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EFFECT OF INCANDESCENT DISPLAY LIGHT AND ETHIOPIAN SPICES ON THE QUALITY OF RAW BEEF

By

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DEDICATION

To

My mother Elfinesh Belayneh

And

My father Mengistu Zerihun

Who had dedicated for the education of their children and

could see only flower, but not lucky enough to see the fruits

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LIST OF ABBREVIATIONS/ACRONYMS

ABE	African birds´ eye
ABTS	2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)
AIT	Ally Isothiocyanate
BF	*Biceps Femoris*
BHA	Butylated Hydroxyl Anisole
BHT	Butylated Hydroxyl Toluene
CFU	Colony Forming Units
CIE	Commission Internationale de l'Eclairage
CMYK	Cyan Magenta Yellow Black
COMb	Carboxymyoglobin
CRI	Color Rendering Index
DFD	Dark, Firm and Dry
DMb	Deoxymyoglobin
DPPH	2, 2-Diphenyl-1-Picrylhydrazyl
DW	Dry Weight
FL	Fluorescent
GC-MS	Gas Chromatography-Mass Spectrometry
HSB	Hue, Saturation and Brightness
HSL	Hue, Saturation and Luminance
IC_{50}	Inhibition Concentration by 50%
INC	Incandescent
JPEG	Joint Photographic Experts Group
K/S	Kubelka-Munk scattering (S) and absorbance (K) coefficients
LD	*Longissimus Dorsi*

LPGMS Low Pungency Ground Mustard Seed

MAP Modified atmosphere packaged

Mb Myoglobin

MDA Malondialdehyde

MH Metal halide

MMb Metmyoglobin

nm nanometer

OMb Oxymyoglobin

PCA Plate Count Agar

PSE Pale, Soft and Exudative

RGB Red Green Blue

ROS Reactive Oxygen Species

SM *Semimembranosus*

SPME Solid Phase Micro Extraction

TAB Total Aerobic Bacteria

TBA Thiobarbituric Acid

TBARS Thiobarbituric Acid Reacting Substances

TEAC Trolox Equivalent Antioxidant Capacity

TIFF Tagged-Image File Format

UFA Unsaturated Fatty Acid

ABSTRACT

Meat purchasing decisions are influenced by color more than any other quality factor when selecting beef at retail, because consumers use color as an indicator of freshness and wholesomeness. Due to this, meat retailers usually keep the meat display area well lighted to attract consumers. In Ethiopia almost all butcher shops are displaying the beef carcasses under high power (400 watt) incandescent lamps through an open window of display booth. Displaying carcasses under this condition might have a potential to accelerate lipid oxidation and the formation of dark brown muscle color. Moreover, at these butcheries, many people frequently consume raw meat (*kurt siga* or *kitfo)* by using Ethiopian spices sauce prepared from the powders of chili pepper*,* African birds eye and mustard seeds. Therefore, this study focused on the evaluation of the effect of incandescent display light (at room temperature) and Ethiopian spices on the color and oxidation stability of raw beef.

The beef samples which used in this study were bought from butcher shops (Addis Ababa, Ethiopia and Ghent, Belgium) from muscle of *semitendinosus, longissimus dorsi* and *triceps brachii.* The spices samples which are commonly consumed with raw beef were chili pepper (c*apsicum annuum)*, African birds eye (c*apsicu*m *minimum)* and mustard seed (b*rassica nigra),* were purchased from *Merkato* market, Addis Ababa. The analyses undertaken in this research were color change of displayed raw beef slices (L*, a*, b*, Chroma, hue angle & ΔE, by colorimeter and image analysis), lipid oxidation (TBARS), myoglobin species content (oxymyoglobin and metmyoglobin), total aerobically count, temperature elevation, antioxidant activities (free radical scavenging/DPPH, reducing power, TEAC/ABTS), total phenol, total flavonoids and SPME-GC-MS to identify aromatic compounds of mustard seeds.

The raw beef slices exposed under incandescent light continuously at 6280±210 lux showed higher TBARS value, ΔE, hue angle & MMb%, and lower L*, a*, b*, Chroma & OMb% value than the raw beef slices kept in the dark at room temperature as the light exposure time increased. These results are a clear evidence of the influence of high power incandescent display light and the light exposure time on color and oxidation stability of raw beef. Moreover, the covered raw beef samples have more color stability than the opened displayed raw beef samples. However, the covered raw beef samples exposed to incandescent light exhibited significantly lower oxidation stability and higher temperature on meat surface compared to open displayed

raw beef samples. One of the interesting result was that the yellowness (b*) value of raw beef sample exhibited greater value than the redness (a*) value of covered displayed beef samples compared to opened displayed beef samples. At starting time (0hr) a* value was significantly higher ($p<0.05$) than b* value but after display, the covered sample acquired higher b* value than a* value in all the displayed periods. This study also revealed that the color stability of displayed raw beef was highly related to the muscle type. Among the selected three beef muscle types the *semitendinosus* showed better color stability than the *longissimus dorsi* and *triceps brachii* muscle types and the *triceps brachii* exhibited the lowest color stability.

The specific wavelengths of the light spectrum upon meat color stability were evaluated by filtered out short wavelength 450 nm (blue), medium wavelengths (green & yellow) and long wavelength 650 nm (red). The display light from the shorter wavelengths (450 nm) exhibited a higher discoloration on raw beef. However, the display light from the long wavelength (red, 650 nm) was reduced the discoloration of displayed raw meat. The ΔL*, Δa* and ΔE* values of short wavelength of visible light (blue spectra at 450 nm) was significantly higher ($p < 0.05$) than long wavelength (red, 650 nm). In addition, the rate of decrease of the color parameter L*, a*, b* and chroma values was higher in transparent (4.99±0.06 lux) and yellow filters (4.50±0.06 lux), which have higher illuminance than others.

It was also observed that the selected spices extracts were found to exhibit effective lipid antioxidant activity of raw beef samples which were stored in a dark refrigerator for two weeks. All these spices extract enhanced color stability in raw beef especially until one week of storage period in a dark refrigerator. In addition, all spices extract performed well in preventing the growth of aerobic bacteria on the surface of spices treated raw beef samples during two weeks storage period and they were able to maintain fresh color as compared to the controls which were not treated by the spices. However, the dosage of spices extracts had no significant difference on color and oxidation stability of raw beef samples.

The spices analyzed were rich in total phenol and flavonoids with high antioxidant activities. Mustard seed, African bird‘s eye and chili pepper showed IC_{50} value of 85 μg/ml, 172 μg/ml and 186 μg/ml respectively, in DPPH method. The TEAC value of chili pepper, African bird‘s eye and mustard seed were 5.75±0.07, 5.75±0.04 and 5.22±0.09 mgTE/g, respectively. The reducing power of the spices extracts increased with increasing concentration and these extracts exhibited

the following order: mustard seed > pepper > African bird‘s eye. Total phenolic and total flavonoid contents of spices extracts showed the following order: chili chili pepper > African bird‘s eye > mustard seed. The chili pepper (*capsicum annuum*) exhibited the highest total phenolic and total flavonoid contents, 16.81±0.25 mg galic acid equivalent per gram of dry weight and 8.74±0.91 mg catichin equivalent per gram of dry weight respectively. The mustard seed extracts were lowest comparing to the two spices, 10.03±0.52 mg galic acid equivalent per gram of dry weight and 0.36±0.26 mg catichin equivalent per gram of dry weight respectively. The germinated mustard seeds exhibited more antioxidant activity, and total phenol and flavonoids contents than the raw/dry mustard seeds and showed a consistent trend, where 3 days germinated > 1day germinated > raw mustard seeds. In the analysis of aroma compounds of mustard seeds, several sulfur contained compounds were identified by SPME-GC-MS. Within the sulfur contained compounds, the ally isothiocyanate was the most dominant.

In conclusion, high power incandescent display light decreased meat color quality faster than meat samples stored in the dark. In addition, the display light from the shorter wavelengths exhibited a higher discoloration on raw beef samples than the long wavelength. Hence, the use of display light sources with a reduced radiation in the short wavelength range is necessary to reduce the deterioration in quality of displayed meat. Generally, use of high intensity lighting for meat display may result in more and faster meat sales, but if sales do not keep pace with discoloration of meat, loss of profit may result. Moreover, the Ethiopian spices are rich in phenolic compound and demonstrated good antioxidant activity. Hence, people eating them with raw beef may benefit as they can to protect themselves against oxidative damage. These spices can be used as a source of antioxidant for production of shelf stable food products and also as a source of healthy compounds for the development of dietary supplements via advanced research and development activities.

CHAPTER 1. INTRODUCTION

The appearance of food products plays a major role in their acceptability and the decision to purchase a product (Barbut S., 2001).The sensory properties used by consumers most often to assess meat quality are appearance (color), texture (tenderness) and flavour/aroma (Liu *et al*., 1995). Meat purchasing decisions are influenced by color more than any other quality factor when selecting beef at retail (Liu *et al*., 1996), because consumers use discoloration as an indicator of freshness and wholesomeness. Due to this, meat retailers usually keep the meat display area well lighted to attract consumers by different types of light sources. Actually, lighting is essential for marketing and presentation of meat, both for traditional and for case-ready sales, but it can speed up meat discoloration. As a result, nearly 15% of retail beef is discounted in price due to surface discoloration, which corresponds to annual losses of retailer around $1 billion (Smith *et al*., 2000).

In Ethiopia almost all butcher shops are displaying the beef carcasses under high power incandescent lamps through an open window of display booth to attract consumers. Displaying carcasses under this condition might have a potential to accelerate lipid oxidation and the formation of an undesirable brown muscle color on carcasses. Because, several studies showed that displaying meat cuts under high intensity lights are the cause of qualities losses of meat; especially by accelerate the formation of an undesirable brown muscle color and lipid oxidation (Lavelle *et al*., 1995; Chiang & Ringkob 1999; Greer 1984). However, these studies are confined in the refrigerator display light effects; there is no study on the effects of high intensity display light on the qualities of meats which displayed in an open window/at room temperature as Ethiopian butcher shops.

Most of Ethiopian popular traditional foods are prepared by spices for flavor, color and to enhance appetite. For example, the spice sauce that is prepared from spiced powder of chili pepper, African bird‘s eye and mustard seed, used for consuming raw and roasted meat. This spice sauce is popular and preferred by several people throughout the country as appetizer for consuming raw beef. We anticipated that this sauce could be a rich source of important phytochemicals.

It is important to note that studies on the potential of these spices on the preservation of raw meat and their antioxidant potential scarce. Therefore, the aim of this study is to evaluate the effect of incandescent display light and Ethiopia spices on the quality of raw beef based on:- color and lipid oxidation stability and specific wavelength of light. The spices extract as a preservative and their effect on the quality raw meat was also investigated.

1.1. Background

Ethiopia ranks first in Africa and 10th in the world in its livestock population. The estimated number of cattle in this country is 49.3 million in 2008/2009 (CSA, 2010). This livestock potential contributed to keep the Ethiopian tradition of eating raw meat. The most popular raw meat products in Ethiopia are *kurt or tire siga and kitffo.* These are traditional foods for a large section of the population; which are prepared from raw meat of cattle and consumed with spices in large quantities all over the country. Most of the people usually purchase raw beef from the butcher shops popularly known as *lekuanda bets*. These butcher shops (*lekuanda bets)* are displaying the beef carcasses through an open window of a display booth under high power of incandescent lamps. These high power incandescent lamps emit heat, as well as radiation that are transformed into heat when absorbed by meat, and can cause the surface temperature of displayed carcasses to be much higher than the temperature of the display booth. Higher temperatures at the meat surface speed up deteriorative factors on meat, such as lipid oxidation, discoloration and microbial development (Chiang & Ringkob 1999; Greer 1984).

The role of lighting for displaying of meat is to show the true quality of the meat, without detracting from appearance or deceiving the customer about meat quality. In view of the importance of color to beef acceptability, brighter display lighting from incandescent lamps are preferable (Barbut S., 2003) and essential for marketing and presentation of meat. But, marketing advantages of brighter display lighting must be weighed against more rapid color deterioration under more intense lighting.

Many types of spices are produced in Ethiopia, some species are indigenous such as *Afromomum kororima* (korerima), *Piper nigrum* (Long Pepper), and *brassica carinata* (Ethiopian Mustard), and others have been introduced. In this country, the habit of eating raw meat is strongly associated with special spices sauce that prepared for eating raw meat from the mixture of food spices such as*, mitmita* (a spicy mixture of bird‘s eye chillie/ *capsicum frutescens*, Ethiopian

cardamom, black cumin, and bishop's weed mixed with salt)*, awaze or paste berbere* (a mixture which includes seeded pods of chili pepper/*capsicum annuum*, ginger, garlic and red onion) and *senafitch (*powder of mustard seed/*brassica nigra*).

1.2. Statement of the Problem

In Ethiopia the butcher shops are mostly using more than two incandescent lamps (200watts each lamp) to display beef carcasses, within on average of 1.5 m by 1.5 m area of display booth; the lamps distance are averagely 0.5 m from the carcasses (based upon the survey study on some butcher shops in Addis Ababa). Using these high power incandescent lamps at short distance from display carcasses can accelerate the formation of undesirable changes in color, texture and oxidation of carcasses that significantly affect consumer acceptance. In addition, both emitted and radiant heat from those high power incandescent lamps can increase the surface temperature of meat and affect the color. As a result the price of retail beef would significantly reduce and discarding rate of discolored meat will increase in the butcher shops. Several studies conducted in this area have shown the adverse effect of high intensity light on quality of meat, such as lipid oxidation, discoloration and surface temperature elevation of displayed meat (Lavelle *et al*., 1995; Chiang & Ringkob 1999; Greer 1984). However, those studies are focused on only in refrigerator display cases. It is important, therefore, to investigate the effect of incandescent light on quality of meat displayed in an open window/at room temperature (as Ethiopian butcher shops case).

Oxidation is a leading cause for quality deterioration during displaying, processing and storage of muscle foods. Numerous factors can affect lipid oxidation including light, oxygen concentration, temperature, presence of prooxidants, degree of unsaturation of the fatty acids and the presence of enzymes (Jakobsen & Bertelsen, 2000). Quality losses in oxidized meat products are generally characterized by flavour deterioration, discoloration, destruction of nutrients, and possible formation of toxic compounds (Kong *et al*., 2010).

The most efficient and practical way to prevent oxidative and colour deteriorations in muscle foods is to incorporate antioxidants into the product formulations (Franco *et al*., 2012; Kong, Zhang & Xiong, 2010). Due to potential health risk with synthetic antioxidants, there is a growing interest in the use of natural antioxidants, particularly those that are extracted from spices and herbs (Moure *et al.,* 2001; Mathew & Abraham, 2006). For example, extracts from

tea, rosemary, clove, sage, and oregano have been shown to decrease lipid oxidation as effectively as certain synthetic antioxidants in cooked meat products (Mansour & Khalil, 2000; McCarthy *et al*., 2001; Tang *et al*., 2001) and on the inhibition of lipid oxidation in beef steaks (Djenane *et al*., 2003). In addition, Formanek *et al.* (2003) studied on rosemary extracts in irradiated ground beef and found that both lipid oxidation and colour change were inhibited by the presence of rosemary. However, there is little information about the antioxidant potential for species grown in Ethiopia and there are no or few studies about the preservative potential of these extract to prevent oxidative and color deteriorations of raw meat.

To systematically approach the problem, this study will focus on the following research questions:

- Does the incandescent display light affect the color and lipid oxidation stability of raw beef displayed openly at room temperature?
- What is the effect of covering raw beef samples on the color and lipid oxidation stability during displaying?
- Which muscles types of beef have color stability during displaying?
- Is there correlation between contents of myoglobin forms and color change of meat in displaying of raw beef steaks by incandescent light?
- Which types of spectral colours or wavelengths of display light contribute for discoloration?
- Do the selected spices extracts have a potential on retarding of lipid oxidation, discoloration and microbial load of raw beef? Can they use for preservation of raw beef?
- What are the antioxidant and polyphenol contents of the three spices (chili pepper, African bird‘s eye and mustard)?
- Is there any correlation between polyphenol contents and antioxidant activity of the selected spices?
- Does the germination process increase the antioxidant capacity of mustard seeds?
- What volatile constituents are contributing for the aroma of the selected spices?

1.3. Significance of the Study

In Ethiopia, the trend of displaying the beef carcasses is through an open window of a display booth under high power of incandescent lamps. Displaying carcasses under this condition

believed to be producing adverse effect on quality of displayed meat, such as discoloration, lipid oxidation and increment of temperature on meat surface that create favorable condition for bacteria development (Lavelle *et al.,* 1995; Chiang & Ringkob 1999; Greer 1984). It is important, therefore, to identify the adverse effects on quality of displayed meat and to recommend the optimized displaying condition and light source that will reduce the discoloration, lipid oxidation and surface temperature of displayed raw beef.

Ethiopia is a homeland for many spices, such as korarima (Aframonum Korarima), long pepper, Black cumin, Bishops weed (_Nech azmud') and coriander. As a result, the history of spice use in Ethiopia is an ancient one and spices have always been and remain as basic food items in the diet of the Ethiopian people. Moreover, many species of spices are grown in Ethiopia, which are used in our daily diet mostly for flavour and color; beyond this these are believed to be very rich sources of antioxidants. However, there is a lack of information that confirm the antioxidant capacity of some spices which grown in this country. Therefore, this study will address the detail information on the antioxidant activity and total polyphenol contents of the three types of spices (*Capsicum annuum, Capsicu*m *minimum* and *Brassica nigra)* which are grown in this country and commonly consume with raw beef. In addition, evaluate the antioxidant activity of Ethiopian spices extract on inhibition of lipid oxidation and colour change of raw beef. Moreover, the researchers believe that the findings of this study will be valuable to employ these spices extracts as a natural additive in foods to preserve both industrially processed and traditionally prepared meat products in the country. Generally, this research will contribute additional scientific data in meat science as well as the data gathered from the result of this study will serve as a guide of other researchers and university students who want to study in this area.

1.4. Objectives of the Study

1.4.1. General Objective

The general objective of the present study is to evaluate the effect of incandescent light on the quality of raw beef based on color and lipid oxidation stability, specific wavelength of light and myoglobin types. It is also to evaluate the preservative effect of of Ethiopian spices extract and their contribution as antioxidant and polyphenol containing products.

1.4.2. Specific Objectives

- Determine the color and lipid oxidation stability of raw beef with respect to exposure time under incandescent light and suggest optimum condition thereto.
- Estimate the relative contents of myoglobin, oxymyoglobin and metmyoglobin of raw beef steaks exposed to incandescent display light and correlate the results obtained with that of the color change of raw beef.
- Evaluate the effect of covering of raw beef during displaying on color and oxidation stability.
- Assess the color stability of different muscle types exposed to incandescent light.
- Investigate the impact of specific wavelengths of visible light on the color change of raw beef and use the results to evaluate the various display light sources currently used by Ethiopian butcher shops.
- Evaluate the potential of using Ethiopian spice extracts as a preservative of raw meat stored in dark refrigerator.
- Evaluate the effect of different dosages of Ethiopian spices on color and lipid oxidation stability of raw beef samples during the exposure to incandescent light.
- Analyze the antioxidant activity and total phenol and flavonoid contents and compare the contribution of the spices towards retarding lipid oxidation and discoloration of raw beef.
- Evaluate the effect of germination of mustard seed on antioxidant activity and total polyphenol contents and recommend optimum conditions to be applied especially by Ethiopian spices shop (*baltina* shop) and butcheries.
- Investigate the volatile aroma constituents of Ethiopian spices by using SPME-GC-MS.

CHAPTER 2. LITERATURE REVIEW

2.1. Meat and Quality of Raw Meat

2.1.1 Meat

The term *meat* mostly applied to the edible portions of domestic mammals such as cattle, calves, sheep, lamb, and swine. The meat of cattle is known as beef; calves, as veal; sheep, as mutton; lambs, as lamb; and swine, as pork. The term *meat* also can be applied to the edible portions of poultry and wild birds and to the portions of other animals such as reptiles that are eaten by humans. Generally, meat consists of skeletal muscle, with varying amounts of fat and connective tissue, but internal organs are also used, these include the liver, kidneys, testicles, brain, heart, and stomach. It is a nutritious food, containing quantities of essential amino acids in the form of protein. It also contains B group vitamins (niacin and riboflavin), iron, phosphorus, ash, and calcium. Certain meats, especially liver, contain vitamins A and D (Fortomaris *et al*., 2006)

2.1.2 Beef Muscle

Muscles can be classified as glycolytic (white) or oxidative (red) due to their dominating energy metabolism, which is based on the proportion of glycolytic and oxidative fibre types in the muscle (Cassens *et al*., 1968; Beecher *et al*., 1965). Glycolytic muscles require a rapid source of energy, and glycolysis is the predominate metabolic pathway used by these muscles. Glycogen and many of the enzymes related to glycolysis are abundant in these muscles. Oxidative muscles rely primarily on oxidative metabolism, which requires a large amount of myoglobin for oxygen storage. As compared with white fibers, the red muscle fibers tend to be smaller in diameter, richer in mitochondria, myoglobin and lipid, and more generously supplied with blood due to higher capillaries (Foegeding, Lanier & Hultin, 1996). Moreover, the proportion of different fiber types may vary markedly in different species and different muscles, depending on physiological function (Essén-Gustavsson, 1992). The variation in fiber types between species is caused by both genetic and environmental factors. There are marked differences in fiber types, fiber morphology and metabolic profiles between muscles both within and between animals (Essén-Gustavsson, 1992). Among beef muscles, inside part of *biceps femoris* (BF) is oxidative (Essén-Gustavsson *et al*., 1994), whereas *longissimus Dorsi* (LD) (Karlsson *et al*., 1993) and *M. semimenbranosus* (SM) (Kiessling & Hansson, 1983) are glycolytic within the same breed. However, the fiber composition in a muscle may differ between breeds (Ruusunen & Poulanne,

1997), and the metabolic profile of a muscle with similar fiber type composition may differ between breeds (Essén & Fjekner, 1985). The metabolic profile may also differ within a muscle. The inside part of BF was classified as red muscle with a high oxidative enzyme activity and the outside part of BF as white muscle with a low oxidative enzyme activity (Beecher *et al*., 1965).

The methods of cutting carcasses of meat animals into parts, and the names given to the different cuts, vary locally but the common names of beef muscles are as listed in Table 2.1.

Table 2 1 Common Names for Beef Muscles

Muscle	Common Name
Adductor	Top (inside) round
Biceps femoris	Bottom (outside) round
Cutaneousomo brachialis	Shoulder Rose
Deep pectoral	Brisket
Deltoideus	Outside chuck (chuck)
Gastrocnemius	Round heel
Gluteus medius	Top sirloin
Gracilis	Inside round cap
Longissimus dorsi	Ribeye; Loin eye
Longissimus dorsi (chuck)	Chuck Eye
Longissimus lumborum	Loin Eye
Longissimus thoracis	Ribeye
Multifidus Dorsi	Subeye
Obliquus internus abdominus	Sirloin butt flap
Psoas major	Tenderloin
Quadriceps femoris	Knuckle; Sirloin tip
Rectus abdominus	Flank
Rectus femoris	Knuckle center
Rhomboideus	Hump meat
Semimembranosus	Top (inside) round
Semitendinosus	Eye of round
Spinalis dorsi	Rib cap
Superficial pectoral	Brisket
Supraspinatus	Mock tender; Chuck tender; Scotch tender
Tensor fascia latae	Tritip
Teres major	Shoulder Tender; Petite Tender
Trapezius	Outside chuck
Triceps brachii	Clod heart; Shoulder center; Shoulder top
Vastus lateralis	Knuckle side

Source: Calkins C. & Sullivan G. (2007)

2.1.3 Quality of Raw Meat

The quality of meat has recently attracted consumer concern (Ngapo *et al*., 2004a). Today, besides concerns about production methods, quality is a contentious issue as a consequence of the absence of a single common definition. For example, quality now refers to all or some of the following: (i) carcass characteristics and composition, such as carcass uniformity and consistency, lean yield and fat, (ii) meat characteristics such as color, marbling, pH, PSE (pale, soft and exudative) score, DFD (dark, firm and dry) score, (iii) eating quality characteristics includ tenderness, juiciness and flavor, (iv) processing and retailing characteristics, such as drip and cook losses as well as shelf life, and (v) nutritional characteristics such as protein, vitamin and mineral contents (Bredahl, Grunert, & Fertin, 1998; Grunert, Bredahl, & Brunsø, 2004).

Appearance has a great influence on how meat, including beef, is valued by the consumer. However, appearance quality dimensions cannot be easily evaluated at the point of purchase because the views on quality are based on a form of conditioning where consumers associate available cues with the quality of the product. The notion is that quality cues can be intrinsic or extrinsic (Olson, 1978). Intrinsic quality cues are part of the physical product such as color or fat content (Hurling & Shepherd, 2003; Ngapo, Martin, & Dransfield, 2004b). Extrinsic quality cues are everything else that is related to the product or its production process, such as price and packaging (Cardello, 1995; Verbeke & Viaene, 2000).

Meat color is one of the most important factors used by consumers when selecting beef at retail (Liu et al., 1996). Therefore, improving color stability of meat and extending its display life are a matter of concern to retailers.This research paper also more emphasized on color change of raw beef in association with Incandescent display light.

2.2 Meat Pigments and Color of Raw Beef

Meat purchasing decisions are influenced by color more than any other quality factor because consumers use discoloration as an indicator of freshness and wholesomeness. As a result, nearly 15% of retail beef is discounted in price due to surface discoloration, which corresponds to annual revenue losses of $1 billion (Smith, et al, 2000). An economic improvement associated with product that achieves its color life potential depends on our knowledge of pre- and postmortem myoglobin chemistry.

The initial impression of the quality and acceptability of a food product is judged on the basis of its visual appearance. Thus, the naturally occurring food pigments, which are the primary components absorbing visible light energy, provide coloration and represent important quality constituents of foods.

Myoglobin and hemoglobin are the pigments giving meat the red color (Govindarajan, 1973; Giddings, 1977). Myoglobin is distributed uniformly throughout the muscle, and its role is to store and to facilitate the diffusion of oxygen from the capillaries to the intracellular structures, where the oxygen is used for oxidative processes (Stryer, 1981; Ledward, 1992). Hemoglobin, which is contained in the red blood cells, serves as the oxygen carrier in blood (Stryer, 1981).

The myoglobin content in muscle depends on species, breed, sex, age, type of muscle, level of training (Ledward, 1992) and altitude (Gimenez *et al*., 1977). Myoglobin is present in the sarcoplasmic fraction of the muscle (Govindarajan, 1973) and evenly distributed across the muscle fibers (Swatland, 1984). Consequently, the myoglobin content is higher in a red muscle, such as the inside part of porcine *M. biceps femoris* (BF), compared with white muscles, such as porcine *M. longissimus dorsi* (LD), and the outside part of porcine BF (Beecher *et al*., 1965).

The color of fresh meat is determined by the concentration and chemical nature of the hem proteins present (Govindarajan, 1973) and the muscle structure (Offer *et al*., 1989), which is influenced by temperature/pH history of the muscle post slaughter (Ledward, 1992). In most meats, myoglobin is the main heme pigment although hemoglobin may be present in significant concentrations and to some extent mitochondrial cytochrome (Hamm, 1975; Ledward, 1992).

Fresh and cured meat color both depend on myoglobin, but are considerably different from each other in terms of how they are formed and their overall stability. The relative proportions of the three myoglobin forms deoxymyoglobin, oxymyoglobin and metmyoglobin affect the color beef. A bright pink (red) colour is related to oxymyoglobin, while the colour of myoglobin is purple and metmyoglobin is more greyish or brownish pink (Lindahl, L. & Tornberg, 2001).

Discoloration of fresh prepackaged meat or "loss of bloom," as it is called by the trade, means that meat loses its bright red appearance. The oxidation of the central iron atom within the heme group is responsible for discoloration, a change from red OMb to brownish MMb (Faustman *et al*., 2010). When ferrous heme iron oxidizes to its ferric form, oxygen is released and replaced by

a water molecule (O'Sullivan, 2003). The rate of discoloration in meat is muscle-specific. Muscles that contain greater relative proportions of red fibers, and thus more lipid and greater oxygen consumption rates, appear to discolor more quickly (O'Keeffe & Hood, 1982).

2.2.1. Fundamental Myoglobin Chemistry

Knowledge of fundamental chemistry is very important when any one engaged in meat color research, because myoglobin is the primary protein responsible for meat color (Faustman, 2010), although other heme proteins such as haemoglobin and cytochrome may also play a role in beef, lamb, pork, and poultry color (Mancini & Hunt 2005). The pigment of myoglobin that determines the color of fresh meat undergoes various chemical reactions to form other pigments. These reactions are triggered by light, heat, and oxygen availability. Myoglobin can oxidize to metmyoglobin during retail display, resulting in development of an undesirable brown muscle color (Zerby *et al*., 1999).

Myoglobin is the water-soluble protein containing the 8 α-helices of myoglobin, a prosthetic heme group containing a centrally located iron atom is positioned in the protein's hydrophobic core. Of the six bonds associated with this iron atom, the iron binds with four nitrogens in the centre of the protoporphyrin ring, the 5^{th} attaches to the proximal histidine-93, and the 6^{th} site is available to reversibly bind ligands including diatomic oxygen, carbon monoxide, water, and nitric oxide (Stryer, 1981). The iron atom can be in several redox states with the ferrous (Fe^{2+}) and the ferric (Fe^{3+}) redox states being most important in relation to fresh meat color. The iron redox state and the type of ligand bound at the 6th coordination site determine meat color (Govindarajan, 1973) through four chemical forms of myoglobin, which are deoxymyoglobin (DMb), oxymyoglobin (OMb), metmyoglobin (MMb) and carboxymyoglobin (COMb); see Figure 2.3. Deoxymyoglobin results in a dark purplish-red or purplish-pink color typical of the interior color of fresh meat (Figure 2.1). Deoxymyoglobin contains ferrous iron (Fe^{2+}) at a vacant 6^{th} coordination site without O_2 attachment. Very low oxygen tension (<1.4mm Hg) is necessary to maintain DMb within vacuum packages or the interior of muscle (Brooks, 1935).

Oxygenation of DMb from atmospheres or surface area will gives a bright-red color by the formation of oxymyoglobin (OMb) (Figure 2.2, left), which has diatomic oxygen (O_2) attached to the 6th coordination site of ferrous iron (Fe^{2+}). Oxymyoglobin, commonly known as the fresh

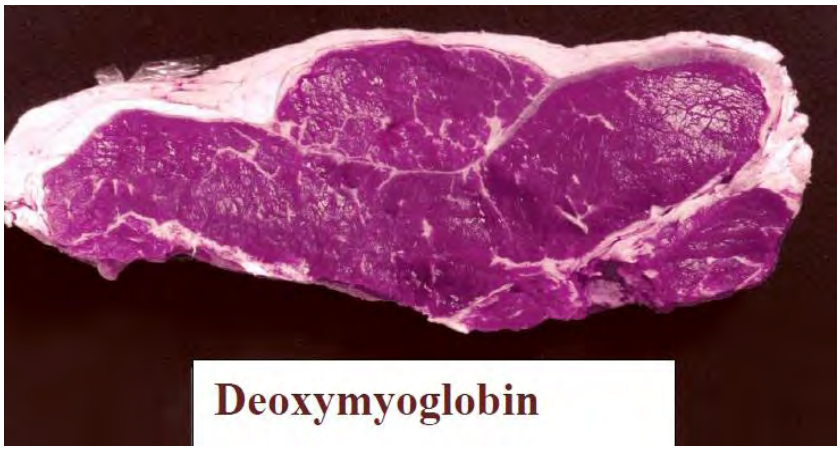


Figure 2.1 Fresh cut meat surface. The meat pigment is Deoxymyoglobin.
Source: Boles J. and Pegg R. (2014)

meat color, is the most desirable color for fresh meats. Maintaining this color requires that the meat surface be free from any contamination which would cause a chemical reaction results in the formation of the brown pigment metmyoglobin. Also, oxygen must be available at a sufficient concentration in order to combine with the DMb to form oxymyoglobin. When the environment is devoid of oxygen and carbon monoxide attaches to the vacant 6^{th} position of DMb, a carboxymyoglobin (COMb) formation will occurs and produce a stable bright-red color on a meat surface (Mancini A. & Hunt C., 2005).

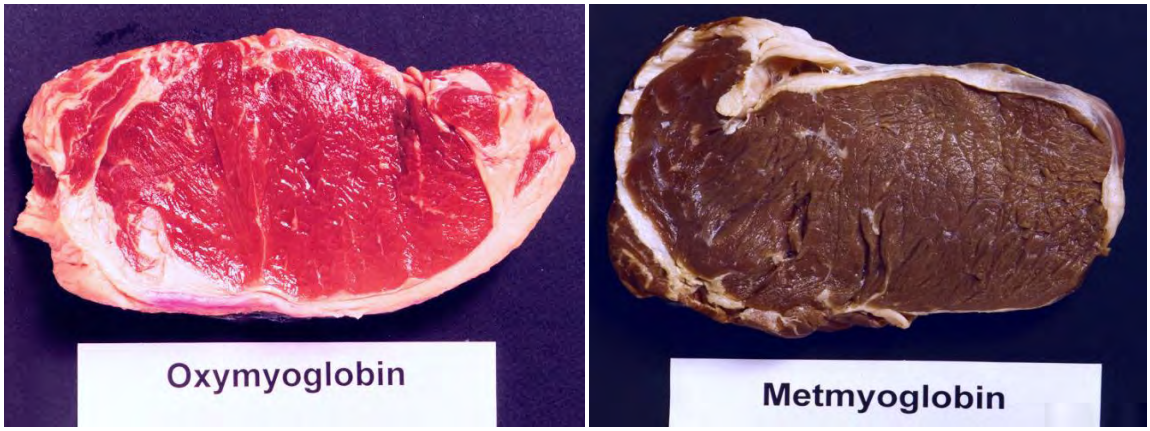


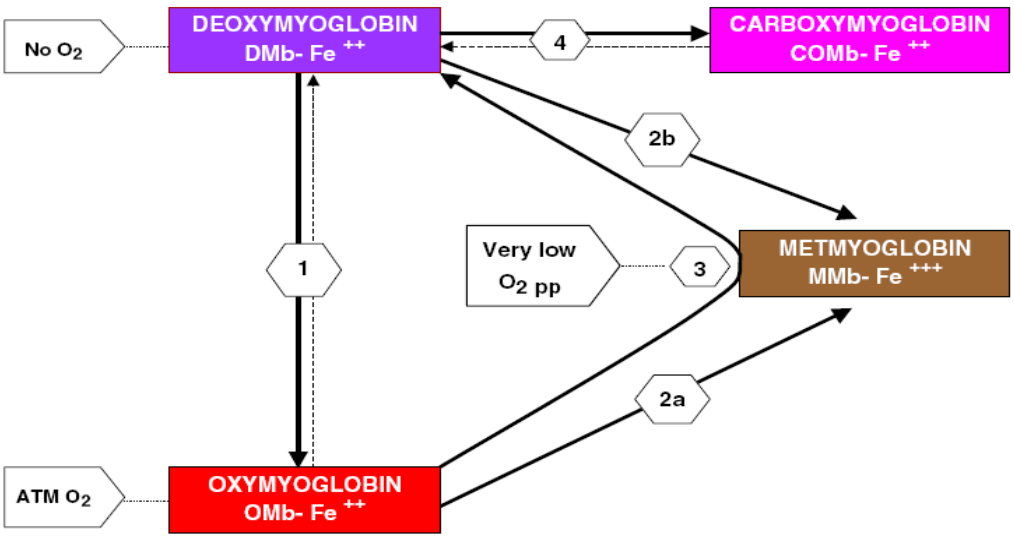
Figure 2.2 Striploin steak allowed blooming to oxymyoglobin (left) and striploin steak that has been stored and metmyoglobin has formed (right). Source: Boles J. and Pegg R. (2014)

Metmyoglobin is oxidized to brown color (Figure 2.2, right) form of myoglobin and it contains ferric iron (Fe^{3+}). Water is the ligand at the 6^{th} position of the iron in MMb. Metmyoglobin

formation depends on numerous factors including oxygen partial pressure, temperature, pH, meats reducing activity, and in some cases, microbial growth (Wallace, *et al*., 1982). Typically, MMb forms easily at low concentrations of oxygen (<7mmHg or about 1 to 2% oxygen) (Livingston & Brown, 1982) such as when meat pieces are stacked one on top of another. Oxygen partial pressure can also be reduced when aerobic bacteria use up the oxygen and it is unavailable to react with the myoglobin.

2.2.2. Dynamics of Myoglobin Redox Form Interconversions

In meat myoglobin, the red iron-containing pigment which is responsible for meat color mainly exists in different conformations as illustrated in Figure 2.3 by Mancini A. & Hunt C. (2005). Deoxymyoglobin oxygenation or blooming (reaction 1 in Figure 2.3) depends on time, temperature, pH, and competition for oxygen by mitochondria (AMSA, 2012). More specifically, the competition for oxygen between myoglobin and mitochondria determines oxygen penetration beneath the meat's surface, which significantly affects the intensity of surface color.



Rx 1 (Oxygenation): DMb + O_2 → OMb
Rx 2a (Oxidation): OMb + [oxygen consumption or low O_2 partial pressure] – e^- → MMb
Rx 2b (Oxidation): [DMb – hydroxyl ion – Hydrogen ion complex]+ O_2 → MMb + O_2^-
Rx 3 (Reduction): MMb + Oxygen consumption + metmyoglobin reducing activty → DMb
Rx 4 (CarboxyMb): DMb + carbon monoxide → COMb

Figure 2.3 Visible myoglobin redox interconversions on the surface of meat.
Source: Mancini A. & Hunt C., (2005)

2.2.3. Effect of Meat pH on Color Stability of Fresh Meat

The rate and extent of postmortem muscle pH declines have a great impact on the color of meat. According to Boles J. and Pegg R. (2014) the normal pH decline in postmortem beef muscles is from approximately 7.0-7.2 down to near pH 5.5-5.7 over about 24 hrs. They also revealed that, if the postmortem beef muscle pH declines to the normal pH of 5.5-5.7 within 45 min or less, the muscle will appear very pale and soft (PSE). If the pH does not drop much in postmortem, the meat will be dark with a dull, dry surface (DFD). As the ultimate pH increases, the meat gradually becomes darker as shown in Figure 2.4.

