Evaluation of the role of marbling texture on beef palatability

by

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Abstract

The objective of this research was to evaluate the role of marbling texture on beef palatability, muscle histology, and collagen characteristics of beef strip loin steaks. Beef strip loins (n = 117) were selected from three quality grade treatments [Top Choice (Modest^{00} – Moderate^{100} marbling), Low Choice (Small^{0} – Small^{100} marbling), and Select (Slight^{0} – Slight^{100} marbling)] to equally represent three different marbling texture groups: fine, medium and coarse, via visual appraisal with the USDA marbling texture standards. Consumers (n = 104) rated all marbling texture groups similar ($P > 0.05$) for tenderness, juiciness, flavor, and overall liking, as well as rated a similar ($P > 0.05$) percentage of samples from each marbling texture group acceptable for each palatability trait. Moreover, consumers indicated no preference ($P > 0.05$) among marbling texture groups for visual desirability or likelihood to purchase. There were no differences ($P > 0.05$) among marbling texture treatments for Warner-Bratzler shear force, slice shear force, and pressed juice percentage. However, trained sensory panelists rated coarse marbled steaks higher ($P < 0.05$) than fine or medium marbled steaks for both beef flavor intensity and sustained juiciness as well as higher ($P < 0.05$) for initial juiciness than medium textured steaks. This minimal impact on palatability was further supported through evaluation of muscle histology and collagen traits. Marbling texture did not affect collagen characteristics, as coarse marbled steaks were similar ($P > 0.05$) to both fine and medium marbled steaks for soluble collagen, insoluble collagen, and total collagen content. Furthermore, all marbling texture groups (fine, medium, and coarse) performed similarly ($P > 0.05$) during the peak thermal transition phase of the perimysial fraction of collagen. However, marbling texture impacted ($P < 0.05$) adipocyte cross-sectional area, where coarse steaks had larger adipocytes in comparison to fine marbled steaks, but medium marbled steaks were similar ($P > 0.05$) to both

coarse and fine marbled steaks. Similarly, quality grade affected adipocyte size, as Top Choice and Low Choice possessed larger ($P < 0.05$) adipocytes than Select steaks. However, marbling texture did not impact ($P > 0.05$) perimysial thickness. Additionally, marbling texture did not affect the percentage of myosin heavy chain (**MHC**) Type I fibers within each steak. However, medium marbled steaks possessed a greater ($P < 0.05$) percentage of MHC Type 2A fibers than both fine and coarse marbled steaks. The opposite trend was displayed in the percentage of MHC Type IIX fibers, as fine and coarse marbled steaks possessed more ($P < 0.05$) MHC Type IIX fibers in comparison to medium marbled steaks. There were no differences ($P > 0.05$) among quality grades for fiber type or marbling texture and quality grade for fiber cross-sectional area. Results from this study indicate marbling texture has minimal impact on eating quality and muscle histology; therefore coarse marbled carcasses should not be excluded from current and future branded beef programs.

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Dedication

This thesis is dedicated to my parents, Steven and Dawn. Thank you for your love and support through this process.

Chapter 1 - Review of Literature

Palatability defined

Palatability is defined as the overall eating experience surrounding a food product; in beef products, this typically focuses on tenderness, juiciness, and flavor, in addition to their interaction (Smith and Carpenter, 1974; Platter et al., 2003; Drey and O'Quinn, 2017). After reviewing 11 studies focusing on consumer preferences of beef with similar 100 mm line scales, Drey and O'Quinn (2017) developed a model of consumer overall liking, where tenderness was responsible for 42%, juiciness was responsible for 7%, and flavor was responsible for 48% of consumer overall liking ($r^2 > 0.99$). Additionally, Drey and O'Quinn (2017) reported that samples that are deemed unacceptable for a single palatability trait dramatically increase the probability of overall failure (tenderness: 69% likely to fail for overall liking, juiciness: 66%, or flavor: 76%). Similarly, O'Quinn et al. (2012) reported strong, positive correlations of tenderness ($r = 0.76$), juiciness ($r = 0.73$), and flavor ($r = 0.88$) to consumer overall liking ratings of beef strip loin steaks of varying fat levels. This was further echoed in several different muscles, including the longissimus lumborum (**LL**), psoas major (**PM**), semimembranosus (**SM**), and gluteus medius (**GM**), where Legako et al. (2015) reported similar correlations for tenderness ($r = 0.79$), juiciness ($r = 0.75$), and flavor ($r = 0.85$) to consumer overall liking. This indicates that these traits and each interaction are drastically important to the consumer's eating experience (Drey and O'Quinn 2017).

In past research, tenderness had been considered the most important palatability trait and has received the most attention from a research standpoint (Dikeman, 1987; Savell et al., 1987; Morgan et al., 1991; Miller et al., 1995; Huffman et al., 1996; Miller et al., 2001; Platter et al., 2003). However, as today's beef supply has become more reliably tender through advancements

in technology and genetic selection, consumers have begun to place a greater emphasis on flavor and juiciness (Guelker et al., 2013; Igo et al., 2013). When asked which palatability trait was the most important when eating beef, consumers have shifted from tenderness to flavor. In past studies of the late 1990's- early 2000's, tenderness had been rated most important 51% of the time and flavor only 39% of the time (Huffman et al., 1996). However, more recently, Lucherk et al. (2016) and Corbin et al. (2015) determined that approximately 50% of consumers identify flavor as the most important, compared to tenderness at 39.3% (Lucherk et al., 2016) and 30.8% (Corbin et al., 2015). Flavor has been reported to be highly correlated to overall palatability, once tenderness is deemed acceptable (Goodson et al., 2002; Behrends et al., 2005; O'Quinn et al., 2012; Legako et al., 2015; Lucherk et al., 2016).

Marbling texture defined

Marbling texture is defined as the size of individual marbling flecks present within the muscle. This is often assessed in the ribeye muscle during evaluation for USDA quality grade. Marbling texture groups are defined by the USDA-AMS-LS-SB-02 marbling texture reference card, which identifies marbling textures into three categories: fine, medium, and coarse. In addition to visual appraisal, instrumental grading systems possess the ability to assess marbling texture within the ribeye (McKenna et al., 2016). It is not clear when the marbling texture standard began, as marbling texture is not mentioned in the USDA Grading Standards, and therefore not referenced within the history of the grading system (USDA, 2016b). For all certified programs requiring a fine or medium marbling texture specification, the USDA-AMS-LS-SB-02 marbling texture card is used to assess and assure the specification is met for the particular branded beef program (USDA, 2016a). Additionally, marbling texture is considered as a factor within evaluation of meat products, as fine, more evenly distributed marbling is

preferred in all beef products over coarse textured marbling, according to the American Meat Science Association's (**AMSA**) Meat Evaluation Handbook (Smith and Griffin, 2001).

Marbling texture effects on palatability

Only one study has investigated marbling texture's role in beef palatability (Moody et al., 1970). In this study, fine marbled beef ribs were lower in Warner-Bratzler shear force (**WBSF**) scores and lighter in color in comparison to coarse marbled beef ribs. However, in this study, the authors used roasts instead of steaks for WBSF determination. Additionally, these roasts were oven roasted with 2.54 cm diameter cores removed from the longissimus for WBSF determination. However, in trained sensory panel evaluation, differences in flavor, sensory tenderness, juiciness, and overall satisfaction were not different. This sensory sample was obtained from between the 6th and 8th ribs. The authors speculated the differences in WBSF may have been attributed to increased levels of perimysial connective tissue in coarse marbled ribs, however, they did not measure this attribute (Moody et al., 1970). In more recent research, perimysial connective tissue thickness has not been a reliable indicator of tenderness (Brooks and Savell, 2004). The research presented by Moody et al. (1970) has been the basis for the specification for fine or medium textured marbling in 75% of the 119 branded beef programs currently supervised by the USDA (USDA, 2017). As the first and one of the largest branded beef programs, Certified Angus Beef (**CAB**) requires fine or medium textured marbling (Bass, 2016). The basis for this marbling texture specification suggests fine or medium textured marbling offers a more even distribution of the marbling, resulting in a more consistent product throughout and is rooted in the findings of Moody et al. (1970) (Bass, 2016). Due to this program's level of success, coupled with the fact that it was the first branded beef program, it

may have resulted in a ripple effect, causing other and future programs to adopt this specification in their requirements, regardless of scientific-based justification.

In a study evaluating the effects of marbling and maturity on beef sensory evaluation of steaks, Goll et al. (1965) observed a significant, negative correlation (r = -0.359) between marbling distribution and texture (as one trait) and WBSF of rib steaks from the 12th rib, in a method similar to Moody et al. (1970), using three 2.54 cm cores. However, instead of oven roasting, Goll et al. (1965) used broiling as a cooking method. Finer, more evenly textured marbled steaks were associated with higher tenderness ratings in trained sensory panels (Goll et al., 1965). Furthermore, Goll et al. (1965) also reported significant, positive correlations for initial (r = 0.366) and residual tenderness (r = 0.299) in trained panel evaluation of these steaks with marbling distribution and texture, as well as positive correlations for juiciness (r = 0.132) and flavor (r = 0.169). Despite these positive correlations between palatability traits and fineness of marbling, the authors concluded there was not a strong enough correlation to indicate marbling texture and distribution as a predictor of palatability.

Marbling texture

Other studies have reported marbling texture scores; however, no comparisons between texture groups were made with palatability ratings in most of these published reports (Cross et al., 1975; Cross, 1977; Dubeski et al., 1997; Mello et al., 2012a; Mello et al., 2012b; Durunna et al., 2014). When evaluating the effect of amount, distribution, and texture of marbling on cooking properties of beef, Cross (1977) used four marbling texture groups (very fine, fine, coarse, and very coarse). The author determined there were no significant differences between marbling texture groups in percent fat or percent moisture. However, very fine marbled ribs exhibited a significantly lower cook loss in comparison to fine marbled ribs, however, very fine

marbled ribs were similar to both very coarse and coarse ribs for cooking loss (Cross, 1977). This indicates that despite visual marbling texture differences, marbling texture does not have a significant impact on the amount of moisture or lipids available for palatability traits such as juiciness and flavor.

From a physiological standpoint, marbling texture has been evaluated as an effect of various feeding regimes, finishing weights, and genetic influence (Dubeski et al., 1997; Mello et al., 2012a; Mello et al., 2012b; Durunna et al., 2014). However, the effects of diet and genetics are not clear due to conflicting results among studies. Marbling deposition is impacted by breed composition of cattle, where Bos taurus breeds, especially British breeds, have increased marbling levels in comparison to their Bos indicus counterparts (Wheeler et al., 1994). However, few studies have evaluated breed impact on marbling texture. In a Canadian study evaluating feeding cattle barley grain and silage to heavier weights to improve marbling traits for various carcass grading systems, Dubeski et al. (1997) reported Angus yearling heifers to have coarser marbling in comparison to Hereford, Hereford x Angus cross, and Holstein heifers. However, this breed effect did not occur in an additional experiment in calf-fed feedlot heifers (Dubeski et al., 1997). Similarly, in a German study where cattle were fed a combination of corn and grass silage in addition to barley grain, Albrecht et al. (2006) reported larger marbling flecks present in German Angus cattle in comparison to Holstein and Galloway cattle from 12-24 mo of age. Additionally, Holstein cattle produced a greater amount of marbling flecks in comparison to the German Angus cattle, which indicates that Holstein cattle produced carcasses with finer, more distributed marbling. In a similar study comparing Holstein and Japanese Black cattle harvested at 26 mo of age, Albrecht et al. (2011) reported Japanese Black cattle had larger marbling flecks and a higher percent marbling fleck area, which resulted in a higher intramuscular fat content.

However, the breeds were similar for number of marbling flecks possessed. In terms of intramuscular adipocyte size, no differences were reported between Japanese Black or Holstein cattle at 26 mo of age. Collectively, these studies indicate that breeds and genetic backgrounds of cattle do play a role and can have a significant impact on marbling texture, with Angus and Japanese Black cattle possessing larger, coarser flecks of marbling. Moreover, Holstein cattle produce ribeyes with smaller flecks of marbling and in some instances, more individual flecks of marbling.

In addition to genetics, feeding regimes also have a significant effect on marbling deposition. It is well documented that finishing cattle on high concentrate, grain based diets improves marbling levels and therefore quality grades (Tatum et al., 1980; Dolezal et al., 1982; Savell and Cross, 1988). When feeding calf-fed feedlot heifers to three weights, Dubeski et al. (1997) found no significant differences in marbling texture between the three different end point weights. However, heifers fed a restricted diet were coarser in marbling texture compared to cattle fed to a higher plane of nutrition (Dubeski et al., 1997). In an additional experiment using yearling feedlot heifers, Dubeski et al. (1997) reported no differences in marbling texture between the two ending weights nor between accelerated and restricted feeding programs. Contrastingly, when evaluating the effects of calving season and feeding system on the carcass characteristics of crossbred steers, Durunna et al. (2014) determined significant differences in marbling texture for feeding systems. Calves that were fed at a slow rate exhibited a lower percentage of coarse marbling flecks when compared to calves fed in a rapid feeding system, which is contrasting to the findings of Dubeski et al. (1997), which found that restricted fed cattle displayed a greater amount of coarse marbling. Additionally, when evaluating the effects of feeding modified distillers' grains with solubles on marbling attributes of beef cattle, Mello et

al. (2012a) determined USDA Choice carcasses were significantly coarser in marbling compared to USDA Select carcasses from cattle fed similar amounts of modified distillers' grains with solubles. In a similar study, Mello et al. (2012b) determined that cattle fed differing levels of wet distillers' grains did not exhibit significantly different marbling textures amongst dietary treatments. These results indicate that diet and feeding regime's impact on marbling texture is variable and may impact different groups of cattle differently, which indicates more research in needed to offer a definite effect on marbling texture.

Marketing effects of marbling texture

In the United States, fine or medium marbling is a specification for 75% of the branded beef programs supervised by the USDA-Agricultural Marketing Service (USDA, 2016a). Within these programs' specifications, each requires marbling texture to be assessed using the USDA-AMS-LS-SB-02 marbling texture card. However, within the USDA beef grading standards, marbling texture is not mentioned once as a determining factor of quality grade (USDA, 2016b). Goll et al. (1965) reported a weak, positive correlation ($r = 0.142$) between marbling distribution and texture with marbling scores, which indicates that coarser marbled cattle will achieve higher quality grades.

In Japan, fine marbling is preferred, as consumers prefer their beef to be marbled like frost and is known as "ko-zashi" (Motoyama et al., 2016). Beef with fine marbling sells at a much higher premium than coarser marbled beef. Coarse marbling is known as "oo-zashi" and priced lower in comparison to their fine marbled counterparts (Motoyama et al., 2016). Similar to the United States grading standards, marbling texture is not mentioned within the Japanese beef grading standards (Polkinghorne and Thompson, 2010). Additionally, according to the Japanese Ministry of Agriculture, Forestry and Fisheries, the Japanese equivalent to the USDA,

there are no premiums for finely marbled beef in comparison to coarsely marbled beef in market reports, though antidotal evidence suggests otherwise (Motoyama et al., 2016). Due to this anecdotal preference for marbling texture, cameras and indices are being developed to more objectively measure marbling texture (Gotoh et al., 2014)

Marbling effects on palatability

Intramuscular adipose tissue, commonly known as marbling, is located between the perimysial connective tissue along muscle bundles (Moody and Cassens, 1968). Adipocytes arise from multipotent mesenchymal stem cells present within the skeletal muscle during fetal muscle development (Du et al., 2010; Du et al., 2013). These multipotent stem cells are primarily composed of cells from two lineages: myogenic and adipogenic-fibrogenic (Du et al., 2013). Recently, studies have shown that intramuscular adipocytes and fibroblasts are developed from common progenitor cells (Joe et al., 2010; Uezumi et al., 2011; Du et al., 2013). The majority of these cells will be differentiated into skeletal muscle tissue, however, some will instead differentiate into adipocytes, creating the basis needed to create intramuscular fat, or marbling (Du et al., 2010). In beef cattle, intramuscular adipocytes are the last to be formed, but are detectable at 180 d of gestation (Du et al., 2013). As cattle mature and age, adipocytes grow through post-natal hypertrophy, however, it is challenging to feed cattle to an optimum combination of both an ideal quality grade and an ideal yield grade, as subcutaneous fat is deposited prior to marbling and continues to be deposited while marbling is also deposited (Du et al., 2013). However, no research has investigated the effects of manipulating the process of adipogenesis on marbling texture or the size of flecks deposited.

Marbling is one of the two main components of quality grades, which are the United States' industry standard of palatability. For similar maturity levels, different levels of marbling

indicate a different quality grade. It has been well documented in published literature that increasing marbling levels also increases the proximate fat percentage, as Standard possesses the lowest percentage of fat (1.3-2.5%), followed by Select (2.5-4.5%), Low Choice (4.5-5.8%), Top Choice (the upper 2/3rds of the Choice grade) (6.0-9.0%), and Prime, which possesses the highest percentage of fat (10.4-14.8%) (Gilpin et al., 1965; Parrish et al., 1973; Savell et al., 1986; Luchak et al., 1998; Dow et al., 2011; Smith et al., 2011; O'Quinn et al., 2012; Emerson et al., 2013; Hunt et al., 2014; Corbin et al., 2015; Legako et al., 2015; Lucherk et al., 2016).

Marbling has been well established in literature as an indicator of palatability of beef products (Luchak et al., 1998; O'Quinn et al., 2012; Corbin et al., 2014; Lucherk et al., 2016). Marbling aids in both tenderness and juiciness through the lubrication theory, which states that marbling present in and around the muscle fibers and perimysial connective tissue during mastication, and therefore, results in a more tender and juicy product (Smith and Carpenter, 1974; Savell and Cross, 1988). Additionally, tenderness is aided by marbling through two other theories: the bite and the strain theory (Smith and Carpenter, 1974; Savell and Cross, 1988). The bite theory proposes that marbling reduces the effort required to shear or bite through a piece of cooked meat, due to fat being less dense than denatured and coagulated protein (Smith and Carpenter, 1974; Savell and Cross, 1988). Moreover, the strain theory suggests that as marbling is deposited within the endomysium and perimysium, it places tension on those connective tissue layers, resulting in reduced strength and splintering, which creates a more tender product (Smith and Carpenter, 1974; Savell and Cross, 1988). This has been confirmed from a histological view, as Nishimura et al. (1999) observed splintering of the endomysium and perimysial connective tissue layers in high marbled Japanese Black cattle at 32 mo of age, which was reflected as a more tender product. Furthermore, in addition to an increase in eating quality, increased

deposition of marbling has been correlated to reduced WBSF values and increased tenderness ratings (Ueda et al., 2007; Dubost et al., 2013; Wilfong et al., 2016).

Moreover, marbling has been reported as a main contributor to sustained juiciness of meat products (Pearson, 1966; Smith and Carpenter, 1974; Savell and Cross, 1988). Juiciness is enhanced by marbling (Smith and Carpenter, 1974; Savell and Cross, 1988). The increased water-holding capacity acts similarly to the lubrication theory, as the extra lipid and water work to lubricate muscle fibers and therefore reduce toughness (Smith and Carpenter, 1974; Savell and Cross, 1988). Finally, marbling also impacts juiciness by increasing salivary flow during mastication through the increased initial juiciness from cooking (Smith and Carpenter, 1974; Savell and Cross, 1988).

Flavor is also significantly impacted by marbling, but it is a complicated relationship. Marbling is responsible in part for the species-specific flavors present within a meat product, especially in pork and beef (Smith and Carpenter, 1974; Savell and Cross, 1988). However, increased marbling levels have not been repeatedly shown to result in increased volatile flavor compounds produced as a result of cooking (Cross et al., 1980; Mottram et al., 1982; Mottram and Edwards, 1983; Legako et al., 2015). Additionally, in the longissimus, consumer flavor ratings have been typically moderately correlated (r = 0.25; 0.37; 0.27) with intramuscular fat percentage (O'Quinn et al., 2012; Hunt et al., 2014; Legako et al., 2015). Contrastingly, in trained panel evaluation, Emerson et al. (2013) reported a strong correlation between instrumental camera marbling scores and buttery/beef fat flavor (r = 0.84).

