Prediction of Beef Tenderness using Hyperspectral Imaging

Farzad Saadatian

Department of Bioresource Engineering

McGill University

Sainte-Anne-de-Bellevue, Quebec, Canada

A thesis submitted to the Graduate and Postdoctoral Studies Office in partial fulfillment of the requirements for the degree of Master in Science

April 2014

ABSTRACT

Cuts from different types of beef muscles [e.g., *infraspinatus* (Top blade, TB), *gluteus medius* (Top sirloin, TS), *psoas major* (Tenderloin, TL), and *longissimus thorasis* (Rib eye, RE)] were dry-aged for up to 21 days and then subjected to near-infrared hyperspectral imaging to gauge its usefulness in evaluating beef tenderness. Imaged in reflection mode using a hyperspectral (900 nm$< \lambda <$1700 nm) imaging system, samples were then cooked (grilled one side to internal temperature of 40ºC, turned and grilled to a final internal temperature of 71ºC) and examined for tenderness by the Warner-Bratzler shear force (WBSF) method. Stepwise regression of mean spectral data collected from the samples was used to determine wavebands that can be used assess beef tenderness. Multiple Linear Regression (MLR) was used to assess the relative advantage of the selected wavebands to predict tenderness of beef samples. An overall correlation coefficient (R) for each muscle type (R=0.89 for TS, R=0.86 for RE, R=0.81 for TB, and R=0.83 for TL) shows the possibility of using hyperspectral imaging for predicting beef tenderness.

RÉSUMÉ

Différentes coupes de muscle de bœuf [e.g., *infraspinatus* (lame supérieure, LS), *gluteus medius* (haut de surlonge, HS), *psoas* (Filet, FL), et *longissimus thorasis* (entrecôte, EC)] furent âgées à sec pour 21 jours, puis soumises à une imagerie hyperspectrale en infrarouge proche pour évaluer l'utilité de telles mesures dans l'évaluation de la tendreté du bœuf cuit. Des images d'échantillons crus furent prises avec un système d'imagerie hyperspectral (900 nm < λ <1700 nm) en mode réflexion. Les échantillons ont ensuite été cuits puis la tendresse de la viande évaluée selon la méthode de force de cisaillement Warner-Bratzler (WBSF). Une régression par étapes de la moyenne des données spectrales provenant des échantillons crus servit à reconnaître les gammes de longueurs d'onde permettant une discrimination utile de la tendreté de la viande cuite. Une Régression Linéaire Multiple (RLM) servit à évaluer l'avantage relatif de la sélection d'une bande de longueur d'onde particulière pour évaluer les échantillons de viande de bœuf. Un coefficient de corrélation globale (R) pour chaque type de muscle (R = 0.89 pour HS, R = 0.86 pour EC, R = 0.81 pour la LS, et R = 0.83 pour FL) évoque la possibilité d'employer un système d'imagerie hyperspectrale pour prédire la tendresse de la viande bovine.

ACKNOWLEDGMENTS

Foremost, I would like to express my sincere gratitude to my supervisor Prof. Michael O. Ngadi for his continuous support, patience, care, and kindness during my Masters program. His guidance and immense knowledge helped me tremendously during my research and thesis writing.

I respectfully express my deepest gratitude to my co-supervisor, Prof. Shiv O. Prasher, for his patience and valuable suggestions in the course of this research work. My sincere thanks also go to Dr. Li Liu and Dr. Hui Huang for their advice and help. Thanks to all wonderful people that I have met at the Macdonald Campus who have made this experience so enjoyable for me; our entire research group, Dr. Chen Zheng, Mr. Hernan Rey-Sanchez, Mr. Jamshid Rahimi, Mr. Peter Adewale, Mr. Adeyemi Olutoyin, Mr. Chijioke Ejebe, Mr. Ogan Mba and Ms. Tina Beigi for their generous help and creating so many wonderful and unforgettable moments during these two years. I also thank Mr. Yvan Gariépy for the French translation of the abstract.

Finally, I thank my mother who had to be both a mother and father. She supported me with her strength and her courage, thus providing me a lesson for life. Thanks to my brothers for giving me the confidence and motivation to pursue my Masters degree in Canada.

CONTRIBUTION OF AUTHORS

This thesis was written according to the rules and regulations of the Faculty of Graduate Studies and Research of McGill University and consists of two manuscripts, both of which I am the primary author. The manuscripts were co-authored with Dr. Michael Ngadi, my thesis supervisor, and Dr. Shiv Prasher, my thesis co-supervisor. The thesis was also reviewed by Dr. M. Ngadi and Dr. S.O. Prasher.

Chapters 3 and 4 were presented at the Northeast Agricultural and Biological Engineering Conference 2013 (NABEC). Manuscripts of these chapters are under preparation and will be submitted for publication in a scientific journal in 2014.

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NOMENCLATURE

°C	Centigrade
HSI	Hyperspectral Imaging System
MLR	Multivariate Linear Regression
NIR	Near Infrared
p	p-Value
R	Correlation coefficient
RE	Rib eye
RMSE	Root Mean Square Error
SAS	Statistical Analysis Software
TB	Top blade
TL	Tenderloin
TS	Top sirloin
V	Validation set
Λ	Wavelength

I. GENERAL INTRODUCTION

To optimize quality and reduce economic losses during the meat production process some non-destructive online facilities must be implemented to assess ongoing changes in meat characteristics. Traditionally, the quality of meat has been identified through its appearance, color, texture and consistency compared to standard quality charts developed by specialists. Current methods applied to assess beef tenderness are normally conducted on cooked samples, but the ability is lacking to predict post-cooking tenderness when the meat is still raw. The challenge is how to quickly and accurately predict tenderness of different dry-aged beef cuts in a non-destructive way.

Time-consuming traditional methods are not appropriate for online assessment of different levels of beef quality; therefore, a quick, accurate and non-destructive method appropriate for online application needs to be developed. An emerging technology for defining food products' physical and chemical characteristics is the safe, fast and non-destructive process of near infrared (NIR) hyperspectral imaging. This multi-analytical method allows concurrent determination of different food characteristics (Shackelford, Wheeler et al., 2004) and provides high precision with little (if there is any) samples preparation (Liu, Chen et al., 2000; Liu, Lyon et al., 2003; Savenije, Geesink et al., 2006).

Recent studies of online food quality inspection systems have emphasized the potential useful applications of hyperspectral imaging, a cutting edge analytical technology which integrates non-contact spectroscopy with digital imaging to obtain both spectral and spatial information regarding the material of interest. The detailed information about the object drawn from the hyperspectral image is termed a "hypercube," being a 3-D (*e.g*., 2 spatial, 1 spectral) image which provides a great deal of subtle information on the object's physical and chemical characteristics (Qiao, Ngadi et al., 2007). Therefore, such a technology could obviously have a bright future in the food inspection and agricultural industries.

The use of NIR hyperspectral imaging in predicting the quality attributes of beef has been studied before (*e.g*., Cluff, Naganathan et al., 2008a; Naganathan, Grimes et al., 2008; Naganathan, Grimes et al., 2008b; ElMasry and Sun, 2010; ElMasry, Iqbal et al., 2011; ElMasry, Sun et al., 2012; Kamruzzaman, ElMasry et al., 2012; Wu, Peng et al., 2012). This study, however, focuses specifically on the prediction of beef tenderness of four muscles types after different dry-aging times.

1.1 Objectives

The overall aim of the proposed research was to predict post-cooking tenderness of different raw dry-aged beef cuts using hyperspectral imaging. The specific objectives were to:

1) Conduct tenderness profiling of different beef muscles,
2) Determine suitable spectral regions and spectral/image features that are indicative of meat tenderness and that can be used to develop predictive models through selection of important wavebands for beef tenderness, and
3) Assess the application of hyperspectral imaging of raw beef, combined with advanced imaging and data analysis techniques, for the prediction of tenderness in different cooked dry-aged beef cuts.

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II. LITERATURE REVIEW

2.1 Meat industry

The red meat industry is one of the Canadian food manufacturing industry's largest sectors, in the onset 2012, there were 12.5 million heads of cattle on 95105 Canadian farms and ranches (Statistics Canada, 2013). Canada was ranked among the top 10 of the world exporters of beef and cattle in the year 2012 (Pereltsvaig, 2013). At the provincial level, Alberta leads in the total number of cattle slaughtered with 67% of the entire Canadian beef market (UFCW Canada, 2011). Ontario is Canada's second largest beef producer with 20% of total slaughters and Quebec is third with 12%. Saskatchewan, British Columbia and Manitoba are also contributors with a combined total of 1.4% (Figures 2.1).

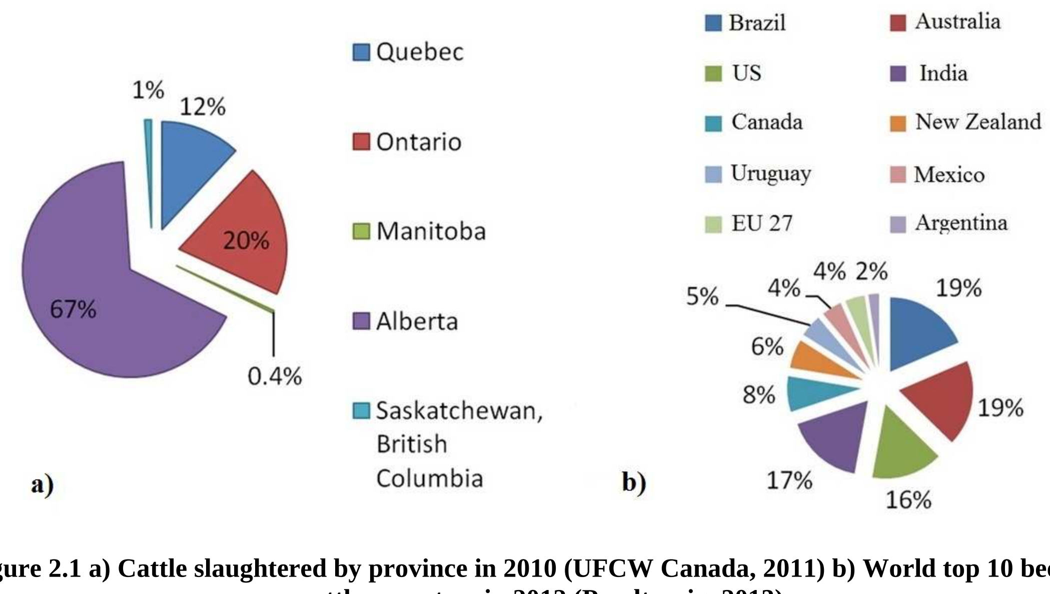

Figure 2.1 a) Cattle slaughtered by province in 2010 (UFCW Canada, 2011) b) World top 10 beef and cattle exporters in 2012 (Pereltsvaig, 2013)

Data on the global consumption of beef, poultry and pork in 2009 shows that while Australians consumed roughly equal portions of beef and poultry, Canadians ate beef and pork in equal amounts (Figure 2.2; Pereltsvaig, 2013). In the 10 years from 2010 to 2020, the per capita consumption of beef in Canada is expected to slightly decrease from 12.96 kg per person to 11.84 kg per person; while the per capita consumption of pork is expected to increase slightly to 12.09 kg per person (CMC, 2012).

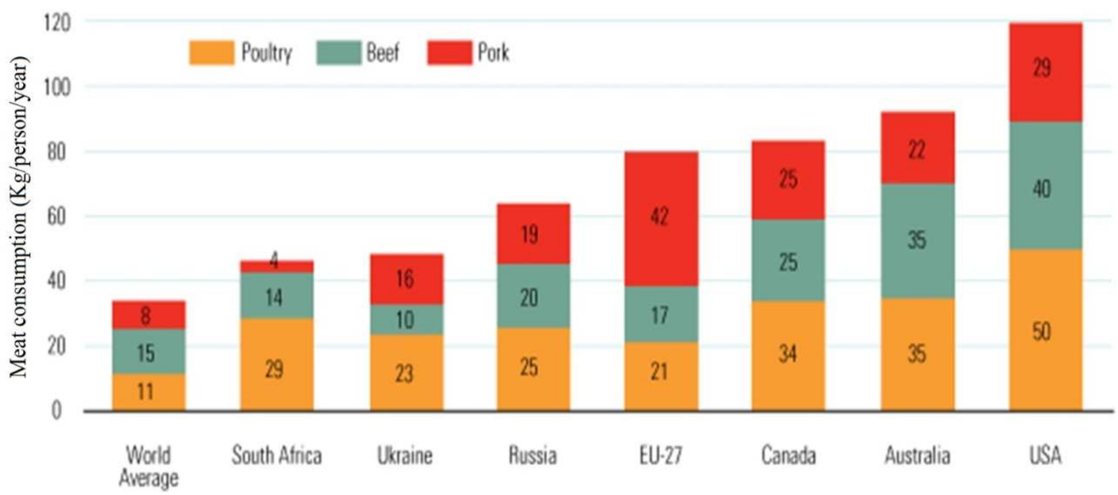

Figure 2.2 Meat consumption (Kg/person/year) in selected countries in 2009 (Pereltsvaig, 2013)

2.2 Meat quality

Generally, meat quality is a term used to describe the overall meat characteristics including its physical, chemical, morphological, biochemical, microbial, sensory, technological, hygienic, nutritional and culinary properties. Meat quality determination traditionally remains a difficult task given the variability in individual consumers' perception of the meat. Meat quality after cooking depends largely on characteristics such as tenderness, juiciness and flavor.

