PREDICTING BEEF TENDERNESS USING NEAR-INFRARED SPECTROSCOPY

By

SARAH RACHELE RUST

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NOMENCLATURE

a*	measures red and yellow
b*	measures blue and green
°C	degree Celsius
cm	centimeters
d	day
h	hour
kg	kilograms
L*	measures black and white
MARC	Meat Animal Research Center
NCBA	National Cattlemen's Beef Association
NIR	Near-Infrared Reflectance
pH	partial concentration of hydrogen
R^2	correlation
RGB	red, green, blue
SSF	Slice-shear force
USDA	United States Department of Agriculture
WBSF	Warner-Bratzler shear force

FORMAT OF THESIS

This Thesis is presented in the Journal of Animal Science style format, as outlined by the Oklahoma State University Graduate College style manual. The use of this format allows for independent chapters to be prepared suitable for submission to scientific journals.

CHAPTER I

INTRODUCTION

Beef tenderness is a primary consideration in consumer satisfaction (Savell et al., 1987, 1989). This fact is confirmed by a positive relationship between the price of a cut of meat and its relative tenderness (Koohmaraie et al., 1994). A "teeter-tooter" relationship exits, in that the beef industry has struggled with the challenge of a balancing act producing lean beef products that are consistent in their palatability characteristics. Working with products that originate from multiple breeds and thousands of producers, who use different production systems, the variability within the carcass population is immense. Despite its significance as a quality indicator, tenderness is not a factor directly affecting product value for beef producers and packers. There is growing recognition that beef tenderness must be incorporated in the quality grading process if true, value-based marketing is to develop. In attempt to wrap their hands around the beef tenderness issue, the National Cattlemen's Beef Association (NCBA) brought together a group of industry experts that represented all segments of the beef production chain. Topics such as factors influencing cooked beef tenderness, prediction of tenderness, and current technologies to estimate cooked beef tenderness were addressed. The "take home message" of the conference was simple and straightforward. "In order to improve beef tenderness and ultimately overall quality, identification of tough carcasses must be issue number one in the beef industry."

The primary method used today as a marketing tool to differentiate between tenderness levels is USDA Quality Grading. The reason this method is implemented today, and has been for years, is because it is a non-destructive, on-line method that is the most applicable across all marbling scores. Certainly, cost of USDA Quality grading (approximately $45/hr/grader) is positive for this palatability-estimating program. However, using USDA Quality grades to sort carcasses into projected eating groups presents a challenge, because the lion's share of U.S. fed beef presents a narrow range of quality-grade variation. Roughly, 80% of production exhibits limited diversity of marbling scores, grading either USDA Select or Low Choice, and consumer dissatisfaction with eating quality of beef generally relates to carcasses within that range (Belk et al., 2000). This posses a problem and has led to the questioning of the economical value in Quality grading. What makes Quality grading such a challenge is that it is an indirect measure of tenderness.

Industry professionals have laid out the groundwork with regard to instrument technology and its guidelines. In order for tenderness prediction to be accepted, it must meet the following guidelines: it must be accurate and repeatable in classifying carcasses into tenderness groups (Tender, Intermediate, and Tough), it must be a rapid, non-destructive method for on-line evaluation, and it should be a direct measure of tenderness at the consumer's level.

Instrument grading has potential to improve objectivity of predictions of palatability and cutability by employing technology to sort carcasses into quality groups. Three technologies that have been developed to determine beef tenderness are Meat Animal Research Center Slice Shear Force, the Colorado State University BeefCam, and the Wulf Colorimeter developed at South Dakota State University. However, none of these instruments have succeeded in meeting all specified criteria. Therefore, the objective of this project was to predict beef tenderness from fresh meat by non-destructive means for on-line evaluation.

CHAPTER II

REVIEW OF LITERATUE

In 1994, the Beef Product Technology Subcommittee of the National Live Stock and Meat Board held the National Beef Instrument Assessment Plan (NBIAP) Symposium to assess then-existent capabilities to evaluate cutability/quality/palatability of live cattle, beef carcasses and/or beef primal/subprimal cuts (Smith, 1999). The priority order for allocation of research funds in the National Beef Assessment Plan (National Live Stock and Meat Board, 1994) was: (A) For Applied Research- (1) Video Image Analysis, (2) Total Body Electrical Conductivity, (3) Tender Tec Probe, (4) Swatland's Probe, and (5) Real-Time Ultrasound For Seedstock, and (B) For Basic Research- (1) Ultrasound for Tenderness, (2) Elasticity for Tenderness, (3) Swatland's Probe, (4) Ultrasound for Cutability, and (5) Total Body Electrical Conductivity (Smith, 1999).

A number of studies have shown that consumers can differentiate beef that varies in tenderness and are willing to pay some level of premium for guaranteed tenderness (Boleman et al., 1997; Lusk et al., 2001; Shackelford et al., 2001). Improving product quality and consistency, with respect to tenderness, has been identified by the NCBA as a critical element in the industry's efforts to increase beef market share. The beef industry focus on improving product consistency and tenderness is predicated on information suggesting that 1) taste and tenderness of beef are two primary drivers of consumer purchase decisions, 2) consumers are not satisfied with consistency of quality or tenderness, 3) improvements of tenderness and consistency of eating quality would motivate consumers to increase beef consumption (Moeller and Courington, 1998). Inconsistency in beef tenderness is due to a combination of the inability to routinely produce tender meat and the ability to identify cuts from tough carcasses (Koohmaraie et al., 1994). As part of its effort to implement value-based marketing, the beef industry began investigating use of instruments with the ability to more precisely sort carcasses on the basis of cooked beef palatability. Despite the best efforts of the beef industry and USDA to continually improve beef carcass Quality grades, new instrument-based technologies are necessary as branded beef programs continue to become the marketing methodology of choice (Belk et al., 2000).

In an experiment at Kansas State University, where consumers had only their own taste tests to rely on, 69% of the surveyed participants preferred "guaranteed tender" steaks when deciding between a guaranteed tender steak and a probably tough steak (Mintert et al., 2000). When consumers were informed that one steak was "guaranteed tender" and the other steak was "probably tough," 84% of the participants preferred the "guaranteed tender" steak. Results from this study demonstrated that providing consumers with tenderness information has value and would help ensure a satisfactory dining experience. When participants were informed which steaks were tender and tough, more participants preferred the "guaranteed tender" steak. Participants who were told which steaks were "guaranteed tender" and "probably tough" bid a premium for the tender ribeye steak that was about $0.82 per pound greater than the participants who relied only on their own taste test to differentiate steaks. This investigation demonstrates that some consumers are willing to pay a large premium as high as $2.67 per pound or more to obtain a "guaranteed tender" instead of a "probably tough" steak (Mintert et al., 2000).

In order for a value-based marketing system to work, both the producer and the packer must be confident that the subjectivity of the assignment of USDA Quality grades and Yield grades has been minimized. Instrument grading has the potential to improve objective of predictions of palatability and cutability by employing technology to segment or sort carcasses into quality and/or yield groups. If researchers can develop a more consistent system of predicting meat tenderness, the beef industry can advance in quality assurance (Tatum et al., 1997).

Because meat tenderness has such a great influence in the beef industry, several direct and indirect methods have been demised to predict cooked meat tenderness. The two most commonly used methods are Warner-Bratzler shear force (WBSF), and slice shear force (SSF). Presently, the WBSF is easily the most widely used and accepted quantitative method to objectively measure tenderness of cooked meat. The U.S. Meat Animal Research Center has shown that beef longissimus WBSF measurements at the time of carcass grading can serve as a valid predictor of cooked beef longissimus WBSF following 14 d of postmortem aging (Shackelford et al., 1997). The shear force value given by this device is the amount of force required to shear a one-half inch fore of meat sample, which is reported in pounds or kilograms (McKenna, 2003). Initially the Warner-Bratzler shear force values were used to establish differences in tenderness from one cut of meat to another. However, this did not determine if a cut of meat was tender or not; it just determined the difference in tenderness among samples (McKenna, 2003). The d 1 shear is a much better predictor of aged longissimus shear force than any visual, physical, or chemical measurement, which makes it a suitable tenderness-grading criterion (Koohmaraie et al., 1994). The U.S. Meat Anima Research Center has outlined a system for measuring beef longissimus tenderness under commercial processing conditions using a simplified method of WBSF determination (Shackelford et al., 1999). Although, this method is used frequently in research, its efficiency has hindered its ability to be implemented in a plant environment. In 1994, at the National Beef Tenderness Plan Conference in Denver Colorado, the need to standardize the protocol for WBSF determinations was identified. The growing interest in genetic information on tenderness via WBSF prompted the need for consistent WBSF determinations across institutions for comparative evaluation (NCBA, 1994). Wheeler et al. (1994, 1996) demonstrated that differences in protocol could result in spurious variation in WBSF values. Proper execution of a standardized protocol is imperative for obtaining accurate and repeatable shear force measurements (Wheeler et al., 1997).

