Relationship Between Heifer Carcass Maturity and Beef Quality Characteristics

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Authorization to Submit Thesis

This thesis of Kacie C. Salove, submitted for the degree of Master of Science with a Major in Animal Science and titled “Relationship Between Heifer Carcass Maturity and Beef Quality Characteristics,” has been reviewed in final form. Permission, as indicated by the signatures and dates given below, is now granted to submit final copies to the College of Graduate Studies for approval.

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Abstract

Our objective was to determine the relationship between maturity and beef quality characteristics of the *longissimus lumborum* (LM) and *biceps femoris* (BF) of heifer carcasses under 30 months of age verified by dentition. Few differences in quality characteristics occurred among A, B, and C maturity carcasses. In LM and BF steaks, B maturity steaks had less of lipid oxidation by the fourth day of retail display ($P < 0.01$). LM steaks from B maturity carcasses tended to have worse overall acceptability ($P = 0.08$) and juiciness ($P = 0.09$) than C maturity steaks. C maturity LM steaks had higher levels of 3-hydroxy-2-butanone, pentanal, and toluene ($P < 0.02$). However, no differences in sensory analysis occurred between A maturity and advanced maturity BF or LM steaks. In conclusion, our results indicate that advanced physiological maturity does not decrease palatability of carcasses from cattle under 30 months of age.

Key words: beef, carcass, heifer, maturity, palatability

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Dedication

To Sam for learning and growing with me along this journey.

To my family for always supporting and believing in me.

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List of Abbreviations

AMSA	American Meat Science Association
AMS	Agricultural Marketing Service
BF	*Biceps femoris*
BSE	Bovine spongiform encephalopathy
CIE	Commision International de I'Eclairage
CJD	Creutzfeldt-Jakob Disease
FSIS	Food Safety Inspection Service
HCW	Hot carcass weight (lbs.)
IMPS	Institutional Meat Purchase Specifications
KPH	Percent kidney, pelvic, and heart fat
LM	*Longissimus lumborum*
LSM	Least Square Mean
MDA	Malondialdehyde
NAMP	North American Meat Processors
NBQA	National Beef Quality Audit
NCBA	National Cattlemen's Beef Association
REA	Ribeye area ($in.^2$)
SRM	Specified risk material
TBARS	Thiobarbituric acid reactive substances
USDA	United States Department of Agriculture
WBSF	Warner-Bratzler Shear Force

CHAPTER 1

Review of Literature

The US Grading Standards for Beef

In 1917 tentative standards for grades of dressed beef began being utilized nationally (USDA, 2016). These standards were created to allow for uniformity when marketing beef throughout the United States. After suggestions on modifications were made by producers, processors, retailers, and consumers, the Standards for Grades of Carcass Beef were published in 1926. These standards allowed for voluntary beef grading to occur, beginning May of 1927.

Voluntary beef grades are utilized to predict the amount of boneless, closely trimmed retail cuts from the four primal cuts (chuck, rib, loin, and round) of a beef carcass, as well as palatability of the product. These grades are referred to as Yield Grades (YG) and Quality Grades (QG), respectively. Carcasses can undergo yield grading or quality grading individually, but more commonly the combination of the grades are applied to each carcass. Employees of the United States Department of Agriculture (USDA) facilitate the voluntary grading, which is provided at no cost to the producer.

Beef yield grades (1-5) are determined based on the following measurements taken from each carcass: adjusted 12^{th} rib fat thickness (in.), percent kidney, pelvic, and heart fat (KPH), hot carcass weight (HCW, lbs.), and ribeye area (REA, $in.^2$). Rib fat thickness is measured perpendicular to the subcutaneous fat surface between the 12^{th} and 13^{th} rib interface at approximately three-fourths of the lateral length of the ribeye from the backbone. Adjustments to this measurement can be made based on fat thickness in other locations, such as the brisket, round, loin, and cod or udder. Twelfth rib fat thickness measurements are

adjusted up if proportionately more fat is found in these areas and adjusted down if the carcass is relatively trimmer in these areas. Percent KPH is determined by the amount of fat found near the kidney, heart, and in the pelvic cavity; these are then expressed as a percentage of the hot carcass weight. The ribeye area is measured at the 12^{th} and 13^{th} rib interface where the carcass is ribbed; this area can be estimated subjectively or measured with a grid or camera instrument. The following equation is used to determine official yield grades:

Yield Grade = 2.50 + (2.50 x adj. fat thickness) + (0.20 x percent KPH) + (0.0038 x HCW) – (0.32 x REA)

Numerically lower yield grades (1 and 2) represent carcasses with a thin layer of subcutaneous fat over the rib, loin, and round, and small amounts of KPH fat. These carcasses are not only trim but are also expressive in their muscling. Yield Grade 3 carcasses are usually entirely covered with fat so that lean is difficult to see underneath and are moderate in muscle. Higher numerical yield grades (4 and 5) represent carcasses with a thick layer of fat, higher percentages of KPH, and less than adequate muscle expression. Pricing of carcasses is affected by premiums and discounts given for yield grades. Generally, Yield Grade 1 carcasses will receive a premium, while Yield Grade 4 and 5 carcasses are discounted.

Quality grades of carcasses are determined based on a relationship between marbling and maturity (Figure 1.1). Marbling scores are measured by the amount of intramuscular fat located at the 12^{th} and 13^{th} rib interface of the ribeye. Seven degrees of marbling are utilized in the beef standards to determine quality grade, in descending order they are: slightly abundant, moderate, modest, small, slight, traces, and practically devoid. The 8 quality

grades available for beef carcasses, in descending order are: Prime, Choice, Select, Standard, Commercial, Utility, Cutter, and Canner

Historically, the corresponding age ranges for maturity groups (A-E) have been assumed to generally follow: A – 9-30 months, B – 30-42 months, C – 42-72 months, D – 72-96 months, and E – older than 96 months (Tatum et al., 2007). One of three methods are currently used to determine maturity of beef carcasses: dentition, documentation, or physiological maturity. Determination of age by dentition is conducted by FSIS upon harvest. Cattle that have two permanent incisors or less are considered to be under 30 months of age, while the eruption of a second set of permanent incisors determines that the carcass is 30 months of age or older (USDA-FSIS). Age verification documents from any USDA-approved program can also be utilized to confirm that carcasses under 30 months of age. Lastly, visible indicators of physiological maturity such as rib shape, rib size, ossification of thoracic cartilaginous buttons and vertebral column, lean color, and texture of lean at the 12-13th rib interface can be evaluated by USDA graders to determine maturity.

Carcasses under 30 months of age fall into the youngest maturity category (A maturity). These carcasses are eligible for the Prime, Choice, Select, or Standard quality grades, depending upon marbling scores; A maturity carcasses are the only maturity group that can achieve the Select quality grade. Carcasses over 30 months of age based on dentition will qualify for B-E maturity. B maturity carcasses are also eligible for Prime, Choice, and Standard quality grades, while C maturity and older carcasses, often referred to as hardbone carcasses, can only quality grade Commercial, Utility, Cutter, or Canner. Similar to yield grades, premiums and discounts are given based upon quality grades. Prime carcasses are the highest quality grade and receive the largest premiums. Carcasses that meet the requirements

for certified programs such as Agribeef's Double R Ranch Signature, which only utilizes carcasses in the upper one-third of Choice, also receive premiums. Select and lesser quality grades are typically discounted, with hardbone carcasses receiving substantial discounts.

Since the publication of the standards, 13 revisions have been made based on the release of scientific research or changes to the beef industry. In April of 2016, a petition from the National Cattlemen's Beef Association (NCBA), the National Association of State Departments of Agriculture, the U.S. Meat Export Federation, and the American Farm Bureau Federation was received by the USDA Agricultural Marketing Service, requesting an amendment to the beef standards. This amendment would allow for the use of dentition, determined by USDA Food Safety Inspection Service (FSIS), or documentation of chronological age when determining maturity groupings of fed steer and heifers for quality grading. Previously, the standards only utilized physiological maturity (skeletal and lean maturity) of carcasses when determining official quality grades. These physiological maturities were thought to be related to ranges of chronological ages of cattle. However, research published by Acheson et al. (2014) and Semler et al. (2016) reported that carcasses under 30 months of age based on dentition, regardless of their physiological maturity, had similar palatability.

In August of 2016, a notice in the *Federal Register* asked for public comments on the above listed amendment to the beef standards. Of the comments, 179 favored the revision, while 53 commenters did not support the changes. The majority of comments received were from producer organizations, such as state beef councils, cattlemen's associations, and Farm Bureau Federations. However, individual producer and consumer comments were also received. Following the solicitation for comments, the USDA posted another notice in July of

2017, informing the public of the proposed changes that would occur in the revision to the beef standards. The proposed change stated that dentition and chronological age documentation can be used to classify carcasses under 30 months of age as A-maturity regardless of physiological maturity, with the exception of carcasses showing physiological maturity characteristics of a D or E maturity carcass. During the commenting period of this notice, 21 comments were received in which the majority were positive. This amendment to the beef standards went into effect across the United States in December of 2017.

Physiological Maturity and Chronological Age

Prior to the most recent amendment of the beef standards, carcasses were designated A through E maturity based only on skeletal and lean maturity (physiological maturity). This meant that carcasses could be verified by FSIS to be under 30 months of age based on dentition for food safety reasons, but when determining quality grade, the carcass would be downgraded due to their advanced physiological maturity. The majority of all conventionally produced heifer and steer carcasses are A-maturity (Garcia et al., 2008). O'Connor et al. (2007) found that the probability of an animal aged 18 months or younger producing a B maturity carcass was approximately 1%. However, the same study revealed that cattle between 22 and 24 months of age had a 9% chance of producing B maturity carcasses and a 3% chance of producing C maturity carcasses.

In 2011, the in-plant portion of the National Beef Quality Audit (NBQA) revealed that 7.2% of the fed-steer and heifer population produced B maturity or older carcasses. Of the carcasses that graded Standard, 46.7% had Small marbling scores, but were downgraded from low Choice due to the designation of B maturity (Moore et al., 2012). During the summer of

2017, carcasses that graded Standard received a $36.00 per hundredweight deduction, while C maturity carcasses (hardbone) were deducted $39.00 per hundredweight (USDA, 2017).

According to an economic loss study conducted by USDA-Agricultural Marketing Service (AMS; Wise, 2016), 1.68% of cattle (*n* = 21 million) under 30 months of age by dentition produced B maturity or older carcasses based on physiological maturity. Of this, 1.3% would have been eligible for the youngest maturity category (A maturity) if dentition had been utilized. When determining quality grades that could have been received by these cattle, 4.5% would have graded Prime, 63.6% Choice, and 31.9% Select, with 24.4% of the Choice category grading as average or high Choice. Carcasses within the top two-thirds of Choice as well as Prime carcasses would have received premiums, had their maturity been determined using dentition. The Agricultural Marketing Service estimated that producers would have gained approximately $55 million if dentition had been utilized to determine quality grade (USDA-AMS, 2017).

Estrogen has long been known to stimulate skeletal ossification. Increases in endogenous estrogen occurs at puberty in female mammals. In the presence of estrogen, chondrocytes at the growth plate of long bones undergo apoptosis, accelerating epiphyseal fusion and causing bone maturation (Weise et al., 2001). Adolescent females will often show accelerated skeletal maturity compared to adolescent males (Grumbach and Auchus, 1999). In the in-plant survey of the last five NBQAs, heifer carcasses were found to have significantly higher overall maturity as compared to steers (Boleman et al., 1998; Mckenna et al., 2002; Garcia et al., 2008; Moore et al., 2012; Boykin et al., 2017). Likewise, 16 to 27-month-old heifers are seven times more likely to produce B maturity carcasses and eleven

times more likely to produce C maturity or older carcasses compared to their steer counterparts (Tatum, 2011).

Reproductive status of heifers can greatly affect physiological maturity (Waggoner et al., 1990; Shackleford et al., 1995; Field et al., 1996). In some instances, heifers will be pregnant during feeding and at harvest. Producers may also cull open heiferettes (single-calf heifers) that go into the fed-beef system. Waggoner et al. (1990) found that heiferettes compared to open 2-year old heifers had significantly higher skeletal maturity. Furthermore, Field et al. (1996), reported that 77.8% of single-calf heifers were B maturity or older, while only 37.5% and 5.6% of virgin and spayed heifers showed advanced physiological maturity, respectively, although all cattle in this study were within 45 days of age from each other. Shackelford et al. (1995) utilized 249 heifers and cows ranging from 1.7 years to 12.9 years in chronological age and determined that physiological maturity scores did not accurately match chronological age. In this experiment, cattle as young as 40 months of age were graded E maturity based on skeletal ossification.

Estrogen from anabolic implants can also accelerate skeletal ossification in fed-steers and heifers. Skeletal maturation due to implants is directly associated with estrogenic effects of zeranol and estradiol (Turner et al., 1981; Vanderwert et al., 1985; Apple et al., 1991). Tatum et al. (2006) reported that approximately 97% of cattle in feedlots are implanted one or more times during finishing. Roeber et al. (2000) determined steers implanted with combination or estrogenic implants had higher degrees of skeletal ossification as compared to steers that were not implanted. Platter et al. (2003) found steers that were implanted 4 or 5 times in their lifetime had significantly higher overall maturities compared with steers implanted 3 or less times. Implanted steers and heifers between 16 to 27 months of age are

three times more likely to produce B maturity carcasses and two times more likely to produce C maturity carcasses as nonimplanted cattle in the same age range (Tatum, 2011).

Dentition and Chronological Age

Dentition has been an accepted form of estimating chronological age of cattle for many years. Furthermore, USDA-FSIS has utilized dentition to identify beef carcasses over 30 months of age since 2004. Cattle that display the eruption of a third permanent incisor are considered to be over 30 months of age. These older cattle are separated from cattle under 30 months of age to prevent risks related to exposure of specified risk materials (SRMs). SRMs are tissues from cattle that may cause prion contamination. Prions are misfolded proteins that can build up in the central nervous system and cause spongiform encephalopathies, such as bovine spongiform encephalopathy (BSE) in cattle and Creutzfeldt-Jakob Disease (CJD) in humans. Tonsils and the distal ileum of the small intestine are SRMs removed from all cattle. However, in cattle over 30 months of age additional SRMs are: the skull, brain, trigeminal ganglia, eyes, spinal cord, dorsal root ganglia, and vertebral column. These tissues are removed from carcasses during fabrication and must go to inedible rendering. To avoid cross contamination, FSIS personnel label carcasses over 30 months of age with colored dye over the vertebrae so that it is easily visible during fabrication (USDA-FSIS, 2004).