2.2.1 The US beef grading system

The United States Department of Agriculture (USDA), has separated the beef carcass into different classes according to the maturity and the amount of fat in the meat (marbling or intramuscular fat). Under the USDA classification, the eight quality grades of beef, from high to low are: USDA prime, USDA choice, USDA select, USDA standard, USDA commercial, USDA utility, USDA cutter, and USDA canner. While the first 5 grades are usually sold to customers for common consumption, the last three grades are commonly used in the production of canned goods and products.

- USDA Prime is the superior grade and exhibits great tenderness, juiciness, flavor and has a fine texture. It has the highest degree of fat marbling and is derived from younger beef. It only accounts for about 2.9% of all graded beef. That is why Prime is generally featured at the most exclusive upscale steakhouse restaurants. Prime graded beef is excellent for dry-heat cooking method.
- USDA Choice is the second highest graded beef. It has less fat marbling than Prime and it accounts normally for 50% of all graded beef. Choice is a quality steak particularly if it is a cut that is derived from the loin and rib areas of the beef such as a tenderloin filet or rib steak. It normally can be cooked by dry as well as moist heat methods. Generally USDA Choice will be less tender, juicy and flavorful with a slightly more crude texture than Prime.
- USDA Select is generally the lowest grade of beef one will find at a supermarket or restaurant. It is tougher, less juicy and less flavorful since, with very little marbling, it is leaner than Prime and Choice. The texture of Select is generally

more coarse. To avoid excessive drying it is usually suggested to cooked it using a moist heat method.

- U.S. Standard and U.S. Commercial grades are very low in fat and are produced from older animals. Therefore, their quality is lower than that of USDA Select. Due to its lesser tenderness, it is sold at low prices in retail markets.
- Utility, Cutter, and Canner Grades are rarely used in foodservice operations and are primarily used by processors and canners.

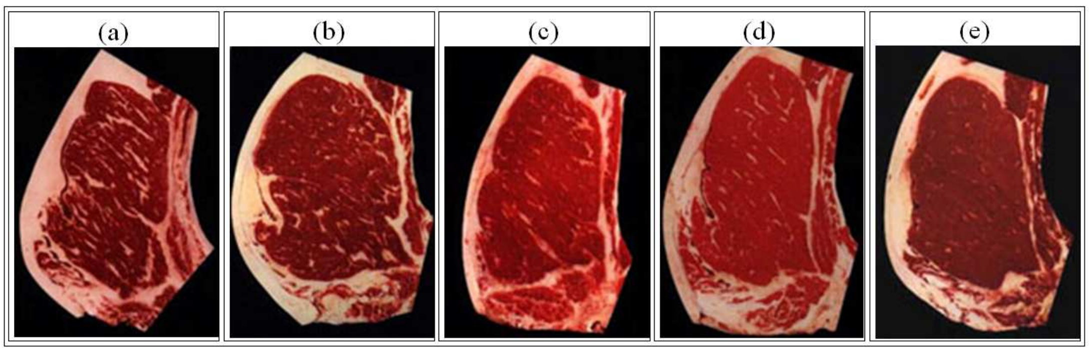

Figure 2.3 Photographic Standards For USDA Quality Grades (USDA, 1996, 1997)

(a) Prime (b) Choice (c) Select (d) Standard (e) Commercial

2.2.2 The Canadian beef grading system

Thirteen grades, from high to low quality, have been categorized in the Canadian system: Canada A, Canada AA, Canada AAA, Canada Prime, Canada B1, Canada B2, Canada B3, Canada B4, Canada D1, Canada D2, Canada D3, Canada D4, and Canada E. The first four grades namely A, AA, AAA and Prime have the highest quality in the Canadian system and included 88% of all beef grades in 2008. B grades in Canada system belong to young (< 30 months) carcasses which have lesser quality requirements than those of the A, AA, AAA or Prime grades. These represented 1% of all beef carcasses graded in 2008. The four D grades of Canada are grades from mature cow carcasses, and represent 10% of total carcasses. The E grade belongs to the meat of mature or young bull carcasses revealing masculinity and represented 1% of the graded carcasses in 2008. Table 2.1 compares the Canadian and American grading systems.

Table 2.1 Grading systems in Canada and US

Canada	UNITED STATES
Canada Prime	USDA Prime
Canada AAA	USDA Choice
Canada AA	USDA Select
Canada A	USDA Standard

2.3 Tenderness

Tenderness is one of the most important meat palatability attributes, and consumers are willing to pay more for beef which is tender (Lusk et al., 2001) as well as juicy and flavorful (Winger and Hagyard, 1994). In recent years, the meat industry has made great progress in improving tenderness and flavor through genetic improvements and meat science technology. The extensive body of research on beef tenderness has shown that many different factors influence beef quality (Koohmaraie, 1994; Brewer and Novakofski, 2008; Lepetit, 2008). In particular, tenderness has been linked to the animal's age, meat marbling, muscle location and aging (Ramsbottom et al., 1945).

2.3.1 Factors affecting tenderness

2.3.1.1 Animal's age

One of the most important factors affecting meat tenderness and general quality is the age of the animal (Shorthose and Harris, 1990). The greatest tenderness and quality of beef is achieved with cattle under 36 months of age; thereafter the meat becomes tougher. The United States Department of Agriculture (USDA) has five maturity groupings::

A — 9 to 30 months

B — 30 to 42 months

C — 42 to 72 months

D — 72 to 96 months

E — more than 96 months

More tender beef is found in young animals because of their greater enzymatic activity compared to older animals (Bouton et al., 1978; Shorthose and Harris, 1990). Normally, the amount of connective tissue (collagen) increases with the age of the animals, thus lessening meat tenderness and requiring greater cooking time using techniques such as braising and casseroling to reduce the meat's resistance (Warren and Kastner, 1992).

2.3.1.2 Marbling

Marbling arises from white flecks of fat within the meat muscle (intramuscular fat). Beef cuts with high levels of marbling are more likely to be tender, juicy and flavorful than cuts with low levels of marbling (Blumer, 1963; Wheeler et al., 1994). There is a direct correlation between the amounts of marbling in younger beef with the beef's quality grade. Younger beef has a lighter texture and red color which is associated with greater tenderness, juiciness and flavor. Hence, based on the USDA classification, Prime grade is the most tender, most flavorful, has a finer texture and contains more marbling due to the younger age of the animal. According to the USDA quality grade, beef with a high amount of intramuscular fat and marbling tends to be more tender, juicy and flavorful than meat containing less fat. Obviously, it is more likely to be accepted by the consumers due to its high quality.

Meats with much more marbling are more tender. Previous research has shown that there is a direct relationship between tenderness and the degree of marbling from one part of muscle to another part (Francis, Romans et al., 1977; Savell, Branson et al., 1987). Actually, marbling plays a strong role in tenderness considering tenderness variability within the range of 5 to 10% (Blumer, 1963; Pearson, 1966; Jeremiah, 1978).

Under the Select and lower choice grades described by the USDA's beef quality evaluation system, over 75 % of the meat from cattle of a given maturity cattle falls within a narrow range of marbling scores (Stolowski et al., 2006). Therefore, marbling alone has a limited ability to distinguish carcasses into groups indicative of differences in marbling values and tenderness at the consumer level (Boleman et al., 1998; Reuter et al., 2002).

2.3.1.3 Muscle location

Generally speaking, locomotory muscles (*e.g.,* leg muscles) are less tender, but more flavorful than other muscles (*e.g.,* back muscles) (Lehmann and Schindler, 1907). The hindquarters are normally more tender than other parts. Other factors that can affect tenderness include maturity and body type of the animal (Ramsbottom et al., 1945; Zinn et al., 1970). Extremely tender and expensive, the tenderloin is a small cut from a very limited portion of the cattle carcass. Given its high quality and ability to be served after a minimal cooking time, it is much more in demand by the consumer than other cuts. However the generally tougher cuts like chuck roast or beef shoulder can be made more tender, juicy, and flavorful as well as provide a high nutritional value when they are properly prepared using a roasting pot. Because of the greater size of the chuck roast on a beef carcass and the greater time and effort required to cook it properly, such cuts do not fetch the same market price per kilogram compared to tenderloin.

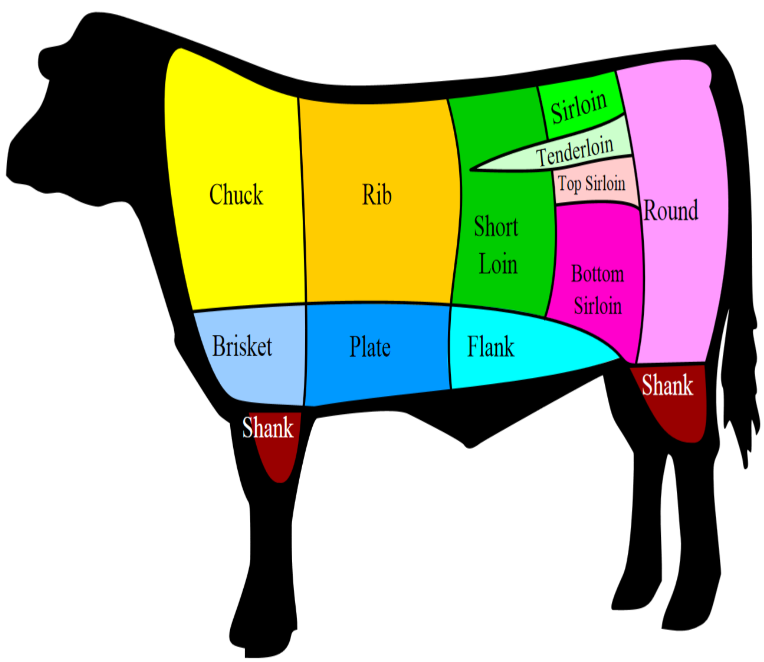

Figure 2.4 Muscle cuts of cattle (Levy, 2008)

2.3.1.4 Postmortem aging

Postmortem meat properties for all muscles of a carcass can be improved in terms of tenderness and flavor, especially in the case of cuts from the rib and loin, through aging of the meat. The natural process of aging tenderizes beef through muscle fiber relaxation and connective tissue breakdown, thus also improve the meat palatability attributes (Huff and Parrish, 1993; Huff-Lonergan et al., 1996). The relaxation of animal muscle fibers which usually occurs after the demise of the animal goes through a number of stages: in the first 12 hours muscles fibers will increase in size, then as the pH in the muscles fibers changes, they slowly begin to relax. This happens in all types of animals with muscle fibers, including fish (Parrish et al., 1973a).

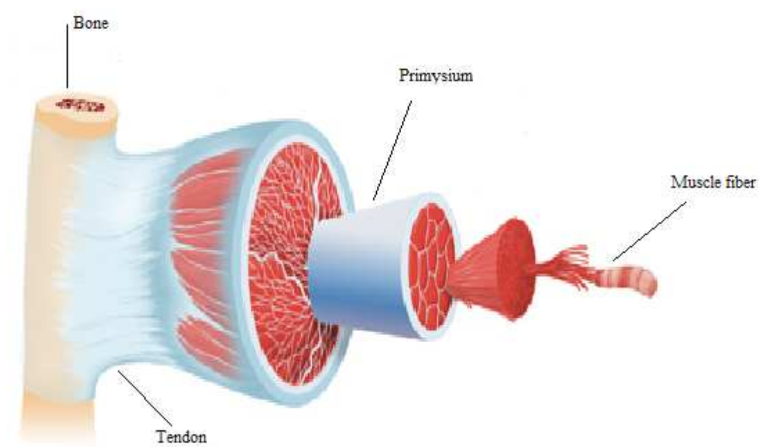

Figure 2.5 Structure of a skeletal muscle (Anonymous, 2012)

Proteolysis is a process in which natural enzymes break down a specific amount of protein in muscle fibers. This process, which is often named myofibril tenderization, takes place from 1 to 7 days postmortem. The rate of tenderness improvement declines as time passes (Fig 2.6). In practice, improvement of tenderness of rib and loin cuts over the first 7 to 10 days usually occur slowly (Morgan et al., 1991; Koohmaraie et al., 1994;).