While developing a method for on-line assessment of meat tenderness, Shackelford et al. (1999) developed a simplified technique for measuring longissimus shear force that is referred to as slice shear force (SSF); this method seems to be more accurate than WBSF. The SSF method is used to segregate carcasses into expected tenderness outcome groups. This method utilizes a classification system that includes three tenderness stages in which the highest grade consists of carcasses that are already at acceptable tenderness before aging, with a mean d-14 shear force value of 4.1 kg, which is identified as "tender". The middle grade consists of carcasses that are not tender before aging, but that will probably be tender after postmortem aging with a mean d-14 shear force value of 5.1 kg, which is identified as "probably tender." The lowest tenderness grade is made up of carcasses that are extremely tough before postmortem aging and that will probably still be tough after extensive postmortem aging, with a mean d-14 shear force value of 7.3 kg, which is identified as "probably tough" (Shackelford et al., 1999). The high level of accuracy of SSF for sorting carcasses into tenderness groups is in agreement with previous data (Shackelford et al., 1999, 2001). It appears that accurate postmortem longissimus tenderness classification would enable the beef industry to market certain cuts based on tenderness (Tatum et al., 1999; Wheeler et al., 2000). It has been determined that beef longissimus when measured directly by WBSF or SSF on d-1 postmortem is strongly related (R^2=.75) to the ultimate tenderness of the longissimus muscle following d-14 of postmortem aging. Analysis of data indicates the accuracy of segregation of carcasses into expected aged longissimus muscle tenderness groups was 85%, which was higher than the present beef Quality grading system (60%). Although Shackelford et al. (1999) showed the system to be effective; samples were collected in a commercial packing plant and transported back to the U.S. Meat Animal Research Center facilities for SSF testing. An on-line system has not yet been developed and the invasiveness of the procedure is still a hindrance to high speed requirements of current packing plants (Belk et al., 2000).

Currently, prediction of cooked beef palatability relies on marbling scores, taken at the 12th-13th rib cross-section of the longissimus combined with physiological maturity, which is used to segregate and price beef carcasses based on USDA beef Quality Grades (Belk et al., 2000). Numerous investigations of the relationship between marbling and beef palatability have shown that although there is a positive relationship between marbling degree and tenderness, this relationship is weak at best (Koohmaraie, 1995). The decision to include marbling as a primary value-determining characteristic in beef carcass assessment was based on the premise that marbling is associated with eating quality (Belk et al., 2000). Smith et al., (1987) illustrated how marbling effectively sorts carcasses on the basis of expected eating quality when the sample population spans the entire range of possible quality grades experienced in the U.S. beef supply. Wheeler et al. (1994) reported that marbling explained about 5% of the variation in palatability traits and that there was both tough and tender meat within each marbling degree. Although, this system results in general categorization according to tenderness differences, product value is lost due to inaccuracy of sorting methodology, and because "inferior" products have been produced and must be sold at discount prices (Tatum et al., 1997). Because consumers are willing to pay more for steaks that are known to be tender, there is economic incentive for predicting meat tenderness (Koohmaraie 1994). This makes it essential to develop methods to impartially predict meat tenderness to supplement or replace the current USDA Beef Quality Grading system.

Tenderness of cooked beef is determined by five structural and historical differences (related to chemical and physical composition of muscles, and to the historical architecture of the sarcomeres, myofibrils, muscle fibers, muscle bundles, and muscles) and by, at least, seven animal and carcass characteristics related to the genetics of the animal and to the environment to which the animal has been exposed. Structural differences among muscles that help determine the tenderness include: amount of connective tissue, sarcomere length, sarcomere degradation, size and dispersion of marbling deposits, and activity of endogenous proteolytic enzymes (Tatum et al., 1997). The seven animal and carcass characteristics that are related to the genetics of the animal and the environment to which the animal is exposed, and that help determine the relative tenderness of cooked beef are: physiological age/maturity of cattle/carcass, external fat thickness of cattle/carcass, amount of marbling in the muscle of carcass biological types of cattle, temperament and handling/stress of cattle, management of cattle relative to castration, use of growth implants and intramuscular injections, and feeding of vitamin D_3 (Tatum et al., 1997).

Pre-Harvest Techniques Associated with Tenderness

Factors that can be biased by procedures during the pre-harvest stage are genetics and management. The best estimate indicates that, within a single breed, genetics control approximately 30% of the variation in beef tenderness, which represents the heritability. Therefore, within a breed, 70% of the variation in beef tenderness is explained by environment and non-additive gene effects (Koohmaraie et al., 1995). The management and handling factors that can affect tenderness include: castration of bulls, dietary management, implanting strategy, time on feed, age of slaughter, and handling of cattle between shipping and the time they are stunned and bled (Dikeman, et al., 2003). Generally, longissimus steaks from bulls are less tender than steaks from steers. Bulls castrated after six months of age may be less tender or more variable in tenderness than those from bulls castrated at a very young age.

Whether cattle are background on roughage before finishing on a high grain diet or fed a high grain diet shortly after weaning through the finishing state there is a minimum direct effect on tenderness. As long as cattle are fed high grain rations for a minimum of 100 days before slaughter, their carcasses are generally more tender (Dikeman, et al., 2003). Some biological types of cattle need to be fed longer than 100 d to attain adequate finish and marbling. The number of days should be matched with the biological type of cattle, so that they reach the target fat thickness and/or percentage of Choice grade carcasses. Several research studies have shown that cattle should be fed to achieve 0.84 cm or more of fat cover in order to avoid possible decreased tenderness associated with unusually rapid chilling (Dikeman, et al., 2003). Whether cattle are backgrounded or not may have more of an 'indirect' effect on tenderness because of differences in age at slaughter rather than true nutritional effect.

Another valuable management strategy affecting tenderness is implanting cattle with anabolic implants to improve growth rate, feed efficiency, and carcass composition. Limited research has shown that implants containing estradiol and trenbolone acetate implanted within 70 d of harvest can have a detrimental effect on tenderness compared with the less aggressive implants not containing trenbolone acetate, and/or compared with implanting more than 70 d before slaughter (Dikeman, et al., 2003).

Tenderness may also be improved by incorporating supra nutritional levels of vitamin D_3 in the diet for several days before harvest. The results are an increase in plasma calcium concentration, which may activate the calpain proteolytic enzyme system to improve tenderness (Dikeman, et al., 2003).

Stressful environmental conditions and handling of cattle in a way that excites them can have negative effects on meat color and tenderness. Stressed cattle can have a higher incidence of dark cutters and borderline dark cutters. Borderline dark cutting carcasses have an intermediate pH (5.9 to 6.2) that is not optimum for enzymatic tenderization of meat (Dikeman et al., 2003).

Genetic-make up of cattle is another indicator of tenderness. On average, some breeds of cattle produce more tender meat and some produce less tender meat relative to other breeds as reported by (Koohmaraie et al., 1994). Mean shear force and variation in shear forces increase as the percentage of Bos Indicus inheritance increases (Crouse et al., 1989). Furthermore, meat from ½ or greater Bos Indicus cattle is usually significantly less tender than meat from cattle with less than ½ Bos Indicus (Koohmaraie et al., 1994).

Genetic maps are rapidly being constructed as a basis for identification of markers associated with Quantitative-Trait-Loci for use in Marker-Assisted-Selection in cattle breeding programs (Koohmaraie et al., 1994). Several hundred markers spaced randomly throughout the cattle genome have been identified, sequenced, and used to trace the heritance of DNA segments from parent to offspring in cattle families designed for development of a linkage map. A linkage map characterizing heterozygous, well-spaced markers enables efficient selection of markers for identification of Quantitative-Trait-Loci's segregating in cattle resource populations (Koohmaraie et al., 1994). Resource populations are well defined large families of animals having traceable heritage through pedigree analysis and segregating alleles of genes affecting phenotypic characteristics of interest like meat tenderness. Evidence is growing that we will be successful in identifying markers with proximity to loci having substantial effect on economically important traits (Koohmaraie et al., 1994). Markers for human disease and plant disease have already been discovered and used for beneficial purposes. A region on pig chromosome 4 was shown to contribute to breed differences in growth rate, fatness, and length of small intestine. A region on cattle chromosome 1 may contain genes responsible for "polledness" (Koohmaraie et al., 1994). Base on these discoveries, it is reasonable to assume that Marker-Assisted-Selection for economically important traits will be implemented in the beef cattle selection programs. Experiments are already underway at the U.S. Meat Animal Research Center to identify markers for beef tenderness and other traits; however it is important to recognize that even with the genes regulating tenderness at hand, not all the variation in meat tenderness can be controlled. The reason is that tenderness is a trait that is highly affected by factors other than additive genetics (Koohmaraie et al., 1995).