Multiple studies have determined that once the first set of permanent incisors have erupted, the animals chronological age is approximately 23-24 months of age. It has also been noted that by the eruption of the second set of permanent incisors the animals chronological age can range from 26-36 months of age. Factors that can affect eruption of permanent incisors include malnutrition, breed, and sex (reviewed by Lawrence et al., 2001). Because of the variation that can occur, the current FSIS regulations take a conservative

approach. So, although carcasses with eruption of the third or fourth permanent incisors may be under 30 months of age, they are considered over 30 months of age by inspection personnel.

Prior to the United States allowing for the use of dentition to determine age of cattle, many other countries were utilizing this method, including Brazil, Australia, and South Africa (Lawrence et al., 2001; Duarte et al., 2011). Brazil's beef carcass grade system breaks cattle into age groups by 0, 2, 4, 6, or 8 permanent incisors. Duarte et al. (2011) determined that this system effectively separated carcasses according to tenderness, with cattle having 4 permanent incisors or less producing beef acceptable in tenderness. Semler et al. (2016) recently compared cattle under 30 months of age and over 30 months of age based on dentition determined by FSIS. In this experiment, the *Longissimus* (LM) from cattle over 30 months of age with a slight degree of marbling (Select Quality Grade) had significantly higher ($P < 0.05$) slice shear force values compared with LM from cattle under 30 months of age with slight, small, and modest or moderate degrees of marbling. Cattle over 30 months of age also had higher levels ($P < 0.05$) of grassy and bloody/serumy flavors according to trained sensory panelists. Semler et al. (2016) concluded that dentition more correctly separated carcasses based on tenderness and flavor as compared to carcass physiological maturities. Similarly, Lawrence et al. (2001) stated that sorting cattle based on the number of permanent incisors would group cattle into more precise age categories.

Meat Tenderness

Warner-Bratzler shear force (WBSF) is commonly used to provide an objective assessment of product tenderness. Consumer sensory panels are also valuable in confirming product tenderness. Shackelford et al. (1991) was able to determine that panelists would

consider WBSF values of 4.6 kg or less to be ‘slightly tender’. While Miller et al. (1995) determined that steaks transitioned from tender to tough at WBSF values of 4.3 to 5.2 kg. Standard specifications are in place for marketing tenderness of beef retail cuts (ASTM, 2011). According to designation F2925-11, Minimum Tenderness Threshold Value (MTTV) for WBSF is 4.4 kg. This indicates that for product to be labeled as “Certified Tender” it must have at or below 4.4 kg WBSF values. Product labeled as “Certified Very Tender” is required to have WBSF values of 3.9 or below

Collagen plays an important function in animal muscle, as it surrounds muscle fibers and bundles and gives structure to the muscle as well as assists in contraction (Bailey, 1985). Of the 28 types of known collagen, two compromise the majority of collagen found in skeletal muscle, Type I and Type III (McCormick, 1994; Ricard-Blum, 2011). Collagen that makes up the perimysium, surrounding bunches of muscle fibers, is predominantly Type I, while Type III collagen is located in higher concentrations in the epimysium, surrounding the muscle, and endomysium, surrounding individual muscle fibers (Gillies et al., 2011). Changes in type of collagen present and cross-linking is also affected by sex, individual muscle, as well as management factors (McCormick, 1994). Collagen makes up approximately 2% of muscle (Bailey, 1985; McCormick, 1994). Structural muscles tend to have less collagen, while locomotive muscles will have larger amounts of collagen. Colle et al. (2015, 2016) determined that of four muscles (*biceps femoris*, *gluteus medius*, *longissimus lumborum*, and s*emimembranosus*) aged for 14 days, the b*iceps femoris* (BF) had the most insoluble collagen (11.86 mg collagen/g meat) and the *longissimus lumborum* (LM) had the least (5.65 mg collagen/g meat). Consequently, the LM, a postural muscle, is more tender than the BF which is frequently used for movement.

Meat from youthful animals is tender due to the fact that collagen is heat labile and able to be gelatinized when cooked. As animals increase in chronological age the tenderness of product from their carcasses decreases (Breidenstein et al., 1968; Berry et al., 1974; Miller et al., 1983). Through an animal's lifetime, the proportion of muscle that is collagen remains fairly constant. However, shifts in types of collagen in skeletal muscle occur (Type I and III) and the proportion of heat-stable collagen cross-links increases (Bailey, 1985). This maturation of collagen is due to a decrease in collagen synthesis (McCormick, 1994). Mature collagen is less soluble and also more thermally stable, leading to less acceptable tenderness (Bailey, 1985). During the cooking process these heat-stable cross-links shrink, causing increased fluid loss and shrinking of the cut as a whole.

Collagen analysis can be conducted on cooked or raw product. Research by Rhee et al. (2004) utilized cooked shear force cores from steaks for collagen analysis. This approach allowed for direct comparisons between shear force values and collagen content of steaks. Among the 12 different muscles in this study, WBSF and collagen content were correlated. Likewise, in cooked meat samples, insoluble collagen was found to responsible for 26.0 and 34.8% of variation of overall tenderness and amount of perceived connective tissue determine by sensory panelists (Jeremiah et al., 2003). However, other studies report that variations in collagen content are better accounted for utilizing raw product. Dubost et al. (2013) determined that tenderness of raw meat varies largely due to collagen content, whereas in cooked product neither insoluble collagen content or cross-linking influenced tenderness.

One method of determining collagen content in cooked beef is by measuring hydroxyproline content (Kolar et al., 1990; Eilert and Mandigo, 1993). Hydroxyproline is an amino acid derived from the hydroxylation of proline. Hydroxyproline is only found in

collagen and elastin and helps to form its helical shape. Therefore, hydroxyproline accurately represents connective tissue content of muscle. The method begins with separation of supernatant and pellet fraction of samples, followed by hydrolysis of fibers with sulfuric acid and heat, oxidation with Chloramine-T, and formation of a reddish-purple colored end product with 4-dimethylaminobenzaldehyde. Soluble and insoluble collagen are assumed to weigh 7.52 and 7.25 times the measured weight of hydroxyproline, respectively. These factors were used to calculate collagen content (Cross et al., 1973; Goll et al., 1963; Field, 1996).

Flavor and Palatability

Although tenderness is frequently the most important factor to consumers when eating beef, the characteristic of second highest importance was found to be flavor (Miller et al., 1995; Huffman et al., 1996). Flavor is considered a mixture of tastes (sweet, salty, savory, bitter, and umami) and aromas (Brewer, 2007; Legako et al., 2016). Many volatile flavor compounds occur in beef products (acids, alcohols, aldehydes, ketones, pyrazines, pyridines, pyrroles, etc.; Shahidi, 1994). Flavor can differ in cooked beef because of live animal factors as well as processing, packaging, and cooking methods following harvest (Brewer, 2007).

One of the largest influencers of beef flavor is feed source, such as the differences in flavor between grass-fed and grain-fed beef. Consumers in the United States tend to favor the flavors and aromas of grain-fed beef. Consumer panelists didn't find grass-fed product to lack flavor, but rather noted the presence of an off-flavor (Brown et al., 1979). Cattle that are grass-fed tend to have higher concentrations of polyunsaturated fatty acids such as α-linolenic acid while grain-fed cattle tend to have more oleic and linoleic acid (Elmore, et al., 2004). Flavor compounds derived from poly-unsaturated fatty acids, such as 4-heptenal, 2,4-

heptadienal, and 2,6-nonadienal, are found in higher quantities in grass-fed beef as compared to grain-fed beef (Larick et al., 1987). These compounds contribute to off-flavors described as grassy or gamey (Vatansever et al., 2000). Compounds such as hexanal, 2-heptenal, and 2,4-decadienal are found in higher quantities in grain-fed beef when compared to grass-fed beef (Larick et al., 1987).

Marbling plays an important role in flavor acceptability of beef. Smith et al. (1983) determined that significant differences ($P > 0.05$) in mean flavor desirability ratings occurred between LM steaks with moderately abundant (Prime) marbling and moderate (high Choice) or lesser marbling scores. Similar differences ($P > 0.05$) in flavor desirability ratings occurred between LM steaks with moderate and modest (average Choice) or lower marbling, and between slight (Select), traces (Standard), and practically devoid (Standard) marbling. Legako et al. (2015) determined that volatile flavor compounds octanal and nonanal were lower ($P < 0.05$) in Prime vs Standard Quality Grade LM steaks. These compounds are known to generate a soapy or grassy flavor in cooked beef product (Hodgen, 2008).

Smith et al. (1982) determined that sensory panel flavor ratings decreased ($P < 0.05$) from 5.72 (5 = slightly desirable in flavor) to 4.08 (4 = slightly undesirable in flavor) from A to E skeletal maturity. This same research also found that with each increasing category of skeletal and overall maturity, ratings for amount of connective tissue and tenderness worsened ($P < 0.05$). According to Jeremiah et at. (2003) insoluble collagen not only has a negative effect on palatability and texture of product, but also on consumers perceptions of flavor desirability. This illustrates that carcasses of older chronological ages are more likely to be associated with adverse flavors. However, research by Acheson et al. (2014) determined that during sensory analysis, trained panelists could not detect differences in meaty/brothy,

buttery/beef fat, bloody/serumy, livery/organy or gamey flavors between LM steaks from A and B-C maturity carcasses under 30 months of age. Similarly, Semler et al. (2016) reported that trained sensory panelists found samples from A and B-D maturity LM steaks to be similar in 6 flavor profiles (beefy/brothy, buttery/beef fat, livery/organy, grassy, or gamey flavor).

As animals increase in age, skeletal muscle pigment concentrations increase. During the cooking process, iron is released from heme pigment. This free iron can lead to lipid oxidation which causes warmed-over flavor in cooked product that is then stored (Igene et al., 1979). Calkins and Cuppett (2006) determined that between 30-40% of beef samples from cows had metallic and sour notes, and that 10-20% contained bloody, bitter, salty, and rancid flavors. Yancey et al. (2006) determined that myoglobin and iron content of lean is related to livery flavors detected by sensory panels. Conversely, Calkins and Cuppett (2006) determined that heme iron did not have a strong relationship with detection of livery flavors. Although differences in flavor due to physiological maturity of carcasses have been determined by sensory panels, research that attributes these differences to specific volatile flavor compounds is lacking.

Postmortem aging of product has been found to have positive effects on flavor and overall palatability (Smith et al., 1978). Colle et al. (2015) determined that no differences ($P > 0.05$) in LM sensory panel flavor occurred when aging for 2 to 63 days, however flavor was the most liked trait when product was aged for 14 and 21 days. Similarly, no differences were determined in BF flavor when aging up to 63 days, but consumer panelists determined that flavor was the most liked trait up to 42 days of age (Colle et al., 2016). Conversely, Spanier et al. (1997) determined that top rounds aged 14 days lead to an increase of negative flavors, such as cardboard, bitter, painty, and sour. While fluid loss has been found to increase with

extended postmortem aging in vacuum sealed bags, juiciness scores of steaks from the BF and *Semimebranosus* were not affect by aging up to 63 days (Colle et al., 2016)

Color and Shelf-Stability

Meat color is regularly utilized by consumers to help in product selection. Beef color is affected predominantly by myoglobin, the pigment that gives meat a red color. However, multiple other factors can affect meat color, such as muscle location and fiber type, marbling, pH, packaging, and length and temperature of storage. Myoglobin is a protein in muscle which contains iron and can take four primary forms depending on what ligand is bound to the iron; these forms are oxymyoglobin, deoxymyoglobin, metmyoglobin, and carboxymyoglobin. Deoxymyoglobin causes a deep purple color in lean tissue and is usually seen when very little oxygen is present. Examples of this include vacuum packaged meat, or in deep portions of muscles where very little oxygen is present. Oxymyoglobin is formed when oxygen binds to myoglobin and forms the well known bright cherry red color. Metmyoglobin occurs when ferrous iron (Fe^{2+}) is oxidized to ferric iron (Fe^{3+}). This occurs when low levels of oxygen are present and forms a brown color. This can commonly be seen in overwrapped packages of hamburger; if a label is present on the oxygen-permeable overwrap, a brown spot will be found when the overwrap is removed. The final type, carboxymyoglobin, is formed when the ligand that binds to the iron in myoglobin is carbon monoxide. Carbon monoxide is used at 0.4% in modified atmosphere packaging and produces a bright red, shelf-stable product (AMSA, 2012; USFDA, 2004).

Fresh beef color can be determined utilizing subjective and objective methods. Subjective measurements assist in describing changes in color that might be seen with the eye. These measurements allow for comparisons between objective measurements, and consumer

preferences for color (AMSA, 2012). Colorimeters or spectrophotometers utilize reflected light and convert it to Commision International de l'Eclairage (CIE) a*, b*, and L* values. a* values measure green (-a) to red (+a), b* values blue (-b) to yellow (+b), and L* values lightness from 0 (black) to 100 (light; AMSA, 2012).

Acheson et al., (2014) determined that LM from B-C maturity carcasses had lower L* values and higher a* values versus A maturity carcasses. Semler et al. (2016) similarly found that the LM from B-D maturity carcasses had lower L* and b* values versus A maturity carcasses. As animals mature, lean color darkens due to increased amounts of heme pigment. These subjective measurements, indicating a darker, more red colored lean, are likely related to this increase in myoglobin content.

Muscles can be classified as high, intermediate, low, or very low color stability (McKenna et al., 2005). These authors found that color stability is best determined by both metmyoglobin reductase activity and oxygen consumption rate of the muscle. Oxymyoglobin is noted for its bright red cherry color, therefore, myoglobin content was found to be strongly related to a* values of beef. McKenna et al. (2005) found that a* values of beef were more associated with color stability than b* or L* values.

