31

Addis Ababa University
(Since 1950)

EFFECT OF MUSCLE STORAGE ON BEEF OXIDATION

By

Temnet Amanuel

Advisor

Melese Abdisa (PhD)

A Thesis Submitted to Addis Ababa University College of Natural Science in Partial Fulfilment of the Requirements for the Degree of Master of Science in Food Science and Nutrition.

May, 2014

Addis Ababa

Effect of Muscle Storage on Beef Oxidation

By

Temnet Amanuel

A Thesis Submitted to Addis Ababa University College of Natural Science in Partial Fulfilment of the Requirements for the Degree of Master of Science in Food Science and Nutrition.

Examining board

Dr. Melese Abdisa (Advisor)
Dr. Abiy Zegeye (Examiner)
Dr. Gezahegn Mamo (Examiner)
Mr. Aynadis Tamene (Chairman)

3/11/2014

Declaration

I Temnet Amanuel do hereby declare that:

(a) This thesis has been written by me and that it is a record of my original research work and has not been presented for a degree in any other University.

(b) All sources of materials used for the thesis have been duly acknowledged.

Name: Temnet Amanuel

Signature: _____

Place: Addis Ababa, Ethiopia

Date of submission: _____

The thesis has been submitted for examination with my approval as supervisor

Name: Melese Abdisa (PhD)

Signature: _____

Date _____

Table of contents

List of tables

Table **Page**

List of figures

Figure page

List of symbols, notation and definition

$^{\cdot}OH$ - Hydroxyl radical

A – Absorbance

a* - Redness

ADP - Adenosine Diphosphate

ANOVA - Analysis of Variance

ANRS - Amhara National Regional State

AOAC - Association of analytical communities

ATP - Adenosine triphosphate

b* - Yellowness

BHA – Butylated hydroxyl anisole

BHT – Butylated hydroxyl toluene

C – Celcius

CIE - Commission international d'Eclairage

CLAs - Conjugated linolelc acid

CMYK - Cyan, Magenta, Yellow and Black

CO – Carbon monoxide

CVC - Computer vision system

DeoxyMb - Deoxymyoglobin

DFD - Dark, Firm and Dry

DI - Digital images

DNA - Deoxyribonucleic acid

ESR - Electron spin resonance

Fe^{2+} - Ferrous iron

Fe^{3+} - Ferric iron

FTIR - Fourier transform infrared

H_2O - Water

H_2O_2 - Hydrogen peroxide

Hb – Hemoglobin

HCl – Hydro chloric acid

HeNe - Helium Neon

HHE - 4-Hydroxy-2-hexenal

HNE - 4-Hydroxy-2-nonenal

$HO^{\cdot}$ - Hydroxyl radical

$HO_2^{\cdot}$ - Hydroperoxyl radicals

HOBr - Hypobromous acid

HOCl - Hypochlorous acid

IF – Infraspinatus

JPEG - Joint Photographic Experts Group

L* - Lightness

LD – Longissimus dorsi

LDL - Low-density lipoprotein

LP - Lipid peroxidation

MAP – Modified atmospheric packaging

Mb – Myoglobin

MDA - Malondialdehyde

MetMb – Metmyoglobin

MFD - Meat flavor deterioration

NaOH – Sodium hydroxide

NO - Nitric oxide

O_2 – Oxygen

$O_2^{\cdot-}$ - Superoxide anion

$ONOO^-$ - Peroxynitrite

OSI - Oxidative Stability Instrument

OxyMb – Oxymyoglobin

p-AnV - P-anisidine value

ppm – parts per million

PSE - Pale, Soft and Exudative

PUFA – Polyunsaturated fatty acids

Q.F. - Quadriceps femoris

R – Rhomboideus

RGB - Red, Green and Blue

RNA - Ribonucleic acid

RNS - Reactive nitrogen species

$RO^{\cdot}$ - Alkoxyl radical

$RO_2^{\cdot}$ - Peroxyl radical

ROOH - lipid hydroperoxide

ROS - Reactive oxygen species

TBA – Thiobarbituric acid

TBARS - Thiobarbituric acid reactive substances

TBHQ - Tert-butyl hydroquinone

TCA - Trichloroacetic acid

TCA cycle - Tricarboxylic acid cycle

UV-Vis - Ultraviolet–visible spectroscopy

WBSF – Warner bratzler shear force

WOF – Warmed over flavour

ACKNOWLEDGMENTS

First and foremost I want to thank God for bringing me to this point in my life. Without my trust in Him, I would have never made it here considering all that I have dealt with for the last three years. So to God be glory.

I extend my deepest gratitude to Dr. Melese Abdisa for serving as my Academic Adviser and for all of his guidance and direction over the past three years. I have had the opportunity to learn and grow from the experiences I have been through.

I wish to express my sincerest appreciation to my examiners Dr. Abiy Zegeye and Dr. Gezahegn Mamo. Especially, I acknowledge my heartfelt and special thanks to Dr. Abiy Zegeye for his competent guidance, constructive criticism and patience.

I extend my special thanks for my family, which I could not have done this without their love and support. Especially my parents my mom Tsehai, thank you for your patience and encouragement needed through this endeavour. Thanks to my dad, Amanuel and my brothers Hago and Eyu for their complete support, guidance, faith and love. You guys have been my strongest supporting foundation and my biggest fans. I am forever indebted to you for all you have done for me.

I also owe great gratitude for all Food Science and Nutrition Laboratory staff, Mrs. Woyneshet Abera, Mr. Muluneh Kefelae, Mr. Debebe Hailu for all they have done for me. You are wonderful friends and incredible asset to our Center, and I feel that I can speak for all when I say

we would be lost without your technical and moral support and resourceful assistance during the entire laboratory analysis.

Thanks to my fellow graduate students, past and present. We were brought together because of a common interest for food science, but we leave here as great friends. Thanks to everyone who helped along the way. Life is about team work. Through these activities, I have learned a great deal about team work and friendship. I would be nowhere if not for all of the wonderful people around me.

I would like to thank AAU Physics Department, thank you for the hours of time and work you spent helping me complete this project, to the Ethiopian Health and Nutrition Research Institute (EHNRI) for allowing me to carry out the research in the laboratory of the Institute and to Ashraf plc. for all that they have done in helping me prepare and complete this project.

Finally, but definitely not least, I would like to thank the Center for Food Science and Nutrition of Addis Ababa University staff and my teachers Mr. Tilahun Bekele, Dr. Ashagrie Zewdu, Mr. Aynadis Tamene, Dr. Kaleab Baye and Mr. Dawood Gashu for all rounded assistance and support they have given me.

Abstract

Lipid oxidation in meat and meat products is the major biochemical process that occurs during storage and cooking of beef leading to quality deterioration: undesirable odour and flavour called rancidity. Rancidity may give rise to toxic compounds like malondialdehyde. The objective of this work was to determine the effect of muscle storage for different duration on quality and oxidation of four beef muscles (*Quadriceps femoris*, *Longissimus dorsi, Infraspinatus* and *Rhomboideus)*. Beef muscle samples were vacuum packaged in polyethylene bag and stored at sub zero (4 °C) temperature for day 0, 7, 14 and 21 before analysis were made for different parameters of study. Measured responses were determination of thiobarbituric acid reactive substance (TBARS), total pigment, heme iron (mg/kg), concentration of metmyoglobin, L*a*b* colour values, pH value, proximate composition and sensory evaluation. The TBARS mean values showed an increasing trend with increased storage time, while total pigment and heme iron decreased. The formation of metmyoglobin increased with increased storage time. The color parameters (L*, a*, b*) showed a decrease with increasing storage time regardless of muscle type. The pH values tend to increase with increasing storage time. Muscle storage types significantly affected mean proximate values. Sensory analyses revealed deterioration in flavour, color, aroma, texture and overall acceptance. It was concluded that muscle storage time affects meat.

1. Introduction

Meat (carcass) can be defined as all animal tissues suitable for consumption. Commonly meat comes from domesticated farm and aquatic animals. Meat provides high quality protein (essential amino acids), vitamins and minerals such as iron and zinc. Meat is not only a rich source of iron, but it provides the most bioavailable form of iron (hem-iron) and also enhances non-heme iron absorption especially from cereal and legume-based meals. As these nutrients are required for proper growth and maintenance, they make meat highly nutritious food for consumption. Meat is also composed of water, fat, and other water-soluble organic material. Meat fat is important in human nutrition with n-3 polyunsaturated fatty acids (PUFA) and conjugated linolelc acid (CLAs). Rich nutrient matrix meat is the first-choice of animal protein for many people all over the world (Ajiboye, *et al.* 2011; Dave and Ghaly, 2011).

Beef carcass is structured from more than a hundred different muscles which have different properties (Klont, *et al.* 1998). The difference will result in various outcomes in processing characteristics and consumer acceptability. For instance the contact between different muscle types and locations with oxygen could contribute to meat oxidation and/or discoloration, which accounts for about half of the color changes in meat. It also causes undesirable sensory characteristics like off-flavor and may also lead to the formation of toxic compounds. The aroma changes resulting from the formation of new volatile odorous compounds formed, during prolonged storage time. Furthermore, flavor modifications and color darkness is due to hydroxyl acids and condensation reaction between oxidation products and proteins, respectively. A new texture might also be attributed to the oxidative induction of protein crosslinks. Finally, nutritive value and safety of the meat will be impaired (Kanner and Rosenthal, 1992).

Well balanced and good nutrition is getting more attention as a result of poor diet and sedentary life style all over the world. It has shown that meat is considered as a nutritious food because of its varied and dense nutrient composition. In order to best qualify for consumption, besides its nutritive value, meat needs to have attractive sensory properties. In public health point of view, meat industries are trying to reduce the amount of fat contained in the meat to make it healthier for consumption, which also address lipid oxidation of meat during storage. However, lipid oxidation will not be minimized by reducing the fat content of the meat alone; the type of fat and processing method will also dictate the extent of quality and safety deterioration resulted due to lipid oxidation (Cascone, 2006). Furthermore, species/breed, age, sex, cut of meat, surface drying of the meat and surface spoilage can cause change in the color and auto-oxidation of meat.

The color and appearance of the meat are the major indicators of quality and freshness, which dictates consumer's outlook or satisfaction, hence the purchasing decision. Meat color is largely determined by the content of myoglobin and its derivative. Prolonged exposure of meat to air can show color and flavour change on the meat surface due to the reaction between myoglobin and oxygen, and reaction of free radicals with oxygen (Cascone, 2006).

In Ethiopia slaughter stocks are presented for slaughter for formal or informal slaughter. After slaughter, carcass is loaded to vehicle and handed over to butchery for retailing. Retail display of carcass under incandescent light in an open butchery room could make it susceptible to oxidation by light, temperature and microorganisms. As mentioned by (Rowe, *et al.* 2004) beef is the vulnerable type of meat for lipid oxidation. Amount of endogenous catalysts such as myoglobin and free ionic iron, reducing compounds, antioxidants (for example, carnosine and related dipeptides), and catalase are the basic conditions that control the rate of lipid oxidation in meat.

Knowledge about carcass musculature is important especially in the area of meat marketing and restaurant businesses to meet consumer demand at most. In fact, lipid oxidation is causing health risk on human like the formation of atherogenetic agents, which considered as having the properties of mutagenic and carcinogenic properties (Karwowska and Dolatowski, 2007).

As a result, this study investigated the effect of muscle location on oxidation of meat during storage. Longissimus dorsi, Infraspinatus, Quadriceps femoris and Rhomboidus muscles of beef located at different quarters were used in the study. They were hypothesised to have no differences in heme pigment and lipid content, and could have differences in oxidation.

1.1. Statement of problem

Despite the fact that beef is nutritious, it could be prone to spoilage due to handling of the carcass on shelf consequent to oxidation. Thus, oxidation causes nutritional deterioration of the meat as well as formation of undesirable sensory characteristics and toxins. On the other hand, beef producers need to add value while consumers are interested to buy quality meat. Therefore, it was imperative to identify sources of beef quality deterioration and the extent to which these quality compromising factors affect quality. Such investigation requires envision of beef into its different location in order to optimise retail conditions of beef muscles so as to curtail the problem of oxidation and add value. Also it is reasonable to assume that muscles have differences in their biological makeup because of this they need to be treated on an individual basis when fabricating and merchandising individual retail cuts or portions from muscles of the chuck and round (Bratcher, 2004).

1.2. Significance of the study

The findings from this study contribute to the better understanding of different muscle cuts from local breeds and there-by contribute towards the reduction of beef oxidation. In doing so it helps in extending the duration of healthy stay of the beef on shelf, thus enables value addition. Further understanding of interactions among retail processing, inherent characteristics of beef and consumer interest could provide platform for further study.

1.3. Objectives

Therefore, this study generally assessed the impact of beef muscle storage on retail processing with the following specific objectives.

Specific objectives

1. To study impact of muscle storage on beef quality
2. To determine conditions that might assist in lipid oxidation of muscle food
3. To optimize the causes of meat discoloration and degradation for consumer acceptability.

1. Literature review

1.1. Different types of muscle locations in beef

Muscle type is the major factor controlling the rate of discolouration of beef muscle on exposure to oxygen, accounting for almost half of the variances in colour stability. According to research findings oxidation rate of muscle longissimus was found to be very stable than most of muscles in beef (Klont, *et al.* 1998).

Beef muscle in a carcase can be divided in to 9 prime cuts (Fig 1.); these are chuck, brisket, shank, rib, plate, flank, sirloin, short loin and round (hip). Muscle types are different in their toughness as the result of fiber types, connective tissue, etc. (Dinh, 2008).

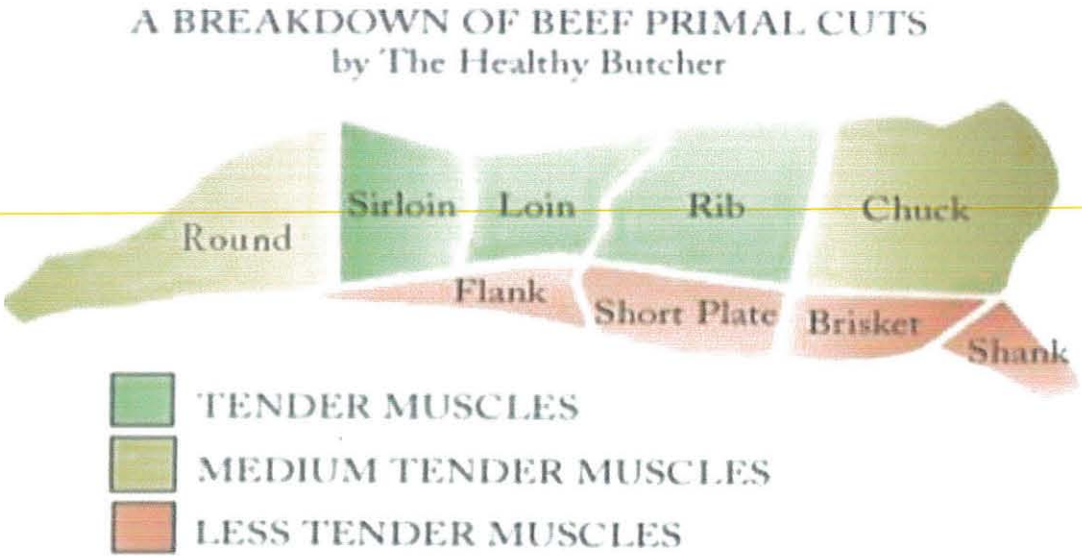


Figure 1. Anatomical locations of different beef cuts (Wadhwani, 2008)

Different cuts of meat have different shelf-life, which is influenced by intrinsic biochemical nature of different muscle types (AMSA, 2012).

2.2. General Information on Beef Chuck Muscles

In beef chuck or shoulder there are about thirty muscles. Infraspinatus, longissimus dorsi and rhomboiues are the three chuck muscles that were tested in this study. The anatomical locations of muscle infraspinatus and rhomboideus are shown in fig. 2. Whereas portion of muscle longissimus dorsi presented in fig. 3. Muscles located on chuck have intermediate toughness when compared to the other parts of beef cuts (Wadhwani, 2008).

I. **Infraspinatus**– it is a large muscle which almost fills the infraspinatus fossa ventral to the scapular spine (Wadhwani, 2008). Infraspinatus is found under the thoracic group and located at the scapular cartilage. Its functions include abduction of the arm and rotating it out-wards. According to (Jones, *et al.* 2007) it also acts as a lateral ligament. The general movement of cattle involves not extending their front limb to any great extent as a result the infraspinatus muscle is not used extensively in locomotion of cattle, hence the front limbs mostly move in a forward/backward movement (Searls, *et al.* 2005).

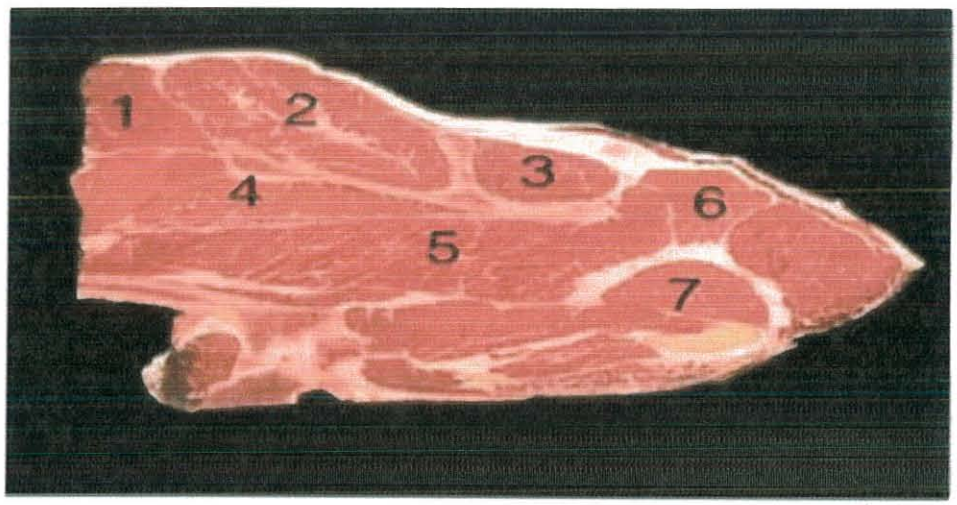


Figure 2. Beef chuck localization within the chuck; cross-section at the 2nd rib (Wadhwani, 2008)
Key

1 = Triceps brachii 3 = Supraspinatus 5 = Serratus ventralis 7 = Complexus
2 = Infraspinatus 4 = Subscapularis 6 = Rhomboideus

II. **Longissimus dorsi** – also known as longissimus thoracis et lumborum by its full name. It is along muscle which forms the large round eye of meat in rib and loin steaks or chops. It is also defined as a muscle lying ventrally to the transverse processes of the vertebra (Wadhwani, 2008).

The muscle originates at the last cervical and the first thoracic vertebrae. It lies bilateral to the dorsal spinous processes and dorsal to the ribs and transverse spinous processes and it inserts onto the ribs, the transverse and mammillary processes of the thoracolumbar spine as well as to the tuber, the crest and the ventral surface of the ilium and the first three sacral vertebrae (Scheven, 2010).