Post Harvest Techniques Influencing Beef Tenderness

Aside from reducing variation in tenderness, other methods have been developed to help eliminate and improve tenderness in the post-harvest stages. It has been determined that connective tissue and marbling together account for only approximately 20% of the observed variation in cooked meat tenderness. In 1984, a project was initiated at the U.S. Meat Animal Research Center to determine factors regulating tenderness of aged beef. Based on the results, it has been hypothesized that the difference in the rate and extent of postmortem tenderization is responsible for the other 80% of variation in the tenderness of aged beef (Koohmaraie et al., 1994). There are at least eight postmortem interventions that will increase the tenderness of beef: high temperature conditioning of carcass, suspension of the carcass by the pelvis, electrical stimulation of the carcass, infusion of calcium into carcass/cuts, wet or dry aging of carcass/cuts, blade/needle tenderization of cuts, marinating in salt/acid solutions, and use of tropical plant enzymes (Tatum et al., 1997). As cooking progresses, contractile proteins in the meat become less tender, and the major connective tissue protein becomes more tender. The degree of doneness also affects tenderness. As the lean is heated, the contractile proteins toughen and moisture is lost. Both decrease tenderness (Epley, 2003).

Instruments have been developed for use in determining carcass merit of seedstock, for sorting feeder cattle, for evaluating slaughter cattle, and for predicting composition of carcasses and tenderness of steaks/roasts from carcasses. Ultrasound technology is of substantial value for characterizing differences in cattle. A number of studies have shown that consumers can differentiate beef that varies in tenderness and are willing to pay some level of premium for guaranteed tenderness. Sorting beef carcasses from young cattle for quality has long been based on the relationship between marbling scores of the 12th rib cross section of the longissimus and cooked beef palatability. Many instruments have attempted to predict tenderness, but no instrument has yet to fulfill every aspect to successfully and efficiently read tenderness. This is probably the biggest challenge of all, inventing a machine that can accurately and quickly predict tenderness at a consistent rate.

Instrument Grading

Ultrasound Technology

Ultrasound technology has been investigated and/or is actually being used in applications for: (a) evaluating seedstock, (b) sorting feeder cattle into outcome groups, (c) identifying the harvest dates for slaughter cattle and (d) predicting quality, palatability, and cutability of carcasses. Ultrasound technology has been used to estimate ribeye area and external fat thickness of seedstock for more than three decades (Smith, 1999). Ultrasound technology used to estimate the amount of intramuscular fat or marbling (Science, 1994) which was conducted by Iowa State University scientists. Numerous seedstock producers now employ such technology and some breed associations (Hough, 1998) use ultrasound technology to compute carcass expected progeny differences for breeding animals. One system using ultrasound technology to predict feeder cattle performance and to sort feeder cattle into outcome groups was the ACCU-TRAC system of the Micro Chemical Company in Amarillo, Texas (Smith, 1999). As a part of the Washington State University Strategic Alliance Project, the AgCanada Ultrasound System (Smtih, 1999), the Brethour Ultrasound System (Smtih, 1999), and the Scofield Cattle Scanning Video Image Analysis System (Smtih, 1999) were compared for accuracy. The Brethour Ultrasound System optimized percentages of cattle that produced U.S. Choice carcasses, minimized percentages of carcasses that were too light or too heavy, and achieved the highest merit score (92.7) and monetary sorting response ($19.89) among the three systems tested as reported by Smith (1999).

Ultrasound technology is used by beef industry companies to reduce the guesswork of figuring the optimum time in the feedlot to finish fattening cattle (Meat Marketing and Technology, 1999). Smith reported (1999) that Dr. Lynn Locatelli (Benkelman, Nebraska) said, "ultrasound scans can be used to evaluate the fat and marbling under the hide, to determine when no further economic gains are likely from further feeding, and with knowledge of amounts of backfat and marbling 60 to 70 d prior to slaughter to adjust rations and feeding schedules to finish the animal at an optimum level." Tatum et al. (1990) ultrasonically evaluated steers on the day before harvest for fat thickness and ribeye area. For fat thickness, 63% of the ultrasound measurements taken at the $12^{th}/13^{th}$ rib were within 0.254 cm of the carcass values, and 92% were within 0.508 cm. For ribeye area, 33% of the ultrasound measurements were within 1.27 square cm of carcass values and 61% were within 2.54 square cm (Tatum et al., 1990).

Smith (1999) reported that ultrasound technology has been investigated for use in estimating Yield grade and Quality grade of beef carcasses but results have been disappointing. Scientists at the University of Illinois attempted to develop a data acquisition system for ultrasound grading of beef carcasses, but the system was very slow and did not predict cutability or palatability with accuracy or precision that was equal to that achieved by application of USDA Yield grades and Quality grades.

Tender Tec

A number of invasive (probe-type) technologies have been developed and proposed for use in predicting tenderness of steaks and roasts by measuring resistance to needle penetration into the ribeye muscle of a carcass. The first such probe was called the Armour Tenderometer. This system utilized a group of probes that were inserted into the longissimus following carcass chilling. It measured the force required to penetrate the muscle and used this information to predict cooked meat tenderness. Carpenter et al., (1972) concluded that the Armour Tenderometer effectively categorized USDA Choice beef carcasses into tenderness desirability groups. Huffman (1974), however, reported that Armour Tenderometer readings were poorly related to WBSF values (R^2= .22), there was no relationship between Armour Tenderometer readings and trained taste panel scores for tenderness (P >.05) taken from 192 carcasses ranging in USDA Quality Grade from Prime to Standard. More recently, Harris et al. (1992), using data from the study by Smith et al. (1984), reported that the Armour Tenderometer readings predicted sensory panel tenderness ratings and shear force values for steaks from 384 steer/heifer carcasses with less than 1% and less than 2% accuracy, respectively. George et al. (1997a) conducted three experiments in an attempt to validate the accuracy of Tender·Tec in predicting the tenderness of steaks from youthful beef carcasses. Tender·Tec failed to consistently detect, tenderness differences in steaks derived from youthful carcasses, and is thus of limited value as an instrument for improving the consistency and uniformity of the U.S. beef supply (George et al., 1997a).

George et al. (1997b) conducted a study to validate the accuracy of Tender·Tec in predicting the tenderness of steaks from mature beef carcasses; Tender·Tec detects differences among carcasses in muscle connective tissue characteristics, so its use is inherently limited as a predictor of cooked steak tenderness to use on mature (heiferette/cow) rather than on youthful (heifer, and probably steers) and particularly as cooked degree of doneness increases. It appears unlikely that mechanical assessment of raw postmortem muscle will ever be useful as a predictor of palatability of that same muscle after it has been cooked (George et al., 1997b). George et al. (1997c) concluded that even though the Tender·Tec probe detected some differences in connective tissue contributions to rib steak tenderness, it was no better than USDA Quality grade at segmenting A-maturity carcasses into anticipated tenderness outcome groups, and thus its applicability as a grading instrument may be limited to use on more mature beef carcasses. Because of the low correlation with ultimate meat tenderness and palatability, and the apparent ineffectiveness of this technology, it has since been abandoned as a tenderness-predicting tool (Belk et al., 2000).

Video Image Analysis

Video image analysis measures color of the entire exposed surface of the longissimus muscle at the 12th rib. Early work using Video image analysis technology to measure beef muscle color was marginally successful (Li et al., 1999). The early video image analysis systems used the computer compatible RGB color measurements computed from the video images to determine the lean color of beef longissimus muscle. Although, the RGB colors were correlated with tenderness, attempts to sort carcasses into different palatability classes using these color measurements were unsuccessful (Li et al., 1997). However, these results did prove that computer software could be written that would accurately segment a video image of a ribeye, via image processing techniques, into lean, fat, and connective tissue components and conduct analysis of color and other attributes generated by color measurements on each of these components, independently (Belk et al., 2000).

In 1996 , Colorado State University initiated work with Hunter Associates Laboratory to develop a video image analysis system that could measure beef carcass lean and fat color using the L*, a*, and b* color scale. Color measurements from the video image analysis system and expert quality grading factors were used to sort steaks based on palatability. The results showed that the probability of encountering a tough (WBSF ≥ 4.5 kg) after 14 21 d of aging was reduced from .18 to .25 and .15 to .02 for USDA Choice and USDA Select steaks, respectively (Belk et al., 1997). Belk et al., (1997) reported that the data confirmed that (1) color is related to subsequent cooked palatability of beef carcasses, independently of differences in marbling or carcass maturity, and (2) video image analysis technology is capable of ascertaining color attributes of beef ribeyes, using color information to augment USDA quality grades, and thereby improve the accuracy of quality grades in sorting carcasses based on expected eating palatability across a narrow ranges of marbling scores (Belk et al., 2000).

With these preliminary results in mind Colorado State University and Hunter Associates Laboratory began development of a prototype portable video imaging system called BeefCam™, which contained hardware and software that was designed for beef carcass lean and fat color in a packing plant environment. A study conducted by Wyle et al., (1999a) used the BeefCam™ system, either alone (Model I) or in conjunction with USDA Quality Grade (Model II) to certify carcasses as being tender (WBSF< 4.5 kg) or tough (WBSF ≥ 4.5 kg). The use of Model I resulted in 51.9% of the carcasses evaluated being characterized as tender, and 92.2% of those that were certified were actually tender. Using Model II, 53.4% of the carcasses evaluated (n=500) were certified as being tender and 94.4% of those certified were actually tender.