As marbling levels increase, it is well documented in published literature that both trained panel ratings and consumer overall liking ratings also increase (Davis et al., 1979; Smith et al., 1985; Savell et al., 1986; O'Quinn et al., 2012; Emerson et al., 2013; Corbin et al., 2015; Lucherk

et al., 2016). However, to be acceptable for palatability, marbling levels must be above 3% in uncooked cuts from the rib and loin, which is indicative of the Slight marbling score (Savell and Cross, 1988). Both consumers and trained panelists rated steaks or cuts below 3% intramuscular fat as significantly drier, tougher, and less flavorful compared to steaks above the 3% threshold (Savell and Cross, 1988). However, this acceptability level was determined from a view of how much fat should be available in meat products to satisfy the requirements of a quality eating experience that is balanced with an amount of fat that falls below the limit for health issues driven by increased cholesterol (Savell and Cross, 1988). Despite being above the 3% threshold, there is still a chance that steaks can fail for overall liking. When evaluating 11 consumer studies on similar 100 mm line scales, O'Quinn (2016) reported Standard and Select steaks had the lowest percentage of steaks rated as acceptable for overall liking (72.04%; 74.75%) and were lower than Low Choice (83.08%), Premium Choice (upper 2/3rds of Choice grade) (86.83%), and Prime (91.37%) steaks. This indicates that the 3% fat window for acceptability is not absolute, and also indicates that as marbling level increases, consumers are more likely to rate samples higher for individual palatability traits and overall liking.

When evaluating consumer preferences of marbling levels, it is well established that steaks with increased levels of marbling are preferred in comparison to less marbled steaks for palatability traits (Savell et al., 1987; Savell and Cross, 1988; O'Quinn et al., 2012; Hunt et al., 2014; Corbin et al., 2015; Legako et al., 2015; Lucherk et al., 2016). The effects of marbling score and quality grade have been evaluated over a wide variety of muscles, including in the LL, GM, PM, SM, and serratus ventralis (**SV**) (Hunt et al., 2014; Legako et al., 2015). As marbling levels increase, consumer ratings of each palatability trait within each muscle improve, with the exception of the SM (Hunt et al., 2014; Legako et al., 2015).

Similarly in trained panel evaluation, Davis et al. (1979) observed that Choice strip steaks with higher marbling levels and increased fat percentages were more likely to be rated as tender. Steaks rated as very tender possessed approximately 7.6% fat whereas slightly tough steaks possessed only 4.4% fat (Davis et al., 1979). Additionally, Emerson et al. (2013) reported significantly higher ratings of tenderness, juiciness, meaty/brothy flavor, and buttery/beef flat flavor as marbling levels increased in steaks. This resulted in positive correlations with juiciness ($r = 0.67$), tenderness ($r = 0.63$), and overall sensory experience ($r = 0.84$). Similarly, in the longissimus thoracis (**LT**), increased intramuscular lipids also resulted in increased sensory tenderness and overall liking ratings (Dubost et al., 2013).

In regards to instrumental measurements of palatability, marbling level has an inverse relationship with WBSF and slice shear force (**SSF**). Warner-Bratzler shear force was developed in 1932 as a mechanical determination of tenderness. Samples are cooked, then cooled overnight (Bratzler, 1932). Following cooling, steaks have 6 1.27 cm cores removed parallel to the muscle fibers, which are then sheared perpendicular to the muscle fibers (Bratzler, 1932). Contrastingly, SSF is performed on a warm sample just after cooking (Shackelford et al., 1999a). The sample from SSF is removed from the lateral end of the steak and cut with a double bladed knife at a 45 degree angle through a cutting guide, then sheared perpendicular to the fibers (Shackelford et al., 1999b). This method was developed as a more efficient, rapid method to be used in processing plants in comparison to WBSF (Shackelford et al., 1999a). However, of the two methods, the most universally recognized is WBSF, as most packers are disinclined to remove a valuable portion of the loin that could be instead sold for profits (Derington et al., 2011).

Many studies have evaluated the effect of marbling level on these measurements over the complete range of quality grades from Prime to Standard (Tatum et al., 1980; Savell et al., 1987).

Within these studies, WBSF was reduced by 20.5-34.9% as quality grade increased from Select to Prime (Tatum et al., 1980; Smith et al., 1985; Savell et al., 1987; Emerson et al., 2013). Additionally, Emerson et al. (2013) reported significant, moderately negative correlations of WBSF (r = -0.48) and SSF (r = - 0.45) to marbling levels. This was a result of a 34.9% reduction in WBSF value and a 35.2% reduction in SSF value with an increase in marbling score from Traces (Standard) to Moderately Abundant (Prime) (Emerson et al., 2013). Similarly, Lucherk et al. (2016) reported when cooked to a medium degree of doneness, Prime and Top Choice steaks were lower in SSF values in comparison to Select and Standard steaks.

Due to the increased and more reliable eating experience from higher marbling levels in steaks, steaks that possess increased marbling scores are awarded a premium. From 2012-2014, on the packer level, Prime beef was rewarded with an average of $13.64/cwt premium (Tatum, 2015). Similarly, carcasses within the upper 2/3rds of the Choice grade also received a premium of $3.63/cwt average premium (Tatum, 2015). As the basis of the average U.S. beef pricing grid, there were no premiums or discounts for Low Choice carcasses (Tatum, 2015). However, Select carcasses were discounted an average of $10.09/cwt (Tatum, 2015). Standard carcasses, which are not typically marketed on a retail basis, were the most heavily discounted at $24.27/cwt (Tatum, 2015).

To no surprise, this increase in carcass price translated to an increase in retail prices of graded product. In retail studies assessing beef product prices in Denver, CO, Oklahoma City and Tulsa, OK, Ward et al. (2008) determined that USDA Prime steaks and roasts were priced $1.37/lb (18.9%) more in comparison to those with no quality designation. Choice steaks and roasts were also priced higher than those with no quality designation, however it was a smaller premium of $0.70/lb (10.7%). Similarly, Killinger et al. (2004b) used experimental auction

techniques and reported that in both Chicago and San Francisco panelists were willing to pay more for Prime steaks in comparison to steaks with lower marbling levels. However, in comparison to Ward et al. (2008), consumers in San Francisco were willing to pay $1.47/lb more for Prime steaks (Killinger et al., 2004b). However, consumers in Chicago, a Midwestern city more similar to Oklahoma City and Tulsa for quality grade purchasing decisions, the magnitude of willingness to pay for Prime steaks was reduced to $0.24-$1.13/lb premium. Platter et al. (2005) also reported consumers were willing to pay more for higher quality steaks. For Select strip steaks, consumers were willing to pay approximately $5.37/kg, but this price increased with marbling levels (Platter et al., 2005). Consumers were willing to bid 31.5% more for Prime steaks for a total price of $7.84/kg, or a premium of $2.47/kg when compared to Select steaks (Platter et al., 2005). Similarly, Umberger et al. (2000) reported consumers that preferred Choice beef for flavor and overall acceptability were willing to pay $1.30/pound more for Choice steaks. Additionally, consumers who preferred Select steaks for flavor and overall acceptability were willing to pay an additional $1.63/pound for those steaks (Umberger et al., 2000). These results indicate that consumers are willing to pay more for their preferred meat products, regardless if they prefer steaks with more marbling or less marbling. It is important to note that in each of these studies, consumers were blinded to the visual aspects of the steak, including color, marbling levels, and external fat, so these prices were solely based on palatability attributes.

Consumer visual ratings of marbling levels

In the United States, marbling levels are typically ranked below price and color as purchasing priorities for consumers (Lusk and Fox, 2000; Claborn et al., 2011; Lucherk et al., 2016; Wilfong et al., 2016). However, this reduced priority toward purchasing steaks based on marbling level is not just limited to the United States. Consumers in the United Kingdom prefer

steaks within the Slight marbling score in comparison to those present in the Modest (Lusk et al., 2003). This may be attributed to the demonization of animal fat from cattle and pigs in human diets (Frank et al., 2016). These animal fats have been cast in a negative light due to increased saturated fat levels in comparison to other species such as fish, which contain higher levels of healthier fats, such as omega-3 fatty acids (Frank et al., 2016).

Due to this health-related phobia, consumers may visually prefer steaks with less marbling, but from a blinded palatability standpoint, prefer steaks of higher quality grades due to increased eating quality and satisfaction. In addition to health concerns, consumers may also be uninformed as to what quality grades indicate and how the grades impact eating experience. On a grade name basis, DeVuyst et al. (2014) reported 57.1% of consumers thought Prime was the leanest quality grade and 43.9% of consumers thought Select was the fattest grade. However, when asked which grade was the juiciest, the majority of consumers were able to identify grades correctly. Approximately 55.6% of consumers selected Prime as the juiciest with 46% of consumers choosing Select as the driest grade (DeVuyst et al., 2014). When viewing pictures of ribeyes depicting Prime, Choice, and Select quality grades, 54.4% of consumers indicated the Prime ribeye to be Select and 53.5% of consumers indicated the Select ribeye to be the Prime grade (DeVuyst et al., 2014). This misunderstanding of grades contributes to consumer visual preferences for Select steaks (Lusk and Fox, 2000; Killinger et al., 2004a; Claborn et al., 2011).

From a visual standpoint, consumers tend to prefer less marbled steaks in direct contrast to consumer palatability ratings (Lusk et al., 2003; DeVuyst et al., 2014). Killinger et al. (2004a) asked consumers in Chicago and San Francisco to rate steaks in a retail case that were contrasting in both marbling levels and color. Consumers preferred steaks with low levels of marbling in comparison to high marbled steaks. Additionally, those consumers were willing to

pay $1.12 more per 0.45 kg for the low marbled steaks. In contrast, consumers with a greater marbling preference were only willing to pay an extra $0.80 per 0.45 kg for steaks with a higher level of marbling. This sentiment towards a preference of lower levels of marbling was echoed in a study done by Lusk and Fox (2000), where marbling was determined to be less important to consumers in comparison to hormone use (23.34%), tenderness (23.71%), and price (24.57%) of beef ribeye steaks.

Additionally, in a study evaluating the purchasing habits of consumers of strip loin steaks of varying quality grades, Claborn et al. (2011) reported that out of 161 consumers, 95 consumers purchased Select steaks in comparison to both Choice (n = 40) and CAB (n = 56) steaks. Furthermore, behind price (61%) and color (17%) of the product, the reduced amount of marbling was reported as a priority (11.8%) for consumers' purchasing decisions (Claborn et al., 2011). However, for over one-third of consumers purchasing CAB steaks, the increased level of marbling was the largest factor for purchasing (Claborn et al., 2011). This same effect was seen in a study conducted by Umberger et al. (2000), where both Chicago and San Francisco based consumers preferred Choice steaks for overall acceptability, however, Chicago consumers were only willing to pay an additional $0.25/pound for high marbled steaks, despite their typical preference for Choice steaks. In comparison, San Francisco consumers, who typically prefer Select steaks, were only willing to pay an additional $0.03/pound for Choice steaks (Umberger et al., 2000).

Muscle fiber morphometrics effect on beef palatability

From a biological standpoint, several factors have been linked to a reduction in beef palatability, specifically fiber-cross sectional area. As fiber cross-sectional area increases, there is a negative correlation of fiber size with sensory tenderness scores (r = -0.25; -0.12; -0.14)

from trained sensory panelists (Seideman et al., 1987; Crouse et al., 1991; Chriki et al., 2013). Similarly, several studies have reported a positive correlation (r = 0.16-0.37) between fiber cross-sectional area and WBSF values in the longissimus (Tuma et al., 1962; Chriki et al., 2013; Ebarb et al., 2016). Tuma et al. (1962) also reported a negative significant correlation in trained sensory panel ratings of tenderness with fiber diameter in the longissimus dorsi (**LD**; r = -0.41). In Waygu cattle, a breed well known for marbling, Duarte et al. (2013) reported greater muscle fiber diameter in the sternomandibularis muscle in comparison to Angus cattle. Albrecht et al. (2011) reported similar results in Japanese Black cattle at 14 mo of age displaying a greater fiber cross-sectional area in comparison to Holstein cattle. Contrastingly, at 26 mo of age, Albrecht et al. (2011) reported Japanese Black cattle to have reduced fiber size in comparison to their Holstein counterparts.

Individual fiber types also readily affect fiber cross-sectional area. Within the adult bovine muscle structure, there are three main fiber types present based on individual contraction speed and metabolic processes (Schiaffino et al., 1989; Chikuni et al., 2004; Schiaffino and Reggiani, 2011). Each fiber type is defined by the myosin heavy chain isoforms (**MHC**) (Schiaffino et al., 1989). In adult beef cattle, this consists of MHC Type I, slow-twitch, oxidative fiber, MHC Type IIA, a fast twitch transitional, oxidative-glycolytic fiber, and MHC Type IIX, a fast-twitch glycolytic fiber. Typically, the largest fiber is MHC Type IIX, followed by MHC Type IIA, and MHC Type I as the smallest fibers (Maltin et al., 2003). Type I fibers are typically the smallest due to their oxidative nature (Maltin et al., 2003). Hwang et al. (2010) reported in Hanwoo cattle, a Korean breed known for their extreme marbling, there was a negative correlation (r = -0.40) between WBSF and MHC Type I fiber cross-sectional area, however, both MHC Type IIA and MHC Type IIB fibers were positively correlated (r = 0.21; 0.45) with

WBSF. This supports the theory that as fiber cross-sectional area increases, there is a simultaneous increase in WBSF (Hwang et al., 2010). Additionally, the fiber type profile is muscle dependent (Kirchofer et al., 2002). In previous research using succinate dehydrogenase staining and ATPase assays, the LD has been classified as a white muscle, as it possesses a greater percentage of α-white (MHC Type IIX) fibers at 43.2-46% in comparison to both β-red (MHC Type I) at 29.3-35% and α-red (MHC Type IIA) at 21.8-24.7% (Hunt and Hedrick, 1977; Kirchofer, 2002). This signifies the LD is a muscle with a faster contraction speed and more glycolytic in its metabolism in comparison to other muscles, such as the PM, which has a higher percentage of β-red fibers (52.4%) and lower levels of both α-red (14.9%) and α-white fibers (32.7%) in comparison to the LD (Hunt and Hedrick, 1977; Kirchofer et al., 2002). However, when using immunofluorescence staining techniques in the LD, both Phelps et al. (2014) and Ebarb et al. (2016) reported a greater amount of MHC Type IIA fibers in comparison to MHC Type IIX fibers, which indicates the LD may be considered a red muscle that is more oxidative in its metabolic nature.

Adipocyte size

Adipocytes are the basis for intramuscular fat, or marbling (Moody and Cassens, 1968). As adipocytes increase in size, it results in increased marbling levels, and therefore, increased quality grades (Moody and Cassens, 1968). Moody and Cassens (1968) determined that as marbling score increased from Traces to Moderate, there was a significant increase in adipocyte cell size. Similarly, Cianzio et al. (1985) reported a strong, positive significant correlation between marbling score and adipocyte diameter (r = 0.73) as well as total adipocyte number (r = 0.68). In a German study using Oil Red O staining and computer analysis to measure adipocyte size, Yang et al. (2006) used F_2 German Holstein and Charolais crossbred bulls to evaluate the

correlation between fat depositions and intramuscular adipocyte traits. Intramuscular fat content was strongly, positively correlated (r = 0.71) to intramuscular adipocyte area, which indicates that fat content increases as the adipocytes increase in overall size. A similar correlation was reported (r = 0.70) between intramuscular fat content and the proportion of marbling fleck area. However, intramuscular adipocyte size was only moderately positively correlated (r = 0.44) to number of marbling flecks. Additionally, a similar correlation existed with intramuscular adipocyte area to proportion of marbling fleck areas (r = 0.62) As fat deposition increases, adipocytes increase in both size and number, which contributes to increased marbling levels and quality grade through increasing both size and number of flecks present for evaluation.

In research, there have been a variety of different methods employed to measure the area of adipocytes, including an ocular grid (Moody and Cassens, 1968), Oil Red O staining, (Albrecht et al., 2006; Yang et al., 2006; Albrecht et al., 2011), and Masson's trichrome staining (Yan et al., 2010). Masson's trichrome staining method for adipocyte size and number has been fairly limited in use within animal science, as it was developed to distinguish between collagen and smooth muscle in tumors, as well as to detect collagen infiltration in human organs, such as effects from cirrhosis (Foot, 1933). In animal science, it has been primarily used to detect infiltration of collagen and adipocytes in white striping of chicken breasts (Kuttappan et al., 2013) and adipocyte size as a measurement of maternal obesity in ewes (Yan et al., 2010).

Perimysial connective tissue effects on palatability

The perimysial layer of connective tissue is located surrounding the bundles of muscle fibers and is primarily composed of collagen, with a small amount of elastin (Nishimura, 2010). This connective tissue layer is highly variable between breeds, age, species, and muscle (Morgan et al., 1991; Brooks and Savell, 2004; Purslow, 2005). Additionally, the perimysial layer creates

the texture of the muscle, as muscles such as the semitendinosus have a much coarser texture in comparison to the finer muscle texture of the longissimus dorsi or psoas major (Light et al., 1985; Brooks and Savell, 2004; Purslow, 2005). This connective tissue layer is responsible for the reduced tenderness seen as "background" or "sustained" tenderness (Smith and Carpenter, 1974). However, as a sole indicator of beef tenderness through WBSF, Brooks and Savell (2004) found perimysial thickness to be poorly correlated (r = 0.17) to WBSF, but positively correlated to muscle (r = 0.47).

In a study evaluating the effect of marbling on the structural changes of perimysial connective tissues in Japanese Black cattle, Nishimura et al. (1999) compared the LT and semitendinosus (**ST**). This study reported in the longissimus, adipose tissues were deposited between muscle bundles, creating a fractionated perimysial and endomysial structure as cattle increased in age from 20 mo to 32 mo (Nishimura et al., 1999). Within the perimysial structure of the LT, Nishimura et al. (1999) observed the perimysium diverged into separate, thinner collagen fragments. However, this was not reflected in the ST, where the both the perimysium and endomysium remained intact and more rigid than that of the longissimus. However, the fractioning of the connective tissue layers did not occur in cattle at 9 or 20 mo of age. This fractioning only occurred when cattle increased in age to 32 mo of age (Nishimura et al., 1999). This indicates that the increased deposition of marbling through increased time on feed resulted in the splintering of both the endomysium and perimysium.

When evaluating the effect of intramuscular lipids on the biochemical structure of intramuscular connective tissue, Dubost et al. (2013) determined intramuscular lipids enhanced meat tenderness and juiciness of the LT, semimembranosus, and the biceps femoris. However,

these intramuscular lipids did not provide a significant effect on collagen content (Dubost et al., 2013).

Collagen

Connective tissue in muscle, including collagen, is developed through the process of fibrogenesis (Du et al., 2013). Fibrogenesis is at its most active during the fetal stage of development as it forms both the perimysium and endomysium present in skeletal muscle (Du et al., 2013). As the most abundant protein in animal systems, collagen has a significant effect on meat tenderness (Weston et al., 2002). This is due to collagen's influence as a structural protein in connective tissue, as it is a major element of tendons, ligaments, bones, and cartilage (Bailey et al., 1985; Purslow et al., 2005). Collagen is present in muscle in varying amounts and kinds, due to different muscle types and activity of muscles, but is generally present in skeletal muscle at 1-15% dry matter (Bendall, 1967; Blanco et al., 2013). Generally speaking, locomotion muscles, such as those of the chuck and round have a greater presence of collagen in comparison to supportive muscles of the back, such as the LD (Light et al., 1985; Blanco et al., 2013). The primary purpose of collagen within skeletal muscle is the basic support of muscle fibers, which allows muscles to contract (Bailey, 1985).