The color changes observed with PSE and DFD meat are mostly due to structural changes in muscle (Seideman etal., 1984). Some of the changes are due to the rate of myoglobin oxygenation. The changes in pH affect the charge on the proteins making up the muscle (Sammel et al. 2002b). These changes alter the spacing between the fibers of the meat, and the change in structure affects how light is reflected and absorbed, and thus affects the visual appearance (Swatland H.J., 2005).

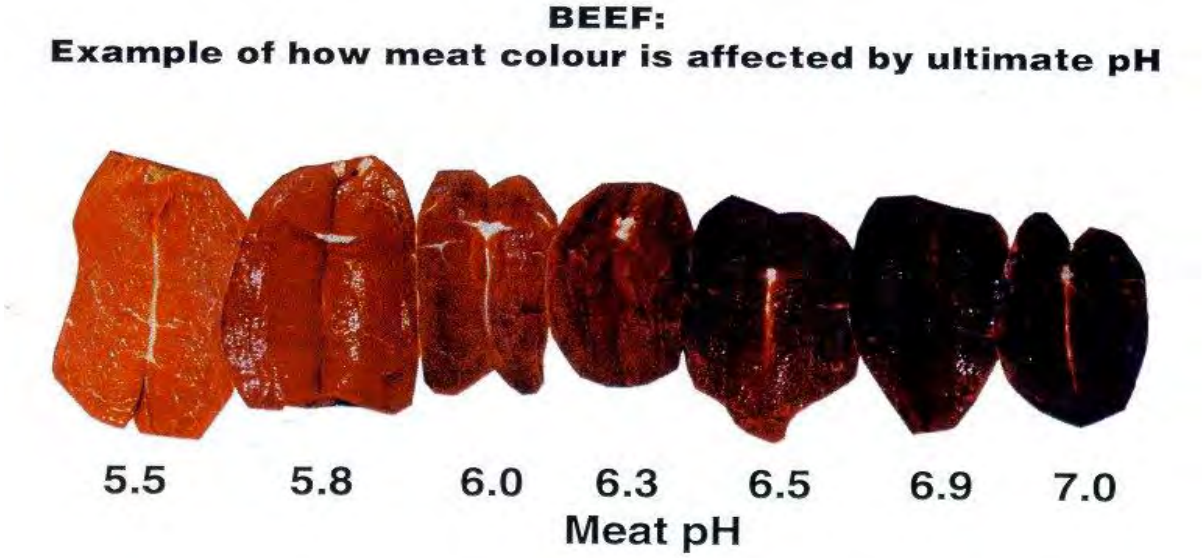


Figure 2.4 Effect of ultimate meat pH on the color of beef.
Source: Boles J. and Pegg R. (2014)

High ultimate pH can affect the color stability of fresh meat because it affects enzyme activity and the rate of oxygenation. Reducing enzymes are necessary to convert metmyoglobin back to oxymyoglobin (Abril *et al*., 2001). The high ultimate pH has a dry surface and this inhibits the penetration of oxygen into the meat and thus slows down the oxygenation process (Swatland H.J., 2005).

2.2.4. Factors Affecting Meat Color

The literature clearly documents that many factors affect meat color. Extrinsic factors such as animal genetics (Brewer *et al*., 2004), gender, age, diet (French et al., 2000; Baublits *et al*., 2004; Realini, *et al*., 2004), time-on-feed, seasonality, ante mortem stress, carcass weight, many postmortem conditions such as method of immobilization, rate of chilling, carcass spacing and alignment and application of antimicrobial interventions; postmortem processing and packaging methods, time and temperature of storage (Sammel *et al*., 2002b), extent of exposure to oxygen (Livingston & Brown, 1982) and especially postmortem age of the product usually influence meat color by influencing the intrinsic factors of meat color.

Intrinsic factors such as pH, muscle type (Sammel *et al*., 2002), areas within a muscle, muscle fiber composition, myoglobin concentration, disruption of various subcellular components related to meat color chemistry, water holding capacity, microbial load (Bala, 1977; Sammel *et al.,* 2002), and temperature history (MacDougall, 1982), meat‘s use of oxygen (Mancini & Hunt, 2005) and meat‘s ability to reduce MMb (Chan *et al.*, 1995).

2.2.4.1 Chilling Rate on Meat Color

The rate at which the temperature of muscle on a beef carcass declines affects color stability of meat products. Sammel *et al*., (2002) reported that the beef carcass surface which decrease in temperature at a slower rate could proceed through glycolysis faster. Rapid rates of glycolysis lead to faster pH declines which denature proteins and open up muscle structure causing light scattering effects that are negative to meat color (MacDougall, 1982). Furthermore, Sammel *et al.*, (2002) found chilling rates decreased aerobic reducing activities and affected the color stability of meat. Therefore, muscles with more rapid chilling rates will have increased redness, decreased discoloration, and greater consumer appeal.

2.2.4.2. Bacterial Contamination on Meat Color

Bacterial contamination of a product affects fresh meat color as reported in literatures. For examples, beef steak inoculated with *Pseudomonas fragi* expressed greater discoloration compared to control samples in work of Bala, (1977). Aerobic bacteria such as *Pseudomonas*, *Achromobacter* and *Flavobacterium* metabolize oxygen and reducing the oxygen pressure at the meat surface resulting in an increase in metmyoglobin content (Renerre, 1990). Robach & Costilow, (1961) also reported the beef steaks inoculated with aerobic bacteria had oxygen uptakes greater than 160µl/30min compared to control steaks at approximately 20µl/30min. Myoglobin denaturation can occur when proteolytic enzymes from bacteria come into contact with the pigment (Lawrie, 1985). Moreover, in study of Robach & Costilow, (1961), sonically treated cell-free *Pseduomonas geniculata* populations were inoculated onto beef steaks that discolored faster than the controls suggesting that the bacteria‘s enzymes caused discoloration. Lawrie also suggests discoloration of fresh meat can be related to pigments produced by microorganisms. A green discoloration may appear on fresh meat from the interaction of the hydrogen peroxide by-products from bacteria and myoglobin producing Choleglobin (Lawrie, 1985). Nicol, Shaw, and Ledward (1970) found an increase in green discoloration with the presence of hydrogen sulfide produced by bacteria on meat products with pH values 6 or greater. Interventions to control microbiological growth through the use of products such as potassium sorbate, sodium acetate, sodium tripolyphosphate, and/or tetrasodium pyrophosphate can prevent metmyoglobin formation (Renerre, 1990).

2.2.4.3. Muscle Metabolism

Meat is a very complex tissue because it is composed to birefringent myofibrils, or variable sarcomere length, arranged in precise ways and it contains numerous membrane boundaries that may reflect light (Swatland H. and Iriel M., 1992). Cellular biochemistry of carcass muscles differs, influencing postmortem metabolism of skeletal muscles. The influence of beef muscle source not only on fresh color but also cooked color has been widely investigated. McKenna et al. (2005) examined various biochemical mechanisms influencing color stability in 19 beef muscles, whereas other researchers (Mancini *et al*., 2009; Suman *et al*., 2009) reported that color stability of beef muscles respond differently to MAP systems and to browning induced by cooking.

The role of mitochondria in meat color has received significant attention, and the mechanisms through which mitochondria influence myoglobin redox stability have been investigated. The influences of vitamin E (Tang *et al.,* 2005a), lipid oxidation (Tang *et al*., 2005a), oxygen consumption rate (Mohan *et al.,* 2010c; Tang *et al*., 2005b), and metabolites (Ramanathan *et al*., 2009; Tang *et al*., 2005c) on the interactions between mitochondria and myoglobin suggest that both the electron transport chain and reductase enzymes in the outer membrane can reduce MetMb and, therefore, are involved in color stability. In general, the color varies between beef muscles as a consequence of differences in pigment content and muscle metabolism (Brewer *et al.,* 2001; Rosenwold &Andersen, 2003a; Lindahl, *et al*., 2001; Lindahl *et al*., 2005a).

2.2.4.4. Oxygen Consumption

Oxygen uptake in *post rigor* muscle results from tissue respiration, reaction with heme pigments and dissolution into tissue fluids (DeVore & Solberg, 1974). *Post mortem* muscle is not inert, and mitochondria continue to metabolise oxygen (Madavi & Carpenter, 1993; Tang *et al*, 2005). Mitochondrial activity in *post mortem* muscle is enhanced by high pH values (Bendall & Taylor, 1972) and high storage temperature (Bendall, 1972), which simultaneously results in higher oxygen consumption. Moreover, the oxygen consumption rate is influenced by muscle type due to differences in muscle metabolism as shown in studies on beef by Madavi & Carpenter, (1993). Although, as a consequence of respiration the oxygen consumption rate declines with time *post mortem* in porcine (Atkinson & Follet, 1973) and bovine muscle (Madavi & Carpenter, 1993; Tang *et al*., 2005b). This decline is due to loss in structural integrity of the mitochondria (Tang *et al*, 2005b), which subsequently allows oxygen to penetrate further into the muscle (Millar *et al*., 1994) and a more pronounced blooming (Lindahl *et al*., 2005c) that gives rise to a more red colour (Rosenvold & Andersen, 2003a).

2.2.4.5. Packaging

Packaging is a vital component of meat products as it provides protection from physical, chemical, and biological hazards as well as containing the product, communicating to consumers as a marketing tool, and providing ease of use and convenience (Yam, Takhistov, & Miltz, 2005). A variety of packaging options exist adding complexity to fresh meat color. Color will vary between meat products packaged in a modified atmosphere (MAP) or placed on a tray and

overwrapped with PVC plastic wrap. The most common styles of packaging meat products for retail display are PVC wrap on a Styrofoam tray and MAP (Charles, Williams, & Rodick, 2006).

2.3. Physics of Light and Color

Light is electromagnetic radiation transports energy through space. What we see as visible light is only a tiny fraction of the electromagnetic spectrum, extending from very low frequency radio waves through microwaves, infrared, visible and ultraviolet light to x-rays and ultra-energetic gamma rays. Our eyes respond to visible light; detecting the rest of the spectrum requires a resource of scientific instruments ranging from radio receivers to scintillation counters. Visible light is the small part of the spectrum of electromagnetic radiation between approximately 400 and 700 nanometers. Each wavelength has a ―spectral signature", or color, ranging from violet at 400 through indigo, blue, green, yellow and orange to red at 700 as shown in Figure 2.5. The combination of these colored wavelengths creates white light. Colored light can be described as the presence of certain wavelengths and the absence of others.

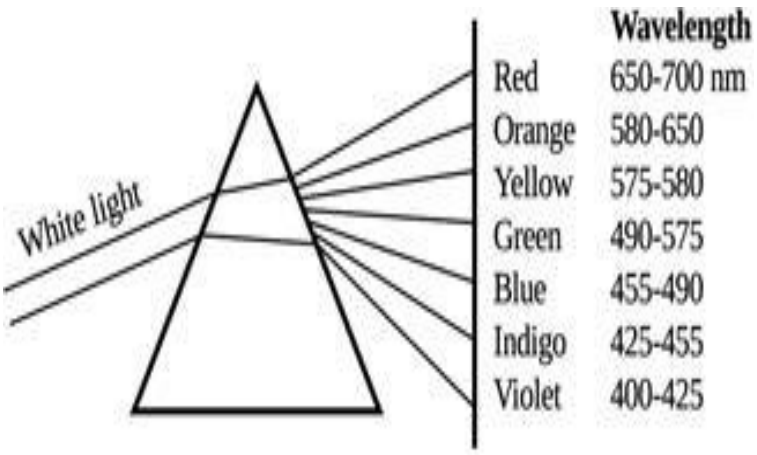


Figure 2.5 White light split into its components by a prism.
Source: AMSA, (2012)

Color is a sensation of light incident to the eye that arises from the interaction between light and matter. Primary colors or additive primaries are the three colors of light (red, green, and blue) that produce all the colors in the visible spectrum when added together in different combinations. Adding equal parts of red, blue, and green light produces white. The complete absence of red, blue, and green light results in black.

2.3.1. Color Perception of Meat

In total darkness, we cannot know color; if there is no object, color does not exist. The three elements of light, vision and object are necessary for us to perceive object color. In detail, perceiving an object and identifying the color of that object involves a complex set of circumstances consisting of the object, its surroundings, and the detector that perceives the object and translates the stimuli into a perception of color. To perceive color, a detector capable of this perception is necessary. That is the human eye or instrumentation such as a colorimeter or spectrophotometer. For human sensory response and color detection, the eye and brain should work coordinately to detect and interpret stimuli to distinguish color.

The eye or mechanical device, does captures wavelength of light reflected from an object such as meat (Figure 2.6). In case of eye, it relays on the brain for interpretation. In the case of mechanical device, it uses a light source that illuminates the sample and is then reflected through red, green, and blue filters onto the microprocessor that can convert the reflected values to numerical color values.

The humans can only detect light in the visual spectrum, which ranges from 390 to 750 nm. In this narrow range of the electromagnetic spectrum, the eye has the ability and the brain the capacity to separate wavelengths into color groups. For instance, red color is associated with light of approximately 650 to 700 nm wavelengths. Green color is associated with approximately 490 to 575 nm, and blue is associated with wavelengths between 455 and 490 nm (Figure 2.5).

For color to be detected, light must reflect off the object being viewed and return to the eye. To have color development, the light illuminating the object must have the spectral range to allow reflectance of corresponding wavelengths that the eye can detect and the brain interpret as color. Therefore, with full visual spectral light comes the possibility for an infinite number of colors to be developed. When light strikes meat, it will be absorbed, reflected, or scattered. The wavelength of light that are absorbed are not perceptible to the eye because they are retained by the object (for example, meat; Figure 2.6). However, the reflected light is perceived by the eye, captured and transmitted to the brain. Because the eye is trichromatic, the brain interprets the intensity of the blue, green, and red stimuli that the eye captures and interprets it as a color. So, to separate meat color, the source of light must contain the wavelengths capable of being reflected off meat surfaces, or color will not be perceptible to the eye or instrumental detector.

For sensory and instrumental evaluation of meat, the light source must be standardized (AMSA, 2012). Overall, for humans to see the true color of an object, a balanced light source should be used. For example, using a light source, such as the incandescent lamp, will make fresh meat appear more bright red than a fluorescent lamp (Barbut S. 2001) with a lower red output (Figure 2.7).

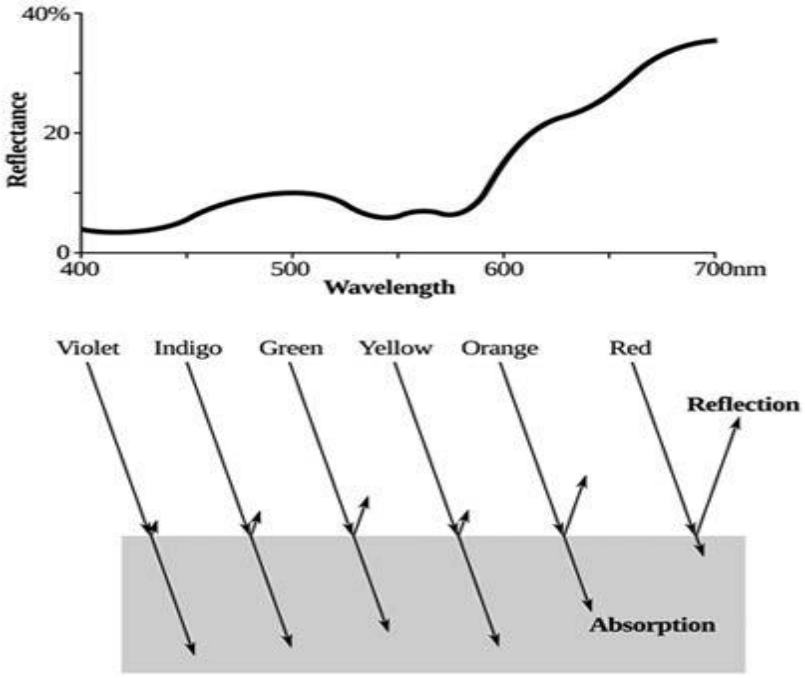


Figure 2.6 Spectra reflectance of a slice of beef meat (top). Relative reflectance of the different wavelengths (bottom).
Source: AMSA, (2012)

Meat color can be perceived differently for the same cut under different conditions. Conditions that can influence meat color are the light source (illuminant), observer differences, size differences in cut or object, smoothness of the surface (for example, using sharp or dull knife to cut meat), background differences, and directional differences (Madhavi & Carpenter, 1993).

Wavelengths of light reflected from meat develop the perception of color, so the light source becomes an issue in the development and perception of color. Light sources or illuminants come in many different types, sunlight, fluorescent light, incandescent light, among many others, and even within types of illuminants, lighting sources can differ greatly. Each light source contains a different spectral light composition. For example, Figure 2.7 illustrates the output of two light

sources. The fluorescent bulb has major peaks in the indigo, green, and orange areas but very low output in the red area; thus, a consumer panel perceived the beef, pork, and chicken cuts as brown. In contrast, when the panel was presented the same meat under incandescent lighting, the consumer panel scores were much more pink/red (Barbut S., 2001).

A balanced light source will have an equal/balanced output of different wavelengths (for example, sunlight). The appearance of displayed meat under different light sources would be different due to variation of output of different wavelengths of the sources. Therefore, when choosing a light source for research, the type of lighting and the light source must remain constant to properly compare color. Along with light source, intensity of light is also important in perceiving color; neither too bright nor too dim is good when viewing meat. Approximately 1630 lux is commonly used to compare meat samples for color (Lavelle C., Hunt M. & Kropf D. 1995).

Observer differences are another condition that can affect color perception. Each individual's eyes are slightly different in sensitivity to color vision. This is perhaps the most difficult to control of all the conditions that affect color perception. Screening for color vision perception can aid in selecting panelists capable of discerning meat color differences (Wiegand and Waloszek, 2003).

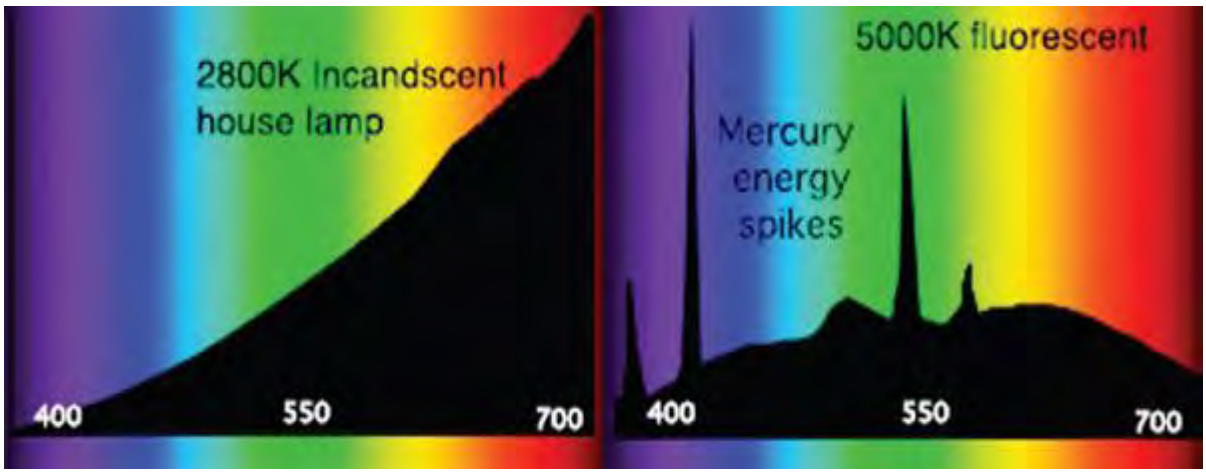


Figure 2.7 Spectral power distribution of an incandescent light bulb (left) & fluorescent bulb (right).
Source: AMSA, (2012)

Size differences in meat cuts can also affect how color is perceived because of the amount of light reflected to the eye. For larger cuts, more light is reflected to the eye, and color is often perceived as being brighter and more vivid (Renerre, M. 1990).

Background differences will also affect color perception (Konica Minolta sensing, 2007). Cuts viewed against a bright background often appear to have duller color, whereas cuts viewed against a dark background often appear brighter. Care should be taken to standardize the background so that comparative color determinations can be made. Also, background color becomes important in meat photography, where light backgrounds can give a false impression of dull or pale color whereas dark backgrounds tend to capture meat color vividness best (Ringkob T., 1997). In addition, the angle from which the cut is viewed and the incident angle of light from the illuminant source will both affect color perception (AMSA, 2012). This is particularly important when gloss occurs, which may impede the ability to view the sample.

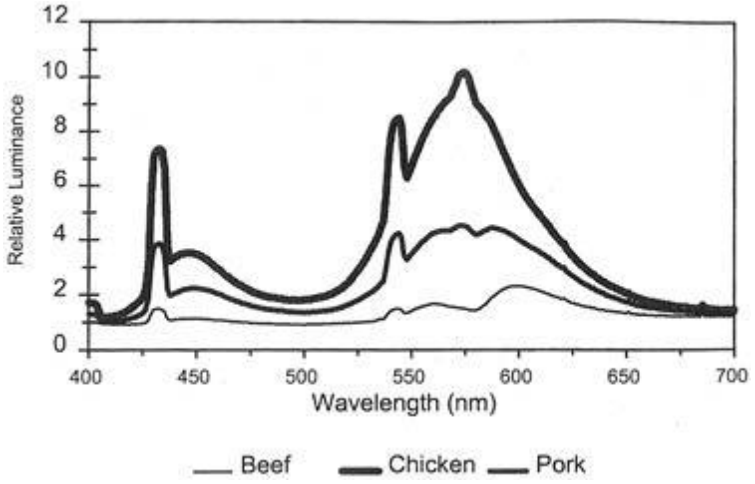


Figure 2.8 Relative luminance of fresh meat cuts positioned under cool white fluorescent light bulb.
Source: Barbut S., (2001)

Figure 2.8 illustrates that the fluorescent light bulb has major peaks in the indigo, green, and orange colors. The minimal light output towards the end of the visible spectrum results in poor appreciation of the red color of the beef and pork meat cuts.

2.3.2. CIE L*a*b* Color Space

Color is a subjective psycho-physical characteristic as it exists only in the observer's eyes and brain. It is necessary to find out parameters in order to measure, classify and reproduce it, because verbal expression of color is very complicated and difficult and communicating color can be quite challenging (Konica Minolta sensing, 2007). To facilitate color communication, tools have been developed. The Munsell system was invented by the American artist A. H. Munsell; it uses color chips to mix then match to that of a specimen. In 1931, the Commission Internationale de l'Eclairage (CIE) developed the tristimulus values XYZ (Figure 2.9) and latter developed the CIE L*a*b* color space in 1976 (Figure 2.10). The L*, a*, b* model is an international standard for color measurement developed by the Commission Internationale de Eclairage (CIE) in 1976. Although there are different color spaces, the most used in food is the L*a*b* color space due to the uniform distribution of colors, and because it is very close to human perception of color (Leo´n *et al*., 2006). Perceptible color has hue, lightness, and saturation properties. Hue is the color description as we communicate it in language (red, yellow, green, blue, etc.). Lightness describes the brightness or darkness of the color. Saturation refers to how vivid or dull the color is.

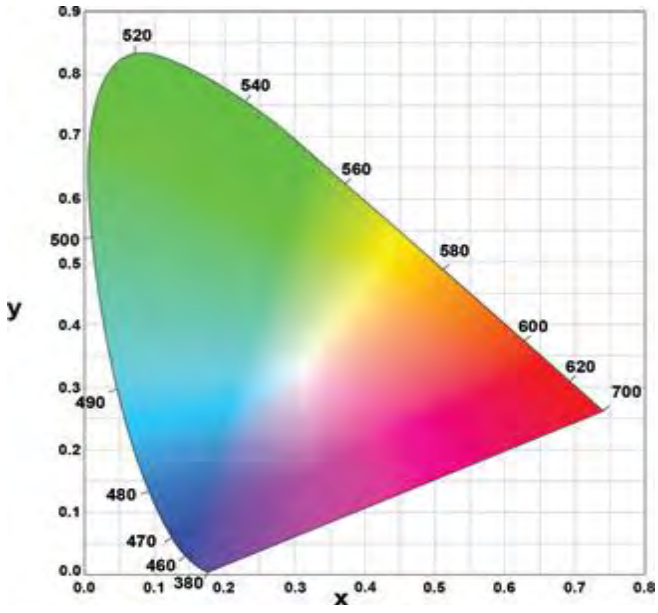


Figure 2.9 Illustration of the CIE 1931 color space.
Source: AMSA (2012)

The L*, a*, b* values are often used in food research studies. Currently, food color is measured in terms of CIE L*, a*, b* values, hue angle and chroma. The reason the CIE L*a*b* system is developed that the XYZ colorimetric distances between the individual colors do not correspond to perceived color differences. For example, the difference between green and greenish-yellow is relatively large, whereas the distance distinguishing blue and red is quite small. The CIE solved this problem in 1976 with the development of the three-dimensional Lab color space (CIELAB color space). In this system, the color differences one perceives correspond to distances when measured colorimetrically.

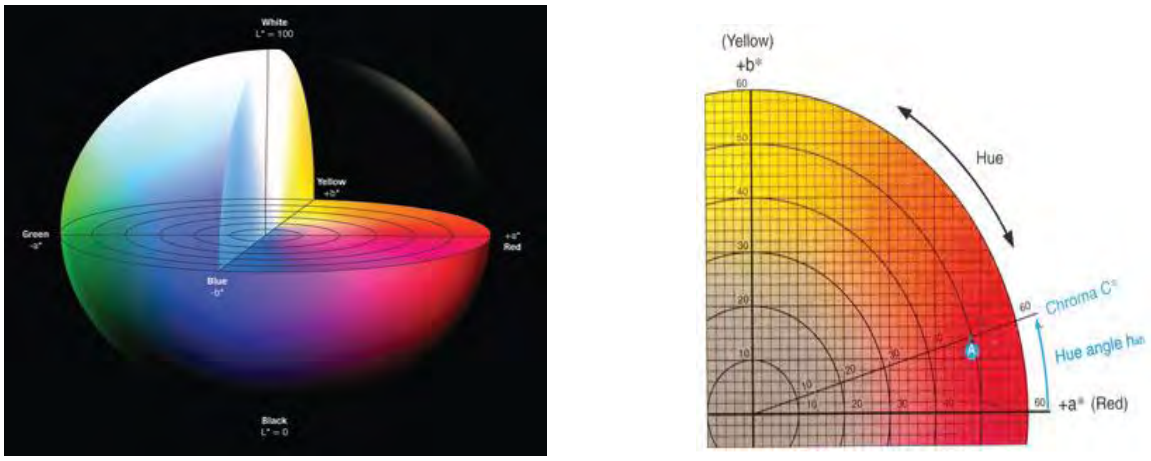


Figure 2.10 Representation of color solid for CIE L*a*b* color space in1976 (left) and an illustration of hue angle and chroma within part of a chromaticity diagram (right).
Source: Konica Minolta sensing, (2007)

The CIE L*a*b* color space (1976) allowed color to be expressed in a three dimensional space (Figure 2.10, left). Because of the optic response of the human eye to blue, green, and red, calculations converted these responses to L*, a*, and b* values (CIE, 1976). When combined, these establish a three dimensional color space. For the color space, a* values are represented on the X axis, the b* values on the Y axis and the L* values on the Z axis. In the center of the color space is neutral gray. Along the a* axis, a positive a* represents red, and a negative a* represents green. Along the Y axis, a positive b* represents yellow, and a negative b* represents blue (Papadakis *et al*., 2000). The third dimension L* is represented numerically where 100 is white, and 0 is black (Figure 2.10, left). For this color space, a* and b* values can be plotted to establish the color or hue of a meat sample (Figure 2.10, right). Using the L* value, lightness or

darkness of the sample can be determined. Therefore, using trigonometric functions, the incident angle a sample deviates from the X axis can be calculated to determine the hue angle (color) of the sample, and the distance of the sample from the origin of the XYZ lines can be calculated to determine the saturation or vividness of the sample (Konica Minolta sensing, 2007).

Hue angle is calculated as h_{ab} = arctangent (b*/a*). For example, with a CIE b* value of 14.12 and a CIE a* value of 47.63, the hue angle would be 16.51 based upon illustration by AMSA, (2012) in Figure 2.11(right). Therefore, the plot of a* and b* points and the corresponding angle will establish the color of the sample. Likewise, since colors become more vivid around the periphery of the color space, the farther the a* and b* plot points are from the origin, the more vivid the color. Chroma (saturation index) can also be calculated from the a* and b* values as $(a^{*2} + b^{*2})^{½}$. For example, with a* value of 47.63 and b* value of 14.12, the chroma (saturation index) would be 49.68 based upon illustration by AMSA, (2012) in Figure 2.10 (right).

In a publishing system no device is capable of reproducing the full range of colors viewable to the human eye (Adobe phtoshope CS3, 2007). Each device operates within a specific color space that can produce a certain range, or *gamut*, of colors. A color model determines the relationship between values, and the color space defines the absolute meaning of those values as colors. Some color models (such as CIE L*a*b) have a fixed color space because they relate directly to the way humans perceive color. These models are described as being *device-independent* (Yam L. and Papadakis E. 2004). Other color models (RGB, HSL, HSB, CMYK, and so forth) can have many different color spaces. Because these models vary with each associated color space or device, they are described as being *device-dependent* (Leo´n *et al*., 2006). The CIE L*a*b* color model is based on the human perception of color. The CIE L*a*b* numeric values describe all the colors that a person with normal vision sees. Because L*a*b* describes how a color looks rather than how much of a particular colorant is needed for a device (such as a monitor, desktop printer, or digital camera) to produce colors (Adobe phtoshope CS3, 2007).

2.4. Meat Color Measurement

Meat color evaluation is an essential part of meat research and product development. There are three meat color evaluation methods mostly apply in meat research especially in meat color evaluation, which are an instrumental measurement (colorimeter or spectrophotometer), visual appraisal through panelists and image analysis. When done properly, both visual, instrumental

and image analysis appraisals of color are powerful and useful research tools for meat scientists. However, these evaluations must be conducted using carefully designed procedures to avoid biased data.

2.4.1. Instrumental meat color measurement

Color is a matter of perception and subjective interpretation. Even if two similar colors look the same to human eye, instrumental color measurement can point out slight differences. Instrumental color measurement is an objective color characterization method that works well alone or in combination with visual color data. To obtain standardized color measurements of the samples, as an alternative to human perception by itself, instrumental analysis should be performed. The instrumental analysis is the best approach to determine specific color attributes that directly impact the overall color of the meat. These attributes, besides the redness (a^*), also include lightness (L^*), yellowness (b^*), trueness of red through the hue angle, vividness of the color through the saturation index, and oxymyoglobin proportions through the reflectance ratio.

Instrumental color measurements, the colorimeter and the spectrophotometer use their own light sources and certain illumination conditions (for example, illuminants A, C, or D65) (Konica Minolta sensing, 2007). Various light sources can be used (for example, tungsten, and deuterium). Instruments differ in the way they measure reflected light. The tristimulus method uses a light source that illuminates the sample and is then reflected through red, green, and blue filters onto photo-detectors as shown in Figure 2.11 (left). The microprocessor can convert the reflected values to XYZ or CIE L*a*b* values. The spectrophotometer illuminates the sample, and the reflected waves are either scanned or read simultaneously by a photo diode array (Figure). These values are sent to a microprocessor and can be presented as the reflected spectra, converted to XYZ values as shown in Figure 2.11 (right). Both colorimeters and spectrophotometers are useful to track color changes in meat over time since they are non-destructive tests.

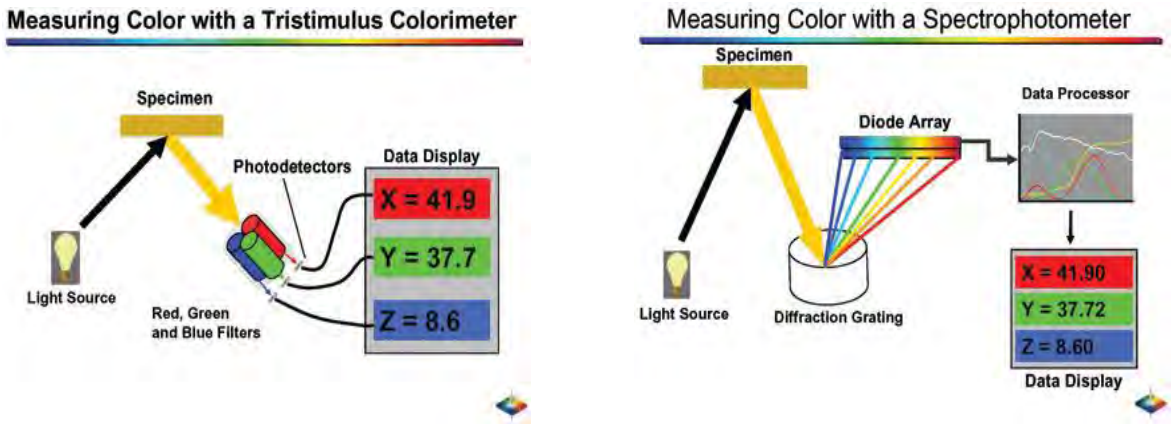


Figure 2.11 Illustration of a tristimulus colorimeter (left) and a spectrophotometer (right). Source: HunterLab (2011).

Some reflectance spectrophotometers are designed to scan wavelengths (colors) reflected from the surface using a diffraction grating, whereas others detect ranges of reflected light through the use of photo diode arrays such as, a type of photo-detector capable of converting light into either current or voltage, depending upon the mode of operation (Konica Minolta sensing, 2007). Reflectance measurements of colour are usually used to assess the color characteristics of meat surfaces (Seideman *et al*., 1984). For research purposes the change in color during shelf display is measured by a change in light reflectance at specific wavelengths (630nm and 580nm). These measurements are used to calculate the oxymyoglobin/metmyoglobin (oxy/met) ratio and this ratio varies between 7 for very red meat to 1 for very brown meat (Lindahl, 2001).

2.4.2. Visual Meat Color Measurement

Visual appraisals of meat color are the fundamental standard of color measurements because they closely relate to consumer evaluations and set the benchmark for instrumental measurement comparisons (AMSA, 2012). Like all sensory evaluations using human panelists, visual color panels are not easy to conduct because human evaluation may not be replicable from day to day and is influenced by personal preference, lighting, visual deficiencies of the eye, and environmental appearance factors other than color (Konica Minolta sensing, 2007). Moreover, meat color cannot be stored, maintained, or reliably reproduced over time. However, through proper panel management, sample presentation, and data collection procedures, visual appraisals of color can provide accurate and repeatable objective data.

According to meat measurement guide line of AMSA, (2012), color panels can be broadly classified as trained visual color panels or consumer panels. Trained, descriptive visual color panels are most commonly used in meat color research and can be regarded as objective instruments. Trained descriptive panelists undergo rigorous screening and training to obtain quantitative ratings of samples on anchored scales. These panelists should not be ask to rate personal preferences or acceptability of the samples they evaluate. Consumer panelists, on the other hand, are useful for providing information using hedonic scales of their preferences and the acceptability of the product‘s attributes. The particular research question determines which type of panel can provide data that addresses that research question.

2.4.3. Image Analysis on Meat Color Change

Image analysis technology has significant promise for assessing meat quality objectively and effectively. Many efforts on using image analysis technique for pork or beef color assessment (Larraínet *et al*., 2008; O‘Sullivana *et al*., 2003; Ringkob, 1997; Lu *et al*., 2000), color scores of beef fat (Chen, 2010), marbling measurement (Faucitano *et al*., 2005; Yoshikawa *et al*., 2000; Gerrard *et al*., 1996) and quality evaluation (Jackman *et al*., 2008; Tan, 2004) have been reported.

In food science/engineering research, it is often necessary to analyze the surface color of food samples both qualitatively and quantitatively. Qualitative analysis may involve visual inspection and comparison of the food samples. Quantitative analysis may involve obtaining color distribution and averages. According to Yam and Papadakis (2004), most commercial color measurement instruments are not well suited for food science and food engineering research, because they are designed mainly for quality control. Since those instruments can only provide average values, it would be rather difficult and time consuming if they were used for point-by-point measurement at many locations to obtain color distribution (Chen, 2010). Moreover, some of these instruments require the food sample to be homogenized using a blender or grinder to achieve uniform color. The blending or grinding not only takes time, but also renders the food sample no longer useable for other purposes.

Rapid advances in hardware and software for digital image processing motivated several studies on the development of computer vision systems to evaluate quality of diverse raw and processed foods (Gerrard, Gao & Tan, 1996). Image analysis is recently described as a highly promising

approach for objectively assessing meat freshness and for the on-line quality control of industrial meat products (Du & Sun, 2004; O¨ tles & O¨ nal, 2004; Wyle *et al*., 2003).

In university research laboratories, computer vision systems have been developed for product quality inspection and grading. Studies were carried out to analyze visual characteristics of products ranging from fruits and vegetables (Tao *et al*. 1995) to meats (for example degree of marbling and color of steaks, Gerrard, Gao, & Tan, 1996).

In the digital imaging method, the required equipment and software costs are low, the experimental setup and operating are simple, and the measurements and analysis are often adequately sophisticated for food science/engineering research. Moreover, the camera took measurements are more representative measurement compared to the colorimeter (O'Sullivan M. *et al*., 2003; Chen, 2010).

The images of the food products can be displayed on computer screen or printed on paper for qualitative analysis of color and structure. Quantitative information such as color distribution and averages (in terms of L^*, a^* and b^* values) can also be determined readily (Yam & Papadakis 2004) by using many photo processing and editing software packages, such as, Adobe Photoshop, Elements, Lightroom, and Picasa which are popular photo management programs. For example, image analysis using Adobe Photoshop CS3 software shown in Figure 2.12 the L*, a*, and b* values are measured on the digital image of the sample visualized on the monitor by pointing the cursor at the center of the area to be evaluated and by clicking on it. The measurements are taken on three different homogeneous areas for each sample.