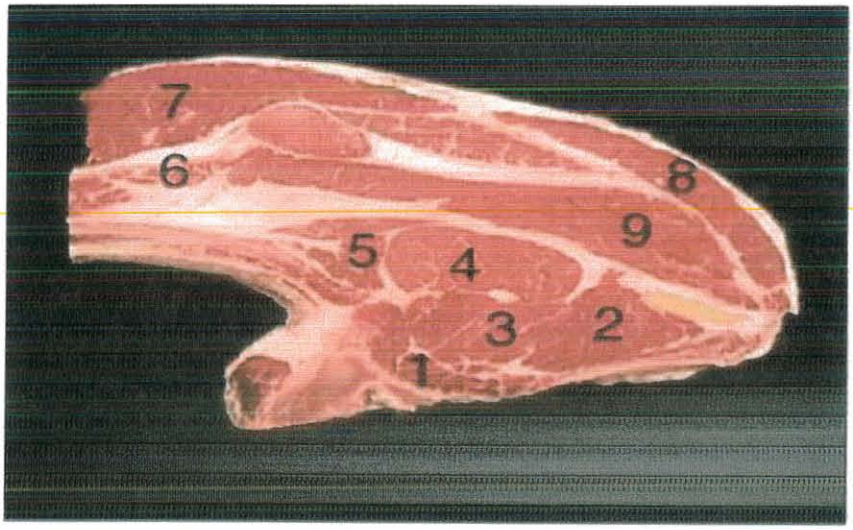


Figure 3. Beef chuck localization within the chuck; cross-section at the 5th rib (Wadhwani, 2008)

Key

1 = Multifidus dorsi	4 = Longissimus dorsi	7 = Latissimus dorsi
2 = Spinalis dorsi	5 = Longissimus costarum	8 = Trapezius
3 = Complexus	6 = Serratus ventralis	9 = Rhomboideus

III. **Rhomboideus**– It is ventral to trapezius. The trapezius is located superficially between the left and right scapular blades (Wadhwani, 2008). Rhomboideus arises on the ligamentum nuchae from the second cervical thru the eighth thoracic vertebrae. Its function includes moving the scapula forward and upward (Jones, *et al.* 2007).

2.3. General Information on Beef Round Muscles

Adductor, biceps femoris, quadriceps femoris, gastrocnemius and shank muscles are the major muscle types which are present in the round of beef (Kirchofer, *et al.* 2002).

I. **Quadriceps femoris** - is one of the major muscles in beef round; it forms the main muscle bulk on the cranial aspect of the thigh, but is covered by the tensor muscle of the fascia lata, the sartorius and the medial femoral fascia. Quadriceps femoris is a large muscle which is composed of four muscles known as vastus intermedius, vastus lateralis, vastus medialis and rectus femoris (Fig. 4) (Budras, *et al.* 2011).

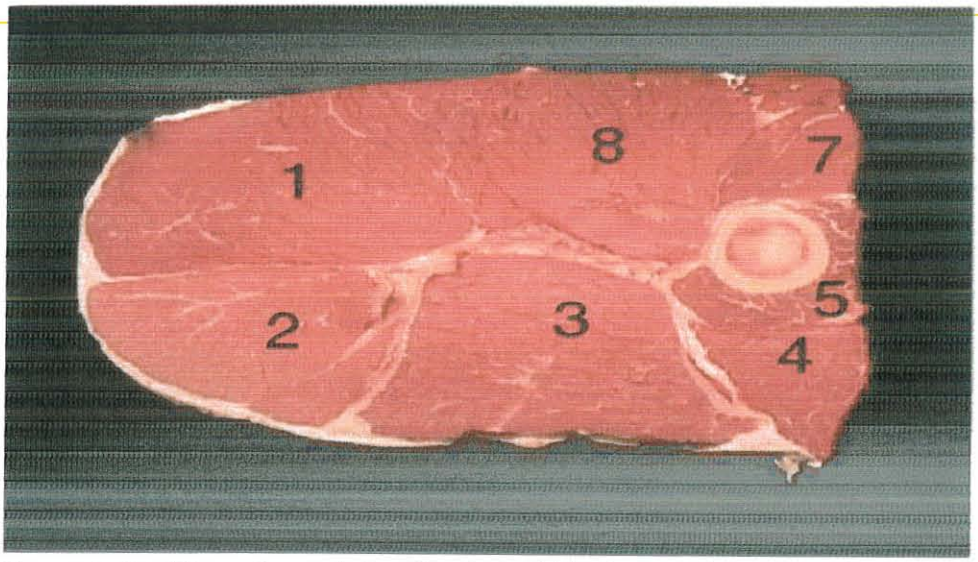


Figure 4. Beef Round muscle localization within the round, cross section (Wadhwani, 2008)

Key

1 = Semimembranosus	3 = Biceps femoris	5 = Biceps femoris	7 = Vastus medialis
2 = Semitendinosus	4 = Vastus lateralis	6 = Rectus femoris (not shown)	8 = Adductor

Rectus femoris originated just at cranial to the acetabulum of the ilium, which also crosses the hip joint. Due to this rectus femoris can flex the hip joint. The other three heads (vastus lateralis, vastus intermedius and vastus medialis) originated on the femur without crossing the hip joint, so they are only involved in the act to extend the stifle joint (Fig. 5) (Budras, *et al.* 2011).

The main role of quadriceps femoris is that, it's the main extensors of the stifle and the straight muscle; in addition it also flexes the hip. In general it is involved in almost all activities involving the leg, like standing and walking (Jones, *et al.* 2007).

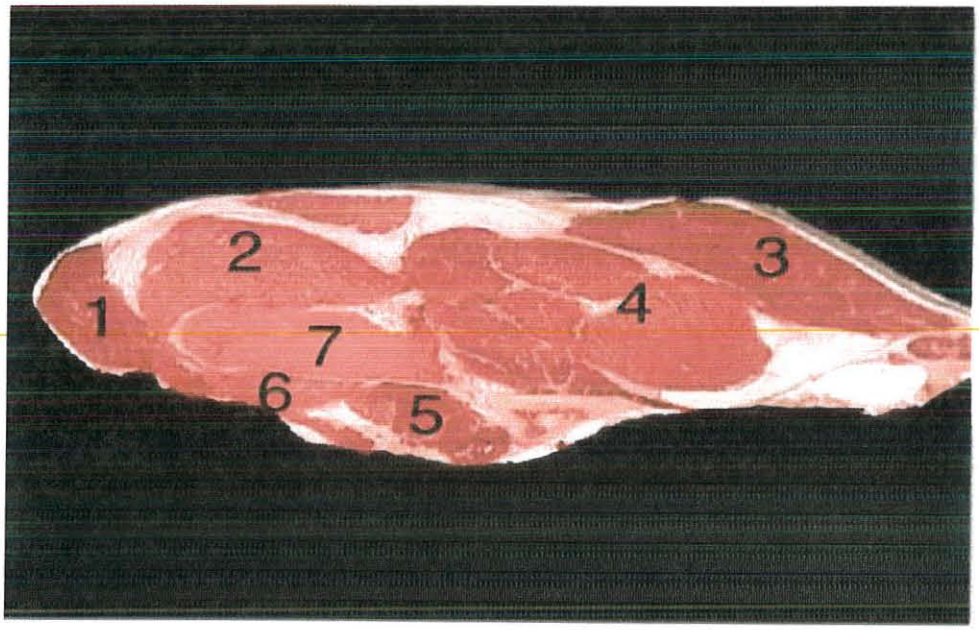


Figure 5. **Beef Round muscle localization within the round, cross section (continues) with rectus femoris shown (Wadhwani, 2008)**

Key

1 = Semimembranosus	3 = Bicepsfemoris	5 = Vastus intermedius	7 = Vastus medialis
2 = Semitendinosus	4 = Vastus lateralis	6 = Rectus femoris	8 = Adductor

2.4. Conversion of Muscle to Meat

When an animal dies, a complex process of metabolic, physical and structural alterations occur to muscles; that result in the conversation of muscle to meat. This conversion doesn't happen suddenly, this delay can take from several hours to days to terminate all living thing functions and become meat. The first change is the exhaustion of available energy. When the muscle tissue tries to keep homeostasis (maintaining systems of the body for life to maintain a balanced internal environment) it will attempt to stay on producing energy the form of ATP. First muscle will use creatine phosphate used up for the rephosphorylation of ADP to ATP. When the creatine stores are run-out, the catabolism of glycogen provides energy through the glycolytic metabolic pathway (Rathgeber, 1999; Anderson, 2011).

When aerobic metabolism through TCA cycle and electron transport chain starts to decline, then circumstances start to favour the production of lactic acid. When blood circulation stops, it makes sure that there are no means of oxygen and energy delivered to the muscles, this brings the change from aerobic metabolism (TCA cycle and electron transport chain) to anaerobic metabolism (lactic acid fermentation) (Anderson, 2011). In general in the process involved in the conversion of muscle to meat, muscles use glycogen to produce ATP and lactic acid is produced (Raines, 2008).

In the process of anaerobic metabolism, pyruvate is produced by glycolysis and changed to lactic acid. In the condition of living muscle tissue, lactic acid plays role in slowing of acidosis, muscle fatigue and of a substrate for metabolism by the mitochondria. But in postmortem muscle tissue, mitochondria become functional for a limited period of time because of its vulnerability to degradation by caspases. With the absence of blood circulation, the role of mitochondria and removal of lactic acid presented by the decline of pH to approximately 5.4-5.8 occurs in the muscle tissue. This change also goes with the rise in ionic strength and the failure to keep

reducing conditions within the muscle. These changes end up producing many effects on the proteins of the muscle tissue. Some of the changes include a decline in pH, shortening of the sarcomere, and a release of Ca^{2+} from the sarcoplasmic reticulum, cause a toughening and subsequent tenderization of postmortem muscle tissue muscle (Rathgeber, 1999; Anderson, 2011).

2.5. Rigor mortis

Immediately after death, the muscle is soft, limp, and dry and can be reversibly extended by using low load (5-15kPa). Cadaveric rigidity (rigor mortis) occurs after a few hours. The onset of rigor depends on the species type and on the types of muscle. For instance the speed of rigor development in beef is 10-24 hours, in pork 4 – 18 hour and in chicken 2 – 4 hours. Cessation of blood circulation ends the O_2 supply to muscle. The continual of metabolism in anaerobic environment causes the beginning of biochemical and structural changes in the muscle tissue. These changes cause rigor mortis (rigidity of muscles) (Dransfield and Sosnicki 1999; Dave and Ghaly, 2011).

Rigor mortis depends on ATP deficiency and also on the internal and external conditions. For instance at the time of slaughter, if the animal used up the energy reserve (ATP and glycogen) rigor happens faster, than animal which was resting at the time of slaughter (Behrends 2004; Pethick, *et al.* 2005). Pre and post slaughter practices bring an effect on quality of meat which affects the retail experience.

2.6. Retail management and color of beef

The purchasing decisions of customers can be different when they come to buy meat, one of the major reason can be quality perception. Quality can be perceived mainly by color. For customers the bright red color of meat serves as a sign for freshness, wholesomeness, and good eating quality (Min and Ahn, 2005; Steele, *et al.* 2012).

The major sensory quality characteristics appearance/color, texture and flavor are the main quality attributes that affect consumer acceptance of meat and lipid peroxidation is the primary cause of these quality deteriorations in meat and meat products. The shelf life and quality of meat products are strongly influenced by the initial meat quality, additives, packaging parameters, and storage conditions. If these conditions are not practiced properly they end up having a negative impact on the quality of meat (Min and Ahn, 2005).

The process of lipid peroxidation starts immediately after slaughtering, and during certainly post-slaughtering events. The conversion of muscle to meat has biochemical changes like post mortem aging cause the destruction of the balance between prooxidant and antioxidant factors. The rate and extent of lipid peroxidation in muscle tissues appears to be dependent on degree of muscle tissue damages during pre-slaughtering events such as stress and physical damage and post-slaughtering events such as early post mortem, pH, carcass temperature, shortening, and tenderizing techniques such as electrical stimulation. Moreover, different processing factors manipulate the amount of lipid peroxidation in meat; composition of raw meat, aging time, cooking or heating, size reduction processes such as grinding, flaking, and emulsification, deboning, especially mechanical deboning, additives such as salt, nitrite, spices, and antioxidants, temperature abuse during handling and distribution, oxygen availability, and prolonged storage (Min and Ahn, 2005).

2.7. Causes of meat spoilage

Pre-slaughter and post-slaughter handling of animal and meat plays an important role in meat quality. The amount of stress to the animal pre-slaughter decreases the level of glycogen this will change the pH of meat either to higher or lower levels depending on the production of lactic acid. The breaking down of glycogen in the animal body leads to the production of lactic acid through

anaerobic glycolytic pathway. pH levels between 6.4-6.8 brought Dark, Firm and Dry (DFD) meat. A long term stress causes DFD and a short term stress results in a Pale, Soft and Exudative (PSE). PSE meat has a pH of 6.2 which is lower than normal this result in breakdown of protein which provides an optimum condition for the growth of bacteria, the texture and color of the DFD, PSE and normal meat (Dave and Ghaly, 2011).

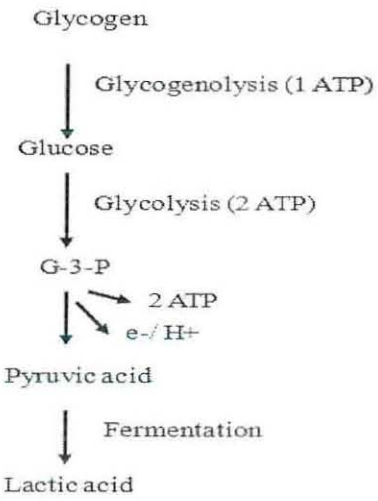


Figure 6. Anaerobic glycolytic pathway (Dave and Ghaly, 2011)

The four main mechanisms of meat and meat spoilage after slaughter and during processing and storage are

- Microbial spoilage
- Protein oxidation
- Lipid oxidation and
- Autolytic enzymatic spoilage (Dave and Ghaly, 2011)

2.8. pH and color change of meat

The level of pH in meat is very important in processing, because it can have influence on appearance/color, texture/tenderness and flavour (Ke, *et al.* 2009; Istrati, *et al.* 2011). Many factors which occur ante and postmortem happened as a result of change in the meat pH (Raines, 2008).

pH value around 7.0 (6.6-7.5) favors the optimum pH value for growth of microorganisms. Few microorganisms' grow below pH 4.0. Meat and seafood have final ultimate pH above 5.6 and above, which make these products susceptible for bacterial, mold and yeast spoilage (Anderson, 2011).

pH of muscle depends on animal diet and stress, which affects the glycogen content present at slaughter time. If the glycogen amount exhausted before slaughter, decrease in pH will slow down and higher than normal ultimate pH will arise, glycogen is a specific kind of carbohydrate which serves as source of energy for living animal. This has direct relation with the amount of final pH attained upon completion of rigor mortis (Magoro, 2007). The final pH of beef which is gotten after completion of rigor mortis will be around 4.8-7.2 depending on the glycolytic potential at the time of slaughter though the normal range of pH is from 5.4 to 6.0 (Quasem, *et al.* 2009).

The pH and color of meat are well related. Dark Firm Dry (DFD) and Pale Soft Exudative (PSE) meats are two conditions of meat related with the amount of pH in thc animal muscle (Magoro, 2007).

2.8.1. Dark Firm Dry (DFD)

DFD occurs when the animal is exposed to chronic or long term stress before slaughter, like transportation of animal for a long distance, long hours of fasting and overcrowding of the animals for a long time. DFD beef is the result of long term stress before slaughter which leads to reduction of stored glycogen, hence less glycogen is available post-mortem disturbing the normal process of acidification and causing the pH of meat high. DFD is defined as meat which has ultimate pH of ≥ 6 after 12- 48 hours of postmortem (Adzitey and Nurul, 2011).

In DFD where meat have higher amount of pH value proteins will be able to bind more strongly with water then allowing less free water. When there is a combination of water with protein, the muscle fibers will get swollen this will leave less space between muscle fibers. As a result meat that has higher amount of pH value is dry and will have darker appearance hence there is less free water to reflect light. In addition to this at higher muscle pH, enzymes that use oxygen are more active, which causes less oxygenation of the surface myoglobin and a darker color (Page, *et al.* 2001; Patten, *et al.* 2008).

DFD is one of the reasons for consumer rejection because customers relate DFD meat with old animal, spoilage, undesirable flavor, toughness and poor shelf life (Miller, 2007).

2.8.2. Pale Soft Exudative (PSE)

The opposite of DFD meat is PSE meat. This condition happens to animals which were exposed to acute stress before undergoing slaughter. Some conditions which cause short term stress to animals include use of electric goads, fighting among animal just before sticking, beating of animals prior to slaughter and overcrowding in the animals. PSE is defined as meat which has pH < 6 after 45 minutes of postmortem. In PSE the pH of the meat falls very rapidly while the carcass is still warm. The combination of high temperature with lower pH results in denaturation of some muscle proteins which cause a decrease in water holding capacity. PSE meat is characterized by its pale color, soft mushy texture and a very wet surface. It has a low water binding properties and loses weight (water) rapidly during cooking (Miller, 2007; Adzitey and Nurul, 2011).

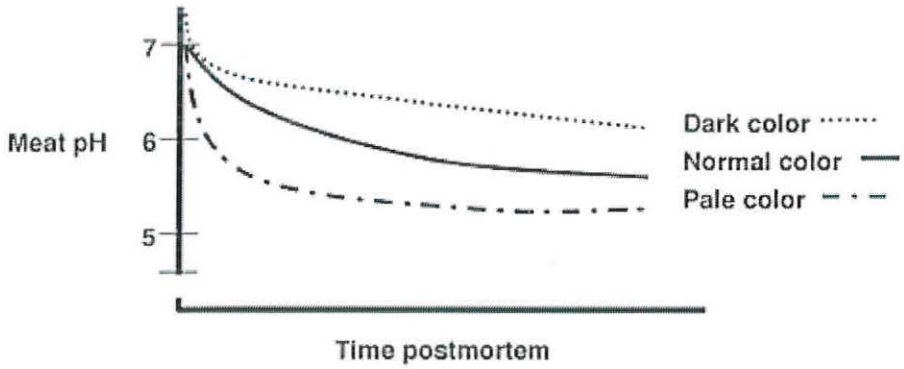


Figure 7. Post-mortem pH decline patter and associated with meat (Raines, 2008)

2.9. Meat surface color

Connective tissue, structure and intramuscular fat gave meat its optically non- homogeneous surface. Intramuscular fat got color which is different from the lean part of meat. In general, the color of the lean part in meat considered as the 'color of the meat'. Only when the matrix has a homogenous surface then the measured area can be considered as the color of the entire surface but this is not always the case with meat (Wulf and Wise, 1999).

Color of meat is one of the most important organoleptic characteristics. The customers' decision to purchase meat mostly depends on the color of meat because they tend to relate the color of meat with flavor, tenderness, safety, storage time, nutritional value and satisfaction level. Color is subjective psycho-physical property which exists in between the observer's eyes and brain, meaning it is not a characteristic proper to the object under observation, it is important to find out parameters in order to measure, classify and reproduce it. There are many ways of objective measurments of color of food, some of them are done on the extraction of pigment concentreation. But this extraction methods are time consuming and tedious. To overcome this problem various methods have been invented. For inistance, there are methods which measure

the light reflected from the food surface. It has been found that these scattering values are related to sensory scores (Nollet and Toldrá, 2010).