Wyle et al. (1999a) determined the relationship between shear force values of 949 steaks and USDA Quality Grades of the carcasses from which the steaks were obtained; percentages of tough steaks among those from carcasses of Upper-2/3 Choice, Lower-1/3 Choice, and Select were 5.6%, 12.4%, and 29.4%, respectively. Each of the 949 carcasses in the Wyle et al. (1999a) study were evaluated by use of the Hunter Lab BeefCam; preliminary analyses suggest that the BeefCam is capable of identifying most of the carcasses that yield tough strip loin steaks. Wyle et al. (1999b) used the Hunter Associates Laboratory BeefCam to evaluate an additional 348 Beefmaster steer carcasses in two feeding trials. In Trial I, the BeefCam correctly identified 150 of 156 carcasses that produced tender rib steaks and 5 of 10 carcasses that produced tough steaks; in Trial II, BeefCam correctly identified 139 of 150 carcasses that produced tender rib steaks and 2 of 8 carcasses that produced tough steaks. BeefCam inappropriately classified very small numbers of carcasses as "likely to produce tough beef" when the opposite in fact was true (Wyle et al., 1999b).

In a study conducted by Wheeler et al., (2002) the Beef Cam was used on 769 carcasses with 13.8% "tough", which resulted in a 7.8% error rate for certification as "tender" with 51.9% of carcasses certified as "tender" (Belk et al., 2000). Results indicated that the BeefCam performed slightly bettering Phase 2 than in Phase I, it was less accurate at sorting carcasses for tenderness than it had been in preliminary experiments. Wheeler et al., (2002) stated that this may be due to total number of observations and/or because the percentage of "tough" samples was too small in some preliminary data sets to get an accurate evaluation of the technology. In this study, Wheeler et al., (2002) concluded that the BeefCam performed poorly.

Color of muscle

Jeremiah (1991) used steaks from 3,435 carcasses and determined that use of a colorimeter or of muscle pH readings could sort carcasses into tenderness/toughness categories with good precision. Wulf et al. (1996) concluded that, of the factors they adequate length of postmortem aging and/ studied, the most rapid strategies for improving beef tenderness would be to ensure and/or to eliminate carcasses with dark-colored lean from the steak and roast market. Wulf et al. (1997) correlated L*, a*, and b* colorimeter readings for carcass ribeye muscles to the tenderness of longissimus steaks from those carcasses and reported that a classification system (darkest/bluest 25%, middle 50%, lightest/yellowest 25%) based on colorimeter readings resulted in classes of beef that were 15, 3, and 0% tough, respectively. Tatum et al. (1997) sorted beef carcasses using Hunter b* values and successfully identified carcasses likely to produce tough top sirloin and top loin steaks in four postmortem-aging (3, 7, 14, 21 d) groups.

Near-Infrared

Near-infrared (NIR) spectrometer measures the reflectance of light in the near-infrared region. The graph is reflectance or absorbance plotted against wavelengths. A series of studies involving NIR spectroscopy have been conducted that report moderate to promising results in regard to predicting current status of tenderness. Analysis of NIR reflectance analysis has been used to predict beef longissimus tenderness. Some studies have been conducted using laboratory spectrometers that require special sample preparation, and others have used fiber optic reflectance probes that can more easily be used in a processing plant environment (Subbiah et al., 2003). Broad-range spectroscopy includes light reflected in the visible region of the spectrum which gives an objective measurement of color of food objects, whereas NIR spectroscopy contains information about physical and chemical properties (Subbiah et al., 2003). Hildrum et al., (1994) reported that the near-infrared reflectance spectra of beef muscles changed during aging. Given that a variation in the rate of aging causes most of the variation in tenderness of longissimus steaks from the carcasses of young, grain-fed cattle (Whipple et al., 1990; Shackelford et al., 1991), near-infrared spectroscopy may be able to predict variation in tenderness of longissimus steaks (Park et al., 1998). In an experiment conducted by Park et al., (1998), near-infrared reflectance spectra (1,100 to 2,498 nm) were collected on beef longissimus thoracis steaks for the purpose of establishing the feasibility of predicting meat tenderness by spectroscopy. Partial least squares analysis and multiple linear regression were used to predict longissimus Warner-Bratzler shear force values from spectra of steaks from 119 beef carcasses. Overall, absorption was higher for tough steaks than for tender steaks. This data indicated that near-infrared reflectance is capable of predicting Warner-Bratzler shear for values of longissimus steaks (Park et al., 1998). Another study (Byrne et al., 1998) reported success in forecasting tenderness. Through the utilization of a spectrometer equipped with a fiber optic probe utilized to predict beef tenderness of 70 heifers carcasses. A 10-factor model based on 1 d spectra successfully predicted 14 d WBSF with R^2=0.68. However, other studies from the same research group (Venel et al., 2001) reported failure in predicting current tenderness. Three studies reported failure in forecasting tenderness (Rodbotten et al., 2000).

CHAPTER III

PREDICTING BEEF TENDERNESS USING NEAR-INFRARED SPECTROSCOPY

S.R. Rust, J.B. Morgan[1], J. Subbiah[1], G. Kranzler[1], and T.L. Wheeler[2]

[1]Oklahoma State University, Stillwater, OK 74075
[2]Meat Animal Research Center, Clay Center, NE

ABSTRACT

The objective of this multiple-phased study was to determine the accuracy of an on-line near-infrared spectral reflectance system to predict 14 d aged, cooked-beef tenderness. In Phase I, 278 carcasses (133 US Select, 145 US Choice quality graded) were selected from two commercial beef processing facilities. Following carcass selection longissimus muscle sections (rib 9^{th}-12^{th}) were individually identified, vacuum-packaged, and transported to the Oklahoma State University Meats Laboratory. On d-3, 2.54-cm thick steaks (n=3) were fabricated and stored in refrigerated conditions (1°C). Following a 30-minute oxygenation period, an NIR spectral scan was obtained on the 12^{th}-rib longissimus steak. Steaks were individually vacuum-packaged and aged for 14-d prior to conducting slice shear force (SSF) analysis. In Phase II and III, 476 carcasses (258 US Select, 218 US Choice) were immediately scanned following carcass presentation to in plant USDA grading personnel. In a similar fashion, all longissimus steaks were aged for 14-d prior to cooking (70°C) and conducting SSF. Of the Phase I and II samples, 39 (6.77%) were categorized as "tough" (i.e. ≥ 25 kg slice shear force after 14 d of postmortem aging). Of the 39 "tough"

samples, 20 (3.7% error rate) were correctly placed in the 90% certification level. Another 10 "tough" samples were placed in the 80% certification level (2.0% error rate). The difference in mean longissimus slice shear force value (2.67 kg) between certified "tender" and not certified tender was significant ($P < 0.05$) for spectral analysis. Removing the toughest 10% of the Phase I and II population improved the mean SSF in excess of 6.5 kg. A similar trend was observed, in that the predicted tough samples (Phase III) were removed from the population, improvements were made in the certified tender population. Third-party verification utilizing 200 Phase III longissimus steaks (100 US Select and 100 US Choice) were delivered to the U.S. Meat Animal Research Center (MARC) for SSF measurements. Accompanying the steaks were scan values estimating d-14 SSF. The NIR system was able to successfully sort tough from tender carcasses up to 70% certification levels. We concluded that NIR offers an in-plant opportunity to sort carcasses into tenderness outcome groups for guaranteed tender branded beef programs.

INTRODUCTION

Beef tenderness is a primary consideration in consumer satisfaction. A "teeter-totter" relationship exits, in that the beef industry has long since struggled with the challenge of a balancing act producing lean beef products that are consistent in their palatability characteristics. Working with products that originate from multiple breeds and thousands of producers who use different production systems, the variability within the carcass population is immense.

Despite its significance as a quality indicator, tenderness is not a factor directly affecting product value for beef producers and packers. There is growing recognition that beef tenderness must be incorporated in the quality grading process if true, value-based marketing is to develop. In order to improve beef tenderness and overall quality, identification of tough carcasses must be issue number one for the beef industry. The primary method used today as a marketing tool to differentiate between tenderness levels is the indirect method of USDA Quality grading.

Several technologies have been developed to evaluate beef tenderness. Near-infrared (NIR) spectroscopy is a rapid, non-destructive system that measures reflected light containing information about properties such as meat tenderness (Park et al., 2001; Byrne et al., 1998). The objective of this experiment was to investigate the feasibility of using NIR spectral reflectance to predict tenderness in a beef processing-plant environment.

MATERIALS AND METHODS

Description of the Instrument: The infrared spectrometer used in this study (FiledSpec Pro Jr, Analytical Spectral Devices) can collect light in the visible and NIR regions (400-2,500 nm). A fiber optic contact probe was used to transmit light reflected from the beef surface to three internal detectors. The detectors consisted of a silicon photodiode array, a thermoelectrically (TE) cooled Indium Gallium Arsenide (InGaAs), and a TE-cooled extended InGaAs to measure the

350-1,000 nm, 1,001-1,670 nm, 1,671-2,500 nm wavelength domains, respectively.