Normally, pH of beef is approximately 5.5. However, when pH is 5.8 to 6.0, product can be darker in color and less tender (Wulf et al., 2002). Beef carcasses with a pH above 6.2 are characterized by a dark lean color, they are often referred to as 'dark cutters'. Product from these carcasses are considered undesirable by consumers (Lawrie, 1958). Because this product has higher water content and more desirable pH for microorganisms, microbial growth may also occur at a faster rate than product with a normal pH (Wulf et al., 2002).

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CHAPTER 2

Relationship Between Heifer Carcass Maturity and Beef Palatability

Abstract

Our objective was to determine the relationship between heifer carcass maturity and beef palatability of the *longissimus lumborum* (LM) and *biceps femoris* (BF). Left sides of A (n=30), B (n=30), and C (n=30) maturity heifer carcasses under 30 months of age by dentition were used. Carcasses were selected to ensure similar marbling scores across maturity groups (Small to Modest). Beef strip loins (LM) and outside rounds (BF) were obtained from these carcasses. Steaks were used to measure color stability, lipid oxidation (thiobarbituric acid reactive substances; TBARS), Warner-Bratzler shear force (WBSF), soluble and insoluble collagen, and consumer sensory perceptions. Heifer carcass maturity did not affect pH, fluid loss, WBSF or collagen content of LM or BF steaks ($P > 0.29$). In LM and BF steaks, a maturity x day of retail display interaction occurred for TBARS, in which B maturity steaks had lower levels of lipid oxidation compared with A and C maturity steaks from the fourth day to the end of the retail display ($P < 0.01$). Nevertheless, LM steaks from B maturity carcasses tended to have lower overall acceptability ($P = 0.08$) and juiciness ($P = 0.09$) than steaks from C maturity carcasses, but steaks from B and C maturity carcasses did not differ from LM steaks obtained from A maturity carcasses. No differences in tenderness or flavor were observed due to maturity ($P > 0.24$). Similarly, maturity had no effect on sensory characteristics of BF steaks ($P > 0.30$). In conclusion, our results indicate that advanced physiological maturity does not decrease palatability of strip loin or outside round steaks from carcasses of heifers under 30 months of age.

Key words: beef, carcass, heifer, maturity, palatability

Introduction

The majority of conventionally raised heifer and steer carcasses fall within the A maturity category (Garcia et al., 2008). However, O'Connor et al. (2007) found that cattle between 22-24 months of age have a 9% probability of producing B maturity carcasses and 3% probability of producing a C maturity carcass. Estrogen accelerates skeletal ossification and increases the physiological maturity of heifers, compared to steers of a similar age (Shackleford; 1995; Field, 1997; Grumbach and Auchus, 1999). Tatum (2011) found that heifers under 30 months of age are 7 times more likely to produce B maturity carcasses and 11 times more likely to produce C maturity or older carcasses, than steers. Additionally, Moore et. al (2012) reported that 46.7% of carcasses that graded Standard had Small marbling but were downgraded from low Choice based on designation as B maturity. In the summer of 2017, carcasses that quality graded Standard received an approximately $36.00 per hundredweight discount, while C maturity or older carcasses received a discount of $39.00 per hundredweight, relative to Choice carcasses (USDA, 2017). The current research was conducted to determine if these discounts were warranted based on beef quality characteristics of carcasses from heifers verified to be under 30 months of age by dentition.

Historically, USDA-AMS only allowed the use of physiological maturity as an indicator of carcass maturity when determining quality grade (2016). When using physiological maturity, carcasses are designated to maturity groups A through E based on the degree of visible maturity (vertebral ossification, rib shape and size, and lean color and texture; USDA, 2016). Approximate ages associated with each of these maturity groups are; A - 9-30 months, B – 30 – 42 months, C – 42-72 months, D – 72-96 months, and E – > 96 months (Tatum, 2007). Recently, the USDA-AMS implemented new standards that allow for

the use of age documentation, dentition, or physiological maturity to classify beef carcasses into maturity categories for quality grading (USDA-AMS, 2017). Cattle that have two permanent incisors or less are considered to be under 30 months of age, while the eruption of a second set of permanent incisors determines that the carcass is 30 months of age or older. The USDA – Food Safety and Inspection Service (FSIS) has utilized dentition to determine chronological age since 2004. A summary of the findings presented herein was submitted on October 24, 2016 during the comment period for the modernization of the beef standards.

Materials and Methods

Institutional Animal Care and Use Committee approval was not needed as no live cattle were used in this experiment. The University of Idaho Institutional Review Board certified this project as Exempt for human subject participation in consumer sensory analysis.

Carcass Selection

The left sides of 90 beef heifer carcasses were selected on four days over a three-month period at a commercial beef processing facility in 2015 and 2016. Selected carcasses from heifers were finished in feedlots in the Western United States and Canada. All carcasses were determined to be from heifers less than 30 months of age based upon dentition, though chronological age was unknown. Thirty carcasses within each physiological maturity category of A^{00}-A^{99} (A), B^{00}-B^{99} (B), and C^{00}-C^{99} (C) were selected. Carcasses were also selected to ensure marbling scores of $Small^{00}$ (SM) to $Modest^{99}$ (MT). This resulted in carcasses of low or average Choice, Standard, Commercial Quality Grades (USDA, 2016).

Initial selection of carcasses was determined based on overall maturity evaluations from USDA graders, as well as marbling scores collected by an USDA-approved instrument grading system (e+v Technology GmbH & Co. KG, Oranienburg, Germany). Upon selection,

carcasses were moved to stationary rails and carcass data (skeletal and lean maturity, marbling score, Quality Grade, hot carcass weight, ribeye area, 12th rib fat thickness, and Yield Grade) were collected by trained University of Idaho personnel. Kidney, pelvic, and heart fat (KPH) was removed at the plant prior to selection of the carcasses and so could not be measured. An average of 2.5% KPH was used to determine the final Yield Grade for each carcass. Outside rounds (IMPS 171B) and strip loins (IMPS 180; LM) from the left side of each carcass were purchased from AB Foods (Toppenish, WA) and transported to the University of Idaho Meat Science Laboratory.

Product Preparation

Subprimal cuts were removed from vacuum packaging following a 14-day aging period at 0°C. The ischiatic heads were removed from the outside rounds to produce trimmed outside round flats (IMPS 171D; BF) as described in the Institutional Meat Purchase Specifications (2014). Five 2.54 cm steaks were then cut from the LM (IMPS 1180) and BF (IMPS 1171D). Steaks were assigned to analyze retail shelf-life, lipid oxidation, Warner-Bratzler shear force (WBSF), insoluble and soluble collagen, and consumer acceptability.

Retail Shelf-Life

Steaks used for retail display were weighed, placed in white Styrofoam trays with the freshly cut surface exposed, and overwrapped with oxygen permeable PVC film (Koch Industries, Inc. #7500-3815; Wichita, KS). Once steaks had bloomed for at least 60 minutes, two objective color measurements per steak were taken using a Hunter MiniScan EZ (Restin, VA). These measurements represented day 0 of retail display. Subsequent measurements were taken on days 1 through 6. The Hunter MiniScan was equipped with a 25 mm-diameter measuring area and a 10° standard observer. The MiniScan was set to illuminant A and

Commission International de l'Eclairage (CIE) L*, a*, and b* values were recorded. Each day prior to use the machine was calibrated against black and white calibration tiles. Subjective color measurements were taken daily by three evaluators following Section 7 Appendix C of the American Meat Science Association guidelines (AMSA, 2012). Oxidized lean color, amount of browning, discoloration, surface discoloration, and color uniformity were measured, and evaluators were calibrated on day 0 of every retail display.

Steaks were displayed in a glass retail case (Model GDM-69, True Manufacturing Co., O'Fallon, MO) kept at approximately 2°C. The retail display case utilized natural white Hg 40w lights and the average light intensity was 409 lx. Steaks were rotated following daily measurements to minimize lighting and temperature effects due to location. Prior to the retail display steaks were weighed and following the seven-day display they were re-weighed to determine retail fluid loss.

Lipid Oxidation

Thiobarbituric acid reactive substances (TBARS) were measured to determine the extent of lipid oxidation (Appendix E). The end (~1 cm) of the steak was discarded before samples were taken from the top half of the steak avoiding the edge. Samples were ~ 0.5 cm wide, ~ 2.0 cm long, and ~ 1.27 cm thick. This initial sample represented day 0 of retail display. On days 2, 4, and 6 of retail display, TBARS were again taken to measure lipid oxidation. TBARS analysis followed the protocol defined in the Meat Color Measurement Guidelines (AMSA, 2012). During the 7-day period, steaks were displayed as listed above.

Cooking

Steaks utilized for Warner-Bratzler Shear Force (WBSF) and collagen analysis were cooked following 14 days of aging. Steaks were weighed prior to and following cooking to

measure fluid lost during the cooking process. Steaks were cooked on open-hearth broilers to an internal temperature of 40°C, then were flipped and cooked to a final internal temperature of 71°C. Temperatures of steaks were measured using hypodermic temperature probes (Omega Engineering Co.) coupled with a 12-channel scanning thermocouple thermometer (Digi-Sense, Cole-Parmer Instrument Co.). Probes were inserted into the geometric center of the steak.

Warner-Bratzler Shear Force

Prior to coring for WBSF, steaks were cooled at 4 °C overnight. Six cores (1.27-cm diameter) were mechanically removed per steak, parallel with muscle fiber orientation, using a drill-mounted coring device (GR Manufacturing, Manhattan, KS). Shear force was then determined by shearing cores once through the center, perpendicular to muscle fiber orientation, using a Warner-Bratzler shearing machine (GR Manufacturing, Manhattan, KS). The six shear values were averaged to determine a shear force (kg) for each steak.

Collagen Solubility

Remaining portions of WBSF steaks were diced, placed in 50 ml centrifuge tubes and frozen at -20°C until collagen analysis could be completed. Twenty-four randomly selected LM and BF steaks from each maturity category (A, B, and C) were used to determine soluble and insoluble collagen, as described by Colle et. al (2015; Appendix F). Total collagen was determined by adding soluble and insoluble collagen values.

pH

A portable pH meter (Model SevenGo, Mettler Toledo, Woburn, MA) equipped with an InLab Solids Pro puncture-type electrode was used to measure pH. The pH meter was calibrated each day prior to use with standard pH 4.0 and pH 7.0 buffers. LM and BF steak

pH was measured prior to steaks being cooked for consumer sensory analysis. The pH was taken at the edge of the steak. This edge was removed prior to cubing for sensory analysis to avoid potential effects of probe insertion on palatability.

Consumer Sensory Perceptions

Separate panels were conducted for strip loin and outside round steaks. Steaks used for sensory panels were individually vacuum packaged after aging and frozen at -20 °C until needed. Steaks were thawed at 4 °C for 24 hr prior to the panel and then cooked as previously described. Four (1.27-cm × 1.27 cm × steak thickness) cubes were obtained per steak and placed in covered cups assigned with a random number. Panelists were asked to evaluate samples for overall acceptability, tenderness, juiciness, and flavor using a 9-point scale (9 = like extremely, extremely tender, extremely juicy, and like flavor extremely, respectively; 1 = dislike extremely, not at all tender, extremely dry, dislike flavor extremely, respectively). Panelists (n = 72) evaluated 5 samples from A, B, and C maturities using an incomplete block design. Additionally, for each sample panelists were asked if they would be willing to purchase the product, if an off-flavor was present, and what the most and least liked attribute was (tenderness, juiciness, flavor, or texture/mouth feel), if applicable.

Statistical Analysis

Data were analyzed using the Mixed Model procedure of the Statistical Analysis System (SAS Institute, Inc., Cary, NC). Subprimal cuts (LM and BF) served as experimental units and maturity and day of retail display, as well as their interaction, served as fixed variables. All color and TBARS measurements were analyzed as repeated measures. Consumer sensory panel analysis was set up using an incomplete block design. Differences in

least square means were compared using the DIFF option. Significance was determined at $P \leq 0.05$, and data were considered trending at $P \leq 0.10$.

Results and Discussion

Characteristics of Selected Carcasses

Carcass measurements used to determine quality grade and yield grade of heifer carcasses ($n = 90$) can be found in Table 2.1. By design, skeletal maturity differed ($P < 0.01$) between A, B, and C maturity heifer carcasses. Skeletal maturity of carcasses ranged from A^{20} to C^{90} with a mean of B^{55}. However, lean maturity measurements were considerably more youthful than skeletal maturity, with a range of A^{20} to B^{90} and a mean of A^{78}. These results are similar to those of Boykin et al. (2017) who determined, based on National Beef Quality Audit data from 2016, that the mean lean maturity score of U.S. fed beef ($n = 8{,}741$) was A^{55}, while skeletal maturity was A^{69}. Likewise, Semler et al. (2016) determined that the overall mean skeletal and lean maturity for beef carcasses ($n = 600$) from fed cattle under and over 30 months of age was B^{54} and A^{50}, respectively. As animals increase in chronological age, myoglobin content in muscle increases, leading to a darker red oxygenated lean color (Breidenstein, 1968; USDA, 2016). In the current research, C maturity carcasses had lower lean maturity values than B maturity carcasses ($P < 0.05$). While this result was unexpected, chronological age likely had little effect on lean color among carcasses from these heifers under 30 months of age.

When determining overall maturity, the US grading standards for carcass beef (2016) state that when differences occur between skeletal and lean maturity, more emphasis is placed on skeletal maturity. The standards also indicate that overall maturity must not differ from the skeletal maturity by more than one full grade. In the current research this accounts for

numerically lower degrees of overall maturity for B and C maturity carcasses compared with A maturity carcasses. As expected, A, B, and C maturity carcasses differed ($P < 0.01$) in overall maturity (Table 2.1). Mean overall maturities for A, B, and C maturity carcasses were A^{74}, B^{20}, and C^{00}, respectively.

Marbling scores from the 90 heifer carcasses ranged from SM^{30} to MT^{70} with a mean of SM^{99}. By design, carcass marbling scores were similar ($P = 0.57$) across A, B, and C maturity groups (Table 2.1). The USDA Quality Grade distributions of the 90 carcasses collected were 26.7% Average Choice, 26.7% Low Choice, 13.3% Standard, and 33.3% Commercial (Table 2.2). Had these carcasses been graded using the modernized beef grading standards, 52.2% would have been categorized as Average Choice based on their marbling scores (Table 2.2).