2.10. Different muscle locations and their color differences

Different muscle locations have different oxygen demand based on their functions. The various colors between different muscle types occur because of variation in oxygen consumption rate and myoglobin autoxidation rate in each muscle type (Klont, *et al.* 1998). Hence the concentration of myoglobin differs from one muscle type to the other. In addition to this when animal gets older the myoglobin concentration also tend to increase. More intense color concentration is found in areas where there is high concentration of myoglobin. Myoglobin concentration also differs in between species for example beef has more myoglobin than pork or lamb, which results in more intense color (Boles and Pegg, 2005).

2.11. Myoglobin

Myoglobin is a globular heme protein found in the muscle of meat-producing animals. It is made up of a single polypeptide chain, globin, consisting of 153 amino acids and a prosthetic heme group, an iron (II) protoporphyrin-IX complex. The heme group gives myoglobin and its derivatives their distinctive color. It has been known to be a major contributor to the color of muscle, depending upon its redox state and concentration. Myoglobin concentration is affected by both genetics and environment. The heme is attached to the protein by a histidine amino acid and the 5^{th} bond from iron. The 6^{th} bond is relatively free to bind oxygen, nitric oxide, carbon monoxide or other compounds that affect color. A second histidine on the protein chain on the other side of the heme is important to stability of fresh meat color. The color of this pigment can be changed by the bound atoms or compounds to it (AMSA, 2012).

Mb (myoglobin) is a monomeric heme protein with a heme prosthetic group and a globin (protein) moiety. The globin chain consists of eight helical segments forming a coiled structure

enwrapping the heme, and the ability of Mb to bind oxygen is due to the presence of heme located within the heme crevice. The globin chain confers water solubility to the heme group and protects the heme iron from external environments/oxidation so that the protein can maintain its functionality. The resonant nature of the conjugated double bonds in the heme group is responsible for the ability of Mb to absorb visible light and thus serve its function as a pigment (Baron and Andersen, 2002).

The heme group contains an iron atom that can exist in a reduced (ferrous/Fe^{2+}) or oxidized (ferric/Fe^{3+}) form. The iron can accept six electrons in its outer orbit and can thus form six coordinate bonds. Four of the bonds are with pyrrole groups of the heme porphyrin ring and one is with proximal histidine (position 93 in the globin chain), which connects heme to the globin chain. Additionally, another histidine residue (distal histidine at position 64 in the globin chain) is in the vicinity of the heme but not bonded with the heme. The sixth position of heme iron is available for binding with oxygen or other small ligands, such as carbon monoxide (CO) or nitric oxide (NO). The spacial arrangement of distal histidine and heme limits the size of the ligands occupying the sixth coordinate in native Mb and protects the heme by preventing its interactions with large biomolecules (Suman and Joseph, 2013).

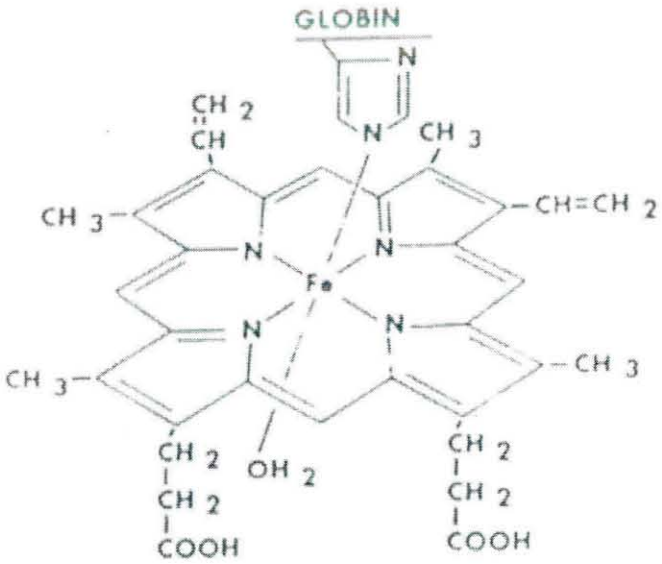


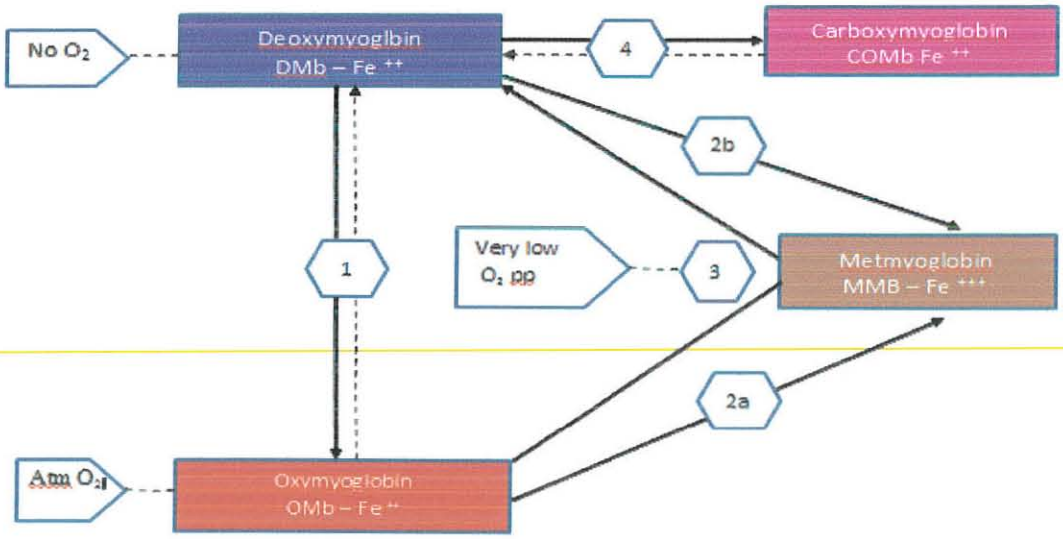
Figure 8. Chemical structure of myoglobin (Chaijan, 2008)

The function of myoglobin in live muscle and meat is different in a living animal it functions as the oxygen binder and delivers oxygen to the mitochondria, enabling the tissue to maintain its physiological functions. In meat the main purpose of myoglobin is to serve as the major pigment responsible for the red color. Although bleeding of food animals at harvest removes the majority of blood, some residual blood is trapped inside the arteries and veins within the large skeletal muscles, resulting in the presence of hemoglobin in meat. Hemoglobin and cytochrome pigments also contribute for the color of meat but in a of meat color but only in smaller amount (Chaijan, 2008; Suman and Joseph, 2013).

2.11.1. Myoglobin oxidation

The greater amount of myoglobin in the muscle the intense the color of meat. There are three states of myoglobin: oxymyoglobin (OxyMb), metmyoglobin (MetMb) and deoxymyoglobin (DeoxMb). When we cut in to muscle we expose the myoglobin to oxygen. The reaction of the meat with oxygen takes about an hour and the meat becomes bright red or known as "bloom colour". Then the myoglobin becomes oxymyoglobin, the color which is associated with fresh

meat. When a meat stored under refrigerator for several days the oxymyoglobin becomes metmyoglobin. Metmyoglobin is the brownish color of discolored meats, it comes from the oxidation of the iron portion of myoglobin and it's unattractive to customers. Appearance of metamyoglobin in meat which is on retail can be because of prolonged exposure to ultraviolet light from florecent light, fluctuation of temperature in retail and microbial growth. Deoxymyoglobin is the myoglobin which is bound to water (or with oxygen removed), is a purplish in color, and is commonly seen in vacuum-packaged meat. Deoxymyoglobin can be change in to oxymyoglobin in the presence of oxygen (Carpenter, *et al.* 2001).



R× 1 (oxygenation): DMb + O_2 → OMb

R× 2s (oxidation): OMb + [oxygen consumption or low O_2 partial pressure] - e' → MMb

R× 2b (oxidation): [DMb – hydroxyl ion – Hydrogen ion complex] + O_2 → MMb + O_2^-

R× 3 (Reduction): MMb + Oxygen consumption + metmyoglobin reducing activity → DMb

R× 4 (CarboxyMb): DMb + carbon monoxide → COMb

Figure 9. Visible myoglobin redox interconversions on the surface of meat (Mancini and Hunt, 2005)

Oxidation of central iron atom within the heme group is the reason for the discoloration, which causes change from red OxyMb to brownish MetMb. When ferrous heme iron oxidizes to its

ferric form, oxygen is released and replaced by a water molecule (Behrends, 2004). The amount of discoloration in meat is dependent on the muscle types. Muscles that contain greater relative proportions of red fibers, thus more lipid and greater oxygen consumption rates, appear to discolor more quickly (Chaijan, 2008).

Temperature, pH, MetMb reducing activity, partial oxygen pressure and lipid oxidation are factors which affect OxyMb oxidation. Higher temperature, lower pH values and the presence of non-heme iron support the oxidation of OxyMb. MetMb reducing activity can be enzymically or non-enzymically based and favors maintenance of ferrous forms of myoglobin in meat. Partial oxygen pressures (pO_2) in which a complete vacuum exists or in which oxygen saturation is attained favor ferrous myoglobin forms. Low non-zer pO_2 favors MetMb formation. Studies show that lipid oxidation and OxyMb have correlation with each other (Faustman, *et al.* 2010).

2.11.2. Myoglobin-initiated lipid oxidation

Lipid oxidation in meat is assumed to be non enzymatic and that myoglobin and heme compounds are the major prooxidant in muscle food. It has been shown that H_2O_2 acting as an oxidizing agent caused changes in the oxidation state of the iron in myoglobin, and induced the formation of red-brown color. The interaction of H_2O_2 with metmyoglobin led very rapidly to generation of an active species, which could initiate lipid peroxidation (Chaijan, 2008).

All of the above changes result in formation of free radicals and oxidation of the iron part in the myoglobin. Various muscle types build up metamyoglobin at different rate as a result different muscle cuts exhibit uneven discolorations (AMSA, 2012).

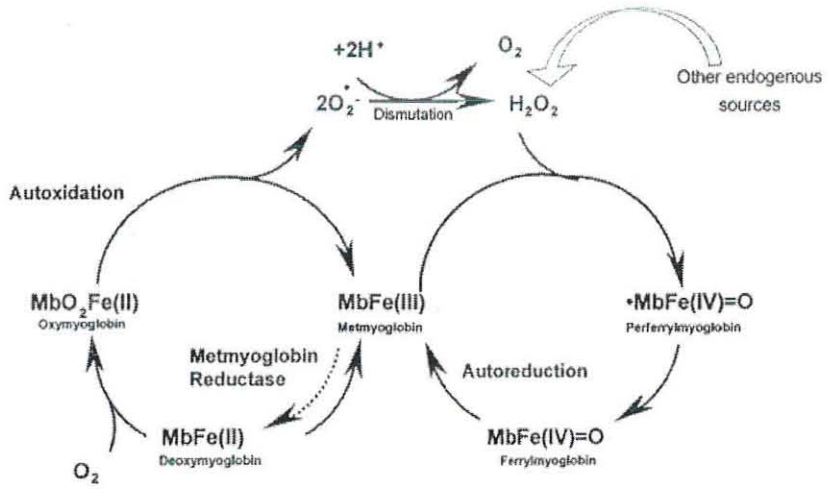


Figure 10. Myoglobin-Induced Lipid Oxidation (Baron and Andersen, 2002)

Through different stages of meat production like processing, distribution and storage meat undergoes chemical and microbiological deteriorations. The most important contributors of pigment oxidation are temperature, relative humidity, oxygen partial pressure, light, and lipid oxidation. This reaction goes together with rancidity caused by fat generally (AMSA, 2012).

2.15. Lab model

Computer imaging (digital imaging) anlaysis detects color change on beef surface. In this research it is also observed that the the instrumental color measurments taken from digital images (DI) of meat sample are related with sensory-panalist scores. Accelerated development of hard ware and soft ware for DI in processing and reduction of equipment costs in these past few years made this technology accessible and cost-effective (Larraín, 2007).

Measuring the color of meat using CVS (computer vision system) have some advantages over the measurement of colorimeter. The CVS system can analyze the entire surface of the meat and their characteristics and defects. It also assesses the color with a non-destructive and objective method. But when using a colorimeter it usually analyzes "spot' (with a surface about 2-5 cm^2)

colorimeter cannot be able to measure a non- homogenous meat surface, with in a single measurement (Wulf and Wise, 1999).

Now a days color of food is measured by CIE L*a*b* values, hue angle and chrome. The L*a*b*, or CIE Lab, color space is an international standard for color measurement, adapted by the commission international d'Eclairage (CIE) in 1976. L* is the lightness component ranging from 0-black to 100-white, a* positive values indicate redness, and negative values indicate greenness, b* positive values indicate yellowness negative values indicate blueness. The L*a*b* color is device independent, providing consistent color regardless of the input or output device such as digital camera, scanner, monitor and printer. The L*a*b* values are mostly used for food research studies (Wulf and Wise, 1999).

The Lab color is the color system which is closely resembles the human vision unlike the RGB (Red, Green and Blue) and CMYK (Cyan, Magenta, Yellow and Black) color models. The L component resembles the human perception of lightness. Due to this it can be used to make accurate color balance relations by adjusting output curves in the a and b components, or to adjust the lightness contrast using the L component (Nollet and Toldrá, 2010). In (Larraín, 2007) it is hypothesised that L*,a*,b* values can be used for indetection of color change in beef and other muscle foods.

2.16. Optical properties of meat

There are many techniques which are used to measure the tenderness of muscle; one of the promising methods is optical measurement. Optical method has many advantages like being non destructive, fast, inexpensive and considered suitable for online measurement (Yao, *et al.* 2006).

The color of meat can be evaluated by two methods the first is by chemically, by analyzing the pigments present and physically by measuring the interaction of light. When looking at light-

muscle interaction, light gets absorbed and scattered in the muscle. The reflection of light is dependent on both absorbing and scattering properties. The chemical composition of the tissue determines the absorbing properties and the scattering properties are determined by the structural composition of the meat (Yao, *et al.* 2006; Price, 2008).

Color essentially needs source of light that illuminates an object. Which as a result, adjusts the light and reflects (transmits) in to an observer. Then the observer senses the reflected light, and the combined elements provide the stimulus that the brain converts into our perception of color. Color has three quantitatively definable dimensions these are hue, chrome and lightness (Nollet and Toldrá, 2010).

Light get absorbed in the muscles by pigments such as myoglobin and its derivatives and the scattering of light in to different directions occurs due to the strike of light in to muscles microstructural components like connective tissue and the sarcomere, which are the fundamental structural and contractile units in skeletal muscles. Measurement of reflectance at the sample surface is as the result of both scattering and absorption processes involved in light-muscle interactions. The measured diffuse reflectance reveals those photons which survived absorption and have been scattered diffusely in the meat and have eventually escaped from the meat surface (Xia, *et al.* 2008).

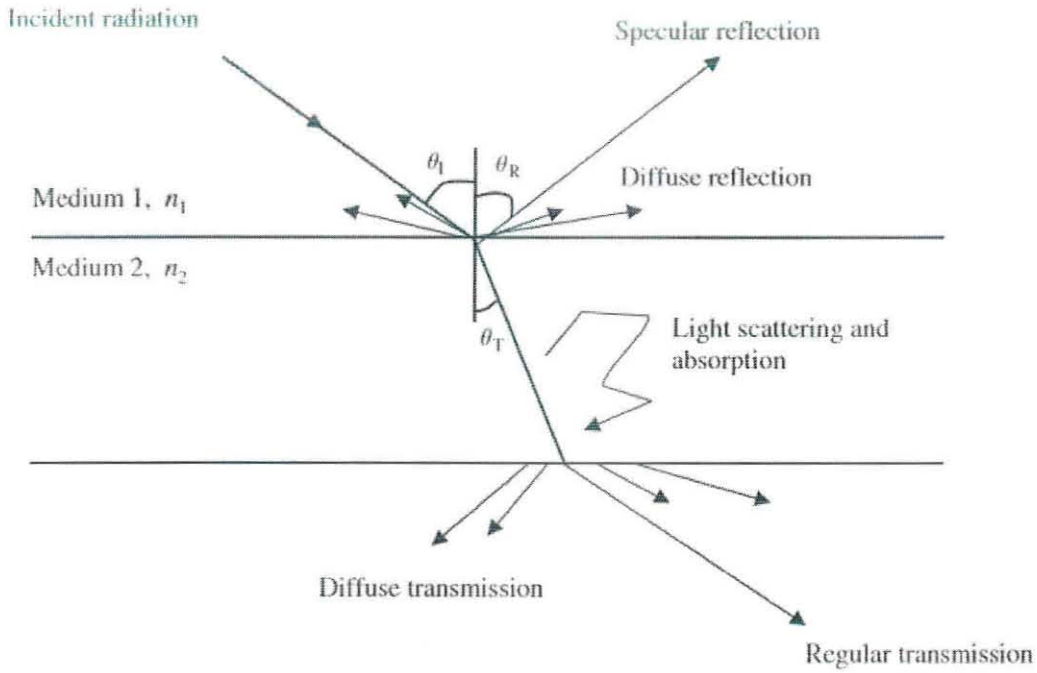


Figure 11. Schematic representation of interaction of light with matter, θ_I = angel of incidence, θ_R = angel of reflectance, θ_T= angel of transmittance, n_1, n_2 = refractive index of medium 1 and 2, respectively (Jha, 2010)

Major meat microstructures include microstructures like the collagen structures, sarcomere length and myofibrillar structures (connective tissue and myofibrillar proteins). These structures are also closely related to beef tenderness but the chemical compositions such as water, myoglobin and its derivatives doesn't have too much connection with beef tenderness. Light scattering property of meat is also dependent on these primary components (microstructures) of meat. Hence, light scattering property of meat can also help in determination of meat tenderness and changes on the scattering profiles can indicate the change on tenderness property of the meat. optical scattering and absorption properties completely describe the light propagation processes in tissue (Xia, *et al.* 2007; ElMasry and Sun, 2010).

2.13. Iron distribution in tissue

Iron in living things is find in to in five distinct pools, which include transport, storage, oxygen carrying, functional, and low molecular weight irons, represented by transferrin, ferritin, hemoglobin/myoglobin, iron-dependent enzymes, and small transit pool of iron chelates, respectively. Haemoglobin consist two-third of iron in the body, with smaller amounts of myoglobin, various iron-containing enzymes, and transferrin. Iron which is not used for these are stored in intracellular storage protein, ferritin and hemosiderin. The concentration of free ionic iron is extremely small. Animal species, muscle type, and anatomical location of muscle determine the amount of myoglobin and haemoglobin in muscle tissue. Both myoglobin and hemoglobin-bound iron accounted for 73.3, 47.0, and 28.5% of total iron concentration in beef, pork, and chicken thigh meat, respectively. Myoglobin (70%) is the predominant iron compound in beef. Myoglobin accounts for most of heme iron in beef and pork (Chaijan, 2008; Min, *et al.* 2008).

Researches have shown that heating or addition of H_2O_2 caused the release of heme iron due to oxidative cleavage of porphyrin ring of heme. Transferrin and ferritin are major iron transport and storage proteins, which are capable of binding two and ~2500 Fe (III) ion at a time, respectively. The amount of ferritin-bound iron in beef, pork and chicken thigh muscles are 1.2%, 4.6%, and 11.1% respectively. Ferritin palys a role in oxidative deterioration by releasing Fe (II) in the presence of reducing compound such as O_2- and ascorbate, heating, or refrigerated storage (Chaijan, 2008).