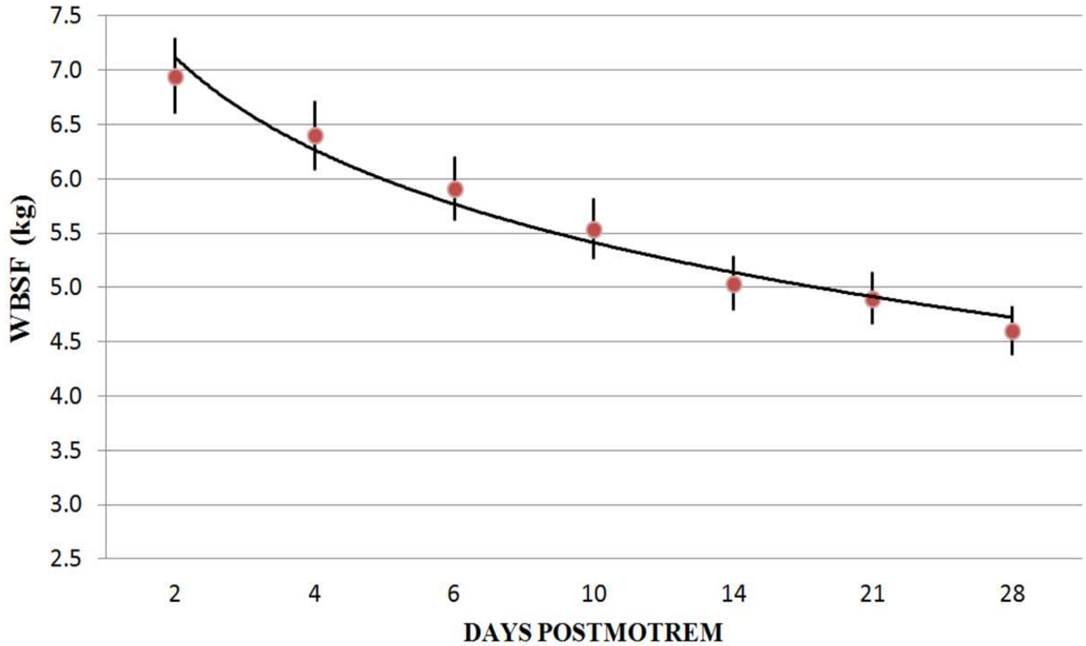

Figure 2.6 Tenderness improvement by aging (i.e. decrease in toughness measured by Warner-Bratzler shear force (WBSF) (Gruber, 2006)

2.3.1.4.1 Wet aging

In wet aging, meat and its juices are vacuum packed in plastic, and refrigerated at 0-2° C when moved from the packing plant to the retailer. This kind of packing assists moisture absorption and migration in the meat which, in turn, aids in the enzymatic break down of the connective tissues, thereby leading to an overall improvement in meat tenderness and juiciness. Under this method, humidity and air velocity are not of importance for proper aging since the beef is vacuum packaged. This method is the predominant method of post-mortem aging today and it is less expensive than dry aging (Laster et al., 2008).

2.3.1.4.2 Dry aging

In dry aging, entire carcasses or wholesale cuts (without covering or packaging) are hung for 21 to 28 days under specific conditions: a very clean environment, temperature between 0-2° C, humidity between 85 and 100%, air velocity from 0.5 to 2.5 m/s. All these conditions are very important in the proper postmortem aging of carcasses. This processing method takes a long time and only good quality meat can be used, thereby resulting in a much better flavor as compared to wet aging (Bischoff, 1984; Miller et al., 1985; Parrish et al., 1991). Under these conditions, the connective tissues in the muscles eventually break down, resulting in more tender beef (Campbell, Hunt et al., 2001).

2.3.2 Measuring of beef tenderness

Based on consumer opinion, tenderness has been defined in terms of how easily the teeth can sink into the piece of steak or how long it takes to chew meat before swallowing. A number of objective methods exist to measure tenderness or its opposite, toughness. Amongst these, the commonly-used method, i.e. the Warner-Bratzler shear force (WBSF) method (Bratzler, 1932; Bouton, Ford et al., 1975; Shackelford, Wheeler et al., 1999), measures the force required to shear through a sample of cooked beef using an Instron machine. The cooking step is very important as there is a strong relationship between increased degree of doneness and shear force and/or decreased tenderness (Ritchey and Hostetler, 1964; Parrish et al., 1973b; Lorenzen et al., 1999; Jeremiah et al., 2003). In order to define tenderness differences between samples, and make valid comparisons between evaluations done in different laboratories, the American Meat Science Association published testing standards in their 1978 publication *Guidelines for*

Cookery and Sensory Evaluation of Meat, which were revised in 1995 (AMSA, 1995). Under these standards, steaks used for WBSF measurements are usually cut into 2.54 cm (1.0 in) thicknesses. The internal temperature of the sample influences tenderness, so it must be the same for all samples. Frozen samples should be thawed until an internal temperature of between 2 to 5°C is reached. The steak is placed on a grill and cooked on one side to an internal temperature of 40ºC, turned and cooked to a final internal temperature of 71ºC. Six cores, each 1.27 cm (0.5 in) in diameter, are removed from each sample, parallel to the longitudinal orientation of the muscle fibers and then sheared perpendicular to the muscle fiber orientation (Fig 2.7).

Figure 2.0 7 Tenderness assessment cores from one meat sample

The individual peak shear force value is recorded for each core test. The Warner-Bratzler shear force should be reported as the mean of all core values (Wheeler, et al., 1994; AMSA, 1995; Wheeler et al., 1996; Wheeler et al., 1997; Shackelford et al., 2004).

The WBSF method for evaluating of beef tenderness is time consuming (it may take several hours or days) as well as being destructive in nature.

2.4 Prediction of beef tenderness

Many different methods for predetermining tenderness are currently available. Based on various regulations and processing protocols, many of methods to evaluate eventual tenderness have been tested over the last ten years (El Karam et al., 1997; Liu et al., 2003; Vote et al., 2003; Abouelkaram et al., 2006). One of these methods involves imaging techniques used in the visual evaluation of meat quality.

2.4.1 Computer vision

Computer vision systems promise to provide a flexible and safe method to evaluate the quality of various types of meat product, in different products. Computer imaging could be used in the food industry to aid or supplement human graders. (Gunasekaran, 1996; Brosnan and Sun, 2004; Sun, 2011). These systems have wide applications, including the analysis of surface weaknesses and color classifications, while determining the visible characteristics and features of the samples examined. Some commercial technologies are using this system to measure the overall quality of products and in the grading process. Belk et al. (2000) developed a computer-linked video imaging system (Beef Cam) to assess beef palatability, and used it to predict the tenderness of beef steaks on-line (Li et al., 1999; Vote et al., 2003; Tan, 2004; Tian et al., 2005). A number of studies have shown that textural features calculated from muscle images can be practical indicators for beef tenderness (Du et al., 2008; Jackman et al., 2009; El Jabri et al., 2010).

Unfortunately, such systems may be inappropriate for certain industrial applications involving similar colors or complex classifications, where it would be incapable of predicting certain quality characteristics (e.g. chemical composition), inefficient in detecting hidden defects, and unable to reveal sufficient information to detect internal characteristics (Brosnan and Sun, 2004; Du and Sun, 2004).

2.4.2 Spectroscopy

Food and particularly meat quality includes several attributes and features, rather than a single characteristic (Abbott, 1999; Noh and Lu, 2005). One of the safest and most successful techniques for providing detailed information during the quality assessment of food products is to measure the optical properties of the products. Optical features, which can be measured using spectral detection equipment, are based on reflectance, transmittance, absorbance, or scatter of polychromatic or monochromatic radiation in the ultraviolet (UV), visible (VIS), and near-infrared (NIR) regions of the electromagnetic spectrum. A quality index for the product can be based on the relationship between the spectral response and a specific quality attribute of the product, usually a chemical component (Park et al., 2003).

Based on the radiation of the sample within a controlled wavelength and a measure of the response from the sample (Sun, 2009), spectroscopy has been widely applied as an analytical method for meat product evaluation. Under optical spectroscopy, the sample is stimulated by irradiating it from a light source, and the sample's light response (e.g., transmission, absorbance, and/or reflection) can be measured by a sensor.

The near-infrared spectroscopy (NIRS) techniques, which show a potential to simultaneously measure multiple quality characteristics, have obtained substantial attention in the past ten years as a non-destructive method to assess meat quality (Liu et al., 2003; Sun, 2009). Applications of NIRS has gained positive attention in the field of food product quality analysis, and is now widely used to predict the quality of fresh meat. It could also potentially serve as a quick and efficient method to assess meat tenderness (Park et al., 1998; Liu et al., 2003; Shackelford et al., 2005; Andrés et al., 2008; Ripoll et al., 2008). Unfortunately, the NIRS technique is limited in that the measurement taken considers the amount of light reflected or transferred only from a specific area of a sample, ignoring a great deal of quality characteristic information available from the samples (Prieto et al., 2009).

2.4.3 Hyperspectral Imaging

Hyperspectral imaging combines the advantages of computer vision and spectroscopy in capturing both spectral and spatial information from an object at the same time, something which is not possible to achieve with routine imaging or spectroscopy. Hyperspectral imaging sensors measure the radiance of the materials within each pixel area through a very large number of contiguous spectral wavelength bands (Manolakis, Marden et al., 2003). With hyperspectral imaging, quality attributes of fruits and vegetables such as strawberry [*Fragaria* × *ananassa* Duchesne] and cucumber [*Cucumis sativus* L.] have been correctly predicted (Liu et al., 2005; Ariana et al., 2006; Liu et al., 2006; ElMasry et al., 2007; Gowen et al., 2007), and the technique has also been used to evaluate quality of pork and beef meat (Qiao et al., 2007; Cluff et al., 2008; Naganathan et al., 2008a; Naganathan et al., 2008b; ElMasry et al., 2012).

2.4.3.1 Hyperspectral imaging system

With line detection technology, hyperspectral imaging systems can capture visual images from hundreds of narrow bands from the visible to the infrared spectral regions, and build a hyperspectral image termed a 'hypercube' of dimensions (x,y,λ), where *x* is the direction of the conveyor movement, *y* is perpendicular to the direction of movement of the conveyor belt, and λ is the wavelength.

A typical hyperspectral imaging system consists of a camera for capturing spatial data, a spectrograph for spectral data, a lens designed to provide a good view of the object, a light source, a conveyor to holding and move the samples, and a computer to save and process the hypercube data (Fig 2.8).

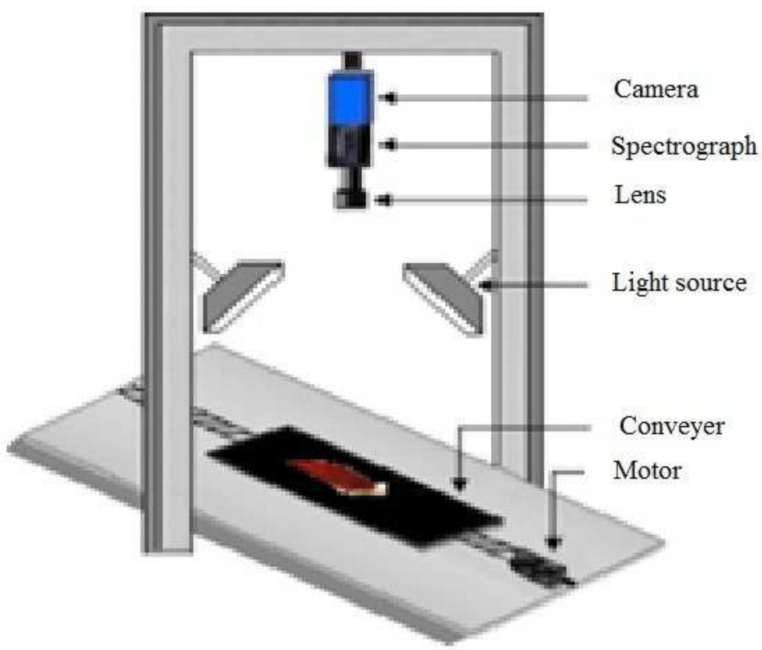

Figure 2.08 Hyperspectral Imaging System (Sun, 2010)

After capturing images of the sample, the images are analyzed (Figure 2.9) for whatever quality assessment is needed,

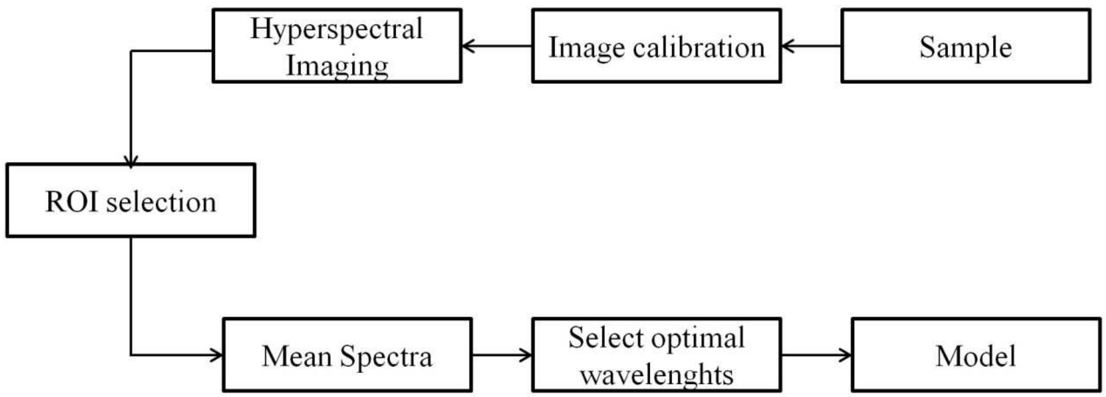

Figure 2.0 9 Analysis the Image (Sun, 2010)

In order to correct the spectral images, a dark image *B* is obtained by covering the lens with a cap and a white image *W* is obtained by taking an image from a standard white reference. The relative reflectance *I* of each image is the calculated as (Liu et al., 2010) :

$$I = \frac{I_0 - B}{W - B} \quad (1)$$

where I_0 was the reflectance of the original image plane.