Carcass measurements used to determine Yield Grade (hot carcass weight, adjusted 12^{th} rib back fat, and ribeye area) did not differ ($P \geq 0.20$) between A, B, and C maturity carcasses (Table 2.1). As animals increase in age, an increase in carcass weight, ribeye area, and lipid deposition are usually observed (Duarte, 2011). However, since cattle in the current study were all verified to be under 30 months of age by dentition, and therefore are of similar age, we would expect for A, B, and C maturity carcasses to have comparable mean hot carcass weights, ribeye areas, and adjusted 12^{th} rib back fat measurements.

Longissimus lumborum and Biceps femoris Quality Characteristics

Increases in physiological maturity have been associated with decreases in beef tenderness (Berry, 1974; Miller, 1983; Weston et al., 2002). This decrease in tenderness is associated with more mature collagen crosslinks and more heat stable collagen (Tatum, 2011). Shorthose and Harris (1990) determined that age-associated toughening of beef was more

pronounced in collagen rich muscles than muscles having lower collagen concentrations. In the current research, LM and BF subprimals were obtained to represent product that contain relatively low and high levels of connective tissue, respectively. Mean total collagen content of LM and BF steaks was 7.9 ± 2.8 and 10.5 ± 3.1 mg collagen/g meat, respectively. However, no differences ($P \geq 0.47$) in insoluble or total collagen were found between A, B, and C maturity carcasses from LM or BF steaks (Table 2.3).

Furthermore, no differences ($P \geq 0.29$) in WBSF values of LM or BF steaks from A, B, or C maturity carcasses were found (Table 2.3). Steaks with WBSF values below 4.6 kg are typically considered tender by consumers (Shackelford, 1991). In the current research, mean overall WBSF values for LM and BF steaks were 3.9 kg and 3.7 kg, respectively. Although mean WBSF measurements of BF steaks were numerically lower than LM steaks, Rhee et al. (2004) demonstrated that the BF ranked last among 11 muscles in trained sensory panel tenderness, but also ranked fourth in tenderness based on WBSF. Our data are consistent with Rhee et al. (2004) who concluded that WBSF is more useful to study changes in tenderness within muscles than among different muscles. Similarly, sensory panel tenderness scores in the current experiment were lower for BF than LM.

The pH of meat can play a large role in fluid loss, meat color, and tenderness. Muscle pH is approximately 7.0 and after death normally declines to 5.4 ~ 5.5 (Wulf, 2002). If ultimate pH remains above 6.0, a product defect referred to as a dark cutter or dark, firm and dry (DFD) may result. These products are noted for their dark color, high water holding capacity, and also a decrease in product tenderness (Lawrie, 1958; Wulf et al., 2002; Bass et al., 2010; English et al., 2016). Because of these factors, dark cutting carcasses were avoided for this experiment. No differences were found ($P \geq 0.60$) in pH of LM or BF steaks from A,

B, or C maturity carcasses (Table 2.3). Mean pH values for LM and BF steaks were 5.50 and 5.51, respectively, with the highest steak pH recorded at 5.71. Since meat pH did not differ among maturity groups, difference reported for lean color are likely due to factors other than pH.

Over the 6-day retail display, interactions were detected between day of retail display and maturity for objective color measurements of LM and BF steaks (Table 2.4 & 2.5). Redness (a*) and lightness (L*) of many steaks increased from day 0 to 1 of retail display, even though steaks were allowed to bloom for at least 60 minutes prior to taking the day 0 objective color measurement. McKenna et al. (2005) also reported increasing a* values from day 0 to day 1 of retail display. They attributed the increase in redness to high levels of oxygen consumption that occur early in display and do not allow for the steak to fully bloom. On day 0 of retail display, B maturity LM steaks had higher ($P < 0.01$) a* values than C maturity LM steaks (Table 2.4). Similarly, on the final day of retail display, B maturity LM steaks had a redder lean color than both A and C maturity LM steaks. These results confirm carcass lean maturity values, where B maturity carcasses had a darker red lean color as compared to C maturity carcasses. However, BF steaks from B maturity carcasses had lower ($P < 0.01$) a* values (less red lean color) than C maturity BF steaks on the second day of display and had lower a* values than A maturity BF steaks on the last day of retail display (Table 2.5). B maturity LM steaks had higher L* values ($P < 0.01$) than A maturity LM steaks on day 0 of retail display. On day two of retail display, C maturity LM steaks had significantly higher b* values versus A maturity steaks. A similar change in b* values occurred in BF steaks, where C maturity steaks were more yellow in color than A and B maturity steaks.

Subjective color measurements (oxygenated lean color, amount of browning, discoloration, surface discoloration, and color uniformity) did not differ among A, B, and C maturity LM steaks ($P \geq 0.37$; Table 2.6). However, a maturity by day of retail display interaction was observed for oxygenated lean color of BF steaks ($P = 0.04$; Table 2.7). On the third day of retail display, B maturity BF steaks had higher (worse) oxygenated lean color scores than C maturity steaks, while A maturity steaks did not differ from either. Similarly, on the fourth day of retail display B maturity BF steaks had higher oxygenated lean color scores than A and C maturities (5.82 vs. 5.32 and 5.24, respectively).

Oxygen on the cut surface of steaks can lead to autoxidation of lipids through the presence of free-radicals. Other factors that can lead to lipid oxidation are heat and light, catalysts, fatty acids present, and pH. A commonly used assay to quantify lipid oxidation is thiobarbituric acid reactive substances (TBARS) analysis. This measures malondialdehyde (MDA) which is an end product of lipid oxidation A maturity by day of retail display interaction was observed ($P < 0.01$) for TBARS values (lower mg MDA/kg of meat) of LM and BF steaks. Less lipid oxidation occurred at day four and six of retail display in B maturity LM steaks than A and C maturity LM steaks (Table 2.8). Throughout the 6-day retail display period, lipid oxidation of LM steaks from B maturity carcasses did not increase, whereas A maturity LM steaks showed increased oxidation on the last day of retail display, and C maturity LM steaks lipid oxidation increased significantly by day 4 of retail display. Similarly, B maturity BF steaks had significantly less lipid oxidation by day four and six of retail display than BF steaks from A and C maturity carcasses. Mean lipid oxidation of all steaks was below 1.0, except for A maturity BF steaks on the final day of retail display. This is noteworthy since this level of lipid oxidation has been associated with off-flavors

(McKenna, 2005). However, in normal retail display settings, steaks overwrapped in oxygen permeable film are usually removed from the shelf within 72 hours due to oxidation of myoglobin that results in a brown color considered unacceptable by consumers (Delmore, 2009). By 72 hours of retail display, LM and BF steak lipid oxidation levels were well below 1.0.

During the LM sensory panel, no differences were detected ($P \geq 0.25$) in tenderness or flavor between steaks from A, B, or C maturity carcasses (Table 2.9). However, overall acceptability ($P = 0.08$) and juiciness ($P = 0.09$) tended to be higher in LM steaks from C maturity carcasses than B maturity, but steaks from B and C maturity carcasses did not differ from those of A maturity carcasses. Similarly, Acheson (2014) and Semler (2016) found no differences ($P > 0.05$) in LM tenderness, juiciness, or flavor between steaks from A and B or older maturity carcasses. Additionally, in the current research LM steaks from C maturity carcasses received the highest percentage of consumer panelists indicating a willingness to purchase the product. Consumers did not report differences ($P \geq 0.31$) in acceptability, tenderness, juiciness, or flavor for BF steaks from A, B, or C maturity carcasses (Table 2.9). An off flavor was detected by 22.7, 25.2, and 23.3 percent of panelists who evaluated BF steaks from A, B, and C maturity carcasses, respectively, while 51.7, 45.8, and 42.4 percent of panelists indicated a willingness to purchase the product. Consumer panel preferences and demographics are listed in Table 2.10 and 2.11, respectively.

The devaluation of carcasses that are under 30 months of age by dentition, but downgraded due to advanced physiological maturity, was typically in excess of $20 per hundredweight prior to the modernization of the carcass beef standards. Recently, Acheson et. al (2014) used A and B-C maturity steer and heifer carcasses from cattle under 30 months

of age to determine if differences occurred in sensory properties of the LM. The authors concluded that carcasses with similar marbling scores from grain-finished cattle younger than 30 months of age by dentition have similar palatability regardless of maturity. Semler et. al (2016) furthered this research by using steer and heifer carcasses to determine if dentition of carcasses 30 months or older and carcasses under 30 months better segregated carcasses based on sensory properties. Similarly, these authors found few differences in palatability of carcasses under 30 months of age regardless of physiological maturity.

The current research specifically utilized heifer carcasses, since they tend to show advanced physiological maturity at younger chronological ages compared to steers. However, other factors such as pregnancy, spaying, usage of anabolic implants, as well as severity of anabolic implants can also influence degree of skeletal maturation (Tatum, 2011). Little background information is known on the 90 selected heifer carcasses, with the exception that heifers were not spayed, but did receive anabolic implants and were grain-finished. These factors are typical of fed-heifers in the Western United States and gave our research an ideal population to utilize. Very few differences were determined between LM and BF steaks from A, B, and C maturity carcasses under 30 months of age. These results support prior research (Acheson, 2014; Semler, 2016), in that carcasses from cattle harvested at less than 30 months of age based on dentition produce beef that provides the same shelf-life and eating experience regardless of its physiological maturity. Furthermore, these results align with the recent modernization of the US standards for grades of carcass beef, which states all carcasses under 30 months of age by dentition should be considered A maturity.

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Chapter 3

The Relationship of Heifer Carcass Maturity and Beef Flavor

Abstract

The objective was to determine if carcass maturity of heifers under 30 months of age affects consumer acceptability, heme pigment concentration, and flavor compound development of strip loins (LM) aged for 28 days and outside rounds (BF) aged for 14 days. LM and BF steaks from 90 heifer carcasses were used to measure lipid oxidation, total heme pigment, consumer sensory perceptions, and volatile flavors. Lipid oxidation of LM steaks did not differ ($P > 0.86$) among maturity groups. No differences ($P > 0.27$) in heme pigment were determined among A, B, and C maturity LM or BF steaks. Consumer panelists also found no differences ($P > 0.34$) in flavor, juiciness, tenderness or overall acceptability of LM steaks from A, B, and C maturity carcasses. C maturity LM steaks had higher ($P < 0.02$) levels of 3-hydroxy-2-butanone, pentanal, and toluene versus steaks from A and B maturity carcasses ($P < 0.02$). No differences were detected in other volatile flavor compounds analyzed for LM or BF steaks from A, B, and C maturity heifer carcasses ($P > 0.10$). In conclusion, our results indicate that advanced physiological maturity does not decrease positive flavor attributes of strip loin or outside round steaks from carcasses of heifers under 30 months of age.

Key words: beef, carcass, heifer, maturity, flavor

Introduction

Beef flavor is one of the most important factors in a consumer's positive eating experience. However, understanding and managing the flavor of beef is complex. Positive beef flavors can be described as beefy, brown/roasted, bloody/serumy, fat-like, sweet, salty, and umami, while flavors generally considered negative are metallic, liver-like, sour,

barnyard, musty-earthy, and bitter. Lipid oxidation, pH, and myoglobin content can also affect the flavor of beef. Yancey et al. (2006) studied volatile flavors, heme pigment, total iron, and lipid oxidation to determine their relationship to livery flavors in three muscles from A and B maturity beef carcasses. These authors concluded that myoglobin content as well as volatiles such as hexanal, limonene, and nonanal are related to the presence of a liver-like flavor. Similar research was conducted by Glascock et al. (2014) who utilized different beef muscles that varied in quality grade, pH, amount of connective tissue, cooking method, and end cooking temperature. This research determined that non-heme iron and myoglobin content was not related to differences in beef flavor, and that 15 volatiles, including 3-methyl-2-butanone, limonene, and benzene, accounted for over 50 percent of overall consumer liking.

As animals increase in maturity changes in flavor may occur. Recently, Acheson et al. (2014) determined that LM steaks with Slight marbling from A maturity carcasses had higher incidences of bloody/serumy flavor than steaks from B-C maturity carcasses. Similarly, Semler et al. (2016) determined that LM steaks from cattle over 30 months of age had higher bloody/serumy flavor and grassy flavor, while no differences in flavor were detected between physiological maturity groups (A and B-D). In Chapter 2, no differences in flavor were detected among A, B, or C maturity LM or BF steaks from heifer carcasses under 30 months of age. However, overall acceptability and juiciness of B maturity LM steaks tended to be lower.

Historically, USDA-AMS only allowed the use of physiological maturity as an indicator of carcass maturity when determining quality grade (2016). Recently, the USDA-AMS implemented new standards that allow for the use of age documentation, dentition, or physiological maturity to classify beef carcasses into maturity categories for quality grading

(USDA-AMS, 2017). The current research was conducted to determine if beef flavor characteristics of carcasses from heifers verified to be under 30 months of age by dentition differ due to physiological maturity.

Materials and Methods

Institutional Animal Care and Use Committee approval was not needed as no live cattle were used in this experiment. The University of Idaho Institutional Review Board certified this project as Exempt for human subject participation in consumer sensory analysis.

Product procurement

Outside (bottom) rounds (IMPS 171B) and strip loins (LM; IMPS 180) from the left sides of 90 beef heifer carcasses verified by dentition to be under 30 months of age were selected. The ninety carcasses were selected to ensure 30 carcasses within each physiological maturity category of A^{00}-A^{99} (A), B^{00}-B^{99} (B), and C^{00}-C^{99} (C). This product was procured as part of the previous research project, which examined the relationship between heifer carcass maturity and beef quality characteristics.

Product Preparation

Retail cuts were removed from vacuum packaging following a 14-day aging period at 0°C. The ischiatic head was removed from the outside rounds to produce trimmed outside round flats (IMPS 171D; BF) as described in the Institutional Meat Purchase Specifications (2014). Five 2.54 cm steaks were then cut from the LM and BF. Remaining posterior portions of the LM were vacuum sealed and aged for another 14 days at 0°C. Subsequently, two 2.54 cm steaks were cut from the LM. Steaks were assigned to analyze lipid oxidation, total heme pigment, consumer sensory perceptions, and volatile flavor compounds.