Of the more than twelve types of collagen known, only types I, III, IV, V, and VII are present in skeletal muscle of animals (Light and Champion, 1984; Shoulders and Raines, 2009). Collagen is primarily composed of glycine, which makes up approximately one-third of the amino acid composition of collagen. Additionally, hydroxyproline and proline also constitute another third of the amino acid profile (Hill, 1966). This, coupled with the fact that hydroxyproline does not occur in large amounts in other tissues, is why hydroxyproline assays are typically used to quantify collagen amounts in muscle (Shoulders and Raines, 2009). Within

each type of collagen, collagen fibrils are composed of structural units known as tropocollagen (Shoulders and Raines, 2009). Tropocollagen is synthesized from a combination of three alpha chains, which form a very strong triple helical structure (Shoulders and Raines, 2009).

Collagen is a main contributor to beef tenderness. Due to intermolecular cross-links which strengthen as animals age, these crosslinks become thermally resistant to degradation during cooking (Cross et al., 1973; Marsh, 1977). Total collagen is weakly, negatively correlated (r = -0.14) to trained sensory panel tenderness scores (Seideman et al., 1987). Similarly, Cross et al. (1973) determined there were no significant correlations between total collagen and connective tissue rating. However, when evaluating the impact of exogenous growth promotants, such as implants and beta-agonists, Ebarb et al. (2016) reported a significant, positive correlation of insoluble and total collagen with WBSF at 21 d of aging. Collagen content of muscle can be broken into two different fractions: soluble and insoluble (Marsh, 1977). However, insoluble collagen, the fraction that is unable to be gelatinized by the presence of water and high temperatures, is also significantly positively correlated (r = 0.13 - 0.23) to higher WBSF values, which results in tougher meat (Li et al., 2010; Blanco et al., 2013) Soluble collagen, however, is a measurement of collagen present that is susceptible to gelatinization, and therefore can help aid in tenderness of a product if the proper temperature and cooking method are employed (Smith and Carpenter, 1974). However, Cross et al. (1973) reported a significant correlation between the percentage of soluble collagen (r = 0.31) and the amount of connective tissue rating by trained sensory panelists. Li et al. (2010) reported significant, negative correlations (r = -0.25) between collagen solubility and WBSF.

Collagen content of skeletal muscle is affected by different factors, including age, breed, muscle, and marbling content (Cross et al., 1973). When evaluating the effects of marbling level

on collagen content of Polish Lowland x Limousin heifers, Oler et al. (2015) reported heifers with a greater marbling score exhibited significantly less soluble collagen, but there were no significant differences reported in total collagen content. Similarly, when comparing Angus and Nellore cattle, Martins et al. (2015) reported no significant differences in collagen content of the longissimus, despite the stark marbling differences observed between the particular breeds in question. When comparing Angus and Waygu cattle, Duarte et al. (2013) reported Waygu cattle possessed a significantly higher amount of total collagen, however, Angus cattle possessed significantly more soluble collagen. This study was done using the sternomandiublaris muscle, which may have played a role in the contrasting results to those seen in the longissimus in other studies.

When aging longissimus steaks from crossbred steers for either 2 or 27 d, Tullio et al. (2014) reported no significant differences in insoluble or total collagen between aging periods. However, there was a significant increase in soluble collagen as the aging period increased. When comparing the longissimus to the semimembranosus, a muscle with significantly less marbling and higher amounts of connective tissue, the semimembranosus possessed significantly higher amount of insoluble collagen and total collagen. In contrast, the longissimus possessed a significantly higher amount of soluble collagen (Tullio et al., 2014).

Transition Temperature of Collagen

In addition to intrinsic factors such as marbling levels, muscle, and connective tissue amount, steak palatability can also be dramatically affected by cooking temperature. There are three major phases that can cause toughening of meat products that occur and affect different tenderness aspects of the steak (Li et al., 2010; Tamilmani and Pandey, 2016). The first stage begins between 40-58°C and consists primarily of the degradation of myosin; the second stage

occurs at 65-67°C and consists of collagen and sarcoplasmic proteins (Tamilmani and Pandey, 2016). Actin is the protein in meat systems that takes the longest and hottest temperature to degrade at 71-83°C (Tamilmani and Pandey, 2016). In the first stage, toughening is attributed to the thermal degradation of collagen, followed by the degradation of myofibrillar proteins, which causes shortening and hardening of the muscle fibers, resulting in a tougher steak (Bouton et al., 1981). Transition temperature of collagen is used to observe when collagen begins to change from a solid state to a melted, denatured state (Tamilmani and Pandey, 2016). Li et al. (2010) observed the perimysial fraction of connective tissue required a higher temperature for denaturation (65°C) in comparison to the endomysial fraction of muscle (50°C). However, no research has evaluated the effects of increased marbling on transition temperature of collagen, as it is primarily used to quantify denaturation of the intermuscular collagen proteins, through thermal degradation of proteins as well as quantify fat composition and stability for processed meat products (Tamilmani and Pandey, 2016).

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Chapter 2 - Marbling texture effects on beef palatability

Abstract

The objective of this study was to evaluate the effects of marbling texture on consumer and trained sensory panel ratings of beef strip loin steaks from three USDA quality grades. Beef strip loins (n = 117) were selected from three quality grade treatments [Top Choice (Modest^{00} – Moderate^{100} marbling), Low Choice (Small^{0} – Small^{100} marbling), and Select (Slight^{0} – Slight^{100} marbling)] to equally represent three different marbling texture groups: fine, medium and coarse, via visual appraisal. There were no quality grade × texture interactions ($P > 0.05$) for all of the traits evaluated. Consumers (n = 104) rated all marbling texture groups similar ($P > 0.05$) for tenderness, juiciness, flavor, and overall liking, as well as rated a similar ($P > 0.05$) percentage of samples from each marbling texture group acceptable for each palatability trait. Moreover, consumers indicated no preference ($P > 0.05$) among marbling texture groups for visual desirability or likelihood to purchase. However, trained sensory panelists rated coarse marbled steaks higher ($P < 0.05$) than fine or medium marbled steaks for both beef flavor intensity and sustained juiciness, as well as higher ($P < 0.05$) for initial juiciness than medium textured steaks. There were no differences ($P > 0.05$) among marbling texture treatments for Warner-Bratzler shear force, slice shear force, and pressed juice percentage. Results from this study indicate marbling texture has no impact on consumer evaluations of eating quality and only minimal effects on trained sensory panel palatability ratings and therefore provides no palatability-based evidence for the exclusion of coarse marbled carcasses from current and future branded beef programs.

Key words: beef, consumer**,** marbling texture, palatability, quality grade, sensory

Introduction

According to the USDA beef grading standards, USDA quality grade consists of both marbling level and maturity (USDA, 2016). Traditionally, marbling texture has not been a consideration of quality grades and is not mentioned in the official USDA beef grading standards (USDA, 2016). Despite this, 75 percent of the 119 branded beef programs under the supervision of the USDA Agricultural Marketing Service require marbling to meet a fine or medium textured specification to qualify (USDA, 2017). This results in lost profits for both packers and producers. However, this influence from marbling texture is not just limited to the United States. Anecdotal evidence suggests that in Japan, fine, frost-like marbling is preferred and priced higher than their coarse marbled counterparts (Motoyama et al., 2016). However, similar to the United States, marbling texture is not mentioned in the Japanese beef grading standards (Polkinghorne and Thompson, 2010). Researchers in Japan are developing camera systems to objectively measure marbling texture in Wagyu cattle through the "New Fineness Index", which measures the perimeter of marbling flecks (Gotoh et al., 2014).

Currently, only one published study has assessed the effects of marbling texture on beef palatability. Moody et al. (1970) reported coarse marbled ribs had significantly higher Warner-Bratzler shear force values from 2.54 cm cores in comparison to fine marbled rib roasts. However, when evaluated by trained panelists, there were no differences reported for flavor, tenderness, juiciness, or overall satisfaction (Moody et al., 1970). Other researchers have collected data on marbling texture; however, comparisons were commonly not made among the marbling texture groups for most of these studies (Goll et al., 1965; Cross et al., 1975; Cross, 1977; Dubeski et al., 1997; Mello et al., 2012a; Mello et al., 2012b; Durunna et al., 2014). Therefore, the objective of the current study to evaluate the effects of marbling texture on

consumer and trained sensory panel ratings of beef strip loin varying in marbling texture (fine, medium, and coarse) from three USDA quality grades.

Materials and Methods

The Kansas State University (**KSU**) Institutional Review Board approved all procedures for use of human subjects in sensory panel evaluations (IRB: #7740.3).

Sample Collection and Preparation

Beef strip loins (*n* = 117, IMPS #180) were collected from a Midwestern beef processor to equally represent three marbling textures (fine, medium, and coarse) from three quality grade treatments [Top Choice (Modest^{00} – Moderate^{100} marbling), Low Choice (Small^{0} – Small^{100} marbling), and Select (Slight^{0} – Slight^{100} marbling)]. Kansas State University trained research team members collected carcass grade data and segregated carcasses into three marbling texture treatments based on the USDA Marbling Texture reference card (USDA-AMS-LS-SB-02). Carcasses were visually scored for marbling texture using a 9-point scale where 1 = extremely fine marbling and 9 = extremely coarse marbling, with scores of 1-3 within the fine classification, 4-6 within the medium classification, and 7-9 within the coarse classification. In order for beef to have been selected for use in the study, 75% of the marbling within the ribeye had to meet the USDA visual standard. Following collection, strip loins were transported to the KSU Meat Laboratory for steak fabrication. Strip loins were fabricated into 2.54 cm thick steaks from anterior to posterior. The first "face" steak of each strip loin was used for instrumental color, proximate analysis, and pH. The next four steaks were assigned to histology analysis (steak 2), consumer sensory analysis (steak 3), trained sensory panel evaluation (steak 4), and objective tenderness and juiciness testing (steak 5). Each steak was vacuum packaged, aged for 21 d at 2 - 4° C, and frozen at -20°C until subsequent analysis.

Consumer Sensory Panel Evaluation

For consumer sensory panel evaluation, 104 consumers were recruited from Manhattan, KS and the surrounding areas. Consumers were monetarily rewarded for their participation. Steaks evaluated by consumers were thawed at 2 - 4°C for 24 h preceding each panel. Immediately prior to cooking, external fat and accessory muscles were removed. Steaks were cooked on a clamshell grill (Cuisinart Griddler Deluxe, East Windsor, NJ) to a medium degree of doneness (71°C) with internal temperatures monitored using a thermometer (Thermapen Mk4, ThermoWorks, American Fork, UT), and final peak temperatures were confirmed using a probe thermometer (Model 450-ATT, Omega Engineering, Stamford, CT). Steaks then were sliced into 1 cm × 1 cm × steak thickness cubes and 2 cubes were immediately served to consumer sensory panelists.

Panels took place at the KSU campus where consumers were placed into a lecture-style classroom and supplied with a ballot, napkins, toothpicks, expectorant cup, plastic knife and fork as well as unsalted crackers, apple juice, and water to use as palate cleansers between samples. Each paper ballot contained a demographic survey, consumer purchasing motivator sheet, and nine sample ballots. Each sample ballot consisted of 100 mm line scales for overall liking, tenderness, juiciness, and flavor liking with verbal anchors at each end and the midpoint, where 0 = extremely dislike/extremely tough/extremely dry; 50 = neither like or dislike/neither tough or tender/ neither dry or juicy; 100 = extremely like/extremely tender/extremely juicy. Each trait was also rated as satisfactory or unsatisfactory with yes/no questions. Additionally, consumers were asked to rate each sample's perceived quality level as unsatisfactory, everyday quality, better than everyday quality, or premium quality. At the beginning of each panel, panelists were

given verbal instructions for panel procedures and filling out each sheet of the ballot. Each panelist was served nine samples, one from each treatment, in a randomly assigned order.

Each panelist was also given an electronic tablet (Model 5709 HP Steam 7; Hewlett-Packard, Palo Alto, CA) with a digital survey (Version 2417833; Qualtrics Software, Provo, UT) that included the digital image of the bloomed face steak from each of the nine samples to be evaluated during the panel. The picture of each steak was edited to a dimension of 2.54 × 6.35 cm that showed the center of the steak to remove any muscling or external fat differences. Additionally, if any image had a darker color, lightness was adjusted to remove to reduce color variation and bias as much as possible. Consumers were asked to rate the appearance of each steak with no regards to color on a 100 mm line scale with verbal anchors at each end and midpoint (0 = dislike extremely; 50 = neither like or dislike; 100 = like extremely), as well as asked to indicate how likely they would purchase the steak pictured, disregarding color, on a similar line scale (0 = extremely unlikely; 50 = neither likely or unlikely; 100 = extremely likely). Visual evaluations were completed prior to serving of cooked samples and each image was uniquely identified, with no identifiable connection to the cooked sensory samples.

Trained Sensory Panel Evaluation

Sensory panelists were trained according to the American Meat Science Association (**AMSA**) sensory guidelines (AMSA, 2015) and similar to the methods and anchors described by Lucherk et al. (2016). Panelists evaluated samples on 100 mm continuous line scales for initial and sustained juiciness, myofibrillar tenderness, connective tissue amount, overall tenderness, beef flavor intensity, and off flavor intensity using the digital survey methods described above. Each scale was verbally anchored at both end and midpoints with descriptive terms (0 = extremely dry/tough/none/unbeef-like/bland, 50 = neither dry nor juicy, neither tough nor tender,

and neither unbeef-like or beef like. 100 = extremely tender/juicy/abundant/beef-like/intense). Additionally, for off-flavor intensity, a box identified as "not applicable" was available for samples where no off-flavor was detected. Thirteen panels were conducted with eight panelists during each session. Each session consisted of 9 samples, one from each treatment in the study. Steaks were cooked using the procedures previously described for consumer sensory evaluation to a medium degree of doneness (71°C) with internal temperature monitored using thermocouples (30 gauge copper and constantan; Omega Engineering, Stamford, CT). Panelists were served in individual booths under low-intensity red incandescent lights. During each panel session, panelists had deionized water, apple slices, and unsalted crackers for palate cleansers, as well as an expectorant cup and napkin.

Slice Shear Force

Slice shear force (**SSF)** values were determined using the protocol of Shackelford et al. (1999). Raw weights were taken prior to cooking for cook loss analysis. Steaks were cooked to a medium degree of doneness (71°C), then allowed to rest for 3 m, and reweighed. After the resting period, 1 cm of the lateral portion of the steak was removed to reveal the orientation of the muscle fibers. After the muscle fiber orientation was revealed, a 5 cm portion of the steak was cut and using a double bladed knife and cutting guide, a 1 cm slice of the steak was removed at a 45° angle, and sheared using a SSF machine (GR-152; Tallgrass Solutions, Manhattan, KS) to measure the peak force (kg) required to shear through the center of the slice.

Pressed Juice Percentage

The protocol developed by Woolley (2014) was used for pressed juice percentage (**PJP**) determination. After removal of the 5 cm portion used for SSF, a 1 cm portion of the steak was removed immediately medial to the SSF sample removal (Woolley, 2014). Using a double

bladed knife, the 1 cm section was cut into three 1 cm portions, individually weighed on 2 pieces of filter paper (VWR Filter Paper 415, 12.5 cm, VWR International, Radnor, PA), and pressed at 78.45 N for 30 s using an Instron Model 5569 machine (Instron, Canton, MA). After the sample was pressed, a final weight was taken without the compressed sample. The three measures were averaged across for the PJP value for one steak. Pressed juice percentage was quantified as a percentage of the weight lost as a result of compression.

Warner-Bratzler Shear Force

After removal of both SSF and PJP samples, the remainder of the steak, including the dorsal and ventral pieces remaining after SSF sample removal, was chilled at 2 - 4°C for 24 h prior to Warner-Bratzler Shear Force (**WBSF**) analysis using the protocol described by AMSA (2015). Six cores (1.27 cm diameter) were removed parallel to the muscle fiber orientation and sheared perpendicular to the muscle fiber orientation using an Instron Model 5569 (Instron, Canton, MA). Measurements were recorded as peak force (kg) and averaged across the 6 cores for each steak.

Proximate Analysis, Instrumental Color, and pH

Instrumental color and pH measurements were obtained during fabrication prior to vacuum packaging and aging. Immediately after cutting, the face steak used for pH and instrumental color analyses was allowed to bloom for 20 m. Then, pH was measured at the geometric center of the steak with a meat pH meter (model HI 99163; Hanna Instruments, Smithfield, RI). After the blooming period, a HunterLab Miniscan EZ spectophotometer (Illuminant A, 2.54-cm diameter aperture, 10° observer; Hunter Associates Laboratory, Reston, VA) was used to obtain L*, a*, and b* measurements. Three color measurements were taken from each steak and recorded.

Following the 21 d aging period, the face steaks were also used for proximate analysis were diced and frozen in liquid nitrogen, then ground using a Waring blender (Waring Products Division; Hartford, CT), and stored at -20°C until further analysis. Proximate analysis was performed at a commercial research lab (Ward Laboratories, Kearney, NE). Samples were analyzed for percent moisture (method 935.29; AOAC International, 2012), crude protein (method 990.03; AOAC International., 2012), percent fat (method 920.39; AOAC International), and ash (method 942.05; AOAC International, 2012).

Statistical Analysis

Statistical analysis was performed using the PROC GLIMMIX procedure of SAS (SAS Version 9.4; Cary, NC). Data were analyzed as a completely randomized design with a 3 × 3 factorial arrangement, with quality grade, marbling texture and the quality grade × texture interaction serving as fixed effects. Panel number was used as a random effect and steak peak temperature was used as a covariate. A model with a binomial error distribution was used for all acceptability data. For all analyses, the Kenward-Roger adjustment was used. Differences were considered significant at $\alpha < 0.05$.

Results

For all traits evaluated and analyses performed, there were no marbling texture × quality grade interactions ($P > 0.05$).

Carcass Data Results

Marbling texture had no effect ($P > 0.05$) on lean, skeletal, and overall maturity, in addition to USDA marbling score (Table 2.1). Furthermore, marbling texture had no effect ($P > 0.05$) on preliminary fat thickness, adjusted fat thickness, hot carcass weight, or the percentage of kidney, pelvic and heart fat. However, coarse marbled carcasses had higher ($P < 0.05$) numerical

yield grades than both fine and medium marbled carcasses, which were similar ($P > 0.05$) in yield grade. There were no ($P > 0.05$) quality grade effects for lean, skeletal, or overall maturity. However, as expected, Top Choice carcasses displayed a higher ($P < 0.05$) USDA marbling score than Low Choice, which was higher ($P < 0.05$) than Select carcasses. Additionally, Select carcasses had less ($P < 0.05$) preliminary and adjusted fat thickness than Top Choice or Low Choice carcasses, which were similar ($P > 0.05$) for both traits. There were no quality grade effects ($P > 0.05$) on ribeye area, hot carcass weight or percentage of kidney, pelvic, and heart fat. Due to the reduced amount of fat thickness, Select carcasses exhibited lower ($P < 0.05$) numerical yield grades in comparison to both Top Choice and Low Choice carcasses, which were similar ($P > 0.05$) in yield grade.

Consumer Panel Demographic Characteristics and Purchasing Motivators

The demographic characteristics of the 104 consumers who participated in the sensory evaluation are presented in Table 2.2. The majority of participants were Caucasian/White (92.9%) and from households of 2 (22.3%) or 4 (23.3%). Additionally, 67.3% of consumers were male, whereas 32.7% were female. There was an even split of consumers that were married (50.0%) and single (50.0%). Most of the consumers were 20-29 years of age (34.6%) with an annual household income of $50,000- $74,999 (28.9%) and some college/technical school (45.5%). When consuming meat, 61.2% of consumers preferred the flavor of beef and 52.9% of consumers ate beef 1-3 times per week. Additionally, when consuming beef, most consumers considered flavor the most important palatability trait (50.0%), followed by tenderness (37.5%).