The region of interest (ROI) of beef samples includes the muscle area without the peripheral and intramuscular fat, or the connective tissue and surrounding fat. The ROI of each hypercube was obtained using a segmentation algorithm (Liu et al. 2012). After ROI selection, the mean reflectance spectrum of each side of the beef sample was calculated and the average spectrum of both sides used as the final spectral features for the given sample. Mean spectra represented the independent variables and the measured value of quality traits the dependent variables for a model. The final models were then developed using machine learning methods.

2.4.3.2 Preprocessing of spectral data

The most widely used pre-processing techniques for NIR spectral measurements (in both reflectance and transmittance mode) can be divided into two categories: scatter correction methods and spectral derivatives.

2.4.3.2.1 Standard normal variate (SNV) transformation

One of the standard scatter correction methods, the standard normal variate (SNV) transformation eliminates the slope variation from spectra caused by scatter and variation of particle size (Barnes et al., 1989; Candolfi et al., 1999). The transformation is performed on each spectrum separately by subtracting the spectrum mean and scaling with the spectrum standard deviation:

$$X_{ij,SNV} = \frac{(X_{ij} - \overline{X_i})}{\sqrt{\frac{\sum_{i=1}^{p}(X_{ij} - \overline{X_i})^2}{p-1}}} \qquad (2)$$

where P is the number of variables in the spectrum

x_i is the mean of spectrum *i*,

and $X_{ij,SNV}$ is the transformed element for original element x_{ij} .

2.4.3.2.2 Derivatives

It is further possible to remove overlapping peaks and correct the baseline using derivative spectra. The derivative draws the overlapping peaks apart and the linear background becomes a constant value in the first derivative spectrum and zero in the second derivative spectrum (Osborne et al., 1993). In the second derivative the peaks alter to troughs, whereas in the first derivative they become zero. Unfortunately, the differencing operation magnifies the noise and increases the complexity of the spectrum. The derivative of mean spectra was calculated as:

$$D(I_i) = \frac{M_{i+1} - M_i}{I_{i+1} - I_i} \tag{3}$$

where i is the number of wavelengths i = 1,2,3,...,n.

M_{i+1} and M_i are the mean reflectance at wavelengths I_{i+1} and I_i, respectively.

The second derivative of the mean spectrum was a 1 × (n-2) vector.

2.4.3.3 Analysis of spectral data

Several criteria are applied to select appropriate spectral regions for calibration. Stepwise regression, one of the methods widely used to narrow down the wavelengths used in building a model for a given parameter of interest (e.g. tenderness), finds the best combination of independent variables (wavebands/wavelengths) to predicts the dependent variable (Cluff et al., 2008). In the processing of stepwise regression not all independent variables may remain in the equation, independent variables entered the regression equation one at a time based upon statistical criteria, then at each step of the analysis, the predictor variable that contributes the most to the prediction equation in terms of increasing the multiple correlation R, is entered first. This process continues only if additional variables add anything statistically to the regression equation. At each round the significance of already accepted independent variables is tested and those whose significance falls below a certain retention threshold are removed. When no additional predictor variables add anything statistically meaningful to the regression equation, and no more are removed, the analysis ends. Thus, not all predictor variables will become or remain part of the equation in stepwise regression.

2.4.3.4 Modeling

Multiple regression finds the relationship between a dependent (predicted) variable and several independent (predictor) variables, where all predictor variables are entered into the regression equation at once. The end result of multiple regression is the development of a regression equation (line of best fit) between the dependent variable and several independent variables.

$$Y = a_0 + \sum_{n=1}^{i=N} a_{\mathrm{i}} X_i \qquad (4)$$

where a_0, a_{i} are regression coefficients

N is the number of selected predictor

X_i is the i^{th} element of a vector of selected independent variables

and Y is the predicted variable.

In the present study, stepwise multiple regression and multiple linear regression (ElMasry et al., 2007) were used to select important wavebands and establish appropriate models for beef tenderness prediction, respectively.

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CONNECTING TEXT

A comprehensive review of literature showed that tenderness is a very important attribute for beef quality and consumer satisfaction. Aging is one of the factors which has an effect on tenderness.

Chapter 3 addresses the first objective of the thesis, i.e., assessing the effect of three different aging durations on tenderness profiling of different beef muscles. This chapter was presented at the Northeast Agricultural and Biological Engineering Conference in 2013. A paper based on this chapter will be submitted for publication. The manuscript is co-authored by my supervisor Dr. Michael Ngadi and a research associate Dr Li Liu. The format of the original manuscript has been modified to remain consistent with the thesis format.

III. EFFECT OF AGING ON BEEF TENDERNESS

3.1 ABSTRACT

Two hundred and sixty beef samples were used to assess the effect of aging time on beef tenderness. Samples of four different types of muscle, namely *infraspinatus* (TB), *gluteus medius* (TS), *psoas major* (TL), and *longissimus thorasis* (RE), were aged for 14 or 21 days at 2ºC and then frozen at -18ºC until analyzed. Cooked samples (to end-point of 71ºC) were measured by Warner-Bratzler shear force (WBSF), and significant aging effects were found. Among the four muscle types, tenderloin was the most tender, whether fresh, or aged 14 or 21 days. For each muscle type, aging duration (14 *vs.* 21 days) had no significant effect on beef tenderness ($p > 0.1$), indicating that no significant increase in beef tenderness occurred by keeping it more than 14 days.

3.2 INTRODUCTION

From a consumers' point of view, tenderness is, amongst all meat texture characteristics, one of the most important palatability attributes (Love,1994). Indeed, several studies have shown that consumers are willing to pay more for a cut of tender beef (Boleman, Boleman et al., 1997; Lusk, Fox et al., 2001; Miller, Carr et al., 2001; Platter, Tatum et al., 2005). Tenderness is also related to juiciness and flavor (Winger and Hagyard,1994).

In recent years, the meat industry has made a great progress in improving tenderness and flavor through genetic improvement and meat science technology. A number of studies of beef tenderness (Koohmaraie, Kent et al., 2002; Oliete, Moreno et al., 2005; Laville, Sayd et al., 2009; Parish, Rhinehart et al., 2009), have shown that tenderness is tied to a number of factors, including post-mortem aging. A strong correlation exists between connective tissue content and tenderness (Mitchell, Hamilton et al., 1928; Mackintosh, Hall et al., 1936).

Meat aging is a process involving post-mortem proteolysis of myofibrillar proteins in muscles. Tenderisation begins shortly after slaughter and increases after the *rigor mortis* phase (Koohmaraie, 1996). To improve the consistency of meat quality with respect to tenderness, beef should be aged at least 14 days (in practice carcasses tend to be aged for only 5 days).

3.3 MATERIALS AND METHODS

3.3.1 Samples and dry-aging

A total of 10 carcasses aged between 403 and 536 days were selected from a slaughterhouse (VG Meats, Simcoe, Ontario, Canada) over three successive months: 3 steer carcasses for the first and second months, and 2 steer and 2 heifer carcasses for the third month. In each month, the predefined numbers of carcasses were slaughtered on the first Thursday. The carcasses were chilled and electrically stimulated after slaughter. At 24 h post-mortem, subprimals were removed from each carcass and packed as individual muscles, namely *infraspinatus* (Top Blade, TB), *gluteus medius* (Top Sirloin, TS), *psoas major* (Tenderloin, TL), and *longissimus thorasis* (Rib Eye, RE). The samples were sliced (one inch thickness) from each muscle by a mechanical slicer on the first Friday, namely fresh samples, third Friday, namely 14 days dry-aged samples, and fourth Friday, namely 21 days dry-aged samples of the month. The details on how to collect the fresh and dry-aged samples have been extensively discussed in Appendix 2. A total of 260 slices, including 59 TB, 60 TS, 69 TL, and 72 RE, were vacuum packed and kept frozen during shipping to the Macdonald Campus of McGill University (Sainte-Anne-de-Bellevue, QC, Canada). The frozen beef samples were received on Monday mornings and stored at 4°C.

3.3.2 Preparation of steaks and cooking

Steaks for WBSF measurement were thawed at 4°C for approximately 24h after receiving. Internal temperature was between 2ºC and 5ºC before cooking. The steak was placed on a grill (model GRP90WGRCAN, George Foreman, Middleton, WI) and cooked on one side to an internal temperature of 40ºC, turned and cooked to a final internal temperature of 71ºC (removed from the heat at 71ºC). Only one steak was cooked per grill at any one time. Temperature was monitored with iron-constantan thermocouple (10 cm spear point, K-type) with a diameter of 20 mm and a maximum error of 0.5ºC. The thermocouple was inserted horizontally into the geometric center of the steak and attached to a voltmeter (Fisher Scientific, Pittsburgh, PA).

After cooking, the steak was placed in a labeled polyethylene bag and immediately immersed in an ice bath to arrest further cooking. It was then stored overnight in a cooler at 2ºC.

3.3.3 Warner-Bratzler Shear Force (WBSF)

For WBSF determination, a minimum of six cores with 127 mm (0.5 in) in diameter were removed from each sample using a hand-held coring device (core borer). Cores were removed parallel to the longitudinal orientation of the muscle fibers and then sheared perpendicular to the muscle fiber orientation. Shearing was conducted using an automated testing machine (model 4500, Instron Corp., Canton, MA) with a Warner-Bratzler shear attachment (Figure 3.1) and crosshead speed set at 0.20 m min^{-1}. Each core was sheared once in the center of the core to avoid involving the hardened part on the outside of the steak. Individual peak shear force value was recorded for each core tested (Figure 3.2). Warner-Bratzler shear force was reported as the mean of all core values (Table 3.1).

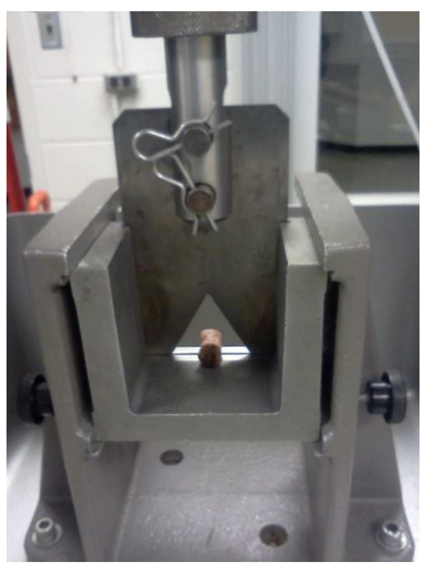


Figure 3.1 Warner-Bratzler shear attachment

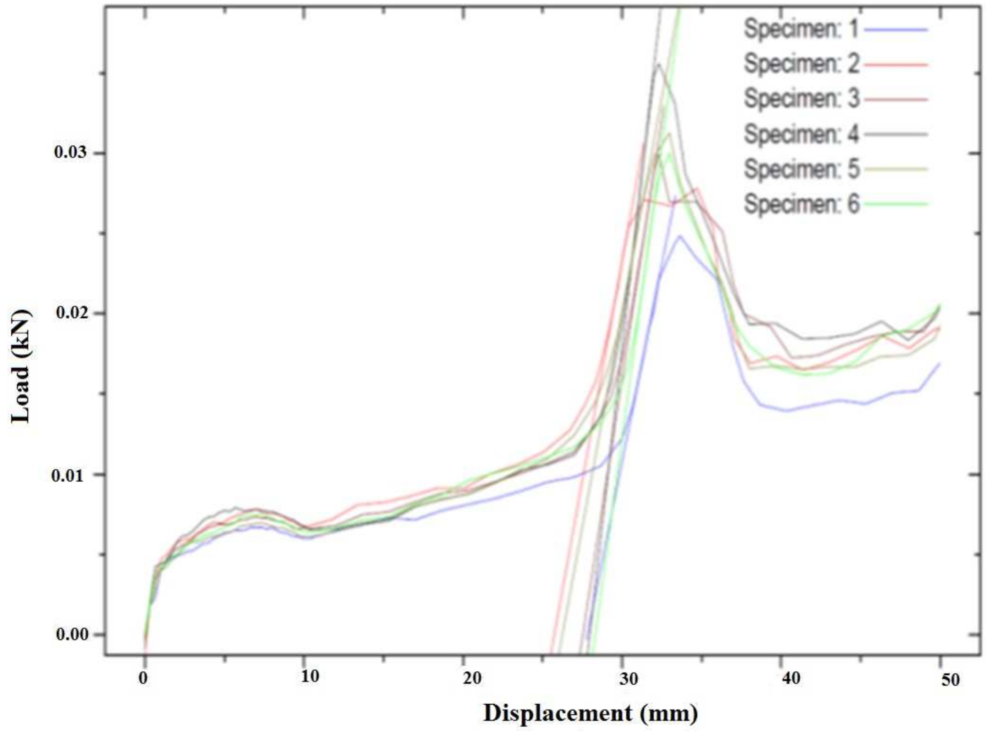


Figure 3.2 Instron result

Table 3.1 Shear force value for each core

Sample	Displacement at Maximum Load (mm)	Load at Maximum Load (N)	Slope (ManYoung) (N mm^{-1})	Energy to Break Point (J)
1	33.660	24.860	4.932	0.537
2	34.860	28.260	5.449	0.646
3	32.460	30.290	6.465	0.651
4	32.260	35.610	8.738	0.689
5	32.790	31.270	5.358	0.645
6	33.130	30.520	7.294	0.641
Mean	33.193	30.135	6.373	0.635
Std. Dev.	0.956	3.543	1.443	0.051

3.3.4 Statistical analysis

Statistical analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC). Summary statistics (mean, standard deviation and range) were computed using the MEANS procedure, Proc GLM, and Tukey test in order to determine the effects of aging on each group of muscles and also to assess any interaction between aging and muscle type. Alpha was set at 0.05.

