	0.0546
Methyl pentanoate	0.029	0.025	0.036	0.031	0.044	0.032	0.041	0.039	0.036	0.027	0.0084
Methyl hexanoate	0.380	0.401	0.484	0.383	0.605	0.528	0.535	0.500	0.489	0.414	0.0953
Methyl octanoate	0.119	0.111	0.115	0.097	0.178	0.195	0.159	0.191	0.149	0.126	0.0529
Furans											
2-Pentylfuran	0.002	0.001	0.004	0.000	0.009	0.004	0.002	0.002	0.009	0.004	0.002
Hydrocarbons											
Toluene	0.084	0.071	0.082	0.093	0.093	0.077	0.085	0.087	0.080	0.076	0.012
Ketones											
3-Hydroxy-2-butanone	0.066	0.079	0.033	0.063	0.054	0.028	0.021	0.028	0.031	0.044	0.023
2,3-Octanedione	0.008	0.007	0.014	0.009	0.033	0.023	0.029	0.020	0.027	0.028	0.01
Phenols											
Phenol	0.007	0.005	0.007	0.009	0.009	0.004	0.009	0.006	0.008	0.002	0.003
4-Methylphenol	0.059^{b}	0.069^{b}	0.101^{a}	0.057^{b}	0.081^{ab}	0.076^{ab}	0.101^{a}	0.085^{ab}	0.109^{a}	0.058^{b}	0.011
Pyrazines											
3-Ethyl-2,5-dimethylpyrazine	0.005	0.004	0.000	0.004	0.003	0.009	0.004	0.000	0.000	0.005	0.004

[1]NT: non-tenderized; BT: blade tenderized.

[ab]Means with different superscripts within the same row differ ($P < 0.05$).

Table 8.2 Mean volatile compound concentrations (μg/g) from cooked[1] *gluteus medius* steaks subjected to aging and tenderization[2] treatments on

	Aging Time (d)										
	5		19		33		47		61		
	NT	BT	NT	BT	NT	BT	NT	BT	NT	BT	SEM
Alcohols											
1-Octen-3-ol	0.016	0.007	0.024	0.016	0.028	0.010	0.019	0.031	0.033	0.015	0.008
Aldehydes											
Hexanal	0.204	0.112	0.273	0.147	0.214	0.097	0.163	0.271	0.211	0.109	0.062
Heptanal	0.058	0.029	0.074	0.045	0.074	0.026	0.037	0.065	0.081	0.033	0.016
Benzaldehyde	0.010	0.013	0.014	0.007	0.020	0.010	0.016	0.015	0.026	0.016	0.004
Octanal	0.085	0.055	0.140	0.075	0.161	0.056	0.086	0.125	0.210	0.069	0.033
Nonanal	0.166	0.081	0.330	0.155	0.282	0.082	0.125	0.233	0.375	0.106	0.090
Carboxylic acid methylesters											
Methyl butanoate	0.089	0.052	0.127	0.142	0.223	0.145	0.112	0.153	0.071	0.080	0.047
Methyl pentanoate	0.012	0.007	0.019	0.023	0.037	0.021	0.016	0.024	0.029	0.006	0.009
Methyl hexanoate	0.138	0.070	0.193	0.225	0.441	0.237	0.169	0.264	0.080	0.106	0.085
Methyl octanoate	0.021	0.005	0.039	0.041	0.079	0.029	0.026	0.043	0.013	0.009	0.021
Furans											
2-Pentylfuran	0.000	0.002	0.006	0.000	0.007	0.000	0.003	0.005	0.009	0.002	0.002
Hydrocarbons											
Toluene	0.080	0.069	0.226	0.098	0.136	0.081	0.086	0.105	0.123	0.079	0.047
Ketones											
3-Hydroxy-2-butanone	0.046	0.072	0.059	0.087	0.067	0.071	0.047	0.028	0.086	0.067	0.023
2,3-Octanedione	0.007	0.003	0.013	0.006	0.015	0.003	0.007	0.016	0.009	0.003	0.005
Phenols											
Phenol	0.010	0.011	0.007	0.008	0.014	0.008	0.007	0.011	0.014	0.010	0.003
4-Methylphenol	0.048^{c}	0.062^{bc}	0.098^{a}	0.057^{bc}	0.084^{ab}	0.063^{bc}	0.076^{abc}	0.092^{ab}	0.100^{a}	0.060^{bc}	0.012
Pyrazines											
2,5 Dimethylpyrazine	0.018	0.023	0.025	0.020	0.065	0.030	0.038	0.020	0.067	0.028	0.011
3-Ethyl-2,5-dimethylpyrazine	0.003	0.009	0.016	0.011	0.029	0.019	0.025	0.005	0.033	0.018	0.005

[1]Steaks cooked to an internal temperature of 60 °C.

[2]NT: non-tenderized; BT: blade tenderized.

[abc]Means with different superscripts within the same row differ ($P < 0.05$).

Table 8.3 Mean volatile concentrations (μg/g) from external[1] and internal[2] locations of *longissimus lumborum* steaks cooked to various degrees of doneness.