In addition to a demographics page, consumers were also asked to rate 15 different purchasing motivators for beef products (Table 2.3). Price, size, weight, thickness, and steak color were rated most important ($P < 0.05$) among the purchasing motivators. Moreover,

marbling level, familiarity with cut, and nutrient content were rated as more important ($P < 0.05$) than local, antibiotic use, growth promotant use, animal welfare, packaging type, natural or organic claims, or brand of product.

Consumer Sensory Panel Results

Marbling texture had no effect ($P > 0.05$) on the palatability traits evaluated (Table 2.4). Consumers rated all marbling texture treatment groups similar ($P > 0.05$) for tenderness, juiciness, flavor liking, and overall liking. Additionally, marbling texture did not impact ($P > 0.05$) the CV for consumer panelists' ratings of juiciness, tenderness, flavor liking, or overall liking. Furthermore, when asked to rate each sample as acceptable or unacceptable for each palatability trait, consumers found a similar ($P > 0.05$) percentage of samples from each marbling texture treatment acceptable (>83% for all traits) (Table 2.5).

Consumers rated Low Choice steaks similar ($P > 0.05$) to Top Choice steaks for all palatability traits evaluated. Low Choice steaks were rated higher ($P < 0.05$) than Select for tenderness, flavor liking, and overall liking scores; however, were similar ($P > 0.05$) to Select for juiciness ratings. Moreover, both Top Choice and Low Choice were rated greater ($P < 0.05$) for flavor liking than Select samples. When consumers were asked to rate samples as acceptable or unacceptable, no differences ($P > 0.05$) were found among quality grades for the percentage of samples rated acceptable for tenderness, juiciness, and overall. However, a lower percentage ($P < 0.05$) of Select samples were rated acceptable for flavor than either Top Choice or Low Choice.

Marbling texture did not affect ($P > 0.05$) the percentage of steaks rated at certain quality levels when consumers rated each sample as unsatisfactory, everyday quality, better than everyday quality, or premium quality (Table 2.6). However, there was a quality grade effect ($P <$

0.05) on the percentage of steaks rated as unsatisfactory. Select steaks were rated as unsatisfactory a higher ($P < 0.05$) percentage of the time than Low Choice steaks. However, Top Choice steaks were similar ($P > 0.05$) to both Low Choice and Select for the percentage of steaks rated as unsatisfactory quality. There were no significant differences ($P > 0.05$) among quality grades for the percentage of steaks rated as everyday quality, better than everyday quality or premium quality.

When asked to visually appraise the desirability of raw steaks of each treatment, consumers rated all marbling texture treatments similar ($P > 0.05$) for the desirability of the appearance of the pictured steak (Table 2.7). This trend continued when the consumers were asked about purchase intent, where marbling texture also had no impact ($P > 0.05$) on the consumers' willingness to purchase. Additionally, quality grade did not affect ($P > 0.05$) the consumer panelists' ratings of the desirability of appearance of the steak or purchase intent.

Trained Sensory Panel Results

Panelists rated coarse marbled steaks higher ($P < 0.05$) than medium marbled steaks for initial juiciness, but rated them similar ($P > 0.05$) to fine marbled steaks for the same trait (Table 2.8). Coarse marbled steaks were also rated higher ($P < 0.05$) for sustained juiciness than both fine and medium marbled steaks. Additionally, beef flavor intensity of coarse steaks was higher ($P < 0.05$) than both fine and medium textured steaks. Fine and medium marbled steaks were rated similar ($P > 0.05$) for sustained juiciness and beef flavor intensity. All marbling texture treatments were rated similar ($P > 0.05$) for myofibrillar tenderness, connective tissue amount, overall tenderness, and off-flavor intensity. Furthermore, marbling texture did not affect ($P > 0.05$) CVs for the trained panelist ratings of initial juiciness, sustained juiciness, myofibrillar

tenderness, connective tissue amount, overall tenderness, beef flavor intensity, or off-flavor intensity.

Top Choice steaks were rated higher ($P < 0.05$) for both initial and sustained juiciness than Select steaks, but were similar ($P > 0.05$) to Low Choice steaks for the same traits. Furthermore, panelists rated all quality grades similar ($P > 0.05$) for myofibrillar tenderness, connective tissue amount, overall tenderness, and off-flavor intensity. However, Top Choice and Low Choice steaks were similar ($P > 0.05$) and more intense ($P < 0.05$) in beef flavor than Select steaks.

Instrumental Tenderness and Juiciness Analyses

Marbling texture did not impact ($P > 0.05$) WBSF, SSF, PJP, or cooking loss (Table 2.10). Additionally, there were no differences ($P > 0.05$) in CV values for both WBSF and SSF. Select steaks exhibited higher ($P < 0.05$) WBSF values than both Top Choice and Low Choice steaks, with Top Choice and Low Choice similar ($P > 0.05$) for WBSF (Table 2.4). There were no quality grade effects ($P > 0.05$) for PJP or SSF. Low Choice steaks had a lower ($P < 0.05$) percentage of cooking loss than Select steaks. Top Choice steaks were similar ($P > 0.05$) to both Low Choice and Select steaks for cooking loss percentage.

Proximate Analysis, Instrumental Color, and pH Results

There were no marbling texture effects ($P > 0.05$) for the percentage of moisture, protein, and ash measured (Table 2.10). Conversely, coarse marbled steaks exhibited a higher ($P < 0.05$) percentage of fat than both fine and medium marbled steaks, which were similar ($P > 0.05$) for fat percentage. As expected, there was a quality grade effect ($P < 0.05$) for fat content (Top Choice > Low Choice > Select). The inverse was observed for protein content, with Select steaks having the most ($P < 0.05$) protein, followed by Low Choice and Top Choice.

There were no marbling texture effects ($P > 0.05$) for all instrumental color traits evaluated (Table 2.10). There was a quality grade effect on L* values, where Top Choice steaks were lighter ($P < 0.05$) than Select steaks, but similar ($P > 0.05$) to Low Choice steaks. Low Choice steaks were similar ($P > 0.05$) in L* values to both Top Choice and Select steaks. There were no quality grade effects ($P > 0.05$) for a* values. Additionally, Select steaks were more blue ($P < 0.05$) than Top Choice or Low Choice steaks. There was no quality grade effect ($P > 0.05$) for pH values (Table 2.3). Coarse marbled steaks had a higher ($P < 0.05$) pH than both fine and medium marbled steaks, though all treatments differed by less than 0.10 units.

Discussion

Branded beef programs are an important contributor to the beef industry. Through branded programs, consumers are delivered a high quality, consistent eating experience (Wilfong et al., 2016). This consistency is due to required specifications which segregate beef into consistent groups. However, it is important to continually evaluate if these specifications provide the quality of eating experience in which they are designed to ensure. Additionally, marbling texture is discriminated against in at both the grading chain and in meat evaluation, as fine marbling is rewarded and preferred at both levels (Smith and Griffin, 2001). Despite being a requirement for 75% of the 119 branded beef programs overseen by the USDA-Agricultural Marketing Service, marbling texture's impact on beef palatability has only been evaluated by one study done by Moody et al. (1970). Marbling texture is impacted by a variety of factors, including breed (Albrecht et al., 2006; Yang et al., 2006; Albrecht et al., 2011) and diet (Dubeski et al., 1997; Mello et al., 2012a; Mello et al., 2012b; Durunna et al., 2014). Although not a part of the formal USDA grading standards, a finer marbling texture has been linked to a higher quality eating experience, specifically as it relates to tenderness (Moody et al.,1970).

Additionally, anecdotal evidence indicates that fine marbling is more evenly dispersed, which results in a more consistent eating experience for a steak (Bass, 2016). This implies that consumers receive a similar amount of marbling within each bite with fine marbling as opposed to coarse marbling.

Consumer Sensory Analysis

Within the current study, consumers did not prefer a certain marbling texture for any of the palatability traits evaluated. Previously, there have not been any studies that have examined the influence of marbling texture on consumer sensory ratings. However, the role of marbling levels in consumer sensory analysis has been well established. In previous literature, as marbling levels and quality grade has been positively associated with increased palatability scores (O'Quinn et al., 2012; Corbin et al., 2014; Lucherk et al., 2016). These studies used a wider range of quality grade treatments, from Prime to Select. However, the results of the current study contrast those of previous findings. With consumer studies, however, it is important to note the random effect of consumers. In comparison to trained panelists, consumers may not be able to detect differences in narrower ranges of quality grade treatments, which may explain why no differences were reported between Top Choice and Low Choice treatments. Despite this, consumers were able to determine differences in flavor and overall liking between the Choice and Select treatments.

Consumer Visual Analysis

In the current study, consumers did not visually prefer any marbling texture or quality grade. Previously, no research has investigated the impact of marbling texture on consumer purchasing decisions; however, quality grade and marbling levels have been well studied. When compared to previous studies using consumer visual panels, the results of the current study

contrast with those previously reported (Killinger et al., 2004; Claborn et al., 2011; DeVuyst et al., 2014). Killinger et al. (2004) reported consumers from were willing to pay more for steaks with a low level of marbling and bright cherry-red color in comparison to those with high levels of marbling and similar color. Similarly, Claborn et al. (2011) reported a consumer preference towards reduced marbling levels when purchasing steaks. However, in these two studies, raw steaks were viewed in a retail-style case in comparison to the cropped pictures of the raw steaks used in the current study. The picture survey method was used to remove any differences or bias in muscling or external fat on steaks. Additionally, Killinger et al. (2004) evaluated consumers' responses in Chicago and San Francisco on a willingness to pay basis, rather than questions about the steak's appearance alone, as was done in the current study. It is not clear how the differences in methodology may have impacted consumer visual ratings, however, our results indicate consumers do not prefer a certain marbling texture or quality grade visually.

Trained Panel Analysis

In trained panel analysis of the current study, coarse marbled steaks were juicer and more flavorful in comparison to their fine and medium counterparts. In contrast to the current study, when served to trained sensory panelists, Moody et al. (1970) reported no differences in flavor, tenderness, juiciness, and overall satisfaction. Contrastingly, Goll et al. (1965) reported a positive correlation between ($r = 0.366$; 0.299) for initial and residual tenderness and more finely, evenly distributed marbling.

In the current study, Top Choice steaks were rated higher for all palatability traits evaluated in comparison to Select steaks. This is in agreement with past research, as it is well established that as marbling levels increase, there is a concurrent increase in trained panel ratings of tenderness, juiciness, and flavor (Davis et al., 1979; Emerson et al., 2013).

Shear Force Analysis

In the current study, marbling texture did not impact either WBSF or SSF values. In previous research, Moody et al. (1970) previosly reported increased WBSF values in coarse marbled roasts in comparison to fine marbled roasts. In the study done by Moody et al. (1970), samples were taken from the anterior end of the LT in wholesale rib for each of the panels and cooked in an oven at 148°C as a rib roast. Although not measured, the authors speculated this difference in shear force was due to perimysial thickness. Similarly in a study by Goll et al. (1965), a negative correlation between marbling texture and distribution and WBSF ($r = -0.359$) was reported when evaluating the effects of marbling on beef palatability, indicating that as steaks increased in fineness, there was a reduction in WBSF. As opposed to today's conventional WBSF methodology using 1.27 cm cores, the authors used a much larger core, 2.54 cm, which required a greater force to shear, regardless of marbling texture. Furthermore, only two cores were removed from the center of the longissimus and sheared three times, in comparison to the six representative cores sheared in the present study. The authors attributed this difference in tenderness to thickness of the perimysial layer of connective tissue. However, more recent studies have indicated that perimysial thickness is not a good indicator nor highly correlated ($r = 0.21$) to tenderness, specifically WBSF values (Nishimura et al., 1999; Brooks and Savell, 2004; Purslow, 2005).

When evaluating the effect of quality grade and marbling levels on WBSF and SSF in the current study, there was a reduction in WBSF as marbling levels increased. However, this was not reflected within SSF values, as there were no differences present between quality grade treatments. In previous research, WBSF values were reduced by 20.5-34.9% as quality grade increased from Select to Prime (Tatum et al., 1980; Smith et al., 1985; Savell et al., 1987;

Emerson et al., 2013). Additionally, a similar reduction of 35.2% was observed for SSF as marbling levels increased from Standard to Prime (Emerson et al., 2013).

Proximate Analysis

In the previous study of Moody et al. (1970), there were no differences found between fine and coarse marbled ribs for both percent ether extracted fat or for the percentage of moisture. However, within the current study, there were differences in the percentage fat found between marbling texture groups, as coarse marbled steaks exhibited a greater percentage of fat in comparison to both fine and medium marbled steaks. This indicates coarser, larger flecks of marbling possess a greater amount of fat in comparison to finer, smaller flecks distributed throughout the steak. Within the USDA grading standards, marbling fleck size is viewed equally for quality grade comparison. Our results indicate coarse marbled steaks also possess larger intramuscular adipocytes in comparison to fine marbled steaks (chapter 3), which could also contribute to increased fat percentage.

Results from this study indicate marbling texture has minimal impact on beef palatability traits. Coarse marbled steaks provide an equivalent eating experience to steaks with fine and medium marbled steaks. Therefore, coarse marbled carcasses should not be discriminated against in the assessment of USDA quality grades or excluded from branded beef programs.

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Table 2.1 Least squares means of beef carcass measurements of carcasses of varying marbling texture and quality grade treatments.

Treatment	Lean Maturity[1]	Skeletal Maturity[1]	Overall Maturity[1]	USDA Marbling Score[2]	Preliminary Fat Thickness, cm	Adjusted Fat Thickness, cm	Ribeye Area, cm^2	Hot Carcass Weight, kg	Kidney, Pelvic, Heart Fat, %	Yield Grade	Marbling Texture Scores
Marbling Texture											
Coarse	151	163	159	512	1.35	1.57	86.8	401.9	2.8	3.7[a]	8.2
Medium	147	162	155	508	1.19	1.37	89.2	388.3	2.9	3.3[b]	4.9
Fine	152	163	159	506	1.19	1.37	85.5	371.3	2.8	3.3[b]	1.9
SEM[3]	2.1	2.3	1.8	1.8	0.03	0.32	0.3	9.2	0.1	0.1	0.1
P-value	0.16	0.87	0.31	0.08	0.32	0.21	0.30	0.07	0.48	0.04	< 0.01
Quality Grade											
Top Choice[4]	150	162	157	676[a]	1.30[a]	1.55[a]	86.9	392.6	2.8	3.6[a]	5.0
Low Choice	150	164	158	475[b]	1.45[a]	1.63[a]	86.2	396.4	3.0	3.8[a]	5.1
Select	150	162	157	374[c]	0.99[b]	1.17[b]	88.5	372.5	2.7	3.0[b]	4.8
SEM	2.1	2.3	1.8	1.8	0.03	0.03	0.3	9.2	0.1	0.1	0.1
P-value	0.98	0.79	0.92	< 0.01	< 0.01	< 0.01	0.61	0.15	0.13	< 0.01	0.16
Texture × QG											
P-value	0.60	0.50	0.71	0.71	0.54	0.29	0.33	0.61	0.98	0.24	0.60

[1]100 = A; 200 = B.

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

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

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

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

Table 2.2. Demographic characteristics of consumers (n = 104) who participated in consumer sensory panels.

Characteristic	Response	Percentage of Consumers
Gender	Male	67.3
	Female	32.7
Household size	1 person	10.7
	2 people	22.3
	3 people	15.5
	4 people	23.3
	5 people	10.7
	6 people	5.8
	>6 people	11.7
Marital Status	Single	50.0
	Married	50.0
Age	Under 20	9.6
	20-29	34.6
	30-39	18.3
	40-49	16.4
	50-59	11.5
	Over 60	9.6
Ethnic Origin	African-American	2.0
	Asian	3.1
	Caucasian/White	92.9
	Hispanic	2.0
	Native American	0.0
	Other	0.0
Annual household income	Under $25,000	6.7
	$25,000 - $34,999	8.7
	$35,000 - $49,999	6.7
	$50,000 - $74,999	28.9
	$75,000 - $100,000	25.0
	More than $100,000	24.0
Education level	Non-high school graduate	0.0
	High school graduate	8.1
	Some college/ technical school	45.5
	College graduate	24.2
	Post graduate	22.2
Beef consumption per week	None	0.9
	1-3 times	52.8
	4-6 times	37.5
	7 or more	8.7
Most important palatability trait	Flavor	50.0
	Juiciness	12.5
	Tenderness	37.5
Degree of doneness preference	Very rare	0.0
	Rare	3.9
	Medium-rare	41.8
	Medium	25.2
	Medium-well	22.3
	Well-done	4.9
	Very well-done	1.9
Preferred meat product for flavor	Beef	61.2
	Chicken	13.6
	Fish	0.9
	Lamb	7.8
	Mutton	0.0
	Pork	8.7
	Shellfish	3.9
	Turkey	0.9
	Veal	0.9
	Venison	1.9

Table 2.3. Beef strip loin steak purchasing motivators[1] of consumers (n = 104) participating in consumer sensory panels.

Trait	Importance
Price	78.0[a]
Size, weight, thickness	72.9[a]
Steak Color	71.9[a]
USDA Grade	63.5[b]
Marbling level	60.5[bc]
Familiarity with cut	59.1[bc]
Nutrient content	54.1[cd]
Eating satisfaction claims	48.6[ed]
Local	43.3[ef]
Antibiotic use in animal	43.2[ef]
Growth promotant use	42.0[fg]
Animal welfare	40.3[fg]
Packaging type	38.6[fg]
Natural or organic claims	36.3[gh]
Brand of product	31.5[h]
SEM	2.8
P-value	< 0.01

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

Table 2.4. Least squares means for consumer panel ratings[1] of grilled beef strip loin steaks of three marbling texture treatments and three USDA quality grades (n = 104).

Treatment	Tenderness	Juiciness	Flavor Liking	Overall Liking
Marbling Texture				
Fine	66.6	63.8	65.0	67.7
Medium	63.0	60.9	62.1	64.2
Coarse	63.7	61.9	63.3	64.9
SEM[2]	2.2	2.2	1.8	1.8
P-value	0.29	0.53	0.35	0.22
Quality Grade				
Top Choice[3]	64.6[ab]	63.2	64.3[a]	66.1[ab]
Low Choice	67.5[a]	63.7	66.3[a]	68.3[a]
Select	61.2[b]	59.6	59.8[b]	62.4[b]
SEM	2.2	2.2	1.8	1.8
P-value	0.04	0.24	0.01	0.02
Texture × QG				
P-value	0.51	0.46	0.78	0.62

[1]Sensory scores: 0 = extremely tough/dry/dislike flavor/dislike overall, 50 = neither dry nor juicy/neither tough nor tender, 100 = extremely juicy/tender/like flavor/like overall.
[2]SE (largest) of the least squares means in the same main effect (marbling texture or quality grade).
[3]USDA marbling score of Modest[00]-Moderate[100].
[ab]Least squares means in the same main effect (quality grade or marbling texture) without a common superscript differ ($P < 0.05$).

Table 2.5. Percentage of beef strip loin steaks of varying marbling texture and quality treatments rated acceptable for tenderness, juiciness, flavor, and overall liking (n = 104).

Treatment	Tenderness Acceptability	Juiciness Acceptability	Flavor Acceptability	Overall Acceptability
Marbling Texture				
Fine	87.9	86.4	87.5	88.5
Medium	86.0	85.7	85.8	85.0
Coarse	86.6	83.7	85.1	85.2
SEM[1]	2.7	2.6	2.4	2.5
P-value	0.78	0.63	0.68	0.38
Quality Grade				
Top Choice[2]	85.8	84.7	87.6[a]	87.5
Low Choice	89.2	87.6	88.7[a]	87.8
Select	85.2	83.4	81.4[b]	83.2
SEM	2.8	2.6	2.8	2.7
P-value	0.29	0.35	0.03	0.20
Texture × QG				
P-value	0.59	0.50	0.38	0.40

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

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

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

Table 2.6. Percentage of beef strip loin steaks of varying marbling texture and quality grade treatments identified as different perceived quality levels by consumer panelists (n = 104).