2.14. Iron in lipid peroxidation

Iron is the most abundant transitional metal in biological systems and almost all iron occur bund to protein. Free iron amount is less mainly because of two reasons: 1) Fe^{3-} is not water soluble, and 2) Fe^{2-} participates in the generation of free radicals. Iron has the possibility of various

oxidation states that is from -II to +VI but Fe (II) and Fe (III) are the forms which are found mainly in biological system. Different ligand environments allow the potential of iron for various oxidation state, reduction potential, and electron spin configuration which helps it to serve in multifunctional roles as a protein cofactor. Metal-binding proteins in biological system are usually classified by the functional role of metal ion: structural, metal-ion storage and transport, electron transport, dioxygen binding, and catalytic protein. However, these different purposes allow it to catalyze the detrimental oxidation of biomolecules such as DNA, protein, lipid, etc. (Min and Ahn, 2005). Dietary protein deficiency, dietary iron loading, low concentrations of iron-binding proteins, or cell injury results in an increase in extracellular or intracellular iron concentrations causes production of ROS, lipid oxidation and oxidative stress (Fang, *et al.* 2002).

The structure and chemistry of the iron atom have an impact on the reactions and color changes that myoglobin undergoes. The oxidation of ferrous-oxymyoglobin (Fe^{2+}) to ferric-metmyoglobin (Fe^{3+}) is responsible for discoloration of meat during storage. Ferrous iron (Fe^{2+}) can react with molecular oxygen to produce superoxide anion ($O_2°$-) with concomitant oxidation to ferric iron (Fe^{3+}). Hydrogen peroxide (H_2O_2), which may be produced by dismutation of $O_2°$-, can react with Fe^{2+} to produce hydroxyl radical (OH°). This reaction termed the Fenton reaction is the principal mechanism for myoglobin oxidation.

$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2°$-

$2O_2°- + 2H+ \rightarrow H_2O_2 + O_2$

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH°$

Figure 12. Reactive oxygen spieces generated by Fenton reaction (Chaijan, 2008)

2.17. Lipid oxidation in meat

Lipid oxidation in muscle foods is initiated by stressors arising from both internal and external sources. Factors such as pre-slaughter stress, physical damage, post-slaughter carcass

temperature, pH value, extent of thermal processing, grinding, deboning, storage and the use of additives such as salt, nitrite and antioxidants can all affect the rate and extent of lipid oxidation in muscle foods (Cascone, 2006; Ke, *et al.* 2009).

The oxidative deterioration of lipids containing a number of carbon-carbon double bonds can be defined as lipid peroxidation (LP). The most important stressors are the reactive oxygen species (ROS) including hydroxyl radicals, peroxyl radicals, superoxide anions, hydrogen peroxide, and nitric oxide. ROS can form a reaction with protein and lipids (Rowe, *et al.* 2004).

Frozen meat tends to stay oxidatively stable however processes like mincing, cooking and various processes before the chilling stage destroys the cell membranes in the muscle which assist in the reaction of unsaturated fatty acids with prooxidants like non-heme iron thus speeding up the rancidity and quality deterioration progress of meat by lipid oxidation. Lipid peroxidation, the oxidative deterioration of the polyunsaturated lipids of food brings about the development of hydroperoxides to short-chain aldehydes, ketones, and other oxygenated compounds, which accounts responsible for the formation of rancidity in food. Oxidation reaction affects the color, the flavour, the smell and declines the nutritional quality and safety of the meat. Malondialdehyde (MDA), a major degradation product of lipid hydroperoxides. This compound is of particular concern since it has been shown to be mutagenic. Carcinogenic and implicated in other pathological processes such as the formation of fluorescent pigments typical of cellular aging (Boe, 2012).

Oxidative deterioration is a major quality loss in muscle foods. The disadvantage of oxidative effect includes off-flavor development, discoloration, nutrient loss, and health risks including economic loss (Klont, *et al.* 1998).

2.18. Mechanism of lipid oxidation in meat

Autoxidation of lipid and formation of free radical are natural phenomena that cause oxidative deterioration and off-flavour production in meat. Autoxidation is the main chemical process of lipid oxidation in muscle food, the reaction of unsaturated lipids with oxygen to form hydroperoxides involving three free radical mechanisms which are initiation, propagation and termination (Dave and Ghaly, 2011).

A. Initiation

The initiation state will begin with catalysts like heat, metal ion and irradiation acts as catalysts to form free radicals. The reaction of free radicals with oxygen form peroxyl radicals as follows

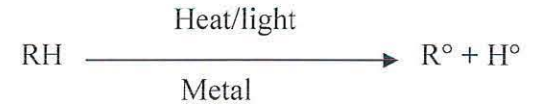


Figure 13. Initiation reaction of lipid oxidation (Mohamed, *et al.* 2008)

B. Propagation

During this stage the peroxyl radicals react with other molecules and form more new free radicals, and these new free radicals will also produce other free radicals this is called a chain reaction.

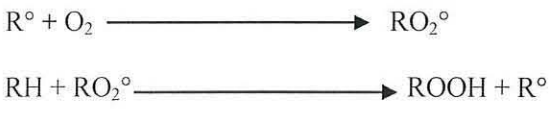


Figure 14. Propagation in lipid oxidation reaction (Mohamed, *et al.* 2008)

C. *Termination*

Terminations of free radicals occur when free radicals react with elements but stops forming free radical.

$R^{\circ} + R^{\circ} \longrightarrow R\text{-}R$

$RO_2^{\circ} + RO_2^{\circ} \longrightarrow ROOH + R^{\circ}$

$\longrightarrow RO_2R$

Figure 15. Termination in lipid oxidation reaction (Mohamed, *et al.* 2008)

Oxidation of lipids and production of free radicals occurs naturally and it affects fatty acids and causes oxidative deterioration and development of off-flavours in meat. After post-slaughter, when blood circulation and metabolic processes stop oxidation starts to happen. Antioxidants can inhibit oxidation by removing free radicals (Mohamed, *et al.* 2008; Dave and Ghaly, 2011).

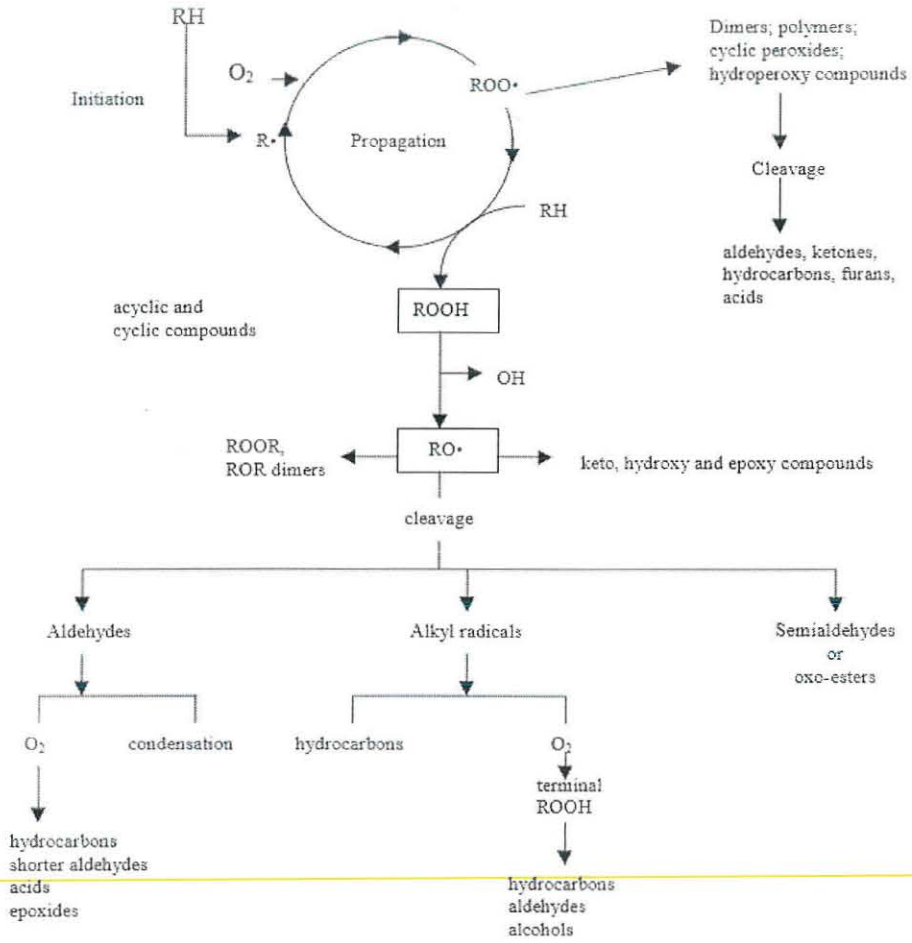


Figure 16. Generalized scheme for autoxidation of unsaturated lipid fatty acids and its consequences (Sickler, 2000)

2.18.1. Free radicals

Free radicals can be defined as molecules with unpaired electron on the outer most shell. They are generally unstable and very reactive. Once formed these highly reactive radicals can start a chain reaction. Examples of oxygen free radicals are superoxide, hydroxyl, peroxyl ($RO_2^{\bullet}$), alkoxyl ($RO^{\bullet}$), and hydroperoxyl ($HO2^{\bullet}$) radicals. Nitric oxide and nitrogen dioxide ($^{\bullet}NO_2$) are two nitrogen free radicals. Oxygen and nitrogen free radicals can be converted to other non-radical reactive species, such as hydrogen peroxide, hypochlorous acid (HOCl), hypobromous acid (HOBr), and peroxynitrite ($ONOO^-$). ROS, reactive nitrogen species (RNS), and reactive

chlorine species are produced in animals and humans under physiologic and pathologic conditions. Thus, ROS and RNS include radical and non-radical species (Fang, *et al.* 2002).

Table 1. Important reactive oxygen species and lipid aldehydes and their common abbreviations

Reactive Oxygen Species and Aldehydes

Radicals	Non-radicals
Hydroxyl ($HO^{\bullet}$)	hydrogen peroxide (H_2O_2)
Superoxide ($O_2^{\bullet-}$)	singlet oxygen (O_2)
Lipid peroxyl ($ROO^{\bullet}$)	lipid hydroperoxide (ROOH)
Alkoxyl ($RO^{\bullet}$)	4-Hydroxy-2-hexenal (HHE)
Peroxyl ($ROO^{\bullet}$)	4-Hydroxy-2-nonenal (HNE)

Source (Boe, 2012)

2.18.2. Antioxidants

Antioxidants are substances that delay onset of, or slow down rate of oxidation. They work either by inhibiting the formation of free radicals in the initiation step or interrupting propagation of the free radical chain. Food antioxidants can be applied in fresh and further processed meat to prevent rancidity and improve color stability (Ismail, *et al.* 2009).

Antioxidant additives are classified as primary (long term) or secondary (processing) antioxidants. Primary antioxidants can be natural (rosemary extract, tocopherols, smoke, etc) or synthetic [BHA (butylated hydroxyl anisole), BHT (butylated hydroxyl toluene), TBHQ (tert-butyl hydroquinone) etc.]. Primary antioxidants form a reaction with free radicals formed in fat oxidation and break the chain reaction formed by the free radicals. Secondary antioxidants have the role of oxygen scavenging properties, synergists and speeds up curing reaction. Examples of secondary antioxidants are citric acid, ascorbic acid, ascorbates, erythorbates, phosphates, lactates, starter cultures, etc. They play a role of oxygen scavenging by removing it, chelate and

create low redox potential/reducing conditions that enhance the curing reactions. The proper use of antioxidants is important to get effective results, in meat promoting curing reaction and prevent rancidity development. The specific antioxidant should be employed for a specific meat system based on any flavor attributes, solubility, type of fat, and type of oxidation (Ismail, *et al.* 2009; Dave and Ghaly, 2011).

2.19. Products of lipid oxidation

Without oxygen life cessed to exist though it causes damage to various cells. The toxicity of oxygen is caused by increased production of ROS. Although ROS are produced under normal physiology, they don't excide the capacity of, natural defence systems in body. The reduction of oxygen molecule by way of one-electron reduction processes produces short-lived but highly reactive oxygen products such as hydroxyl radical (-OH), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroperoxyl radical (HO_2^-), and iron-oxygen complexes (ferryl and perferryl radical), all of which may directly or indirectly participate in lipid peroxidation processes in meat and meat products (Min and Ahn, 2005).

Hydroperoxides are products of primary lipid autoxidation (with general formula ROOH). They are compounds which are non-volatile, odourless, tasteless and relatively stable at moderate reaction conditions like low temperature and absence of metal ion, but these conditions are not found in muscle foods so the hydroperoxides becomes susceptible to further free radical chain reaction such as isomerization and decomposition. Hydroperoxides decompose readily by hemolytic cleavage to form alkoxyl ($RO^\bullet$) and peroxyl ($ROO^\bullet$) radicals (Cascone, 2006).

$2\ ROOH \rightarrow ROO^\bullet + RO^\bullet + H_2O$

Decomposition of lipid hydroperoxides is a complex process which produces many chemicals that may have biological effect and result flavour deterioration in fat-containing foods. The radicals which are formed from this are highly reactive and decomposed quickly by the

homolytic scission of a carbon-carbon bond or oxygen-oxygen bond to produce compounds of various chemical nature including aldehydes, ketones, esters, alcohols, hydrocarbons, furans, epoxides and lactones as well as polymers. These secondary product of lipid oxidation are flavor-active, particularly aldehydes, that have a low threshold values in parts per million or even parts per billion levels, as a result they are accountable for the development of warmed-over flavor (WOF) and meat flavor deterioration (MFD) (Boe, 2012).

Table 2. Consequences of Lipid Oxidation Activity

Loss of flavor
Development of off-flavors (rancidity)
Color changes: • Myoglobin (red) ⟶ Metmyoglobin (brown) • Loss of carotenoid
Accumulation of oxidation products with potentially detrimental health implication
Protein denaturation
Functionality changes
Loss of nutrient value

Source (Boe, 2012)

The product of secondary lipid oxidation depends on the types of fatty acids present in lipid foods and also the random nature of free radical reactions. These products affect organoleptic properties (color, flavor, taste, texture and safety) and degradation of nutritional value and their wholesomeness, further more they are also associated with biological oxidation and may also interact with other foods components and change their functional and nutritional properties (Boe, 2012). Measurement of secondary oxidation products of lipid oxidation is suitable since these products are generally odour-active, where the products of primary lipid oxidation products are

colourless and flavourless. Examples of secondary lipid products include aldehydes, ketones, hydrocarbons and alcohols, among others (Ferioli, 2007).

Among secondary lipid products MDA (Malondialdehyde) is one of them. This is a compound formed by degradation of fatty acids with three or more double bonds, it is the principal and the most studied product of lipid oxidation and can be considered as the major indicator of the extend of lipid oxidation, both for the early appearance as oxidation occurs and for the sensitivity of the analytical method (Boe, 2012).

2.20. Methods for estimating lipid peroxidation

There are many physical and chemical tests, including instrumental analyses, which have been used in the laboratories and industry for determination of lipid oxidation. To list some of the methods the weight-gain and headspace oxygen uptake method for oxygen absorption; chromatographic analysis for changes in reactants; iodometric titration, ferric ion complexes, and Fourier transform infrared (FTIR) method for peroxide value spectrometry for conjugated dienes and trienes, 2- thibarbituric acid (TBA) value, p-anisidine value (p-AnV), and carbonyl value; Rancimat and Oxidative Stability Instrument (OSI) method for oil stability index; and electron spin resonance (ESR) spectrometric assay for free-radical type and concentration (Shahidi and Zhong, 2005).

2.20.1. TBA reactive substances (TBARS), mainly Malondialdehyde (MDA)

TBA is the most common method used in the determination of lipid oxidation of food products. This assay measures the pink chromophore by using spectrophotometry, with an absorption maximum at 530–532 nm, which is formed by the reaction of Thiobarbituric Acid (TBA) and secondary products, such as malondialdehyde. TBARS (Thiobarbituric acid reactive substances) values are reported as milligrams of malondialdehyde (MDA) equivalents per kilogram of tissue

or samples and have been correlated with off flavor scores (Bess, 2011). But it should be also noted that other oxidized products can react with TBA reagent and produce a pink color hence the term thiobarbituric acid reactive substances (TBARS) is used instead of MDA (Sun, *et al.* 2011).

The TBA test is used frequently to assess the oxidative state of a variety of food systems although there are limitations to this method; the TBA test provides an outstanding means for evaluating lipid oxidation in foods (Boe, 2012).

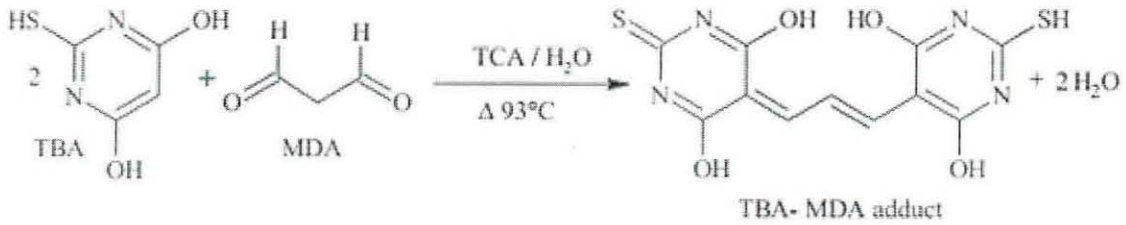


Figure 17. Reaction of thiobarbituric acid (TBA) and Malonaldehyde (MDA) (Ferioli, 2007)

In general, MDA can react with several functional groups on molecules including proteins, lipoproteins, RNA and DNA. Checking the level of MDA in food stuffs and in biological materials can be used as an important indicator of extent of lipid peroxidation (Boe, 2012).

2.21. Health and nutritional implication of lipid oxidation products

These days the nutritional and toxicological effects of lipid oxidation in food, in relation with human health has given much interest. Although the product of primary lipid oxidation may not be toxic, toxic effects can be found on secondary lipid oxidation products. Furthermore the interaction of lipid oxidation products and food components particularly, vitamin and proteins, produce major nutrition and sensory problems (Boe, 2012).

Recent studies has showen that dietary lipid peroxidation products reduce activities of glucose-6-phosphate dehydrogenase and other enzymes involved in fatty acid synthesis in the liver. Also

oxidized fat can lead to cellular damage in various organs, cardiac fibrotic lesions, hepatic bile duct lesions and altered fatty acid composition of tissue lipids. In relation to this, animals which are fed oxidized oils reported to show increased liver and kidney weights (W¹sowicz, *et al.* 2004; Boe, 2012).

The primary products hydroperoxides are mostly decomposed in the stomach, but in a small amount they can be converted to the corresponding hydroxyl fatty acids that were absorbed and transported to the blood. As a result they can promote thrombosis, accelerate progression of atherosclerosis and participate in the development of cancer in human (Boe, 2012).

Secondary lipid oxidation products, like aldehydes are absorbed, in free form or conjugated with amino acids, from gastrointestinal tract to plasma and incorporated into liver and muscle. These compounds can react with protein and DNA to form cross-link bounds that have been show mutagenic and cytotoxic activity (Fang, *et al.* 2002; Boe, 2012) .