Lipid Oxidation

To determine lipid oxidation of LM steaks, samples were collected for thiobarbituric acid reactive substances (TBARS) analysis. TBARS analysis followed the protocol defined in the Meat Color Measurement Guidelines (AMSA, 2012). The lateral end (~1 cm) of the steak was discarded before samples were taken from the top half of the steak avoiding the edge. Samples were ~ 0.5 cm wide, ~ 2.0 cm long, and ~ 1.27 cm thick. Steaks were then cooked and served for consumer sensory analysis.

Heme Pigment

Samples utilized for analysis were removed from LM and BF steaks prior to consumer sensory analysis and frozen at -20°C until needed. Total heme pigment was quantified as described by Warris (1979), with slight modifications. Duplicate samples (n = 60 per subprimal) were pulverized using a 1.5 cup food processor and then 2 g were homogenized for 20 seconds in 10 ml of 0.04 M phosphate buffer (pH 6.8). Homogenate was subsequently held in an ice bath for 1 hr. Samples were then centrifuged at 6,500 x g for 10 minutes at 4°C. Following centrifugation, a 1 ml aliquot of the supernatant was removed and mixed with 0.1 ml of 6.6 mM potassium ferricyanide and 8.8 mM sodium cyanide. Samples were then centrifuged at 30,000 x g for 1 hr at 15°C. Duplicate 200 ml aliquots were then placed into a 96-well plate and absorbance was measured at 540 nm. Total heme pigment concentration was expressed as mg of total pigment/g of tissue, and was calculated as follows:

A_{540}/[11,300 L*mol^{-1}*cm^{-1}(0.6217 cm)] [(17,500g/mol*0.0132 L*1000 mg/g)/2 g]

11,300 is the molar extinction coefficient for cyanmetmyoglobin (Drabkin, 1950)

0.6217 cm is the light path

17,500 g/mol is the molecular weight of myoglobin (Low & Rich, 1973)

0.0132 L is the equivalent volume of diluted aqueous sample

2 g is weight of sample used for extraction.

Correction factor = A_{540} x 16.44

Cooking

LM and BF steaks were utilized for volatile flavor analysis. Steaks were weighed prior to and following cooking to measure fluid lost during the cooking process. Steaks were cooked as described in the previous chapter.

Consumer Sensory Perceptions

LM steaks used for taste panels were individually vacuum packaged and frozen at -20°C until needed. Steaks were thawed at 4°C for 24 hr prior to the panel and then cooked. LM steaks were sampled, served to panelists, and rated as described in Chapter 2. Sensory panel data from BF steaks is also reported in Chapter 2.

Volatile Flavors

During sensory panel cooking, approximately 10 g samples of cooked steak were removed and placed in 50 ml centrifuge tubes (polypropylene; VWR, Radnor, PA, USA) then put into dry ice until the end of the panel. These samples were frozen at -80°C until volatile flavor analysis could be completed. Beef volatile flavors were analyzed following procedures by Legako et al. (2015), with slight adjustments. Samples were allowed to thaw overnight at 4°C. Approximately 6 grams of sample were mechanically pulverized using a 1.5 cup food processor and then placed into 15 ml clear glass vials with polytetrafluoroethylene septum screw caps (Supelco, Bellefonte, PA, USA). Vials were then placed into a water bath for 5 minutes at 65°C. Following, a manual solid phase microextraction (SPME) fiber (85 µm carboxen polydimethylsiloxane; Supelco, Bellefonte, PA, USA) was inserted into the headspace above the sample for 10 minutes.

Volatiles were analyzed using gas chromatography-mass spectrometry (GC-MS). Cryogenic focusing was conducted by cooling a 20 cm portion of the GC column with

nitrogen gas cooled by passage through a coil of copper tubing submerged in liquid nitrogen. The column was cooled for 5 minutes prior to injection of the SPME fiber. The fiber was then withdrawn from the headspace and injected into the GC injection port for analysis. Volatile compounds were desorbed from the SPME fiber at the GC-MS inlet at 250°C. The fiber was exposed for 5 minutes while the column remained enveloped in cooled nitrogen gas. Following, nitrogen gas flow was stopped, and the oven heating program of the GC-MS began. The SPME fiber remained exposed within the inlet for the first three minutes of the oven method. The oven temperature started at 35°C and was held there for 5 minutes, then an 8°C per minute temperature ramp to 220°C began. Once at 220°C, a 20°C per minute ramp to 290°C was used. The oven method ended with a 5-minute hold at 290°C.

The MS was utilized to quantify analytes and to produce spectrum to identify peaks (Figure 2.1). A library of stored spectra was utilized to identify peaks. Only confidence scores of 90% or higher were utilized in matching unknown peaks to analytes. Area under the curve was measured in total ion count (TIC).

Statistical Analysis

Data were analyzed using the Mixed Model procedure of the Statistical Analysis System (SAS Institute, Inc., Cary, NC). Subprimal cuts (LM and BF) served as experimental units and maturity served as the fixed variable. Consumer sensory panel analysis was set up using an incomplete block design. Differences in least square means were compared using the DIFF option. Significance was determined at $P \leq 0.05$, and data were considered trending at $P \leq 0.10$.

Results and Discussion

Lipid oxidation of LM steaks aged for 28-days did not differ by maturity ($P = 0.86$; Table 3.1). The mean TBARS value was 0.158 mg malondialdehyde (MDA) per kg of meat while values ranged from 0.038 to 0.880 mg MDA per kg of meat. According to Yancey et al. (2005), rancid flavors increase as product is aged longer. Lipid oxidation likely also occurred at higher levels in some samples because of loss of vacuum seal during storage. A small number of vacuum bags at the taste panel were noted to have oxygen penetration. However, no differences in flavor or overall acceptability of LM steaks were noted by consumer panelists ($P > 0.05$).

Heme pigment did not differ ($P \geq 0.27$; Table 3.1) among A, B, or C maturity LM or BF steaks. Mean heme pigment values of LM and BF steaks were 6.25 and 6.15 mg total pigment per g of tissue, respectively. As cattle increase in age, heme pigment levels in lean tissue increase (USDA, 2016; Smith, 1988). This leads to a darker colored lean in animals of older chronological age. To determine lean maturity, a scale of youthful, bright red lean to darker lean is utilized to categorize carcasses into A-E maturity for grading purposes. Although carcasses in this research were considered A, B, and C maturities, nearly all exhibited youthful lean maturity scores, which is consistent with cattle of young (under 30-months) chronological age.

During sensory panel evaluation of LM steaks aged 28 days, consumer panelists found no differences ($P \geq 0.34$; Table 3.2) among A, B, and C maturity steaks for acceptability, tenderness, juiciness, or flavor. Interestingly, in LM steaks aged for 14 days, there was a tendency for B maturity steaks to have lower ratings for juiciness and overall acceptability versus A and C maturity steaks. Extended aging of the LM, even as short as 28 days, can

improve characteristics such as tenderness, juiciness, and flavor (Colle et al., 2015; Colle et al., 2016). These data suggest that additional aging may eliminate the tendency for B maturity LM steaks to have lower acceptability and juiciness than A and C maturity LM steaks. Consumer panel preferences and demographics are listed in Table 3.3 and 3.4, respectively.

Numerous volatiles are present in and affect the flavor of beef. In the current research, eight volatiles in LM steaks were analyzed and 6 volatiles were analyzed for BF steaks. No significant differences ($P > 0.10$; Table 3.5) in LM steaks from A, B, or C maturity carcasses were determined for benzene, 3-methyl butanal, 4-methyl heptane, hexanal, or octane. In C maturity LM steaks, 3-hydroxy-2-butanal, pentanal, and toluene had larger peak areas than cooked samples from A or B maturity steaks. Limonene and toluene were determined to be associated with liver-like samples by Yancey et al. (2006) and Hodgen et al. (2006). Hexanal is used as an indicator of deterioration of beef flavor because of its rapid increase compared to other volatiles. Heptanol, nonanal, and pentanal are associated with off-flavors due to lipid oxidation. (Shahidi and Pegg, 1994). Although C maturity LM samples were higher in pentanal and toluene, which have been associated with oxidized or liver-like flavors, consumer panelists were unable to determine differences in flavor, and did not detect an increased incidence of off-flavors due to maturity. No differences ($P > 0.12$; Table 3.5) were detected in volatile flavor compounds measured in cooked BF steaks from A, B, and C maturity carcasses. Percentage of all volatiles present in LM and BF steaks are listed in Table 3.6. These data suggest that the occurrence of volatile flavor compounds may vary by muscle. The volatiles are further broken down into A, B, and C maturities for LM and BF steaks in Tables 3.7 and 3.8, respectively.

Results of this research, as well as prior research conducted by Acheson et al. (2014), and Semler et al. (2016), determine that very few differences in flavor can be detected between A, B, and C maturity carcasses verified by dentition to be under 30 months of age. These findings support the amendment to the US Beef Grading Standards to include carcasses verified to be under 30 months of age by dentition or age verification in the youngest maturity category (A maturity).

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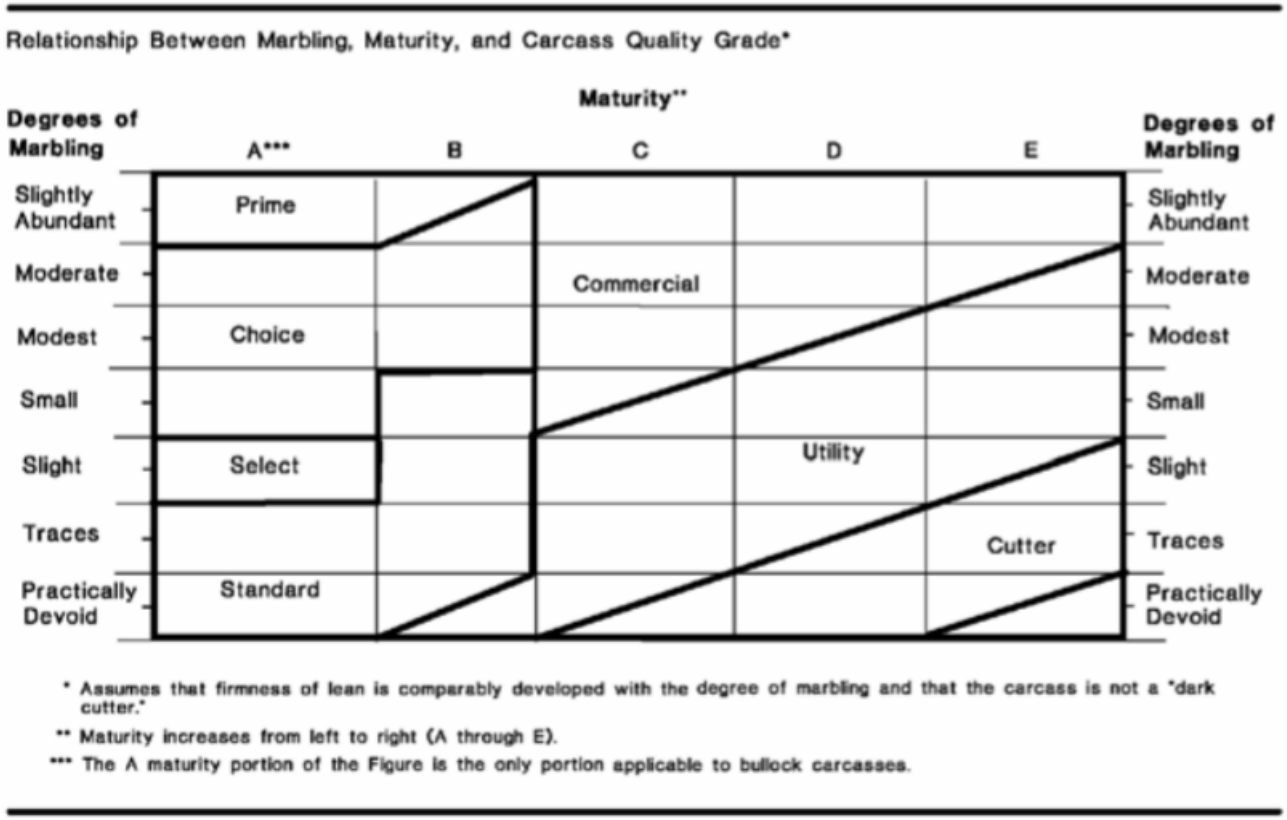


Figure 1.1. Relationship between marbling, maturity, and carcass quality grade.

Figure 2.1. Outside round roast with the side muscle removed (IMPS 171D). Steaks were cut perpendicular to the to the grain of the muscle (IMPS 1171D) and are also referred to as a "western griller steak".

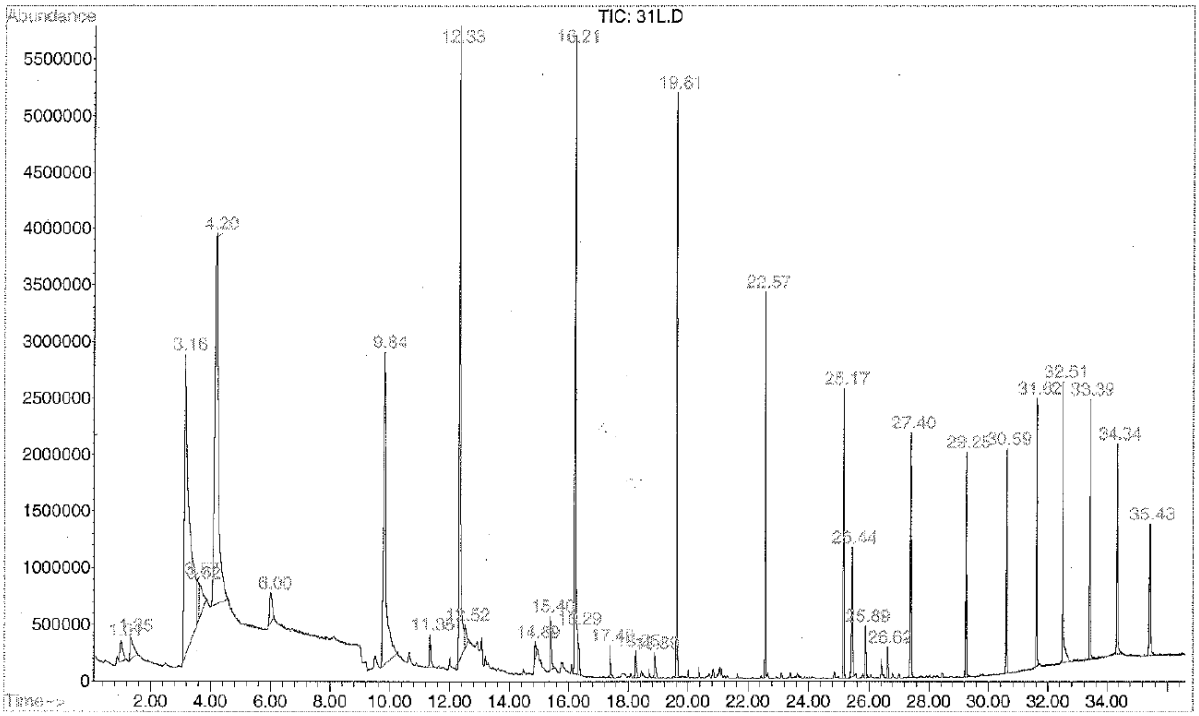


Figure 2.2. Integrated spectrum of volatiles from an LM sample shown in TIC x time point in the oven method.