3.4 RESULTS AND DISCUSSION

The mean (n = 6) WBSF load measurement was used to assess beef tenderness. The WBSF value has a reverse relationship with tenderness; i.e., the higher the WBSF is, the less tender is the beef. Summary statistics of the results are shown in Table 3.2 and Figure 3.3.

Table 3.2 Warner-Bratzler Shear Force values of beef tenderness

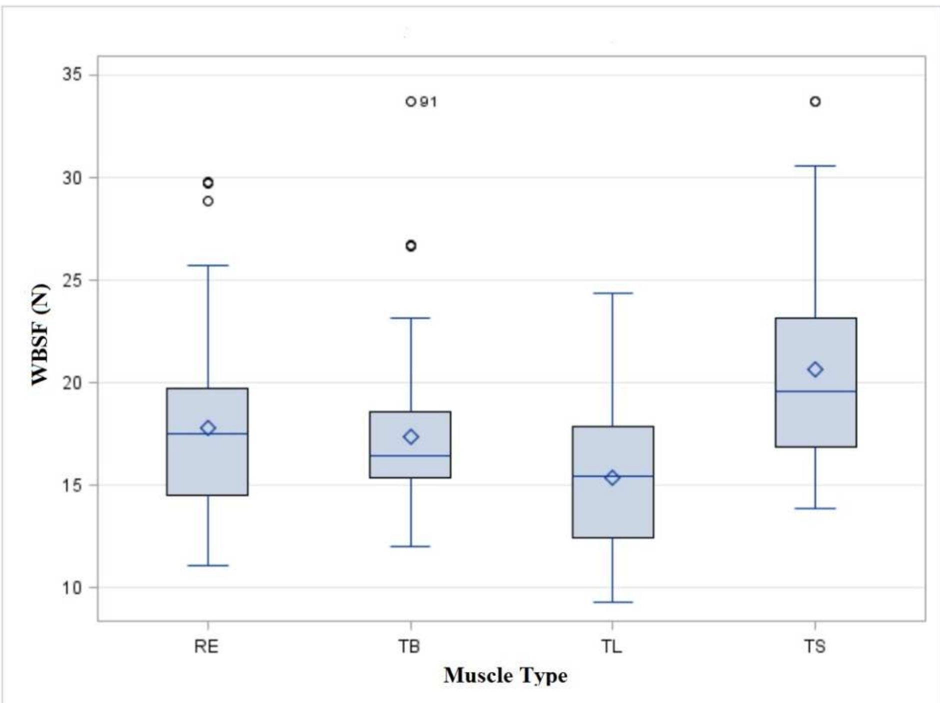
Muscle Type	Aging (days)	Mean± Standard deviation (N)	Minimum (N)	Maximum (N)	Sample Size
RE	0	19.77 ± 3.49^{a}	13.33	29.79	26
	14	16.53 ± 3.53^{b}	11.48	25.28	23
	21	16.84 ± 3.53^{b}	11.07	25.73	23
TB	0	19.60 ± 4.62^{a}	13.97	33.70	19
	14	16.50 ± 3.70^{b}	11.74	20.77	20
	21	16.15 ± 4.19^{b}	12.25	22.70	20
TL	0	17.04 ± 3.01^{a}	10.74	24.36	23
	14	$15.35\pm3.33^{a,b}$	9.51	21.19	23
	21	13.73 ± 2.72^{b}	9.30	20.58	23
TS	0	23.69 ± 4.71^{a}	16.37	33.73	20
	14	$20.27\pm4.30^{a,b}$	14.31	30.54	20
	21	17.92 ± 3.99^{b}	13.89	28.76	20

RE: rib eye; TB: top blade; TL: tenderloin; TS: top sirloin

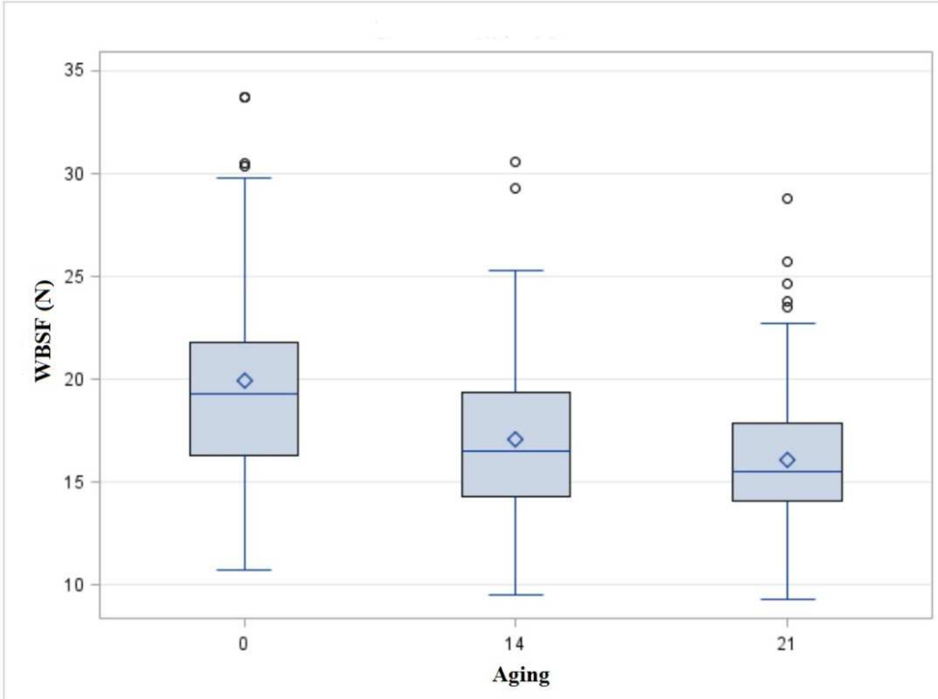
* Values followed by different letters for each type of muscle differ significantly at the 0.05 level.

The main observations from Table 3.2 are as follows. In general, for 14-day and 21-day aged samples, different muscle types showed no significant difference ($p>0.05$) between each other in terms of tenderness. The tenderness of dry-aged RE and TB muscles (14 or 21 days) was statistically greater than that of fresh (non-aged) beef. For TL and TS muscles, there was no significant difference in tenderness ($p>0.05$) between fresh and 14-day aged, as well as between 14-day and 21-day aged; however, we observe a statistically significant difference between 21-day aged and fresh beef ($p<0.05$).

Figure 3.3 (a) and (b) show the comparison of the means of tenderness in terms of muscle type and aging, respectively.



(a)



(b)

Figure 3.3 Distribution of Tenderness (a) Muscle type (b) Aging

(RE: rib eye; TB: top blade; TL: tenderloin; TS: top sirloin)

O: Outlier; ◊: Mean; ┴: Lower adjacent; ┬: Upper adjacent; ─: Median

Note that WBSF has a reverse relation with the tenderness; the less the WBSF is, the more tender the meat would be. It is easy to verify from Figure 3.3 (a) that TL and TS have the least and the highest WBSF means, respectively, which means that TL is the most tender muscle and TS is the least tender muscle. Moreover, there is not a significant difference between WBSF of RE and TB. The main takeaway from Figure 3.3 (b) is that the WBSF decreases in aging, which means that aging increases the tenderness of the muscle. However, the increase of tenderness is more significant from fresh beef to 14-day aged beef comparing to that from 14-day to 21-day aged beef. This result is consistent to the results reported in the related literature (Oliete, Moreno et al., 2005, Laville, Sayd et al., 2009).

Finally, we examine the impact of aging on different muscle types in Figure 3.4. Verify that the rate of decline in WBSF (and increase in tenderness) with aging mainly depends on the type of muscle. Specifically, there is no significant improvement in tenderness between RE and TB if we keep them more than 14 days, however, the tenderness of TS and TL can be improved by aging from 14 to 21 days.

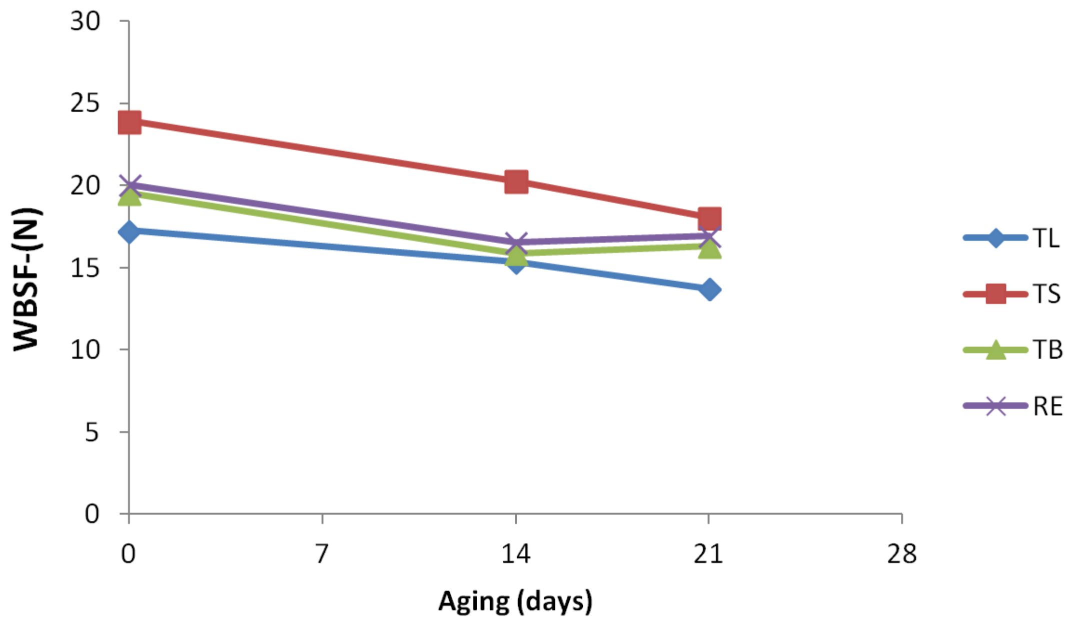


Figure 3.4 Warner-Bratzler Shear Force for RE: rib eye; TB: top blade; TL: tenderloin; TS: top sirloin at different aging times.

3.5 CONCLUSION

The improvement of beef tenderness by aging was confirmed by showing that the Warner-Bratzler Shear Force was decreased by aging. Our statistical analysis shows that the impact of dry-aging on the tenderness of the beef depends on both the type of muscle (RE, TB, TL, and TS) and the length of dry-aging (fresh, 14-day, and 21-day). Specifically, there is no significant difference between 14-day and 21-day dry-aging for all muscle types in terms of tenderness; however, we can significantly improve the tenderness of RE, TB and TS muscles by either 14-day or 21-day dry-aging. The results for the TL muscle were different, specifically, we could not verify significant difference in tenderness between fresh and 14-day aged, as well as between 14-day and 21-day aged; but, there was a significant difference between 21-day aged and fresh TL. Finally, our results indicate that there is not a significant interaction between the main parameters of the model, i.e., aging and type.

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CONNECTING TEXT

In chapter 3, the effect of dry aging on the tenderness of four different muscles was measured. Chapter 4 addresses the second and third objectives of the thesis, *i.e.*, prediction of beef tenderness using hyperspectral imaging and the identification of spectral regions suitable for this purpose.

This chapter was presented at the Northeast Agricultural and Biological Engineering Conference in 2013. A summary of this chapter has been submitted to The Canadian Meat Science Association (CMSA) newsletter. Moreover, a paper based on this chapter will be submitted for publication. The manuscript is co-authored by my supervisors Dr. Michael Ngadi and Dr. Shiv O. Prasher and a research associate, Dr Li Liu. The format of the original manuscript has been modified to remain consistent with the thesis format.