The presence of MDA in foodstuffs can be representative of rancid food. This aldehyde is considered as indicator of lipid peroxidation but it's also a highly toxic molecule. The interaction of MDA with DNA and protein is referred as potentially mutagenic and atherogenic. Furthermore MDA affects the cardiovascular system, in vivo it has shown that it reacts with primary amines in lipoproteins (LDL) generating lysine-lysine cross-links. These reaction products are responsible for the initiation of the atherosclerotic lesion. Moreover malondialdehyde, can react with the free amino groups of proteins and lipids in the cell membranes to form cross-linked products between the various components of the membrane, which assist in rigidity of the membrane (Cascone, 2006).

2.22. Interaction of lipid oxidation products with food components

Initiation of lipid oxidation in food is by free radical and/or singlet oxygen mechanisms which generate a series of autocatalytic free radical reactions. These autoxidation reactions cause the breakdown of lipid and the formation of a wide variety of oxidation products. Type and amount of these products can differ widely between foods and they depend on the composition of the food as well as many environmental factors (Boe, 2012).

The toxicological significance of lipid oxidation is resulted by reaction of secondary lipid oxidation products with other food components. These interactions can lead to limiting the bioavailability of lipid breakdown products or can lead to the formation of toxic products derived from non-lipid sources (W¹sowicz, *et al.* 2004; Cascone, 2006).

Secondary lipid oxidation products can be absorbed and may cause an increase in oxidative stress and deleterious changes in lipoprotein and platelet metabolism. On the other hand primary products of lipid oxidation – hydroperoxides are able to decompose lipid soluble vitamins (vitamin A, E, D and K) or its provitamins (carotenes) and, also water-soluble vitamin C (W¹sowicz, *et al.* 2004).

Moreover the interaction of lipid oxidation products with protein and other food components has major effect on oxidative and flavor stability and texture during processing, cooking and storage. Degradation of protein-containing foods affects the nutritional value of food. It can also generate brown color appearance and unpleasant odours and taste (Boe, 2012).

3. Material and Methods

3.1. Location of the study site

Beef samples were collected from Bahir Dar town of Amhara National Regional state (ANRS), Ethiopia. It is located approximately 578 km north- northwest of Addis Ababa. The major farming system of the area is mixed crop – livestock system. Cattle breed in the area is said to be Fogera breed.

The town is provided with one municipal abattoir which gives slaughter service to the butchers retailing meat. In the city, there is also private abattoir which is dedicate for slaughter and meat export. It is owned by Ashraf PLC, Sudanese meat exporting company. In the town there are about 38 butcheries which are distributed in the 9 *kebeles* of the town.

3.2. Description of the abattoir

The public abattoir operates at medium capacity of throughput for slaughter. Estimated number of heads slaughtered per day is 150 cattle and 200 shoats. Slaughter stock is brought to the lairage of the abattoir; await slaughter for about 12 hours in the lairage with no feed provision. Antemortem examination is conducted during this period. Animals unfit for slaughter are either sent to recovery room or rejected as unfit for slaughter.

Once antemortem inspection is conducted, slaughter stock fit for slaughter are channelled to sticking room for exsanguination. Then carcass dressing and fabrication proceeds. Meanwhile, postmortem inspection of carcass is conducted by inspector. Gross or parts of carcasses unfit for consumption are condemned. Carcasses fit for consumption are allowed to exit and loaded for distribution to butcheries.

The abattoir does have standard sanitary operational procedure for the facilities and operators of the slaughter. However, no certification was documented in any forms of internationally recognized standards of HACCP or ISO 2200.

3.3. Description of the butcheries

About 38 butchers were distributed throughout the city. These formed the sampling frame of the sample collection for the study. Among the butchers three were randomly (lottery method) selected and visited. All the butchers hang the carcass against white painted wall under incandescent light. Meat starts from the time of the receipt. Carcass is received by butchers after about 2 hrs of completion of slaughter. Among the visited butcheries, two of the butchery men wore white protective clothing while the third did not. In all cases, no head covers and hand gloves of the butchery men were observed. Retail facilities and operation table seems subject to regular cleaning. However, the butcheries had no protective features for flies or insects attracted by the carcass via windows or doors. Consequently there might be a possibility of contamination of the carcass which could lead to safety and quality compromise. The butchery men responded saying that they didn't undergo any form of formal training in aseptic handling and carcass fabrication.

3.4. Purchase and preparation of the muscles for the study

General facts of the area reveal that beef animals for slaughter belong to indigenous Fogera breed. Slaughter animals were purchased by the butcheries from terminal markets of the area and submitted to the abattoir for slaughter service and carcass delivery.

Three carcasses from the randomly selected butcheries were the basis of muscle samples selected for the study. Selected muscles were made to represent entire quarter of the carcass in order to be biologically meaningful. Accordingly, for muscles from four locations were sampled. These

were Rhomboideus (hump meat), Infraspinatus (top blade, flat iron, or triangle), Longissimus dorsi (rib eye, strip, loin eye), and Quadriceps femoris (knuckle, sirloin tip). One kilogram of each muscle was purchased at about 5 hours after slaughter.

After purchase, the samples were aseptically transported for 15 minutes to Ashraf meat export PLC for packaging. Once in Ashraf meat export abattoir, pH measurements were taken for day 0 and the samples were vacuum sealed in poly ethylene bag using Multivac Packaging machine (Multivac C500, Multivac Inc., Kansas City, MO) and labeled. Following the vacuum package, day zero sample was immersed in a liquid nitrogen for about 30 seconds. Days 7, 14 and day 21 samples were aged at 4 ^{0}C temperature in a chilled box (during transportation) and refrigerator once in the laboratory. All the samples, either after immersion in a liquid nitrogen or under simulated condition, were transported to the laboratory of Center of Food Science and Nutrition. Once at the center, samples executed experimental period were stored in deep refrigerator at -20 ^{0}C until analysis.

3.5. Sample preparation

Appropriate size samples were taken from each muscle types for each test days and freeze dried in a freeze drier (Christ Beta 1-8 LD plus) for the studied parameter. In the freeze drying procedure, the meat samples were ground using a blender (Waring commercial grinder). Then the ground samples were labelled and placed in small glass plates separately. After that the labelled meat samples were placed in a freeze drying machine until they become dry. Freeze dried meat samples were ground to powder using a mortar. Powders were sealed in plastic bags and analysed for determination of crude protein and fat.

3.6. Determination of moisture content

Moisture of beef was determined in duplicate using oven drying method (AOAC, 2000) using the official methods 925.09 the empty dish was dried in air oven at 105 ^{0}C for an hour. Then the container was placed in desiccators to cool for 30 min and then weighted. Next chopped samples were mixed thoroughly and homogenized; 5 g of the chopped beef was put in to the dried dishes. Then the dishes with their samples were left in the air oven to get dry for about 4 hrs. at 105 ^{0}C until their weight remains constant, and taken out of the oven to cool at room temperature in desiccators then weighted.

Calculations

$$\text{Moisture content (\% by mass)} = \frac{(W_2 - W_3) \times 100}{W_1}$$

Where: W_1 = weight of wet sample

W_2 = weight of dish plus weight of wet sample

W_3 = weight of dish plus sample after drying

3.7. Determination of crude fat content

Ether extract values were obtained from duplicate samples from each muscle type. Approximately 2 g of dried samples were added into extraction thimbles, and then covered with about 2 cm layer of fat free cotton. The thimbles with sample were placed into a Soxhlet Extraction Chamber. Cooling water was switched on, and 50 ml of diethyl ether was added to the extraction flask through the condenser. The extraction was conducted for 4 hrs. at 55 ^{0}C. The extraction flask with fat was removed from the extraction chamber and placed in the drying oven at 92 ^{0}C for about 30 minutes, cooled to room temperature in desiccators for about 30 minutes and weighed.

Calculation: -

$W = W_2 - W_1$

Crude fat in g/100g fresh sample = (W*100)/Ws

W = weight of fat (g); W_2 = weight of extraction flask after extraction (wt. of flask and fat);

W_1 = weight of extraction flask before extraction (wt. of flask); Ws = weight of dried sample.

3.8. Determination of crude protein content

Duplicate samples were analyzed by Kjeldahl method (AOAC, 2005) official method 979.09. This method is basically divided in to three parts: (1) digestion, (2) distillation and (3) titration. Half a gram of dried meat was weighted in to Kjeldahl digestion flask and then digested by heating at 37 ^{0}C for three hours in the presence of 6ml HCl, 3.5 ml H_2O_2, 3 g of catalyst mixture (ground 10 g of $CuSO_4$ with 150 g of K_2SO_4). After digestion is completed the sample was cooled for 30 minute at room temperature and neutralized by addition of 25 ml NaOH (40%) and diluted using 25 ml distilled water. The digestion produces ammonium sulfate using the nitrogen from the beef protein. The addition of NaOH converted the NH_2SO_4 to ammonia gas.

Then 25 ml of distilled water, 25 ml of Boric acid and 3 drops of Methyl red indicator was added to 250 ml receiving flask. Then the flask was connected to the distiller for distillation. The distillation process was stopped when the volume of receiving flask reached between 200-250 ml. Finally, the nitrogen content was estimated by titration of the ammonium borate formed with 0.1N hydrochloric acid. In the procedure multiplication factor of 6.25 is used.

Crude protein = N x 6.25

3.9. Determination of total ash content

In this study, the total amount of mineral content in beef muscles was determined by dry ashing method, according to (AOAC, 2000) using the official methods 923.03 according to the procedure, porcelain crucible were cleaned and dried in an oven for 30 minute at 105 ^{0}C. Next 2 g of dried sample (from the moisture determined samples) was weighed in to ashing dish (porcelain) and charred on hot plate under a fume hood. The temperature (150 °C – 300 °C) was increased slowly until smoking ceased and the sample was totally charred. Then, the samples were changed to ash in a muffle furnace at 550 ^{0}C for 5 hours and taken out of the furnace and cooled at room temperature for an hour. Then the crucibles were reweighed with ash.

At the ashing stage water and other volatile materials are vaporized and CO_2, H_2O and N_2 was formed by organic substances burned in the presence oxygen.

The ash content of the beef was calculated as

$$\% \text{ Ash} = \frac{(W_2 - W_3) \times 100\%}{W_1}$$

Where W_3 = weight of the empty crucible

W_2 = weight of the crucible with ash

W_1 = weight of the original sample

3.10. Determination of pH

pH from each sample was measured on day 0, 7, 14 and 21. Duplicate, chopped samples (10 g) were homogenised in 100 ml of distilled water for 1 min using a homogenizer. Then, the pH of the supernatant was measured using a digital pH meter (Seggern, *et al.* 2005). The electrode on the pH meter was kept in the supernatant until a stable pH value was read. The pH meter was calibrated using pH 4.0 and 7.0 buffers.

3.11. Determination of total pigment

Samples were analyzed in duplicate from each muscle using the method according to (Wadhwani, 2008). In the procedure, a total of 5 gm ground beef is placed in 100 ml beaker to which were added, 20 ml of acetone, 1 ml of distilled H_2O and 0.5 ml of concentrated HCl (37 %). The solution was then mixed with glass rod in the beaker. The beaker was covered with parafilm and kept in the dark for an hour at 4 °C. The sample was then filtered through Whatman #1 filter paper. The filtrate absorbance was measured at 640 nm with UV-VIS spectrophotometer (Bechman DU-64). A solution with 80% acetone, 2% HCl and 18% H_2O is used for the blank. The µg/g of total heme pigments was calculated by multiplying the absorbance by 680:

Total pigments (ppm; µg/g) = Absorbance at 640 nm x 680

3.12. Determination of heme iron content

Heme iron was calculated by the modified technique of (Ozer and SariçObaN, 2010); the iron content was estimated with the factor of 0.0882 µg/µg hematin. The value obtained using procedure from section 3.11 was used for total pigment.

Heme iron (ppm; µg/g) = total pigment (ppm; µg/g) × 0.0882 or
Heme iron (mg/kg) = total pigment (mg/kg) × 8.82/100

3.13. Determination of metmyoglobin content

The analysis of metmyoglobin content was performed as described by (Ozer and SariçObaN, 2010). Duplicate minced beef samples (5 g) were placed into a 50 ml polypropylene centrifuge tube, and 25 ml of ice-cold phosphate buffer (pH 6.80, 40 mM) was added into the tube. For the preparation of phosphate buffer we used Na_2HPO_4 (sodium hydrogen phosphate) and NaH_2PO_4

(Sodium dihydrogen phosphate). Then mixture in the tube was homogenised for 10 s at 1,500 rpm with a stirrer (IKA® Eurostar power control-visc stirrer). The homogenised sample was allowed to stand at 4°C for 1 h and centrifuged at 3000 rpm. The supernatant was filtered through Whatman #1 filter paper and the absorbance was read at 572, 565, 545, and 525 nm by scanning the visible spectrum with a spectrophotometer. The percentage of metmyoglobin was calculated using the following equation:

% Metmyoglobin =

$$[-2.51(A572/A525) + 0.777(A565/A525) + 0.8(A545/A525) + 1.098] \times 100$$

3.14. Determination of lipid oxidation (TBARS)

Beef steaks were analysed in duplicate for thiobarbituric acid reactive substances (TBARS) as described by (Wadhwani, 2008). Evaluation was made on days 0, 7, 14, and 21 to provide measurement of lipid oxidation. Samples (0.5 g) weighed in test tube with 2.5 mL stock solution, containing 0.375% TBA, 15% TCA and 1.0 N HCl. Sample tubes were heated in hot boiling water bath for 10 min until pink color developed and then cooled under running tap water. Samples were centrifuged at 5500 rpm for 25 min. Absorbance of supernatant was read at 532 nm using UV-VIS spectrophotometer (Bechman DU-64) against a blank sample having all reagents except the beef sample. The malondialdehyde (MDA) concentration was calculated using an extinction coefficient of 1.56 X 10^{5} M^{-1} cm^{-1}. The MDA concentration was converted to Thiobarbituric acid (TBA) number (mg MDA/Kg meat sample) as shown below:

TBA # (mg/kg) = sample A 532 X (1M MDA chromagen / 1.56 X 10 5) X
[(1 mole / L) / M] X (0.003L / 0.5g meat) X (72.07g MDA / mole MDA) X (1000g / kg)

3.15. Determination of meat surface color

Color change measurement was evaluated according to the method of (Yam and Papadakis, 2004). The method uses a combination of digital camera, computer and graphics software Photoshop (Adobe system) to measure and analyse the surface color of beef steak.

A digital camera with resolution of 15 mega-pixels was used to measure color change by capturing the color image of the meat samples under proper lighting in a dark room. Briefly, the camera was set up on a tripod and the lens faced downwards towards the sample supporting plane. The distance from the bottom of the camera lens to the sample surface was set as 35 cm. Images of displayed beef samples were captured on a black background matter under the following camera setting; manual mode, no flash and resolution of 1024 x 1680 pixels. After zooming the lens to make the sample cover the whole field of view and focusing, the image of each sample was taken and stored in JPEG (Joint Photographic Experts Group) format for image processing and analysis. Photographing was carried out on day 0, 7, 14 and 21. The beef samples were taken out of their vacuum pack and they were allowed to bloom for 30 minute. The L*a*b* values were measured using graphics software Adobe Photoshop CS3.

3.16. Optical scattering property

A Helium Neon (HeNe) laser with 632.8 nm was mounted at 90° to the sample surface to collect the backscattered light from meat. The collection fiber was positioned for scanning via a translation stage so that reflectance could be measured at different positions along the scanning plane. The scanning plane of the collection plan was parallel to the plane of incidence, but was offset by a small distance of 1.5 mm to avoid collision with the incident fiber. Detection was made by photo detector, mounted on a traveling stage. The scattering light then acquired and processed by a personal computer (Yao, *et al.* 2006). Unfortunately detecting the scattering light

from the meat at a specific point was not possible because the detector used was large and thus blocked the incident light from reaching the sample hence the detector was placed far away from the sample. This leads to the collection of scattered light from larger area of the sample. Thus, results that are obtained are generalized scattering properties of meat sample.

3.17. Sensory evaluation

The sensory evaluation used humans as instrument to assess the color, texture, aroma and overall attributes of the meat samples.

Beef steaks were evaluated by 15 semi-trained panellists over the 4 different storage periods for all muscle types. The sessions were held either in mid-morning or mid-afternoon. Panellists were laboratory workers which are accustomed to sensory evaluation tests, furthermore they were consumers. They also received introductory training prior to analysis.

Packaging systems individually opened with a scissor, and the panel evaluated the color, odor, texture and over all acceptability of the steaks. The samples for evaluation were presented at room temperature (≈25 °C), in plastic trays and were identified with three-digit random numbers. Place where the sensory cession conducted was well ventilated and free of noise and odors. Panellists ranked each package using a five point scale (1 = dislike extremely to 5 = like extremely).

3.18. Statistical analysis

All the data except optical scattering was analyzed either by one way Analysis of Variance (ANOVA) for the treatments or independent t-test. Statistically significant means were separated using Duncan Multiple Range mean separation technique. Significance was declared at $p < 0.05$. Computation was done using SPSS Version 20. All measurements were reported as Mean ± SE. Optical data was collected and illustrated by figures.

4. Result and Discussion

4.1. Moisture content

Moisture content of infraspinatus and quadriceps femoris significantly reduced between day 0 and day 21 (Table 3). In both muscles', moisture on day 21 decreased compared to day zero. Non significant mean moisture difference was observed for muscles longissimus dorsi and rhomboideus.

Table 3. Mean moisture (%) and fat (%) content of the beef samples over test days

Muscle	Moisture %		Fat %	
	Day 0	Day 21	Day 0	Day 21
Infraspinatus	75.90 ± 0.38^{b}	73.81 ± 0.48^{a}	7.95 ± 0.28^{a}	7.74 ± 0.20^{a}
Quadriceps femoris	77.38 ± 2.12^{b}	73.99 ± 0.33^{a}	7.23 ± 0.22^{a}	7.08 ± 0.14^{a}
Longissimus dorsi	75.40 ± 0.43^{a}	74.29 ± 0.45^{a}	8.52 ± 0.29^{a}	8.42 ± 0.18^{a}
Rhomboideus	25.36 ± 1.13^{a}	23.28 ± 0.81^{a}	66.28 ± 2.78^{a}	64.71 ± 2.42^{a}

Mean values having different superscripts within a row are significantly different at $p < 0.05$. Sample size, (n=3)

This result is not consistent with the results of Yuksel *et al.* (2011). They reported that significant differences in moisture loss depend on the storage duration. But in their finding it is reported that longissimus muscle have low drip (moisture) loss and quadriceps muscle have high amount of moisture loss among the muscle groups relative to days of storage Yuksel, *et al.* (2011) which agrees with the current result. Several authors have postulated that an increase in marbling score is related with an increase in fat content and a decrease in moisture content (Brackebusch, *et al.* 1991). Therefore, in this study also it was noted that inherently marbled Rhomboideus had the least moisture content and highest fat content respective to each test day. Furthermore, it has shown that moisture content of meat is inversely proportional to its lipid content (Patten, *et al.* 2008; Karakok, *et al.* 2010). In the present study, no such relationship was noted. However,

plausible assumption for non-statistical difference noted in this experiment for mean moisture content of both Longissimus dorsi and Rhomboideus could be the lesser fat content attributed to the breed of slaughter stock. Drip loss typically attributed to marbled muscles may not have been pronounced as well (Yuksel, *et al.* 2011).