Inside the instrument, diffraction grating split the reflected light into narrow wavelength bands. A 512-channel silicon photodiode array was geometrically positioned to receive light within a narrow bandwidth (1.4 nm) in the region of 350-1,000 nm. The photodiodes converted the accumulated light to an electronic signal. The signal was digitized by and transferred to the computer. Spectral resolution in this region was 3 nm.

The two InGaAs detectors were the scanning type. They differed from the first sensor in that they measured wavelengths sequentially, rather than simultaneously. Each sensor consists of a concave holographic grating and a single TE-cooled InGaAs detector. The gratings are mounted on a shaft which oscillates with a period of 200 ms (100 ms/scan). As the grating oscillates, the detector measures different wavelength bands. The resolution in these spectral regions is 30 nm (ASDI, 2002). The spectrometer is carried in a backpack with the laptop computer positioned ahead of the operator. The contact probe provided broadband light from an internal tungsten-halogen light source.

Meat Samples: Beef ribeye roll samples (n=768) were collected from two regional packing plants (n=304, Sam Kane's, Corpus Christi, TX; and n=464 Excel Packing, Plainview, TX). It should be noted that the US Quality grades of the tested carcasses (approximately 50% US Select and 50% US Choice) were selected primarily to reflect typical Quality grade distributions of U.S. beef processing plants. This project was conducted in three phases. In Phase I (Laboratory Scanning), following 48-h chill (1°C) carcasses passed the quality grading stand where they were selected for inclusion in the investigation. Selected carcasses were individually identified and moved off onto separate rails by USDA Quality grade. Approximately 100 carcasses per grade per plant were selected. Carcass grade data factors were collected for preliminary yield grade, adjusted fat thickness, ribeye area, kidney, pelvic, and heart fat percentage, lean maturity, skeletal maturity, marbling score, and quality grade as evaluated and stamped by USDA graders (USDA, 1997). Hot carcass weight and carcass identification numbers were recorded from plant tags.

Following grade data collection, Phase I carcasses were fabricated and individually identified ribeye rolls (IMPS# 112) were collected, vacuum-packaged, and packed into refrigerated chests with ice packs and transported back to the Oklahoma State University Food and Agricultural Products Research Center. At approximately 72-h postmortem, a 2.54-cm longissimus steak was generated from the anterior end of each subprimal using a band saw, individually identified, placed on a plastic tray, and allowed to "bloom" for 30 minutes. After pH and color (L* a* b*) values were collected, trays were transported to the Oklahoma State University Biosystems & Agricultural Engineering Machine Vision Laboratory for spectral scanning under controlled conditions. Following scanning, transported steaks vacuum-packaged, aged for 14 d, and frozen (-2.0°C) until further analysis.

In Phase II, carcasses (n=276) from two commercial plants were selected at the grading stand and moved onto separate rails by USDA Quality grade. In-plant scanning was performed on each carcass (US Choice=100, US Select=100). Carcass grade data factors were collected in a manner similar to Phase I. After scanning the individually identified carcasses, ribeye rolls were collected during fabrication, vacuum-packaged, and transferred to Oklahoma State University. Once again, at 72 hours postmortem, a steak (2.54 cm thick) was uniformly fabricated from each ribeye roll. Following blooming, pH and Hunter color values were obtained from the steak surfaces. Following baseline data collection, steaks were individually vacuum-packaged and allowed to age for an additional 11 days.

The concluding portion of the project (Phase III) consisted of a third-party verification stage that included overnight shipping longissimus steaks (n=200) to U.S. Meat Animal Research Center (MARC) in Clay Center, Nebraska for slice shear force measurements. Accompanying the 14-d aged and frozen steaks, an NIR predicted tenderness classification rating was provided to the MARC personnel. These tenderness ratings were established from the in-plant spectral scans obtained from the two cooperating beef processing facilities. The OSU spectrometer was based on Phase I & II and was used to predict slice shear-force values. The predicted values were then compared with the slice shear-force values supplied by MARC.

It should be noted that the SSF steaks at MARC were cooked with a belt grill (TBG060 Magigrill, MagiKitch'n Inc) operating at the highest temperature setting using the "very rapid" cooking protocol that Shackelford et al. (1999a) developed for tenderness classification. The steaks were thawed (5°C) until the internal temperature of 5° was reach and cooked to a final temperature of 70°C. After the steaks exited the belt grill, they were held at room temperature for two minutes for post cooking temperature rise to be complete.

Slice Shear Measurement: Steaks for slice shear-force (SSF) assessment were thawed for 24 h at 1 to 2°C and cooked on a belt-fed impingement oven (Model 1132-000-A, Lincoln Foodservice Products, Inc.). Preliminary test cooking was conducted to determine appropriate cooking times to reach 71°C internal temperature. SSF was measured after the cooked steaks were allowed to chill for 24 hours at 4°C. Using the procedures as outlined by Shackelford et al. (1999a), a first cut was made approximately 1 cm from the lateral end of the cooked steak. The SSF sample was removed at an angle of 45° using a knife with two parallel blades separated by a 1 cm space. This procedure generated a cooked meat sample measuring 5 cm in length by 1 cm in thickness and 2.5 cm in width. This sample location was selected so that limited connective tissue would be located within the slice-shear sample. SSF was measured using a flat, blunt-end blade (slice-shear attachment) mounted on an Instron Universal Testing Machine (Instron Corp, Canton, MA). Force required to shear the muscle fibers of the slice was recorded as a slice shear force. Higher SSF values indicated "tougher" beef.

Instrument Operation-Optimization of Parameters: Integration time (IT) is an important parameter for the photodiode array detector. Integration time, also known as exposure time, is the time during which the photodiode array accumulates the signal. In general terms, the higher the IT, the greater the signal. However, the photodiode array begins to saturate if the signal exceeds the dynamic range of the detector. Therefore, optimization of the IT is required. In this experiment, the contact probe was placed on a white reference plate (Spectralon® Diffuse Reflectance Targets, LabSphere, Inc., North Sutton, NH) that reflected light across the spectrum of interest. The spectrometer automatically adjusts the IT to allow maximum signal without saturation.

A certain amount of electric current is generated by thermal electrons, called dark current, and is added to the signal generated by reflected light. Dark current is a property of the detector and associated electronics (not the light source) and varies with temperature. It also varies linearly with the integration time for the photodiode array. Dark current was produced by the detector when the mechanical shutter blocked the entrance slit of the spectrometer. This signal is detected from all readings to eliminate the effect of temperature variation. Dark current was read every 5 minutes during spectral collections.

Another factor, that was used to optimize instrument spectral capabilities, was a white reference plate. Because a white surface reflects nearly 100% of incident light, the resulting is a measure of incident light intensity (I_o). White reference spectra were collected every 5 minutes during carcass spectral readings. To avoid soiling the white plate, it was protected with a 1.59-mm cover glass made of fused quartz borosilicate. This glass has more than 90% transmission over the spectral range of interest.

Reflectance: The spectrum reflected from the sample (I) must be collected under conditions similar to those used for the white reference. Because a cover glass was placed over the white reference plate, a glass plate with identical specifications was placed over the sample. By dividing the reflected spectrum by incident light (white reference spectrum), reflectance (I/I_o) was obtained. Reflectance is the property of the material alone, which is the fraction of incident light that is reflected from beef surface. In addition, for a given scan, 10 spectra were collected consecutively and averaged to minimize the effect of electronic noise. Three spectra were collected at 3 locations near the lateral end of the longissimus muscle, in an effort to avoid connective tissue.

The median of three spectra was calculated and saved as a reflectance spectrum for that sample. Median calculations aid in the prevention of outlier data points such as spectra over a thick marbling spot or connective tissue, or low signal at water absorption bands. Reflectance (R) was converted to absorbance (1/R) by log transformation. This transformation is commonly employed to linearize the relationship between the concentration of an absorbing compound and the absorption spectrum.

Model Development: Each spectrum has 2,150 data points, or independent variables, and the SSF is the dependent variable. In order to accommodate this scale of variables, a multivariate dimensionality reduction technique was employed to avoid over-fitting. Partial least squares (PLS) regression was used to produce new features. These features are linear combinations of original spectral data points yielding new factors that are not correlated and that explain most of the variation in both the dependent and independent variables (PLSplus, 2000). Absorbance spectra in the region of 400-1,500 nm were used to predict slice shear force. Spectra beyond 1,500 nm were not found to be useful. The model was developed with Unscrambler software (Camo, Inc., Corvallis, OR). Cross-validation (Esbensen, 2001) was employed to select the number of PLS factors included in the models.

Evaluation of Statistical Model: Our evaluation of system performance followed procedures described by Wheeler et al. (2002). They assessed performance of three instrumented tenderness prediction systems on the basis of progressive certification of steak sample "tenderness" in 10% certification increments. We classified any steaks with 14-d SSF greater than 25 kg as "tough" and the rest as "tender." In the description that follows, "observed values" refers to the reference slice shear force values. "Predicted values" refers to the 14-d shear-force predicted by the spectral reflectance system.