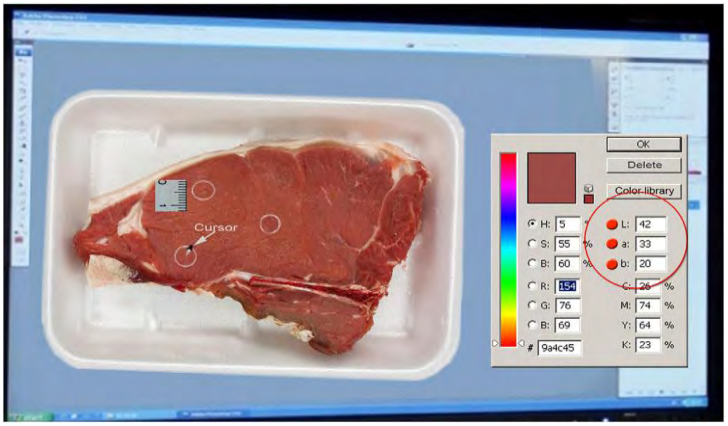


Figure 2.12 Image analysis using Adobe Photoshop CS3 software.
Source: Girolami *et al*., (2013)

In quantitative analysis of color, the L*, a* and b* values are preferable because they are device independent, providing consistent color regardless of the input or output device such as digital camera, scanner, monitor and printer (O'Sullivan *et al*., 2003) and cover a larger gamut than RGB and CMYK (Yam & Papadakis 2004). In addition, The L*a*b* color model is based on the human perception of color and its numeric values describe all the colors that a person sees with a normal vision (Adobe phtoshope CS3, 2007).

A high-resolution digital camera should be used to measure color by capturing the color image of the food sample under proper lighting. When capturing color images, proper light source is important since the color of the food sample depends on the part of spectrum reflected from it (Yam & Papadakis 2004). Hence, the spectral power distribution of the illumination must be standardized. The CIE (1978) has defined several standard illuminants, which are specified by their color temperatures. The standard illuminants commonly used in food research are A (2856K), C (6774K), D65 (6500K), and D (7500K). The light sources C, D65, and D are designed to mimic variations of daylight. The angle between the camera lens axis and the lighting source axis should be around 45^0, because the diffuse reflection responsible for the color occurs at 45^0 from the incident light (AMSA, 2012). Furthermore, the light intensity over the food sample should be uniform.

2.4.3.1. Photography of Meat

The color of displayed meat can be change in short time intervals. Therefore, documenting meat color in photographs is an important component of meat color research. Red, the primary color of meat, is difficult to reproduce accurately in photographs. Therefore, capturing realistic images of red meats and their varying degrees of discoloration is challenging. Special equipment is needed for packaging, lighting, cameras, image processing, and transfer of images to print or computer (AMSA, 2012).

The following guide line recommended by AMSA, (2012) for photography of meat:

A. Packaging

Although the best color reproductions are obtained with unpackaged meat, with care, meat packaged in glossy films can be photographed. For meat packaged in modified atmosphere packages, the effects of headspace and moisture under the top film must be considered. The use

of anti-fog films for meat photography is recommended. If packaged meat is to be photographed, white or black trays are recommended.

B. Lighting and Background Conditions

Photography of meat is best done in rooms completely shielded from daylight. Proper lighting is essential to successful food photography. Light sources can either be camera flashes, permanent lighting, or both. The position of the light should be 45° to the meat from two opposite sides to minimize reflection on the surface. When selecting background for the meat or package, use a material with different color than the subject. This will ease the photo editing process if the subject of the photo must be isolated.

C. Camera and Lens Selection

The digital camera should have a resolution of at least 10 megapixels and should have a direct image output for data transfer. On the camera, selecting the white balance or the light source must be possible. Cameras must be able to capture images as raw file formats, which can then be used for further image processing. Images can be in the formats RAW, JPEG, or TIFF. RAW files can capture up to 12 bits per color (red, blue, green, for 36 bits per location), whereas JPEG files can capture exactly 8 bits per color. A tripod to support the camera and adjust the distance to the objects is important for securing high quality, clear images. A remote control for the camera is valuable to avoid vibrations and unclear images.

2.4.4 Equations for Quantifying Myoglobin Redox Forms on Fresh Meat

The estimation of myoglobin forms is also essential to determine color deterioration on raw meat. The relative contents of Mb, OMb and MMb can be estimate based on reflectance measurement on meat surface. With this nondestructive sampling method, repeated measurements over time can be performed on the same sample. Moreover, the procedure is rapid and easy to perform (Mancini *et al*., 2003).

Several methods exist for estimating the amounts of the three chemical states of myoglobin (Mb) on the surface of meat. However, the two reflectance methodologies are mostly used for quantifying myoglobin redox forms. One involves using surface reflectance to calculate *K/S* ratios at isobestic wavelengths for each myoglobin redox form (Francis and Clydesdale, 1975). The other method uses selected wavelengths with a correction factor (Krzywicki, 1979) to

calculate percentages of DMb and MMb and determines OMb by difference from 100%. Estimating DMb, OMb, and MMb is essential for basic studies of meat pigment stability. However, Ledward (1970) warns that reflectance estimates of the pigment chemical forms were accurate only to ± 6 or 7%.

2.4.4.1 The K /S Method of Isobestic Wavelengths

Reflectance at wavelengths that are isobestic (equal reflectance for two or more of the three myoglobin forms; see Figure 2.13) is measured on the meat sample surface and converted to *K/S* values (Judd & Wyszecki, 1963). Converting reflectance to *K/S* values makes data more linear and helps account for the scattering (*S* = scattering coefficient) and absorptive (*K* = absorbance coefficient) properties of meat (Francis & Clydesdale, 1975). The sample *K/S* values are put into equations requiring reference values for 100% of the three primary meat pigment forms.

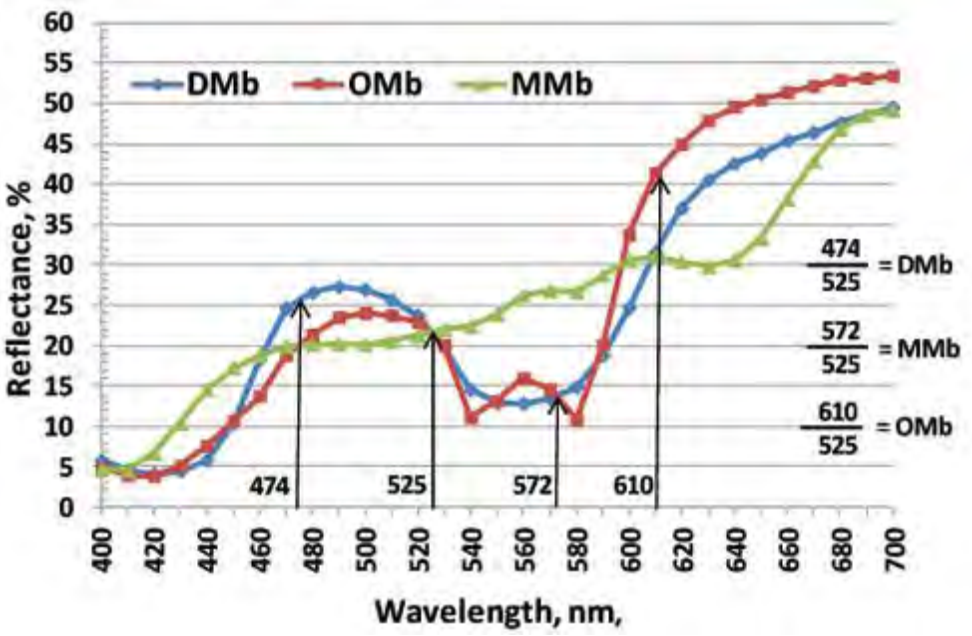


Figure 2.13 Reflectance and isobestic wavelengths use for quantitative determination of myoglobin redox forms.
Source: AMSA, (2012)

Once myoglobin is converted to 100% of each pigment form, record the reflectance at 474, 525, 572, and 610 nm (Mancini *et al*., 2003). Then convert reflectance percentages to *K/S* values using the following equation, $K/S = (1 - R)^2 \div (2R)$, where R = % reflectance. Many reflectance instruments only record reflectance values at 10-nm intervals. Thus, it will be necessary to

integrate the reflectance at 474 using 470 and 480 nm, at 525 using 520 and 530 nm, and at 572 using 570 and 580 nm (Lindahl, 2001).

These 100% reference *K/S* values can then be substituted into the appropriate equation along with sample *K/S* values to calculate the percentage of DMb, OMb or MMb on the sample surface. Equations for myoglobin form estimation were summarized as shown below in Hunt (1980).

$$\%OMb = \frac{\frac{K/S\ 610}{K/S\ 525}\ for\ 100\%\ MMb - \frac{K/S\ 610}{K/S\ 525}\ for\ sample}{\frac{K/S\ 610}{K/S\ 525}\ for\ 100\%\ MMb - \frac{K/S\ 610}{K/S\ 525}\ for\ 100\%\ OMb} \times 100$$

$$\%MMb = \frac{\frac{K/S\ 572}{K/S\ 525}\ for\ 100\%\ DMb - \frac{K/S\ 572}{K/S\ 525}\ for\ sample}{\frac{K/S\ 572}{K/S\ 525}\ for\ 100\%\ DMb - \frac{K/S\ 572}{K/S\ 525}\ for\ 100\%\ MMb} \times 100$$

$$\%DMb = \frac{\frac{K/S\ 474}{K/S\ 525}\ for\ 100\%\ OMb - \frac{K/S\ 474}{K/S\ 525}\ for\ sample}{\frac{K/S\ 474}{K/S\ 525}\ for\ 100\%\ OMb - \frac{K/S\ 474}{K/S\ 525}\ for\ 100\%\ DMb} \times 100$$

2.4.4.2 Calculating Myoglobin Forms via Selected Wavelengths

An alternative to using *K/S* ratios for determining myoglobin forms was presented by Krzywicki (1982). Because 100% conversion of the pigments is not necessary with this method, it does have an advantage. Deoxymyoglobin and MMb are determined and OMb is calculated indirectly by subtracting their combined percentages from 100%. The method is based on the concept of reflex attenuance (A) which is the logarithm of the reciprocal of reflectance. The reflectance is measured at the isobestic wavelengths 474, 525, and 572 nm and at 730 nm, which is referred to as the reflectance of pigment-free meat. Some instruments do not measure reflectance at 730 nm, in which case a reading at 700 nm or any wavelength closer to 730 nm, can be used (Mancini et al., 2003).

Conversion of the reflectance (R) to reflex attenuance (A) is by using Equation 1 and inserts the A-values in Equation 2 to calculate MMb and in Equation 3 to calculate DMb. Oxymyoglobin is calculated using Equation 4:

Equation 1: $A = \log 1/R$

Where R = reflectance at a specific wavelength expressed as a decimal (0.30 rather than 30%)

Equation 2: $$\%MMb = \left\{1.395 - \left[\frac{(A572 - A730)}{(A525 - A730)}\right]\right\} x\ 100$$

Equation 3: $$\%DMb = \left\{2.375\ x - \left[1 - \frac{(A473 - A730)}{(A525 - A730)}\right]\right\} x\ 100$$

Equation 4: %OMb = 100 – (%MMb + %DMb)

Table 2.2 Details on the calculation of various color parameters (AMSA, 2012)

Color parameter	**Purpose of calculation**
630 nm ÷ 580 nm or 630 nm – 580 nm	Larger ratios and differences indicate more redness due to either OMb or DMb; a ratio of 1.0 would indicate essentially 100% MMb. This parameter has been used to follow color change during display.
650 nm ÷ 570 nm	Ratio values of ≈1.1 = no cured color; ≈1.6 = moderate fade; ≈1.7 to 2.0= noticeable cured color; ≈2.2 to 2.6 = excellent cured color
570 nm ÷ 650 nm	where small values indicate less fade.
537 nm ÷ 553 nm	Ratio to establish nicotinamide hemochrome as a pink color defect in uncured cooked light poultry meat. Higher ratios equal more nicotinamide hemochrome.
474 nm ÷ 525 nm	Isobestic wavelengths of OMb and MMb for calculating DMb
572 nm ÷ 525 nm	Isobestic wavelengths of DMb and OMb for calculating MMb
610 nm ÷ 525 nm	Isobestic wavelengths of DMb and MMb for calculating OMb
a* ÷ b* or b* ÷ a*	Larger ratios of a*/b* (or decreases in b*/a*) indicate more redness and less discoloration

Chroma (saturation index)	$C = (a^{*2} + b^{*2})^{1/2}$ with larger values indicating more saturation of the principle hue of the sample. Very useful to indicate intensity of whatever the hue is on the product.
Hue angle	Hue angle = [arctangent (b*/a*)]. Larger values indicate less red, more MMb. Very useful to indicate shifts in color over time toward discoloration.
Delta E	Total color change over a selected period of time. Generally calculated as $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ Useful parameter to show total color differences over time. Various periods of time can be selected and compared

These parameters do not all have to be measured; but one can select the ones most pertinent for the objectives of the study (Krzywicki, 1979). Ideally, L* a*b* data will correlate nicely with each other and their calculated parameters, may tell the complete color history and also reflects important color changes for treatments (Setser, 1984).

2.5. Lipid Oxidation of Beef

Lipid oxidation is a general term that is used to describe a complex sequence of chemical changes that result from the interaction of lipids with oxygen. Oxidative processes result in lipid and protein (including pigment) degradation are one of the primary mechanisms by which quality deterioration limits shelf life in meat and meat products (Faustman et al., 2010). In raw meat products, the substrates necessary for this deteriorative reaction include unsaturated fatty acids, oxygen, light and chemical species that accelerate oxidation (Kanner et al., 1988); these are abundant in meat displayed aerobically or in modified atmosphere packaging. Primary products of lipid oxidation include chemical species formed during the initiation and early propagation steps. Alkyl, alkoxy and peroxy radicals may all be produced and readily abstract protons from neighboring molecules (Faustman et al., 2010). Peroxides are commonly formed as primary products and can subsequently undergo scission to form lower molecular weight secondary oxidation products including aldehydes, ketones and epoxides.

Lipid peroxidation is one of the primary mechanisms of quality deterioration in foods and especially in meat products. The changes in quality can be manifested by deterioration in flavor, color, texture, nutritive value and the production of toxic compounds. Polyunsaturated fatty acids in meat membrane lipids, especially those with four or more double bonds, are particularly susceptible to oxidation (Allen & Cornforth, 2006). A study of the kinetics of lipid autoxidation reported that relative oxidation rates of UFA are as follows: C18:3>C18:2>> C18:1 (Cosgrove *et al*., 1987). In general, the higher the degree of unsaturation, especially the polyunsaturation, is the higher the rate of oxidation.

Furthermore, the relationship between lipid and pigment oxidation is significant (Kanner & Harel 1985). Lipid oxidation and myoglobin oxidation in meat lead to off-flavor development and discoloration, respectively. These processes often appear to be linked and the oxidation of one of these leads to the formation of chemical species that can exacerbate oxidation of the other. For example, free radicals produced by lipid oxidation can accelerate oxymyoglobin oxidation to metmyoglobin (Faustman et al., 2010). Similarly, hydrogen peroxide activates metmyoglobin to form ferrylmyoglobin radicals, which are lipid oxidation catalysts in muscle foods (Kanner *et al.* 1988).

2.5.1. Lipid Oxidation Mechanisms

Lipid oxidation is often referred to as Autoxidation. The term Autoxidation used to describe the self-perpetuating of free radicals from unsaturated fatty acid in the presence of oxygen that occurs during lipid oxidation. Autoxidation is a natural process that takes place between molecular oxygen and unsaturated fatty acids. Autoxidation of unsaturated fatty acids occurs via a free radical chain mechanism consisting of basic steps of initiation [Eq. (1)], propagation [Eqs. (2) to (3)], and termination [Eqs. (4) to (6)] as shown below.

Initiation starts with the abstraction of a hydrogen atom adjacent to a double bond in a fatty acid (LH) molecule/moiety, and this may be catalyzed by light, heat, or metal ions to form a free radical. The resultant alkyl free radical (L^{*}) reacts with atmospheric oxygen to form an unstable peroxy free radical ($LOO^{*)}$, which may in turn abstract a hydrogen atom from another unsaturated fatty acid to form a hydroperoxide (LOOH) and a new alkyl free radical. The new alkyl free radical initiates further oxidation and contributes to the chain reaction. The chain

reaction (or propagation) may be terminated by formation of nonradical products resulting from combination of two radical species.

$$\text{Initiation: } LH \longrightarrow L^{*} + H^{*} \quad (1)$$

$$\text{Propagation: } L^{*} + O_2 \longrightarrow LOO^{*} \quad (2)$$

$$LOO^{*} + LH \longrightarrow LOOH + L^{*} \quad (3)$$

$$\text{Termination: } L^{*} + L^{*} \longrightarrow LL \quad (4)$$

$$L^{*} + LOO^{*} \longrightarrow LOOL \quad (5)$$

$$LOO^{*} + LOO^{*} \longrightarrow LOOL + O_2 \quad (6)$$

Prooxidants (singlet oxygen, transition metals, radiation and lipooxygenase) are found in virtually all food systems and biological systems, are compounds or factors that cause or accelerate lipid oxidation. Prooxidants can accelerate lipid oxidation by direct interaction with unsaturated fatty acids to form hydroperoxids or by promoting formation of free radicals (McClements & Dacker 2008).

2.5.2. Radiant Energy on Lipid Oxidation

The mechanism of light to cause or accelerate lipid oxidation (Photochemical effect) is described as the following:

To better understand the mechanism, a briefly review of the wave-particle duality of light first described by Einstein in 1905 is necessary. Light is a form of electromagnetic energy. Electromagnetic radiation has a dual wave-particle nature. While we may often think of light as being comprised of a continuous spectrum of different radiant wavelengths, it is vital to also consider the more particulate properties of light, including the existence of light as quanta of energy referred to as photons. A photon can be absorbed by a molecule only if the photon energy is equivalent to the energy difference between the molecule‘s current energy state and an allowed higher-energy level known as the excitation state. When light is absorbed by a meat pigments, its particle nature is important.

Photochemical effect is thought to be the most common mechanism by which light exposure causes or accelerate lipid oxidation on meat or generate free radicals (Rokyta *et al*., 2004). It is associated with both long-duration exposure times as well as lower-wavelength light exposure.

Visible and invisible radiation from light sources has a harmful effect on the shelf life of food. It is not only the UV and infrared cycles of the spectrum that damage food. There is considerable literature that connects the photochemical oxidation of most food groups to the wavelengths of visible radiation that these foods are exposed to (Rokyta *et al*., 2004).

The heme proteins/ the pigment myoglobin of meat are theorized to mediate the light-induced effect on the meat. Light with wavelengths in the high-energy portion of the visible spectrum interacts with the pigment myoglobin (chromophore) molecules contained within the meat. A chromophore is a region in a molecule in which the energy difference between two different molecular orbitals falls within the range of the visible spectrum. Visible light that hits the chromophore can thus be absorbed by exciting an electron from its ground state into an excited state.

The exposure of radiant energy can cause the generation of free radicals in one of two ways (Rokyta et al., 2004). In the first mechanism of free radical generation, absorption of radiant energy causes excitation of electrons from the ground state to the excitation state. However, the excitation state is unstable and because of this volatility the raised level of energy in the excitation state can be dissipated in one of several ways. While some atoms will simply release the quanta of energy that they previously absorbed and return the excited electron to the ground state, other interactions may lead to the formation of free radicals or reactive oxygen species. Free radicals form after the higher energy level of the excitation state is used to split the bond in another molecule either by direct electron exchange or direct hydrogen exchange. In the second mechanism, the absorption of radiant energy leads to the direct transfer of energy from the excited chromophore to oxygen, creating a singlet oxygen species. Once generated, free radicals can attack many molecule types, thereby causing adverse effect on meat. Muscle tissues in which there is a large concentration of cell membranes are particularly vulnerable to free radicals; the attack of free radicals on polyunsaturated fatty acids results in lipid peroxidation that breaks down membranous structures. Lipid peroxidation is propagated as a chain reaction and can cause extensive damage.

2.5.3. Effect of Lipid Oxidation on Fresh Meat Color

Many meat color studies include measures of lipid oxidation, because myoglobin oxidation is often closely linked with lipid oxidation. Aldehyde products of lipid oxidation initiate

conformational changes in myoglobin, causing increased heme oxidation and browning (Alderton *et al*., 2003). Hemin released from fish hemoglobin during storage also stimulates lipid oxidation (Grunwald and Richards, 2006). Similarly, ionic iron released from heme during heating may stimulate lipid oxidation, as measured by the assay for thiobarbituric acid reactive substances (Igene *et al*., 1985). The extent of lipid oxidation can be measured using many techniques, including headspace analysis of volatile oxidation products (Watanabe *et al*., 2008), and sensory evaluation, but the TBARS test is most often used in meat products.

2.5.4. Lipid Oxidation Measurement

In the presence of an initiator such as light, heat, or metal ions, unsaturated lipids can generate different types of free radicals (Youssef, 2011; McClements & Dacker, 2008). Quantifying these free radicals to estimate the extent of lipid oxidation is very difficult and need very sophisticated instruments. Because, free radicals have a very short half-life (Rokyta *et al*., 2004), which makes them very hard to measure in the laboratory. A commonly used alternate approach measures markers of free radicals rather than the actual radical. These markers of oxidative stress are measured using a variety of different assays. These assays are described below:

a) Conjugated dienes (CD) are often measured as indicators of free radical production. Oxidation of unsaturated fatty acids results in the formation of CD. The CD formed is measured and provide a marker of the early stages of lipid peroxidation (Ronald, 2001).

b) The peroxide value (PV) of an oil or fat is defined as the quantity of peroxide oxygen present in the sample. The primary products from autoxidation are hydroperoxides, which are often simply referred to as peroxides. Peroxides in general can be measured by titrimetric methods based on their oxidation potential to oxidize iodide (I^-) to iodine (I_2). The amount of iodine present is determined by titration with a standard sodium thiosulfate solution using a starch indicator, thereby reflecting how much peroxide is present in the oil or lipid extract (Marmesat *et al*., 2009). The results are generally expressed as milliequivalents active oxygen (i.e., peroxide) per kilogram lipid (meq/kg). This method is easy and produces consistent results (Ronald, 2001).

c) Thiobarbituric acid reacting substances (TBARS) have been widely accepted as a general marker of free radical production (O'Keefe & Pike, 2010). When a fatty acid is peroxidized it is broken down into aldehydes, which are excreted. The most commonly measured TBARS is

malondialdehyde (MDA). Malondialdehyde (MDA) is a major degradation product of lipid hydroperoxides has attracted much attention as marker for assessing the extent of lipid hydroperoxidation.

Generally, more than one method should be used to determine the extent of oxidation to enhance validity; it is recommended that one method measure primary products of lipid oxidation (e.g., peroxide value, conjugated dienes) and the other method measure secondary products (e.g., VOC, *p*-anisidine value, TBARS, malonaldehyde/high-performance liquid chromatography assay, malonaldehyde/gas chromatography assay) O'Keefe & Pike 2010).

2.6. Displaying of Meat

Display is defined as the offering of product under lighting in the retail case, usually under refrigeration, but there is also display aerobically. Display is not the same as storage, which implies keeping the product in the dark and usually not for sale (Kropf, 1998).The key to retail meat display is to present meat products in an attractive and saleable format using bright light. Effective sales depend on fresh appearance and acceptable product quality. Visual appearance and color are important factors in consumer selection of beef (Hutchings, 1999).

2.6.1. Meat Display Lighting

Food store managers usually keep the meat display are a well-lighted to attract consumers; the light source used in a meat display case can have a significant effect on the customer impression and decision to purchase fresh meat, so that different types of light sources can be used. The three main sources include incandescent (INC), fluorescent (FL), and metal halide (MH). These sources have different spectral distribution patterns (Bickford and Dunn, 1972; Philips, 1991), and the decision to install each one depends on various factors (e.g., energy efficiency, heat output, cost of the light bulb, and its life expectancy). For example, FL bulbs radiate about one-fifth of the heat produced by INC bulbs of the same light output (Kropf, 1980), and, therefore, FL bulbs can be placed inside the display cooler. A source such as MH is the most efficient one to illuminate a large area, but it does not produce a full visible spectrum.

2.6.1.1. Incandescent Display Light

Many different processes convert energy into visible radiation (light). One basic process is Incandescnce. Solid and liquids emit visible radiation when they are heated to temperature above

1000K, the intensity increases and the appearance becomes whiter as the temperature increases. This phenomenon is known as incandescnce or temperature radiation, eg. an incandescent lamps.

An incandescent lamp produces light by using electric current to heat a metallic filament to high temperature about 3000^{o}C. A tungsten filament is used because of its high melting point and low rate of evaporation at high temperature. The filament is coiled to shorten the overall length and to reduce thermal loss and it enclosed in glass bulb (Figure 2.14) filled with inert gas at low pressure because it permits operation at higher temperature compared to vacuum, resulting in a smaller evaporation rate of the filament.

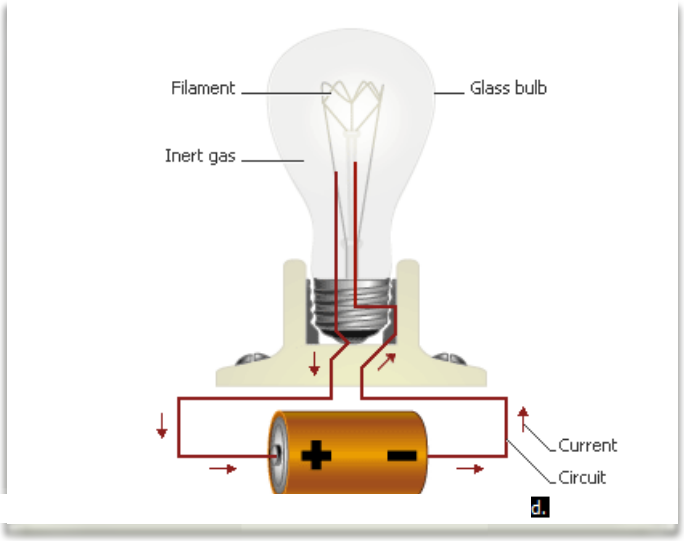


Figure 2.14 Parts of incandescent lamp
Source: Microsoft Encarta (2008)

2.6.2. Retail Display Lighting Effect on Meat Shelf Life

Type of light source, illuminance level and duration of exposure, temperature elevation, color rendering, specific light wavelengths and other factors affect the appearance and discoloration of meats.

2.6.2.1. Type of light source on appearance of meat

Light is a combination of colors emitted from a light source. Light determines how all things look. The same product can look different under different light sources. Different lighting can affect the perceived color of meat as well as various lighting can make meat look redder, bluer, greener, yellower, or grayer.

Food store managers usually keep the meat display area well lighted to attract consumers (Barbut, 2001). In addition, they mostly assign a relatively large display space to high value meat cuts (Kropf, 1980) and use bright lights from different types of light sources.

In work of Barbut (2003), meat color varied under the three light sources (INC, FL & MH) and the color of beef was most preferred under INC light, due to the appearing red color. When beef was presented under FL light, it was significantly less desirable than under INC, due to the lack of redness in the FL light source (Barbut, 2003). Furthermore, in regard to color balance and color rendering of displayed meats, many studies (Barbut, 2001, Kropf, 1984) reported that the incandescent lamps generally are preferable in terms of color if not on the basis of radiant heat, than fluorescent lamps. Light reflectance data indicated that the reason was a lack of red light in the bulb output of FL light and the INC produces a so-called full spectrum, which provided a full natural color to the product, i.e., there was adequate reflection in the yellow (550 to 600 nm) and red (650 to 700 nm) ranges (Barbut, 2003). Moreover, some incandescent lamps use dichroic cold mirror reflectors to direct the light beam forward while letting the infrared power pass backwards through the reflector. These would be the preferred lamps when incandescent lighting is used on meat displays. In general, light sources that emit higher proportions of red light are desirable for meat product display.

2.6.2.2. The Intensity and Duration of Display Illumination on Quality of Meat

Another important consideration is the intensity and duration of display illumination. Too great of light intensity accelerates discoloration of meat (Kropf *et al*., 1984, Lavelle, Hunt & Kropf, 1995), whereas too low intensity does not adequately illuminate the product. Optimizing the variables of lighting type and intensity results in the best appearance of meat product and increases display life.

The retail case life of fresh beef is usually limited to 2 to 3 days due to the development of undesirable surface discoloration (Greer 1984). In the work of Schwab (1981) discoloration was closely related to foot-candle intensity and length of light exposure time. Wambura & Verghese (2011) also reported that the location of the meat sample from the lamp played a major role in the change in temperature that means the meat samples closer to the INC lamp and prolonged treatment time resulted in higher temperatures than their counterparts.

2.6.2.3. Temperature Elevation on Meat Surface

Radiant heat from intense display lighting increases the temperature on the meat surface (Chiang & Ringkob 1999). Temperature of the meat surface increases proportionally with increased light intensity under both incandescent and fluorescent lights. As cited in Kropf (1998) Santa Maria reported that the temperature elevation of about 7^0C and 6^0C respectively at the meat surface from incandescent and the cool white fluorescent lights compared to temperatures of sample kept in the dark. An estimated 1^0F temperature rise has been reported for each 10 foot-candles of incandescent lighting for display cases with 70 cubic feet per minute air velocity (Kropf 1998).

The heat that elevates the meat surface can originate from the absorption of light by the meat (Photothermal effect) and/ or from lamp heat. Photothermal effect occurs by the transfer of radiant energy, a photon, from light to the meat, based upon the following mechanism:

A photon can be absorbed by a molecule only if the photon energy is equivalent to the energy difference between the molecule‘s current energy state and an allowed higher-energy level known as the excitation state. For wavelengths of light at the upper end of the visible spectrum, as well as wavelengths of light near infrared, a vibration and rotational energy states predominate over the excitation states (Youssef, 2010). Therefore, rather than attain their excitation states, molecules in the muscle tissue tend to gain both rotational and vibration energy. This increase in mean kinetic energy is dissipated as molecules collide with each other and their temperature increases. The ability of light to cause an increase in mean kinetic energy is inversely proportional to the wavelength of the light. This relationship between light and energy is described by the equation:

$$E = hc/\lambda$$

Where energy (E) equals Planck‘s constant (h) multiplied by the speed of light (c) divided by the wavelength of light (λ). The shorter the wavelength has the greater potential to increase kinetic energy and the rise in temperature for a given exposure time. In a closed system, there is a proportional relationship between exposure time and thermal effect; in an open system, the amount of energy required to produce a given thermal effect increases for longer exposure times as energy in the form of heat dissipates to the surrounding environment during the exposure.

Higher temperatures at the meat surface speed up deteriorative influences on meat color such as oxidation and microbial metabolism (Chiang & Ringkob 1999; Greer 1984). The growth rate of typical beef spoilage bacteria can double as the temperature of incubation increases from 1 to 5° C and can triple with a further increase to 10°C (Greer 1984). Increased bacterial growth means reduced case life.

In the display case study of Greer G. (1984) a beef steak was illuminated with 150 watt incandescent, cool-beam floodlights to give a light intensity of about 80 foot-candles at the meat surface to illuminate the display case for 12 hours. During this 12 hour period of illumination steak surface temperatures were found to be higher than in the absence of illumination. Thus, although display illumination enhances the appearance of beef it is detrimental to keeping quality.

2.6.2.4. Photochemical Effects

Photochemical effects are caused by certain wavelength energies that excite one or more molecules and initiate or catalyze such reactions as oxidation which leads to a change in the meat pigment, myoglobin, causing discoloration. Meat discoloration is associated with the accumulation of metmyoglobin on the meat surface as reported by Kannan, Kouakou & Gelaye, (2001). In addition, wavelengths that are absorbed cause photochemical effects, resulting in greater destruction of meat pigments, but wavelengths that are primarily reflected produce less of a photochemical effect.

2.6.2.5. Color Rendition

Color rendition means how closely the spectral energy distribution of the lighting matches the color (light reflecting pattern) of the meat. Light source with a closer fit to the reflecting pattern for a meat product will come closer to bringing out the true appearance of that product. Light sources that have relatively low emission in the red part of the spectrum (eg. fluorescent lamps) and high emission in the blue part will result in an undesirable less red meat color. Conversely, a display light producing too high of a proportion of red emission may contribute to a deceivingly red appearance of meat. Barbut (2001) noted that beef inside round steak appeared redder under incandescent lighting than fluorescent or metal halide lighting, with each used at a 760 lux light intensity.

Light sources are characterized by several systems as to their color rendering properties. Color rendering index (CRI) is based on emissions at eight specific wavelengths and is a widely accepted system. Since some of the wavelengths do not relate well to meat color, this system has limited value in describing appropriate meat display lighting, even though the Lighting Handbook (IESNA, 2001) suggests use of fluorescent lamps with high CRI plus a "strong content of red wavelengths". Kropf (1980) suggested that a color temperature, in degrees Kelvin, to be a more meaningful indicator of recommended display lighting. A wide range of degrees Kelvin, from 2600 (describing a warm light with a higher proportion of red) to 4200 (describing a colder appearing, bluer, less red light), is appropriate for meat display lighting.

2.6.2.6. Specific Light Wavelengths on Discoloration of Meat

The available litrature contains few reports of work directly related the spesific wavelength of radiant energy that cause changes in muscle color. Kropf (1980) has attempted to determine effects of specific regions of the color spectrum upon meat color stability. He demonstrated that oxymyoglobin, the pigment responsible for the red color of fresh meat, is damaged by visible spectrum wavelengths at 545 and 582 nm, while metmyoglobin, which gives meat a brown color, absorbs radiation at 504 and 630 nm wavelengths. Furthermore, in Satterlee and Zachariah (1972) model studies using beef, lamb or pork myoglobin, led them to conclude that "low wavelength light" encouraged oxidation. Therefore, rate of discoloration would increase. But wavelengths of 625 nm or longer are less absorbed by oxymyoglobin, which means these would be less prone to promote discoloration. On the bases of work with colored filteres, Iversen (1985) reported that film with all wavelengths below 550 nm blocked out was effective in slowing oxidation and discoloration. However, as sighted on Setster *et al*., (1973), Fownsend & Bratzler (1958) suggested that 560-630 nm light was the region of the spectrum most damaging to frozen meat pigments. Incontrast, Solburg & Franke (1971) found no one wavelength among six selected wavelengths of visible light spectrum resulted in greater damage to meat pigments than any other wavelenghts.

2.7. Light Measurement and Terminology

Light is electromagnetic radiation. What we see as visible light is only a tiny fraction of the electromagnetic spectrum, extending from very low frequency radio waves through microwaves,

infrared, visible and ultraviolet light to x-rays and very high frequency gamma rays. Our eyes respond to visible light; detecting the rest of the spectrum requires a scientific instruments.

Radiometry is the science of measuring light in any portion of the electromagnetic spectrum. In practice, the term is usually limited to the measurement of infrared, visible, and ultraviolet light using optical instruments (Ian Ashdown, 2002).

Photometry is the science of measuring visible light in units that are weighted according to the sensitivity of the human eye. It is a quantitative science based on a statistical model of the human visual response to light that is, human perception of light. The only difference between radiometric and photometric theory is in their units of measurement (Ian Ashdown, 2002).

Table 2.3 The four basic concepts of practical light measurement

Radiometricaly		photometricaly	
quantity	**unit**	**quantity**	**unit**
Radiant flux (Radiant power)	watt	Luminous flux (Luminous power)	Lumen
Radiant Intensity	Watt per steradian	Luminous Intensity	candela
Radiant Flux Density (Irradiance)	Watt/m^2	Luminous Flux Density (illuminance)	Lux /foot-candel
Radiance	watts per square meter per steradian	Luminance	Candela per square metre (cd/m^2)

Source: Ian Ashdown, (2002)

2.7.1. Radiant Energy

Light is radiant energy. Electromagnetic radiation (which can be considered both a wave and a particle, depending on how one measure it) transports energy through space. When light is absorbed by a physical object, its energy is converted into some other form. For example, visible light causes an electric current to flow in a photographic light meter when its radiant energy is transferred to the electrons as kinetic energy. Radiant energy (denoted as Q) is measured in joules.

2.7.2. Radiant Flux (Radiant Power)

Energy per unit time is power, which measure in joules per second, or watts. A laser beam, for example, has so many milliwatts or watts of radiant power. Light flows through space, and so radiant power is more commonly referred to as the ―time rate of flow of radiant energy," or radiant flux. It is defined as:

$$\Phi = dQ/dt$$

Where Q is radiant energy and t is time.

In terms of a photographic light meter measuring visible light, the instantaneous magnitude of the electric current is directly proportional to the radiant flux. The total amount of current measured over a period of time is directly proportional to the radiant energy absorbed by the light meter during that time. This is how a photographic flash meter works, it measures the total amount of radiant energy received from a camera flash (Ian Ashdown, 2002).

2.7.3. Radiant Flux Density (Irradiance)

Radiant flux density is the radiant flux per unit area at a point on a surface, where the surface can be real or imaginary (i.e., a mathematical plane). The flux can arrive from any direction above the surface.

Irradiance is defined as: $E = d\Phi/dA$

where Φ is the radiant flux arriving at the point and dA is the differential area surrounding the point.

2.7.4. Radiant Intensity

An infinitesimally small point source of light that emits radiant flux in every direction. The amount of radiant flux emitted in a given direction can be represented by a ray of light contained in an elemental cone. This gives us the definition of radiant intensity:

$$I = d\Phi/d\omega$$

where $d\omega$ is the differential solid angle of the elemental cone containing the given direction. From the definition of a differential solid angle ($d\omega = dA/r^2$), we get:

$$E = d\Phi/dA = d\Phi/r^2 d\omega = I/r^2$$

where the differential surface area dA is on the surface of a sphere centered on and at a distance r from the source and E is the irradiance of that surface. More generally, the radiant flux will

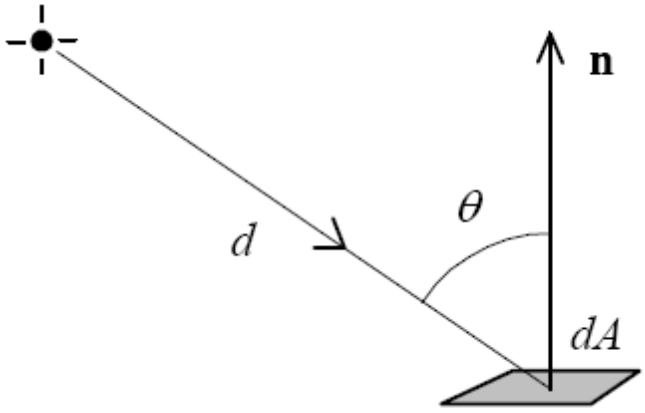


Figure 2.15 Inverse square law for point sources

intercept dA at an angle θ (see Figure 2. 15). This gives us the inverse square law for point sources:

$$E=I\cos\theta/r^2 \text{ and } E_p=I/h^2 \text{ (for perpendicular light source; Figure 2.16)}$$

where I is the intensity of the source in the given direction and d is the distance from the source to the surface element dA.