4.2. Fat content

From Table 3 it can be seen that at day 0 value of fat percentage varies from 7.23 - 66.28%. There was no significance difference ($p < 0.05$) during the storage period. Although, it wasn't statistically significance a decrease in fat percentage was observed throughout the test days.

Moreover the highest marbling score goes to muscle rhomboideus and the least amount belongs to muscle quadriceps femoris (Pedrão, *et al.* 2009). Hence the percentage of ether extract (fat) increased with increased marbling score (Seggern, 2005).

At day 21, the amount of fat percentage shows decrease in each muscle type but the difference is not statistically significant. It has been reported that the value of lipid slowly starts to decline with increased storage time (Hui, 2006). The reasonable explanation for loss of lipid during storage is that some lipid from the samples may be released in a form of drip loss. Vacuum packaging may be one of the most effective packaging materials against drip loss although it doesn't prevent it because drip loss depends on several intrinsic factors like; species, pH and type of muscle (Hui, 2006).

4.3. Protein content

The percentage of protein at the start of trial varied from 18.88 – 22.80% (Table 4). The mean protein content of quadriceps femoris, longissimus dorsi and rhomboideus show no significant difference ($p < 0.05$) through test days. Regardless, slight declining trend of mean protein over

test days was observed. Even though the level variation of protein content looks constant the minimum amount of protein was scored by muscle rhomboideus.

The least amount of protein value in rhomboideus muscle can be explained by inverse relation of marbling score with protein content in muscles (Brackebusch, *et al.* 1991).

Table 4. Mean protein (%) and Ash (%) content of the beef samples over test days

Muscle	Protein %		Ash %	
	Day 0	Day 21	Day 0	Day 21
Infraspinatus	20.06 ± 0.36^{b}	19.13 ± 0.26^{a}	1.04 ± 0.02^{a}	1.15 ± 0.00^{a}
Quadriceps femoris	22.80 ± 0.43^{a}	21.80 ± 0.35^{a}	1.21 ± 0.02^{a}	1.28 ± 0.04^{b}
Longissimus dorsi	21.29 ± 0.26^{a}	20.41 ± 0.26^{a}	1.11 ± 0.05^{a}	1.19 ± 0.03^{b}
Rhomboideus	18.88 ± 0.20^{a}	17.16 ± 1.67^{a}	1.35 ± 0.02^{a}	1.46 ± 0.04^{b}

Mean values having different superscripts within a row are significantly different at $p < 0.05$. Sample size, (n=3)

At day 21 the only muscle type that shows significant decline in % of protein as compared to day 0 is Infraspinatus. Several authors have hypothesized the reason for protein loss, which is purge or drip, mostly of proteins which are water-soluble, sarcoplasmic proteins (Dalton, 2005; Huff-Lonergan and Lonergan, 2005). Hence the decline of protein value in test days in muscle infraspinatus can be explained by drip loss which causes leaching of sarcoplasmic proteins.

4.4. Ash content

The ash percentage at day 0 varies from 1.35 – 1.04 % (Table 4). No significant difference ($p < 0.05$) was noted for mean ash content of muscle infraspinatus between test days. Muscle longissimus dorsi, quadriceps femoris and rhomboideus show a slight increase of mean ash content at day 21 than at the beginning of the trial.

Over 21 days the content of ash shows an increase, this condition can be explained by moisture loss with increased storage time which causes the total content of dry substances to show an increase (Kuzelov, *et al.* 2011).

4.5. pH

The pH variations for meat samples according to storage time are shown (Fig 18). The mean for pH measurement ranges between 5.40 – 5.83 at day 0. The pH values show a significant increase with the increase of storage time, except where rhomboideus of all muscle types did not show significant difference from day 0 to day 7.

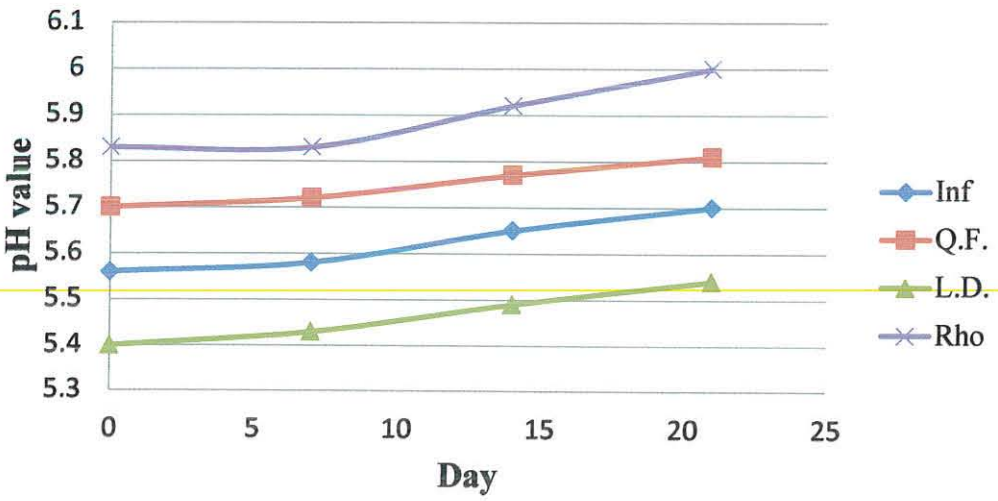


Figure. 18. pH value at different test days, SE 0.01, at $p < 0.05$. Sample size, (n=3)
Q.F. = (Quadriceps femoris) Inf = (Infraspinatus)
L.D. = (Longissimus dorsi) R = (Rhomboideus)

In this study the samples of beef were taken a few hours after slaughter, maybe while still undergoing a stage of post mortem changes. This can be the cause for the initial value of pH being around 5.4 – 5.8 instead of around 7. The highest pH measurement goes to rhomboideus, followed by quadriceps femoris, infraspinatus and longissimus. Storage time had statistically significant ($P < 0.05$) effect on pH values.

The increase of pH through storage time is in agreement with other researches that found out vacuum packed muscles tend to show an increase during storage time (Patten, *et al.* 2008; D'agata, *et al.* 2010). Although this study was not consistent with the results of Stanišić, *et al.* (2012) who reported that the increase in pH doesn't seem to be significantly different through storage time.

While looking at the pH variation among storage time, there are studies which explain the slight and constant increase in pH value. Such an increase in pH is due to progressing alkalization caused by the release of basic products of protein breakdown throughout the post-slaughter endogenous changes (Stanišić, *et al.* 2012). In addition to this, the observed trials of variation of pH values between muscle types might be attributed to the amount of glycogen present at slaughter time. The amount of glycogen in muscle tissue depends on animal species and type of muscle (Przybylski, Monin *et al.* 2006). In studies it is indicated that muscles with high concentration of glycogen have lower pH values (Warriss, 2010). Animal's diet and stress before slaughter affects the amount of glycogen in the muscle which also affects the pH (Patten, *et al.* 2008).

4.6. Concentration of total pigment

Total pigment measurement ranges from 139 - 273.81 μg/g on day 0 (Table 5). Mean total pigment content was significantly reduced between day 0 and day 21 in muscle longissimus dorsi and rhomboideus. Whereas no significant difference was observed in muscle infraspinatus and quadriceps femoris through the storage time.

The variation of total pigment among muscle types can be explained by anatomical location and physiological functions, which result in differences in metabolism which causes unique color biochemistry in each individual muscle type (Suman and Joseph, 2013).

Table 5. Total pigment (μg/g) value of the beef samples over test days

Muscle	Day 0	Day 7	Day 14	Day 21
Infraspinatus	273.81 ± 5.27^{a}	268.03 ± 3.00^{a}	267.30 ± 5.44^{a}	265.44 ± 4.98^{a}
Quadriceps femoris	297.06 ± 4.02^{a}	289.99 ± 5.98^{a}	289.19 ± 8.09^{a}	286.46 ± 6.46^{a}
Longissimus dorsi	173.57 ± 6.54^{c}	161.85 ± 2.24^{b}	161.43 ± 4.69^{b}	160.04 ± 10.04^{a}
Rhomboideus	139.48 ± 4.97^{b}	131.19 ± 1.85^{a}	128.63 ± 5.23^{a}	127.02 ± 3.38^{a}

Mean values having different superscripts within a row are significantly different at $p < 0.05$. Sample size, (n=3)

For muscle longissimus dorsi and rhomboideus at day 0 the total pigment values are higher than the rest of other test days. This means the muscles appear more red at day 0 than the rest of test days (Tambunan, 2008). The rest of other muscles show a higher total pigment value at day 0, although it doesn't show a significant difference when compared with the other test days.

With increased storage time the amount of total pigment shows a decrease, the possible explanation for this is pigment oxidation. It is suggest that total pigment and myoglobin amounts are well related to the variation of lipid peroxidation of stored raw meat (Min and Ahn, 2005).

Furthermore, the difference of total pigment measurement between muscles type can be the result of residual blood (hemoglobin) from one muscle to another, which also causes difference in amount of hemoglobin and myoglobin. One of the main reasons for variation of pigment in muscles is physical activity (Seggern, *et al.* 2005). According to Tambunan (2008) the difference of total pigment between musce types may be due to the amount of myoglobin present in the

muscles. Muscle to muscle differences in myoglobin content is because of muscle fibers that are present.

4.7. Concentration of heme iron

Heme content of the beef samples according to storage time is shown (Table 6). At day 0 the heme content of the muscles varies from 12.29 - 26.19 mg/kg. In general the heme content shows a decrease with increasing storage but this decrease was not significantly different in muscle infraspinatus and quadriceps femoris whereas the difference was significant in muscle longissimus dorsi and rhomboideus at $p < 0.05$.

Table 6. Mean Heme iron (mg/kg) value of the beef samples over test days

Muscle	Day 0	Day 7	Day 14	Day 21
Infraspinatus	24.15 ± 0.46^{a}	23.63 ± 0.26^{a}	23.57 ± 0.48^{a}	23.40 ± 0.43^{a}
Quadriceps femoris	26.19 ± 0.35^{a}	25.57 ± 0.52^{a}	25.50 ± 0.71^{a}	25.26 ± 0.56^{a}
Longissimus dorsi	15.30 ± 0.57^{c}	14.27 ± 0.19^{ab}	14.23 ± 0.41^{ab}	14.11 ± 0.88^{a}
Rhomboideus	12.29 ± 0.43^{b}	11.56 ± 0.16^{a}	11.34 ± 0.46^{a}	11.19 ± 0.29^{a}

Mean values having different superscripts within a row are significantly different at $p < 0.05$. Sample size, (n=3)

The color of meat is dependent on the chemistry of the heme pigment myoglobin (Mohamed, *et al.* 2008a). The total heme content is an indication of myoglobin + hemoglobin content (Wadhwani, 2008).

From table 6 it can be seen that muscle infraspinatus and quadriceps femoris have more heme iron content, which makes them appear more red in color than the rest of the muscles. Red meat has more heme iron level, because heme iron is found in myoglobin and haemoglobin molecules (Met, *et al.* 2013).

It is noted that with increased test days the amount of heme shows a decrease in muscles longissimus dorsi and rhomboideus. This is in agreement with Ozer and SariçObaN (2010) who reported that the drip from meat released during storage contained significant quantities of iron and mainly soluble heme iron.

4.8. Amount of metmyoglobin

The amount of metmyoglobin at the beginning of the trial was lower than the rest of the test days for all muscle types. At day 0, the amount of metmyoglobin measurement varies from 8.90 – 10.35 %. From Table 7 it is evident that metmyoglobin values were significantly influenced by storage time.

Table 7. The Metmyoglobin (%) value of the beef samples over test days

Muscle	Day 0	Day 7	Day 14	Day 21
Infraspinatus	9.00 ± 0.02^{a}	14.03 ± 0.01^{b}	22.60 ± 0.05^{c}	29.53 ± 0.07^{d}
Quadriceps femoris	10.35 ± 0.05^{a}	14.67 ± 0.05^{b}	22.77 ± 0.05^{c}	28.22 ± 0.03^{d}
Longissimus dorsi	8.90 ± 0.06^{a}	13.13 ± 0.06^{b}	20.34 ± 0.06^{c}	25.24 ± 0.04^{d}
Rhomboideus	7.94 ± 0.04^{a}	11.30 ± 0.06^{b}	17.08 ± 0.07^{c}	24.22 ± 0.02^{d}

Mean values having different superscripts within a row are significantly different at $p < 0.05$. Sample size, (n=3)

As expected with increased storage time the amount of metmyoglobin in all muscle types tend to increase too. This finding is consistent with Jeremiah and Gibson (2000) who stated that metmyoglobin formation increased over time, regardless of muscle type (Behrends, 2004). Once formation of metmyoglobin started it also acts as a pro-oxidant for the beginning of lipid oxidation process (Seydim, *et al.* 2006).

It is hypothesized that pigment and lipid oxidation are linked. Muscle which has the highest metmyoglobin content also had the highest amount of lipid oxidation (O'Sullivan, *et al.* 2004).

Furthermore, according to Faustman and Cassens (1991) muscles which have greater oxygen consumption rate discolored more rapidly. The oxygen consumption rate is an important issue for muscle color stability but maintained that enzymatic reducing activity was the most important factor determining metmyoglobin accumulation in different muscles.

4.9. Determination of lipid oxidation (TBARS)

The initial (Day 0) TBARS values of the four muscles were lower when compared to the other test days. Muscle infraspinatus has the highest and muscle longissimus dorsi has the least amount of TBA at day 0. A significant difference ($p < 0.05$) was observed for mean TBARS values within duration of storage, with increased storage time the value of TBARS also show an increase regardless of muscle type (Table 8).

Table 8. TBARS (mg/kg) value of the beef samples over test days

Muscle	Day 0	Day 7	Day 14	Day 21
Infraspinatus	0.57 ± 0.03^{a}	0.64 ± 0.01^{b}	0.76 ± 0.02^{c}	0.83 ± 0.05^{d}
Quadriceps femoris	0.42 ± 0.07^{a}	0.49 ± 0.03^{b}	0.58 ± 0.02^{c}	0.65 ± 0.03^{d}
Longissimus dorsi	0.24 ± 0.02^{a}	0.28 ± 0.04^{b}	0.35 ± 0.05^{c}	0.58 ± 0.06^{d}
Rhomboideus	0.31 ± 0.08^{a}	0.37 ± 0.05^{b}	0.43 ± 0.03^{c}	0.49 ± 0.07^{d}

Mean values having different superscripts within a row are significantly different at $p < 0.05$. Sample size, (n=3)

Lipid oxidation and Mb oxidation were closely related in meat with an increase in one resulting in a similar increase for the other. This was thought to be related to direct Mb oxidation or destruction of Mb reducing systems by free radicals during lipid oxidation (Calkins and Hodgen, 2007). TBARS estimates the formations of secondary reactive lipid oxidation products, such as

aldehydes, which form covalent adducts with myoglobin, accelerating heme oxidation followed by meat discoloration (Joseph, *et al.* 2012).

In the current study the increase of metmyoglobin is in similar trend as of lipid oxidation (see Table 7 and Table 8). When lipids are oxidized, free radicals are produced, which cause a destruction of the pigments, thereby causing increased discoloration and metmyoglobin formation (Behrends, 2004).

The difference between the content of TBARS value between the muscle types might be related to their heme pigment levels. Meat with a higher pigment content is more oxidative, and thus its colour is less stable (Florek, *et al.* 2009).

Comparison of different TBA numbers from other findings is difficult because TBA method has been subjected to several modifications and also the comparison of TBA numbers from different tests did not seem to be meaningful. In TBARS test, due to the possibility of malonaldehyde to react with protein and the interfering action of other compounds, it is generally employed in comparing samples of the same material at different stages of oxidation rather than determining the exact amount of secondary oxidation products (Ferioli, 2007).

It is concluded that, the different TBA determinations can lead to different results and interpretations. However, the TBA evaluation has been a very useful tool to study the effect of several treatments and processes on lipid oxidation (Pensel, 1990).

4.10. Surface color (Lab values)

4.10.1. Hunter L* Color Values (Lightness)

From Fig.19, it can be seen that rhomboideus has the highest amount of L* value (lighter) whereas quadriceps femoris exhibit the lowest score for L* value (darker) at all test days. Hunter L* values are commonly used to evaluate the lightness of steaks. This value is possibly related with the fiber characteristic.

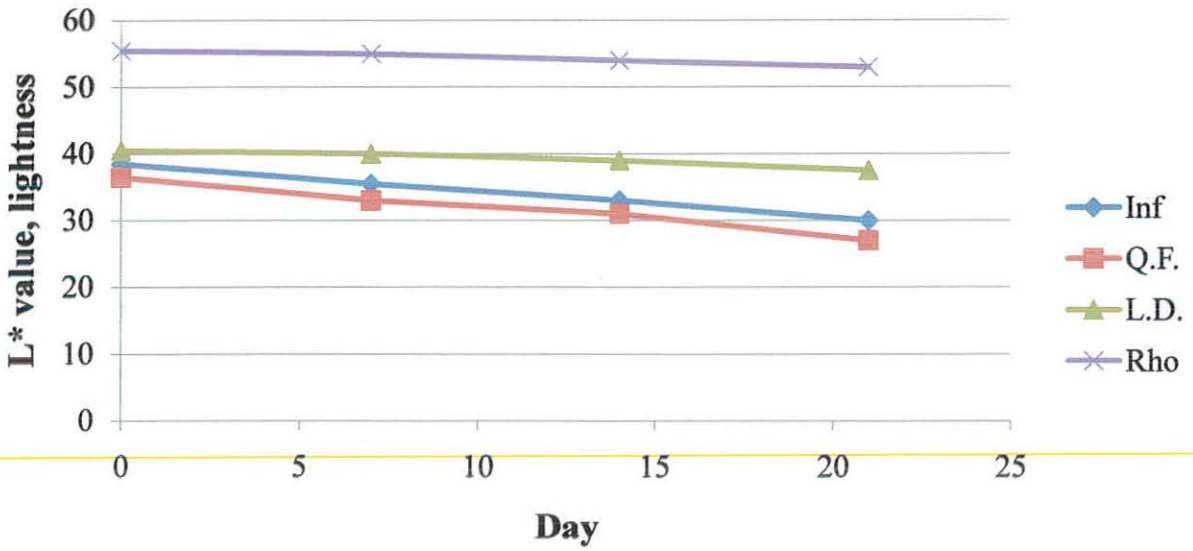


Figure 19. L* values at different test day

Q.F. = (Quadriceps femoris) Inf = (Infraspinatus)

L.D. = (Longissimus dorsi) R = (Rhomboideus)

The L* value show a significant decline ($p < 0.05$) in all muscle types with increased storage time. From this it can be seen that muscles act differently during different storage conditions in terms of their lightness. The reasonable explanation for the decreasing of L* value through test days is, vacuum packaged fresh meat has a dark, purplish red color. The dark, purplish red color occurs because oxygen has been removed from the package and reducing enzymes have converted the meat pigment back to myoglobin (Boles and Pegg, 2005).

4.10.2. Hunter a* Values (Redness)

Throughout the test days the highest amount of a* (redness) value is subjected to muscle quadriceps femoris and the least belongs to muscle rhomboideus. The a* values (redness) tends to be a predictor of color. From Fig. 20 it can be noted that different muscle types have different color stability through storage duration.