Treatment	Unsatisfactory Quality	Everyday Quality	Better than Everyday Quality	Premium Quality
Marbling Texture				
Coarse	7.3	47.8	31.3	11.8
Medium	10.1	50.0	29.2	9.2
Fine	6.9	43.5	32.3	14.2
SEM[1]	1.9	3.0	2.7	2.4
P-value	0.28	0.27	0.72	0.18
Quality Grade				
Top Choice[2]	7.4[ab]	48.0	29.6	12.6
Low Choice	5.9[b]	42.8	35.1	14.0
Select	11.5[a]	50.5	28.3	8.7
SEM	2.1	3.0	2.8	2.4
P-value	0.05	0.16	0.2	0.12
Texture × QG				
P-value	0.74	0.18	0.06	0.14

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

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

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

Table 2.7. Least squares means for consumer panel visual ratings[1] of beef strip loin steaks of varying marbling texture and quality treatments (n = 104).

Treatment	Visual Desirability	Purchase Intent
Marbling Texture		
Coarse	63.4	63.5
Medium	64.8	65.1
Fine	63.1	63.7
SEM[2]	1.5	1.5
P-value	0.68	0.73
Quality Grade		
Top Choice[3]	64.0	65.1
Low Choice	63.6	63.1
Select	63.8	64.1
SEM	1.5	1.5
P-value	0.98	0.65
Texture × QG		
P-value	0.35	0.49

[1]Visual ratings: 0 = dislike extremely/extremely unlikely to purchase; 50 = neither like nor dislike appearance/neither likely nor unlikely to purchase; 100 = like extremely/extremely likely to purchase.

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

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

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

Table 2.8 Least squares means for trained panel ratings[1] of grilled beef strip loin steaks (*n* = 117) from varying marbling texture and quality grade treatments.

Treatment	Initial Juiciness	Sustained Juiciness	Myofibrillar Tenderness	Connective Tissue Amount	Overall Tenderness	Beef Flavor Intensity	Off Flavor Intensity
Marbling Texture							
Coarse	65.5[a]	54.5[a]	73.6	9.2	69.8	42.6[a]	1.8
Medium	60.3[b]	48.5[b]	71.5	8.2	68.4	38.5[b]	1.4
Fine	61.4[ab]	49.5[b]	74.1	8.8	70.8	39.6[b]	1.7
SEM[2]	1.8	2.0	1.6	0.7	1.6	1.1	0.7
P-value	0.04	0.03	0.17	0.55	0.53	0.01	0.88
Quality Grade							
Top Choice[3]	65.8[a]	55.2[a]	74.7	8.3	71.5	42.2[a]	2.0
Low Choice	62.4[ab]	50.6[ab]	73.3	8.2	69.9	40.5[a]	1.3
Select	59.1[b]	46.7[b]	71.2	9.8	67.6	38.0[b]	1.6
SEM	1.8	2.0	1.6	0.7	1.6	1.1	0.7
P-value	0.01	< 0.01	0.34	0.22	0.18	0.01	0.67
Texture × QG							
P-value	0.33	0.38	0.83	0.81	0.89	0.85	0.18

[1]Sensory scores: 0 = extremely dry/tough/none/bland/no off-flavor, 50 = neither dry nor juicy/neither tough nor tender, 100 = extremely juicy/tender/abundant/intense.

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

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

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

Table 2.9. Least squares means of instrumental measures of tenderness and juiciness of grilled beef strip loin steaks (n = 117) from varying marbling texture and quality grade treatments.

Treatment	Warner-Bratzler Shear Force, kg	Pressed Juice Percentage, %[1]	Slice Shear Force, kg	Cook Loss, %[2]
Marbling Texture				
Coarse	2.53	20.44	12.29	15.81
Medium	2.46	21.60	11.57	16.47
Fine	2.37	21.41	12.06	16.47
SEM[3]	0.09	0.40	0.46	0.32
P-value	0.44	0.08	0.53	0.23
Quality Grade				
Top Choice[4]	2.32[b]	21.29	12.09	16.28[ab]
Low Choice	2.35[b]	20.93	11.88	15.64[b]
Select	2.70[a]	21.23	11.96	16.84[a]
SEM	0.09	0.40	0.46	0.32
P-value	< 0.01	0.79	0.94	0.03
Texture × QG				
P-value	0.71	0.10	0.98	0.57

[1]Percentage moisture lost during compression of sample between filter paper at 8 kg of pressure for 30 seconds.
[2]Cook loss = [(raw weight – cooked weight) / raw weight] × 100.
[3]SE (largest) of the least squares means in the same main effect (marbling texture or quality grade).
[4]USDA marbling score of Modest[00]-Moderate[100].
[ab]Least squares means in the same main effect (marbling texture or quality grade) without a common superscript differ ($P < 0.05$).

Table 2.10. Least squares means for proximate analysis, pH, and instrumental color values for beef strip loin steaks (*n* = 117) of varying marbling texture and quality grade treatments.

	%							
Treatment	Fat	Moisture	Protein	Ash	pH	L*[1]	a*[2]	b*[3]
Marbling Texture								
Coarse	6.7[a]	60.2	23.9	1.3	5.64[a]	43.18	25.97	17.96
Medium	5.4[b]	62.0	23.9	1.4	5.59[b]	42.96	25.97	17.42
Fine	5.2[b]	64.2	24.0	1.3	5.57[b]	42.46	25.60	17.79
SEM[4]	0.3	1.2	0.2	0.04	0.01	0.54	0.23	0.22
P-value	< 0.01	0.06	0.90	0.18	< 0.01	0.63	0.43	0.20
Quality Grade								
Top Choice[5]	7.4[a]	61.6	23.4[c]	1.3	5.58	44.06[a]	25.76	18.03[a]
Low Choice	6.0[b]	61.8	23.9[b]	1.3	5.60	42.95[ab]	26.02	17.88[a]
Select	3.8[c]	63.0	24.4[a]	1.4	5.61	41.60[b]	25.75	17.25[b]
SEM	0.3	1.2	0.2	0.04	0.01	0.54	0.23	0.22
P-value	< 0.01	0.67	< 0.01	0.18	0.50	< 0.01	0.66	0.03
Texture × QG								
P-value	0.66	0.52	0.20	0.14	0.08	0.22	0.48	0.09

[1] L*: Lightness (0 = black and 100 = white).

[2] a*: Redness (-60 = green and 60 = red).

[3] b*: Blueness (-60 = blue and 60 = yellow).

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

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

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

Chapter 3 - Marbling texture effects on muscle histology and collagen characteristics

Abstract

The objective of this study was to evaluate the effects of marbling texture (fine, medium, and coarse) on muscle fiber morphometrics, adipocyte cross-sectional area, perimysial thickness, collagen solubility, and thermal transition temperature of perimysial collagen of beef strip loin steaks from three USDA quality grades. Beef strip loins (n = 117) were selected from three quality grades [Top Choice (Modest^{00} – Moderate^{100} marbling), Low Choice, and Select] to equally represent three different marbling texture groups: fine, medium and coarse, via visual appraisal. There were no marbling texture × quality grade interactions for all traits evaluated. Marbling texture impacted ($P < 0.05$) adipocyte cross-sectional area, where coarse steaks displayed larger adipocytes in comparison to fine marbled steaks, but medium marbled steaks were similar ($P > 0.05$) to both coarse and fine marbled steaks. However, marbling texture did not impact ($P > 0.05$) perimysial thickness. Marbling texture did not affect collagen traits, as no differences ($P > 0.05$) were found among marbling texture treatments for soluble collagen, insoluble collagen, and total collagen concentrations. Furthermore, all marbling texture groups (fine, medium, and coarse) were similar ($P > 0.05$) for the peak thermal transition phase of the perimysial fraction of collagen. Quality grade ($P < 0.05$) affected adipocyte size, as Top Choice and Low Choice possessed larger adipocytes than Select steaks. There were no differences ($P > 0.05$) among quality grades for fiber type; nor were differences found among marbling textures or quality grades for fiber cross-sectional area. These results indicate that marbling texture does not contribute to differences in collagen characteristics or fiber cross-sectional area that may impact eating quality of beef strip loin steaks.

Key Words: adipocyte, beef, collagen, histology, marbling texture, quality grade

Introduction

Marbling texture has long been established as a priority in meat evaluation (Smith and Griffin, 2001). This has led to an anecdotal bias towards coarse marbled carcasses at the grading chain. Additionally, of the 119 branded beef programs the USDA-Agricultural Marketing Service supervises, 75% have a specification for fine or medium textured marbling (USDA, 2017). This means carcasses exhibiting coarse marbling in the ribeye at the time of grading are not eligible for these programs, which results in reduced profits in an already thin margin industry. Additionally, the influence of marbling texture is not limited to the United States, as Japanese researchers are working to develop objective camera systems for marbling texture (Gotoh et al., 2014). However, only one study has examined the influence of marbling texture on beef palatability (Moody et al., 1970). Moody et al. (1970) observed that fine marbled roasts possessed lower shear force values in comparison to coarse marbled roasts (Moody et al., 1970). In that study, the authors speculated the differences in shear force were attributed to differences in perimysial thickness. However, the authors did not measure this trait. Additionally, there are other biological factors which impact beef tenderness, including fiber cross-sectional area and collagen solubility. Increased fiber cross-sectional area has been linked to increased instrumental tenderness and reduced sensory tenderness ratings (Seideman et al., 1987; Crouse et al., 1991; Ebarb et al., 2016). Furthermore, intermuscular collagen content also has been implicated in increasing shear force values (Cross et al., 1973; Ebarb et al., 2016). Currently, no research has addressed the effects of marbling texture on muscle histology and collagen characteristics. Other researchers have measured marbling texture; however, no comparisons have been made among the marbling texture groups (Goll et al., 1965; Cross et al., 1975; Cross, 1977; Dubeski et al.,

1997; Mello et al., 2012a; Mello et al., 2012b; Durunna et al., 2014). Therefore, the objective of the current study was to determine the effects of marbling texture on muscle histology, adipocyte cross-sectional area, and collagen traits of beef strip loin steaks.

Materials and Methods

Sample Collection and Preparation

Beef strip loin selection and preparation is described in detail in Chapter 2. Briefly, beef strip loins (IMPS #180; n = 117; 39 / quality grade) were selected through visual appraisal using the USDA-AMS-LS-SB-02 marbling texture reference card. To qualify for selection for a texture treatment group (fine, medium, or coarse), 75% of the marbling at the 12^{th} and 13^{th} rib interface had to meet the standard (USDA-AMS-LS-SB-02) for the group. After selection, strip loins were transported to the Kansas State University (**KSU**) meat laboratory and fabricated into 2.54 cm steaks. During fabrication, the most anterior "face steak" was removed, and the next most anterior steak was used for histology analysis. At fabrication, each steak designated for histological analysis had four marbling flecks selected to best represent the marbling texture treatment. Marbling flecks were removed from each steak and embedded in optimum cutting temperature media (Tissue Tek OCT; VWR; Radnor, PA) and frozen in liquid nitrogen cooled isopentane. Samples were then stored at -80°C until subsequent analysis. After sample embedding, each steak was vacuum packaged and aged for 21 d at 2-4°C. Following the aging period, each histology steak was diced, with 50 g of the diced steak frozen and stored at -80°C for perimysial peak thermal transition temperature, and the remainder of the steak was diced and frozen in liquid nitrogen, then ground using a Waring blender (Waring Products Division; Hartford, CT) and stored at -80°C until further analysis for collagen content analysis.

Histology

Individual marbling flecks were used for muscle histology, perimysial thickness, and adipocyte cross-sectional area analysis. For muscle histology, the protocols of Phelps et al. (2014) were used for muscle fiber type analysis. Seven-micrometer cyrosections were collected on positively charged glass microscope slides. For each marbling fleck, one section was taken for immunofluorescent fiber type staining and another section was taken for Masson's Trichrome staining. For histology analysis, slides were incubated with a blocking solution of 10% horse serum and 0.2% Triton X-100 in phosphate buffer solution (**PBS**) for 30 m at room temperature. Slides were then incubated in the primary antibody solution of 1:500 rabbit α-dystrophin (catalog: #PA137587; Thermo Scientific, Waltman, MA), 1:10 supernatant mouse anti-MHC, slow IgG2b (BA-D5; Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA), and 1:10 mouse anti-MHC all but type IIX, IgG1 (BF-35; Developmental Studies Hybridoma Bank). Slides were then rinsed three times with PBS for five minutes each. After rinsing, a solution of secondary antibodies was applied for 30 m. The secondary antibody solution included 1:100 Alexa-Fluor 488 goat anti-mouse IgG1 (Invitrogen, Grand Island, NY); 1:1000 Alexa Fluor 633 goat anti-mouse IgG2b (Invitrogen); 1:1000 Alexa-Fluor 594 goat anti-rabbit H&L (Invitrogen); and 1:1000 DAPI (catalog: #PI146190; Fisher Scientific; Waltman, MA).

Slides were then coverslipped and imaged using a Nikon TI-U inverted microscope (Nikon; Lewisville, TX) furnished with a X-Cite 120XL epifluorescence illumination system (EXFO; Mississagua, Ontario, Canada) and a DS-QiMC digital camera at 100× magnification. In addition, slides designate for Masson's Trichome were imaged using a with a DS-Fi1 camera at 100× magnification. Photomicrographs were analyzed for muscle fiber cross-sectional area and

myosin heavy chain (**MHC**) type distribution using NIS-Elements Imaging Software (Basic Research 3.3; Nikon Instruments, Inc., Melville, NY). For each marbling fleck sample, a minimum of 3 photomicrographs and 300 fibers were analyzed per section. Images were taken specifically near the marbling fleck. For analysis, fibers that stained positive for BA-D5 antibody were considered as MHC Type I fibers. Fibers that stained positive for the BF-35 antibody and negative for BA-D5 antibody were considered as MHC type IIA fibers. Any fibers that did not stain positive for both BF-35 and BA-D5 were considered as MHC Type IIX fibers (Schiaffino et al., 1989). The α-dystrophin ring around each fiber identified the fiber cross-sectional area.

For Masson's trichrome staining protocol, a Masson's trichrome staining kit was used (SigmaAldrich; St. Louis, MO). Seven-micrometer thick sections were collected on glass frost-free microscope slides. Slides were then incubated with deionized water for 2 m, followed by a Bouin's solution (SigmaAldrich; St. Louis, MO) incubation for 15 m at 56°C. Samples were then successively incubated in tap water, Weigert's Hematoxylin solution, tap water again, deionized water, Beibrich Scarlet-Acid Fuchsin stain, a phosphotungstic and phosphomolybdic acid solution, and aniline blue solution for 5 m each. Following these solutions, slides were then incubated in a 1% acetic acid solution for 2 m, then successively incubated in deionized water, 100% ethanol, and xylenes for 1 m each.

Photomicrographs were analyzed for adipocyte cross-sectional area and perimysial thickness using NIS-Elements Imaging Software (Basic Research 3.3; Nikon Instruments, Inc., Melville, NY). For each marbling fleck, a minimum of 3 photomicrographs per section was analyzed for both adipocyte cross-sectional area and perimysial thickness. A minimum of 200 adipocytes per steak were measured. For perimysial thickness, images were taken around the

marbling fleck. Perimysial thickness was measured every 10 micrometers with a minimum of 10 measurements in each image.

Collagen Solubility

A modified method described by Gonzalez et al. (2014) was used to determine hydroxyproline content of each sample. Briefly, following the 21 d aging period, steaks were diced and frozen in liquid nitrogen, then ground using a Waring blender (Waring Products Division; Hartford, CT) and stored at -80°C until further analysis. Three grams of ground sample in duplicate were mixed with ¼ strength Ringer's Solution. The tubes were then placed in a hot water bath at 77°C for 70 m and stirred every 10 m. Samples were then centrifuged at 20°C for 15 m at 5,200 × *g*. Soluble samples were then decanted into autoclave milk bottles through Fisher 09-795 filter paper (Fisher Scientific; Waltham, MA). Soluble samples were re-centrifuged with ¼ strength Ringer's solution under the same conditions, and then re-decanted. After the sample was filtered, 25 mL of 12 N hydrochloric acid was added to the bottles and sealed. The remaining pellet was transferred into autoclave bottles with the filter paper used for decantation and labeled as the insoluble fraction. The centrifuge tubes were rinsed with 25 mL of 6 N hydrochloric acid and decanted into the bottles.

Following decantation, all samples were autoclaved for 18 h at 121°C at 18-21 psi. One gram of decolorizing charcoal was added to each sample bottle and mixed thoroughly. Following the addition of charcoal, all samples were filtered through Whatman #2 filter paper (Fisher Scientific; Waltham, MA). Sample bottles were rinsed with deionized water to bring the soluble samples to 175 mL and insoluble samples to 300 mL. Each sample's pH was adjusted to 6 using 12 N sodium hydroxide and various concentrations of hydrochloric acid. Soluble samples were diluted to 250 mL and insoluble samples were diluted to 500 mL in volumetric flasks. All

samples were thoroughly mixed and filtered through Fisher 09-795 filter paper into 15 mL centrifuge tubes and frozen at -40°C until reading. Hydroxyproline determination was accomplished using procedures of Bergman and Loxley (1963). A BioTek Eon spectrophotometer (Biotek Instruments Inc., Winooski, VT) was used to read absorbance at 558 nm and oriented using a blank of distilled water and a standard curve created for each group of analyses. To determine total and fractional collagen contents, hydroxyproline content was multiplied by 7.52 for the soluble fraction and 7.25 for the insoluble fraction (Cross et al., 1973).

Transition Temperature of Collagen

The protocol of Light and Champion (1984) was used to extract the perimysial fraction of muscle. In short, 50 g of tissue was diced and blended with cold 0.05 M calcium chloride in a glass Waring blender (Waring Products Division; Hartford, CT). After blending, the mixture was placed through a 1 mm^2 sieve. This process was repeated three times until the perimysium was separated from the endomysial fraction. Following separation, samples were freeze dried for 24 h. After freeze drying, 20 mg of the perimysial fraction was weighed into aluminum pans. Peak thermal transition temperature was analyzed using a digital scanning calorimeter (Shimadzu 201-52943; Shimadzu Scientific Instruments, Kyoto, Japan), and analyzed using TA 60WS software (Shimadzu Scientific Instruments).

Statistical Analysis

Statistical analysis was performed using the PROC GLIMMIX procedure of SAS (SAS Version 9.4; Cary, NC). Data was analyzed as a completely randomized design with a 3 × 3 factorial arrangement, with quality grade, marbling texture and the quality grade × texture interaction serving as fixed effects. For all analyses, the Kenward-Roger adjustment was used.

Results

There were no marbling texture × quality grade interactions for all traits evaluated.

Histology

Marbling texture impacted ($P > 0.05$) adipocyte cross-sectional area (Figure 3.1). Coarse marbled steaks possessed larger ($P < 0.05$) adipocytes than fine marbled steaks. Medium marbled steaks were similar ($P > 0.05$) in adipocyte size to both fine and medium marbled steaks. Similar to marbling texture, quality grade impacted ($P < 0.05$) adipocyte size (Figure 3.2). Top Choice and Low Choice steaks possessed larger ($P < 0.05$) adipocytes than Select steaks. Top Choice and Low Choice steaks were similar ($P > 0.05$) for adipocyte size.

Marbling texture did not impact ($P > 0.05$) fiber cross-sectional area for any of the three MHC (Type I, Type IIA, and Type IIX) isoforms (Figure 3.3). However, marbling texture affected ($P < 0.05$) the distribution of the MHC isoforms (Figure 3.4). Medium marbled steaks possessed a higher ($P < 0.05$) percentage of MHC Type IIA fibers than both fine and coarse steaks, which were similar ($P > 0.05$). A contrasting effect occurred in MHC Type IIX fibers, where fine and coarse marbled steaks possessed a greater ($P < 0.05$) percentage of MHC Type IIX fibers compared to medium marbled steaks. Fine and medium marbled steaks were similar ($P > 0.05$) for the percentage of MHC Type IIX fibers. Quality grade did not impact ($P > 0.05$) fiber cross-sectional area for MHC Type I, Type IIA, and Type IIX (Figure 3.5). Similarly, quality grade did not affect ($P > 0.05$) fiber type distribution (Figure 3.6).