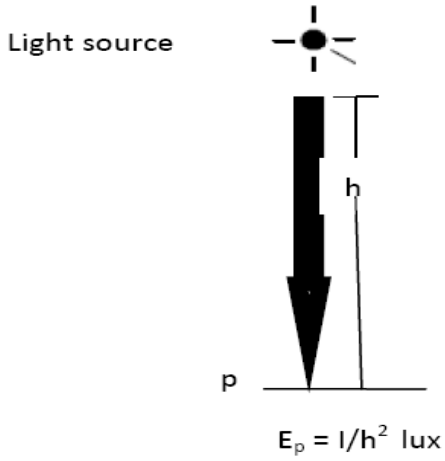


Figure 2.16 The illuminance/irradiance on a point in a plane perpendicular to the direction of light

2.7.5. Luminous Flux Density (Illuminance)

Luminous flux density is photometrically weighted radiant flux density. Illuminance is the photometric equivalent of irradiance. It is measured in lux or lumens per square meter. (A foot-candle is one lumen per square foot) (Ian Ashdown, 2002).

2.7.6. Optical Filters

Most light sources emit a broad range of wavelengths that cover the entire visible light spectrum. In many instances, however, it is desirable to produce light that has a restricted wavelength spectrum. This can be easily accomplished through the use of specialized filters that transmit some wavelengths and selectively absorb or reflect unwanted wavelengths.

A color filter functions by selectively transmitting or blocking (absorbing) spectral elements of a beam of white light emanating from a light source. For example, a Roscolux 27 Medium Red filter will allow red light frequencies to pass through and absorb blue and green.

A Spectral Energy Distribution curve is a graph of the transmission of energy plotted by wavelength. These curves are included in the filters. In Figure 2.17, the curve for R27 shows that frequencies above 620nm will pass through the filter at varying percentages, while the wavelengths below will not. With this information, one can predict what color the filter will render. As a reference, the peak intensity for violet is 440, blue 480, green 520, yellow 570 and red, 650 (Billington, 2014)..

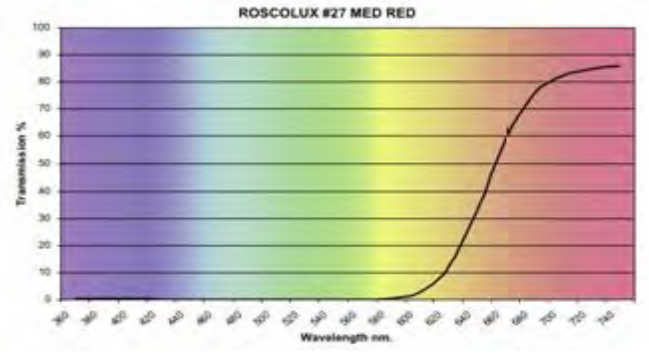


Figure 2.17 A Spectral Energy Distribution curve for Roscolux 27 Medium Red filter
Source: Billington, (2014)

Color filters are usually constructed using transparent pieces of dyed glass, plastic, lacquered gelatin that have been treated to selectively transmit the desired wavelengths while restricting others. The two most common types of filters in use today are absorption filters that absorb

unwanted wavelengths and interference filters that remove selected wavelengths by internal destructive interference and reflection.

A. Absorption filters – these filters absorb unwanted wavelengths as shown in the Figure 2.18 below.

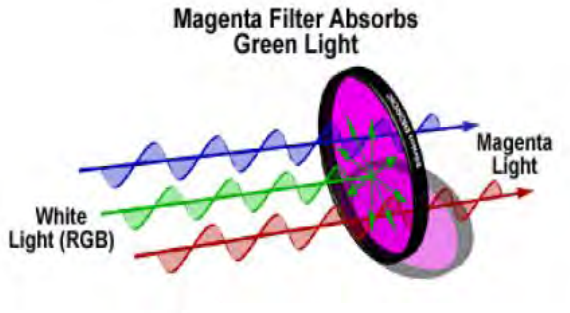


Figure 2.19 Absorption filter

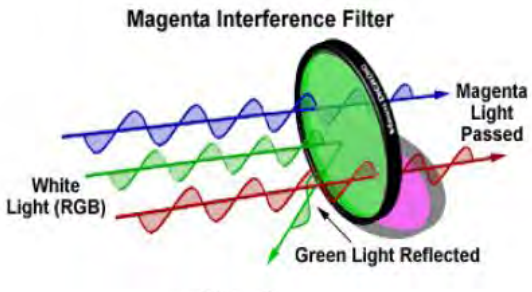


Figure 2.18 Interference Filter

Source: Neaves, Davidson & Abramowitz (2003).

In Figure 2.18, the three incident waves are colored red, green, and blue but are intended to represent all the colors that comprise white light. The filter selectively transmits the red and blue portions of the incident white light spectrum, but absorbs most of the green wavelengths.

B. Interference filters - These filters differ from absorption filters in the fact that they reflect and destructively interfere with unwanted wavelengths as opposed to absorbing them. The term *dichroic* arises from the fact that the filter appears one color under illumination with transmitted light and another with reflected light. In the case of the magenta dichroic filter illustrated in Figure 2.19, green light is reflected from the face of the filter and magenta light is transmitted from the other side of the filter.

C. Dichroic filters are far more accurate and efficient in their ability to block unwanted wavelengths when compared to gel and glass absorption filters (Neaves, Davidson & Abramowitz, 2003). Short and long wavelength pass dichroic filters act as the names imply and allow transmission of only narrow bands of short or long wavelengths, reflecting the unwanted wavelengths. Band pass dichroic filters are the most common and are designed to transmit selected wavelengths in the visible region.

When it uses before a light source, a dichroic filter produces light that is perceived by humans to be highly saturated (intense) in color. When it uses behind a light source, dichroic reflectors commonly reflect visible light forward while allowing the invisible infrared light (radiated heat) to pass out of the rear of the fixture, resulting in a beam of light that is "cooler". Many quartz halogen bulbs have an integrated dichroic reflector for this purpose, being originally designed for use in slide projectors to avoid melting the slides, but now widely used for interior home and commercial lighting (Neaves, Davidson & Abramowitz, 2003).

2.8. Spices as a Natural Antioxidant

Antioxidants are the compounds which inhibit the oxidation reactions caused by free radicals. Free radicals which are also called as Reactive Oxygen Species (ROS) or Active Oxygen Species (AOS), are produced during various metabolic cellular processes. ROS include free radicals such as hydroxyl radicals (OH^{-}), superoxide ions (O^{2-}), nitric oxide (NO^{-}), alkyl oxide (RO^{-}), alkyl carboxylic acid (ROO^{-}) and non-free radicals such as hydrogen peroxide (H_2O_2), singlet oxygen (O^{-}), hypo chloride radicals ($HClO^{-}$) (Halliwell, 1995) and Odukoya et al., 2005). Normal range of production of ROS helps in the regulation of cell proliferation, intercellular signalization, phagocytosis and also synthesis of biologically active compounds. But hyper production of ROS develops oxidative stress which causes cardiovascular diseases, diabetes, tumors, rheumatoid arthritis, epilepsy, mutagenesis, carcinogenesis, arteriosclerosis, Alzheimer‘s disease, tissue injury (Halliwell and Gutteridge, 1989; Alho and Leinonen, 1999).

Spices are the best sources of polyphenol compounds such as flavoids, flavanoids, phenolic compounds, anthocyanins, phenylpropanoids, anthraquinones which are good antioxidants (Graham, 1992; Rice-Evans *et al*., 1996).

Natural antioxidants extracted from spices (Ginger, Garlic, Rosemary, Cardamom, Cinnamon, Clove, Mustard, Sage and Hot Pepper) are reported to be effective additives inhibiting lipid oxidation due to the antioxidative compounds they contain, when used in food systems (Iris *et al*., 2006; Stoilova *et al*., 2007; Dimitrios et *al.,* 2007; Biljiana *et al*, 2008 ;Yara *et al*, 2009; Baohua *et al*., 2010). Furthermore, grape seed extracts have shown both antioxidant and antimicrobial activities on meat (Ahn, Grun, & Fernando, 2002; Jayaprakasha, Selvi, & Sakariah, 2003). Many spices have components that act as antioxidants and protect cells from free radicals.

Some spices have more antioxidant properties than others depending on the type of compounds they contain. Combining spices with other spices or antioxidants such as tocopherols and ascorbic acid produces synergistic effects (Dimitrios et *al.,* 2007). It has been suggested that interactions between structurally different compounds with variable antioxidant activity provides additional protection against increased oxidative stress (Monica *et al.*, 2002). This cooperative effect of the inhibitors during oxidation which results by their reinforcing each other is known as synergism.

Antioxidants from spices and herbs have the potential for large-scale applications. They have been used not only for their flavoring and coloring properties but also for their food-preserving ability for hundreds of years. Many researchers have looked at the potential for spices as important natural antioxidants (Guerra *et al*., 2005; Priyanjali et *al*., 2005; Lars et *al*., 2010). Their antioxidant activity has been attributed to the presence of polar phenolic compounds and essential oils (Aline *et al*., 2008).

Among phytochemicals possessing antioxidant capacity, phenolic compounds are one of the most important groups (Jahangir et al., 2009). Phenolics are characterized by having at least one aromatic ring with one or more hydroxyl groups attached. In excess of 8000 phenolic structures have been reported and they are widely dispersed throughout the plant kingdom (Strack 1997). Phenolics range from simple, low molecular-weight, single aromatic-ringed compounds to large and complex tannins and derived polyphenols. They can be classified based on the number and arrangement of their carbon atoms and are commonly found conjugated to sugars and organic acids.

The most widespread and diverse group of polyphenols in *Brassica* species are the flavonoids (mainly flavonols but also anthocyanins) and the hydroxycinnamic acids (Cartea *et al*., 2011). Flavonoids are polyphenolic compounds comprising fifteen carbons, with two aromatic rings connected by a three-carbon bridge. The basic flavonoid skeleton can have numerous substituents. Hydroxyl groups are usually present at the 4, 5 and 7 positions`(Pereira *et al.,* 2009). Sugars are very common with the majority of flavonoids existing naturally as glycosides. Whereas both sugars and hydroxyl groups increase the water solubility of flavonoids, other substituents, such as methyl groups and isopentyl units, make flavonoids lipophilic (Crozier *et al.* 2009). In plants, flavonoids are involved in such diverse processes as UV protection,

pigmentation, stimulation of nitrogen-fixing nodules and disease resistance (Pereira et al., 2009; Pierpoint 2000).

The phenolic compounds, simple phenolics, phenolic acids, anthocyanins, hydroxycinnamic acid derivatives, flavonoid and tannins, have received considerable attention because of their physiological functions, including free radical scavenging antioxidant (Peng *et al*., 2011) activity. The antioxidant activity of phenolics is mainly due to their redox properties which make them act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelating agents (Izabela *et al*., 2010).

The antioxidant activity of phenolic compounds is related with its chemical structure that confers them redox properties. They can play an important role in adsorbing and neutralizing reactive oxygen species (ROS), quenching singlet and triplet oxygen, or decomposing peroxides. Reactive oxygen species, derived from oxidation processes, are an important part of the defense mechanisms against infection, but excessive generation of free oxygen radicals may damage the tissue. When there is an imbalance between ROS and antioxidant defense mechanisms, the ROS lead to the oxidative modification in cellular membranes or intracellular molecules and result in the peroxidation of membrane lipids, leading to the accumulation of lipid peroxides. This oxidative stress has been linked to cancer, aging, atherosclerosis, inflammation and neurodegenerative diseases such as Parkinson‘s and Alzheimer‘s disease (Shen, *et al*., 2010). Therefore, antioxidants, such as phenolic compounds, are considered as possible protective agents, reducing the oxidative damage from ROS in the human body and retarding the progress of many chronic diseases as well as the oxidation of low-density lipoproteins, which is thought to play an important role in atherosclerosis. The antioxidant ability of flavonoids and phenolic acids is related to the number and position of hydroxyl groups in the molecule; an increase in the number of hydroxyl groups leads to a higher antioxidant activity (Podsedek, A., 2007). Compounds with three hydroxyl groups on the phenyl ring of phenolic acids or the B ring of flavonoids have a high antioxidant activity. The loss of one hydroxyl group decreases activity slightly, whereas the loss of two hydroxyl groups significantly decreases the activity (Fukumoto & Mazza G., 2000).

The antioxidant capacity of Brassica species has been related to its phenolic profile and content, especially flavonoids, since phenolic compounds have demonstrated a higher antioxidant activity

than vitamins and carotenoids (Llorach, R.,et al 2003). Many studies have shown the antioxidant power of particular flavonoids and flavonoid-rich extracts. Flavonoids can also inhibit, and sometimes induce, a large variety of mammalian enzyme systems; some of these enzymes are involved in important pathways that regulate cell division and proliferation, platelet aggregation, detoxification and inflammatory and immune response. Over the last 15 years, numerous publications have demonstrated that besides the in vitro antioxidant capacity, certain phenolic compounds such as anthocyanins, catechins, proanthocyanidins and other non-colored flavonoids may regulate different signaling pathways involved in cell survival, growth and differentiation (Podsedek, A., 2007, Ackland *et al*., 2005). The effects of flavonoids on various stages of the cancer process, on the immune system and on homeostasis in cell systems and animals have been also described (Aron, P.M. & Kennedy, J.A., 2008). However, nowadays, it is widely accepted that if flavonoids have any preventive or curative activity through their ingestion, this effect must involve, not only their antioxidant potential, but also the modulation of multiple cellular pathways that are crucial in the pathogenesis of those diseases (de Pascual-Teresa *et al*., 2010; Skandrani et al., 2010).

Oxidative rancidity greatly affects the quality of food products, especially products with relatively high fat content like meat. To combat oxidative rancidity, antioxidants are often added to these products to help improve the shelf life (Baohua *et al*., 2010). The most common antioxidants in use today are butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT), but consumer concerns over the health risks attributed to these two additives has prompted researchers to investigate antioxidants from other sources such as natural dietary spices and herbs. Cho S.H. *et al*. (2004) explained that lipid oxidation growth in meat products can be controlled or minimized by using either synthetic or natural food additives either by incorporating on the diet of the animal or adding during processing, cooking and packaging.

2.8.1. Antioxidant Measurments

Several analytical methods have been developed to determine the antioxidant capacity of natural substances *in vitro*. They can be categorized into two groups: (i) assays for radical-scavenging ability and (ii) assays for lipid oxidation inhibitory effect. A number of methods have been developed to measure the efficiency of dietary antioxidants either as pure compounds or in food extracts. These methods focus on different mechanisms of the

antioxidant defense system, i.e., scavenging of oxygen and hydroxyl radicals, reduction of lipid peroxyl radicals, inhibition of lipid peroxidation, or chelation of metal ions (Satheesh *et al*., 2010; Ivana *et al*., 2010; Sebranek *et al*., 2005; Yoo *et al*., 2008).

There are different assays used to measure the *in vitro* antioxidant properties of dietary phytochemicals. Some methods determine the ability of antioxidants to scavenge free radicals generated in the reaction medium such as the TEAC (Trolox Equivalent Antioxidant Capacity) (Semra *et al*., 2010); ORAC (Oxygen Radical Absorbance Capacity) (Tomaino *et al*., 2005), FRAP (Ferric-reducing antioxidant power) (Omidreza *et al*., 2005) or the DPPH (2, 2-diphenyl-1-picrylhydrazyl) (Arash *et al*., 2010) measure the scavenging of stable radical species by antioxidants. Other methods evaluate the inhibition of lipid peroxidation by antioxidants, quantifying products such as conjugated dienes (Iris *et al*., 2006), lipid peroxides or hydroperoxides (Shlomit *et al*., 2005), as well as products resulting from the decomposition of lipid peroxides such as malondialdehyde and hexanal determined by the TBARS (Stoilova e*t al*., 2007) and gas chromatography method (Vincent *et al*., 2007), respectively.

2.8.2. The use of spices for delaying of color change and lipid oxidation on meat

Lipid oxidation is a major cause of flavor deterioration in raw meats. Therefore, enhanced oxidative stability is needed for maintaining the safety and quality of meat. As meat and meat products are some of the most important sources of dietary fat, modification of the lipid profile of such products can reduce their nutritional quality. In order to inhibit the development of oxidative reaction in meat products, natural and synthetic antioxidants have commonly been used in the meat industry. Antioxidants are regarded as compounds that are able to delay, retard or prevent oxidation processes. Antioxidants with free radical scavenging activities may help to protect the irradiated beef burger from lipid oxidation (Ahn *et al*., 2007). However, due to concerns about toxicological safety of synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), it may be desirable to replace these conventional antioxidants with natural antioxidative substances (Formanek *et al*., 2001).

Various extracts separated from natural sources (e.g. rosemary, oregano, sage, thyme) have proven to possess strong antioxidant activity due to their high content of phenolic compounds, and they are permitted for use in food to replace synthetic antioxidants such as BHT/BHA (Ahn *et al*., 2007). The antioxidant properties of spices are related mainly to their phenolic

compounds, thus their antioxidant action is similar to synthetic phenolic antioxidants. The antioxidant activity of spices extract has been associated with the presence of several phenolic diterpenes such as carnosic acid, carnosol, rosmanol, and rosmaridiphenol, which break free radical chain reactions by electron donation and metal ion chelation (Georgantelis *et al.*, 2007).

2.8.3. Ethiopian Spices which Consumed Commonly with Raw Beef

Almost all popular traditional Ethiopian foods are prepared by spices, mostly for flavor, color and to improve appetite. For example, the spice sauce that prepared from spiced powder chili pepper, spiced powder of bird's eye chili and powder of mustard seed, used for consuming raw and roasted beef see the Figures 2.20.

Figure 2.20 Raw beef with the spice sauce & enjera

2.8.3.1. Red pepper & Chilli Pepper (Capsicum annuum/ frutescens)

Capsicums are the berries of the genus capsicum (family; solanacea) (Sanatombik and Sharma, 2008).This taxa includes both sweet cultivars eaten mainly as vegetables and hot cultivars often used as a spice (Durucasu and Tokusoglu, 2007). The most common species of capsicum are *capsicum annum*, *Capsicum frutescens*, *Capsicum chinense*, *Capsicum pubescens* and *Capsicum baccatum* (Netha and Reddy, 2000). The genus capsicum comprises greater than two hundred varieties (Contreras and Yahia, 2000).

In terms of its anatomy the pods are roughly triangular in shape in longitudinal cross-section with the base of the triangle at the point of attachment to the peduncle (stalk). The angles within this triangular shape may vary widely, the angle opposite the point of attachment of the peduncle being generally very acute, but becoming obtuse in rare cases, depending on the species (ISO, 1997).

The mature pods may vary in color from dark blackish-red through orange-yellow-green, according to the species. The material pigmentation, particularly red, is affected by exposure to air and light during storage and the intensity decreases with time. Dimensions may vary from 10mm to 120mm in length and 4 mm to 50mm in diameter, depending on the species (ISO, 1997).

A. Health Benefits of Capsicum

Based on the American Dietetic Association definition (Bloch and Thomson, 1995), pepper (Capsicum annuum) can be considered a functional food because it contains high levels of certain compounds that have beneficial effects for humans. Pepper contains vitamins A, B, C, and E and phytochemicals such as phenolic compounds, carotenoids, and capsaicin (USDA Nutrient Data Laboratory, 2007). These compounds are reported to have a multitude of favorable effects for humans, including antioxidant, anticarcinogenic, antimutagenic, antiaging, and antibacterial properties (Chu *et al*., 2002; Ferrari and Torres, 2003; Surh and Seoul, 2002). Antioxidants are of particular interest because they reduce free radicals and reactive oxygen species (ROS).

Carotinoids of capsicum ingested in the diet have important nutritional and physiological functions through their general role as antioxidant and as provitamin A. Thus they have been related with decreased risk of certain types of cancer, anti-ulcer effect, activation of the immunological system (Hornero *et al.*, 2000a). Capsicum lowered gastric ulcer by decreasing acid pepsin and increasing mucous secretion (Das and Deka, 2008). Capsaicin, as the most important among pungent principle, is a powerful irritant of the receptors participating in circulatory and respiratory reflexes and is used in stimulating medicines and food preparation. It gives relief from pain sensations when applied to scar tissue or other pain full conditions (Perva *et al.*, 2004).

The attractive red color is due to the various carotenoid pigments, which include β-carotene with pro-vitamin A activity and oxygenated carotenoids such as capsanthin, capsorubin and crypto-capsin, which are exclusive to this genus and are shown to be effective free radical scavengers (Matsufuji et al., 1998). Red peppers also contain moderate to high levels of neutral phenolics or flavonoids, namely quercetin, luteolin and capsaicinoids (Hasler, 1998). Capsicum cultivars have

been identified as potential vegetables with high antioxidant activity (Deepa, 2006). Wide variations exist among the different cultivars/genotypes of the same fruits and vegetables (Amakura *et al*., 2000; Cordensunsi *et al.,* 2002; Raffo *et a*l., 2002; George *et al*., 2004). Although, hot types with high pungencies had higher total antioxidant capacity, phenolic content, and vitamin C content (Frary *et al*., 2008).

B. Pepper (capsicum) in Ethiopia

Production of pepper is well known in Ethiopia (Rehima, 2006). Pepper (capsicum) is propagated by raising seedlings in nursery. Seedlings are raised starting April and transplanted as the main rainy seasons begins, which is June/July. The seedlings are transplanted 40-50 days after planting. Planting is carried out at the beginning of the main rain season. In areas where rainfall is inadequate, supplementary irrigation is required. About three weeding seasons are recommended during the growth period. Depending on the area, harvesting starts four to five months from transplanting. The red pepper is harvested when it is fully red and starts to dry. After harvesting the pepper is dried. Shade drying is recommended for high quality oleoresin (Roukens, 2005).

Wide variations exist among the different cultivars/genotypes of the same fruits and vegetables. The common four different varieties are known as Mareko fana, Bako Local, Birsheleko, and Alaba; Mareko Fana is the most preferred variety for the oleoresin extraction. The dried pods of many different varieties of Capsicum annum are utilized to prepare caynne pepper, ground red pepper and crushed pepper (EPEO, 2005).

Recently pepper is gaining greater importance in the global market because of its value added (processed) products and diverse uses (Sadhineni and Patil, 2007). Paprika is the powder of dry red pepper (Jaren *et al.*, 1999). Oleoresin is a liquid made by percolating a volatile solvent through a ground capsicum (paprika) and subsequent elimination of the solvent by vacuum evaporation. Oleoresin consists of essential oils, resins and the components that provide pungency (ESEF, 2003a).

In Ethiopia pepper is also processed traditionally at home to prepare Berbere. The pepper is sun dried and mixed with ginger, cardamom, coriander, fenugreek seeds, cloves, cinnamon, garlic, salt, cayenne pepper which are toasted at a low heat for a minute or so on a pan. This mixture

will coarsely grinded and toasted at low heat for few minute. Finally, the mixture will be milled in to powder. This powder is commonly called Berbere. Awaze is a food item prepared from Berbere. Berbere is mixed with water and sometimes alcoholic drinks are added as flavoring agent.

Capsicum annuum (Red Pepper**)**: Red pepper is a spice plant grown and consumed in considerable quantities worldwide and also used as a natural food color. Paprika is a spice made from grinding of dried fruits of *Capsicum annum*. Paprika varieties differ by its color, shape, dimension, flavour, degree of hotness, etc. In Ethiopia, red pepper takes the very special place among paprika varieties which is widely consumed as green or dried ground condiment. *Capsicum frutescens* (African Birdaeye) has high degree of hotness and it also widely consumed as green or dried ground condiment especially with raw and roasted meat in Ethiopia.

2.8.3.2. Mustard

The family *Brassicaceae* consists of 350 genera and about 3,500 species, and includes several genera like *Camelina, Crambe, Sinapis, Thlaspi* and *Brassica* (Cartea *et al*, 2010). The genus *Brassica* is the most important one within the Brassiceae, which includes some crops and species of great worldwide economic importance such as *Brassica oleracea* L., *Brassica napus* L. and *Brassica rapa* L. The same species can be utilized for several uses according to different forms or types. The genus is categorized into oilseed, forage, condiment, and vegetable crops by using their buds, leaves, roots, seeds, and stems. The mustard group which is formed by three species, *Brassica Carinata* (Ethiopean/Abyssinian mustard), *Brassica nigra* (Black mustard) and *Brassica juncea*, is mainly used as a condiment although leaves of *B. juncea* are also consumed as vegetables (Cartea *et al*, 2010).

Mustard seeds are the small round seeds of various mustard plants. The seeds are typically about 1 or 2mm in diameter (Hemmingway *et al*., 1993). Mustard seeds may be colored from yellowish white to black. Mustard seeds generally take three to ten days to germinate if placed under the proper conditions, which include a cold atmosphere and relatively moist soil. Major producers of mustard seeds include Canada (90%), Hungary, Great Britain, India, Pakistan and the United States. Brown and black mustard seeds return higher yields than their yellow counterparts (Paunović *et al*. 2012).

Mustard is a condiment made from whole, powdered or crushed dried seeds of mustard plants: Brassica nigra (black mustard), Brassica juncea (brown mustard) or Sinapis alba (white mustard). The seeds are usually mixed with wine, vinegar, water and various spices to create a paste ranging in color from bright yellow to dark brown. Mustard plants keep attracting a lot of attention since they have been recognized as a functional food for health maintenance and disease prevention (Hemmingway et al., 1993).

The spiciness of mustard is caused by a group of compounds called isothiocyanates. When mustard seeds are crushed and exposed to liquids, an enzyme called myrosinase (thioglucoside glucohydrolase) hydrolyses glucosinolates to release isothiocyanates. The differences in mustard flavour are due to the structural changes of the released isothiocyanates. For example, oriental and brown mustard release a volatile compound, ally isothiocyanate (AIT), which produces a sharp taste sensation and pungent aroma similar to horseradish. Yellow mustard releases a non-volatile compound, 4-hydroxybenzyl isothiocyanate (PHBIT), which elicits a hot mouth feel in condiments.

Mustard products contain a wide range of such active components including isothiocyanates, phenolics, dithiolthiones and dietary fibre. For example, isothiocyanates of essential mustard oil exert pharmacological as well as toxic properties by their goitrogenic, antibacterial, antifungal and antiprotozoal activities (Zsolnia 1966). The mucilage from yellow mustard does not only stabilize prepared mustard products, but also is able to reduce glycemic index in both normal people and diabetic patients.

Mustard seeds are composed of protein (23-30%), fixed oil (29-36%), carbohydrate (12-18%) together with minor constituents including minerals (4%), essential oil (glucosinolates, 0.8-2.3%), phytin (2-3%) as well as phenolic compounds and dithiolthiones (Hemmingway J. et al., 1993). Concerning the fatty acid content we may claim that mustard seeds contain a substantial amount of linoleic acid, which is essential for the human body. In the study of Kisbenedek A. (2013) a smaller amount of saturated fatty acids were found during his analysis, such as palmitic acid and stearic acid. Erucic acid content of the mustard seed in his study was 32.78g/100g.

A. Antioxidant property of mustard seeds

Concern over the toxicity of synthetic antioxidants has resulted in a search for natural antioxidants in foods (Barlow, S.M., 1990). The latter include phenolic compounds, tocopherols, phospholipids and amino acids (Dugan, L.R., 1980). Oilseeds in general, are rich in phenolic compounds that retard oxidation of the oil. Koslowska and coworkers (1983) reported the presence of phenolic acids in rapeseed and mustard in which sinapic acid isomers were the predominant forms present. Recent research by Saleemi et al., (1993) compared the antioxidant properties of low pungency ground mustard seed (LPGMS) with synthetic antioxidants butylated hydroxytoluene (BHT) and tertiary-butyl hydroquinone (TBHQ) on the stability of comminuted pork. Further work by these researchers showed that an 85% methanolic extract from LPGMS exhibited the strongest antioxidant activity due to the presence of much higher levels of phenolics compared to either water or 10% methanolic extracts. In addition, to antioxidant activity, LPGMS also enhanced cook yield without exerting a detrimental effect on the colour quality of the treated meat samples (Saleemi *et al*., (1993).

B. Antimicrobial Properties of mustard seeds

The antibacterial activity of isothiocyanates (the main compound present in mustard seed) was found to be more effective against Staphylococcus aureus (Zsolnia 1966). A study by Delaquis and Mazza (1995) showed the effect of vaporized AIT on food born pathogens was dose dependent and the growth of microorganisms was inhibited at concentrations above 500ng/ml. Some isothiocyanates, such as benzyl isothiocyanates, exhibit antibiotic activity in vitro, and using as pharmaceuticals for the treatment of infections of the respiratory and urinary tracts (Bergmann, M., 1996).

Mustard oil was reported over half a century ago to exhibit antifungal activity (Zsolnia 1966). The antifungal effectiveness of AIT was demonstrated by Zsolnia (1966) and Tsunoda (1994). Evidence accrued to-date points to AIT and other volatile isothiocyanates being specifically effective against the germination and growth of several fruit pathogens (Mari, M., et al., 1993). In the vapour phase, AIT from brown mustard proved a potent antifungal agent when included in modified atmosphere packaging of different food samples (Isshiki *et al*., 1992). AIT was particularly effective against mycotoxin producing molds such as Aspergillus flavus, Penicillium citrinum and Fusarium graminearum (Delaquis, P.J. and Mazza, G., 1995).

2.9 Meat Production and Handling in Ethiopia

In Ethiopia, agricultural products like coffee, cereals, pulses, oilseeds, the stimulant chat, meat, hides, and skin, contribute 45% of the $6.1 billion GDP (US Department of State, 2004). Livestock products alone contribute 40% of the agricultural GDP and 20% of the total GDP (Akiliu, 2002). The country exporting beef, goat and sheep meats in addition to live animals. The meat exportation remained slightly more stable (Ahmed *et al*., 2003) and attracting local and foreign investors.

In 2003, the livestock population in Ethiopia, only including cattle, sheep, goats, and chickens, had reached 95.6 million head. Ninety percent of the 35.5 million cattle, 11.5 million sheep, 9.6 million goats, and 39 million poultry remained in the highlands (FAOSTAT, 2004).

2.9.1. Livestock markets in Ethiopia

The livestock markets especially for cattle will follow a set pattern. First, animals will sell from a farmer to rural traders. The rural traders, or the farmers that prefer not to sell locally, herd their livestock to a local and/or primary market. These market centers are generally concentrated in the rural areas. The animals are then will sell by the rural traders and still possibly farmers in a secondary market in regional towns to larger traders and butchers. Most of these animals will be sold for consumption or used for breeding or draft purposes. The animals used for consumption are resold again by the larger traders at a terminal market to butchers, supermarkets, or occasionally individuals. Large traders are more dominate and manipulate Ethiopia‘s livestock markets (Ahmed, *et al*., 2003).

In today‘s system, from the terminal market live animals are delivered to customers in different ways. A few are sent through official markets, while another unknown amount is for home slaughter and group consumption (*kircha*). Many, however, are sent to the abattoirs by butcheries or supermarkets (Ahmed *et al*., 2003). Most of butcheries in Ethiopia sell only beef; however, a few butcheries may sell sheep and goat meat in addition to beef.

2.9.2. Slaughtering

According to the report of Abbey A. (2004) after the live animals are purchased at the terminal market by butcheries or supermarkets, they are sent directly to the official Addis Ababa Abattoirs Enterprise. The animals are kept in a holding lot for a maximum of three to four hours,

and then sent through the Orthodox, Muslim, or European slaughter facilities. Sheep and goats are slaughtered on a long table, skinned, and vertically hung and inspected by veterinarians from the Ministry of Agriculture. Cattle are brought into the building and are slaughtered on the ground, followed by a haphazard butchery in which the carcasses are cut into four parts. After inspection by the Ministry of Agriculture, the carcasses will distribute to the customers by van cars.

The men and housewives of the middle-income group account for the majority of Addis Ababa butchery customers (Abbey A., 2004). Most customers bought raw meat to prepare and consume within their home. At the larger butcheries, however, many people frequently consume the traditional meal of raw meat or *kitfo*, the higher quality raw meat.

2.9.3. Meat Safety

The export abattoirs are required to inspect all meat prior to shipment in regards to a 1976 proclamation, clarified by a set of guidelines developed by the Ministry of Agriculture (2000). Many of the guidelines, however, are not enforced by abattoirs that produce meat for local consumption. For example, during a tour of the Addis Abattoir facilities that slaughters animals for local consumption, visitor was not required to wear the set forth head covering (Abbey A., 2004), as stated in the guidelines developed by the Ministry of Agriculture (2000). The export abattoirs, however, did strictly enforce the covering statement. Also, much of the building was open air, in violation of the Meat Hygiene Guidelines (2000). Additionally, section 2.1 of the *Meat Inspection Procedures* states that, ―One of the most important functions of ante mortem inspection is to ensure that animals are rested sufficiently so that signs important to inspection disposition are not masked.‖ Most animals, however, travel from the Harar region, at a distance of 500km from Addis Ababa, and are slaughtered within a maximum of four hours as reported by Abbey A., (2004).

Additionally, the guidelines state that raw foods of animal origin should be stored between one and four degrees Celsius. The supermarkets follow this guideline closely. Beef, sheep meat, and goat meat are stored at an average temperature of 1.8^{o}C, while poultry is frozen at -5^{o}C or below (Abbey A., 2004). Addis Ababa butcheries, however, are rarely equipped with refrigeration. One hundred percent of the butcheries stored their meat in a room temperature, open-air environment display as shown in figure 2.21. Moreover, according to the study of Abbey A., (2004) only

8.8% of the butcheries had refrigerators for nightly storage, 91.2% of the meat was stored at room temperature permanently. While supermarkets only kept their refrigerated red meat an average of two days, the butcheries stored their room temperature meat for an average of two and a half days, with a frequent maximum of five days.

From slaughter to resale, there is no formal classification of carcass quality, and therefore little classification of economic quality of Ethiopia‘s meat products. Age, marbling, tenderness, etc. are not considered when setting the price. There is no system of grading or naming meat. A few butcheries and several supermarkets reported that the beef round was the highest quality of meat, but there was no price differentiation except for one supermarket case (Abbey A., 2004). Butcheries also reported that meat for *kitfo* and *kurt* were classified as the highest quality, with still no price differentiation.

The livestock potential of the country contributed to keep the Ethiopian traditional meal of raw meat (*kurt* or *kitfo),* which consume by using spices such as *berbre, mitmita* and *senafich*. Some people who used these spices to consume the traditional meal of raw meat believed that the spices use as antimicrobial in addition to their flavor. But not considered their nutritional value and their use as a functional food beyond their potential on inhibition of lipid oxidation, which relate to their phytochemical compounds such as antioxidants, phenols and flavonoids.

In Ethiopia almost all butcher shops are displaying the beef carcasses under high power incandescent lamps through an open window of display booth to attract consumers as shown in Figure 2.21. Displaying carcasses under this condition might have a potential to accelerate lipid oxidation and the formation of an undesirable brown muscle color on carcasses. Because, these high power incandescent lamps are generate heat in addition to their radiant energy, which have significant impact on the organoleptic of displayed beef carcasses. Furthermore, several studies also showed that displaying meat cuts under high intensity lights are the cause of qualities losses of meat; especially by accelerate the formation of an undesirable brown muscle color and lipid oxidation (Kropf D. (1980); Lavelle et al., 1995; Chiang & Ringkob 1999; Greer 1984).

Figure 2. 21 Displaying of beef carcas in Ethiopia

CHAPTER 3. MATERIALS AND METHODS

3.1. Effect of incandescent display light on color and oxidation stability of raw beef

3.1.1 Sample preparation

For raw beef image analysis, different muscles types of raw beef steaks from *Semitendinosus* (red), *triceps brachii* (red), *Longissimus dorsi* (red) and *Longissimus dorsi* (white/fat) were bought from butcher shop in Addis Ababa, Ethiopia. The raw beef steaks were cut in slices approximately 20 mm thick. The raw beef samples were prepared after 24 hrs slaughter at the Food analysis laboratory in the Center of Food Science and Nutrition, Addis Ababa University.

For measurement of color by colorometre TBARS and TAC, the raw beef sample (rib eye) from *m.longissimus dorsi* was bought from Keurslager Manuel & Anoeshka, Gent, Belgium. The raw beef steak was cut in slices uniformly 15 mm thick and about 35 cm^2 areas by meat slicer machine. The raw beef samples were prepared after 48hrs slaughter at the Food Chemistry and Meat Technology laboratory, Katholieke Hogeschool (KaHo) Sint-Lieven, Technologie campus, Gent, Belgium.

3.1.2 Illumination of the raw beef slices

The raw beef slices were placed in opened petri dishes and illuminated by 200 watt incandescent lamp, at room temperature in dark room to avoid interference of other light source. The control samples were kept in the darkness. All illuminated samples were exposed to lighting continuously at 6280±210 lux at the surface for 48 hrs. Light intensity was measured using a luxometer Chauvin Arnoux 810 (Paris, France). The positions of the samples in the illumination were rotated three times in a day to minimize light intensity differences and possible abuse temperature at the surface of meat.