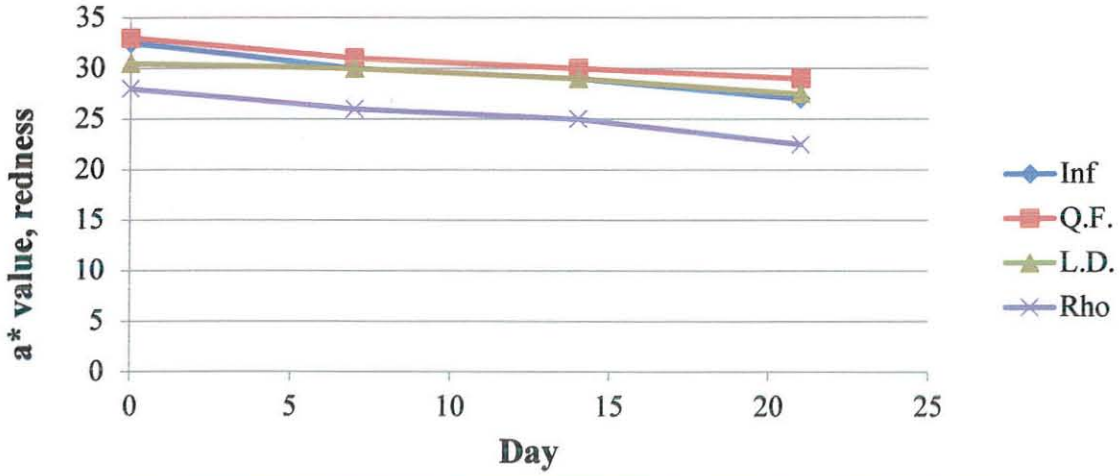


Figure 20. a* values at different test day

Q.F. = (Quadriceps femoris) Inf = (Infraspinatus)

L.D. = (Longissimus dorsi) R = (Rhomboideus)

Studies reported that a* values were negatively related to the duration of storage as shown by a decrease in a* values as time increased (Behrends, 2004). The decrease of a* (redness) value can be the result of gradual accumulation of metmyoglobin on the meat surface, as they are negatively correlated (Florek, *et al.* 2009).

The color stability between the muscle types vary considerable, this can be explained by distribution of muscle fiber across muscles that vary in location and function within the living animal (King, *et al.* 2011).

The current research is in agreement with several authors that reported the stability of muscle longissimus (McKenna, 2003; Behrends, 2004). The stability of this muscle can be related with its least metmyoglobin concentrations (King, *et al.* 2011). In addition the abundance of mitochondria in muscle infraspinatus can be the reason for the rate of oxidation and color instability (Florek, *et al.* 2009).

4.10.3. Hunter b* Values (Yellowness)

On fig. 21 it is indicated that at the beginning of the trial the b* (yellowness) values are higher when compared to the later stages of the experiment. At day 0 the b* values of muscle quadriceps femoris, infraspinatus and longissimus dorsi doesn't seem to vary that much, but through time the values started to show differences in the rate of change in b* value. In all muscles, yellowness decreased over the course of storage period.

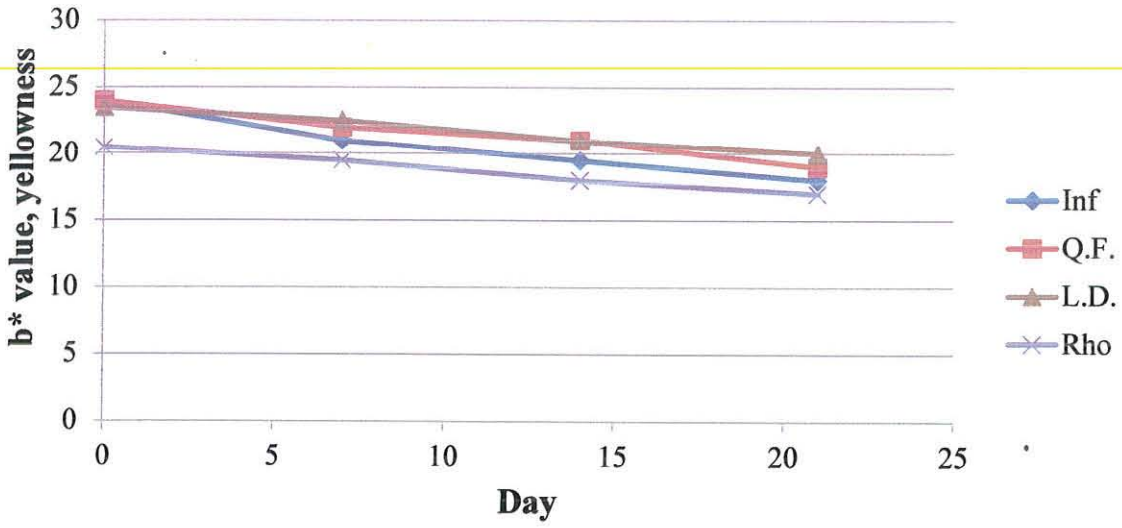


Figure 21. b* values at different test day

Q.F. = (Quadriceps femoris) Inf = (Infraspinatus)

L.D. = (Longissimus dorsi) R = (Rhomboideus)

The muscles tend to show similar b* values at the beginning of the trial but as storage time increases they become more differentiated. This variation can be explained by each individual muscle's own unique biological and biochemical properties as a result various muscle types have different rate of discoloration and shelf-life, for example beef chuck is considered to be the least color-stable muscle (Page, *et al.* 2001).

This study is similar with the finding of Lee, *et al.* (2001) who documented that increased storage time will result in an increase in pH value and decrease in all Lab values of meat samples.

In the current research, it has been observed that declining L*, a*, and b* values are accompanied by increasing muscle pH (see Fig 18). It has been reported that the values of a* and b* are highly related to muscle pH than value of L*. L* value is highly correlated with lean maturity, which shows that lean maturity is more a function of lightness/darkness, whereas muscle pH affects muscle color by altering hue (red, yellow, green, blue, or an intermediate) more than lightness/darkness (Seggern, *et al.* 2005).

4.11. Optical scattering properties

In this study distribution of optical scattering coefficients in the studied muscles has been measured (Figures 22 and 23) at day 0 and day 21 respectively. In Fig. 22 it was shown that at day 0, the scattering coefficient of longissimus dorsi muscle has the highest value among these muscles. Rhomboid and quadriceps femoris muscles have similar scattering distributions intensity. The least intensity of scatter was recorded for muscle infraspinatus.

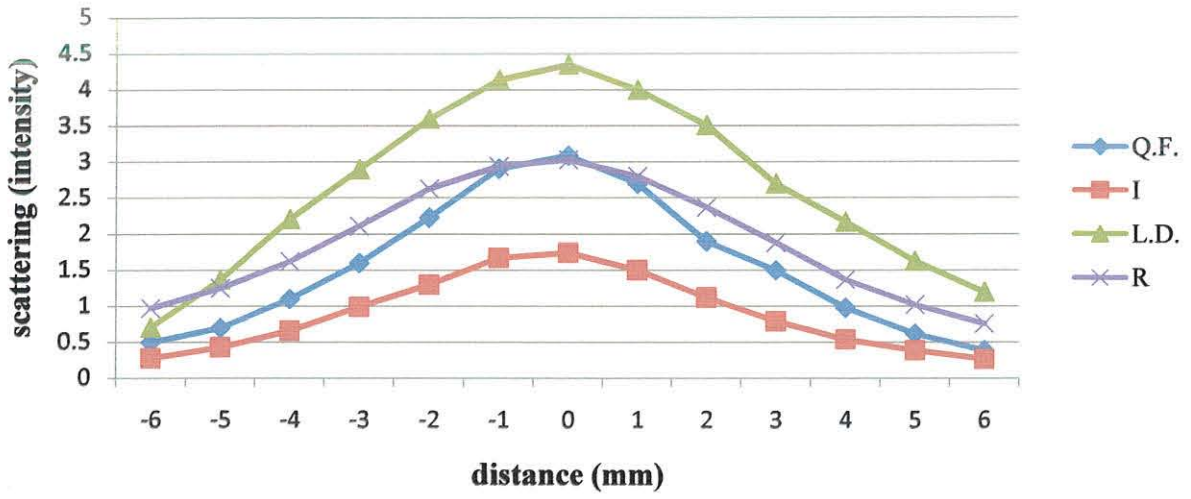


Figure 22. Optical distribution of the muscles on day 0

Q.F. = (Quadriceps femoris) Inf = (Infraspinatus)

L.D. = (Longissimus dorsi) R = (Rhomboideus)

Normally, scattering property in beef muscles is directly related to tenderness property of the muscles. In this study, however, this observation was not evidenced. Nevertheless, this relationship could be affected by a number of pre-slaughter and post-slaughter processing practices. Consequently, this finding report is consistent with the work of Yao, *et al.* (2006), who stated that, there is a correlation between optical scattering and Warner-Bratzler shear force. This correlation indicates that when Warner-Bratzler shear force increases the scattering coefficient also increases.

Based on this correlation, muscle longissimus dorsi muscle has the highest scattering coefficient which also means that it is the toughest muscle from the rest of the other muscles followed by rhomboideus and quadriceps femoris muscles which shows similar scattering distributions in quantity and finally infraspinatus muscle.

Based on Yao and Berg (2008) it can be hypothesized that optical scattering coefficient shows the tenderness-related muscle structural properties such as sarcomere length and collagen content. The relation shown here is mainly brought by variation in collagen content. However, the variations in sarcomere length and proteolysis also play important roles in determining the correlation between scattering coefficients and Warner-Bratzler shear force.

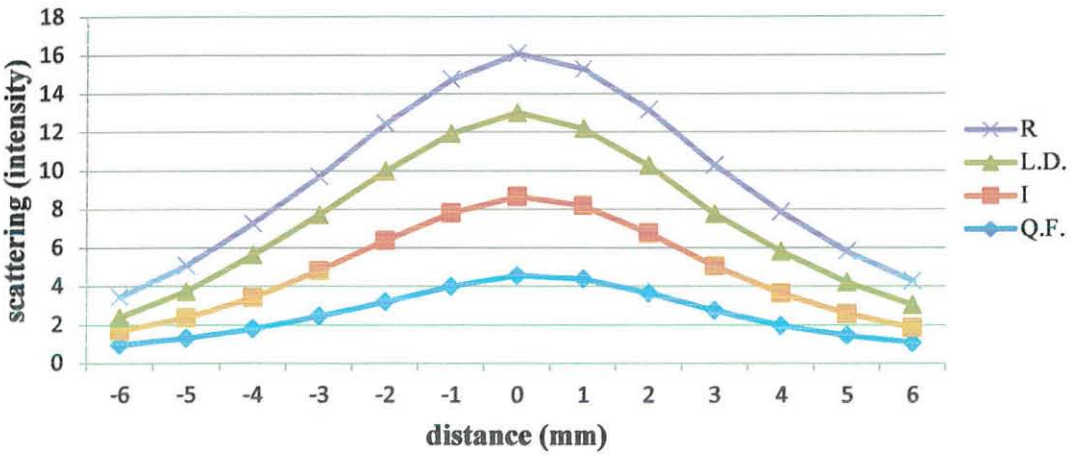


Figure 23. Optical scattering properties of the four muscle types at day 21

Q.F. = (Quadriceps femoris) Inf = (Infraspinatus)

L.D. = (Longissimus dorsi) R = (Rhomboideus)

The reason for muscle rhomboidus to show the highest scattering coefficient at day 21 (Fig. 23) is that it has improved in tenderness more than the rest of the muscles because aging of muscle under controlled environment tends to improve the tenderness characters of muscles. Improvement of tenderness through storage time has been reported in many studies (Dinh, 2008; Anderson, 2011).

While looking at the four muscles, each have different optical scattering properties, this indicate that the fundamental structural and contractile units in each skeletal muscles are different bases on their functions by their anatomical locations in the animal.

In contrast with the previous studies (Xia, *et al.* 2008) states that WBSF (Warner bratzler shear force) have a negative correlation with optical scattering properties of meat. The content of connective tissue and collagen solubility are the main factors which assist in tenderness among muscles. High collagen content results in higher WBSF in addition to this, high collagen content result in higher reduced scattering coefficient.

In general the correlation between optical scattering and beef tenderness depends on the primary mechanism regulating tenderness in a specific muscle. In samples where tenderness variation is due to protein-protein interactions, manifested as sarcomere length, larger optical scattering indicates increased tenderness. Conversely, in muscles whose tenderness variations are due to collagen content, a higher optical scattering implies tougher meat. Finally in muscle samples that are heavily influenced by aging, increased optical scattering is correlated with improved tenderness (Xia, *et al.* 2008).

4.12. Sensory analysis

4.12.1. Sensory Color Evaluation

The highest mean of color score was recorded on day 0. While, the least mean score of this attribute was observed between test days 14 and 21 (Table 9). The initial visual color of samples from quadriceps femoris and longissimus dorsi was relatively higher than those of muscle infraspinatus and rhomboideus.

Table 9. Sensory scores of color value over test days

Muscle	Day 0	Day 7	Day 14	Day 21
Infraspinatus	3.97 ± 0.53^{c}	3.42 ± 0.53^{b}	2.42 ± 0.53^{a}	2.57 ± 0.53^{a}
Quadriceps femoris	4.28 ± 0.75^{b}	4.00 ± 0.81^{b}	3.57 ± 0.53^{b}	2.71 ± 0.48^{a}
Longissimus dorsi	4.28 ± 0.48^{b}	4.14 ± 0.37^{b}	3.57 ± 0.53^{a}	3.14 ± 0.37^{a}
Rhomboideus	4.14 ± 0.37^{c}	3.28 ± 0.48^{b}	3.00 ± 0.57^{ab}	2.87 ± 0.78^{a}

Mean values having different superscripts within a row are significantly different at $p < 0.05$. Sample size, (n=15)

Storage days gave significant effect on mean color scores. The highest means were recorded for test day 0 with significant ($p < 0.05$) decrease up to test day 21. Therefore, the length of time which meat was kept in chilled storage had an effect on the rate of different sensory attributes. This finding is consistent with the results of (McKenna, 2003) who stated muscle infraspinatus is grouped under muscles which have low color stability. Moreover, it has been indicated that muscle infraspinatus can be one of the muscles, with high off-flavour properties, due to its high concentration of mitochondria while compared with the rest of the muscles (Calkins and Hodgen, 2007).

4.12.2. Sensory aroma evaluation

In all the muscle types the highest value recorded at the beginning of the trial and the least score was documented at day 21. During storage time there was a decline on qualities of sensory attribute regarding aroma (Table 10).

Table 10. Sensory scores of aroma value over test days

Muscle	Day 0	Day 7	Day 14	Day 21
Infraspinatus	4.14 ± 0.37^{d}	3.42 ± 0.53^{c}	2.57 ± 0.53^{b}	1.71 ± 0.48^{a}
Quadriceps femoris	4.14 ± 0.89^{c}	3.85 ± 0.69^{ab}	3.14 ± 0.69^{b}	2.00 ± 0.57^{a}
Longissimus dorsi	4.28 ± 0.75^{b}	4.00 ± 0.57^{b}	3.71 ± 0.48^{b}	2.71 ± 0.48^{a}
Rhomboideus	3.85 ± 0.69^{b}	4.00 ± 0.81^{a}	3.57 ± 0.78^{a}	3.14 ± 0.69^{a}

Mean values having different superscripts within a row are significantly different at $p < 0.05$. Sample size, (n=15)

At day 0, muscle longissimus dorsi was rated the highest followed by quadriceps femoris and infraspinatus. The least flavor intensity was recorded for muscle rhomboideus. This finding was in agreement with Calkins and Hodgen (2007); Wadhwani (2008) which states that muscle longissimus dorsi has the highest flavor intensity and that muscle rhomboideus had the lowest flavor intensity when compared to other muscle type. Muscle quadriceps femoris had the highest off flavor when compared to longissimus dorsi and the lowest while compared with infraspinatus, which is in agreement with (Brewer, 2006).

Muscle infraspinatus got the lowest score at the end of the trial. This might be due to its high production of hexanal, which is an indicator of rancid odour and lipid oxidation development (Brewer, 2006). In addition muscle infraspinatus is one of the muscles, with high off-flavour properties (Calkins and Hodgen, 2007).

The reason for the degradation of aroma value at day 21 can be as a result of lipid oxidation. Lipid oxidation can occur in foods containing significant amount of fat, like meat. This process causes aroma changes result from new volatile odorous compounds, flavour changes are caused by hydroxy acids. Further oxidation results in the formation of epoxides, cyclic peroxides and

bicyclic endoperoxides. Some of these compounds have distinct aromas which can affect the sensory aroma scores of muscle foods (Kanner and Rosenthal, 1992).

4.12.3. Sensory texture evaluation

Table 11 shows the texture scores of the samples at different test days. It shows that the highest score for texture is recorded at the beginning of the trial and the scores tend to decline as the duration of storage increases.

Table 11. Sensory scores of texture value over test days

Muscle	Day 0	Day 7	Day 14	Day 21
Infraspinatus	3.85 ± 0.69^{b}	3.42 ± 0.53^{b}	2.42 ± 0.53^{a}	2.42 ± 0.53^{a}
Quadriceps femoris	4.57 ± 0.53^{c}	4.14 ± 0.37^{ab}	3.71 ± 0.48^{b}	3.00 ± 1.00^{a}
Longissimus dorsi	4.28 ± 0.95^{c}	4.00 ± 0.00^{bc}	3.42 ± 0.53^{b}	2.85 ± 0.69^{a}
Rhomboideus	3.71 ± 0.48^{b}	3.57 ± 0.53^{b}	3.14 ± 0.37^{ab}	2.85 ± 0.69^{a}

Mean values having different superscripts within a row are significantly different at $p < 0.05$. Sample size, (n=15)

The texture values of each main effect show decline with increased storage duration. This finding was consistent with Przysiezna (2007) who concluded the texture of meat that has been stored for a prolonged time will be degraded by endogenous proteases and lipid oxidation.

The current research disagreed with published data in which trained panelists found improvement in texture characteristics of meat samples through increased storage time (Stanišić, *et al.* 2012). The reason for disagreement may be that the sensory scores in this study were done with semi-trained consumers; this might cause a difference in the texture sensory scores with personal preference (Wheeler, *et al.* 2004).

It has been reported that muscle infraspinatus is one of the most tender muscle cuts in beef (Rhee, *et al.* 2004). But in the recent study, muscle quadriceps femoris were stated as the most tender muscle of the four samples. This result was likely due to the consumer preference of

muscle cuts. The present study has established that, there is wide variability in the ability of individual semi trained consumers to accurately and repeatedly detect differences in beef steak texture.

The scores for sensory texture values show decline with increasing storage time in all muscle types. According to Dalton (2005) freezing and frozen conditions influence the size and dispersal of ice crystals, which consequently negatively affect the texture of the meat.

4.12.4. Overall sensory evaluation

For overall sensory score at day 0 muscle quadriceps femoris and longissimus dorsi rated the highest followed by muscle infraspinatus; whereas muscle rhomboideus ranked the lowest for overall sensory properties (Table 12).