Samples were first sorted and ordered on the basis of predicted values. For 10% certification levels, 10% of the steaks having the lowest predicted values were classified into a "certified tender" group and the remaining into a "not certified tender" group. The mean observed SSF values were compared for the "certified tender" and "not certified tender" groups using a 't' test for independent samples ($\alpha = 0.05$). First, equality of variance for the two groups was tested. If the variances were equal, a pooled variance estimate was used in the 't' test. If the variances were not equal, Satterthwaite approximation was used to estimate the variance. When there was a significant difference in mean observed shear force values between the two groups, we concluded that the spectral reflectance system had successfully sorted the "tender" from the "tough" samples at that certification level. Any "tough" sample (observed 14-d SSF value $\geq$ 25 kg) in the "certified tender" group was an error. This procedure was repeated for certification levels up to 90%, in 10% increments. A 100% certification level signified classifying as "tough" (without sorting) all samples with observed 14-d slice-shear values greater than 25 kg.

RESULTS ANS DISCUSSION

As anticipated, simple statistics for various carcass and muscle traits to characterize the samples were highly variable in all traits (**Table 1**). It should be mentioned that lean color traits were similar to those reported by Page et al. (2000) for 1,000 carcasses selected to represent the U.S. fed beef population. A great deal of effort was taken to assure that some "tough" ribeye steak samples were included in this investigation. Certainly, in order to best test and challenge the spectral instrument, "tough" samples were needed in the population. According to the most recent National Beef Tenderness Survey (Brooks et al., 2000), only 1.5% of the ribeye steak samples had shear force values that exceeded 4.6 kg. Certainly, many factors could have contributed to this low percentage of "tough" samples. Variables such as ribeye steaks from food service and retail sectors were sampled, the reported postmortem aging time average exceeded 21 d, and an attempt was made to include representatives from the entire national beef processing industry in the survey. In our tested ribeye steak population (**Figure 1**), even following a 14-d postmortem aging period, 6.8% (39 of 568 steaks) displayed SSF values that were classified as being "tough" (i.e., ≥ 25 kg). It should be mentioned that in the instrument-testing project summarized by Wheeler et al. (2002), their initial population included in excess of 14% samples that were classified as "tough". It should be noted that several distinct differences were observed between that particular investigation (Wheeler et al., 2002) and the current study; 1) they utilized strip loin samples, whereas we tested ribeye steaks, 2) a belt grill broiler was utilized by Wheeler and co-workers, whereas an convection impingement oven was utilized in the current study (the validation part of our study involved a belt grill broiler), and lastly, the SSF procedure was used to determine actual tenderness rating whereas Wheeler used the more traditional Warner-Bratzler shear force procedure. However, it appears that both studies were fortunate in that several samples classified as being "tough" following an extended postmortem aging were included in both test populations.

If one further investigates the distribution of tenderness ratings as determined by slice shear force values, it becomes very obvious that ribeye samples originating from Select Quality graded carcasses were "tougher" and more variable in their tenderness ranges than samples from steaks from Choice graded carcasses (**Table 2**). In both phases of the investigation, SSF values exceeding 25 kg was greater for the Select quality samples (i.e., ≥ 9.0%) when compared to only 3.4% and 5.9% "tough" samples from Choice carcasses in Phases I and II, respectively (**Table 2**). There was substantial variation in tenderness for the entire population, with a range in slice shear force from 9.87 to 39.87 kg. In fact, 12 longissimus samples had SSF values that exceeded 28 kg in toughness. It appears that the hurdle of finding "tough" samples was cleared for the purpose of instrument testing. In order to prevent biased comparisons between the three tested tenderness prediction procedures, Wheeler and co-workers (2002) utilized the use of progressive certification as "tender" in 10% increments (10 to 90% certified as "tender"). In our study, 6.8% of the carcasses were tough. If one designs a system that classifies all carcasses as "tender" without even taking a reading, they could claim that their system is 93.2% accurate. This problem stems from the lower number of tough carcasses in testing. Ideally, we would like to have 50% tender and 50% tough carcasses. That is not possible. Then, how do we evaluate an instrument? The solution is progressive certification. Note that in this certification method, we grouped the samples into 10 categories. At each certification level, there are 2 groups – "certified tender" and "not certified tender." Significant differences in mean observed slice shear force indicate successful sorting at that certification level. The best-case scenario would be to have all tough carcasses in the "toughest" category and zero error rate at all certification levels. However, with the Oklahoma State University spectral instrument this was not obtainable; but, compared to the previous tested instruments, progress is being made. In the initial phases of the project, 39 of the 568 carcass samples were categorized as "tough" (i.e., ≥ 25 kg slice shear force at 14 days of postmortem aging). This performance reflects into 6.8% error in certification at the 100% level (**Figure 2**). A very high percentage of the samples were correctly classified as "tender" when the population was categorized in expected certification levels. Of the 39 "tough" samples, 20 "tough" carcasses were correctly placed in the "not certified tender" category. Another 19 "tough" samples were incorrectly placed in the "not certified tender" category (3.7% error rate). At the 80% certification level, 30 tough carcasses (10 more "tough" samples were correctly identified in addition to the 20 "tough" carcasses identified at the 90% certification level) were correctly placed in the "not certified tender" category. Nine "tough" carcasses were incorrectly place in the "certified tender" category (1.98% error rate). In the MARC study, the error percentage of carcasses certified as "tender" that had WBSF of 5 kg at 14 days postmortem. In their population, the error rate for 100% certification using all carcasses was 9.3%. Slice shear force certification levels up to 80% had lower ($P < 0.05$) error rates that did 100% certification.

From a real-world stand point, what do these findings actually mean? It means that the Wheeler et al. (2002), SSF instrument worked average, at best, and that the remaining two tested instruments *were less than acceptable*. For example, of the 400 tested samples, 37 samples (9.3%) exhibited WBSF values > 5 kg at 14 day of postmortem aging (i.e., 100% certification). Using SSF to segment carcasses into a 90% (i.e., predict "toughest" 10% of population) and 80% certification group (i.e., predict "toughest" 20% of population), resulted in error rates (percentage of carcasses certified as "tender" that had WBSF of >5 kg at 14 days postmortem) of 6.4 and 4.1%, respectively. This means that following certification, 23 of 37 and 13 of 37 carcasses were certified as being "tender," but they were actually "tough". The inaccuracy of the U.S. Meat Animal Research Center SSF machine as a predictor of ***aged*** beef tenderness is easily explained. One must remember that the SSF measurements were obtained on day 3 of postmortem aging. Using this value to predict ultimate cooked beef tenderness has some shortcomings in that it is a measurement of tenderness at a given point in time. Postmortem aging and the associated improvements, or even more importantly the carcass-to-carcass variation in postmortem aging and its impact on cooked beef tenderness, are not taken into account by the U.S. Meat Animal Research Center SSF instrument.

In another attempt to better understand the data, we actually separated the predicted SSF estimates from the d-3 spectral scans and segmented them into projected palatability groups (**Table 6**). However, following 14 d of postmortem aging, the actual SSF value was placed in the location of the projected sample number. The column on the left of Table 3 represents the 57 samples that were spectrally predicted on d-3 to be the most "tender" and their respective d-14 slice shear force. The mean SSF value for this group was 14.90 kg. No tough samples (i.e., shaded samples) were included in this subset. As one moves across the table, mean SSF values increase as predicted. The overall mean for the samples predicted as "toughest" was 25 kg, with 20 of the 39 tough samples being correctly placed in this category. One concern is that one of the "tough" samples was predicted to be one of the most tender samples (20% certification), however, this sample is on the boarder line. Even though much progress has been made, much more work is needed.

Regardless of percentage certified, the difference in mean longissimus slice shear force value between "certified tender" and "not certified tender" was significant ($P < 0.05$) for spectral analysis in both USDA Choice and Select carcasses (Table 3). Removing the "toughest" 10% improved the mean SSF in excess of 6.5 kg. A similar trend was observed, in that when predicted "tough" samples were removed from the population, improvements resulted in the "certified tender" population. The magnitude of difference was not greatly improved after the toughest 40% (60% certified as "tender") was segmented as not certified. The SSF values for "certified tender" and "not certified tender" among the USDA Select samples are also significant (**Table 4**). In the Wulf et al. (2000) study the colorimeter appeared to be useful at identifying "guaranteed tender" beef using an independent sample, but not within the narrow range of marbling in USDA Select carcasses.

Table 5 shows the certification table for Phase III (validation) samples. Up to 70% certification levels, significant differences were observed, meaning that the system sorted the "tough" from "tender" carcasses successfully. The implication is that if we remove the top 30% of carcasses sorted 'tough' by this system, the remaining 70% carcasses can be sold as "guaranteed tender" for premium markets such as restaurants. At 20% certification level, the means of two categories were different at $P = 0.06$. The number of samples in the "certified tender" group was 40 and the "not certified tender" group was 160. This large difference in sample sizes of two groups can cause problems in significance analysis. Also, the p-value (0.06) is close to the significance level (P = 0.05) and therefore can be considered as significant for practical purposes.