Collagen Traits

Marbling texture did not impact ($P > 0.05$) the amount of soluble, insoluble or total amount of collagen (Table 3.1). All texture groups (fine, medium, and coarse) possessed a similar ($P > 0.05$) amount of both soluble and insoluble collagen. In addition, each marbling

texture group exhibited a similar ($P > 0.05$) amount of total collagen. Quality grade did not affect collagen content ($P > 0.05$). All quality grades had a similar ($P > 0.05$) amount of soluble, insoluble and total collagen. Similarly, marbling texture did not influence perimysial thickness, as coarse, medium, and fine marbled steaks were all similar ($P > 0.05$) for this trait. Additionally, quality grade did not impact ($P > 0.05$) perimysial thickness, as Top Choice, Low Choice, and Select steaks were similar ($P > 0.05$) for perimysial thickness. Similarly, marbling texture did not impact ($P > 0.05$) peak thermal transition temperature of the perimysial fraction. Quality grade also did not impact ($P > 0.05$) peak transition temperature of the perimysial fraction, as Top Choice, Low Choice, and Select steaks possessed a similar ($P > 0.05$) perimysial thermal transition temperature.

Discussion

Marbling texture has been established as a factor in meat evaluation (Smith and Griffin, 2001). Due to this, bias towards carcasses displaying coarse marbling in the ribeye has existed at the grading chain. However, it is important to assess whether these factors truly play a role in beef palatability. In previous beef palatability research, fine marbled steaks were determined to produce lower shear force values in comparison to coarse marbled steaks (Moody et al., 1970). These authors speculated the differences observed may have been due to differences in perimysial thickness (Moody et al., 1970). However, the authors did not measure perimysial thickness within the study. Perimysial thickness has been linked to reduced tenderness ratings and is linked to the background tenderness seen in beef products (Smith and Carpenter, 1974; Purslow, 2005; Purslow, 2014). In the more than 45 years since this idea was proposed, no other published study has evaluated the impact of marbling texture on collagen characteristics, including perimysial thickness.

Our results oppose the theory proposed by Moody et al. (1970), as no differences were found among marbling texture categories for not only perimysial thickness, but for all of the collagen characteristics evaluated in the current study. This is further supported by similar tenderness ratings among the marbling texture groups as measured by both instrumental (Warner-Bratzler and Slice Shear Force) and sensory panelists (reported in Chapter 2). Other authors have demonstrated the impact of collagen on beef tenderness (Cross et al., 1973; Seideman et al., 1987; Ebarb et al., 2016), but have often limited these comparisons between breeds, muscles, diet, and different maturity groups. Additionally, in the current study, no differences were found among the quality grade treatments evaluated for any of the collagen traits evaluated. These findings for total collagen are similar to previous studies comparing marbling levels and collagen content, where other authors have reported no differences in total collagen among beef from various quality grades (Oler et al., 2015). Similarly, in a study comparing cattle breeds, Martins et al. (2015) reported no differences in collagen content between Angus and Nellore cattle, despite a large marbling difference observed between those breeds. However, Oler et al. (2015) reported that as marbling levels increased in Polish Lowland × Limousin crossbred heifers, the percentage of soluble collagen was reduced. Our results indicate marbling texture has no impact on collagen characteristics nor the collagen-related aspects of tenderness (Chapter 2).

Additionally, by evaluating the thermal transition temperature of the perimysial fraction of the muscle, it further supports the similarity in tenderness observed between marbling texture groups, as there were no differences among marbling texture groups. Peak transition temperature of collagen, particularly the perimysial and endomysial fractions, have been investigated as an indication of tenderness, as it indicates the temperature at which collagen present would denature

(Tamilmani and Pandey, 2016). There has been very limited research to investigate differences within perimysial peak transition temperature, with most published work primarily focusing on differences between muscles, especially the semitendinosus (Li et al., 2006; Li et al., 2010). However, when making comparisons within the same muscle, similar to the current study, most authors have reported no differences (Li et al., 2006; Li et al., 2010). Our results show that the perimysial fraction of muscle, as well as other connective tissue factors are not impacted by marbling texture.

Previously published literature does not include research that has evaluated the effect of marbling texture on adipocyte size. The quality grade effects observed in the current study for adipocyte size are in agreement with Moody and Cassens (1968), who also observed that both Small and Moderate marbled steaks possessed larger adipocytes in comparison to steaks with Traces marbling scores. Larger depositions of marbling, accompanied with larger adipocytes could have contributed to possible splintering and thinning of the perimysium, as reported by Nishimura et al. (1999) in Japanese Black cattle, a breed known for extreme marbling. Because the coarse marbled steaks possessed larger adipocytes than fine marbled steaks, this could have thinned and splintered the perimysium, resulting in the lack of difference observed between these two marbling texture treatments for perimysial thickness.

To further identify any possible histological effects on eating quality as a result of marbling texture, muscle fiber type and cross-sectional area were evaluated. Increased fiber cross-sectional area has previously been linked to increased Warner-Bratzler shear force and sensory tenderness scores (Seideman et al., 1987; Crouse et al., 1991; Chriki et al., 2013; Ebarb et al., 2016). However, in the current study, there were no differences found among marbling texture groups nor among quality grades in fiber cross-sectional area. Nevertheless, marbling

texture impacted fiber type distribution, with medium marbled steaks possessing a greater percentage of MHC Type IIA type fibers and a corresponding reduction in MHC Type IIX fibers. The fiber distribution in the current work differs from previous investigations of the longissimus dorsi, where MHC Type IIX have been reported to be in greater amounts than both MHC Type I and IIA (Hunt and Hedrick, 1977; Kirchofer et al., 2002). However, the results found in the current study are similar to a more recent study by Ebarb et al. (2016), which reported increased amounts of MHC Type IIA fibers in strip loin steaks in comparison to MHC Type IIX. This difference could be attributed to the use of immunofluorescence staining in comparison to ATP-ase activity and succinate dehydrogenase techniques used in previous research.

The results from the current study indicate that marbling texture increases adipocyte cross-sectional area, but has no impact on collagen characteristics or fiber cross-sectional area, two main biological influencers of beef tenderness and eating quality. Therefore, any potential differences in tenderness among different marbling texture classes are not the result of these factors.

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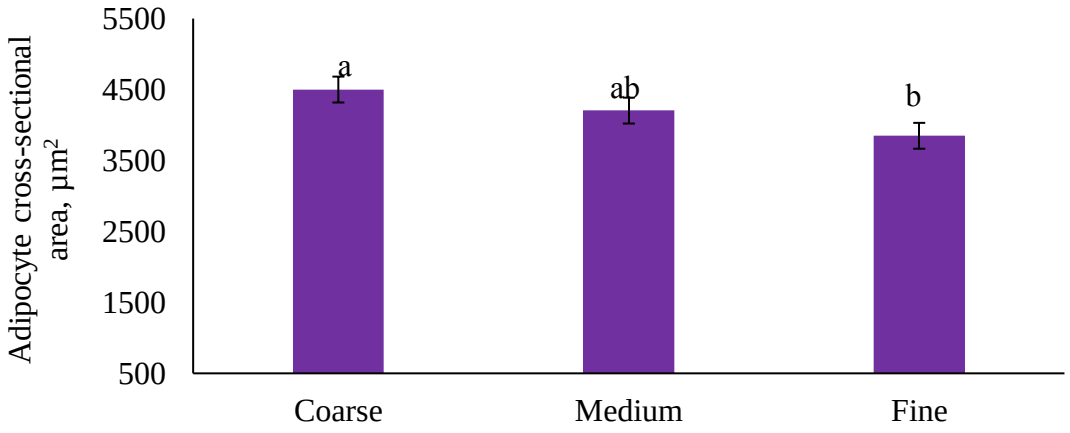
Table 3.1. Least squares means of collagen characteristics of beef strip loin steaks of varying marbling texture and quality grade treatments.

Treatment	Soluble collagen, mg/g	Insoluble collagen, mg/g	Total collagen, mg/g	Perimysial peak transitional temperature, °C	Perimysial thickness, µm
Marbling Texture					
Coarse	1.48	9.49	10.98	48.87	43.23
Medium	1.74	9.81	11.56	54.07	46.26
Fine	1.72	9.92	11.64	47.10	41.44
SEM[1]	0.12	0.34	0.38	2.72	2.20
P-value	0.27	0.65	0.41	0.17	0.31
Quality Grade					
Top Choice[2]	1.50	9.86	11.36	50.29	44.56
Low Choice	1.77	9.61	11.37	47.84	42.54
Select	1.67	9.77	11.44	51.91	43.84
SEM	0.12	0.34	0.38	2.72	2.20
P-value	0.31	0.87	0.99	0.57	0.81
Texture × QG					
P-value	0.19	0.28	0.12	0.47	0.36

[1]SE (largest) of the least squares means in the same main effect (marbling texture or quality grade).
[2]USDA marbling score of Modest[00]-Moderate[100].

Figure 3.1. Least squares means of adipocyte cross-sectional area of beef strip loin steaks of varying marbling texture treatments.

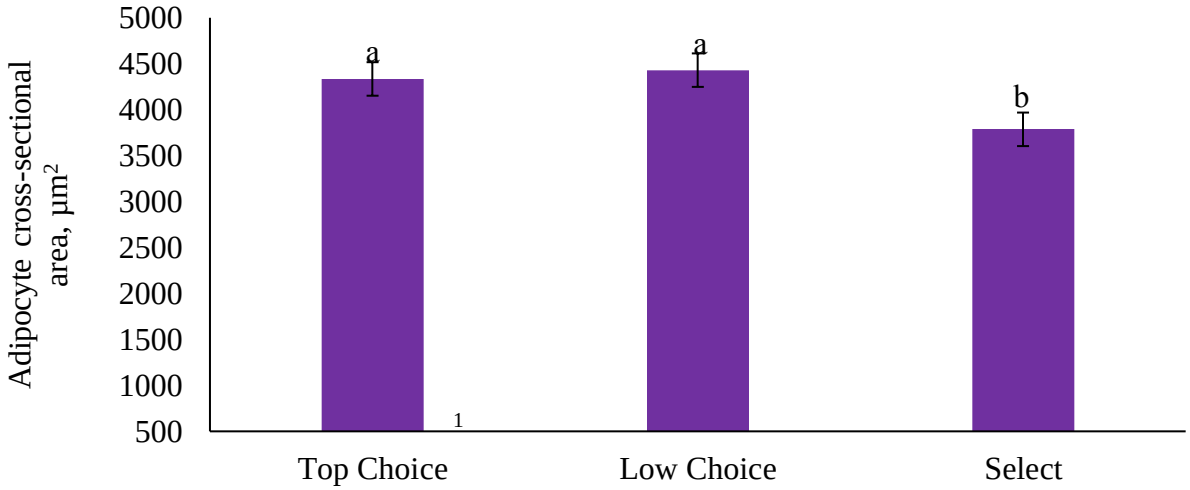
Adipocyte size: $P = 0.04$



[ab]Means without a common superscript differ ($P < 0.05$).

Figure 3.2. Least squares means of adipocyte cross-sectional area of beef strip loin steaks of varying quality grade treatments.

Adipocyte size: $P = 0.03$



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

[ab]Means without a common superscript differ ($P < 0.05$).

Figure 3.3. Least squares means of myosin heavy chain (MHC) cross-sectional area of beef strip loin steaks of varying marbling texture treatments.

MHC Type I: $P = 0.19$
MHC Type IIA: $P = 0.21$
MHC Type IIX: $P = 0.67$

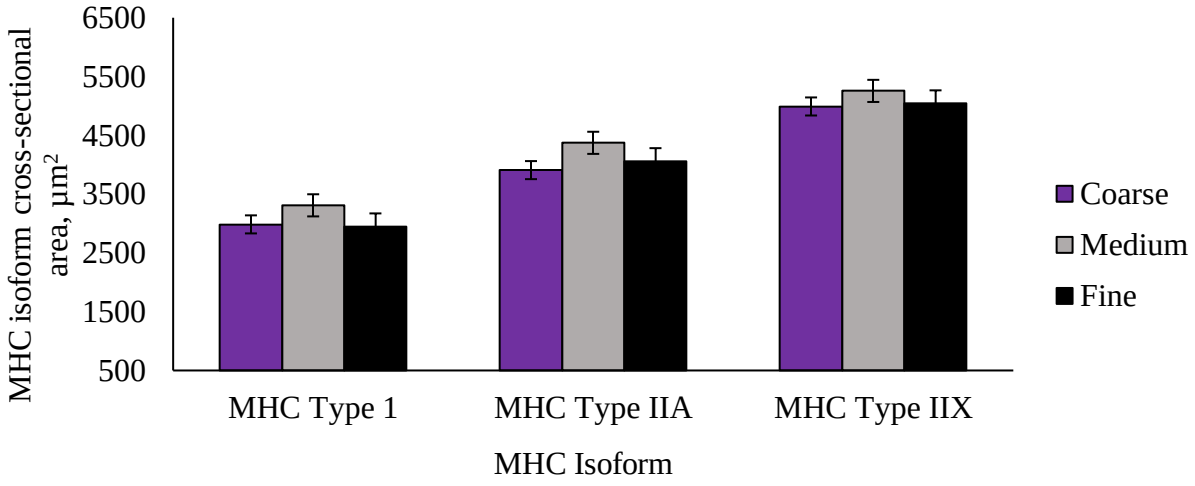
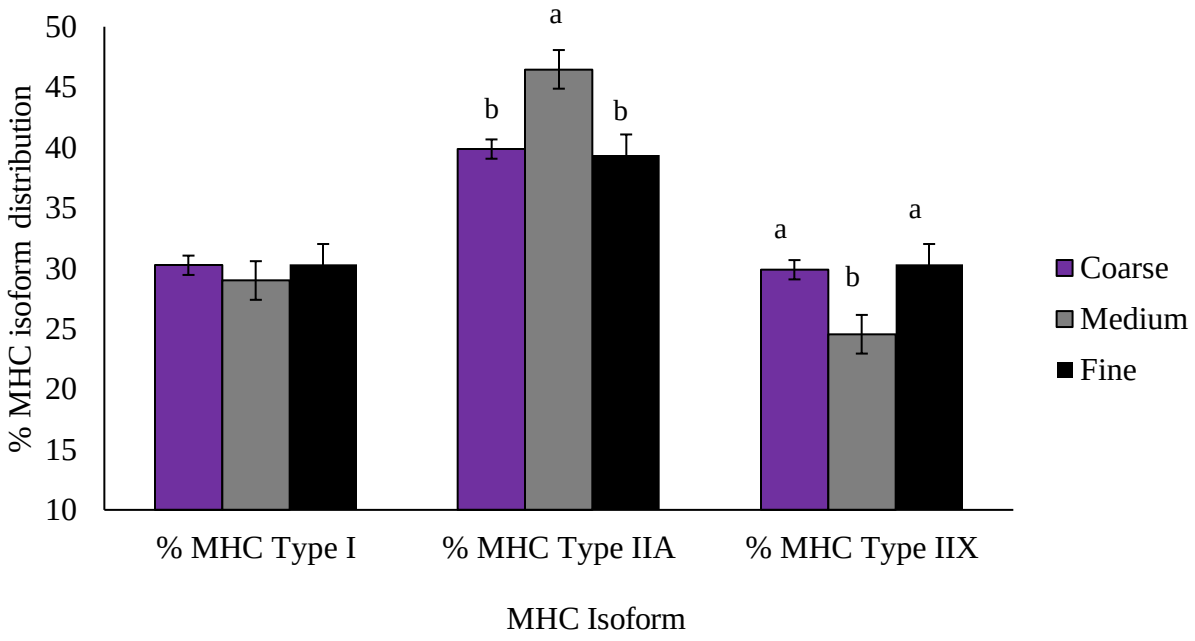


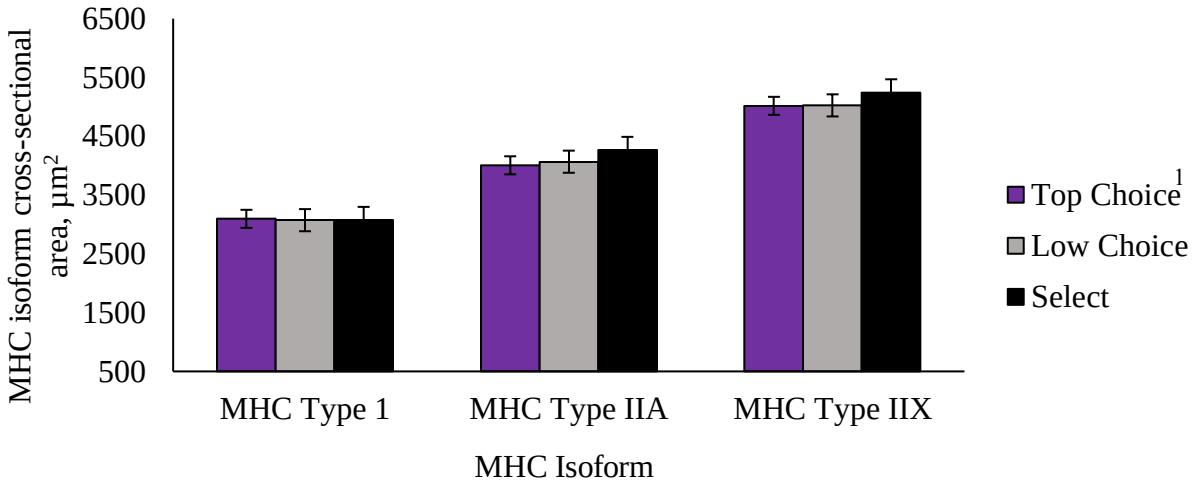
Figure 3.4. Least squares means of myosin heavy chain (MHC) distribution of beef strip loin steaks of varying marbling texture treatments.

MHC Type I: $P = 0.54$
MHC Type IIA: $P < 0.01$
MHC Type IIX: $P = 0.03$



[ab]Means within the same MHC isoform without a common superscript differ ($P < 0.05$).

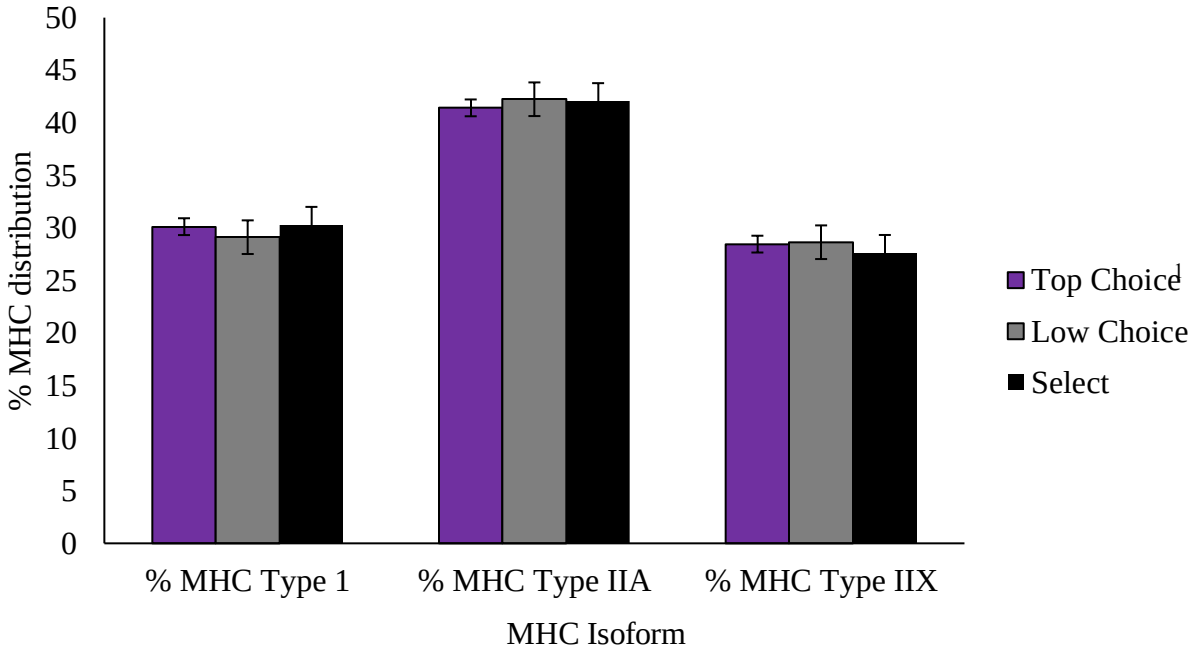
Figure 3.5. Least squares means of myosin heavy chain (MHC) cross-sectional area of beef strip loin steaks of varying quality grade treatments.