Table 2.1. USDA Quality Grade and Yield Grade carcass measurements

	Maturity				
	A	B	C		
	n = 30	*n* = 30	*n* = 30	SEM	*P*-value
Skeletal maturity[1]	167[c]	249[b]	348[a]	4	< 0.01
Lean maturity[1]	177[ab]	188[a]	170[b]	5	0.05
Overall maturity[1]	174[c]	220[b]	300[a]	3	< 0.01
Marbling score[2]	494	502	501	6	0.57
Hot carcass weight, kg	402	419	413	7	0.20
12th rib back fat, in	0.73	0.67	0.72	0.05	0.67
Ribeye area, in^2	14.7	14.7	15.4	0.3	0.24
Yield grade[3]	3.5	3.5	3.4	0.2	0.83

[a-c]Means in the same row within a measurement that do not share a common superscript letter differ.
[1]Maturity: A maturity = 100 – 199, B = 200 – 299, C = 300 – 399.
[2]Marbling Score: Small = 400 – 499, Modest = 500 – 599.
[3]An average of 2.5% kidney, pelvic, and heart fat (KPH) was used to determine Yield Grade.

Table 2.2. Quality Grade distribution by maturity.[1]

	Maturity			
n = 90	A	B	C	All
Utilizing Physiological Maturity				
Average Choice	43.3	36.7	--	26.7
Low Choice	56.7	23.3	--	26.7
Standard	--	40.0	--	13.3
Commercial	--	--	100.0	33.3
Utilizing modernized Standards for Carcass Beef				
Average Choice	43.3	60.0	53.3	52.2
Low Choice	56.7	40.0	46.7	47.8

[1]Carcasses selected to ensure Small to Modest marbling.

Table 2.3. pH, purge, Warner-Bratzler shear force, cook loss, insoluble and total collagen of *longissimus lumborum* and *biceps femoris* steaks

		Maturity				
	n	A	B	C	SEM	*P*-value
Longissimus lumborum						
pH	90	5.49	5.51	5.50	0.01	0.60
Percent purge	90	1.37	1.26	1.33	0.06	0.46
WBSF, kg	90	3.82	4.05	3.89	0.14	0.49
Percent cook loss	90	23.37	24.97	22.79	1.08	0.34
Insoluble collagen, mg/g	24	6.33	7.73	7.44	0.85	0.48
Total collagen, mg/g	24	6.94	8.53	8.32	1.40	0.47
Biceps femoris						
pH	90	5.51	5.51	5.52	0.01	0.65
Percent purge	90	1.74	1.81	1.65	0.10	0.48
WBSF, kg	90	3.70	3.87	3.55	0.14	0.29
Percent cook loss	90	28.99	29.44	28.62	0.72	0.72
Insoluble collagen, mg/g	24	9.54	9.55	9.80	1.14	0.98
Total collagen, mg/g	24	10.60	10.41	10.60	1.25	0.99

Table 2.4. Instrument color of *longissimus lumborum* steaks

n = 90	Day of display	Maturity A	Maturity B	Maturity C	SEM
Longissimus lumborum					
L*	0	37.07^{bwy}	38.69^{ay}	37.71^{abwy}	0.57
P = < 0.01	1	39.73^{z}	40.16^{z}	40.55^{zx}	0.57
	2	36.55^{y}	35.70^{x}	37.23^{yz}	0.57
	3	38.09^{x}	38.20^{y}	38.49^{yx}	0.57
	4	37.94^{x}	38.22^{y}	38.26^{yz}	0.57
	5	37.57^{wx}	37.97^{y}	38.55^{xy}	0.57
	6	37.49^{wx}	38.36^{y}	37.64^{wz}	0.57
a*	0	34.72^{abz}	36.09^{az}	34.33^{by}	0.59
P = < 0.01	1	34.88^{z}	35.19^{z}	35.50^{z}	0.59
	2	31.41^{y}	31.26^{y}	32.77^{x}	0.59
	3	30.73^{xy}	29.74^{x}	30.49^{w}	0.59
	4	31.09^{xy}	30.90^{y}	31.51^{wx}	0.59
	5	29.97^{x}	30.37^{xy}	30.27^{w}	0.59
	6	25.45^{bw}	27.64^{aw}	25.59^{bv}	0.59
b*	0	29.89^{z}	30.88^{z}	29.95^{z}	0.51
P = 0.03	1	28.12^{y}	28.65^{y}	29.06^{z}	0.51
	2	25.93^{bx}	26.37^{abx}	27.53^{ay}	0.51
	3	24.92^{w}	24.02^{w}	24.60^{w}	0.51
	4	25.03^{wx}	24.68^{w}	26.00^{x}	0.51
	5	24.36^{w}	24.82^{w}	25.13^{wx}	0.51
	6	22.67^{v}	22.80^{v}	23.43^{v}	0.51

[a-c]Within a row, means without a common letter differ.
[u-z]Within a column, muscle, and trait, means without a common letter differ.

Table 2.5. Instrument color of *biceps femoris* steaks

n = 90	Day of display	Maturity			SEM
		A	B	C	
Biceps femoris					
L*	0	37.43	38.75	37.47	0.60
P = 0.27	1	38.89	38.99	39.58	0.60
	2	35.52	34.72	35.45	0.60
	3	36.11	35.97	35.85	0.60
	4	35.58	36.33	35.35	0.60
	5	35.48	35.71	35.05	0.60
	6	35.30	36.53	36.66	0.60
a*	0	34.97^{z}	36.19^{z}	35.2^{z}	0.67
P = < 0.01	1	32.93^{y}	32.59^{y}	33.8^{y}	0.67
	2	27.77^{abx}	26.68^{bx}	29.37^{ax}	0.67
	3	24.48^{w}	23.36^{w}	24.48^{w}	0.67
	4	23.85^{w}	23.89^{w}	24.12^{w}	0.67
	5	20.80^{u}	21.48^{v}	21.25^{v}	0.67
	6	22.59^{av}	20.62^{bv}	22.05^{abv}	0.67
b*	0	31.04^{z}	31.88^{z}	31.39^{z}	0.49
P = 0.01	1	28.00^{y}	27.81^{y}	28.98^{y}	0.49
	2	25.49^{bx}	24.77^{bx}	26.89^{ax}	0.49
	3	22.5^{vw}	21.53^{v}	22.34^{v}	0.49
	4	23.01^{v}	22.77^{w}	23.49^{w}	0.49
	5	21.74^{w}	21.53^{uv}	22.23^{v}	0.49
	6	21.62^{w}	20.57^{u}	21.56^{v}	0.49

$^{a-c}$Within a row, means without a common letter differ.
$^{u-z}$Within a column, muscle, and trait, means without a common letter differ.

Table 2.6. Visual Color of *longissimus lumborum* steaks

n = 90	Day of display	Maturity			SEM
		A	B	C	
Longissimus lumborum					
Oxygenated lean color[1]	0	2.9	2.8	2.6	0.1
P = 0.37	1	3.2	3.1	3.0	0.1
	2	3.3	3.2	3.2	0.1
	3	3.8	3.7	3.6	0.1
	4	4.1	4.3	3.9	0.1
	5	4.4	4.5	4.2	0.1
	6	4.6	4.7	4.5	0.1
Browning[2]	0	1.0	1.0	1.0	0.1
P= 0.97	1	1.4	1.3	1.4	0.1
	2	1.5	1.5	1.5	0.1
	3	2.0	1.9	2.2	0.1
	4	2.6	2.6	2.7	0.1
	5	2.9	2.8	2.9	0.1
	6	3.2	3.2	3.1	0.1
Discoloration[3]	0	1.0	1.0	1.0	0.1
P = 0.99	1	1.4	1.3	1.3	0.1
	2	1.4	1.4	1.5	0.1
	3	1.8	1.8	1.9	0.1
	4	2.3	2.3	2.3	0.1
	5	2.5	2.4	2.5	0.1
	6	2.8	2.7	2.8	0.1

[1]1 = extremely bright cherry red, 2 = bright cherry red, 3 = moderately bright cherry red, 4 = slightly bright cherry red, 5 = slightly dark cherry red, 6 = moderately dark red, 7 = dark red, 8 = extremely dark red.

[2]1 = no evidence of browning, 2 = dull, 3 = grayish, 4 = brownish-gray, 5 = brown, 6 = dark brown.

[3]1 = none, 2 = slight, 3 = small, 4 = moderate, 5 = extreme.

Table 2.6. Visual Color of *longissimus lumborum* steaks (Cont.)

n = 90	Day of display	Maturity			SEM
		A	B	C	
Longissimus lumborum					
Surface Discoloration[4]	0	1.0	1.0	1.0	0.1
P = 0.91	1	1.4	1.3	1.4	0.1
	2	1.5	1.4	1.5	0.1
	3	1.8	1.9	1.9	0.1
	4	2.4	2.5	2.3	0.1
	5	2.6	2.6	2.5	0.1
	6	2.9	3.0	2.9	0.1
Uniformity[5]	0	1.3	1.2	1.2	0.1
P = 0.50	1	1.2	1.2	1.2	0.1
	2	1.2	1.2	1.2	0.1
	3	1.2	1.2	1.3	0.1
	4	1.4	1.3	1.4	0.1
	5	1.4	1.2	1.3	0.1
	6	1.6	1.4	1.5	0.1

[4]1 = no discoloration (0%), 2 = slight (1-20%), 3 = small (21-40%), 4 = modest (41-60%), 5 = moderate (61-80%), 6 = extensive (81-100%).
[5]1 = uniform, 2 = slight two-toning, 3 = small amount two-toning, 4 = moderate two-toning, 5 = extreme two-toning.

Table 2.7. Visual color of *biceps femoris* steaks

n = 90	Day of display	Maturity			SEM
		A	B	C	
Biceps femoris					
Oxygenated lean color[1]	0	3.1^{v}	3.1^{u}	2.8^{u}	0.2
P = 0.04	1	3.5^{w}	3.6^{v}	3.2^{v}	0.2
	2	4.3^{x}	3.2^{w}	4.2^{w}	0.2
	3	5.1^{aby}	5.4^{ax}	4.7^{bx}	0.2
	4	5.3^{by}	5.8^{ay}	5.2^{by}	0.2
	5	5.8^{z}	6.1^{z}	5.8^{z}	0.2
	6	6.0^{z}	6.3^{z}	6.0^{z}	0.2
Browning[2]	0	1.1	1.2	1.2	0.1
P= 0.60	1	1.8	2.0	1.8	0.1
	2	3.0	3.1	3.0	0.1
	3	4.0	4.0	3.9	0.1
	4	4.7	4.7	4.6	0.1
	5	5.0	4.9	4.8	0.1
	6	5.0	4.9	5.1	0.1
Discoloration[3]	0	1.2	1.2	1.2	0.1
P = 0.37	1	1.8	1.9	1.7	0.1
	2	2.8	2.7	2.8	0.1
	3	3.6	3.7	3.6	0.1
	4	4.2	4.3	4.2	0.1
	5	4.5	4.5	4.4	0.1
	6	4.6	4.4	4.7	0.1

[a-c]Within a row, means without a common letter differ.

[u-z]Within a column and trait, means without a common letter differ.

[1]1 = extremely bright cherry red, 2 = bright cherry red, 3 = moderately bright cherry red, 4 = slightly bright cherry red, 5 = slightly dark cherry red, 6 = moderately dark red, 7 = dark red, 8 = extremely dark red.

[2]1 = no evidence of browning, 2 = dull, 3 = grayish, 4 = brownish-gray, 5 = brown, 6 = dark brown.

[3]1 = none, 2 = slight, 3 = small, 4 = moderate, 5 = extreme.

Table 2.7. Visual color of *biceps femoris* steaks (Cont.)

n = 90	Day of display	Maturity			SEM
		A	B	C	
Biceps femoris					
Surface Discoloration[4]	0	1.2	1.3	1.2	0.2
P = 0.83	1	1.9	2.0	1.7	0.2
	2	3.2	3.3	3.3	0.2
	3	3.9	4.3	3.9	0.2
	4	4.6	4.8	4.5	0.2
	5	4.8	5.1	4.7	0.2
	6	5.1	5.2	5.2	0.2
Uniformity[5]	0	1.8	1.8	1.8	0.1
P = 0.16	1	2.0	2.1	1.9	0.1
	2	2.7	2.8	2.6	0.1
	3	3.2	3.3	3.1	0.1
	4	3.7	3.7	3.8	0.1
	5	3.9	3.8	3.8	0.1
	6	4.0	3.7	3.9	0.1

[4]1 = no discoloration (0%), 2 = slight (1-20%), 3 = small (21-40%), 4 = modest (41-60%), 5 = moderate (61-80%), 6 = extensive (81-100%).
[5]1 = uniform, 2 = slight two-toning, 3 = small amount two-toning, 4 = moderate two-toning, 5 = extreme two-toning.