As previously observed, in all samples in our study (Choice and Select), segmenting carcasses based on their predicted SSF value is a very effective tool. Utilizing the 70% "certified tender" as a sorting tool for eliminating tough carcasses improved SSF values in excess of 4.0 kg. In fact, in the Select samples in Phase I and II, the mean slice shear force values between the extreme 10% "certified tender" (14.80 kg) and "not certified tender" (21.56 kg) categories is 6.76 kg (Table 4). Previous attempts to segment USDA Select Quality carcasses into palatability outcome groups have failed. It appears that spectral reflectance has promise segmenting less consistent, lower Quality grading carcasses into palatability groups. In this study the Oklahoma State University Spectrometer has been more consistent with sorting US Choice and US Select than any other system reported.

In conclusion, many attempts have been made to develop and implement instrumental methods for predicting meat tenderness. Most of these were developed as laboratory research tools and have varied greatly in their effectiveness. Some of these systems include the TenderTec (George et al., 1997a; Belk et al., 2001), connective tissue probe (Swatland, 1995), elastography (Berg et al., 1999), ultrasound (Park and Whittaker, 1991), image analysis (Li et al., 1999, 2001) and more recently the colorimeter (Wulf and Page,

2000) and SSF (Shackelford et al., 1999a, b, 2001). Today, the beef industry needs an on-line system, not to eliminate USDA Quality grading, but to serve as a complementary tool to assist in quality grading. This is especially true for the lower quality carcasses in the U.S. Standard and Select grades. To date, the OSU Spectral Reflectance systems appears to meet most industry criteria, in that it is an objective, noninvasive, tamper-proof, and accurate system that appears to be applicable across various carcass quality levels in a harsh, packing-plant environment. An NIR reflectance system with contact probe was developed and evaluated on-line. The contact probe provided stable, broadband light and fixed the geometry of light and fiber optic probe in relation to the meat surface. Spectral reflectance values were collected at 3-d post-mortem and were used to predict 14-d slice shear-force tenderness values. A low correlation coefficient between the observed and predicted slice-shear force values indicated that the system did not predict exact tenderness categories with high accuracy. Up to 70% certification levels, the system sorted the carcasses into "tender" and "tough" categories successfully. The practical implication to the beef industry is that at or below 70% "certified tender" carcasses could be sold as "guaranteed tender" to premium markets like restaurants.

IMPLICATIONS

Tenderness is a critical factor in consumer perception of beef palatability. Direct evaluation is absent; because there is currently no accepted method available for predicting tenderness on-line. Carcasses are not priced on the basis of tenderness; therefore producers lack incentive to supply a tender product. As a result, consumer preference is not routed back to the producers. The OSU NIR Spectrometer responds to the need for objective measurement. It is a rapid, non-destructive method for on-line evaluation that is an accurate predictor of tenderness at the consumer level.

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Table 1. Simple Statistics for Carcass Population

Variable	N	Mean	SD	Minimum	Maximum
Hot Carcass Wt, lb	768	760.8	250.1	520.4	1001.7
Adjusted Fat Thickness, in	768	0.48	0.26	0.15	0.95
Ribeye Area, in^2	768	13.28	1.71	8.00	22.00
Kidney, Pelvic, Heart Fat, %	768	2.31	0.55	1.00	4.5
USDA Yield Grade	768	2.5	0.8	0.6	4.6
Selected Maturity[a]	768	162	12	135	240
Lean Maturity[a]	768	167	28	130	270
Marbling Score[b]	768	492	79	230	860
USDA Quality Grade[c]	768	670	64	510	830
Muscle pH, day 3	768	5.53	0.12	5.05	6.61
L*[d]	768	35.96	3.45	25.77	47.48
a*[e]	768	19.16	2.38	12.64	29.01
b*[f]	768	17.63	2.05	11.48	24.86
Slice Shear Force, kg	768	18.15	4.14	9.87	39.87

[a]Maturity: 100=A^{00}, 200=B^{00}, ect.
[b]Mabling: 200=Practically Devoid^{00}, 300=Traces^{00}, 400=Slight^{00}, 500=Small^{00}, 600=Modest^{00}, 700=Moderate^{00}, 800=Slightly Abundant^{00}.
[c]USDA Quality Grade: 500=Standard00, 600=Select00, 700=Choice00, 800=Prime00.
[d]L*:0=black, 100=white.
[e]a*: Lower numbers=more green, higher numbers=more red.
[f]b*: Lower numbers=more blue, higher numbers=more yellow.

Table 2. Quality grade effects on slice-shear force values of longissimus beef steaks (d-14).

Population	n	Mean, kg	SD	Min, kg	Max, kg	(≥ 25 kg)
Phase 1, Select	133	18.20	4.66	9.87	39.87	12 (9.0%)
Phase 1, Choice	145	17.39	3.15	11.21	28.31	5 (3.4%)
Phase 2, Select	158	19.02	4.37	10.11	35.81	15 (9.5%)
Phase 2, Choice	118	17.71	4.28	11.57	39.02	7 (5.9%)

[a,b,c] Values within a column lacking a common superscript are significantly different ($P< 0.05$).

Figure 1. Distribution of slice-shear force values for longissimus steaks included in Phases 1 & 2 (n=568 samples).

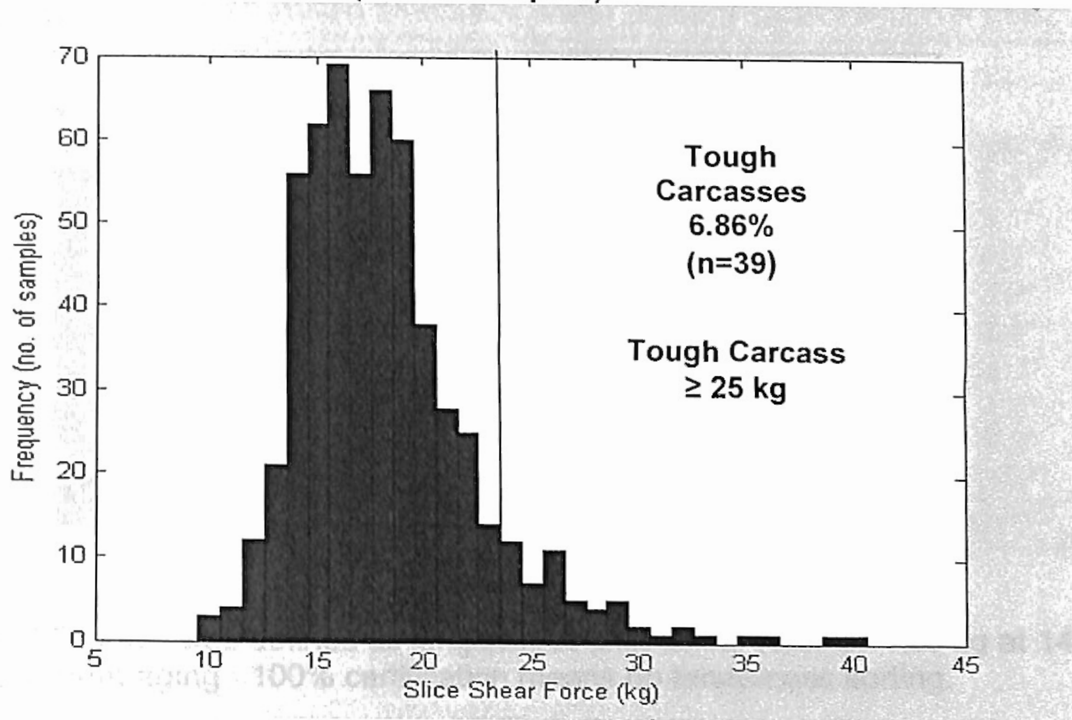
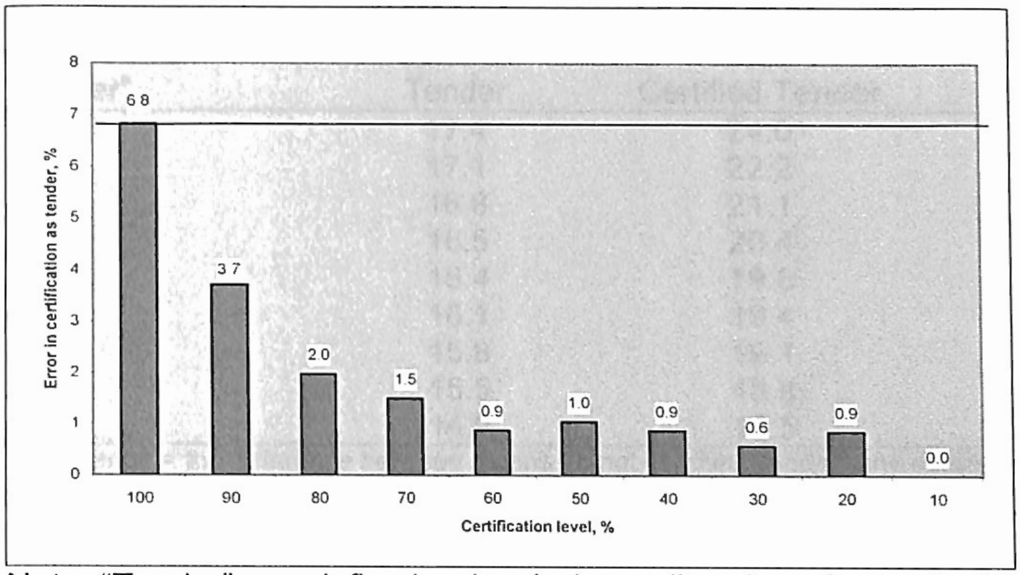


Figure 2. Error rates for certifying choice and select quality carcasses as "tender" in increments of 10% of the sample population in Phase I & II (n=568).