3.1.3 Instrumental color measurement

Color measurement was performed on the cross section of raw beef slices. Measurements were carried out immediately after cutting and after 8, 24 and 48 hrs of exposure to incandescent lamp and air. Color measurements were performed using a Hunter Lab Mini scan Minolta XE plus spectrocolorimeter (light source of D65, standard observer of 10°, 45°/0° geometry, 1 in. light surface) in 6-fold from different position on the surface for all samples at 0, 24 and 48 h. Before

each measurement the apparatus was standardized against a black and white calibration tile. The three color scale indices L* (lightness), a* (redness) and b* (yellowness) and reflectance values at different wave lengths from 400 to 700 nm were obtained from the instrumental measurement. The proportion of the three myoglobin (Mb) redox forms (Mb, Oxymyoglobin (OMb) and metmyoglobin (MMb) at the surface of the samples was also determined from the measurement of reflex attenuance at the iso-bestic point by using the method of Krzywicki (1979):

%Mb = 2.375 {1- (A473 - A730) / (A525 - A730)}

% MMb = 1.395 {1 – (A572 - A730) / (A525 - A730)}

%OMb = 100 – (%Mb + % MMb)

Where: A= reflex attenuance

From the L*a*b* values Chroma (C) and Hue angle (H°) were obtained by using the following equations:

$C = (a^{*2} + b^{*2})^{1/2}$ (a measure of saturation where high values indicate more vivid color)

H^{o} = arctan b*/a* (departure from the true red axis of the CIE color space in degree)

3.1.4 Delta E Color Change

This is also known as a total color change (ΔE) over some specific time. It is a useful parameter to show total color differences over time. Theoretically, ΔEs of less than 1.0 are not detectable unless the samples are side by side. This parameter is also useful for establishing tolerances for variation in color between samples.

The mean measurements were recorded for each color parameter. Total color difference (ΔE) expresses the stability of color, and it is calculated according to the following formula (Szmańko et al., 2006; Wójciak et al., 2012):

$$\Delta E_x = \{(L_o^* - L_x^*)^2 + (a_o^* - a_x^*)^2 + (b_o^* - b_x^*)^2\}^{1/2}$$

Where: ΔE_x (means ΔE_8 or ΔE_{24} or ΔE_{48}) – colour stability of raw beef slices after 8 or 24 or 48hrs exposure to incandescent lamp and air; L_o^*, a_o^*, b_o^* – values of color parameters L*, a*, b* measured on the cross section of raw beef slices immediately after cutting the raw beef steaks and also after 8, 24 and 48hrs exposure to incandescent lamp and air. Larger ΔE_X value means a larger color change that is equal to smaller color stability.

3.1.5 pH measurement

pH values were measured using a pH meter (Testo 252, Lenzkirch, Germany) equipped with a stab glass electrode for the direct measurement of pH in meat and meat products. Before and during the values reading, calibration of the pH meter was realized using standard buffer solution (pH buffer calibration was 7.00 and 4.00 at 20°C). Measurements were carried out in triplicates.

3.1.6 Lipid oxidation measurement

Lipid oxidation was evaluated through the determination of TBARS using the method of Salih et al. (1987). From top side of illuminated raw beef slice 5 g was grinded and homogenized with 30 ml cold perchloric acid ($HClO_4$, 0.6 M) in a plastic pot of 100ml and 1ml of Butylated hydroxytoluene (BHT) (Sigma-Aldrich NV/SA Bornem,Belgium) using ultra-turrax (Ika laboratory technology technology) at 1300 rpm for about 30 s. The slurry was filtered through a folded filter paper. Standard working solution of MDA was prepared following the procedure of Shlafer and Shepard (1984).

Five ml meat samples extract (aliquots) and standard working solution were transferred in heat resistance glass test tubes in duplicate and mixed with 1ml TBA reagent (Sigma-Aldrich NV/SA Bornem, Belgium). The test tubes were placed in boiling water bath for 35 min at 95^{o}C together with tubes for the standard curve. After cooling to room temperature in tap water, the absorbance was measured in a spectrophotometer at 532 nm against a blank. The standard curve was prepared by using dilutions prepared from a 20 times diluted 14.4µl 1, 1, 3, 3-tetramethoxypropane (Sigma T-1642) in 0.6 M $HClO_4$.

TBARS level was determined in duplicate for each samples at times 0, 8, 24 and 48hrs. The MDA concentration was converted to TBA number (µg MDA/g meat sample) as follows:

$$\text{TBARS number (µg MDA/g sample)} = \frac{C \times 31 \times 72}{5 \times G \times 1000}$$

Where: 72=molecular weight of MDA

G=Weight of the sample in g and

C=Concentration of MDA per 5 ml extract based on the regression line of the standard curve shown in Figure 3.1.

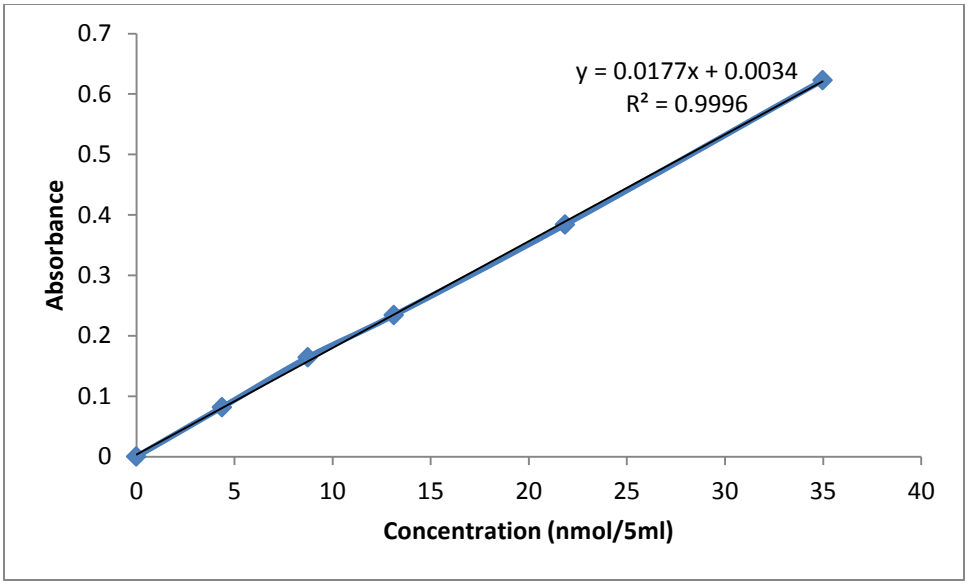


Figure 3.1 The regression line of the standard curve for TBARS assay

The sample was kept as cold as possible during the treatment to prevent oxidation. During the analysis standard curves were prepared for each TBARS measurement to avoid the effect of variations during pipetting, weighing, incubation and other steps. Mean values are reported as µg MDA/g of meat.

3.1.7 Microbial Analysis

Total aerobic bacterial (TAB) counts were done in microbiology laboratory, Katholieke Hogeschool (KaHo) Sint-Lieven, Technologie campus, Gent, Belgium. A small sample was cut using a sterile scalpel and scissors. The samples were placed in Stomacher sterile bags (Stomacher Lab system, Seward) and homogenized for 2 min at high speed with sterile peptone-water in stomacher. A 10-fold serial dilution of each sample was made with peptone sterile water. Plate count agar dishes were inoculated with 0.1 ml of inoculum prepared as already described. The inoculum was spread uniformly over the culture medium using the spiral plater (Eddy Jet, IUL Instruments). The inoculated plates were incubated at 26°C for 48 hrs. Plate count agar dishes were removed from the incubator and viable numbers were determined from plates bearing 20–300 colony forming units (CFU). Results were expressed as number log CFU/g.

3.2. Effect of display condition (covering samples) on color and lipid oxidation stability of raw beef steaks

3.2.1 Sample preparation

The raw beef sample (rib eye) from *m.longissimus dorsi* bought from Keurslager Manuel & Anoeshka, Gent, Belgium. The raw beef steak was cut in slices uniformly 15mm thick and about 35cm^2 areas by meat slicer machine. The raw beef samples were prepared after 48hrs slaughter at the Food Chemistry and Meat Technology laboratory, Katholieke Hogeschool (KaHo) Sint-Lieven, Technologie campus, Gent, Belgium.

3.2.2 Illumination of the raw beef slices for covered and opened samples

The raw beef slices from *m.longissimus dorsi* were placed in a petri dish, half of the samples were covered by transparent cover of the petri dish the other were opened and illuminated by 200 watt incandescent lamp, at room temperature in dark room to avoid the interference of other light source. The control samples were placed in dark at room temperature. All illuminated samples were exposed to lighting continuously at 6280±210 lux at the surface for 20hrs. Light intensity was measured using a luxometer Chauvin Arnoux 810 (Paris, France). The positions of the samples in the illumination were rotated three times in a day to minimize light intensity differences and possible abuse temperature at the surface of meat.

3.2.3 Measurement of temperature elevation on beef steaks.

Change of temperature on the displayed and control raw beef samples was measured using thermocouple that was inserted into a depth of about 3 mm in different portions of the sample. Measurements were carried out in triplicates.

The measurements of different color parameters, pH and lipid oxidation by TBARS assay were done in the same manner in part 3.1.

3.3 Assessment of color stability of different muscles types of raw beef in incandescent display light

3.3.1. Sample preparation for different muscles types

Different muscles types of raw beef steaks from *Semitendinosus* (red), *triceps brachii* (red), *Longissimus dorsi* (red) and *Longissimus dorsi* (white/fat) were bought from butcher shop in Addis Ababa, Ethiopia. The raw beef steaks were cut in slices approximately 20 mm thick. The

raw beef samples were prepared after 24hrs slaughter at the Food analysis laboratory in the Center of Food Science and Nutrition, Addis Ababa University.

3.3.2. Illumination of different muscles types of the raw beef slices

The raw beef slices were placed on a polystyrene tray and illuminated by 400 watt incandescent lamp at room temperature in dark room to avoid the interference of other light source. The control samples were placed in dark place at room temperature. The distance of light source was 0.5 m from the samples, and the positions of the samples during the illumination were rotated three times in a day to minimize light intensity differences and possible abuse temperature at the surface of meat.

3.3.3. Color measurement by image analysis method

Color change measurement was evaluated by image analysis, according to the method used by Yam & Papadakis (2004). It is a simple method that used a combination of digital camera, computer, and graphics software Photoshop (Adobe Systems) to measure and analyse the surface color of beef steaks.

A high-resolution digital camera (Nikon, P90, 20 mega-pixel) was used to measure color change by capturing the colour 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 0.5 m. The image of both control and displayed beef samples were captured on a brawn background under the following camera settings: manual mode, no flash and resolution of 1024×1680 pixels (Chan et al., 2010). After zooming the lens to cover the whole field of view on the sample and focusing, the image of each sample were taken and stored in JPEG format for image processing and analysis.

CIE L*, a* and b* values were measured using a graphics software Adobe Photoshop CS3 for both control and displayed meat samples.

3.3.4. Temperature and pH measurement for different muscles types

The pH values of meat samples were measured using a pH meter equipped with a stab glass electrode for the direct measurement of pH in meat and meat products. Before and during the values reading, calibration of the pH meter was realized using standard buffer solution (pH

buffer calibration was 7.00 and 4.00 at 20°C). Change of temperature on the samples was measured using thermocouple that was inserted into a depth of about 3 mm in different portions of the sample. Measurements were carried out in triplicates.

3.4 Investigation of the impact of specific wave length of visible light on color change of raw beef by using optical filters

3.4.1 Sample preparation for illumination of the raw beef slices under filters

The raw beef sample (rib eye) from *m.longissimus dorsi* bought from Keurslager Manuel & Anoeshka, Gent, Belgium. The raw beef steak was cut in slices uniformly 15 mm thick and about 35 cm^2 areas by meat slicer machine. The raw beef samples were prepared after 48hrs slaughter at the Food Chemistry and Meat Technology laboratory, Katholieke Hogeschool (KaHo) Sint-Lieven, Technologie campus, Gent, Belgium.

3.4.2 Illumination of the raw beef slices under different specific wavelength

The raw beef slices were placed in open petri dishes and illuminated under different specific wavelength of optical filters (Lee filters) from 200 watt incandescent lamp (6.28±0.21 klux), at room temperature inside of black box to avoid the interference of other reflected lights. The filters color, wavelengths and light intensities which illuminated on the surface of raw beef samples are given in Table 3.1. Light intensity was measured using a luxometer Chauvin Arnoux 810 (Paris, France). The positions of the samples in the illumination were rotated three times in the six hours of illumination to minimize light intensity differences at the surface of meat.

Table 3.1 Filters color, wavelengths & light intensities of illuminated raw beef samples

Filter color	**Transparent**	**Red**	**Yellow**	**Green**	**Blue**
wavelength	**400-700 nm**	**650 nm**	**550 nm**	**500 nm**	**450 nm**
Illuminance(klux)	4.99±0.06	1.49±0.01	4.50±0.06	1.58±0.01	1.10±0.01

3.4.3 The color stability of raw beef by using green and red colored incandescent lamps.

The raw beef slices were placed in open petri dishes and illuminated under green (1650±50 lux) and red (700±25 lux) colored 60w incandescent lamps inside of black box to avoid the interference of other reflected lights as shown in Figure 3.2.

Figure 3.2 Illumination of raw beef slices under green and red color lamps inside of a black box

Determination of color change of the illuminated raw beef samples was done by different color parameters similarly as part 3.1.

3.5. Effect of Ethiopian spices extract on color and lipid oxidation of raw beef steaks during storage in dark refrigerator

3.5.1. Sample preparation of Ethiopian spices

Samples of Ethiopian spices (commonly consume with raw beef) were chili pepper (*Capsicum annuum)*, African birds eye (*Capsicu*m *minimum)* and mustard seed (*Brassica nigra),* which were purchased from *Merkato* market, Addis Ababa. The purchased spices were air and sun dried, milled and flour was passed through a sieve of 0.5 mm. The powder of samples were kept in dark glass containers and stored in refrigerator until extracted.

3.5.2. Extraction of Ethiopian spices

Samples were extracted based on the procedures used by Zakia, N. (2013). Briefly, five gram of dried spice powder was extracted by stirring with 50 ml of methanol/water (4:1) at 25^0C at 150 rmp for 24 hrs using temperature shaker incubator (ZHWY-103B) and then filtered through Whatman No. 4 paper. The residue was then extracted with two additional 50 ml portions of methanol/water as described above. The combined methanol/water extracts were evaporated at

40^0C to dryness using rotary evaporator (Stuart R3300) and re-dissolved in water at the concentration of 20 mg/ml and stored in dark brawn glass bottle at 4^0C for further use.

3.5.3. Preparation of raw beef steaks with spices for dark storage

The raw beef sample (rib eye) from *m.longissimus dorsi* was bought from Keurslager Manuel & Anoeshka, Gent, Belgium. The raw beef steak (after 48 hrs slaughter) was aseptically cut into slices uniformly 15 mm thick and about 35 cm^2 areas by meat slicer machine, which were placed inside of petri dishes. Then, raw beef slices were sprayed with a sterile solution of spices extract and water (Control). Each raw beef slices were received 5ml of natural antioxidant extract (chili pepper, African birds eye and mustard seed), except those in Control. The prepared samples were placed in covered petri dishs and stored in a dark at 4°C for 0, 2, 7, and 14 days to analyze: pH, instrumental color, thiobarbituric acid reactive substance (TBARS), and total aerobic bacterial (TAB) count. Both the raw beef samples and spices extract were prepared at the Food Chemistry and Meat Technology laboratory, Katholieke Hogeschool (KaHo) Sint-Lieven, Technologie campus, Gent, Belgium.

Determination of color change by different color parameters, pH, TAC and lipid oxidation by TBARS assay were carried out for the raw beef slices treated by Ethiopian spices extract in the same manner as part 3.1.

3.6 Various dosage of the spices extract on color and lipid oxidation stability of raw beef steaks during exposure to incandescent display light

3.6.1. Preparation of raw beef steaks with different dosage of spices extract

The raw beef sample (rib eye) from *m.longissimus dorsi* was bought from Keurslager Manuel & Anoeshka, Gent, Belgium. The raw beef steak (after 48 hrs slaughter) was aseptically cut into slices uniformly 15 mm thick and about 35 cm^2 areas by meat slicer machine and were placed inside of petri dishes. Then, raw beef slices were sprayed with different dosage of spices extract and water (Control). Half of raw beef slices were received 0.5 ml and the other 1ml of natural antioxidant extract (chili pepper, African birdseye and mustard seed), and controls were treated by the same amount of distilled water. The prepared samples were placed in covered petri dishes and displayed under incandescent light at room temperature for 0, 4 and 20 hrs. Both the raw beef samples and spices extract were prepared at the Food Chemistry and Meat Technology laboratory, Katholieke Hogeschool (KaHo) Sint-Lieven, Technologie campus, Gent, Belgium.

The measurements of different color parameters, pH and lipid oxidation by TBARS assay were done in the same manner as part 3.1.

3.7. Antioxidant activity and total polyphenol content of Ethiopian spices

3.7.1. Sample preparation of Ethiopian spices for analysis of the antioxidant & polyphenols

Samples of Ethiopian spices (commonly consumed with raw beef) were chili pepper (*Capsicum annuum)*, African birdseye (*Capsicu*m *minimum)* and mustard seed (*Brassica nigra),* which were purchased from *Merkato* market, Addis Ababa. The purchased spices were air and sun dried, milled and flour was passed through a sieve of 0.5 mm. The powder of samples were kept in dark glass containers and stored in refrigerator until extracted.

3.7.2. Extraction of spices for analysis of the antioxidant & polyphenols

Samples were extracted based on the procedures used by Zakia, N. (2013). Briefly, five gram of dried spice powder was extracted by stirring with 50 ml of methanol/water (4:1) at 25^0C at 150 rmp for 24 hrs using temperature shaker incubator (ZHWY-103B) and then filtered through Whatman No. 4 paper. The residue was then extracted with two additional 50 ml portions of methanol/water as described above. The combined methanol/water extracts were evaporated at 40^0C to dryness using rotary evaporator (Stuart R3300) and re-dissolved in methanol/water at the concentration of 20 mg/ml and stored in dark brawn glass bottle at 4^0C for further use.

3.7.3 Determination of free radical scavenging activity

The hydrogen atoms or electrons donation ability of the corresponding extracts and some pure compounds were measured from the bleaching of purple colored methanol solution of DPPH (Gursoy *et al*., 2010). The effect of methano/water (4:1) extracts on DPPH radical was estimated according to Kirby and Schmidt (1997). A 0.004% solution of DPPH radical solution in methanol was prepared and then 4 ml of this solution was mixed with 1ml of various concentrations (12 – 416 µg/ml) of the extracts in methanol/water. Finally, the samples were incubated for 30 min in the dark at room temperature. Scavenging capacity was read spectrophotometrically (Perkin Elmer Lamda 950 UV/Vis/NIR) by monitoring the decrease in absorbance at 517 nm. This absorption maximum was first verified by scanning freshly prepared DPPH from 200-800 nm using the scan mode of the spectrophotometer. Ascorbic acid and trolox were used as a standard and mixture without extract was used as the control. Inhibition of free radical DPPH in percent (I %) was then calculated:

$$\textbf{Radical scavenging activity (I \%)} = \frac{A_0 - A_1}{A_0} \times 100\%$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample. The extract concentration providing 50% of radicals scavenging activity (EC_{50}) was calculated from the graph of RSA percentage against extract concentration (Barros *et al*., 2007).

3.7.4 Trolox equivalent antioxidant capacity (TEAC) assay.

The trolox equivalent antioxidant capacity was determined based on the method developed by Re *et al*., (1999), in detail a stable stock solution of $ABTS^{\bullet+}$ was produced by reacting a 7 mmol/L aqueous solution of ABTS with 2.45 mmol/L potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. $ABTS^{\bullet+}$ solution (3.9 mL) was added to 0.1 mL of the test samples and mixed thoroughly. The blank was 3.9 mL of $ABTS^{\bullet+}$ solution with 0.1mL of 80% ethanol. The reactive mixture was allowed to stand at room temperature for 6min and the absorbance was immediately read with a Lambda 950 UV/VIS/NIR spectrophotometer (Perkin Elmer, and USA) at 734 nm. Trolox standard solution (final concentration 3–200 $\mu g ml^{-1}$) in 80% ethanol was prepared and assayed under the same conditions. The absorbance of the resulting oxidized solution was compared with that of the calibrated trolox standard. Results were expressed in terms of trolox equivalent antioxidant capacity (TEAC, mmol trolox equivalents per g dry weight of spice powder). All tests were performed in triplicate.

3.7.5 Determination of total reducing power

Total reducing power was carried out according to the method established by Oyaizu (1986). One ml of different concentrations (31.25 – 500 $\mu g\ ml^{-1}$) of each spice extract was mixed in test tube with a 2.5 ml of 0.2M phosphate buffer (2.5 ml), pH 6.6, and 2.5 ml 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min, to reduce ferricyanide into ferrocyanide. The 2.5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged at 6,000 rpm for 10 min. The upper layer (2.5 ml) was transferred into another tube and mixed with 2.5 ml deionized water and 0.5ml ferric chloride (0.1%) and left to react for 10 min. Finally, the absorbance of the reaction mixture was measured at 700 nm using a UV-VIS spectrophotometer (Perkin Elmer Lamda 950). The ascorbic acids are used for the positive control for all extracts and the reducing power tests are run in triplicate. Reducing power was

given in ascorbic acid equivalent in milligram per gram of dry weight, using the equation obtained from the calibration curve (Figure 3.3):

Absorbance = 3.9844 ascorbic acid (mg) + 0.2562; R^2 = 0.9993.

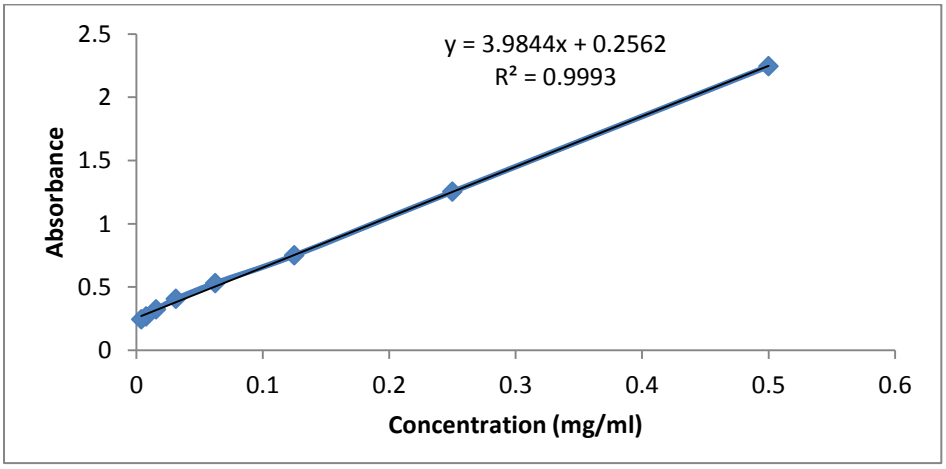


Figure 3.3 The calibration curve of ascorbic acid for determination of total reducing power

3.7.6 The total phenolic content of the spices extract

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method (Afolayan J., Aboyade M. & Sofidiya O., 2008). The spice extract (1ml) was mixed with 2.5mL Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 2mL (75 g L-1) of sodium carbonate.The mixture was vortexed for 15sec and allowed to stand for 30min at 40°C for colour development. The absorbance was measured at 765 nm using a UV-VIS spectrophotometer (Perkin Elmer Lamda 950 UV/Vis/NIR).

Total phenolic content was expressed as mg gallic acid equivalent using the equation obtained from the calibration curve:

Absorbance = 5.2411 gallic acid (mg) + 0.0192; R^2 = 0.9912.

All tests were performed in triplicate.

3.7.7 Total flavonoid content of the spices extract

The Total flavonoids content was determined using spectrophotometric method based on the formation of a flavonoid–aluminum complex (Zhishen 1999). One milliliter of extract was placed in a 10 mL volumetric flask. Five milliliter of bidistilled and 0.3 mL of sodium nitrite

were added and mixed. About 0.6 mL of 10% A1Cl3.6H2O was added after 5 min. Two milliliters of NaOH (1M) was added 5min later in the solution mixture, vigorously. The absorbance was immediately read with a Lambda 950 UV/VIS/NIR spectrophotometer (Perkin Elmer, and USA) at 510 nm. Total flavonoid content was expressed as mg catichin equivalent using the equation obtained from the standard calibration curve prepared from catichin:

$$\text{Absorbance} = 0.4425 \text{ catichin (mg)} + 0.0224;\ R^2 = 0.9915.$$

All tests were performed in triplicate.

3.8 Assessment of the effects of germination on antioxidant activity and polyphenol content of mustard seeds.

3.8.1 Mustard seeds germination

Mustard seeds were sterilised with 70% ethanol for 2.5 min, followed by 2.5% sodium hypochlorite for 15 min (Huang *et al.,* 2003). Ethanol and sodium hypochlorite were removed by rinses of sterile water. After disinfection, mustard seeds were allowed to imbibe water for 24 hrs at room temperature. Then water was removed and seeds were dark-germinated in sterile plates with humidified cotton at 28^{o}C. The cotton was kept moist by spraying with sterile water as needed.

After one and three day germination periods the germinated mustard seeds were dried at 40^{o}C in oven for 48 hrs. The dried germinated mustard seeds were milled and the flour passed through a sieve of 0.5 mm. The powder of germinated mustard seeds were kept in dark glass containers and stored in refrigerator until extracted.

3.8.2 Extraction of the powder of germinated mustard seeds

The powder of germinated mustard seeds were extracted based on the procedures used by Zakia, N. (2013). Briefly, five gram of dried spice powder was extracted by stirring with 50 ml of methanol/water (4:1) at 25^{0}C at 150 rmp for 24 hrs using temperature shaker incubator (ZHWY-103B) and then filtered through Whatman No. 4 paper. The residue was then extracted with two additional 50 ml portions of methanol/water as described above. The combined methanol/water extracts were evaporated at 40^{0}C to dryness using rotary evaporator (Stuart R3300) and re-dissolved in methanol/water at the concentration of 10 mg/ml and stored in dark brawn glass bottle at 4^{0}C for further use.

3.9 Determination of the aroma properties of the spices by SPME - GC-MS

Sample preparation technique was solid-phase micro extraction (SPME) or purge-and-trap. Briefly, one gram of mustard seed (*brassica nigra*) powder and 0.5 g of sodium chloride were mixed with 3 ml of distilled water in vial. This vial was placed in automatic Gerster multipurpose sampler, which injects it automatically in GC-MS (Agilent Technologies, 6890N, Network GC System coupled with Agilent 5973 Network Mass Selective Detector). GC-MS instrument control parameters informations are attached in Appendix B.

The aroma properties of the mustard seed (*brassica nigra*) powder was determined by head space solid phase micro extraction method combined with gas chromatographic-mass spectrometric (GC-MS) techniques in laboratory of aroma research for food industry in KU Leuven, technology campus Ghent, Belgium. The most relevant peaks were identified by comparison of their retention indices with literature data and on the basis of their total mass spectra, by comparison with mass spectra databases (NIST mass spectral library).

The percentage of each aromatic chemical compound was calculated using the following formula:

$$\textbf{\% Individual aromatic chemical compound} = \frac{\textbf{Individual chemical compound peak area}}{\textbf{Total chemical compound peak area}} \times 100$$

3.10 Statistical Analysis

Summary statistics (mean and standard deviation) were computed for each dependent variable. In order to find differences between treatments the time evolution of L*, a*, b*, OMb%, MMb%, chroma, hue angle, TAC and TBARS values were used as a variable. Data obtained from instrumental measurement and chemical analysis was all submitted to General Linear Model (GLM) procedure from SPSS 20.0 (SPSS 2006) statistical software package to determine the effect of light, spices extract and light exposure and dark storage times. The treatments whenever found significant, the tukey test was used for pair wise comparisons among the different treatments at the 5% ($p<0.05$) significant level. The statistical results of all experiment obtained in General Linear Model (GLM) procedure from SPSS 20.0 (SPSS 2006) statistical software package are attached in Appendix A.

CHAPTER 4. RESULT AND DISCUSSION

4.1 Effect of incandescent display light on color and oxidation stability of raw beef

4.1.1 CIE L*, a*, b* values of raw beef samples

Color is one of the most important quality attributes of raw beef since it affects overall quality. The results of color parameters, expressed as CIE L*, a*, b* values, measured on the surface of raw beef slices immediately after cutting of the raw beef steak and after 8, 24 and 48 hrs exposure to high power (200 watt) incandescent lamp and air at room temperature are presented in Figure 4.1.

Comparing measured color parameters of raw beef slices exposure to high power incandescent lamp and control placed in dark at room temperature, we observed that the changes a* (redness) and b* (yellowness) values were significantly less ($p<0.05$) in the control samples placed in dark than the displayed samples as shown in Figure 4.1. Although, the L* (whiteness) values were almost constant, no significant differences were found. The a* and b* values of the displayed samples were exhibited a remarkable decrease throughout all display periods. However, these values were almost stable in the dark storage samples until 24hrs then substantially fall to the end of displayed period (48hrs).

In the present study, we found color stability is more dependent up on photochemical action than storage time. While the color of raw beef slices exposed to lighting continuously at 6280±210 lux at the surface showed a remarkable decrease, the color of raw beef slices stored in dark was relatively stable, from which we noticed that light conditions and higher temperature more contributed for the color change of raw beef. These results were in agreement with those reported in literature, where a decrease of redness a* occurred in slices of dried sausages exposed to air and the most remarkable changes were observed with the slices exposed to the light at 25°C (Rohlík *et al*., 2010). They also explained that the higher temperature and light conditions accelerated the oxidation reaction of haem pigments and therefore caused the discoloration of the samples. Similar result was revealed from previous work by Caraballo *et al*. (1991) on sliced pork bologna. In their study on sliced meat products Nannerup *et al*. (2004) also reported fading of the pink color when illuminating the packages with light intensity of 600 1ux. They reported further discoloration observed when intensity increase to 1200 1ux.

The color of raw beef slices exposed to incandescent light and air for 48 hours showed a substantial fall, due to the degradation of the myoglobin pigments. This result was in agreement with those reported in literature, where duration of exposure to light and air has a significant effect on the color change of meat product (Rohlík *et al*., 2010; Haile *et al*., 2013).

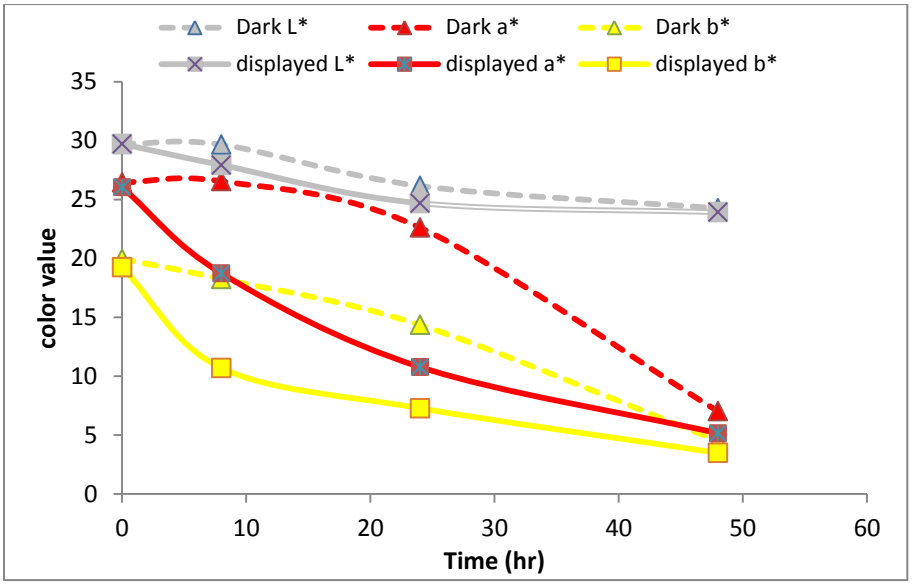


Figure 4.1 L*, a* & b* values of the beef slices displayed under incandescent lamps

4.1.2 Total color difference (ΔE)

The value of ΔE represents relative color changes that an observer might report for the materials after treatment or between time periods (AMSA, 2001). The lower value of ΔE is the more stable color change of meat. The total color difference (ΔE) increased as the display time increased (Figure 4.2). The total color difference (ΔE) of the control was significantly lower ($p < 0.05$) than the displayed samples. This showed the impact of high power incandescent lamp on the total color change of the raw beef during displaying.

According to International Lighting Commission (CIE, 2001), the total color difference (ΔE) between 0 and 2 is unrecognizable, and from 2 to 3.5 recognizable by an experienced observer, whereas the value exceeding 3.5 constitutes a significant color deviation for the observer (Wójciak *et al*., 2012). The criterion classified total color differences (ΔE) relevantly to the human perception of colors. Thus total color differences of the control sample at 8hrs were unrecognizable by an inexperienced observer ($\Delta E_8 = 2.39$) and after 24hrs which were recognizable by an inexperienced observer ($\Delta E_{24} = 7.58$), but relatively stable. However, the total

color differences of the control sample after 48hrs were significant color deviation for the observer ($\Delta E_{48} = 25.34$), as well as the displayed samples after 24 ($\Delta E_{24} = 20.11$) and 48hrs ($\Delta E_{48} = 26.82$), showed significant color deviation (Figure 4.2).

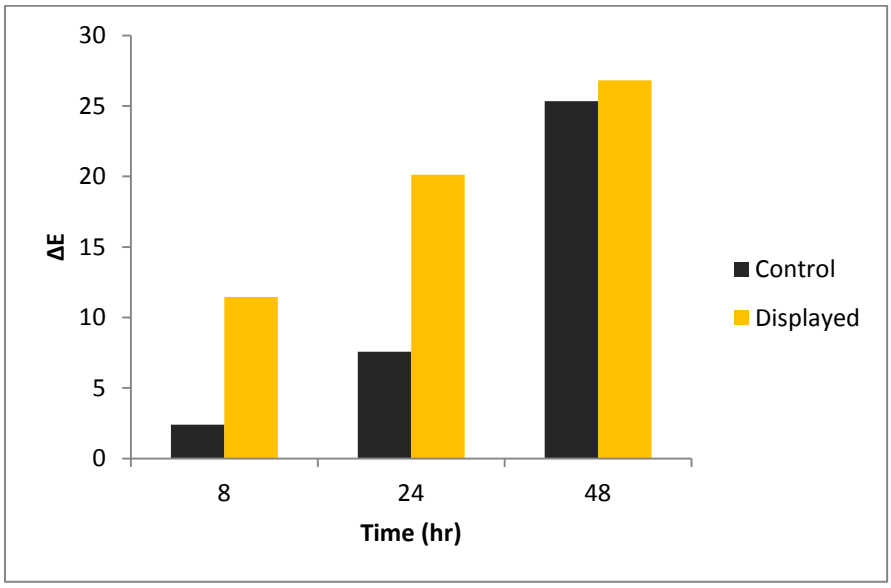


Figure 4.2 ΔE values of the beef slices displayed under incandescent lamps

4.1.3 Chroma, hue angle & pH values of the raw beef displayed under incandescent lamps

Chroma (saturation) values is calculated from a* and b* values, and it is a measure of color intensity where the higher values represents more intense color. Higher chroma values for meat represent maintenance of color intensity (Dawson and Acton, 1995), due to the stabilizing of product redness (a*) by Oxymyoglobin (Pierson *et al*., 1970). In this experiment, the chroma values of the raw beef samples were decreased as the display time increased. Although, the rate of decreasing was high in the raw beef slices exposed to incandescent display light (Table 4.1).

Hue angle combines the a* and b* values to specify in terms of the angle between pure red (Hue angle= 0°) and pure yellow (hue angle= 90°) axes of color space (CIE, 1986). A higher hue angle corresponds to meat browning or increases in yellowness over redness within the meat color range due to the metmyoglobin formation. In this experiment, the hue angle of the raw beef samples was decreased as display time increased in the raw beef slices kept in dark/controls. However, in displayed samples the hue angle value was slightly decreased until 8hrs and gradually increased during the remaining display time (Table 4.1).

The pH value of the raw beef samples showed slight decreasing as as the display time increased. However, there were no significant differences between the raw beef slices exposed to incandescent light and the raw beef slices kept in the dark at room temperature.

Table 4.1 Chroma, hue angle & pH values of the raw beef displayed under incandescent lamps

Measurements	Control in dark				Displayed			
	0 hr	8 hrs	24 hrs	48 hrs	0 hr	8 hrs	24 hrs	48 hrs
Chroma	33.13±0.45	32.22±0.17	26.78±0.10	8.37±0.40	32.40±0.03	21.60±0.82	12.98±0.08	6.24±0.01
Hue angle	37.06±0.28	34.46±0.72	32.36±1.24	32.68±1.62	36.50±0.42	29.82±2.21	33.87±1.58	34.05±2.04
pH	5.89±0.01	5.87±0.01	5.86±0.01	5.49±0.02	5.87±0.01	5.73±0.02	5.64±0.01	5.33±0.02

4.1.4 The OMb & MMb contents of the beef slices displayed under incandescent lamps

The oxymyoglobin content was decreased with increase of treatment time (Figure 4.3). The rate of decreasing content of the displayed sample was higher than the dark storage sample throughout to all the display periods. Regarding to metmyoglobin content, it was increased with increase of treatment period (Figure 4.3). Similarly, the rate of increasing content of the displayed sample was higher than the dark storage sample throughout all display periods.

The relationship between oxymyoglobin content and CIE a* value was also computed. The raw beef slices kept in the dark at room temperature showed to have higher values of redness *a** than those kept at the same temperature but exposed to the incandescent light. Similarly, Figure 4.3 shows the raw beef slices kept in the dark at room temperature showed higher oxymyoglobin content than those kept at the same temperature but exposed to the incandescent light. This showed the positive relation of CIE a* value with the oxymyoglobin content of raw beef sample. In addition the presence of light initiates the haem pigment oxidation and caused the discoloration of displayed raw beef.

We also observed a negative relation between the metmyoglobin content and the CIE a* value of raw beef samples. While the CIE a* value of raw beef slices kept in the dark at room temperature showed higher values than those exposed to the incandescent light, the of raw beef slices kept in the dark at room temperature showed lower metmyoglobin content than those exposed to the incandescent light as shown in Figure 4.3. The increase in MMb% was also negatively correlated to redness ($r = -0.93$). This relation between MMb% and redness was in agreement with study

conducted by Insausti *et al*. (1999) on color stability of beef and Hunt *et al*. (1991). Moreover, a decrease in a*value can be explained by the chemical reaction of the light induced oxidation of the color pigment. The activated state of Mb reacts with triplet oxygen and forms metmyoglobin and other products. Metmyoglobin formation causes the gray brown color of illuminated meat and therefore the decrease in a*value (Andersen & Skibsted, 1992).