Table 12. Overall sensory scores over test days

Muscle	Day 0	Day 7	Day 14	Day 21
Infraspinatus	4.00 ± 0.00^{d}	3.64 ± 0.24^{c}	2.64 ± 0.47^{b}	2.00 ± 0.00^{a}
Quadriceps femoris	4.35 ± 0.55^{c}	4.00 ± 0.50^{ab}	3.64 ± 0.47^{b}	2.57 ± 0.53^{a}
Longissimus dorsi	4.28 ± 0.48^{c}	$4.07 \pm 0.18b^{c}$	3.71 ± 0.39^{b}	3.07 ± 0.18^{a}
Rhomboideus	3.85 ± 0.24^{b}	3.57 ± 0.34^{b}	3.00 ± 0.50^{a}	2.92 ± 0.60^{a}

Mean values having different superscripts within a row are significantly different at $p < 0.05$. Sample size, (n=15)

The highest sensory scores belong to muscle quadriceps femoris and longissimus dorsi because of their high total pigment value (appears more red than the rest of the muscles, see Table 9) which most customers relate with freshness and wholesomeness.

The lowest overall sensory for muscle infraspinatus might be related with its susceptibility to oxidation and development of off-flavor (McKenna, 2003). Likewise the reasonable explanation

for muscle rhomboideus least score of overall acceptability might have to do with its high marbling score. High marbling score is mostly related with unhealthy choice of hence high marbling score of rhomboideus muscle may negatively affected the panelists increased desire for healthy and more nutritious meat cut (Pedrão, *et al.* 2009).

At day 21, the scores of all sensory parameters show significant decline. Lipid oxidation has the potential to cause a decline in quality attributes of muscle foods. The reason for the unattractive, darken color of the muscles is the result of condensation reaction between oxidation products and proteins, the change of aroma caused by new volatile odorous compounds and finally the change in texture might be because of the oxidative induction of protein crosslinks (Kanner and Rosenthal, 1992).

5. Conclusion

Postmortem biochemical processes influence the color of muscle foods, which is a major trait governing consumer perception of wholesomeness. Meat spoilage leads to the development of off- flavours, off-odors and often slime formation due to the breakdown of valuable contents (fat, protein and carbohydrates) which make the product undesirable for human consumption.

Nutritive value degradation and lipid oxidation in muscle infraspinatus, quadriceps femoris, longissimus dorsi and rhomboideus occur in different ways and at different postmortem times. Best keeping quality belongs to muscle longissimus dorsi.

Consumer acceptability rates declines as the storage time increases. This is due to the change in pH, metmyoglobin, lipid oxidation and L*a*b* value. In the four test days the muscles become darker and unattractive to consumers.

The nutritional values of these muscles decreased with increased storage time. The nutritional value of the muscles remained high until day 7. The change in nutritional value was because of oxidation which causes degradation in proximate values. Although the nutritive values of these four muscles does not decline that much, consumers won't prefer them because of the dark appearance of the muscles, which makes them appear spoiled.

Postmortem aging affected all of the muscles evaluated in this study in various degrees. Better understanding of storage condition for each muscle's with their biochemical and physical attributes, the industry can target individual muscles through processing or packaging strategies to help improve maximize color shelf-life and retard rate of oxidation in individual muscles.

6. Recommendation

On the basis of the present study, the following recommendations are forwarded;

1. It is recommended that the four cuts of beef cuts investigated be stored under vacuum seal at -20^{0}C for no more than 7 days with no appreciable loss of keeping quality.
2. Vacuum packaging and freezing doesn't provide protection from lipid oxidation to the muscles for more than 7 days. So, addition of antioxidants may be one option to consider for export abattoirs that require to keep beef muscles under freeze storage for an extended period of time.
3. The storage of the four muscle types under vacuum at -20^{0}C pack should not extend beyond 7 days, as there will be loss of important nutritive values due to drip loss.
4. General observations indicate that hygiene and sanitation conditions in butcheries around Barh Dar town require improvements. The Bahr Dar city administration is recommended to play an active role in promulgating new standards and regulations that take public safety as a priority.
5. Investigation of other packaging and storage conditions is needed to keep beef muscles at desirable sensory and nutritional quality beyond 7 days.

References

Adzitey, F. and H. Nurul (2011). "Pale soft exudative (PSE) and dark firm dry (DFD) meats: causes and measures to reduce these incidences-a mini review." International Food Research Journal **18**(1).

Anderson, M. J. (2011). "Identification of proteins and biological processes associated with tenderness in beef muscles."

Baron, C. P. and H. J. Andersen (2002). "Myoglobin-induced lipid oxidation. A review." Journal of Agricultural and Food Chemistry **50**(14): 3887-3897.

Behrends, J. M. (2004). Metmyoglobin reducing ability and visual characteristics of nine selected bovine muscles, Texas A&M University.

Bess, K. N. (2011). Effects of various salt purity levels on lipid oxidation and sensory characteristics of ground turkey and pork, University of Illinois.

Boe, R. (2012). "Oxidative stability, lipid composition and nutritional value of ruminant meat as affected by animal feeding system, sex and common household treatments."

Boles, J. A. and R. Pegg (2005). "Meat color." Montana State University and Saskatchewan Food Product Innovation Program.

Brackebusch, S., F. McKeith, et al. (1991). "Relationship between longissimus composition and the composition of other major muscles of the beef carcass." Journal of animal science **69**(2): 631-640.

Bratcher, C. L. G. (2004). The Effects of Quality Grade, Aging and Location on Selected Muscles of Locomotion of the Beef Chuck and Round, University of Florida.

Brewer, S. (2006). "The chemistry of beef flavor-executive summary." Online source: http://www. beefresearch. org/CMDocs/BeefResearch/The% 20Chemistry% 20of% 20Beef% 20Flavor. pdf.

Budras, K. D., R. E. Habel, et al. (2011). Bovine anatomy: an illustrated text, Manson Publishing.

Calkins, C. and J. Hodgen (2007). "A fresh look at meat flavor." Meat Science **77**(1): 63-80.

Carpenter, C. E., D. P. Cornforth, et al. (2001). "Consumer preferences for beef color and packaging did not affect eating satisfaction." Meat Science **57**(4): 359-363.

Cascone, A. (2006). Study and prevention of lipid oxidation in meat, Università degli Studi di Napoli Federico II.

Chaijan, M. (2008). "Review: Lipid and myoglobin oxidations in muscle foods." Songklanakarin Journal of Science & Technology **30**(1).

Chaijan, M. (2008). "Review: Lipid and myoglobin oxidations in muscle foods." Songklanakarin. J. Sci. Technol **30**: 47-53.

D'agata, M., R. Nuvoloni, et al. (2010). "Effect of packaging and storage time on beef qualitative and microbial traits." Journal of Food Quality **33**(s1): 352-366.

Dalton, M. A. (2005). The effects of fresh and frozen storage on palatability, oxidative rancidity and color of packaged beef steaks, Oklahoma State University.

Dave, D. and A. E. Ghaly (2011). "Meat spoilage mechanisms and preservation techniques: A critical review." American Journal of Agricultural and Biological Sciences **6**(4): 486.

Dinh, N. T. T. (2008). "Meat quality: understanding of meat tenderness and influence of fat content on meat flavor." Tạp chí Phát triển Khoa học và Công nghệ **9**(12).

Dransfield, E. and A. Sosnicki (1999). "Relationship between muscle growth and poultry meat quality." Poultry science **78**(5): 743-746.

ElMasry, G. and D.-W. Sun (2010). "Meat quality assessment using a hyperspectral imaging system." Hyperspectral imaging for food quality analysis and control: 175-240.

Fang, Y.-Z., S. Yang, et al. (2002). "Free radicals, antioxidants, and nutrition." Nutrition **18**(10): 872-879.

Faustman, C., Q. Sun, et al. (2010). "Myoglobin and lipid oxidation interactions: Mechanistic bases and control." Meat Science **86**(1): 86-94.

Ferioli, F. (2007). "Application of chromatographic and spectroscopic techniques in the evaluation of the lipid fraction of animal products."

Florek, M., Z. Litwinczuk, et al. (2009). "Influence of slaughter season of calves and ageing time on meat quality." Polish Journal of Food and Nutrition Sciences **59**(4): 309-314.

Huff-Lonergan, E. and S. M. Lonergan (2005). "Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes." Meat Science **71**(1): 194-204.

Hui, Y. H. (2006). Handbook of food science, technology, and engineering, CRC press.

Ismail, H., E. Lee, et al. (2009). "Fat content influences the color, lipid oxidation, and volatiles of irradiated ground beef." Journal of food science **74**(6): C432-C440.

Ismail, H., E. J. Lee, et al. (2009). "Effect of antioxidant application methods on the color, lipid oxidation, and volatiles of irradiated ground beef." Journal of food science **74**(1): C25-C32.

Istrati, D., O. Constantin, et al. (2011). "Study of the combined effect of spices and marination on beef meat vacuum packaged." Annals of the University Dunarea de Jos of Galati. Fascicle VI: Food Technology **35**.

Jha, S. N. (2010). Colour measurements and modeling. Nondestructive Evaluation of Food Quality, Springer**:** 17-40.

Jones, S. J., A. Guru, et al. (2007). "Bovine myology and muscle profiling." Cattlemen's Beef Board and National Cattlemen's Beef Association (Colorado, 2005), pp. cover: 1-33.

Joseph, P., S. P. Suman, et al. (2012). "Proteomics of muscle-specific beef color stability." Journal of Agricultural and Food Chemistry **60**(12): 3196-3203.

Kanner, J. and I. Rosenthal (1992). "An assessment of lipid oxidation in foods." Pure and applied chemistry **64**(12): 1959-1964.

Karakok, S. G., Y. Ozogul, et al. (2010). "Proximate analysis. Fatty acid profiles and mineral contents of meats: a comparative study." Journal of Muscle Foods **21**(2): 210-223.

Karwowska, M. and Z. J. Dolatowski (2007). "The effect of natural antioxidants on the oxidative processes in beef." Acta Sci. Pol., Technol. Aliment **6**(1): 17-25.

Ke, S., Y. Huang, et al. (2009). "Impact of citric acid on the tenderness, microstructure and oxidative stability of beef muscle." Meat Science **82**(1): 113-118.

King, D., S. Shackelford, et al. (2011). "Relative contributions of animal and muscle effects to variation in beef lean color stability." Journal of animal science **89**(5): 1434-1451.

Kirchofer, K., C. R. Calkins, et al. (2002). "Fiber-type composition of muscles of the beef chuck and round." Journal of animal science **80**(11): 2872-2878.

Klont, R., L. Brocks, et al. (1998). "Muscle fibre type and meat quality." Meat Science **49**: S219-S229.

Kuzelov, A., N. Taskov, et al. (2011). "Changes in the chemical composition of Musculus quadriceps femoris of beef kept at different temperatures." Biotechnology in Animal Husbandry **27**(3): 1083-1090.

Larraín, R. E. (2007). Dietary High-tannin Sorghum and Oxidative Stability of Muscle and Muscle Foods, ProQuest.

Lee, S., Y. Kim, et al. (2001). "Effect of muscle pH and display conditions on surface color in Hanwoo (Korean Native Cattle) beef." Asian-Australian journal of animal sciences **14**(3): 365-371.

Magoro, M. M. (2007). The Sensory, Nutritional and Shelf-life Attributes of Raw Fresh Meat Sausages Containing Beef Offal, Tshwane University of Technology.

Mancini, R. and M. Hunt (2005). "Current research in meat color." Meat Science **71**(1): 100-121.

McKenna, D. R. (2003). Biochemical and physical factors affecting color charactersitics of selected bovine muscles, Texas A&M University.

Met, A., A. Çelik, et al. (2013). "Investigation of different thawing methods combined with freezing rate on slab shape meat quality."

Miller, M. (2007). "Dark, firm and dry beef." National Cattlemen's Beef Association, Centennial, CO.

Min, B. and D. Ahn (2005). "Mechanism of lipid peroxidation in meat and meat products–A review." Food Science and Biotechnology **14**(1): 152-163.

Min, B., K. Nam, et al. (2008). "Endogenous factors affecting oxidative stability of beef loin, pork loin, and chicken breast and thigh meats." Journal of food science **73**(6): C439-C446.

Mohamed, A., B. Jamilah, et al. (2008a). "A review on some factors affecting colour of fresh beef cuts." J. Food, Agric. Env **6**: 181-186.

Mohamed, A., B. Jamilah, et al. (2008b). "A review on lipid oxidation of meat in active and modified atmosphere packaging and usage of some stabilizers." J Food Agric Environ **6**(3&4): 76-81.

Nollet, L. M. and F. Toldrá (2010). Handbook of muscle foods analysis, CRC Press.

O'Sullivan, A., K. O'Sullivan, et al. (2004). "Influence of concentrate composition and forage type on retail packaged beef quality." Journal of animal science **82**(8): 2384-2391.

Ozer, O. and C. SariçObaN (2010). "The effects of butylated hydroxyanisole, ascorbic acid, and α-tocopherol on some quality characteristics of mechanically deboned chicken patty during freeze storage." Czech J. Food Sci **28**: 150-160.

Page, J., D. Wulf, et al. (2001). "A survey of beef muscle color and pH." Journal of animal science **79**(3): 678-687.

Patten, L., J. Hodgen, et al. (2008). "Chemical properties of cow and beef muscles: Benchmarking the differences and similarities." Journal of animal science **86**(8): 1904-1916.

Pedrão, M. R., F. Lassance, et al. (2009). "Comparison of proximate chemical composition and texture of cupim, Rhomboideus m. and lombo, Longissimus dorsi m. of Nelore (Bos indicus)." Brazilian Archives of Biology and Technology **52**(3): 715-720.

Pensel, N. A. (1990). Influence of experimental conditions on porcine muscle and its effect on oxidation, The Ohio State University.

Pethick, D., D. Fergusson, et al. (2005). "Muscle metabolism in relation to genotypic and environmental influences on consumer defined quality of red meat." Indicators of milk and beef quality EAAP Publication **112**: 95-110.

Price, D. M. (2008). Augmentation of Near-infrared (NIR) and In-plant Beef Video Image Analysis (VIA) Systems to Sort Carcasses Into Tenderness Categories, ProQuest.

Przysiezna, E. (2007). "Effect of chill storage time on proteolysis and lipid oxidation in vacuum-packed muscles from duck." Polish Journal of Food and Nutrition Sciences **57**(4): 457-463.

Przybylski, W., G. Monin, M. Kocwin-Podsiadla and E. Krzecio (2006). "Glycogen metabolism in muscle and its effects on meat quality in pigs-a mini review". Polish Journal of Food and Nutrition Sciences **15**, 257.

Quasem, J. M., A. S. Mazahreh, et al. (2009). "Nutritive value of seven varieties of meat products (Sausage) produced in Jordan." Pakistan Journal of Nutrition **8**(4): 332-334.

Raines, C. R. (2008). Intrinsic factors affecting ground beef color stability, ProQuest.

Rathgeber, B. M. (1999). Protein degradation in tltrkey breast muscle, University of Saskatchewan.

Rhee, M., T. Wheeler, et al. (2004). "Variation in palatability and biochemical traits within and among eleven beef muscles." Journal of animal science **82**(2): 534-550.

Rowe, L., K. Maddock, et al. (2004). "Influence of early postmortem protein oxidation on beef quality." Journal of animal science **82**(3): 785-793.

Searls, G. A., R. J. Maddock, et al. (2005). "Intramuscular tenderness variation within four muscles of the beef chuck." Journal of animal science **83**(12): 2835-2842.

Seggern, D. V., C. Calkins, et al. (2005). "Muscle profiling: Characterizing the muscles of the beef chuck and round." Meat Science **71**(1): 39-51.

Seydim, A., J. Acton, et al. (2006). "Effects of packaging atmospheres on shelf-life quality of ground ostrich meat." Meat Science **73**(3): 503-510.

Shahidi, F. and Y. Zhong (2005). "Lipid oxidation: measurement methods." Bailey's industrial oil and fat products.

Sickler, M. L. (2000). Inhibition of lipid oxidation with phosphates in muscle foods, Virginia Polytechnic Institute and State University.

Stanišić, N., M. Petričević, et al. (2012). "Changes of physical-chemical properties of beef during 14 days of chilling." Biotechnology in Animal Husbandry **28**(1): 77-85.

Steele, K., M. Weber, et al. (2012). "LED lighting extends color shelf life for three beef products compared with fluorescent lighting." Beef Cattle Research, 2012 is known as Cattlemen's Day, 2012.

Suman, S. P. and P. Joseph (2013). "Myoglobin chemistry and meat color." Annual review of food science and technology **4**: 79-99.

Sun, Y.-E., W.-D. Wang, et al. (2011). "Autoxidation of unsaturated lipids in food emulsion." Critical reviews in food science and nutrition **51**(5): 453-466.

Tambunan, R. D. (2008). Determination of cured meat pigments on three cured beef muscles. Seminar Nasional Teknologi Peternakan dan Veteriner, Bogor.

von Scheven, C. C. A. (2010). The Anatomy and Function of the Equine Thoracolumbar Longissimus Dorsi Muscle, Citeseer.

W¹sowicz, E., A. Gramza, et al. (2004). "Oxidation of lipids in food." Pol J Food Nutr Sci **13**: 87-100.

Wadhwani, R. (2008). Cause and prevention of liver off-flavor in five beef chuck muscles, ProQuest.

Warriss, P. D., (2010). Meat science: an introductory text. Cabi.

Wheeler, T., S. Shackelford, et al. (2004). "The accuracy and repeatability of untrained laboratory consumer panelists in detecting differences in beef longissimus tenderness." Journal of animal science **82**(2): 557-562.

Wulf, D. and J. Wise (1999). "Measuring muscle color on beef carcasses using the L* a* b* color space." Journal of animal science **77**(9): 2418-2427.

Xia, J., E. Berg, et al. (2007). "Characterizing beef muscles with optical scattering and absorption coefficients in VIS-NIR region." Meat Science **75**(1): 78-83.

Xia, J., A. Weaver, et al. (2008). "Distribution of optical scattering properties in four beef muscles." Sensing and Instrumentation for Food Quality and Safety **2**(2): 75-81.

Yam, K. L. and S. E. Papadakis (2004). "A simple digital imaging method for measuring and analyzing color of food surfaces." Journal of Food Engineering **61**(1): 137-142.

Yao, G., J. Xia, et al. (2006). Optical scattering coefficients are correlated with muscle structure properties. Optics East 2006, International Society for Optics and Photonics.

Yuksel, S., L. Turgut, et al. (2011). "Fatty acid composition, lean color and drip loss of the different muscles from young Holstein Friesian bulls finished on diets containing varied proportions of wet sugar beet pulp and wheat straw." Bulgarian Journal of Agricultural Science **17**(5): 696-703.

Zaujec, et al. (2012). "Comparison of Meat Quality in Bulls and Cows" Journal of Microbiology, Biotechnology and Food Science 1, 1098-1108.

Appendices

Sensory acceptability test

Evaluate the samples according to the numbers indicated and give value from 1-5 for each attribute based on the key given.

If you have any questions please ask the server.

Keys

5 =Like extremely

4 = Like moderately

3 = Neither like nor dislike

2 = Dislike moderately

1 = Dislike extremely

Attributes	123	456	789	147
Color				
Aroma				
Texture				
Overall acceptability				

Preference ranking test

In front of you there are seven coded samples. Taste each sample.

Please indicate your preference by giving score

123_____456_____789_____147_____

1st choice besides the sample that prefer most

2nd choice besides sample that you prefer second

3rd choice beside the sample that you prefer third

4th choice beside the sample that you least prefer

General Comment_____