IV. HYPERSPECTRAL IMAGING FOR BEEF TENDERNESS ASSESSMENT

4.1 ABSTRACT

Tenderness is one of the principal properties of meat quality. The traditional way to measure tenderness the beef is time consuming and also destructive, and therefore not appropriate for rapidly identifying quality parameters on the processing line, with the minimum of human intervention. The objective of the present research was to measure the tenderness of cooked beef samples obtained from four types of muscles (i.e. *infraspinatus* (TB), *gluteus medius* (TS), *psoas major* (TL), and *longissimus thorasis* (RE)) at three different durations of dry aging (Fresh (0 days), 14 days, and 21 days), using near infrared hyperspectral imaging. Hyperspectral reflectance spectra (900 nm < λ < 1700 nm) were acquired for a total of 260 beef steak samples with dry-ages of 0, 14 or 21 days. After imaging, samples were cooked and the Warner-Bratzler shear force (WBSF), a parameter inversely related to meat tenderness, was measured. After reflectance calibration, a region of interest (ROI) was selected from each acquired hyperspectral image and stepwise regression was applied to the ROI to select wavelengths that were strongly related to cooked meat tenderness. Multiple Linear Regression (MLR) calibration models were developed for quantitative evaluation of beef tenderness. The correlation coefficient (*R*) and the root mean square error (*RMSE*) were employed to evaluate the calibration model's predictive ability for each group. The

calibration model developed predicted tenderness with *R* values of 0.89, 0.86, 0.81 and 0.83 for TS, RE, TB, and TL, respectively. The results revealed that the HSI could be used for non-destructive measurement of beef tenderness in beef having undergone three different durations of aging.

4.2 INTRODUCTION

Meat quality is determined by a combination of factors including intramuscular fat, marbling, water absorption, muscle fiber structure, pH, and the quality at the time of eating, as indicated by tenderness, juiciness and flavor (AMSA, 2001). The visual appearance, texture and color of raw meat are important decision factors for consumers in purchasing meat. These factors are linked to chemical parameters such as marbling, water and protein contents. One of the primary beef quality attributes determining consumers' acceptance of meat is tenderness, and it is therefore of utmost importance for the meat industry to produce meat of good quality which is safe to consume.

Existing meat quality assessment methods still rely largely on visual judgment, which is unfortunately subjective and time consuming. Therefore, there is a crucial need in the meat industry for a fast, accurate and non-destructive approach to determining beef quality (Herrero, 2008). Recently, many objective spectroscopic and imaging methods have been developed and successfully applied to assessing meat quality (El Karam, Berge et al., 1997; Liu, Lyon et al., 2003; Liu, Windham et al., 2003; El Jabri, Abouelkaram et al., 2010); however, these do not provide detailed information about the samples (Vote, Belk et al., 2003; Kumar and Mittal, 2010).

Hyperspectral imaging is an emerging technology now being used for real-time, robust and non-destructive inspection and quality evaluation of food and agricultural products (Lu, Chen, 1999; Mehl et al., 2004; ElMasry et al., 2007; Qiao et al., 2007; Wang, 2007; Barbin et al., 2012). Hyperspectral imaging combines the advantages of conventional imaging and spectroscopy, and simultaneously obtains spectral and spatial information from the object to determine its quality (Qiao et al., 2007; Liu et al., 2010; Barbin et al., 2012). Therefore, it is possible to find out a number of important attributes, characteristics or diagnostic features through the surface reflectance spectra of food products.

Hyperspectral imaging has been reportedly used in determining the meat quality parameters of intramuscular fat, marbling, color, chemical composition, and especially tenderness (Cluff et al., 2008; Gowen et al., 2008; Naganathan et al., 2008). Though some work has been done on meat quality assessment with hyperspectral imaging, few studies have reported on its use in predicting beef tenderness for meat having undergone different periods of aging. The non-destructive nature of hyperspectral imaging is an advantage when determining the quality of raw material and final product (Wold et al., 2006; Folkestad et al., 2008).

4.3 MATERIALS AND METHODS

4.3.1 Sample collection

A total of 10 carcasses (8 steer and 2 heifer) between the ages of 403 and 536 days were selected from a slaughterhouse (VG Meats, Simcoe, Ontario, Canada). For each month, a predefined numbers of bulls were slaughtered on the first Thursday. The carcasses were chilled and electrically stimulated after slaughter. At 24 h post-mortem, subprimals were removed from each carcass and separated into individual muscles: *infraspinatus* (Top blade, TB), *gluteus medius* (Top sirloin, TS), *psoas major* (Tenderloin, TL), and *longissimus thorasis* (Rib eye, RE). Using a mechanical slicer, fresh and dry-aged samples were sliced from each muscle on the first Friday (fresh samples), third Friday (14 days dry-aged samples), and fourth Friday (21 days dry-aged samples) of each month. Slices were vacuum-packed and kept frozen during shipping to the Hyperspectral Imaging Lab, Macdonald Campus of McGill University (Sainte-Anne-de-Bellevue, QC, Canada). A total of 260 raw beef samples were collected and images of each sample were captured before cooking and subsequent measurement of tenderness by the Warner-Bratzler shear force method.

4.3.2 Warner-Bratzler Shear Force (WBSF)

There exist different methods in order to measure either the tenderness or the toughness of meat. One of the most popular techniques is the Warner-Bratzler shear force (WBSF) method (Bratzler, 1932; Bouton, Ford et al., 1975; Shackelford, Wheeler et al., 1999), which measures the force required to shear through a sample of cooked beef using an Instron machine. The data then were analyzed using the MEANS procedure in SAS 9.3 (SAS Institute Inc., Cary, NC). We refer the reader to Section 2.3.2 in Chapter 2 for details of the above technique.

4.3.3 Hyperspectral Imaging System (HSI)

A laboratory near-infrared (NIR) hyperspectral imaging (HSI) system was set up to collect the hyperspectral images of the beef samples. The NIR-HSI system consisted of an InGaAs camera mounted with a line-scan spectrograph (Headwall Photonics, Fitchburg, MA, USA, 900-1700 nm), two 50W tungsten-halogen lamps placed at a 45° angle to illuminate the camera's field of view, a moving conveyor driven by a stepping motor with a user-defined speed (MDIP22314, Intelligent Motion System Inc., Marlborough, CT, USA), a supporting frame, and a computer (Figure. 4.1). The system consisted of a line-scan pushbroom with a 4.8 nm resolution, allowing one to scan the sample line by line and generate a data cube with one spectral and two spatial axes. Each raw beef sample was imaged on both surfaces using the NIR-HSI system.

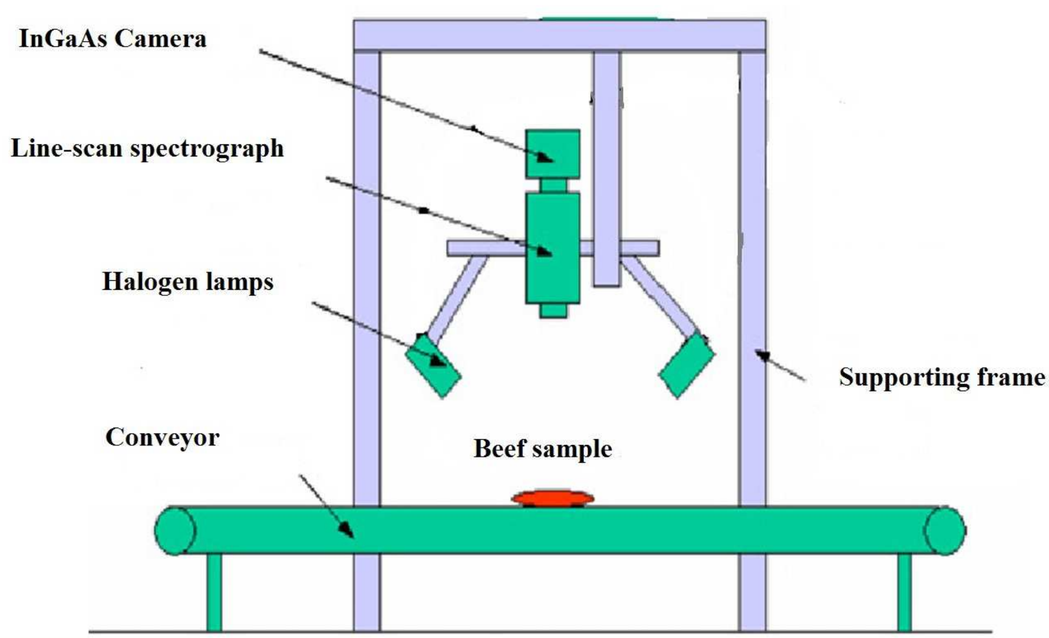


Figure 4.1 Illustration of the near-infrared hyperspectral imaging system

4.3.4 Image correction

Data analysis in this project involved spectral and image analysis for beef tenderness prediction. Each hypercube was corrected from the dark current of the camera prior to segmenting the region of interest (ROI) of each sample. To correct the spectral images, a dark image *B* with about 0% reflectance and a white image *W* with about 99% reflectance were obtained by covering the lens with a cap, and by taking an image from a standard white reference plate (Spectralon, Labsphere, North Sutton, NH, USA), respectively. The relative reflectance *I* of each image was calculated as (Liu et al., 2010):

$$I = \frac{I_0 - B}{W - B} \tag{4.1}$$

Where I_0 is the reflectance of the original image.

A single beef sample was placed on a dark panel to collect the hyperspectral data. Images of both surfaces of the beef sample were taken and saved for subsequent analysis. The images obtained were stored in a data *hypercube*, composed of one spectral and two spatial coordinates.

4.3.5 Data processing

Data analysis and image processing operations followed the procedure outlined in Figure 4.2. All of the acquired data hypercubes were processed and analyzed using MATLAB 7.3.0 (The MathWorks, Inc., MA., USA). Using a method developed by Liu *et al.* (2012), each hypercube's region of interest (ROI) was automatically segmented. Due to the low signal-to-noise ratio at the two ends of the spectral range, only spectral images from 970-1630 nm were used for image analysis. After ROI selection, the mean reflectance spectrum of each side of the beef sample was calculated and the average spectrum of the two sides served as the final spectrum. Each mean spectrum was smoothed through the Standard Normal Variate (SNV) method in MATLAB 7.3.0 (The MathWorks, Inc., MA., USA), and finally the second derivative of the mean spectrum was calculated.

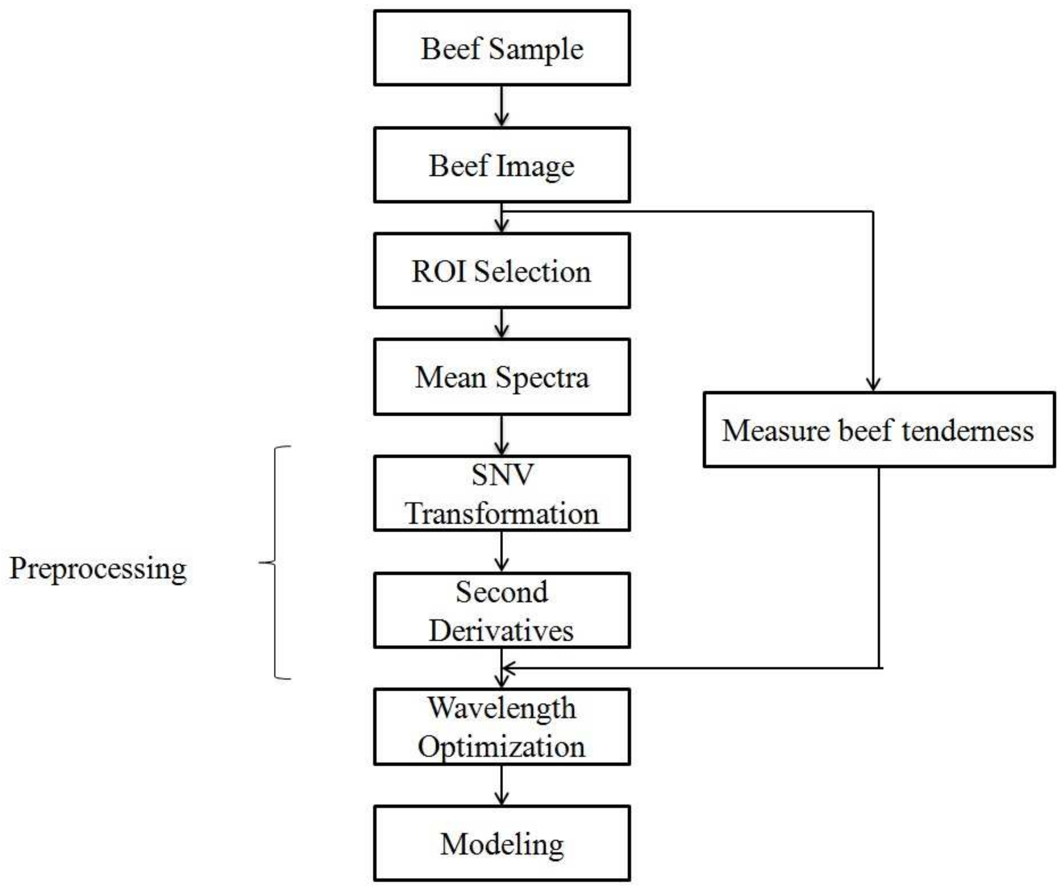


Figure 4.2 Flow chart image analysis

4.3.6 Wavelength optimization

The second derivative of the mean spectrum was used to select the optimal wavelengths, based on a high coefficient of determination (R^2) for the relationship between reflectance and tenderness. Wavelength selection was performed by GLMSELECT in SAS (SAS 9.3, Cary, NC, USA)

4.3.7 Multivariate linear regression

For each muscle type, samples were divided into calibration and validation sets at a ratio of three to one: all samples were arranged in an ascending order according to tenderness values, and then one sample, from four samples, was picked out for the validation set. A predictive model of beef tenderness was created with selected wavelengths of the calibration set based on the Multiple Linear Regressions (MLR) technique using Unscrambler multivariate software (v10.13, Camo, Norway). For the validation set of beef samples, the measured reflectance constituted the MLR model's input when assessing its prediction accuracy for beef tenderness. The correlation coefficient (R) and Root Mean Square Error ($RMSE$) between the predicted and measured tenderness score of the calibration (R_C , $RMSE_v$) and validation (R_V, $RMSE_v$) sets were used to evaluate the prediction models. A good model with high value of R_C and R_V, small values of RMSEv and $RMSE_v$ was obtained in calibration and maintained in validation.