	Degree of Doneness[3]						
	Medium Rare		Medium		Well Done		
	External	Internal	External	Internal	External	Internal	SEM
Aldehydes							
Pentanal	0.046	0.016	0.065	0.025	0.054	0.022	0.0078
Hexanal	0.706	0.170	0.984	0.332	0.714	0.169	0.153
Heptanal	0.064	0.015	0.089	0.029	0.071	0.023	0.011
Benzaldehyde	0.017	0.003	0.034	0.005	0.032	0.006	0.004
Octanal	0.089	0.015	0.159	0.032	0.152	0.027	0.024
Nonanal	0.126	0.074	0.167	0.080	0.139	0.077	0.020
Carboxylic acid methylesters							
Methyl butanoate	0.043	0.040	0.039	0.029	0.030	0.022	0.0046
Methyl pentanoate	0.002[b]	0.006[a]	0.000[c]	0.003[b]	0.000[c]	0.001[c]	0.0007
Methyl hexanoate	0.047[a]	0.051[a]	0.045[a]	0.013[bc]	0.033[ab]	0.000[c]	0.0089
Furans							
2-Pentylfuran	0.010	0.000	0.014	0.000	0.013	0.000	0.002
Hydrocarbons							
Toluene	0.060	0.030	0.069	0.041	0.062	0.037	0.0077
3-Dodecene	0.015	0.015	0.020	0.017	0.016	0.015	0.0021
Ketones							
2-Butanone	0.068	0.035	0.095	0.048	0.086	0.047	0.011
2-Pentanone	0.022	0.013	0.029	0.018	0.027	0.018	0.0026
3-Hydroxy-2-butanone	0.039	0.017	0.038	0.025	0.042	0.019	0.0088
2,3-Octanedione	0.056	0.008	0.086	0.010	0.049	0.004	0.02
Pyrazines							
2,5-Dimethylpyrazine	0.045	0.000	0.139	0.000	0.093	0.000	0.02
Trimethylpyrazine	0.098	0.000	0.227	0.000	0.175	0.000	0.03
3-Ethyl-2,5-dimethylpyrazine	0.023	0.000	0.045	0.000	0.040	0.000	0.005
Pyridinamines							
4-Pyridinamine	0.009	0.000	0.032	0.000	0.021	0.000	0.005

[1]External: samples prepared from outer 6.35 mm of steak.
[2]Internal: samples prepared from portion 6.35 – 12.7 mm from outside surface of steak.
[3]Steaks cooked to different internal temperatures as follows: Medium rare: 63 ºC, medium: 71 ºC, well done: 77 ºC.
[abc]Means with different superscripts within the same row differ ($P < 0.05$).

Table 8.4 Mean volatile concentrations (μg/g) from external[1] and internal[2] locations of *longissimus lumborum* steaks subjected to different postmortem aging times.

	Aging Time (d)						
	5		21		37		
	External	Internal	External	Internal	External	Internal	SEM
Aldehydes							
Pentanal	0.065[a]	0.025[bc]	0.035[b]	0.015c	0.017[c]	0.016[c]	0.0059
Hexanal	0.984	0.332	0.620	0.134	0.207	0.064	0.11
Heptanal	0.089[a]	0.029[bc]	0.042[b]	0.017[cd]	0.017[cd]	0.006[d]	0.006
Benzaldehyde	0.034[a]	0.005[c]	0.028[a]	0.003[c]	0.013[bc]	0.001[c]	0.003
Octanal	0.159	0.032	0.102	0.020	0.047	0.005	0.02
Nonanal	0.167[a]	0.080[b]	0.107[b]	0.082[b]	0.022[c]	0.016[c]	0.013
Carboxylic acid methylesters							
Methyl butanoate	0.039	0.029	0.024	0.021	0.021	0.040	0.0070
Methyl pentanoate	0.000	0.003	0.001	0.003	0.002	0.007	0.001
Methyl hexanoate	0.045[a]	0.013[b]	0.032[ab]	0.013[b]	0.031[ab]	0.047[a]	0.0091
Furans							
2-Pentylfuran	0.014[a]	0.000[d]	0.012[a]	0.002[c]	0.005[c]	0.001[c]	0.002
Hydrocarbons							
Toluene	0.069	0.041	0.034	0.012	0.038	0.031	0.0060
3-Dodecene	0.020	0.017	0.008	0.005	0.003	0.006	0.002
Ketones							
2-Butanone	0.095[a]	0.048[bc]	0.052[b]	0.030[c]	0.030[c]	0.027[c]	0.0082
2-Pentanone	0.029	0.018	0.016	0.011	0.009	0.020	0.005
3-Hydroxy-2-butanone	0.038	0.025	0.020	0.012	0.012	0.014	0.0057
2,3-Octanedione	0.086[a]	0.010[bc]	0.038[b]	0.007[c]	0.017[bc]	0.005[c]	0.01
Pyrazines							
2,5-Dimethylpyrazine	0.139	0.000	0.109	0.000	0.044	0.000	0.02
Trimethylpyrazine	0.227	0.000	0.136	0.000	0.051	0.000	0.02
3-Ethyl-2,5-dimethylpyrazine	0.045	0.000	0.040	0.000	0.015	0.000	0.005
Pyridinamines							
4-Pyridinamine	0.032	0.000	0.029	0.000	0.011	0.000	0.005

[1]External: samples prepared from outer 6.35 mm of steak.
[2]Internal: samples prepared from portion 6.35 – 12.7 mm from outside surface of steak.
[abcd]Means with different superscripts within the same row differ ($P < 0.05$).