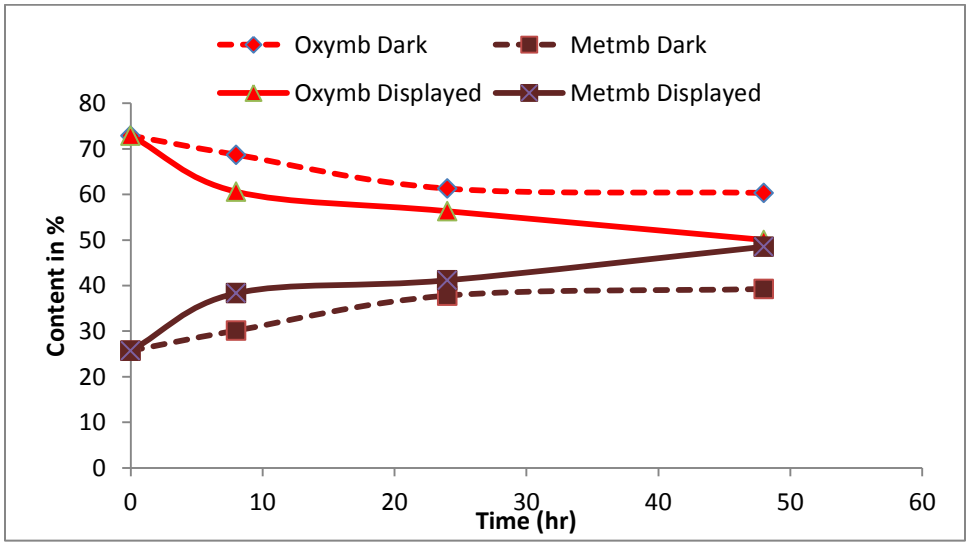


Figure 4.3 Oxymyoglobin and metmyoglobin content of the raw beef steaks

4.1.5 Lipid oxidation of raw beef samples by TBARS

Meat products should be safe, stable within the stipulated time period and the corresponding sensory properties. According to the consumer, color is an important parameter of the meat product quality, because it reflects the composition, the freshness of raw meat and the proper storage conditions. Undesirable color of the meat product is associated with poorer product quality. Many chemical reactions can take place and affecting the color of the meat product. The most important chemical reactions that take place are lipid oxidations which in fact affect the oxidation of haem pigments whose red color turns into brown (Rohlík *et al*., 2010; Georgantelis *et al*., 2007; Parra *et al*., 2012; Haile *et al*., 2013).

Thiobarbituric Acid Reacting Substances (TBARS) value increased linearly with display time (Figure 4.4), in consonance with the suggested mechanism of lipid oxidation (Allen & Foegeding, 1981; Melton, 1983). Periods of exposure to light and self-propagation required in the free radical driven mechanism of auto-oxidation may explain the time dependency of lipid

oxidation. This has implications for shelf life of meat products in retail especially when light can induce free radical formation.

In this study we found a lower TBARS value from raw beef samples stored in dark than those exposed to light. This was possible because the oxidation might have been promoted by the heat generated from incandescent display light. The TBAR results agree with Jakobsen *et al.* (2000) in as much that an increase in TBAR values can occur with increasing display temperatures and duration. On the contrary, Park *et al*. (2007) in their study on pork meat also reported non-significant variation in lipid oxidation level attributes to exposure of light, which was contrary to our findings.

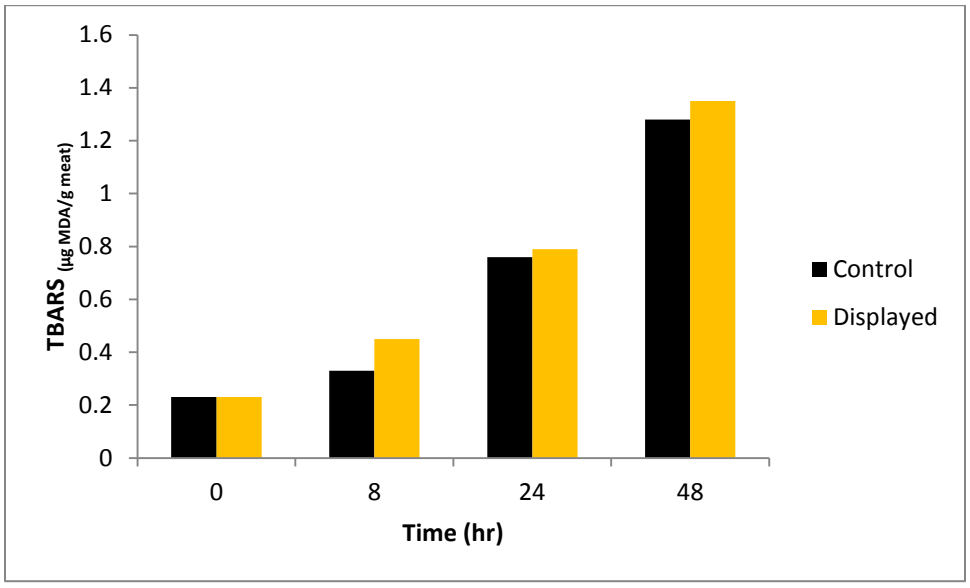


Figure 4.4 TBARS value of the raw beef samples displayed & control

4.1.6 Total aerobic count (TAC) of raw beef samples

The TAC value of raw beef samples was increased linearly with the increase of display and dark storage time (Figure 4.5). When compared the displayed sample with control, the TAC value of the display raw beef sample was lower than the counter control sample; this may be due to the influence of high intensity of incandescent display light on the growth of colonies of microbial or which reduced the population of colonies of microbial during displaying.

In general, the raw beef slices exposed in incandescent light showed higher TBARS value, ΔE, hue angle and metmb%, and lower L*, a*, b*, Chroma & oxymb% value than the raw beef slices kept in the dark at room temperature (Figure 4.1 - 4.4). From these results we noticed a clear

influence of high power incandescent lamp and air exposure on color and lipid oxidation stability of raw beef during displaying.

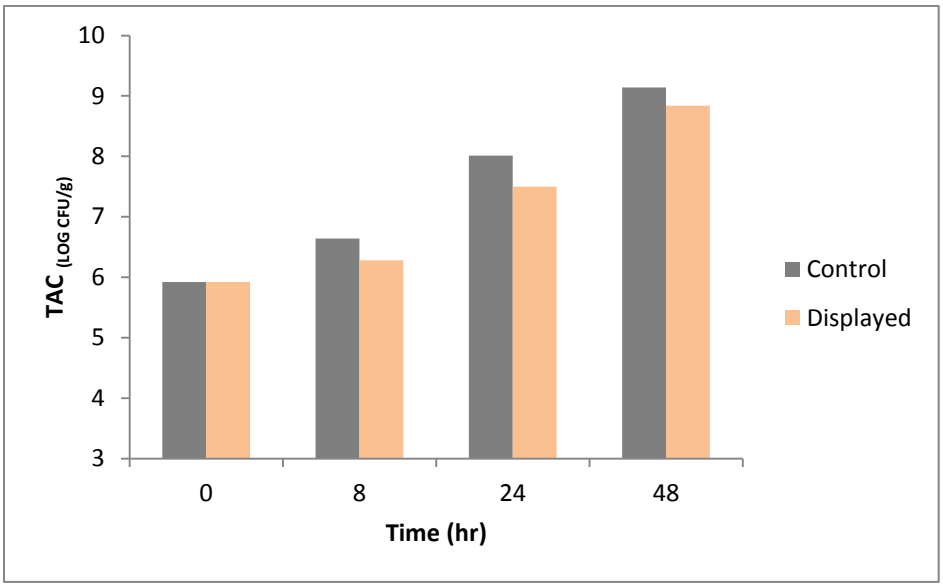


Figure 4.5 TAC value of the raw beef samples displayed & control

4.2 Effect of display condition (covering samples) on color and lipid oxidation stability of raw beef steaks

4.2.1 CIE L*, a*, b* values of the covered & open displayed raw beef samples

All CIE L*, a* and b* values of covered samples showed higher values compared to opened samples in both displaying and dark storage condition especially at the end of treatment period (20 hrs) as shown in Figure 4.6 - 4.8.

The L* value of covered samples exposed to display light was significantly higher than covered sample that store in dark at 4hrs where as they were almost equal at 20 hrs. However, the L* value of opened sample which exposed to display light was significantly lower than the control that placed in dark, throughout all displaying periods (Figure 4.6). The higher value of L* in covered samples than opened samples may be due to the covered samples reserved more water on the surface of raw beef, because dried meat surface showed lower L* value.

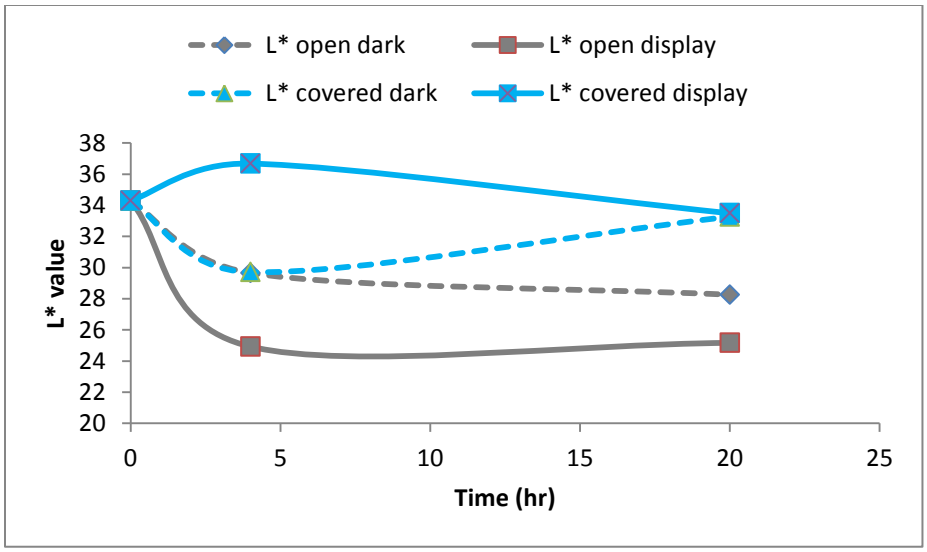


Figure 4.6 CIE L* value of covered and opened displayed & control beef samples

The redness (CIE a*) value of both covered and opened samples those placed in dark were significantly higher than those exposed to incandescent display light throughout all displaying periods (Figure 4.7). Although, the a* value of covered sample was higher than opened sample at 20hrs in both display and dark storage. During the first 4hrs the a* value of all displayed samples were drastically decreased but dark storage samples showed stable tendency and opened sample was highest (a* = 26.55) whereas at 20 hrs covered sample showed highest value (a* = 15.28).

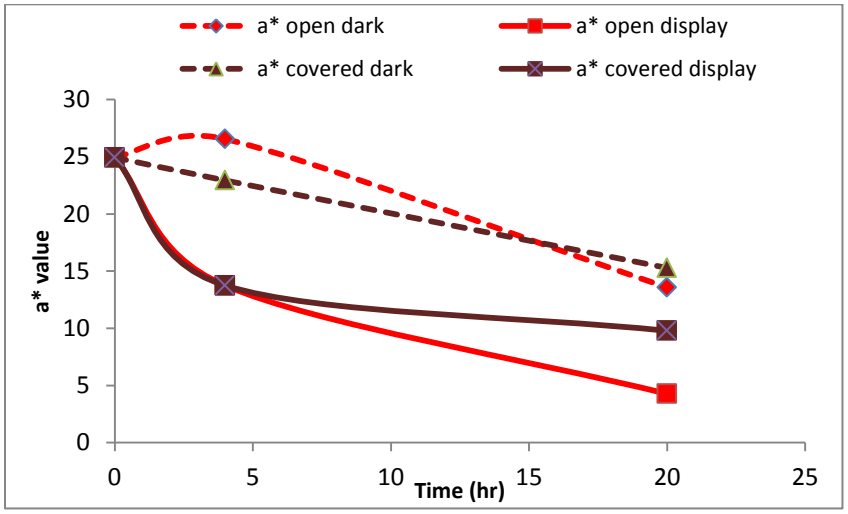


Figure 4.7 CIE a* value of covered and opened displayed & control beef samples

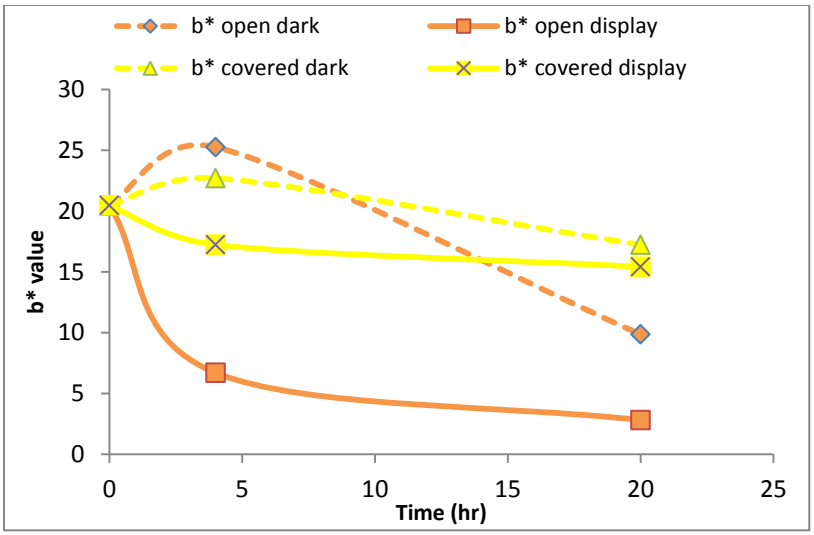


Figure 4.8 CIE b* value of covered and opened displayed & control beef samples

Figure 4.8 depicts the b* values of covered displayed and control beef samples were stable throughout all periods. However, opened samples were significantly decreased especially at 20hrs. Although, until 4 hrs the b* value of dark storage significantly increased to highest value (b* = 25.26). Moreover, the b* values of dark storage dark storage samples were higher than displayed samples. However, among the displayed samples the rate of change of b* value of opened display was significantly greater than covered display samples. In other word, the b* value of covered display sample was significantly higher than opened display samples.

One of interesting result of this experiment was the yellowness (CIE b*) value of raw beef sample exhibited greater value than the redness (CIE a*) value of covered samples compared to opened samples in both displaying and dark storage condition (Figure 4.9). However, this result was more significant in displaying under incandescent light. At starting time (0 hr) a* value was significantly higher than b* value but after treatment especially displaying, the covered sample was acquired higher b* value than a* value in all displayed periods. This result may be due to higher temperature as shown in Figure 4.14 that developed inside of covered petri dish by incandescent display light. In addition, during this experiment we observed the condensed water droplets inside of the cover of petri dish of the covered display samples (Figure 4.15) which indicated the development of higher temperature inside of the petri dish. Therefore, this higher temperature inside of the petri dish might contributed for the higher b* value on raw beef samples, like cooking increase the b* value on cooked meat.

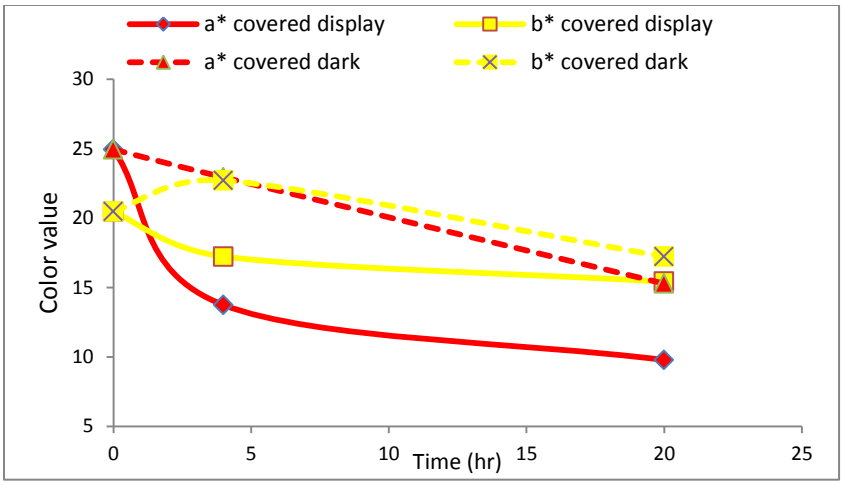


Figure 4.9 Comparison of a* and b* values of covered and opened displayed & control beef samples

4.2.2 The chroma and hue angle values of the covered & open displayed raw beef samples

The chroma of covered samples were exhibited the relative stable condition throughout all periods. However, opened samples were significantly decreased especially at 20 hrs. Although, until 4hrs the chroma of dark storage significantly increased to highest value (C* = 36.66). Figure 4.8 shows that the chroma of dark storage dark storage sample was higher than displayed sample. However, among the displayed samples the covered display sample was significantly higher than opened display samples.

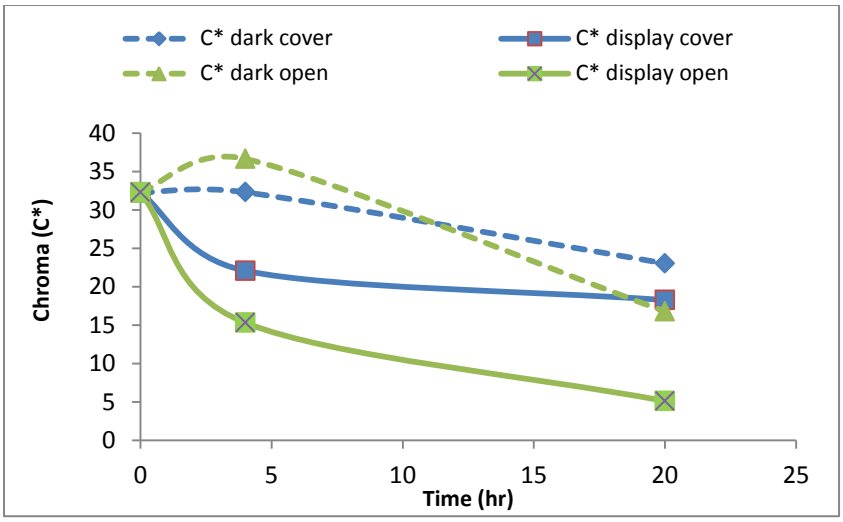


Figure 4.10 Chroma value of covered and opened displayed & control beef samples

A higher hue angle corresponds to meat color increase in yellowness over redness. In this experiment the hue angle of covered samples were higher than opened samples in both display and dark storage condition throughout all periods (Figure 4.11), this indicated the color of

covered samples were more tend to yellowness than redness. This is in agreement with the result to the CIE b* value of covered samples which discussed above.

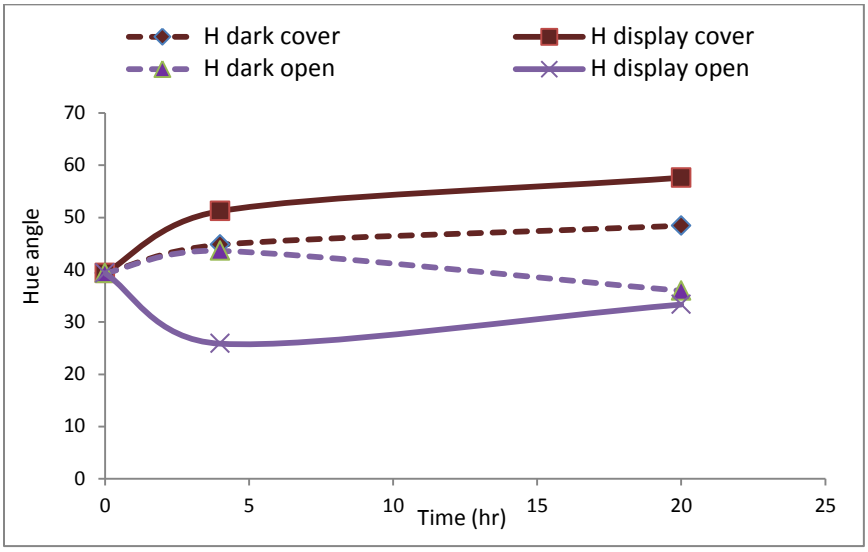


Figure 4.11 Hue angle of covered and opened displayed & control beef samples

4.2.3 Total color change (ΔE) value of the covered and open displayed samples

Total color change or delta E (ΔE) of the covered samples was lower than opened samples in both display and dark storage condition throughout all periods (Figure 4.12). This indicated that the covered samples have more color stability than the opened samples. Among the displayed samples the total color change of covered display sample was significantly lower ($p < 0.01$) the opened display sample throughout all displaying periods.

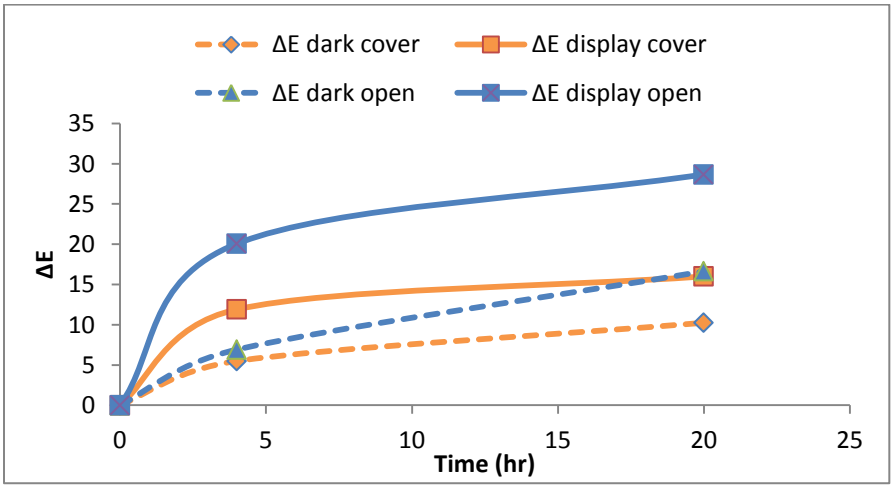


Figure 4.12 Total color change (ΔE) value of covered and opened displayed & control beef samples

4.2.4 The results of oxymyoglobin and metmyoglobin content

The results of oxymyoglobin and metmyoglobin content and pH in transparent covered petri dish and in opened petri dish given in Table 4.2. The oxymyoglobin content was decreased and metmyoglobin content was increased with increase of treatment time in the samples of dark storage in both covered and opened condition. However, the oxymyoglobin content was decreased and metmyoglobin content was increased until 4hrs in all displayed samples then the oxymyoglobin content was increased and metmyoglobin content was decreased at the end of displayed period (20 hrs).

Table 4.2 OMb, MMb and pH values of covered and open displayed beef samples

Samples		Time (hr)	OMb(%)	MMb(%)	pH
covered display sample	Control in dark	0	69.78±0.98	3o.64±0.58	5.78±0.01
		4	60.19±1.04	41.02±0.68	5.77±0.02
		20	56.32±0.98	41.15±0.94	5.71±0.02
	Displayed	0	69.78±0.98	3o.64±0.58	5.81±0.01
		4	56.19±0.96	41.08±0.68	5.80±0.02
		20	61.70±1.12	37.81±0.66	5.73±0.02
opened display sample	Control in dark	0	69.78±0.98	30.64±0.58	5.78±0.01
		4	68.71±1.56	38.13±0.76	5.76±0.02
		20	56.32±1.08	41.15±0.88	5.51±0.03
	Displayed	0	69.78±0.98	3o.64±0.58	5.81±0.01
		4	56.32±1.04	41.15±0.68	5.78±0.02
		20	60.86±1.14	38.31±0.54	5.64±0.01

4.2.5 Lipid oxidation of covered & open displayed raw beef samples by TBARS

As expected, the TBARS value of raw beef samples were increased linearly with the increase of display and dark storage time (Figure 4.13). Even though, the covered samples revealed good color stability by different color parameters, those covered samples exposed to incandescent display light were exhibited significantly lower oxidation stability compared to open displayed samples. At 20hrs the TBARS value of the covered sample stored in dark was lower than the opened sample stored in dark. However, the TBARS value of the covered displayed sample was significantly higher ($p < 0.05$) than the opened displayed sample. This result is a clear indicator of the influence of higher temperature on raw beef oxidation stability. In addition, this result was in agreement with the work of Rohlík *et al*., (2010). They explained that the higher temperature

and light conditions accelerated the oxidation reactions of haem pigments and therefore caused the discoloration of the meat samples. Savanović *et al*., (2014) also reported that at the higher temperature the color changes of dry fermented beef sausage are significantly greater.

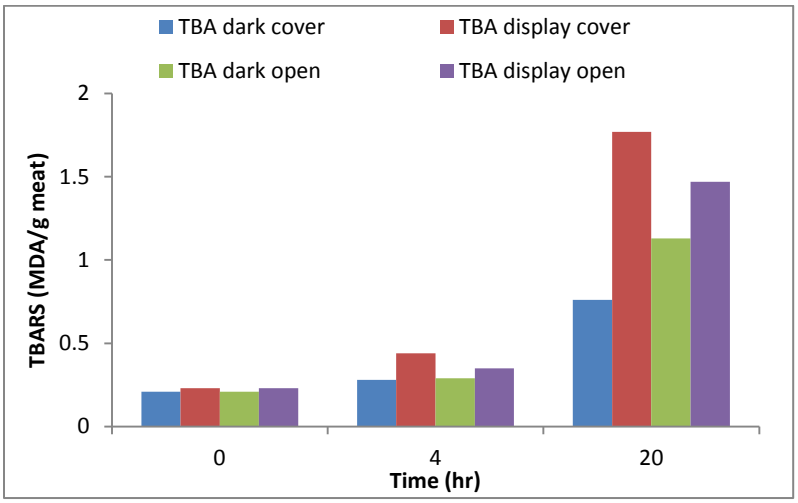


Figure 4.13 TBARS value of covered and opened displayed & control beef samples

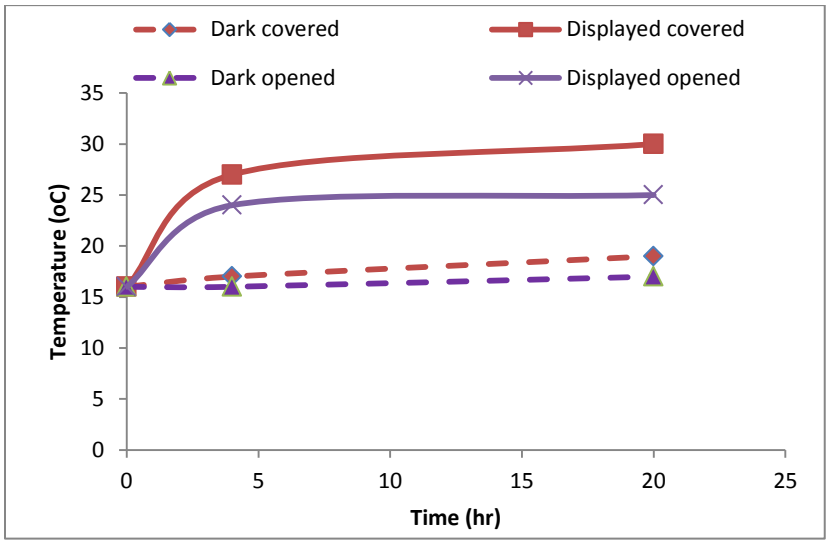


Figure 4.14 Temperature of covered & opened displayed & control beef samples

Figure 4.14 illustrates the temperature of displayed raw beef samples was significantly higher ($p < 0.05$) than the raw beef samples kept in dark at room temperature. Among the displayed raw beef samples the covered display samples exhibited more temperature elevation than the opened display samples. This result was expected because; heat can originate from the absorption of light by the meat and/or by lamp heating. In detail, the light and heat energy that originated from

incandescent light have absorbed by meat surface and reemitted thermal energy with wavelengths in infrared regions. Unlike visible light, infrared wavelength cannot pass out through transparent materials, so that it trapped by cover of petri dish, then developed higher temperature inside of the covered petri dish. This thermal elevation in the covered display samples caused for higher lipid oxidation and CIE b* value in the covered display samples as discussed in above. The pH values of all display conditions were showed insignificant decrease with increase of displayed and dark storage hours.

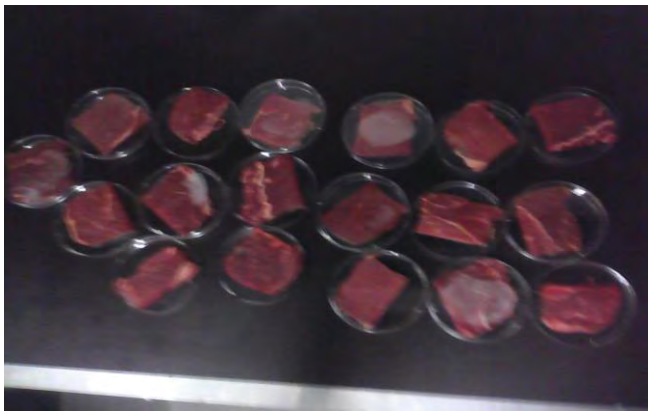


Figure 4.15 Covered and opened display raw beef sliced at 4hrs which showed condensed water droplets inside of the cover of petri dish of the covered display samples

4.3 Assessment of color stability of different muscles types of raw beef in incandescent display light

4.3.1 The results of color variables of different muscles types in response to light exposure

The results of color variables, expressed as CIE L*, a*, b*, chroma, hue angle and delta E values, measured on the cross section of *semitendinosus, triceps brachii, longissimus dorsi* (red) and *longissimus dorsi* (white/fat) muscle types of raw beef slices immediately after cutting of the raw beef steak and after 1, 2 and 3 days exposed to high power (400 watt) incandescent lamp and air at room temperature presented in graphs (Figure 4.18-4.24). In addition, for comparison their color change between the displayed raw beef samples with the samples kept in dark (control), the picture of *semitendinosus* muscle type as an example presented in Figure 4.16 & 4.17.

Figure 4.16 *Semitendinosus* muscle kept in dark at 0hr (left) & after 24 hrs (right)

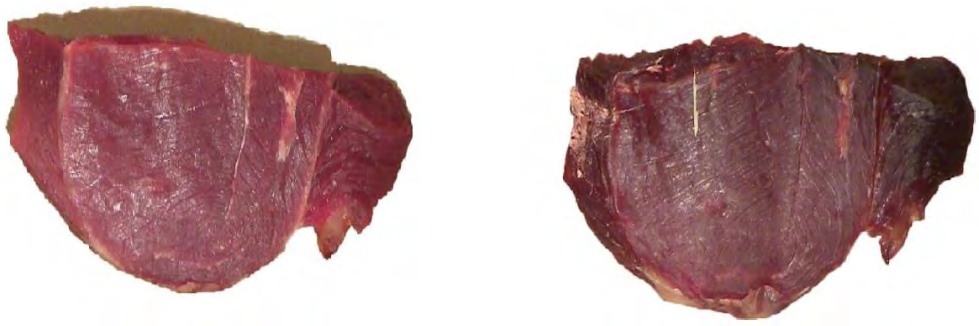


Figure 4.17 *Semitendinosus* muscle type displayed under incandescent lamp at 0hr (left) & after 24 hrs (right)

Overall, a decrease of all color variabes occurred in all raw beef slices as the treatment period increased; the most remarkable changes were observed with the slices exposed to the incandescent light. This is due to the higher temperature and light conditions that accelerated the oxidation reactions of haem pigments and so caused the discoloration of the displayed beef samples. How ever, the color of the dark stored references did not change significantly during 24 h as shown in Figure 4.16.

Values of a* (redness) and b*(yellowness) on the surfaces of all red muscle displayed samples were decreased significantly ($p<0.05$) during 1, 2 and 3 days of exposure to high power incandescent lamp and air, at room temperature. Whereas L* (lightness) value was stable until day 1, after that decreased until the end of display day. When compared the displayed samples to the controls which kept in dark, a* and b* values of the displayed samples were significantly lower ($p<0.05$) than the controls in all red muscle types throughout all display and dark storage periods. However, the L* value of displayed *triceps brachii* and *longissimus dorsi* (red) samples was higher ($p>0.05$) than the control of these muscle types in day 2 and 3 (Figure 4.18).

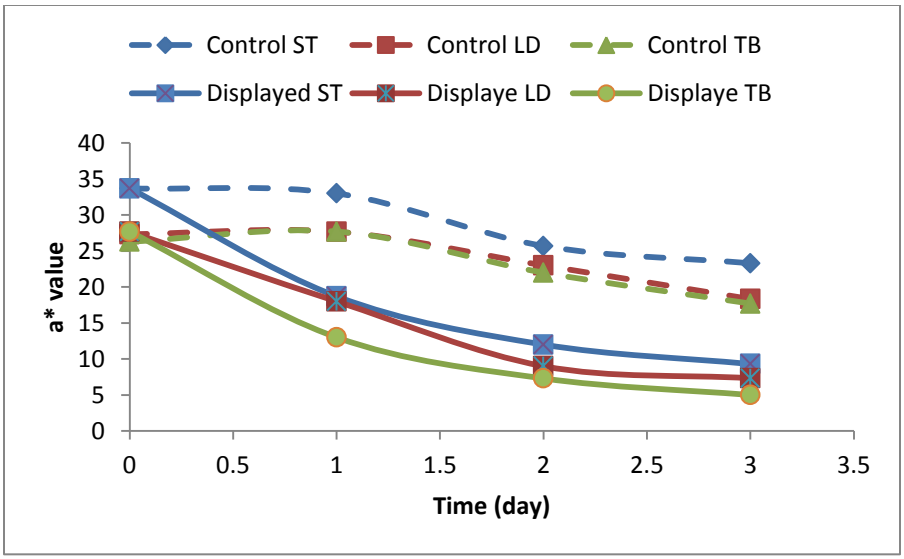


Figure 4.18 CIE a* value for ST, LD & TB muscle types

Among the muscle types the *semitendinosus* muscle type showed the highest CIE L* (lightness), a* (redness), b* (yellowness) and chroma (saturation index) values and lowest value of hue angle compared to the *triceps brachii* and *longissimus dorsi* (red) muscle samples in both displayed and dark storage throughout all display and dark storage periods. Next to the *semitendinosus*

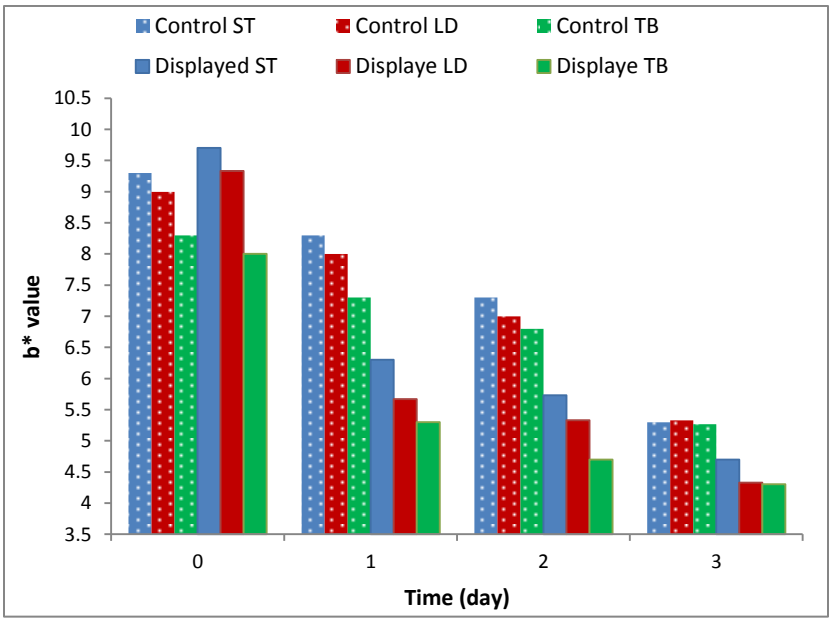


Figure 4.19 CIE b* value for ST, LD & TB muscle types

muscle type, the *longissimus dorsi* (red) muscle samples exhibited better values of color parameters than the *triceps brachii* muscle type as shown in Figure 4.18 - 4.20. However, they showed no difference in terms of chroma and hue angle (Figure 4.21 and Figure 4.22)

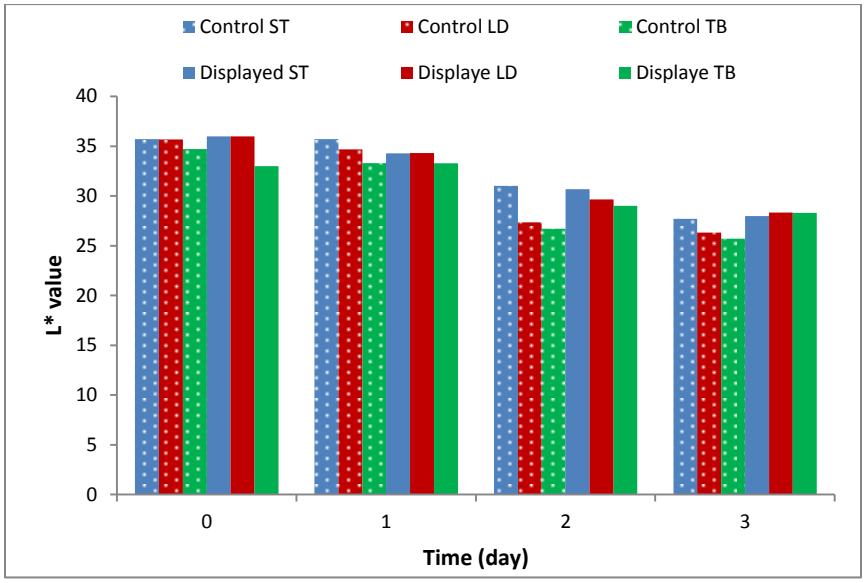


Figure 4.20 CIE L* value for ST, LD & TB muscle types

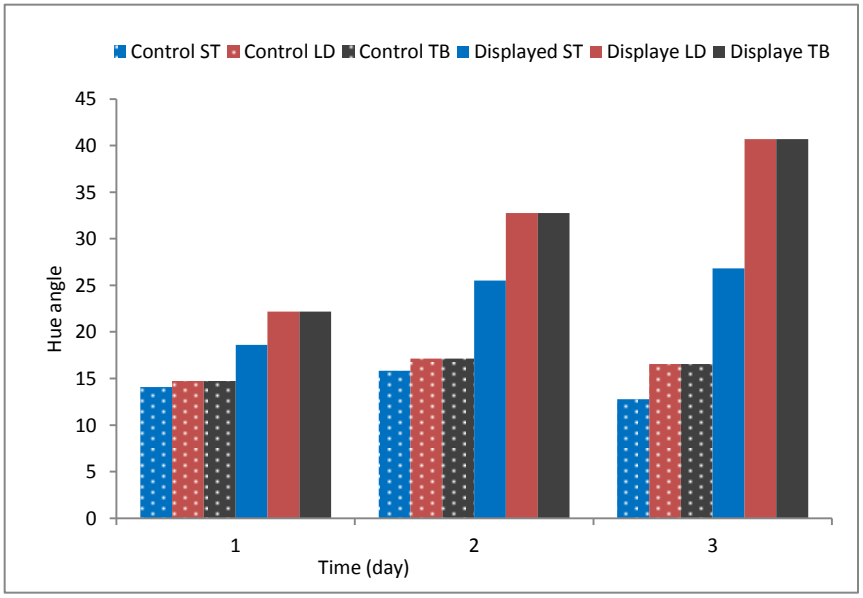


Figure 4.21 Hue angle value of ST, LD & TB muscle types

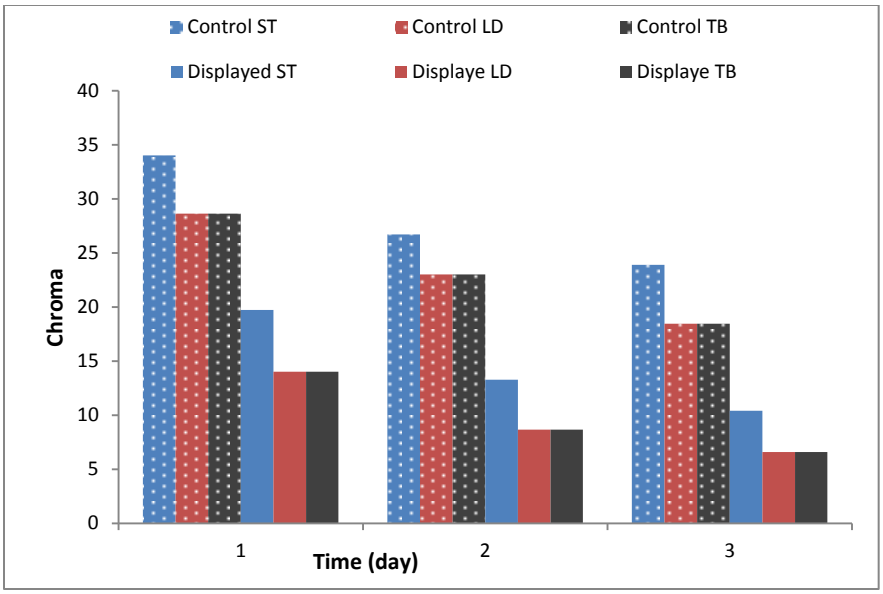


Figure 4.22 Chroma values of ST, LD & TB muscle types

Figure 4.23 depicts the total color change (delta E) value of the *semitendinosus* muscle type was higher than the *triceps brachii* and *longissimus dorsi* (red) muscle samples in both displayed and dark storage throughout all display and dark storage periods. Whereas there was no difference between the *triceps brachii* and *longissimus dorsi* (red) muscle types.