4.4 RESULTS AND DISCUSSIONS

Tenderness of all beef samples (n = 260) were measured by the Warner-Bratzler shear force (WBSF) method and the summary of statistics, i.e., mean, standard deviation and range, is provided in Table 4.1. An example of images collected from both sides of a beef sample is shown in Fig. 4.3 and the corresponding segmented ROI in Fig. 4.4.

Table 4.1 Statistics for cooked beef meat Warner-Bratzler shear force (inverse of tenderness)

Muscle Type	Aging (days)	Mean± Standard deviation(N)	Minimum (N)	Maximum (N)
RE	0	19.77±3.49	13.33	29.79
	14	16.53±3.53	11.48	25.28
	21	16.84±3.53	11.07	25.73
TB	0	19.60±4.62	13.97	33.70
	14	16.50±3.70	11.74	20.77
	21	16.15±4.19	12.25	22.70
TL	0	17.04±3.01	10.74	24.36
	14	15.35±3.33	9.51	21.19
	21	13.73±2.72	9.30	20.58
TS	0	23.69±4.71	16.37	33.73
	14	20.27±4.30	14.31	30.54
	21	17.92±3.99	13.89	28.76

RE: rib eye; TB: top blade; TL: tenderloin; TS: top sirloin

The mean spectrum of the ROI (*i.e.*, the average values of the ROI of image planes over the wavelength range) of each beef sample served as spectral features of the hypercube. The spectral feature of a beef sample was defined as the average profile of the mean spectra for both sides of the beef sample. Figure 4.5 shows typical spectral features for different muscles at different dry ages. Preprocessing by SNV transformation was applied to the means spectra (Fig.4.6), followed by second derivatives for wavelength selection (Fig. 4.7).

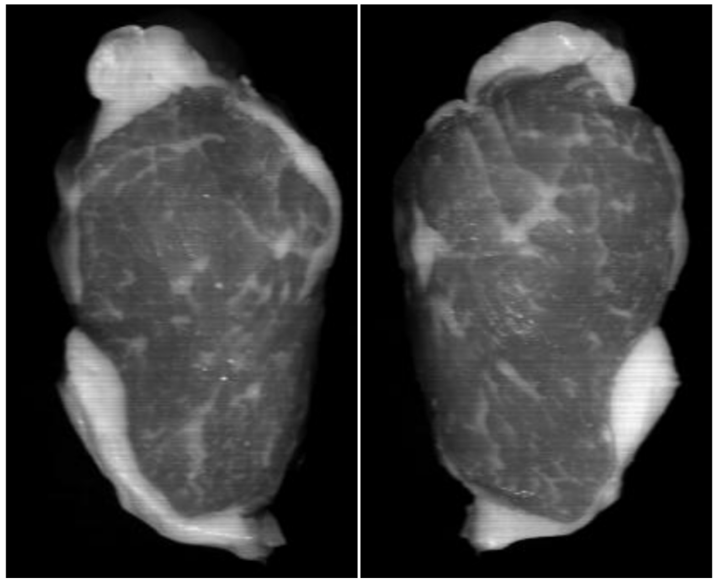


Figure 4.3 NIR (λ = 1086 nm) images of beef meat

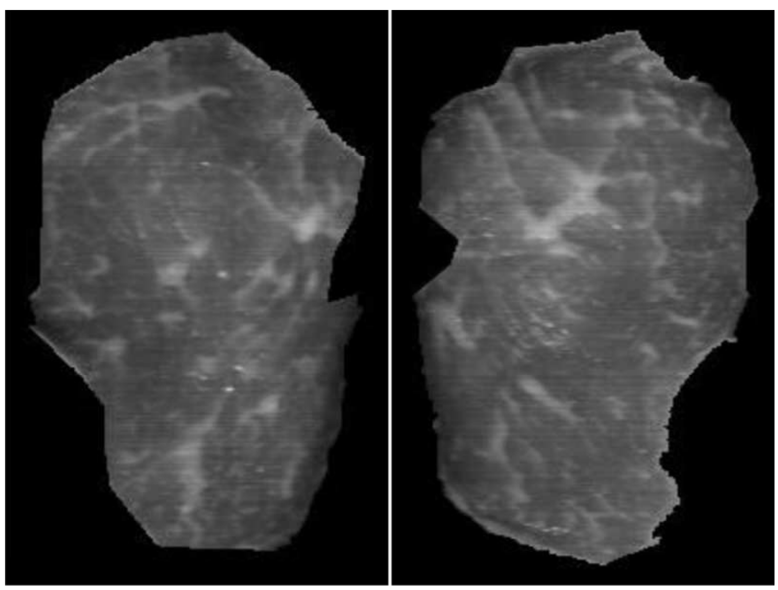


Figure 4.4 ROI of (λ = 1086 nm) NIR images of beef meat

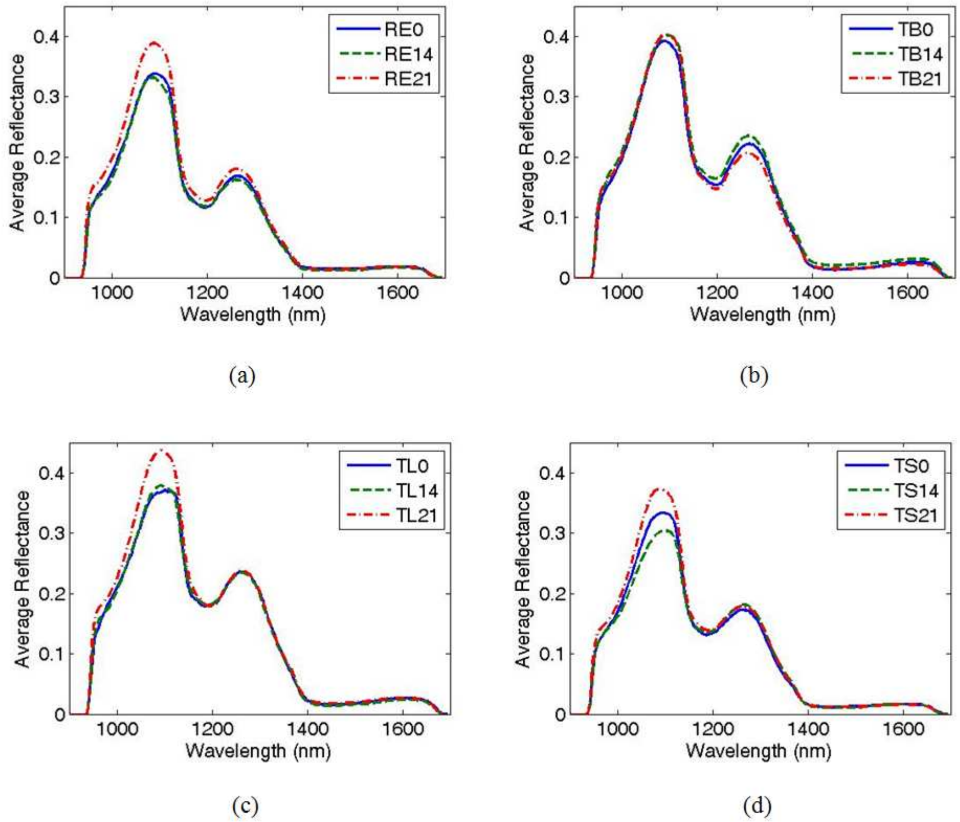


Figure 4.5 Spectral features for muscles (a) RE, (b) TB, (c) TL, (d) TS after different dry aging durations. RE: rib eye; TB: top blade; TL: tenderloin; TS: top sirloin

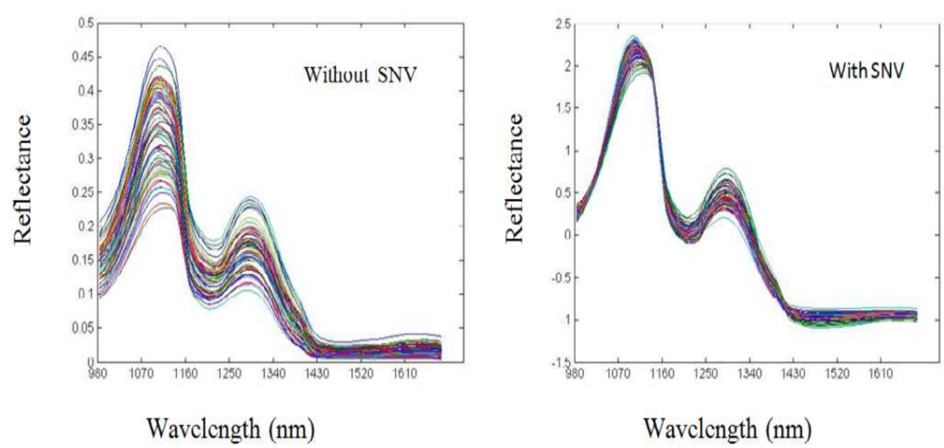


Figure 4.6 SNV transformation of mean NIR spectra for the samples used in this study.

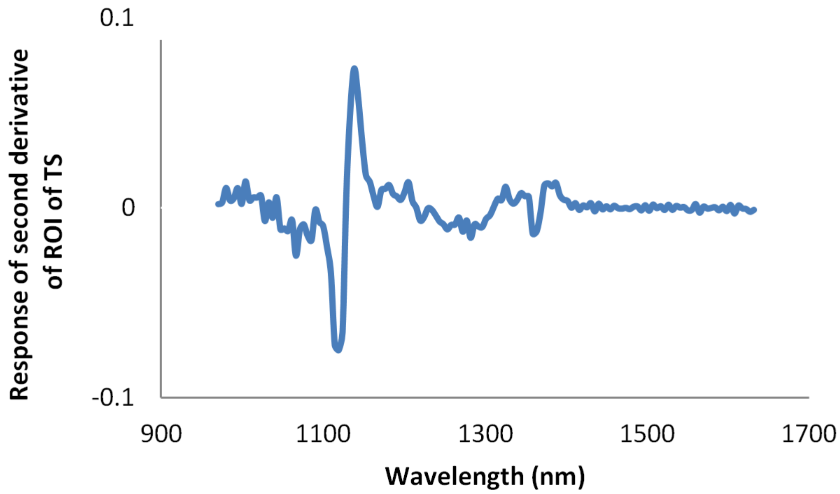


Figure 4.7 Typical second derivatives of mean reflectance spectra

For each muscle type, the wavelength selection was performed separately. Based on the results of the stepwise analysis, effective wavelengths were selected, as shown in Table 4.2.

Table 4.2 Wavebands selected by the stepwise regression operation

Muscle Type	Waveband selected (nm)	Number of wavebands
RE	971,995,1014,1018,1042,1086,1124,1253,1263,1469,1517,1575	12
TB	1081,1100,1215,1320,1340,1359,1368,1392,1479,1541,1565,1632	12
TL	1172,1354,1421,1431,1483,1488,1522,1527,1551	9
TS	980,999,1023,1062,1071,1196,1220,1320,1460,1493	10

RE: rib eye; TB: top blade; TL: tenderloin; TS: top sirloin

A comparison of the Warner-Bratzler shear force (inverse of tenderness) for the calibration and validation sets of different cuts (Table 4.3) shows that for all cut types the range of WBSF in the validation set was covered by the range of the calibration set, indicating an appropriate distribution of samples for modeling.