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

Figure 3.6. Least squares means of myosin heavy chain (MHC) distribution of beef strip loin steaks of varying quality grade treatments.

MHC Type I: $P = 0.44$
MHC Type IIA: $P = 0.93$
MHC Type IIX: $P = 0.90$



[1]USDA marbling score of Modest[00]-Moderate[10]

Appendix A - Immunofluorescence Staining Protocol

Immunofluorescence Staining Protocol Dystrophin, BF-35, BAD5 on Bovine Muscle Cryosections

Blocking Solution:

10% Horse Serum (HS)/0.2% TritonX-100 in PBS (pH of 7.4)

Primary Antibodies:

1) Dystrophin (Prod# PA137587 ThermoFischer)
   - Pierce Anti-dystrophin Rabbit Polyclonal
   - Dilution of 1:500
2) BF-35 (mouse IgG1 DSHB BF-35)
   - Myosin Heavy Chain all but 2X
   - Dilution of 1:10
3) BAD5 (mouse IgG2b DHSB BAD5)
   - Myosin Heavy Chain Type 1
   - Dilution 1:10

Secondary Antibodies:

1) Alexa-Fluor 488 goat anti-mouse IgG1 (Invitrogen Cat # A-21121)
   - Dilution of 1:1000
2) Alexa-Flour 633 goat anti-mouse IgG2b (Invitrogen Cat # A-21146)
   - Dilution of 1:1000
3) Alexa-Flour 594 goat anti-rabbit H&L (Invitrogen Cat # A-11012)
   - Dilution of 1:1000

DAPI (Fisher Scientific # PI46190)

-Dilution 1:1000

Staining Procedure:

- Use PAP pen to make a hydrophobic ring around the edge of each slide while the slide is dry.
- Incubate cultures with Blocking solution (100 µL per section) for 30 min at RT to block nonspecific antigen binding.
  - Use a tip box that has the top wrapped in foil and a very wet paper towel in the bottom to provide the humidity for all the steps where the volume per slide is minimal.
- Remove blocking solution from each slide using pipette tip in the corner of the slide
- Add primary antibody solution (100 µL per section) and incubate at RT in a humidified box for 1 h.
  - Primary antibodies can be combined into a single solution:
    - Remember to account for the volume of both antibodies in your calculations.
- Rinse with PBS for 5 min 3X.

Be Sure to protect slides from light for the remainder of the procedure

- Add secondary antibody and DAPI solution (300 µL per slide or 100 µL/section) and incubate at RT in a humidified box for 30 min.
  - Secondary antibodies and DAPI can be combined into a single solution:
- Rinse with PBS for 5 min 3X.
- Coverslip with 9:1 glycerol/PBS solution.
- Let slides dry sufficiently

- Fingernail polish the edges of the slide to seal it. Once dry, the slides can be stored at room temperature.

NOTE: The fluorescent signal is usually only good enough for analysis for 7-14 days.

Appendix B - Masson's Trichrome Staining Protocol

Trichrome Staining Procedure

Materials

1) Trichrome Stain (Masson) kit (HT15-1KT, Sigma-Aldrich)
2) Bouin's Solution (HT10132-1L)
3) Weigert's Iron Hematoxylin set (HT1079-1SET)
4) Acetic Acid

Preparation

1) Turn on heating block and blue incubator to 56°C
2) Prepare 1 N acetic acid (stock solution)
    a. 5.742 mL acetic acid into 25 mL of MQ water and bring to volume in 100 mL flask
3) Prepare 1% acetic acid (stock solution)
    a. 8.8 mL of 1N acetic acid in 41.2 mL MQ water
4) Prepare working Phosphotungstic/Phosphomolybdic acid solution:
    a. Mix 1 volume of Phosphotungstic acid solution and 1 volume Phosphomolybdic acid solution with 2 volumes of deionized water (Theresia's water)
5) Prepare Weigert's Iron Hematoxylin solution:
    a. Mix equal parts of Solution A and Solution B

Staining Procedure

1) Let slides warm to room temperature for about 5 min
2) Draw hydrophobic rings around individual sections while slides are still dry
3) On slides, incubate sections in DI water for 2 min
4) On slides, incubate sections in preheated Bouin's solution for 15 min at 56°C in blue incubator.
5) On slides, incubate sections in tap water for 5 min
6) In Coplin jar, let slides sit in running tap water for 5 min
7) On slides, incubate sections in Weigert's Hematoxylin solution for 5 min
8) In Coplin jar, let slides sit in running tap water for 5 min
9) On slides, incubate sections in Theresa's DI water for 5 min
10) On slides, incubate sections in Biebrich Scarlet-Acid Fucshin for 5 min
11) On slides, incubate sections in Theresa's DI water for 5 min
12) On slides, incubate sections in working Phosphotungstic/Phosphomolybdic acid solution for 5 m.
13) On slides, incubate sections in Aniline Blue Solution for 5 min
14) On slides, incubate sections in 1% acetic acid for 2 min
15) In Coplin jar, incubate slides in Theresa's DI water for 1 min
16) In Coplin jar, incubate slides in 100% ethanol for 1 min
17) In Coplin jar, incubate slides in xylenes for 1 min
18) Immediately dry slides (do not touch sections), coverslip, and nail polish.

Appendix C - Hydroxyproline Determination as an Estimate of Collagen (Insoluble and Soluble in Meat) Protocol

Hydroxyproline Determination as an estimate of Collagen (Insoluble and Soluble) in Meat

Modified from:

AOCA. 2005. Official method 990.26 18th ed. W. Horwitz and G. W. Latimer.

Bergman, I. and R. Loxley. 1963. Two improved and simplified methods for the spectrophotometric determination of hydroxyproline. Anal. Chem. 35:1961-1965.

Cross, H. R., Z. L. Carpenter, and G. C. Smith. 1973. Effects of intramuscular collagen and elastin on bovine muscle tenderness. J. Food Sci. 38:998-1003.

Hill, F. 1966. The solubility of intramuscular collagen in meat animals of various ages. J. Food Sci. 31:161-166.

A. Extraction/Hydrolysis

Prior to these steps, make sure you have enough supplies and reagents to complete all samples for the extraction group. Also, pre-label tubes for soluble and insoluble fractions to make the process more efficient.

Chemicals and Reagents:

12 N NaOH

480 g Sodium hydroxide (NaOH)

In a hood, add approximately 700 ml of MQ water to a 1000 ml beaker. Place in an ice bath on a stir plate. Slowly add NaOH, allow NaOH to fully dissolve. Transfer to a 1000 ml volumetric flask and dilute to volume.

6 N HCl

Dilute concentrated HCl 1:1 with MQ water

Ringer's solution

3.5 g Sodium Chloride (NaCl)

0.013 g Calcium Chloride (CaCl2) **OR** 0.017 Calcium Chloride Dihydrate ($CaCl_2 \cdot 2H_2O$)

0.18 g Potassium Chloride (KCl)

Place chemicals in a 500 ml volumetric flash and dilute to volume with MQ water.

¼ Strength Ringer's Solution

Dilute Ringer's Solution 1:3 with MQ water.

Protocol:

1. Weigh out 3g ± 0.05g of powdered **RAW MEAT** sample (liquid nitrogen ground) into 50ml polyethylene centrifuge tubes. Record the net weight of the sample. Make duplicate subsamples for each meat sample, 2 for soluble and 2 for insoluble.
2. Add 16 ml of ¼ strength Ringer's Solution.
3. Heat for 70 min in 77°C water bath, stirring every 10 min with a metal spatula (keep spatula in sample). Place distilled DI water bottle in water bath for future steps.
4. Remove the centrifuge tubes from the water bath, rinse the spatulas with a small amount of hot DI water and let samples cool to room temperature in a water bath for 10 min.
5. Centrifuge at 3,200 x *g* (4,000 rpm) for 15 min at 20°C

6. Under the hood, decant the supernatant from **SOLUBLE** samples into labeled (with autoclave tape) screw top milk bottles through Fisher 09-795 filter paper. Decant the supernatant from **INSOLUBLE** samples into a waste beaker through Fisher 09-795 filter paper (make sure to save the filter paper).

***FOR SOLUBLE SAMPLES (steps 7- 11)**

7. Add 8 ml of ¼ strength Ringer's Solution to the residue. Stir with a metal spatula. Rinse the spatula with a small amount of hot distilled water.
8. Centrifuge the samples again with the same conditions
9. Decant the supernatant into the same milk bottles using the sample filter paper.
10. Rinse the filter paper with minimal amount of hot DI water
11. Add 25 ml of concentrated HCL to the bottles. Place screw top caps on bottle (make sure caps are slightly loose before going in autoclave).

***FOR SOLUBLE SAMPLES (steps 12-15)**

12. Using a metal spatula, quantitatively transfer the pellet from the centrifuge tubes into labeled (with autoclave tape) screw top milk bottles. Using half a Kimwipe, wipe all residue left in the centrifuge tube. Place the Kimwipe in the milk bottle with the pellet.
13. Rinse the centrifuge tubes with 25 ml of 6 N HCl and decant into the respective screw top milk bottles.
14. Place the filter paper used from step 6 into the respective screw top milk bottles containing the residue.
15. Place screw top caps on bottle (make sure caps are slightly loose before going in autoclave).

***FOR ALL SAMPLES**

16. Autoclave the bottles containing soluble and insoluble fractions for 18 hours at 121°C at 18-20 psi.
17. Remove milk bottles from autoclave and allow to cool (approximately 30 min).
18. Add 1± 0.05 g of charcoal to each milk bottle and shake until the charcoal and sample are thoroughly mixed.
19. Filter all samples through Whatman #2 filter paper into Erlenmeyer flasks (filter **INSOLUBLE** samples into 500 ml Erlenmeyer flasks and **SOLUBLE** samples into 250 ml Erlenmeyer flasks).
20. With DI water, rinse the milk bottles and caps three times, then rinse charcoal three times. Rinse enough to bring the **INSOLUBLE** sample volume just below 300 ml and the **SOLUBLE** sample volume just below 175 ml. It is very important not to exceed these volumes.
21. Adjust the pH of the filtered samples (both insoluble and soluble) to 6.0 ± 0.1 using 12 N NaOH and various concentrations of HCl. (soluble samples take about 15-20 mL 12 N NaOH, insoluble samples take about 8 ml 12 N NaOH).
22. Dilute the **SOLUBLE** collagen samples to 250 ml and the **INSOLUBLE** samples to 500 ml in volume metrics flasks.
23. Mix thoroughly by pouring solution into a beaker and stir using a stir bar and stir plate for approx. 2 min.
24. After sample is mixed, gravity filter sample into a 15 mL glass culture tube using Fisher 09-795 filter paper. Note: At this point, samples may be held overnight if refrigerated or for 1-2 wk if frozen.

B. Hydroxyproline Assay

Make sure you have enough chemicals, supplies, and reagents to read all the samples for the group. Pre-label the culture tubes to save time prior to reading.

Chemicals and Reagents:

Make the following two solutions prior to beginning hydroxyproline assay

600 µg/mL Stock Hydroxyproline Standard

30 mg hydroxyproline (.03 g)

In a 50-mL volumetric flask, dissolve 30 mg hydroxyproline in MΩ H_2O. Mix thoroughly and transfer to a 50-mL plastic conical tube, and store at 4°C for up to 2 months. (Make sure to get all of hydroxyproline into flask, weigh out hydroxyproline in flask to alleviate any problems with transferring)

Buffer solution

1. In a 1-L glass beaker filled with 500 mL of MΩ H_2O, dissolve while stirring:

30g Citric acid monohydrate

15g Sodium hydroxide

90g Sodium acetate trihydrate

2. Add 290 mL 1-propanol. Mix vigorously. At this point, if this solution is not mixed continually, it will separate into layers.

3. Adjust the pH to 6.0 with concentrated HCL

3. Transfer to 1-L volumetric flask and bring up to volume using MΩ H_2O. Store in a labeled, glass bottle covered in foil at 4°C for up to 1 month. Before using, make sure solution has not separated into layers again.

Make the following solutions same day, and just before adding the solution to the first set of tubes.

Chloramine-T Oxidant Reagent

Wear a mask when weighing out the Chloramine-T.

Dissolve 1.41g chloramine-T in 100 mL of Buffer solution.

DMBA (dimethylaminobenzaldehyde) Color Reagent

In a 100 mL beaker, dissolve 10 g of 4-dimethylaminobenaldehyde in 35 mL of cold 60% perchloric acid. Slowly add, with stirring, 65 mL of 2-propanol (isopropyl alcohol)

Protocol:

1. Set water bath to 60°C and preheat prior to reading (Takes approx. 30-45 min to get to correct temperature)

2. Prepare the 6 µg/mL Working Hydroxyproline Standard: pipet 1 mL of 600 µg/mL Stock Hydroxyproline into a 100-mL volumetric flask. Bring up to volume with MΩ H_2O. Mix thoroughly.

3. Prepare the standard curve following the table below:
   a. Order of the tubes for the standard curve is as follows: Blank, S-1, S-2, S-3, S-4, S-5, S-6, S-7

Standard Number	Volume of 6 µg/mL Working Hydroxyproline standard, mL	Volume of MΩ H_2O, mL	Final Volume, mL	Hydroxyproline Final Concentration, µg/mL

Blank	0.000	2.000	2.000	0.000
S-1	0.025	1.975	2.000	0.075
S-2	0.050	1.950	2.000	0.150
S-3	0.100	1.900	2.000	0.300
S-4	0.200	1.800	2.000	0.600
S-5	0.400	1.600	2.000	1.200
S-6	0.600	1.400	2.000	1.800
S-7	0.800	1.200	2.000	2.400

4. Using a repeater pipet with 50-mL combi-tip attached, add 1.0 mL of Chloramine-T Oxidant Reagent to all standard curve tubes. Vortex to mix (set vortex to 7 or less.) Let stand at room temperature for 20 min.

5. While incubating the standard curve, using the repeater pipet, pipet 1.50 mL of MΩ H_2O into all insoluble culture tubes and 1.0 mL of MΩ H_2O into all soluble tubes.

6. After incubation of chloramine-T, add 1.0 mL of DMBA Color Reagent using a repeater pipet to tubes. Vortex to mix (set vortex to 7 or less), cover with aluminum foil, and incubate in a water bath set to 60°C for 15 minutes (timing is critical).

7. After incubation in water bath is complete, remove tubes and move them to a cold tap water bath for 5 min.

8. Pipet 1 mL from each culture tube into a cuvette and read absorbance of samples against the water BLANK on a UV/Vis Spectrophotometer set to 558 nm. Reading should be completed immediately after pipetted into cuvettes.

9. After the standard curve is read, begin pipetting samples into culture tubes (For insoluble: 1.50 mL MΩ H2O, 0.5 mL of sample; for soluble: 1.0 mL MΩ H2O, 1.0 mL of sample (limit the amount per group to 12 tubes. AFTER standard curve is read, once a group is in the water bath, the next group can start being pipetted out).

10. Follow the same protocol for samples (Chloramine-T incubation, DMBA + water bath incubation, cold water bath incubation, pipette samples into cuvettes, read).

11. Using the standard curve absorbances and known concentrations, the GEN5 software will generate a linear regression equation and calculate the initial concentration of the unknown samples.

    a. Check the standard curve R2 should be 0.995 to 1.0. If not, delete/mask the bad points.
    b. Are the slope and intercept similar to previous hydroxyproline assays?

12. Save program file to computer in specific folder, labeled distinctly. Save to your external storage device for safe keeping.

C. Data Entry

In the GEN5 Program

1. Click on the interplate tab to check R2 and concentration values

2. For R2 value, click on the graphs tab → results: standard curve fitting results → Check R2

3. For concentration values, click on the Statistics tab → Data: concentration → Check CV %, ≤10, click on green X button for excel file → Save to your external storage device.

Using ENTRY SHEET

All gray/red/pink box columns in the entry sheet already have calculation in them. They are also locked so they cannot be changed accidently.

1. Enter the SAMPLE, REP, EXTRACTION WEIGHT, and GROUP information in the "Extraction weights tab" (Note: the "ALL" column will formulate automatically).

2. In the "Conc Entry Sheet" tab manually enter in the ALL, GROUP, SAMPLE, and REP information, but each tube will have 2 entries. So essentially, each samples will have 8 rows devoted to it. EXAMPLE:

All	Group	Sample	Rep	sample weight, g
S 4447-1	1	S 4447	1	3.0144
S 4447-1	1	S 4447	1	3.0144
I 4447-1	1	I 4447	1	3.0462
I 4447-1	1	I 4447	1	3.0462
S 4447-2	1	S 4447	2	3.0429
S 4447-2	1	S 4447	2	3.0429
I 4447-2	1	I 4447	2	3.0151
I 4447-2	1	I 4447	2	3.0151
S 8873-1	1	S 8873	1	3.0311
S 8873-1	1	S 8873	1	3.0311
I 8873-1	1	I 8873	1	3.0105
I 8873-1	1	I 8873	1	3.0105
S 8873-2	1	S 8873	2	3.0472

S 8873-2	1	S 8873	2	3.0472
I 8873-2	1	I 8873	2	3.0407
I 8873-2	1	I 8873	2	3.0407

NOTE: The samples MUST BE IN THE EXACT ORDER as listed above!

Soluble 1
Soluble 1
Insoluble 1
Insoluble 1
Soluble 2
Soluble 2
Insoluble 2
Insoluble 2

3. All gray boxes will be calculated for you.
4. Manually enter INITIAL HYDROXYPROLINE CONCENTRATION, µg/ml [FROM UV/VIS SPEC].
5. On the "Conc Entry Sheet" check CV Soluble and CV Insoluble values. These should be less than 10 between replicates. If they are not, they should be redone (Discuss with PI before redoing).
6. Once CVs are checked as acceptable, calculated values, and CVs should be copied into the "FINAL Collagen Values" worksheet. It should look like:

A	B	C	D	E	F	G	H	I	J	K	L
Sample	Rep	Soluble collagen, mg/g	Insoluble collagen, mg/g	Calculated total collagen	% Soluble collagen	% Insoluble Collagen	CV Soluble	CS Insoluble	CV Total	CV% Soluble	CV % Insoluble
6285	1	1.09	7.43	8.52	12.80	87.20	9.7	5.6	6.2	3.5	0.5
6285	2	1.25	8.04	9.29	13.45	86.55					
8258	1	0.64	6.33	6.97	9.21	90.79	10.7	0.3	1.3	9.3	1.0
8258	2	0.75	6.36	7.11	10.51	89.49					
8873	1	0.96	6.99	7.95	12.05	87.95	8.0	5.9	4.4	12.4	1.5
8873	2	0.86	7.60	8.46	10.11	89.89					
6841	1	0.95	6.82	7.77	12.24	87.76	4.9	1.6	2.0	2.8	0.4
6841	2	1.02	6.98	8.00	12.74	87.26					
4447	1	0.87	7.71	8.59	10.18	89.82	0.7	4.6	4.2	3.5	0.4
4447	2	0.87	7.22	8.09	10.71	89.29					

Appendix D - Perimysial and Endomysial Collagen Extraction From Muscle

Perimysial/Endomysial Collagen Extraction from Muscle

Adapted from Champion and Light (1984)

Reagents:

0.05M Calcium chloride

- 1000 mL D.D.I. Water
- 5.549 g $CaCl_2$
- After mixing, keep in the refrigerator.