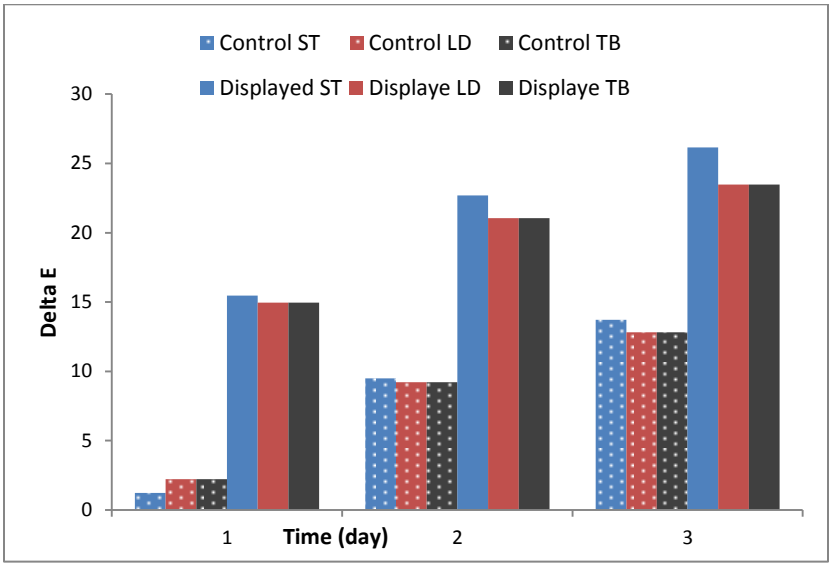


Figure 4.23 Delta E values of ST, LD and TB muscle types

Regarding to the white/fat raw beef samples the CIE L* (lightness) value was increased until day 1then dropped up to the end of treatment period in both displayed and dark storage. The a* (redness) value was stable until day 1 and significantly decreased ($p<0.05$) at day 2 and 3 in both displayed and dark storage. The b* (yellowness) value was almost stable in both displayed and dark storage throughout all display and dark storage periods as shown in Figure 4.24. Like a* value the chroma value was decreased and the hue angle also increased in both displayed and dark storage conditions.

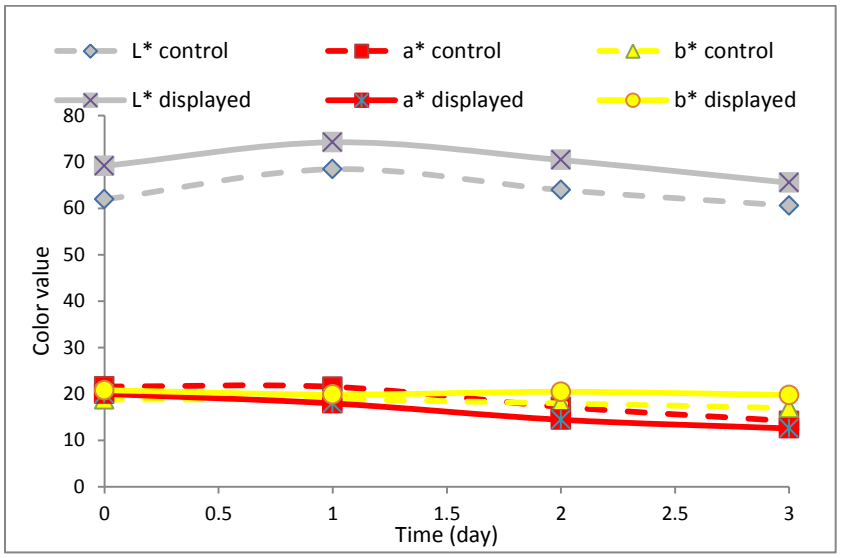


Figure 4.24 L*, a* and b* values for *Longissimus dorsi* (white/fat) muscle type

In conclusion, in this experiment we noticed that the color stability of raw beef depend upon the muscle type, this is in agreement with Insani *et al*., (2008) and Von Seggern et al., (2007). This difference can be associated with the different balance of antioxidative and pro-oxidative compounds that control the oxidation rate of myoglobin (Trout & Gutzke, 1995). For example, Psoas major muscle has poor color stability, which is associated to its high oxidative activity and high myoglobin autoxidation rate (Renerre & Labas, 1987). Among the selected three muscle types we found that the *semitendinosus* muscle type has best color stability than the *triceps brachii* and *longissimus dorsi* (red) muscle types and the *triceps brachii* muscle types have the lowest the color stability. This result is in agreement with the classification of beef color stability by Jacob R, Warner R. and Jose C. (2008). They classified the *semitendinosus* as stable and the *longissimus dorsi* and *triceps brachii* as intermediate.

4.4 Investigation of the impact of specific wave length of visible light on color change of raw beef by using optical filters

Photochemical effects are caused by certain wavelength energies that excite one or more molecules and initiate or catalyze such reactions as oxidation which leads to a change in the meat pigment, myoglobin, causing discoloration. Wavelengths that are absorbed cause photochemical; effects, resulting in greater destruction of heme pigments, but wavelengths that are primarily reflected should have produce less of a photochemical effect.

4.4.1 Color change of raw beef under specific wave length of visible light

In this experiment we tried to evaluate the influence of short wavelength (blue 450 nm) and long wavelength (red 650 nm) and different light intensities on the color stability of raw beef samples which exposed to different wavelengths and intensities of visible light. The illuminating tests were performed at light intensities in the range between 1.10 klux (blue filter) and 4.99 klux (transparent) for six hours.

The L* value was decreased linearly with increase of displayed time in all wavelengths (Figure 4.25, left). However, the rate of decrease of this parameter was highest in transparent (it has highest illuminance/ irradiancy) throughout display time. Figure 4.25 (right) shows ΔL* of short wavelength (blue, 450 nm) was significantly higher than long wavelength (red 650 nm) especially at 6 hrs display time.

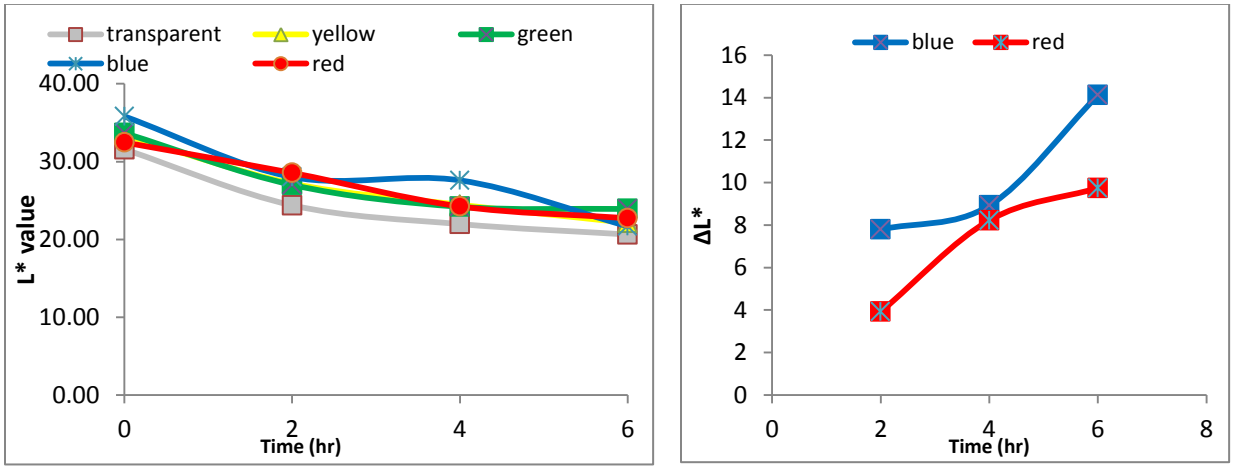


Figure 4.25 CIE L* value for all filters (left) and ΔL* of long and short wavelength (right)

Similarly, the CIE a* and b* values were decreased linearly with increase of displayed time in all wavelengths as shown in Figure 4.26 (left) and 4.27 (left). Although, the rate of decrease of a* and b* values were higher in transparent and yellow filters (which have higher irradiancy) throughout display time. The Δa* of short wavelength was significantly higher ($p < 0.05$) than lower wavelength in all display periods (Figure 4.26, right); Δb* also showed (Figure 4.27, right) the same result but not statistically significant ($p > 0.05$).

Generally, in all wavelengths the color parameter L*, a*, b* and chroma values were decreased linearly with increase of displayed time. However, the rate of decrease of these parameter was higher in transparent and yellow filters (which have higher illuminance), except L* value for yellow filter. This indicated that the higher intensity of light has more impact on the discoloration of raw beef. Regarding to the influence of long and short wavelengths on color stability of raw beef, the short wavelength (blue 450 nm) was exhibited higher color change compared to long wavelength (red 650 nm) based on all CIE color parameters. From these results we noticed that short wavelength was more promote to discoloration of raw beef samples. This result is in agreement with studies (Archer & Bandfield, 1950; Satterlee & Zachariah, 1972; Iversen, 1985), which reported an undesirable influence of short wavelengths on meat color. For example, Archer and Bandfield (1950) filtered out low 400nm (violet & blue) wavelengths, which delayed the onset of discoloration of meat samples. In model studies using beef and pork

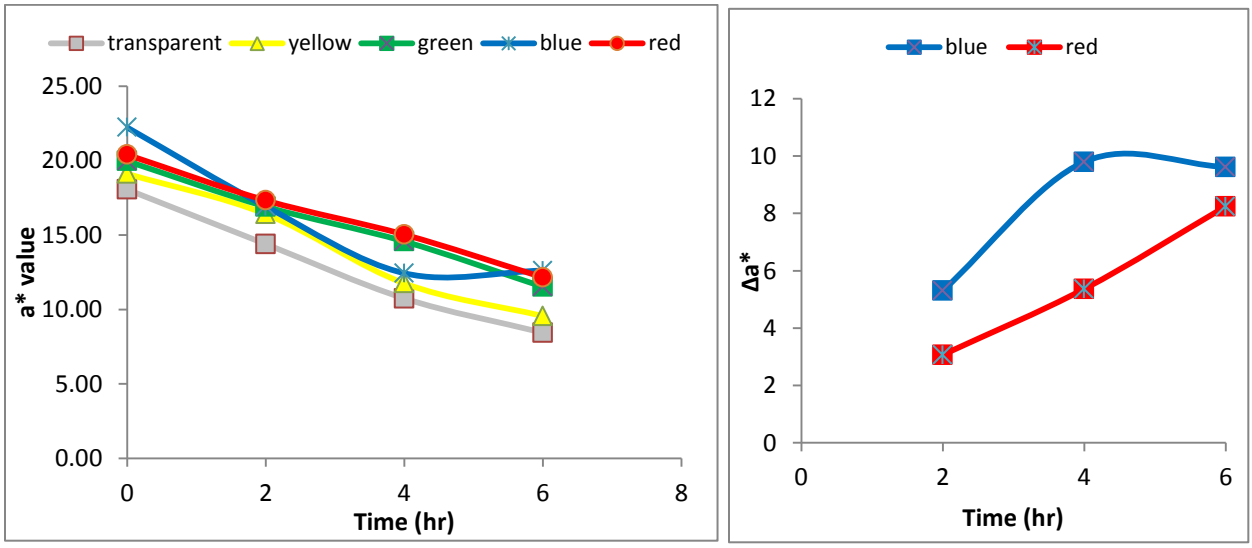


Figure 4.26 CIE a* value for all filters (left) and Δa* of long and short wavelength (right)

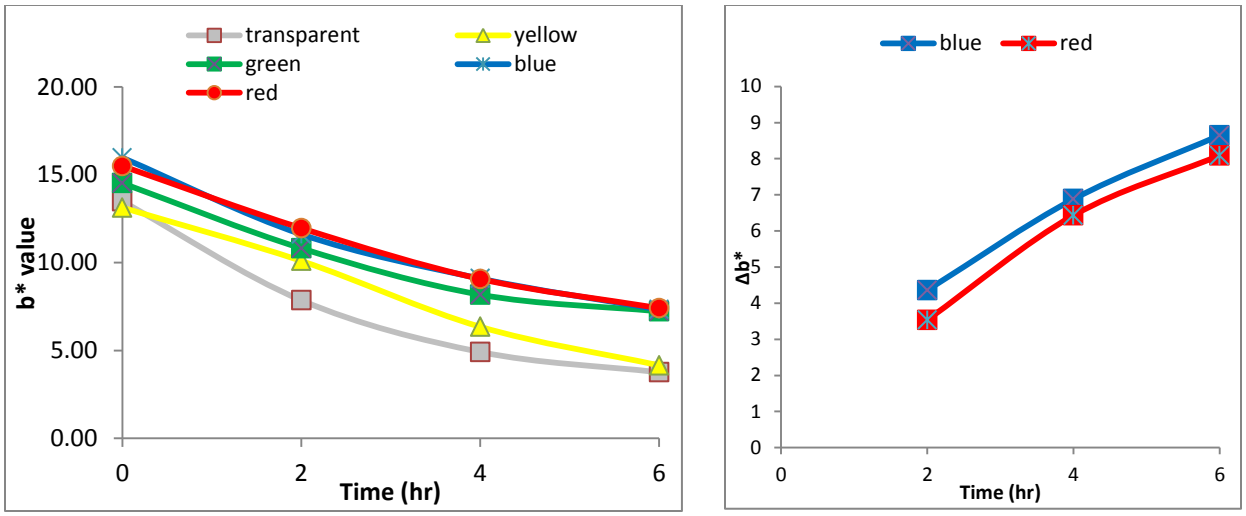


Figure 4.27 CIE b* value for all filters (left) and Δb* of long and short wavelength (right)

myoglobin (Satterlee & Zachariah, 1972) led them to conclude that "low wavelength light" encouraged oxidation. Therefore, rate of discoloration of meat had increaseed. But wavelengths of 625 nm or longer are less absorbed by oxymyoglobin, which means they would be less prone to promote discoloration. Iversen (1985) reported that film with all wavelengths below 550 nm blocked out was effective in slowing oxidation and discoloration.

Display lighting intensity can have an important influence on product display life. The effect of different intensities (100, 150, 200 and 300 foot candles) of display lighting on color fading of wafer sliced cooked and cured beef slices in nitrogen flush packages was studied by Schwab, (1981) and he found that the meat samples discoloration was closely related to foot candle intensity and length of light exposure time.

The color saturated index or chroma (C*) results revealed similar changes as a* and b*, this was expected because the chroma value was calculated from values of a* and b* (Table 4.3). However, the hue angle result was differed from the rest of color parameters in terms of those light intensity dependent results. The hue angles of transparent and yellow filters (which have higher irradiancy) were lower than other filters throughout all display periods (Table 4.3). Although, this parameter showed similar results in terms of short and long wavelength dependent results. Like other color parameters, the hue angle of short wavelength (blue 450 nm) was higher than long wavelength (red 650 nm).

Table 4.3 The chroma, hue angle & ΔE results of raw beef steaks which displayed under different color of optical filters

Filter color & wavelength	Illuminance (k lux)	Time(h)	Chroma	Hue angle (degree)	ΔE
Transparent 400-700nm	4.99±0.06	0	22.55±0.84	36.76±0.40	0
		2	16.40±0.31	28.63±1.02	9.84±0.20
		4	11.81±0.45	24.57±0.76	14.81±0.11
		6	9.24±0.50	23.98±1.61	17.53±0.11
Red (650nm)	1.49±0.01	0	25.62±0.57	37.24±0.29	0
		2	21.06±0.18	34.63±0.73	6.1±0.10
		4	17.56±0.20	31.11±0.72	11.74±0.03
		6	14.24±0.73	31.38±0.55	15.11±0.11
Yellow (550nm)	4.50±0.06	0	23.19±0.10	34.49±0.12	0
		2	19.27±0.33	31.59±0.87	7.44±0.11
		4	13.37±0.35	28.36±1.49	13.43±0.10
		6	10.42±0.11	23.47±0.82	17.31±0.10
Green (500nm)	1.58±0.01	0	24.69±0.38	36.03±0.15	0
		2	20.07±0.16	32.61±0.68	8.11±0.11
		4	16.71±0.16	29.31±0.81	12.62±0.22
		6	13.61±0.15	32.09±0.36	14.82±0.12
Blue (450nm)	1.10±0.01	0	27.38±0.17	35.70±0.28	0
		2	20.53±0.09	34.47±0.84	10.38±0.11
		4	15.43±0.50	36.11±3.60	14.51±0.20
		6	14.59±0.42	30.15±0.45	19.14±0.12

Delta E color change also known as the total color change (ΔE) over a selected period of time is a useful parameter to show total color differences over time. Thus, ΔE is more meaningful than the individual L*, a* and b* values. In this experiment this total color change (ΔE) parameter revealed the clear differences of the short and long wavelengths on the color stability of raw beef samples. As shown in Figure 4.28 the total color change (ΔE) of the short wavelength (blue 450 nm) was highest throughout all display periods. Even if it has lowest light intensity it showed remarkable color change more than even the transparent and yellow filters which have higher light intensity. While the long wavelength exhibited lowest total color change (ΔE) in most of display periods. These results were a clear evidence of the impact of short wavelengths and light intensity on color change stability of displayed raw beef.

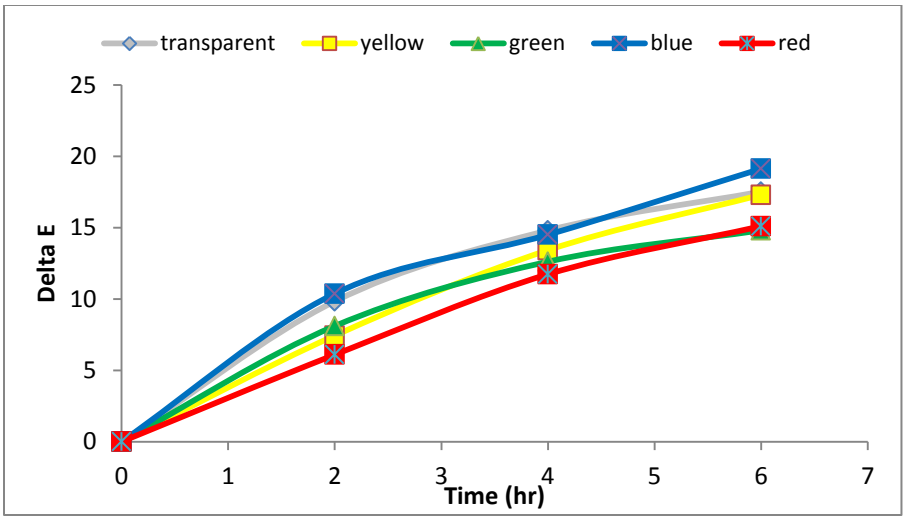


Figure 4.28 Total color change (ΔE) of raw beef slices displayed under different color filters

4.4.2 The color stability of raw beef by using green and red colored incandescent lamps.

Under this experiment we also tried to assess the color stability of raw beef using green and red colored incandescent lamps. Even if both lamps have equal power (60 watt) the light intensity on displayed raw beef samples was varied by far, the green lamp produced more than twice of the red lamp light intensity at the surface of raw beef slices. Though, the values of most of color parameters of green color lamp showed higher color change than red colored lamp throughout 24 hrs display time (Table 4.4). Similarly, in study of Bohner *et al*., (2014) various lamps and the exposure time showed a significant effect on the graying of the cured boiled sausages.

Table 4.4 Colorimeter results of raw beef displayed under incandescent green & red colored lamps

Time (hr)	Lamp color	Light intensity (lux)	L*	a*	b*	C*	Hue angle	ΔE
0	green	1650±50	30.00±0.58	18.14±0.10	12.72±0.78	22.16±0.39	35.01±1.77	0
	red	700±25	31.79±0.85	18.23±0.59	12.74±0.13	22.24±0.42	34.96±1.12	0
6	green	1650±50	22.62±1.13	13.63±0.82	6.50±0.62	15.1±1.00	25.45±0.78	10.67±1.73
	red	700±25	24.74±0.63	16.24±0.81	8.32±0.22	18.25±0.82	27.16±0.57	8.58±0.90
24	green	1650±50	18.16±0.54	4.01±0.26	1.64±0.14	4.33±0.29	22.12±0.34	21.53±0.66
	red	700±25	18.18±0.24	5.52±0.39	2.52±0.69	6.08±0.62	24.19±4.72	21.43±1.32

In general, the display light sources with higher ratio in the shorter wavelengths like the most frequently used daylight fluorescent lamps showed a higher discoloration. The display light

sources with a higher radiation in the medium and long wavelength range of the visible spectra could reduce the discoloration of displayed raw meat (Bohner *et al*., 2014). Further research on new lighting technologies and on the effect of single wavelength bands in the visible spectrum is necessary to reduce the deterioration in quality of displayed meat.

4.5 Effect of Ethiopian spices extract on color and lipid oxidation of raw beef steaks during storage in dark refrigerator

4.5.1 CIE L*, a* and b* values for spice treated beef samples

One of the most important quality attributes of raw beef is color, since it influences consumer acceptability (Bozkurt & Erkmen, 2002; Bozkurt & Bayram, 2006). Color stability during displaying and storage are very important quality attributes of meat products. To investigate the spices treatment effects on color and determine color of raw beef were used color measuring instrument. The use of color measuring instruments such as the widely used CIE L*a*b* color space is recommended (Mancini and Hunt, 2005). This system is very effective for measuring color differences and tracking color changes during processing and storage (Wrolstad *et al*., 2005; Rohlík *et al*., 2010; Haile *et al*. 2013).

Overall, the L* value was increased until the second day of storage and keep stable until the seventh day then its value started decreasing in all samples (Figure 4.29). This is in agreement with results reported by Luciano *et al*. (2009), who studied meats with an initial L* value of 40, which increased to 50 in 14 days of storage and also Esmer, Irkin, Degirmencioglu, and Degirmencioglu (2011) observed an increase of L* values during storage. Contrarily, King, Shackelford, Rodriguez, and Wheeler (2010) found L* values that decreased from 49.7 to 48.6 in 6 days. The L* value was highest in raw beef slice that treated by the extract of chili pepper (L* = 37.77) whereas the lowest value (L* = 23.01) was recorded on the control that treated by water after 14 days storage and its value was significantly lowest than ($p < 0.05$) the L* values of samples which were treated by the extracts of spices. Moreover, the mean L* value for treatments with spice extract was significantly higher than those for control.

Regarding a* and b* values of the spices treatment of raw beef which stored in dark at 4^{o}C for 14 days, both values were similarly increased and got maximum at the second day of storage (Figure 4.30 and 4.31). One explanation for this may be that the loss of mitochondrial respiration during storage resulted in increased oxygen at the surface of the muscle, which might be a

suitable for oxymyoglobin generation (O'Keefe & Hood, 1982), resulting in a higher a* and b* values. Then, these values were decreased to the end of storage period. Again like L* value, the a* and b* values were highest in raw beef slice that treated by the extract of chili pepper (a* = 33.39 and b* = 27.32) at the second day whereas after 14 day the a* and b* values were highest in raw beef slice that treated by the extract of mustard seed (a* = 18.78 and b* = 21.69). In addition, the b* value was significantly greater ($p < 0.001$) than a* value at this storage time.

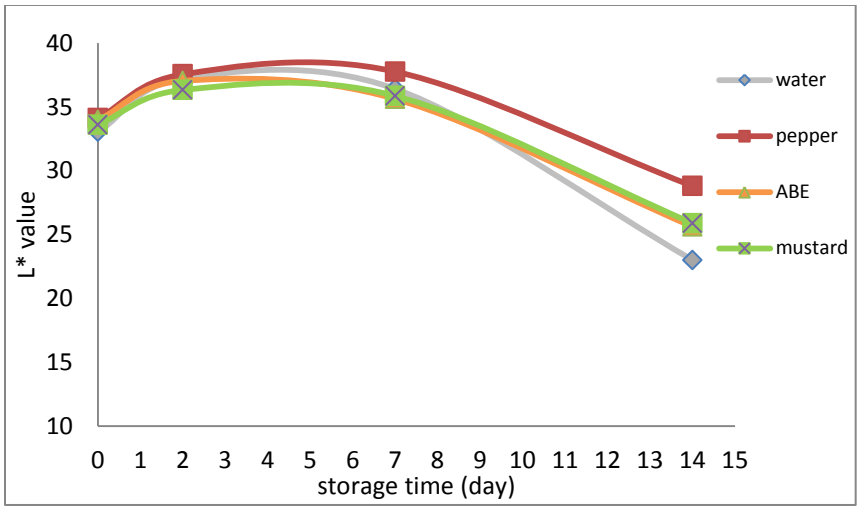


Figure 4.29 L* value of the raw beef slices treated by spices and water/control

Also, like L* value the lowest value (a* = 10.85 and b* = 11.03) was recorded on the control after 14 days storage and its value was significantly lowest than ($p < 0.001$) a* and b* values of samples which were treated by the extracts of spices. This result indicated the antioxidant activity of the spices minimized color changes of raw beef compared to control during dark storage at 4^{o}C. It is also obvious that the redness is higher in samples containing the spice extract due to the capability of this antioxidant to inhibit oxidative reactions, which affects the raw beef pigment. This is in agreement with results reported by Aksu and Kaya (2005) who observed that an interaction (antioxidant addition×storage time) in meat samples produced a faster decrease in a* values in control samples (without antioxidant addition) than in those with added antioxidants. Similar results were observed for irradiated ground beef; the addition of antioxidants was effective in minimizing color changes, at least until the 3rd storage day (Ismail *et al*., 2009).

Overall, the spices extract improved ($P < 0.05$) surface discoloration ratings from day 2 through day 7 of the dark storage period. Whereas, overall color ratings were greater ($P < 0.05$) for raw

beef slices treated with the spices on day 2 of the storage period. This is in agreement with the results reported by Samantha R. *et al*, (2013), who reported that rosemary extract improved beef steaks surface discoloration ratings from day 3 through day 5 of the retail display period. Savanović *et al*., (2014) also reported that the addition of rosemary extract and green tea extract, have a positive effect on color stability of dry-fermented beef sausage during 120 minutes of sausage slices exposure to air.

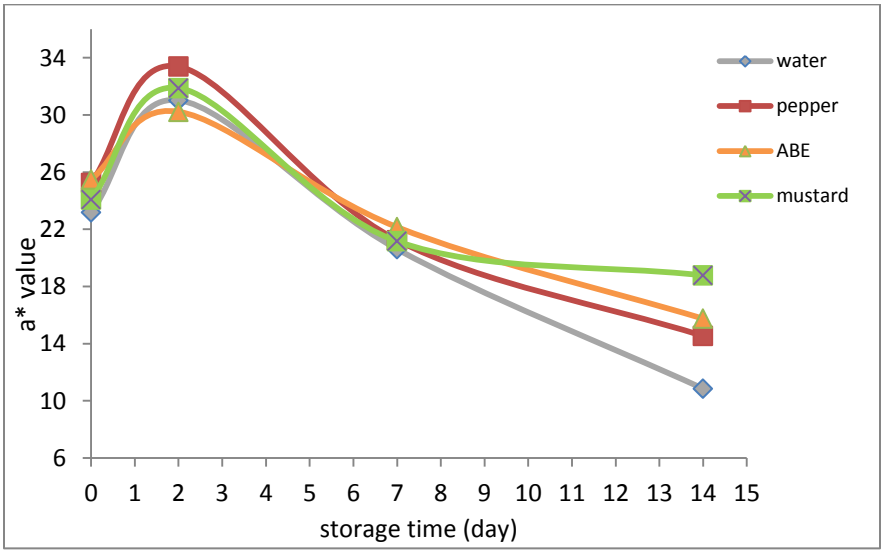


Figure 4.30 CIE a* value of the raw beef slices treated by spices and water/control

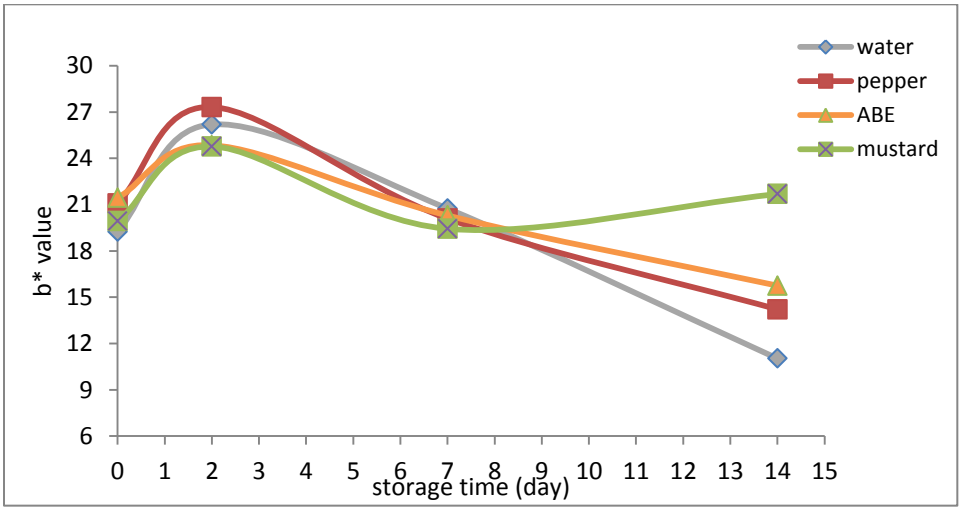


Figure 4.31 CIE b* value of the raw beef slices treated by spices and water/control

4.5.2 Chroma and hue angle, OMb, MMb & pH values of spice treated beef samples

The mean and standard deviation of chroma, hue angle, oxymyoglobin, metmyoglobin and pH values of the raw beef slices treated by spices and water/control which stored in dark refrigerator for two weeks presented in Table 4.5.

The chroma was increased until day 2 and decreased gradually during the remaining days of storage (Table 4.5). Taylor *et al*. (1980) found that meat samples with C* values above 20 were regarded as acceptably bright red by consumer panels. Over all the chroma value was above 20 throughout two weeks storage period for all samples which treated by the spices extract. This indicated that these treatments had good impact on the meat color stability. The hue angle of the raw beef samples was slightly decreased until day 2 and gradually increased during the remaining days of storage (Table 4.5).

The oxymyoglobin content of the raw beef slices treated by mustard extract was relatively the same throughout all storage periods. However, the oxymyoglobin content of the raw beef slices treated by pepper and ABE spices extract and water/control was decreased until day 2 then increased during the rest of storage periods (Table 4.5). Regarding to the pH value, all samples exhibited the increase of pH linearly as the storage time increase.

Table 4.5 Chroma, hue angle, OMb, MMb & pH values of the beef sampls treated by spices

Measurement	Time (day)	Chroma	Hue angle	OMb (%)	MMb (%)	pH
Control by water	0	30.41±1.40	39.29±0.52	55.78±1.11	42.6±1.10	5.47±0.03
	2	40.14±1.31	40.12±0.49	52.13±1.00	46.56±1.01	5.49±0.04
	7	29.97±0.65	42.92±0.39	54.34±1.01	42.27±1.01	5.81±0.01
	14	16.48±0.43	44.35±1.74	56.89±1.06	41.25±0.22	6.56±0.03
Pepper	0	32.87±0.38	39.85±0.51	55.78±1.11	42.64±1.06	5.34±0.02
	2	44.86±1.36	38.61±0.50	55.19±1.06	44.59±0.80	5.51±0.01
	7	28.30±1.30	43.34±0.69	58.74±1.12	42.24±0.89	5.78±0.04
	14	19.63±0.66	45.02±1.27	58.39±0.87	45.22±1.11	6.43±0.04
A.B.E	0	33.32±0.19	40.06±0.08	55.78±1.11	42.64±1.22	5.67±0.02
	2	39.14±0.42	39.42±1.46	53.86±1.76	44.07±1.02	5.88±0.03
	7	30.05±0.39	42.50±0.22	56.75±0.22	42.82±1.08	6.14±0.01
	14	22.28±0.37	45.02±1.47	61.36±1.00	39.73±0.22	7.11±0.03
Mustard	0	30.84±1.14	39.62±0.53	55.78±1.11	42.64±1.11	5.44±0.02
	2	43.37±0.61	37.84±0.63	52.81±0.11	45.85±0.89	5.63±0.04
	7	28.73±0.67	42.56±0.40	55.92±0.67	43.00±0.95	5.81±0.01
	14	28.69±0.47	49.09±1.82	51.77±1.82	44.84±1.11	6.65±0.03

4.5.3 The total color change (ΔE) of spice treated beef samples

Delta E color change also known as the total color change (ΔE) over a selected period of time is a useful parameter to show total color differences over time. The value of delta E (ΔE) represents relative color changes that an observer might report for the materials after treatment or between time periods (AMSA, 2001). Thus, ΔE is more meaningful to represents relative color changes of the treated raw beef steaks between different time of two weeks dark storage periods than the individual L*, a* and b* values. In this study various periods of time were selected and compared to for total color change of raw beef steaks.

The total color difference (ΔE) increased at day 2 and decreased until day 7 then again increased until the end of storage period (day 14) as shown in Figure 4.32. The mean of total color difference (ΔE) of samples those treated by spices extract were significantly lower ($p<0.001$) than control at the end of storage day. The treated raw beef steaks by mustard seed extract was significantly lowest ($p<0.001$) than pepper and ABE at the end of dark storage period (day 14), whereas no significant change ($p>0.05$) between pepper and ABE at this time. From this result we noticed that mustard has more potential as preservative for raw meat. Generally, the total color change of raw beef samples those treated by spices extract were lower than control. Similar results were observed for irradiated ground beef; the addition of antioxidants was effective in minimizing color changes, at least until the 3rd storage day (Ismail, Lee, Ko, & Ahn, 2009).

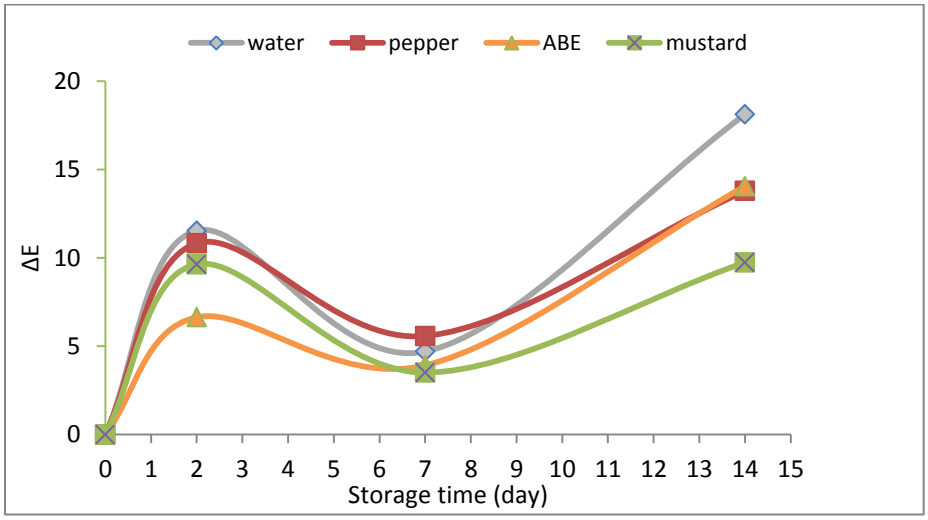


Figure 4.32 Total color change (ΔE) value of the raw beef slices treated by spices and water/control

4.5.4 Lipid oxidation or TBARS values of spice treated beef samples

During the present study the samples were stored maximum for two weeks in dark storage. Gutensperger and Escher (1994) suggested determination of TBARS should be done during the first two weeks of storage as during advancing oxidation the rate of MDA decomposition surpass that of its formation.