Table 4.3 Warner-Bratzler shear force value of calibration and validation sets for 4 cuts of meat

Muscle Type	Sample set	Number of samples	Minimum	Maximum	Mean
RE	Calibration	54	11.07	25.73	17.11
	Validation	18	12.16	26.39	17.59
TB	Calibration	44	11.74	26.62	16.78
	Validation	15	12.25	25.70	17.09
TL	Calibration	52	9.30	24.36	15.49
	Validation	17	9.51	22.69	15.60
TS	Calibration	45	16.37	23.78	20.19
	Validation	15	17.77	20.20	20.11

RE: rib eye; TB: top blade; TL: tenderloin; TS: top sirloin

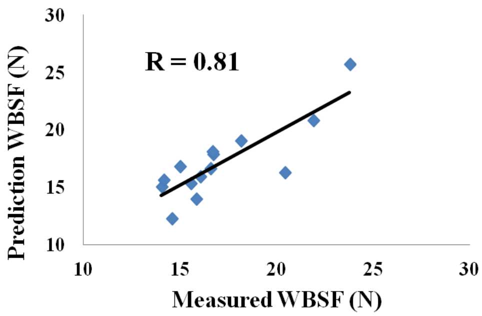
The Multiple Linear Regressions (MLR) has been used to build determination models. The effective wavebands selected by the stepwise approach were respectively set as the independent variable X for development of tenderness determination models. The correlation coefficient (*R*) and Root Mean Square Error (RMSE) of each model and the corresponding prediction results are shown in the Table 4.4. Specifically, the correlation coefficient for calibration and validation sets lies in the range of [0.84-0.92] and [0.81-0.89], respectively. This indicated that our model significantly increased the accuracy of prediction compared to the results reported in the literature, i.e. 0.67 reported in Cluff et al. (2008) and around 0.7 reported in Liu et al. (2003).

Table 4.4 Results of the tenderness prediction models

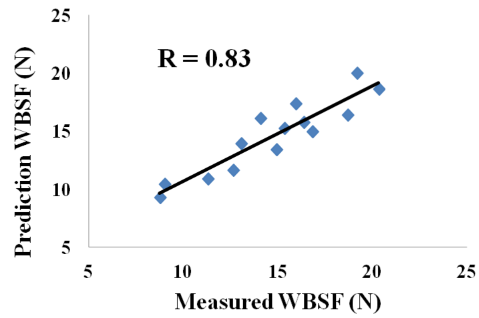
Muscle Type	R_c	$RMSE_c$	R_v	$RMSE_v$
RE	0.88	1.72	0.86	1.76
TB	0.84	1.62	0.81	1.72
TL	0.86	1.86	0.83	1.98
TS	0.92	1.85	0.89	2.2

RE: rib eye; TB: top blade; TL: tenderloin; TS: top sirloin
Note. Indexes "c" and "v" indicate calibration and validation sets, respectively.

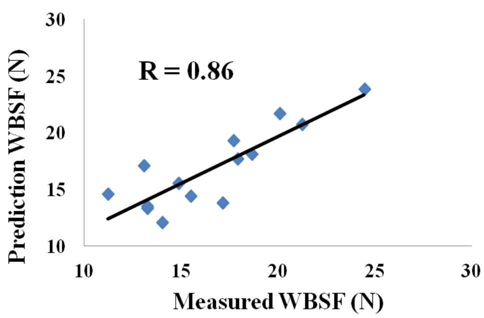
The actual vs. predicted values of the tenderness of four types of muscles are plotted in Figure 4.8. While beef tenderness prediction results for all four type muscles showed good accuracy, prediction accuracy for tenderness of Top sirloin (TB) was greater than that for other muscles. This indicates that overall hyperspectral images in the NIR range had a good explanatory power for beef tenderness. The success of non-destructive detection of beef tenderness made it possible to develop a rapid and accurate on-line system to assess beef tenderness.



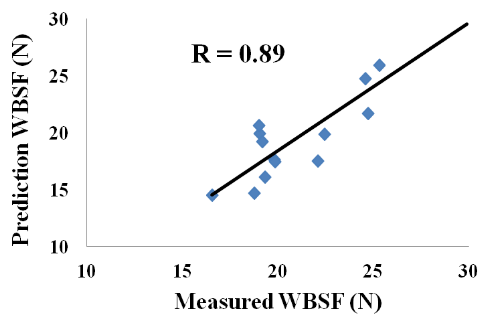
a) Top blade prediction



b) Tenderloin prediction



c) Rib eye prediction



d) Top sirloin prediction

Figure 4.8 Measured vs. predicted value of the Warner-Bratzler shear force (WBSF)

4.5 CONCLUSION

This study was conducted to assess the possibility of using NIR hyperspectral imaging of raw beef, coupled with proper image processing techniques, to predict cooked beef tenderness. To accomplish this purpose, multiple linear regression models at optimal wavelengths were constructed.

Prediction models for beef tenderness of four types of beef muscles after three different aging durations were developed from hyperspectral imaging data in the near infrared region. Prediction accuracies (*R*) were 0.89, 0.86, 0.81, 0.83 for TS, RE, TB, and TL respectively. These results confirmed the potential of hyperspectral imaging as an online, rapid and nondestructive technique for developing predictive models for beef tenderness at three different aging periods. Further work will focus on improving the predictive accuracy, building industrial instruments for objective tenderness evaluation and exploring the potential of other NIR hyperspectral imaging techniques for prediction of beef tenderness.

We believe that our study contributes to the existent literature in the field by providing a prediction method that indicates the tenderness of the beef with an acceptable accuracy. This approach provides more applicable tool to its users comparing to the other models in literature those provide only classification models in which beef can be categorized in small number of classes based on its tenderness.

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VI. SUMMARY AND GENERAL CONCLUSIONS

Previously, studies have demonstrated the promising potential of hyperspectral imaging in inspection of food quality and safety of food; however, with regard to assessing the tenderness of beef, limited research was found.

The current study shows the applicability of hyperspectral observation in the near infrared region for predicting beef tenderness in four types of muscles after three different aging periods. Objectives were met by collecting a total of 260 samples of beef in 3 months. Samples were grouped in two sets, i.e. calibration and validation sets. The calibration models developed using the wavelength region from 900-1700 nm could adequately predict beef tenderness. Multi linear regression models showed good performance in calibration and validation, with $Rc \geq 0.84$, $Rv \geq 0.81$ for tenderness. A stepwise regression approach was used to find the spectral region that was most important in the calibration model. For specific muscles, the most important wavelengths, *i.e.*, those contain valuable information allowing the prediction of beef tenderness, were found.

Prediction models were developed using multiple linear regression (MLR). Using MLR on data from a selected set of wavebands allowed us to develop calibration models which showed a high accuracy in the prediction of beef tenderness. Our results clearly show the potential of using hyperspectral observation to predict beef tenderness.

In further work, hyperspectral imaging of beef samples of more diverse muscle types and after more different aging times should be taken into account to establish a more robust and generalization tenderness determination model. This could provide incentive for optimization of the aging process for beef products.

Appendix 1

Information collected on the carcasses

Carcass number	Day of Birth	Day of Harvest	Difference (days)	Dressed weight (lbs)	Sex
1	May 12, 2011	Sept. 13, 2012	490	745	Steer
2	May 1, 2011	Sept. 13, 2012	501	669	Steer
3	April 25, 2011	Sept. 13, 2012	507	707	Steer
4	May 27, 2011	Oct 4, 2012	496	817	Steer
5	June 5, 2011	Oct 4, 2012	487	859	Steer
6	May 19, 2011	Oct 4, 2012	504	825	Steer
7	May 23, 2011	Nov 8, 2012	534	631	Heifer
8	May 21, 2011	Nov 8, 2012	536	756	Steer
9	Oct. 1, 2011	Nov 8, 2012	403	908	Heifer
10	Aug. 31, 2011	Nov 8, 2012	434	671	Steer

Appendix 2

Protocol for Collection of Beef Samples

1. Three different dry aged (0, 14, 21 days) beef tendernesses will be evaluated in this project.

2. Three, three, and four carcasses will be selected for September, October, and November, respectively. A total of 10 carcasses will be used in this project.

3. All carcasses will be from the same breed.

For Each Month

4. Randomly select 3 carcasses for study (*4 carcasses for November*).

5. Dissect 4 muscles (top blade, top sirloin, tenderloin, rib eye) from each carcass.

<u>After 24 h post-mortem</u>

6. Slice tenderloin and rib eye to ***three*** **1-inch thick slices** (as shown in Fig. 1)**,** top blade and top sirloin to ***two*** **1-inch thick slices** (as shown in Fig. 2) by a mechanical slicer. A total of 30 fresh cut slices (3 carcasses × 2 muscles × (3+2) slices) will be collected. (*For November: Muscles of the 4th carcass are cut as shown in Fig. 3. A total of 40 fresh cut slices will be collected in November*).

7. Vacuum pack the cut slices separately in polyethylene bags and label them (to differentiate carcasses, muscles, and ages).

8. Transport the vacuum packed slices in ice boxes to McGill Univ., Macdonald Campus.

9. Send the carcass information to Dr. Ngadi (or Dr. Liu), including the age of each carcass when slaughtered, the hot carcass weight of each carcass.

10. Continue dry aging the remaining muscles.

<u>After 14-day dry aging</u>

11. Slice tenderloin and rib eye to ***three*** **1-inch thick slices** (as shown in Fig. 1)**,** top blade and top sirloin to ***two*** **1-inch thick slices** (as shown in Fig. 2) by a mechanical slicer. A total of 30 14-day dry aged cut slices will be collected. (*For November: Muscles of the 4th carcass are cut as shown in Fig. 3. A total of 40 14-day dry aged cut slices will be collected in November).*

12. Repeat Steps 7-8.

13. Continue dry aging the remaining muscles.

<u>After 21-day dry aging</u>

14. Slice tenderloin and rib eye to ***three*** **1-inch thick slices** (as shown in Fig. 1)**,** top blade and top sirloin to ***two*** **1-inch thick slices** (as shown in Fig. 2) by a mechanical slicer. A total of 30 21-day dry aged cut slices will be collected. (*For November: Muscles of the 4th carcass are cut as shown in Fig. 3. A total of 40 21-day dry aged cut slices will be collected in November*).

15. Repeat Steps 8-9.

Over the Three Months

16. A total of 300 beef slices (10 carcasses × 2 muscles × (9+6) slices) will be collected from VG Meats, including 100 slices from fresh beef, 7-day dry aged beef, and 14-day dry

aged beef, respectively.

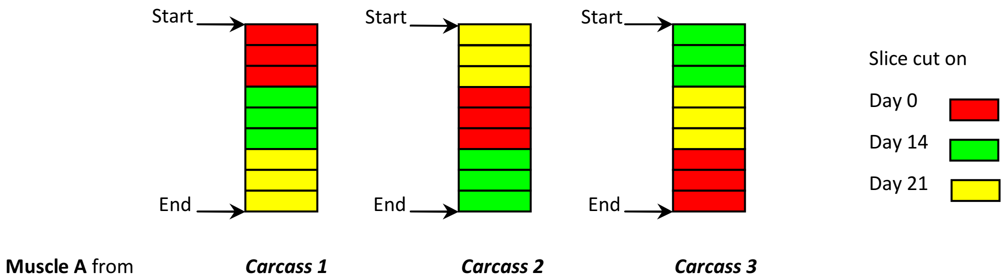

Fig.1. Illustration of cutting muscle A (tenderloin and rib eye) for each group (3 carcasses per month).

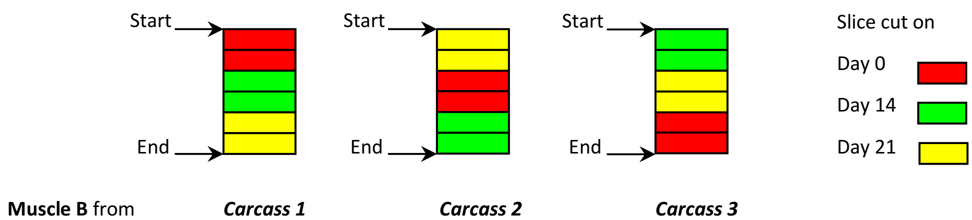

Fig.2. Illustration of cutting muscle B (top blade and top sirloin) for each group (3 carcasses per month).

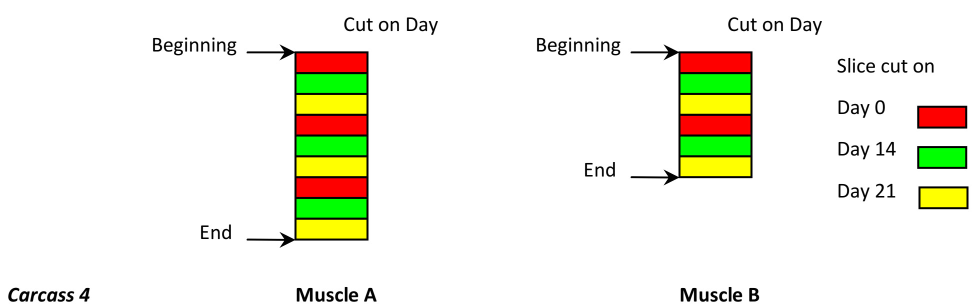

Fig.3. Illustration of cutting muscle A (tenderloin and rib eye) and muscle B (top blade and top sirloin) of the 4th carcass in Group 3 (November).