1. Weigh out approximately 100-g of diced tissue, once weighed, keep tissue in refrigerator or frozen if not extracting the day of weighing.
2. Blend approximately 50-g of diced tissue with 100 mL of the calcium chloride solution for 10-15 s using a Waring blender.
3. Filter the homogenate through 1 mm^2 sieve into a beaker. Collect any material that does not pass through the sieve and set aside the residues for later.
4. Blend the other 50-g of tissue with 100 mL of the calcium chloride solution for 10-15 s using a Waring blender.
5. Filter the homogenate through 1 mm^2 sieve into a beaker. Collect any material that does not pass through the sieve and combine with the residues from before
6. Blend the residues with 100 mL of calcium chloride. Filter using the sieve. Collect any material that does not pass through the sieve. Repeat 2 more times.
7. Any material not passing through the sieve is referred to as the perimysial fraction and the filtered material is the endomysial fraction.
8. Freeze dry the perimysial fraction and 50 mL of the endomysial fraction.

Appendix E - Consumer and Trained Sensory Panel Forms

INFORMED CONSENT STATEMENT

1. I volunteer to participate in research involving Sensory Evaluation of Meat. This research will be conducted by personnel in the Department of Animal Sciences and Industry at Kansas State University.

2. I fully understand the purpose of the research is for the evaluation of beef steaks, pork chops, lamb chops, goat meat, poultry meat, ground meat, and processed meat products from the previously mentioned species for the sensory traits of tenderness, juiciness, flavor intensity, connective tissue amount, off flavor presence, odor, and color and sensory evaluation will last approximately one hour.

3. I understand that there are minimal risks associated with participating and that those risks are related to possible food allergies. All meat products will be USDA inspected and all ingredients are GRAS (generally accepted as safe) by FDA.

4. I understand that my performance as an individual will be treated as research data and will in no way be associated with me for other than identification purposes, thereby assuring confidentiality of my performance and responses.

5. My participation in this study is purely voluntary; I understand that my refusal to participate will involve no penalty or loss of benefits to which I am otherwise entitled and that I may discontinue participation at any time without penalty or loss of benefits to which I am otherwise entitled.

6. If I have any questions concerning my rights as a research subject, injuries or emergencies resulting from my participation, I understand that I can contact the Committee on Research Involving Human Subjects, 203 Fairchild Hall, Kansas State University, Manhattan, KS 66506, at (785) 532-3224.

7. If I have questions about the rationale or method of the study, I understand that I may contact, Dr. Travis O'Quinn, 247 Weber Hall, Kansas State University, Manhattan, KS 66506, at (785) 532-3469 or Sally Stroda, 107 Weber Hall, at 785-532-1273.

I have read the Subject Orientation and Test Procedure statement and signed this informed consent statement, this _____ day of _____, _____.

_____ _____

Printed name Signature

About Yourself

(Please circle the answer that best describes you for each item)

Gender	**Household Size**	**Marital Status**	**Age**	**Ethnic Origin**
Male	1 person	Single	Under 20	African-American
Female	2 people	Married	20-29	Asian
	3 people		30-39	Caucasian/White
	4 people		40-49	Hispanic
	5 people		50-59	Native American
	6 people		Over 60	Other
	Over 6 people			

Annual Household Income	**Education Level**
$25,000 - $34,999	Non-high School graduate
$35,000 - $49,999	High school graduate
$50,000 - $74,999	Some College/Technical School
$75,000 to $100,000	College graduate
more than $100,000	Post graduate

How many times a week do you consume beef?

1 to 3 4 to 6 7 or more

When eating beef, which palatability trait is the most important to you (circle one)?

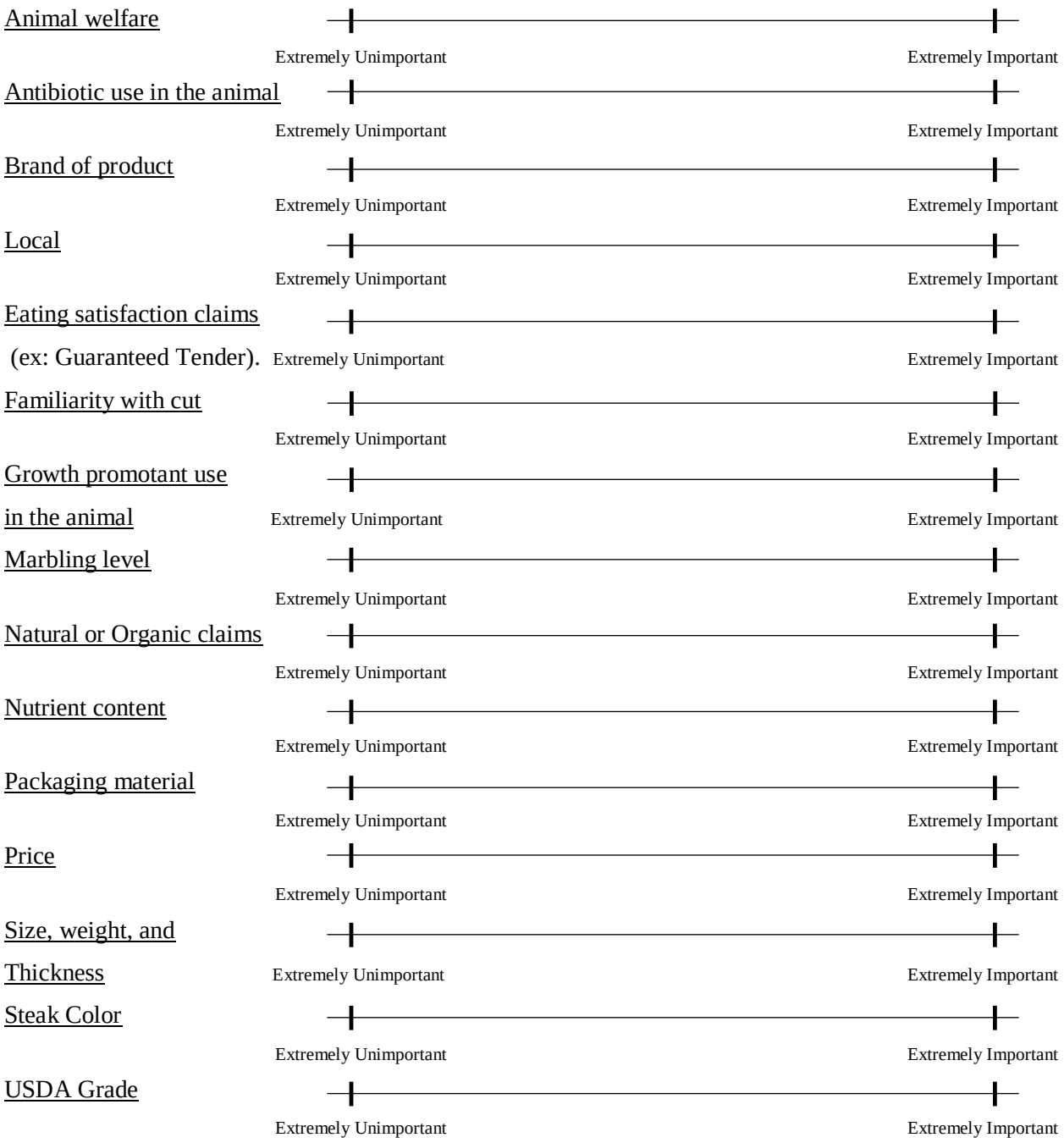
Flavor Juiciness Tenderness

Which meat product do you prefer the flavor of the most (circle one)?

Beef Chicken Fish Lamb Mutton

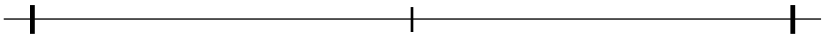
Pork Shellfish Turkey Veal Venison

Please indicate the importance of each trait when purchasing fresh beef steaks:



Consumer ID: _____ Night: _____ Round: _____ Sample ID: _____

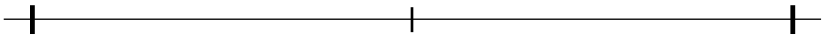
Tenderness:



Extremely Tough | Neither Tough nor Tender | Extremely Tender

Was the steak acceptable for tenderness? Yes _____ No _____

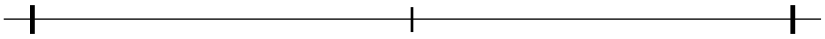
Juiciness:



Extremely Dry | Neither Dry nor Juicy | Extremely Juicy

Was the steak acceptable for juiciness? Yes _____ No _____

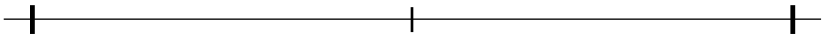
Flavor:



Dislike Extremely | Neither Dislike nor Like | Like Extremely

Was the steak acceptable for flavor? Yes _____ No _____

Overall Liking:



Dislike Extremely | Neither Dislike nor Like | Like Extremely

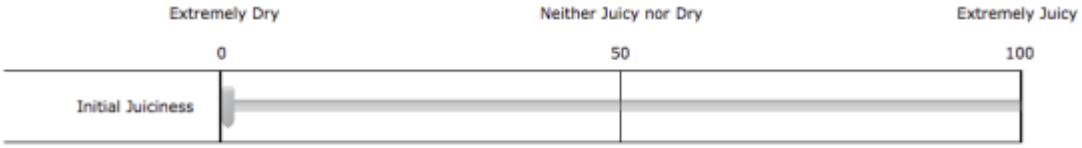
Was the steak acceptable for overall liking? Yes _____ No _____

Please check one of the following to **rate the quality** of the beef sample you have just eaten. Choose only **one** (you must make a choice).

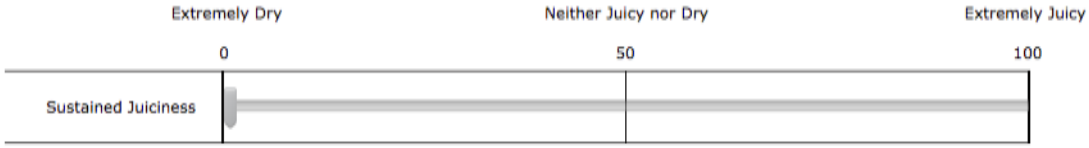
Unsatisfactory	☐	Better than everyday quality	☐
Everyday quality	☐	Premium Quality	☐

Trained Panel Form

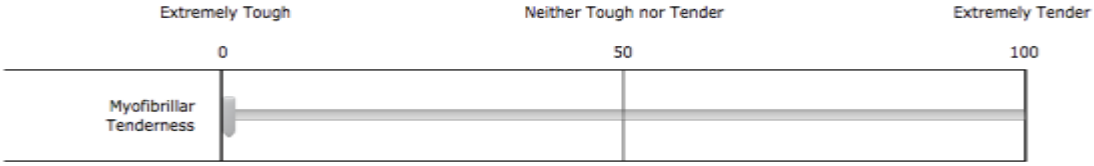
Initial Juiciness



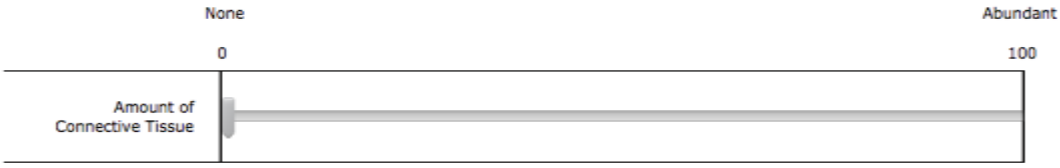
Sustained Juiciness



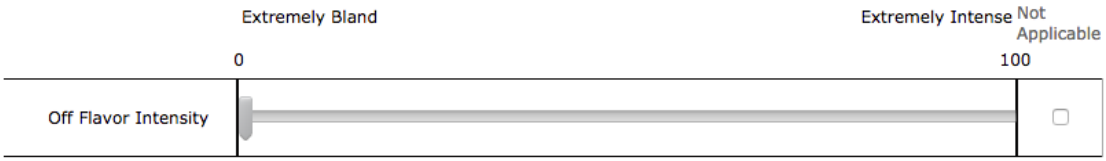
Myofibrillar Tenderness



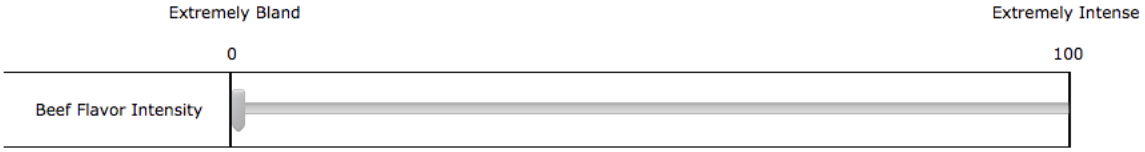
Amount of Connective Tissue



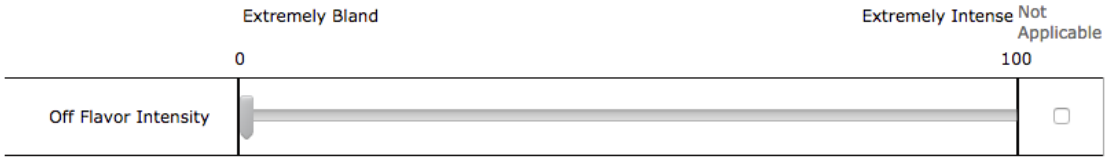
Off Flavor Intensity



Beef Flavor Intensity



Off Flavor Intensity



Off Flavor Description

Appendix F - Data Sheets

Color and pH

Sample ID	Scan Number	L*	a*	b*	pH

Cooking Weights and Temperatures

Steak ID	Raw Weight	Cooked Weight	Peak Temperature

Pressed Juice Percentage

	Sample 1			Sample 2			Sample 3		
Steak ID	**Dry Filter Paper**	**Filter Paper with Sample**	**Wet Filter Paper**	**Dry Filter Paper**	**Filter Paper with Sample**	**Wet Filter Paper**	**Dry Filter Paper**	**Filter Paper with Sample**	**Wet Filter Paper**

Appendix G - Appendix Tables

Table G.1. Least squares means of myosin heavy chain (**MHC**) distribution and cross-sectional area of beef strip loin steaks of varying marbling texture and quality grade treatments.

Treatment	% MHC Type I	% MHC Type IIA	% MHC Type IIX	MHC Type I Cross-Sectional Area, μm^2	MHC Type IIA Cross-Sectional Area, μm^2	MHC Type IIX Cross- Sectional Area, μm^2
Marbling Texture						
Coarse	30.25	39.87^{b}	29.88^{a}	2984.90	3907.87	4990.48
Medium	28.99	46.47^{a}	24.54^{b}	3309.04	4372.13	5255.13
Fine	30.31	39.38^{b}	30.31^{a}	2948.10	4057.85	5041.25
SEM[1]	0.8	1.6	1.7	153.39	188.32	223.47
P-value	0.54	<0.01	0.03	0.19	0.21	0.67
Quality Grade						
Top Choice[2]	30.12	41.42	28.46	3094.95	4005.22	5016.73
Low Choice	29.12	42.24	28.64	3072.21	4066.85	5024.72
Select	30.30	42.07	27.63	3074.89	4265.79	5245.41
SEM	0.8	1.6	1.7	153.39	188.32	223.47
P-value	0.44	0.93	0.90	0.99	0.59	0.71
Texture × QG						
P-value	0.24	0.42	0.75	0.93	0.82	0.46

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

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

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

Table G.2. Least squares means of adipocyte size and perimysial thickness of beef strip loin steaks of varying marbling texture and quality grade treatments.

Treatment	Adipocyte size, μm^2	Perimysial thickness, μm
Marbling Texture		
Coarse	4499.77^{a}	43.23
Medium	4203.27^{ab}	46.26
Fine	3847.94^{b}	41.44
SEM[1]	182.33	2.23
P-value	0.04	0.31
Quality Grade		
Top Choice[2]	4334.39^{a}	44.56
Low Choice	4430.30^{a}	42.54
Select	3786.30^{b}	43.84
SEM	182.33	2.23
P-value	0.03	0.81
Texture × QG		
P-value	0.92	0.36

[1]SE (largest) of the least squares means in the same main effect (marbling texture or quality grade).
[2]USDA marbling score of Modest^{00}-Moderate^{100}.
^{ab}Means within the same main effect (marbling texture or quality grade) without a common superscript differ ($P < 0.05$).

Table G.3. Coefficient of variation of palatability traits of beef strip loin steaks of varying marbling texture and quality treatments evaluated by trained panelists.

Treatment	Myofibrillar Tenderness	Connective Tissue Amount	Overall Tenderness	Beef Flavor Intensity	Off-Flavor Intensity
Marbling Texture					
Coarse	0.18	0.95	0.20	0.31	0.00
Medium	0.19	1.00	0.21	0.29	0.26
Fine	0.17	0.97	0.19	0.31	0.00
SEM[1]	0.01	0.06	0.01	0.02	0.25
P-value	0.37	0.78	0.60	0.53	0.17
Quality Grade					
Top Choice[2]	0.17	0.95	0.19	0.32	0.00
Low Choice	0.17	0.93	0.20	0.29	0.00
Select	0.19	1.05	0.22	0.30	0.45
SEM	0.01	0.06	0.01	0.02	0.26
P-value	0.28	0.25	0.38	0.45	0.26
Texture × QG					
P-value	0.11	0.47	0.28	0.23	0.08

[1]SE (largest) of the least squares means in the same main effect (marbling texture or quality grade).
[2]USDA marbling score of Modest[00]-Moderate[100].

Table G.4. Coefficient of variation of palatability traits of beef strip loin steaks of varying marbling texture and quality treatments evaluated by consumers (n = 104).

Treatment	Juiciness	Tenderness	Flavor Liking	Overall Liking
Marbling Texture				
Coarse	0.33	0.31	0.33	0.32
Medium	0.35	0.32	0.31	0.31
Fine	0.32	0.30	0.30	0.29
SEM[1]	0.02	0.02	0.02	0.02
P-value	0.75	0.31	0.40	0.42
Quality Grade				
Top Choice[2]	0.32	0.31	0.31	0.29
Low Choice	0.33	0.29	0.31	0.30
Select	0.35	0.33	0.33	0.33
SEM	0.02	0.02	0.02	0.02
P-value	0.68	0.82	0.61	0.40
Texture × QG				
P-value	0.73	0.99	0.29	0.28

[1]SE (largest) of the least squares means in the same main effect (marbling texture or quality grade).
[2]USDA marbling score of Modest[00]-Moderate[100].

Table G.5. Coefficient of variation of objective palatability measurements of beef strip loin steaks of varying marbling texture and quality treatments.

Treatment	Warner-Bratzler shear force, kg	Pressed Juice Percentage[1]
Marbling Texture		
Coarse	0.25	0.11
Medium	0.26	0.08
Fine	0.28	0.12
SEM[2]	0.02	0.01
P-value	0.47	0.06
Quality Grade		
Top Choice[3]	0.28	0.09
Low Choice	0.24	0.11
Select	0.26	0.10
SEM	0.02	0.01
P-value	0.23	0.66
Texture × QG		
P-value	0.33	0.74

[1]Percentage moisture lost during compression of sample between filter paper at 8 kg of pressure for 30 seconds.

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

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

Table G.6. Interaction of the coefficient of variation of initial juiciness and sustained juiciness trained panelist ratings of beef strip loin steaks of varying marbling texture and quality treatments.

Treatment	Initial juiciness	Sustained juiciness
Coarse		
Top Choice[1]	0.24[abc]	0.34[ab]
Low Choice	0.18[bc]	0.27[b]
Select	0.25[ab]	0.37[a]
Medium		
Top Choice	0.23[abc]	0.33[ab]
Low Choice	0.22[abc]	0.34[ab]
Select	0.21[abc]	0.31[ab]
Fine		
Top Choice	0.17[c]	0.26[b]
Low Choice	0.27[a]	0.38[a]
Select	0.23[abc]	0.34[ab]
SEM[2]	0.26	0.38
P-value	0.02	0.02

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

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

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