Note: "Tender" was defined as longissimus slice shear force of < 25 kg at 14 d postmortem aging. 100% certification means no tenderness sorting.

Table 3. Effect of percentage certified as "tender" on USDA Choice and Select longissimus steaks 14-d slice shear force (Phase I & II, n = 568).

Percentage Certified as "tender"	Slice Shear Force		
	Certified Tender	Not Certified Tender	Difference[a]
90	17.4	24.0	6.6*
80	17.1	22.2	5.1*
70	16.8	21.1	4.3*
60	16.5	20.4	3.9*
50	16.4	19.8	3.4*
40	16.1	19.4	3.3*
30	15.8	19.1	3.3*
20	15.5	18.8	3.3*
10	14.9	18.5	3.6*

[a]Difference = the difference between means for not certified "tender" and certified "tender."
*The difference between certified "tender" and not certified "tender" was significant ($P < 0.05$).

Table 4. Effect of percentage certified as "tender" on USDA Select longissimus steaks 14-d slice shear force (Phase I & II, n = 291).

Percentage Certified as "tender"	Slice Shear Force		
	Certified Tender	Not Certified Tender	Difference[a]
90	18.32	21.56	3.24*
80	17.83	21.91	4.08*
70	17.26	21.91	4.65*
60	16.95	21.21	4.26*
50	16.69	20.62	3.93*
40	16.40	20.13	3.73*
30	16.23	19.68	3.45*
20	15.69	19.38	3.69*
10	14.80	19.07	4.28*

[a]Difference = the difference between means for not certified "tender" and certified "tender."
*The difference between certified "tender" and not certified "tender" was significant ($P < 0.05$).

Table 5. Mean shear force values of Certified and Not Certified Tender groups for Phase III (validation) samples.

Percentage Certification Level	Slice Shear Force (kg)		
	Certified Tender	Not Certified Tender	Difference
90	14.60	14.80	0.20
80	14.62	14.64	0.03
70	13.99	16.09	2.09*
60	13.71	15.99	2.28*
50	13.44	15.80	2.36*
40	13.10	15.63	2.53*
30	13.01	15.31	2.30*
20	13.39	14.93	1.54
10	13.05	14.80	1.75*

*Difference between "certified tender" and "not certified tender" was significant ($P < 0.05$).

Table 6. Using spectral scan information to segment carcasses into palatability outcome groups.

Most Tender	Predicted Categories for Phase I & II →								Least Tender
12.47[a]	25.05[b]	16.15	18.09	19.08	14.32	17.14	14.18	19.79	25.91
13.38	14.60	16.73	13.15	19.08	20.60	17.55	16.90	19.12	25.96
14.51	18.97	15.97	13.63	22.55	18.06	17.56	21.07	18.67	22.40
15.25	13.44	16.21	18.19	21.45	17.01	18.95	17.79	23.09	19.45
12.58	13.43	19.65	13.59	25.83	19.04	21.56	11.24	23.57	27.16
11.57	18.71	17.47	18.33	22.28	19.86	24.89	15.55	13.73	23.48
15.12	16.52	17.78	14.90	18.36	13.79	14.65	24.31	21.75	16.45
15.61	17.15	16.39	21.12	16.22	13.43	20.03	20.48	13.77	17.90
13.30	15.26	17.17	16.36	18.21	14.21	18.61	15.28	22.18	24.10
12.83	18.57	13.98	20.40	19.11	18.74	14.36	17.40	12.23	31.43
14.32	13.65	12.63	20.51	16.62	11.74	19.83	12.11	16.61	17.99
12.35	12.56	18.30	18.25	21.18	15.03	15.84	16.18	21.55	26.25
13.77	14.51	15.24	15.88	19.39	19.10	21.25	16.73	14.93	23.26
16.21	15.85	14.81	15.43	13.81	20.45	13.56	27.85	16.83	16.04
11.84	15.46	15.51	16.84	17.65	15.88	21.21	19.85	31.59	20.34
18.00	19.11	16.87	18.79	17.56	14.35	21.91	23.27	17.70	22.06
16.38	21.18	17.72	13.78	9.87	15.31	17.23	24.63	19.74	24.02
13.07	11.88	21.97	21.81	20.50	18.41	16.63	29.15	14.76	17.64
20.36	14.24	16.06	19.34	18.17	19.26	17.23	18.71	19.32	29.28
18.13	10.93	15.85	15.01	16.70	16.23	16.21	20.01	26.24	21.34
14.17	16.33	15.44	11.21	16.48	16.88	14.09	20.61	21.64	20.93
14.44	13.50	18.07	15.94	19.89	18.00	17.22	21.79	20.61	19.12
13.36	18.49	13.95	24.28	16.92	15.36	17.19	17.54	19.35	21.89
18.15	10.07	15.51	16.01	14.53	17.15	19.12	15.49	23.68	26.02
14.13	23.87	16.49	18.66	13.93	17.19	14.68	14.96	22.45	29.66
14.92	19.79	23.00	15.20	10.11	23.12	19.17	21.47	19.43	18.61
14.15	16.07	13.58	15.78	15.44	16.22	15.65	26.68	26.45	19.91
14.49	14.86	14.08	13.21	16.59	16.29	17.77	18.71	20.22	20.29
17.67	16.93	19.41	19.63	21.91	21.72	18.31	14.75	15.08	22.94
14.15	13.96	12.24	19.96	19.09	14.73	22.66	16.73	27.21	27.03
15.57	14.52	14.33	17.98	16.12	18.16	14.16	18.11	15.45	17.68
13.22	19.27	18.11	17.65	15.89	20.93	25.07	20.55	17.46	30.12
16.19	11.64	14.27	17.99	17.68	17.87	12.20	15.80	18.89	19.06
16.89	17.05	15.58	15.19	17.56	17.09	19.89	14.26	19.66	19.57
14.79	14.95	15.79	19.06	14.61	20.42	16.88	14.77	16.52	21.86
16.33	14.12	14.30	13.49	16.24	19.39	20.38	18.46	21.52	23.83
14.93	16.36	16.95	14.73	15.92	16.90	14.67	20.94	20.00	17.44
16.58	16.36	16.57	18.92	15.80	22.96	18.05	19.34	17.97	31.91
16.32	13.85	14.33	18.92	18.44	20.71	28.31	19.97	26.90	20.38
15.16	17.79	22.08	15.90	18.27	16.60	18.22	22.09	25.87	23.33
13.79	15.43	15.49	13.02	13.95	19.61	24.07	16.04	15.26	19.38
16.60	13.86	16.10	13.33	14.86	14.18	16.75	18.46	17.77	19.47
14.70	18.81	18.58	21.05	17.29	19.22	25.17	17.69	28.90	22.77
13.81	12.74	14.33	13.67	18.61	17.19	19.64	19.23	19.87	24.62
13.01	14.81	15.76	16.82	16.28	17.12	18.31	17.68	17.65	39.87
18.30	14.11	11.66	20.61	15.84	18.12	17.72	20.80	20.93	18.73
17.67	13.38	14.23	10.95	15.46	15.99	19.66	21.03	18.52	27.61
15.51	14.59	15.70	15.70	15.46	15.10	15.19	17.25	23.57	21.70
14.80	16.47	16.57	18.76	16.66	15.33	15.55	14.77	18.45	26.24
13.05	18.11	20.80	15.68	19.16	14.09	14.33	21.45	21.67	21.72
13.72	17.13	16.19	18.33	15.56	14.91	16.98	18.90	27.81	34.76
13.92	18.51	18.78	25.23	18.72	14.81	22.63	19.98	21.69	35.81
15.74	17.79	13.92	16.32	22.05	16.23	19.04	15.99	19.45	33.31
14.87	20.60	16.94	15.57	21.18	18.40	23.63	18.39	19.63	24.42
13.78	15.09	18.02	17.83	17.05	14.98	20.84	15.58	29.18	28.91
14.41	19.60	20.47	18.60	18.84	18.61	14.42	20.07	16.60	39.03
14.98	16.59		19.27	16.32	16.82	22.59		26.42	25.81
14.90[c]	**16.11**	**16.43**	**17.08**	**17.51**	**17.25**	**18.53**	**18.73**	**20.47**	**24.00**

[a]Actual slice shear force value at 14 day of postmortem aging.
[b]Yellow boxes signify "tough" slice shear force values.
[c]Shaded values represent column means.