Table 2.8. Lipid oxidation of *longissimus lumborum* and *biceps femoris* steaks[1]

n = 90	Day of display	Maturity			SEM
		A	B	C	
Longissimus lumborum	0	0.24^{y}	0.21	0.20^{x}	0.03
P = < 0.01	2	0.22^{y}	0.19	0.23^{x}	0.03
	4	0.25^{ay}	0.22^{b}	0.27^{ay}	0.03
	6	0.33^{az}	0.23^{b}	0.32^{az}	0.03
Biceps femoris	0	0.43^{w}	0.42^{x}	0.40^{w}	0.06
P = < 0.01	2	0.63^{x}	0.50^{x}	0.62^{x}	0.06
	4	0.80^{ay}	0.62^{by}	0.79^{ay}	0.06
	6	1.01^{az}	0.74^{bz}	0.94^{az}	0.06

$^{a\text{-}c}$Within a row, means without a common letter differ.
$^{u\text{-}z}$Within a column, muscle, and trait, means without a common letter differ.
[1]mg malondialdehyde/kg meat

Table 2.9. Consumer panel analysis of *longissimus lumborum* and *biceps femoris* steaks[1]

	Maturity				
n = 90	A	B	C	SEM	*P*-value
Longissimus lumborum					
Acceptability	5.7	5.4	5.9	0.1	0.08
Tenderness	5.7	5.4	5.8	0.2	0.29
Juiciness	5.2	5.1	5.6	0.2	0.09
Flavor	5.5	5.5	5.8	0.2	0.25
Biceps femoris					
Acceptability	5.2	4.9	4.9	0.2	0.31
Tenderness	4.7	4.4	4.5	0.2	0.51
Juiciness	4.6	4.4	4.7	0.2	0.39
Flavor	5.3	5.2	5.2	0.2	0.86

[1]Scale, 9 = like extremely, extremely tender, extremely juicy, and like flavor extremely, respectively; 1 = dislike extremely, not at all tender, extremely dry, and dislike flavor extremely, respectively.

Table 2.10. Consumer panel preferences for *longissimus lumborum* and *biceps femoris* steaks

	Longissimus lumborum Maturity			*Biceps femoris* Maturity		
	A	B	C	A	B	C
Like most[1]						
Flavor	33.3	44.9	41.4	58.7	45.6	44.8
Tenderness	34.2	24.3	27.9	23.1	12.6	16.7
Juiciness	21.6	19.6	23.4	12.5	24.3	22.9
Texture	10.8	11.2	7.2	5.8	17.5	15.6
Like least[2]						
Flavor	33.6	24.8	30.8	16.9	20.7	20.7
Tenderness	25.2	27.4	25.2	44.1	40.5	46.8
Juiciness	27.1	30.1	29.9	21.2	24.0	25.2
Texture	14.0	17.7	14.0	17.8	14.9	7.2
Off flavor[3]						
Yes	24.4	24.2	21.2	22.7	25.2	23.3
No	75.6	75.8	78.8	77.3	74.8	76.7
Purchase[4]						
Yes	57.6	54.6	68.1	51.7	45.8	42.4
No	42.4	45.4	31.9	48.3	54.2	57.6

[1]Percentage of panelists that liked that attribute the most
[2]Percentage of panelists that liked that attribute the least
[3]Percentage of panelists that did or did not detect an off flavor
[4]Percentage of panelists willing to or not willing to purchase the product

Table 2.11. Demographics of consumer panelists[1]

	Longissimus lumborum		*Biceps femoris*	
	n	%	*n*	%
Age				
18-19	2	2.8	1	1.4
20-29	51	71.8	49	70
30-39	7	9.9	8	11.4
40-49	3	4.2	1	1.4
50+	8	11.3	11	15.7
Gender				
Male	23	32.4	25	35.7
Female	48	67.6	45	64.3
Beef meals/wk[2]				
0-1	6	8.3	8	11.3
2-4	41	56.9	45	63.4
5-7	22	30.6	18	25.4
8+	3	4.2	0	0.0
Most consumed[3]				
Ground	42	56.8	50	70.4
Roast	9	12.2	7	9.9
Steak	19	25.7	14	19.7
Other	4	5.4	0	0.0

[1]Rounding prevents all categories from adding to 100.0%.
[2]Please indicate the number of meals a week in which you consume beef: 0-1, 2-4, 5-7, or 8+
[3]Please indicate the form in which you most commonly consume beef: ground, roast, steak, or other

Table 3.1. Lipid oxidation and heme pigment of *longissimus lumborum* steaks aged 28 days and heme pigment of *biceps femoris* steaks

		Maturity				
	n	A	B	C	SEM	*P*-value
Longissimus lumborum						
Lipid Oxidation[1]	90	0.16	0.15	0.17	0.03	0.86
Heme Pigment[2]	60	6.31	6.15	6.29	0.19	0.80
Biceps femoris						
Heme Pigment[2]	60	5.88	6.16	6.41	0.23	0.27

[1]mg malondialdehyde/kg meat
[2] mg of total pigment/g of tissue

Table 3.2. Consumer panel analysis of *longissimus lumborum* steaks aged 28 days[1]

	Maturity				
n = 90	A	B	C	SEM	*P*-value
Longissimus lumborum					
Acceptability	5.9	5.9	5.9	0.2	0.92
Tenderness	5.8	5.7	5.5	0.2	0.41
Juiciness	5.2	5.2	5.5	0.2	0.34
Flavor	5.7	6.0	5.7	0.2	0.39

[1]Scale, 9 = like extremely, extremely tender, extremely juicy, and like flavor extremely, respectively; 1 = dislike extremely, not at all tender, extremely dry, and dislike flavor extremely, respectively.

Table 3.3. Consumer panel preferences for *longissimus lumborum* steaks aged 28 days

	Maturity		
	A	B	C
Like most[1]			
Flavor	40.9	43.8	33.0
Tenderness	30.9	28.6	23.2
Juiciness	19.1	21.9	31.3
Texture	9.1	5.7	12.5
Like least[2]			
Flavor	20.2	25.5	29.0
Tenderness	24.8	26.4	29.9
Juiciness	38.5	30.9	27.1
Texture	16.5	17.3	14.0
Off flavor[3]			
Yes	13.6	19.5	19.3
No	86.4	80.5	80.7
Purchase[4]			
Yes	64.2	67.5	64.4
No	35.8	32.5	35.6

[1]Percentage of panelists that liked that attribute the most
[2]Percentage of panelists that liked that attribute the least
[3]Percentage of panelists that did or did not detect an off flavor
[4]Percentage of panelists willing to or not willing to purchase the product

Table 3.4. Demographics of consumer panelists of *longissimus lumborum* steaks aged 28 days[1]

	n	%
Age		
18-19	8	11.3
20-29	38	53.5
30-39	9	12.7
40-49	2	2.8
50+	14	19.7
Gender		
Male	39	54.9
Female	32	45.1
Beef meals/wk[2]		
0-1	10	13.9
2-4	49	68.1
5-7	13	18.1
8+	0	0
Most consumed[3]		
Ground	52	61.2
Roast	10	11.8
Steak	22	25.9
Other	1	1.2

[1]Rounding prevents all categories from adding to 100.0%.
[2]Please indicate the number of meals a week in which you consume beef: 0-1, 2-4, 5-7, or 8+
[3]Please indicate the form in which you most commonly consume beef: ground, roast, steak, or other

Table 3.5. Volatile flavors of *longissimus lumborum* steaks aged 28 days and *biceps femoris* steaks

	Maturity				
n = 48	A	B	C	SEM	*P*-value
Longissimus lumborum					
Benzene	0.24	5.40	7.03	2.19	0.52
3-Hydroxy-2-Butanone	13.09[b]	11.37[b]	63.17[a]	10.90	< 0.01
3-Methyl Butanal	12.40	18.04	60.73	16.90	0.10
4-Methyl Heptane	4.00	11.36	15.72	5.22	0.31
Hexanal	107.79	81.71	18.74	57.90	0.41
Octane	0.56	3.24	5.98	2.59	0.42
Pentanal	6.33[b]	5.74[b]	54.25[a]	8.90	< 0.01
Toluene	8.67	7.87	34.16	7.47	0.02
Biceps femoris					
Benzene	2.85	0.83	3.87	1.49	0.42
3-Hydroxy-2-Butanone	40.79	11.48	57.29	19.50	0.27
Heptanal	14.01	1.75	10.41	5.89	0.39
Hexanal	123.00	24.07	108.77	35.30	0.12
Octane	8.75	1.80	5.32	3.28	0.40
Pentanal	30.35	2.65	15.03	13.70	0.34

[1]total ion count (TIC) area under the curve (millions)
[a-b]Within a row, means without a common letter differ.

Table 3.6. Percentage of samples in which volatile flavors were found in *longissimus lumborum* steaks aged 28 days and *biceps femoris* steaks (*n* = 48)

Volatile	*Longissimus lumborum*	*Biceps femoris*
Hexanal	97.9%	93.8%
Benzene	79.2%	31.3%
4-methyl heptane	68.8%	22.9%
Toluene	64.6%	12.5%
3-methyl butanal	60.4%	20.8%
Limonene	58.3%	20.8%
3-hydroxy-2-butanone	56.3%	75.0%
Pentanal	38.8%	37.5%
Octane	27.1%	37.5%
Heptanal	18.8%	25.0%
2-methyl butanal	14.6%	6.3%
2-methyl 1-pentene	10.4%	2.1%
Octanal	8.3%	14.6%
2,3-butanedione	6.3%	4.2%
2,4-dimethyl heptene	6.3%	2.1%
2-octanamine	6.3%	2.1%
2-(ethenyloxy) ethanol	4.2%	10.4%
2-butanamine	4.2%	--
Carbon disulfide	4.2%	2.1%
Nonanal	4.2%	18.8%
Piperidine	--	6.3%

Table 3.7. Percentage of samples in which volatile flavors were found in *longissimus lumborum* steaks aged 28 days from A, B, and C maturity carcasses (*n* = 16 per maturity)

Volatile	A	B	C
Benzene	81.3%	75.0%	81.3%
2-(ethenyloxy) ethanol	6.3%	--	6.3%
2,3-butanedione (diacetyl)	6.3%	--	12.5%
2,4-dimethyl heptene	--	12.5%	--
2-butanamine	--	6.3%	6.3%
2-methyl 1-pentene	6.3%	6.3%	18.8%
2-methyl butanal	6.3%	6.3%	31.3%
2-octanamine	--	12.5%	6.3%
3-hydroxy 2- butanone	37.5%	75.0%	56.3%
3-methyl butanal	56.3%	62.5%	68.8%
4-methyl heptane	81.3%	75.0%	50.0%
Carbon disulfide	6.3%	6.3%	--
Heptanal	6.3%	37.5%	12.5%
Hexanal	100.0%	93.8%	93.8%
Limonene	56.3%	62.5%	56.3%
Nonanal	--	12.5%	--
Octanal	6.3%	18.8%	--
Octane	12.5%	37.5%	31.3%
Pentanal	62.5%	62.5%	56.3%
Toluene	56.3%	75.0%	62.5%

Table 3.8. Percentage of samples in which volatile flavors were found in *biceps femoris* steaks from A, B, and C maturity carcasses (*n* = 16 per maturity)

Volatile	A	B	C
Benzene	31.3%	31.3%	31.3%
2-(ethenyloxy) ethanol	12.5%	12.5%	6.3%
2,3-butanedione	--	--	6.3%
2-methyl butanal	6.3%	--	12.5%
3-hydroxy 2- butanone	93.8%	62.5%	68.8%
3-methyl butanal	25.0%	6.3%	31.3%
4-methyl heptane	25.0%	6.3%	37.5%
Heptanal	31.3%	18.8%	25.0%
Hexanal	100.0%	81.3%	93.8%
Limonene	25.0%	12.5%	--
Nonanal	25.0%	18.8%	12.5%
Octanal	25.0%	18.8%	--
Octane	43.8%	31.3%	37.5%
Pentanal	37.5%	50.0%	25.0%
Piperidine	--	12.5%	6.3%
Toluene	12.5%	6.3%	18.8%

Appendix A

Exempt Certification for IRB Project Number 15-867

University *of* Idaho
Office of Research Assurances
Institutional Review Board
875 Perimeter Drive, MS 3010
Moscow ID 83844-3010
Phone: 208-885-6162
Fax: 208-885-5752
irb@uidaho.edu

To: Matthew Doumit

From: Jennifer Walker
Chair, University of Idaho Institutional Review Board
University Research Office
Moscow, ID 83844-3010

Date: 7/28/2015 12:37:02 PM

Title: Relationship Between Heifer Carcass Maturity and Beef Quality Characteristics

Project: 15-867
Certified: Certified as exempt under category 6 at 45 CFR 46.101(b)(6).

On behalf of the Institutional Review Board at the University of Idaho, I am pleased to inform you that the protocol for the above-named research project has been certified as exempt under category 6 at 45 CFR 46.101(b)(6).

This study may be conducted according to the protocol described in the Application without further review by the IRB. As specific instruments are developed, modify the protocol and upload the instruments in the portal. Every effort should be made to ensure that the project is conducted in a manner consistent with the three fundamental principles identified in the Belmont Report: respect for persons; beneficence; and justice.

It is important to note that certification of exemption is NOT approval by the IRB. Do not include the statement that the UI IRB has reviewed and approved the study for human subject participation. Remove all statements of IRB Approval and IRB contact information from study materials that will be disseminated to participants. Instead please indicate, 'The University of Idaho Institutional Review Board has Certified this project as Exempt.'

Certification of exemption is not to be construed as authorization to recruit participants or conduct research in schools or other institutions, including on Native Reserved lands or within Native Institutions, which have their own policies that require approvals before Human Subjects Research Projects can begin. This authorization must be obtained from the appropriate Tribal Government (or equivalent) and/or Institutional Administration. This may include independent review by a tribal or institutional IRB or equivalent. It is the investigator's responsibility to obtain all such necessary approvals and provide copies of these approvals to ORA, in order to allow the IRB to maintain current records.

As Principal Investigator, you are responsible for ensuring compliance with all applicable FERPA

Appendix B

Sensory Panel Consent Form

Sensory Panel Consent Form

Cooking spray used contains soy

1. The University of Idaho Human Assurance Committee has reviewed and found this study to be exempt.

2. The objective of this study was to evaluate the effects of different carcass maturities on beef. The samples will be prepared under the Research Guidelines for Cookery, Sensory Evaluation, and Instrument Tenderness Measurements of Fresh Meat, as outlined by the American Meat Science Association. This taste panel is part of research funded by the Idaho Beef Council.

3. You will be asked to evaluate 6 samples (approximately 1" x ½" x ½") per session for tenderness (1 = extremely tough to 9 = extremely tender), juiciness (1 = dry to 9 = juicy), and flavor (1 = bland to 9 = intense) using a 9 point scale. It is not necessary that samples be ingested. The study should take approximately 15 to 20 minutes.

4. Although there are no or minimal risks associated with the project, it is possible that some samples will have one or more qualities that may not be appealing to you (e.g. tenderness or juiciness that is less than you would prefer).

5. With your help, society can benefit from our attempt to improve the understanding of beef maturity.

6. We anticipate that samples will be well received by panelists. However, if we find during the course of the taste panel that samples are unappealing, we will stop the evaluation process.

7. To maintain anonymity of the data collected during this evaluation, all the information you provide will be placed in a locked file with Dr. Doumit.

8. If you have questions about the taste panel, you can ask the investigator during the evaluation, when the evaluation is complete or at a time you feel is appropriate.

9. Contact information for the University of Idaho faculty member leading this research:
   Dr. Matthew E. Doumit
   University of Idaho
   Department of Animal and Veterinary Science
   Moscow, ID 83844
   208-885-6007

10. During the course of this taste panel, you may terminate participation at any time. If you choose to do so, please notify the investigator that you no longer wish to participate.

11. If you choose to terminate participation in this evaluation, there will be no penalties associated with your withdrawal.

I have reviewed this consent form and understand and agree to its contents.

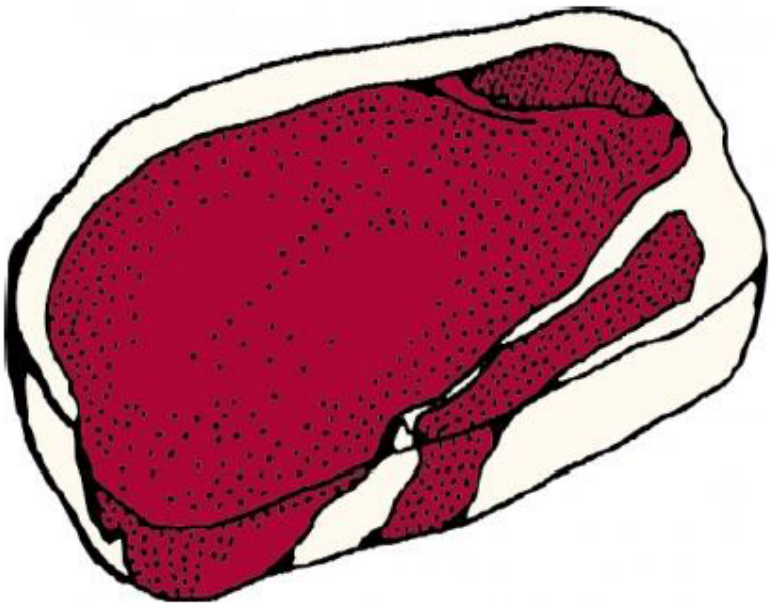
Participant Name: _____ Date: _____

Signature: _____ Date of Birth: _____

Appendix C

Sensory Panel Demographics Questionnaire

EVALUATION OF BEEF QUALITY



Panelist #:_____ Date: _____

Age: _____ Gender: _____

Please indicate the number of meals a week in which you consume beef:

0-1 2-4 5-7 8+

Please indicate the form in which you most commonly consume beef:

Ground Roast Steak Other

Appendix D

Beef Sensory Panel Questionnaire

BEEF SENSORY PANEL QUESTIONNAIRE

Sample ID #:_____

1. **OVERALL ACCEPTABILITY OF SAMPLE:** This is based on your overall acceptability of the sample

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

(Dislike extremely) (Like extremely)

2. **TENDERNESS:** This is based on your overall opinion of the sample's tenderness

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

(Dislike extremely) (Like extremely)

3. **JUICINESS:** This is based on your overall opinion of the sample's juiciness

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

(Dislike extremely) (Like extremely)

4. **FLAVOR:** This is based on your overall opinion of the sample's flavor

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

(Dislike extremely) (Like extremely)

5. **OFF-FLAVOR:** This is based on your ability to detect an off-flavor of the sample

NO YES

6. **CONSUMER SATISFACTION:** Would you be willing to purchase this product?

NO YES

7. **IF APPLICABLE,** please circle the trait you liked **least** about this product.

Flavor Tenderness Juiciness Texture/Mouth Feel

8. **IF APPLICABLE,** please circle the trait you liked **most** about this product.

Flavor Tenderness Juiciness Texture/Mouth Feel

9. **Overall Comments on Product:**

Appendix E

TBARS for Oxidative Rancidity—Rapid, Wet Method

Adapted from Appendix O. TBARS for Oxidative Rancidity—Rapid, Wet Method

Principle

In the presence of thiobarbituric acid (TBA), malonaldehyde and other aldehyde products of lipid oxidation (TBA reactive substances; TBARS) form pink chromogens with maximum absorbance at 532 to 535 nm. However, in the presence of interfering sugars, a yellow chromagen forms, which can be avoided using Tarladgis' (1960) distillation method.

Reagents

1. TBA stock solution: 0.375% thiobarbituric acid, 15% trichloroacetic acid, and 0.25 N HCl.
2. Stock solutions (100 mL) are sufficient for 20 individual tests. Stock solution may be stored at room temperature in the dark (foil-wrapped container).

Procedure

1. Finely chop or mince a portion of the product of interest. Weigh out duplicate 0.5-g samples.
2. Add 2.5 mL TBA stock solution to each sample, giving a dilution factor of 6. Mix well.
3. Heat samples 10 minutes in boiling water in loosely capped tubes (round bottom Pyrex or polypropylene centrifuge tubes). Caution: Tightly capped tubes may burst during heating. Positive samples turn pink during heating.
4. Cool tubes in tap water.
5. Centrifuge at 5,000 × g for 10 minutes at 4°C to obtain a clear supernatant.
6. Carefully pipette a portion of the supernatant to a spectrophotometer cuvette. Take care that the solution remains clear.
7. Measure supernatant absorbance at 532 nm against a blank that contains all the reagents, but not the meat.
8. Calculate the TBA value expressed as ppm malonaldehyde, using 1.56 × 105/M/cm as the extinction coefficient of the pink TBA chromagen (Sinnhuber and Yu, 1958), as follows:

TBARS number (mg MDA/kg) = sample A532 × (1 M TBA chromagen/156,000) × [(1 mol/ L/M] × (0.003 L/0.5 g meat) × (72.07 g MDA/mol MDA) × 1000 mg/g) × 1000 g/kg), or **TBARS value (ppm)** = sample A532 × 2.77.

References

Buege, J. A., and S. D. Aust. 1978. Microsomal lipid peroxidation. Methods Enzymol. 52:302–304.
Sinnhuber, R. O., and T. C. Yu. 1958. 2-Thiobarbituric acid method for the measurement of rancidity in fishery products. II. The quantitative determination of malonaldehyde. Food Technol. 12:9–12.

Appendix F

Collagen Analysis

SOLUTION RECEPIES

Ringers Solution (1L)
-7.0g NaCl
-0.026g $CaCl_2$
-0.35g KCl

Sulfuric Acid 3.5M (2L)
-1675mL H_20
-375mL sulfuric acid
-Add sulfuric acid *slowly* to H_2O on ice while stirring.
**This must be re-calculated based on purity of sulfuric acid.

375/2000 = 0.1875 (correction factor for this calculation)

For example, 98% pure sulfuric acid:
0.1875/0.98 = 0.1913

2000*0.1913 = 382.6mL sulfuric acid + 1617.4ml H_2O

***This solution becomes *extremely hot* when adding acid to H_2O. Prepare *on ice* in the *fume hood*!!!!

Buffer Solution (1L) pH 6.0
-500mL H_2O
-30g citric acid monohydrate ($C_6H_80_7 \cdot H_2O$)
-15g NaOH
-90g sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$)
-Stir all ingredients until dissolved, then add:
-290mL 1-propanol
-Check pH – you will need to add more citric acid to bring pH down to 6.0.
-Bring solution up to volume in graduated cylinder or volumetric flask.

**Solution is stable for 2 months @ 4°C in a dark bottle.

Oxidant solution (100mL)
-100mL Buffer Solution
-1.41g Chloramine-T (Sigma catalog #C9887)

**Solution is stable for 1 week @ 4°C in a dark bottle.

Color Reagent (100mL)
-35mL perchloric acid (chilled) (Sigma catalog #244252)
-10g 4-dimethylaminobenzaladehyde (Sigma catalog #156477)
-Stir until 4-dimethylaminobenzaldehyde is dissolved, then slowly add:
-65mL 2-propanol

***This solution becomes hot when adding 2-propanol to perchloric acid. Prepare on ice in the fume hood!!!!

****Solution is stable for no more than 24 hours.****

Hydroxyproline Standard Solution (100mL)
-60mg hydroxyproline (Sigma catalog #H5534)
-100mL H2O

**Solution is stable for 2 months @ 4°C.

Intermediate Solution (IS) (XmL)
-Intermediate solution is standard solution diluted.
-To make 10ml of IS, add 0.1mL of Standard to 9.9mL H2O.

**Make solution fresh on day of use.

Working Standards (20mL)

Standard	0.3µg/mL	0.6µg/mL	1.2µg/mL	2.4µg/mL	4.8µg/mL	6.0µg/mL
Intermediate Solution	1.0mL	2.0mL	4.0mL	8.0mL	16.0mL	20.0mL
H20	19.0mL	18.0mL	16.0mL	12.0mL	4.0mL	0mL

**Make solution fresh on day of use.

PROTOCOL

Sample Preparation

1. Use frozen samples left over from Warner-Bratzler shear force determination.
2. Temper frozen samples at 4°C overnight.
3. Grind meat sample in 1.5 cup food processor for 1 minute, stir sample, and grind for another 45 seconds.
4. Weigh out 4.0g in duplicate.
5. Add 22mL Ringers solution.
6. Homogenize sample for 20 seconds, rest for 20 seconds, then homogenize for 20 seconds at 18,000rpm.
7. Heat in water bath at 50°C for 15 minutes stirring every 5 minutes.

8. Centrifuge tubes at 5200xg for 10 minutes.
9. Decant supernatant into 125mL Erlenmeyer flasks through filter paper.
10. Add 10mL ¼ strength Ringers solution to pellet and stir.
11. Centrifuge again with same conditions, decant into same flasks.
12. Transfer pellet to another 125mL Erlenmeyer flask along with the filter paper.
13. Add 30mL 3.5M sulfuric acid to insoluble portion.
14. Add 8mL concentrated sulfuric acid to soluble portion.
    Add slowly on ice in the fume hood
15. Place in oven at ~105°C for at least 16 hours with watch glass on top.
16. Carefully remove flasks from oven.
17. Transfer hot hydrolysate to 100mL graduated cylinder.
18. Rinse flask 3 times with water and bring volume up to 100mL.
19. Mix sample by covering with parafilm and inverting 4 times.
20. Filter approximately 20mL into 50mL conical tube.

Assay

1. Pipet each 2mL sample in duplicate into test tubes. (0.12mL hydrolysate + 1.88mL H_2O, you may need to experiment with a dilution that keeps sample readings within the range of the standard curve.)
2. Pipet 2mL of each standard in duplicate into test tubes.
3. Add 1mL of Oxidant solution to each tube. Mix and let stand at room temperature for 20 minutes.
4. Add 1mL of Color reagent to each tube. Mix and heat covered with foil in 60°C water bath for 15 minutes.
5. Cool tubes with H_2O for at least 3 minutes.
6. Pipet 200µL of each sample (and standard) into 96 well plate.
7. Read samples at 558nm.

Appendix G

Total Heme Pigment Quantification

0.04 M Phosphate Buffer, pH 6.8 (1 L)
- 3.46g of Dibasic Potassium Phosphate
- 5.48g of Monobasic Potassium Phosphate
*add phosphoric acid to make more acidic, or sodium hydroxide to make more basic
*measure pH at temperature that will be used (ice cold)
*Shelf-stable at 20° C for ~12 months

6.6 mM Potassium Ferricyanide and 8.8 mM Sodium Cyanide (50 mL)

- 0.1086g of Potassium Ferricyanide
- 0.0216g of Sodium Cyanide
*Make fresh, store in dark bottle

Total heme pigments were quantified as described by Warris (1979), with slight modifications.

1. Collect muscle sample 24-48 hours postmortem and store at < -20°C.
2. Pulverize sample in by cutting and then mashing with mortar and pestle.
3. Weigh out duplicate 2 g samples into 50 mL centrifuge tubes.
4. Homogenize samples in 10 mL of ice-cold 0.04 M phosphate buffer @ pH 6.8 for 20 seconds.
5. Let homogenate stand for 1 hour at 4°C (ice bath).
6. Centrifuge at 6500 x g for 10 minutes at 4°C.
7. Remove 1 mL aliquot of supernatant (collect 24 aliquots before addition of cyanide compounds); mix with 0.1 mL of 6.6 mM potassium ferricyanide and 8.8 mM sodium cyanide.
8. Immediately after, centrifuge samples (24 total) at 30,000 x g for 1 hour at 15°C
9. Place duplicate 200 µL aliquots of supernatant in a 96 well plate and measure absorbance at 540 nm using microplate reader.
10. Total Heme pigment concentration expressed as mg of total pigment/g of tissue was calculated as follows:

A_{540}/[11,300 $L*mol^{-1}*cm^{-1}$(0.6217 cm)] [(17,500g/mol*0.0132 L*1000 mg/g)/2 g]
11,300 is the molar extinction coefficient for cyanmetmyoglobin (Drabkin, 1950)
0.6217 cm is the light path
17,500 g/mol is the molecular weight of myoglobin (Low & Rich, 1973)
0.0132 L is the equivalent volume of diluted aqueous sample
2 g is weight of sample used for extraction.

Correction factor = A_{540} * 16.44