The rate of lipid oxidation can be effectively controlled or minimized, by natural antioxidants addition (Doolaege et al., 2012; Georgantelis *et al*., 2007; Mata *et al*., 2007; Grujić *et al*., 2009).The addition of Ethiopian spices extract to the raw beef steak had a positive effect on the decrease in the TBARS values of the raw beef. The mean TBARS concentration (mg/kg meat) was lower for the treatments with spices extract than control (0.38 vs. 0.44 mg MDA/kg, $P<0.05$). In spices extract, the rate of TBARS formation was lower when compared to samples without spices extract overall 14 days. This showed the spices antioxidant efficiency to retard lipid oxidation of raw beef. Ahn *et al*. (2007) also report that the use of natural antioxidants from grape seed can effectively reduce lipid oxidation. The antioxidant activity of spice extracts has been associated with the presence of phenolic compounds that break free radical chain reactions by hydrogen atom donation (Basaga, Tekkaya, & Acikel, 1997).

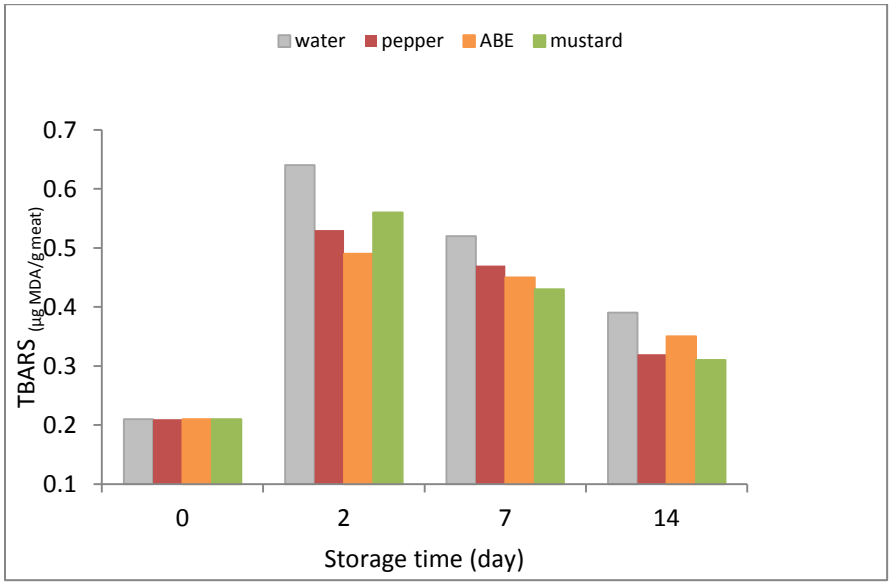


Figure 4.33 TBARS value of the raw beef slices treated by spices and water/control

TBARS values showed significant ($P<0.05$) difference with in the samples storage periods. A TBARS value from 0 days dark storage to second day has increased. This increase at the early period of storage is in agreement with Yu *et al*. (2002) who reported an increase in TBARS value as storage time increase during 3 days of fish oil. However, after day 2 TBARS value was decreased until the end of storage period (Figure 4.33). This decline in TBARS value as dark storage day's increase is in agreement with haile *et al*. (2013) work on dark storage of cooked ham and on dark storage of cooked *pâtés* by Gokalp *et al*. (1983). In contrast, this decline in TBARS value in disagreement with Gonzalez et al. (1992) who reported an increase in TBARS value as storage time increase during 12 days storage of cooked turkey. The most probable reason for this decline in TBARS value as dark storage duration increase could be either due to hydroperoxide decomposition rate being higher than the rate at which it is formed (Georgantelis *et al*., 2007) or the prolonged storage allow the interaction between MDA and spices extract so that make the MDA bound. This interference of other compounds was also reported by Igene and Pearson (1979); Gokalp *et al*. (1983); and Shahidi (1998). The other probable reason for the decrease in TBARS value as dark storage duration increase may be due to the instability or transitory nature of MDA (secondary product from lipid peroxidation) that react with TBA to generate a red color (Wang *et al.* 2002) and underestimate the expected TBARS number. Finaly, secondary oxidative compounds are not only responsible for quality deterioration of precooked meat, but ingested oxidative products can also be a risk for human health (Aikawa and chikuni, 1988). Thus, enhanced oxidative stability is needed for maintaining the safety and quality of raw meat.

4.5.5 Total aerobically count (TAC) of spice treated beef samples

TAC is important indicators of meat quality with respect to microbial growth on stored meat. Bacterial activity is another factor in pigment changes in fresh meat. Meat spoilage was also related to discoloration of meat (Robach and Costelow, 1961). Maintaining the low microbial populations will slow meat discoloration since one effect of bacteria is reducing oxygen tension at the meat surface tissue. Butler et al., (1953) stated that the rate of MMb was greatest during the bacterial logarithmic growth phase. In contrast, Faustman et al., (1990) concluded that color change was not necessarily related to the level of microorganisms in meat.

Results pertaining to the microbial growth on stored raw beef samples treated by the spices extracts were presented in Figure 4.34. It has been found that the spices extract exhibited maximum inhibitory effect than the sample treated without spice extract. Initial microbial count (total aerobic count) of raw beef was around 5.4 log CFU/g. As expected, during storage, bacterial populations increased ($P<0.05$) for all types of samples as the storage days increased. The TAC of samples treated by spices extract were significantly lower ($p<0.05$) than control throughout 14 days dark storage period, whereas no significant difference ($p>0.05$) between spices. The TAC of control samples increased to a maximum of 10.12±0.23 on the day 14, but the TAC of samples treated with spices extracts was lower than this maximum. This was in agreement with Franco D. et al,. (2012) they reported natural antioxidant extracts and MAP maintained low total aerobic counts in beef steaks until the 21 day. Similar reports were also made in ginger extract treated sheep meat (Mendiratta *et al.* 2000) and buffalo meat (Ziauddin *el al. 1995),* at refrigerated storage. The inhibitory effect of hot peppers *(capsicum annuum* L.) on the growth of *Aspergi1lus niger* and *A. flavus* was also observed by Yin and Cheng (1998).

In the current study, all spices extract performed equally well at preventing the growth of aerobic bacteria in raw beef (Figure 4.34) and they were able to preserve fresh color as compared to the control. __Free" iron has been demonstrated to promote Mb oxidation, both directly (Allen & Cornforth, 2006) and indirectly via lipid oxidation (Alderton *et al*., 2003; Kanner, Harel, & Hazan, 1986). One potential source of iron in fresh meats is iron liberated from iron-containing proteins broken down by the growing bacteria. It is possible that these spices compounds are able to prevent browning by chelating the iron released in this manner.

Results of the present investigation indicates that, all the spice extracts used for the study such as chili pepper, ABE and mustard seed possesses the antimicrobial property. Most probably they have different types of important phenolic compounds. The composition, structure and functional groups in these phenolic compounds play an important role in determining their antimicrobial activity. According to Eklund, (1985) there is a relationship between the chemical structures of phenolic compounds and the antimicrobial activity. Generally, the extent of the inhibitory effect of the phenolic compounds could be attributed to the presence of aromatic nucleus containing polar functional groups. The phenolic compounds present in the spices extract may interfere with energy metabolism, disrupt electron transport or nutrient uptake or affect nucleic acid synthesis,

ultimately resulting in cellular death. It is well known that the phenolic OH group is quite reactive and easily forms hydrogen bonds with active sites of target enzyme. In fact, the mode of action of phenolic compounds is generally thought to involve interference with functions of the cytoplasmic membrane, including proton motive forces and active transport (Freese *et al.,* 1973). This suggested that the phenolic compounds of the selected spices extract might significantly contribute to their antibacterial activity. The present study also demonstrated that these extracts

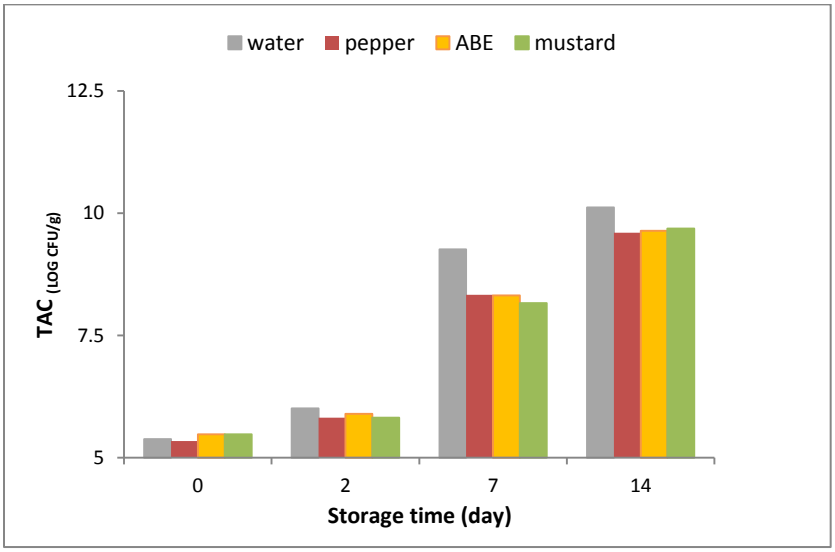


Figure 4.34 TAC value of the raw beef slices treated by spices and water/control

contained high levels of phenolics and possessed strong antimicrobial activity. They could be a potential source for inhibitory substances against some foodborne pathogens as well as antioxidant agents. Therefore, those spices extract which selected in this study may be candidates for future studies of synergism, compatibility, and activity in foods or food-processing systems and mechanisms of activity against specific meat born pathogens.

In conclusion, in this experiment we found that the natural antioxidants such as pepper, African bird´s eye and mustard seed extract were found to exhibit potent lipid antioxidant activity in raw beef. All these spices extract enhanced color stability in raw beef especially stored until one week in dark refrigerator. In addition, all spices extract performed equally well at preventing the growth of aerobic bacteria in raw beef and they were able to preserve fresh color as compared to the control.

4.6. Effect of various dosage of Ethiopian spices extract on color and oxidation stability of raw beef steaks during exposure to incandescent display light

4.6.1 The results of color parameters for beef samples

In this experiment we tried to determine, in one hand, the effect of dosages of Ethiopian spices on color and lipid oxidation stability; on the other hand, the effect of those spices extract on color and lipid oxidation stability of raw beef samples during the exposure of incandescent display light for 20 hrs.

Unlike the dark refrigerator storage experiment, the CIE L*, a* and b* values of the raw beef samples treated by the 0.5 ml spices extract and displayed under incandescent light at room temperature were not exhibited a significant difference with control samples. However, the 1ml spice extract showed relatively higher L*, a* and b* values (Figure 35 – 37) than the control sample especially at end of display time. In addition, the total color change (ΔE) value of the raw beef samples treated by the 0.5 ml spices extract was relatively lower than the control sample at 20 hrs, except mustard seed extracts (Table 4.6). Although, 1ml dosage of the spice extract showed lower ΔE than control in all spice extracts at 20 hrs. Moreover, the total color change (ΔE) was dosage dependent, as the dosage of the spices extract increased the ΔE value decreased in all display hours.

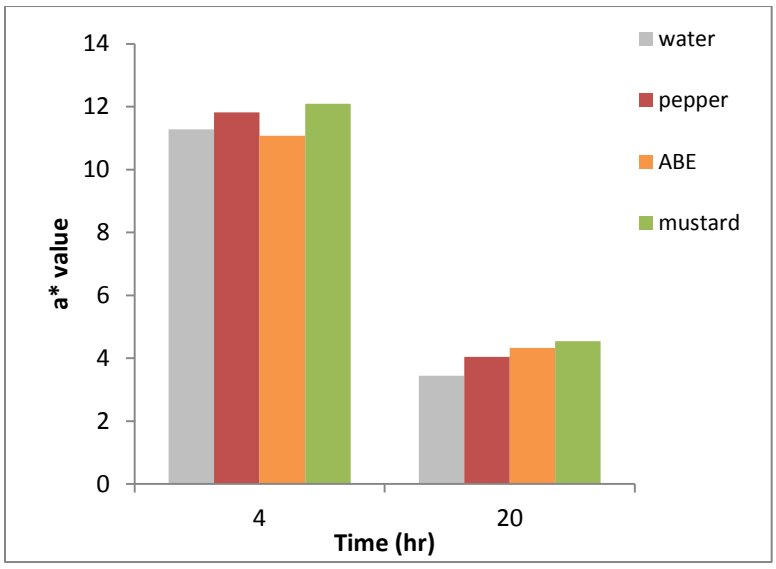


Figure 4.35 CIE a* value for the beef steaks treated by 1ml spices

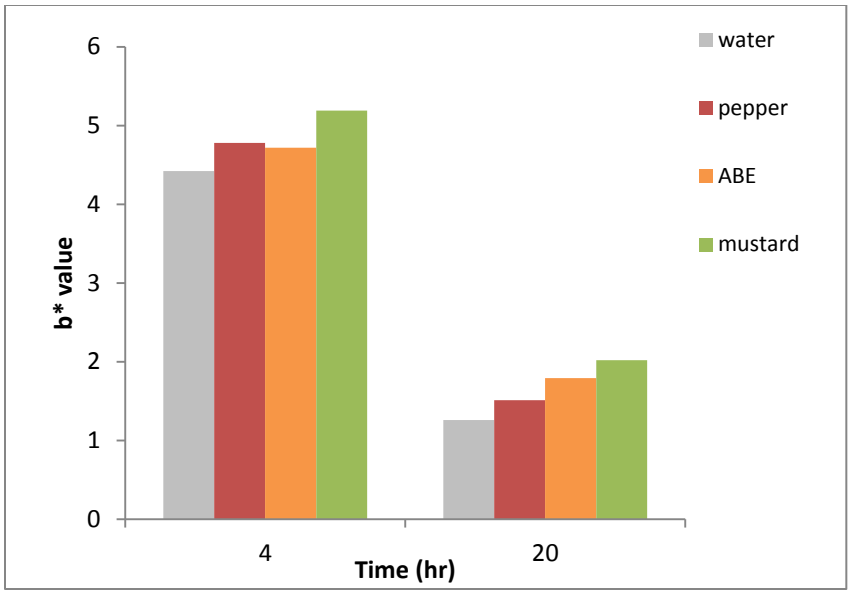


Figure 4.36 CIE b* value for the beef steaks treated by 1ml spices

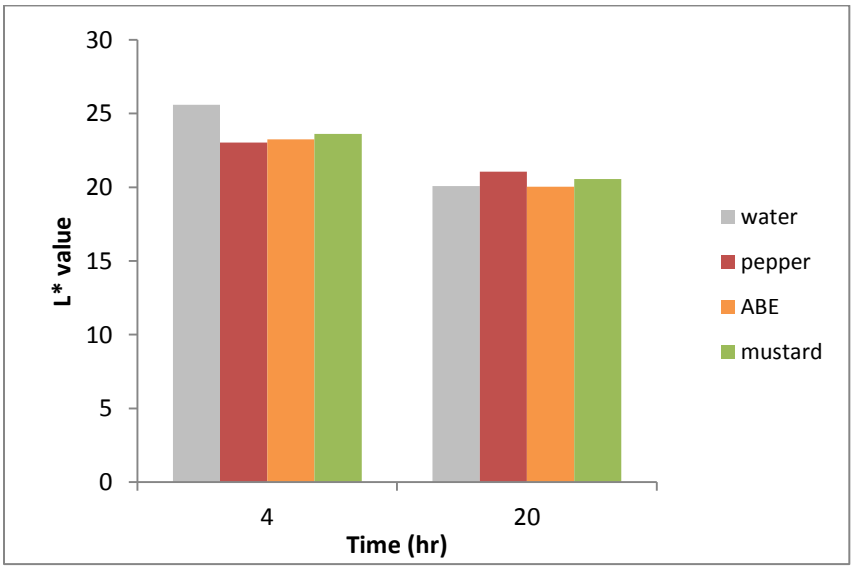


Figure 4.37 CIE L* values for the beef steaks treated by 1ml spices

In this experiment, the values of chroma and hue angle of the raw beef slices treated by 0.5 and 1ml spices decreased as the display time increased. Although, no significant differences in the values of chroma and hue angle exhibited between the raw beef slices treated by 0.5 and 1ml spices as well as among the spices and control (Table 4.6).

Table 4.6 The chroma, hue angle & ΔE results of the raw beef slices treated by 0.5 and 1ml spices

	The beef steaks treated by 0.5 ml spices				The beef steaks treated by 1ml spices		
Types of spice	Time (hr)	C*	Hue (H^o)	ΔE	C*	Hue (H^o)	ΔE
Control by water	0	23.65±1.21	40.66±0.22	0.00	23.75±1.07	40.03±1.00	0.00
	4	13.51±0.29	23.60±0.48	15.44±0.55	12.12±0.44	21.37±1.00	15.6±0.67
	20	4.54±0.39	23.52±2.37	24.05±1.84	3.67±0.18	20.21±2.07	24.88±0.26
Pepper	0	22.50±0.37	42.22±0.43	0.00	23.22±0.37	40.67±0.23	0.00
	4	11.36±0.19	21.81±0.79	17.29±0.57	12.74±0.20	22.04±0.43	15.92±0.13
	20	4.22±0.22	27.15±3.46	22.63±1.05	4.32±0.08	20.60±0.84	22.98±0.36
A.B.E	0	22.39±0.20	40.54±0.36	0.00	22.41±0.62	39.53±0.58	0.00
	4	11.68±0.23	23.00±0.74	15.48±0.55	12.04±0.90	23.05±0.20	14.12±0.88
	20	4.16±0.29	25.69±4.69	22.06±0.53	4.69±0.13	22.42±0.13	21.39±0.94
Mustard	0	23.42±0.37	41.42±0.04	0.00	23.68±0.26	40.72±0.08	0.00
	4	12.57±0.55	23.52±0.82	17.67±0.48	13.17±0.28	32.20±0.73	14.78±0.57
	20	4.11±0.73	24.95±0.73	25.88±0.58	4.98±0.28	23.79±4.78	22.43±0.36

4.6.2 Analysis of Lipid oxidation or TBARS values

The mean and standard deviation of TBARS, oxymyoglobin, metmyoglobin and pH values of the raw beef slices treated by 0.5 and 1ml spices and water/control presented in Table 4.7. The TBARS value of the spice extract treated raw beef samples was significantly lower ($p < 0.05$) than the control samples which treated by only water, in both 0.5 and 1ml dosage (Table 4.7). However, oxidation inhibition power of the spices was not dependent on the dosage of treatment, except the mustard seed extract that showed lower TBARS value in 1ml than 0.5ml dosage treatment, in all display hours. From this result we noticed that the oxidation of raw beef samples containing spice extracts was relatively stable under the incandescent light exposure at room temperature. In the case of the control sample, that the raw beef sample without any spice extract was relatively not stable under the light exposure. This is in agreement with the work of Rohlík B., Pipek P. & Pánek J. (2010).

Like, the values of chroma and hue angle, no significant differences and dosage of spices dependence exhibited in OMb, MMb and pH values of the raw beef slices treated by 0.5 and 1ml spices as well as among the spices and control.

Table 4.7 OMb, MMb, TBARS & pH values of raw beef treated by 0.5 and 1ml spices extracts

Types of spice	Time (hr)	The beef steaks treated by 0.5 ml spices				The beef steaks treated by 1ml spices			
		pH	OMb(%)	MMb(%)	TBARS (µgMDA/g meat)	pH	OMb(%)	MMb(%)	TBARS (µgMDA/g meat)
Control by water	0	5.80±0.01	52.21±0.94	44.29±0.52	0.67±0.01	5.80±0.01	50.84±0.97	44.83±0.54	0.67±0.01
	4	5.83±0.01	65.26±1.12	50.09±0.64	0.91±0.02	5.82±0.01	66.24±1.05	26.70±1.98	0.90±0.02
	20	5.91±0.02	59.56±0.88	38.91±0.50	1.72±0.02	5.87±0.01	58.40±0.74	39.38±0.97	1.70±0.02
Pepper	0	5.80±0.01	53.36±0.91	43.66±0.58	0.67±0.01	5.80±0.01	53.10±0.99	44.34±0.74	0.67±0.01
	4	5.83±0.01	52.81±0.93	39.81±0.63	0.77±0.02	5.83±0.01	51.16±1.32	40.69±1.21	0.79±0.02
	20	5.89±0.01	69.53±1.05	47.26±0.58	1.31±0.03	5.89±0.01	58.73±1.02	39.09±0.90	1.45±0.04
A.B.E	0	5.81±0.01	53.16±0.96	43.97±0.51	0.67±0.01	5.81±0.01	53.14±0.87	44.44±0.69	0.67±0.01
	4	5.80±0.01	52.7±1.21	39.78±0.91	0.78±0.03	5.82±0.01	53.46±1.15	39.77±0.81	0.79±0.02
	20	5.78±0.02	58.83±0.79	39.19±0.77	1.39±0.02	5.87±0.01	58.38±0.88	39.00±1.03	1.43±0.03
Mustard	0	5.79±0.01	54.25±0.97	43.75±0.52	0.67±0.01	5.79±0.01	52.59±0.91	45.02±0.66	0.67±0.01
	4	5.80±0.01	51.79±0.83	41.02±0.49	0.78±0.01	5.83±0.01	53.00±1.23	41.02±1.27	0.75±0.02
	20	5.81±0.01	58.29±1.10	39.12±0.89	1.37±0.04	5.93±0.01	57.87±0.97	39.00±0.94	1.27±0.03

4.7 Antioxidant activity and total polyphenol content of Ethiopian spices

4.7.1 Analysis of the antioxidant activity of Ethiopian spices by different method

Numerous methods and modifications have been proposed to evaluate antioxidant activity and to explain how antioxidants function. Of these, total antioxidant activity, reducing power, DPPH assay, metal chelating, active oxygen species such as H_2O_2, $O^{-2\cdot}$, and $OH^{\cdot}$ quenching assay are most commonly used for the evaluation of antioxidant activities of extracts (Chang *et al*.,, 2002).

4.7.1.1 DPPH free radical scavenging activity

In this study, the radical scavenging of spices extracts was tested using the ‗stable' free radical, DPPH. A freshly prepared DPPH solution exhibits a deep purple color with absorption maximum at 517 nm. The purple color generally fades/disappears when an antioxidant is present in the medium (Gursoy *et al*., 2010).

First, based upon literature review and preliminary work (Figure 4.38), the appropriate solvent for extraction of the spices was selected to be methanol/water (4:1). In addition, the concentration required to scavenge DPPH completely was optimized with pre-tests by tracking the purple to yellow color change of DPPH with increased concentration for each samples. This had been crucial part of the study since setting a much lower or much higher concentration range

might not indicate the difference in scavenging power between samples clearly and more so, the IC_{50} (the concentration required to scavenging 50% of the radical) might be skipped. It was after this optimization process that 12-416µg/ml was chosen and prepared by diluting the stock solution (20mg/ml) with methanol/water (4:1). This concentration optimization process was applied in similar way for reducing, total phenols and total flavonoids assays.

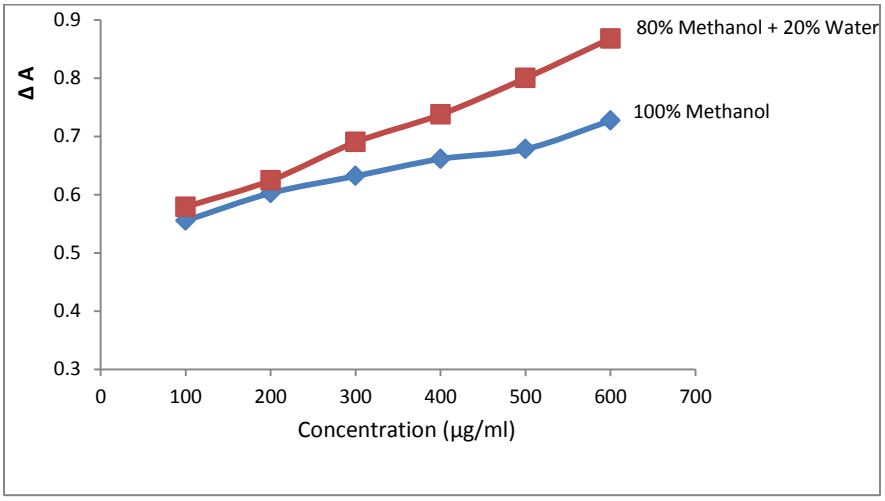


Figure 4.38 Comparison of extraction solvents

Based upon the results of antioxidant presented in Table 4.8, the order of DPPH free radical scavenging activity of spices extracts can be seen as Mixture of spices > mustard > ABE > pepper.

Table 4.8 DPPH free radical scavenging activity

Types of spice	mg trolox equivalent /g DW by DPPH method	mg ascorbic acid equ./g DW by DPPH method
Chili pepper	1.36± 0.02	1.64±0.025
African bird‘s eye	1.4±0.01	1.71±0.01
Mustard seed	1.42±0.02	1.72±0.025
Mixture of spices	1.46±0.03	1.77±0.02

The various antioxidant mechanisms of the spices extract may be attributed to their effectiveness as good scavenger of free radicals, which can calculated from the IC_{50} of their assays. Table 4.9 presents the IC_{50} values for scavenging of free radicals obtained from each spice extract and controls.

Table 4.9 DPPH - IC_{50} values of the spices and standards

Chili pepper	**African bird's eye**	**Mustard seed**	**Ascorbic acid**	**Trolox**
186µg/ml	172µg/ml	85µg/ml	15µg/ml	12µg/ml

Mustard seed, African bird's eye and chili pepper showed IC_{50} value of 85µg/ml, 172µg/ml and 186µg/ml respectively; whereas the standard antioxidants ascorbic acid and trolox showed IC_{50} values 15µg/ml and12µg/ml respectively, in DPPH method. In the work of Badrul *et al* (2011) mustard *(Brassica nigra)* showed IC_{50} value of 63.09µg/ml and the standard antioxidant ascorbic acid showed IC_{50} value 14.45 µg/ml in DPPH method. The ratio of the result was relatively in agreement with our result. Loizzo M. (2011) also reported Ethiopian spice blend *berbere* (*capsicum annuum*) had DPPH scavenging activity with a concentration giving 50% inhibition (IC_{50}) value of 34.8 µg/ml and Ascorbic acid 5.0 µg/ml. The synthetic antioxidant (ascorbic acid and trolox) which was used as positive controls had a superior performance with the least IC_{50} (Table 4.9). Moreover, the radical scavenging activities of spices extracts were estimated by

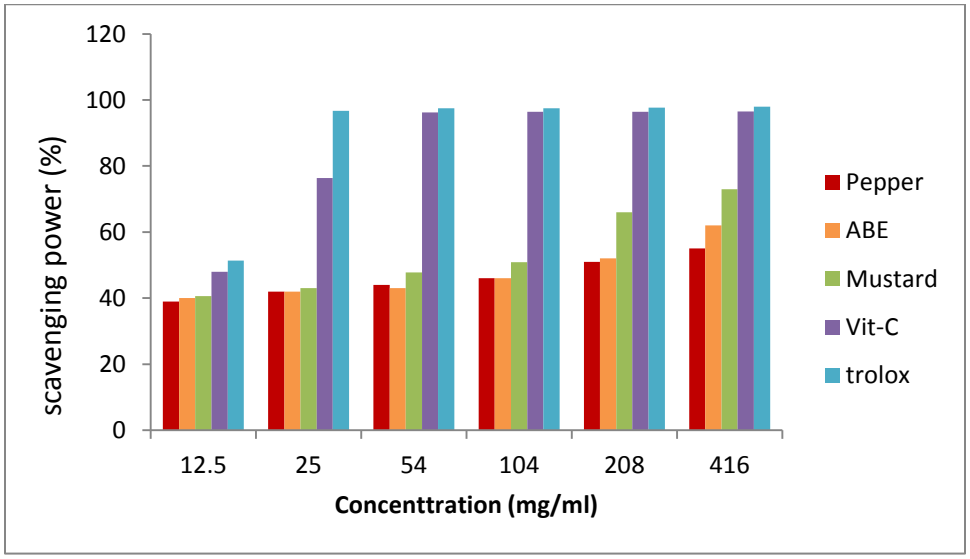


Figure 4.39 The radical scavenging activities of spices and standards by concentration

comparing the scavenging ability of DPPH radical with Vitamin C and trolox. Figure 4.39 depicts at very small concentration the DPPH radical scavenging activities of the spices relatively comparable with the standards. However, the DPPH radical scavenging activities of these extracts were less than the standards at higher concentrations.

4.7.1.2 TEAC /ABTS assay

This method is based on the ability of antioxidant molecules to quench the long-lived $ABTS^{\bullet+}$, a blue-green chromophore with characteristic absorption at 734nm, compared with that of Trolox, a water-soluble vitamin E analog. The addition of antioxidants to the preformed radical cation reduces it to ABTS, determining a decolorization.

The TEAC value of spices extracts were statistically no significant difference between pepper and ABE but they have significant difference with mustard seeds and mixture of spices (Table 4.10). In addition, mixture of the three spices exhibited highest TEAC value and synergetic effect. Romaric et al., (2011) reported that TEAC (1.6 ± 0.1µmol trolox/g) for *Capsicum frutescens* and for sweet pepper (green) (*Capsicum annuum)* TEAC (µmol trolox/g 0.6 ± 0.0); Loizzo M. (2011) also found 2.2 ± 0.7 TEAC value for Ethiopian spice blend *berbere* (*capsicum annuum*); which are lower when compared to our results..

Table 4.10 The TEAC value of spices extract

Samples	TEAC (mg TE/g D.W)	TEAC (m mol TE/g D.W)
Chili pepper	5.75±0.07	2.30±0.03
African bird‘s eye	5.75±0.04	2.30±0.02
Mustard seed	5.22±0.09	2.09±0.04
Mixture of spices	5.80±0.01	2.32±0.00

4.7.1.3 Reducing power assay

Assay of reducing power was based on the reduction of Fe^{3+}/ferricyanide complex to the ferrous form in presence of reductants (antioxidants).The yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of each compound (Ferreira *et al*., 2007). The Fe^{2+} was then monitored by measuring the formation of Perl‘s Prussian blue at 700 nm (Oyaizu 1986).

The reducing power of a compound is used to evaluate its ability to donate electrons (Dorman *et al.,* 2003) and may serve as a significant indictor of its potential antioxidant activity (Meir *et al*., 1995). Reducing powers of chili pepper, ABE, mustard seeds and mixture of spices extracts were 9.62±0.72, 9.37±0.23, 10.02±0.57 and 11.08±0.79 mg ascorbic acid equivalent /g , respectively and exhibited the following order: mixture of spices > mustard seed > pepper > ABE (Table 4.11). The antioxidant activity of the mixture of spices extracts also exhibited synergetic effect in this assay. Moreover, the reducing power of the spices extracts increased with increasing concentration as shown in Figure 4.40.

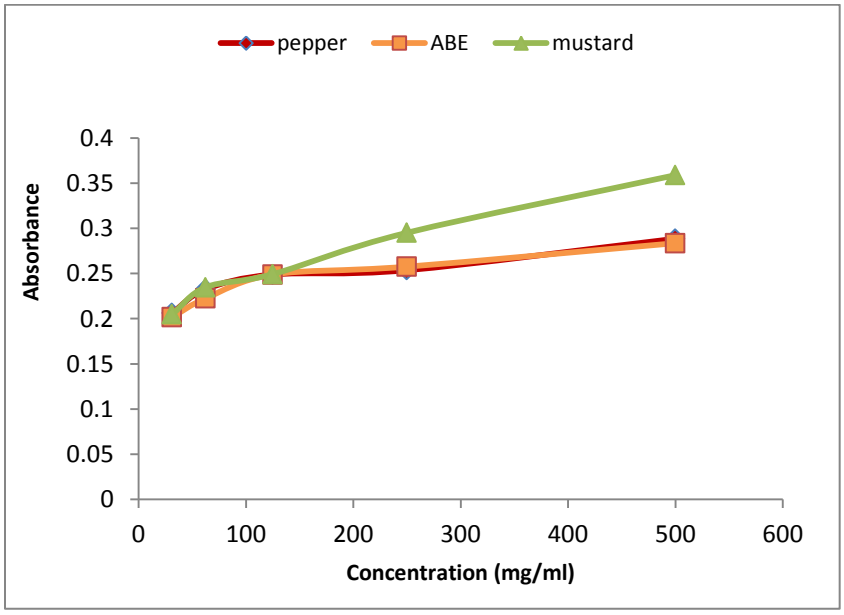


Figure 4.40 Reducing power by concentration of spices

Table 4.11 Reducing powers of the spices extract

samples	mg Ascorbic acid equ. /g DW
Chili pepper	9.62±0.72
African bird‘s eye	9.37±0.23
Mustard seed	10.02±0.57
Mixture of spices	11.08±0.79

4.7.2 Total phenolic and total flavonoid contents of spices extracts

The radical scavenging properties of samples could be due to the present of phenolics compounds (Yahia M., Gutiérrez-Orozco F. & Arvizu-de L.C., 2011). Phenolics are characterized by having at least one aromatic ring with one or more hydroxyl groups attached. Phenolic compounds such as flavonoids, phenolic acids, and tannins are considered to be major contributors to the antioxidant capacity of plants. These antioxidants also possess diverse biological activities, such as anti-inflammatory, antiatherosclerotic, and anti-carcinogenic activities. These activities may be related to their antioxidant activity (Chung *et al.,*1998). Moreover, it had been reported that the antioxidant activity of plant materials was well correlated with the content of their phenolic compounds (Velioglu *et al.,* 1998). Study by Miller and Rice-Evans (1997) also showed that 80% of the total antioxidant activity was contributed by phenolic compounds. Therefore, in this study the total phenolic content of the spices extracts were determined based on the phenolic substances which were oxidized by Folin-Ciocalteu reagent. Folin- Ciocalteu method allows the estimation of the total phenolic content that included the flavonoids, anthocyanins and nonflavonoid phenolic compounds.

Total phenolic and total flavonoid contents of spices extracts were examined and are presented in Table 4.12. A significant difference of total phenolic and total flavonoid contents were detected among spices extracts ($p < 0.05$). The chili pepper (*capsicum annuum*) exhibited the highest total phenolic and total flavonoid contents, 16.81±0.25 mg galic acid equivalent per gram of dry weight and 8.74±0.91 mg catichin equivalent per gram of dry weight respectively.

Table 4.12 Total phenolic and total flavonoid contents of spices extracts

samples	Total phenol content (mg galic acid equ./g DW)	Total flavonoid content (mg catichin equ./g DW)
chili pepper	16.81±0.25	8.74±0.91
African bird's eye	14.17±0.62	4.30±0.61
mustard seed	10.03±0.52	0.36±0.26

The total phenolic and total flavonoid contents of mustard seed extracts were lowest comparing to the two spices, 10.03±0.52mg galic acid equivalent per gram of dry weight and 0.36±0.26mg catichin equivalent per gram of dry weight respectively. Total phenolic and total flavonoid contents of spices extracts exhibited the following order: chili pepper > African bird‘s eye > mustard seed. Badrul *et al* (2011) reported 6.67mg/g equivalent of galic acid and 2.04mg/g of quercetin, respectively. Deepaa *et al*., (2007) also reported for different varaities of red peppers varied from 3.23mg/g to 8.52mg/g equivalent of galic acid. In the work of Naima *et al*., (2013) the pepper total phenol contents values ranged from 6.75 to 13.60mg/100g DW gallic acid equivalent. For *Capsicum frutescens* Romaric *et al*., (2011) reported that 3.34 ± 16.2mg GAE/g fresh weight for total phenol contents. Which are lower compared to our results.

The results obtained in the present study clearly demonstrate the interesting antioxidant properties of Ethiopian spices. Both spices extracts showed the promising free radical scavenging effects as evaluated by DPPH, TEAC/ABTS and Fe reducing tests; both these assays have been frequently used to assess antioxidant activity. Moreover, the spices extract were characterized by the high content of phenols and flavonoids, plant secondary metabolites known for their antioxidant properties. In conclusion, the data showed that these spices may be used not only for raw beef consumption but also as a source of healthy compounds for the development of dietary supplements and to protect against oxidative stress as well as for preservation of raw meat.

To understand the relationship between, antioxidant activity and total phenol and flavonoid content, the correlation coefficient was calculated. The correlation analysis showed a positive relationship between total phenol and flavonoid content and antioxidant activity as measured by TEAC/ ABTS assay. However, the correlation analysis showed a weak relationship between total phenol and flavonoid content and antioxidant activity as measured by DPPH and Fe reducing power assays. The literature reports concerning the relationship of phenols and antioxidant activity are contradictory; some have observed a positive relationship (Wang and Stretch, 2001; Howard, *et al*., 2003), while others report a weak one (Imeh & Khokhar, 2002). The weak correlation in our results could be attributed to variations in antioxidant composition among spices. Such differences could easily be explained on the basis of compositional diversity and presence of less polar analytes and their interactions with polar compounds, influencing

antioxidant activity. Another probable reason may be the Folin's test used overestimates the content of phenolic substances in capsicum, since the reducing agents such as ascorbic acid present in the capsicum can interfere in this assay.

In conclusion, consuming of Ethiopian spices with meat or other foods is advisable because they are rich in bioactive phenolics with high antioxidant activities.

4.8 Assessment of the effects of germination on antioxidant activity and polyphenol content of mustard seeds.

Germination of legume seeds is one of the processing methods for increasing nutritive value (Augustin & Klein, 1989; Ghorpade & Kadam, 1989) and health qualities (Deshpande *et al*., 2000) as sprouts. This process is simple and inexpensive and different seeds have been germinated for human consumption such as legumes (soybean, lentils, beans, chickpeas, peas), cereals (rye, wheat, barley, oats) and, recently, seeds of some other vegetables (alfalfa, radish, mustards, brassica) (Frias *et al*., 2005). Therefore, in this experiment we tried to assess the one and three days germinated mustard seeds on antioxidant activity and polyphenol content and compared with the dormant/raw mustard seeds.

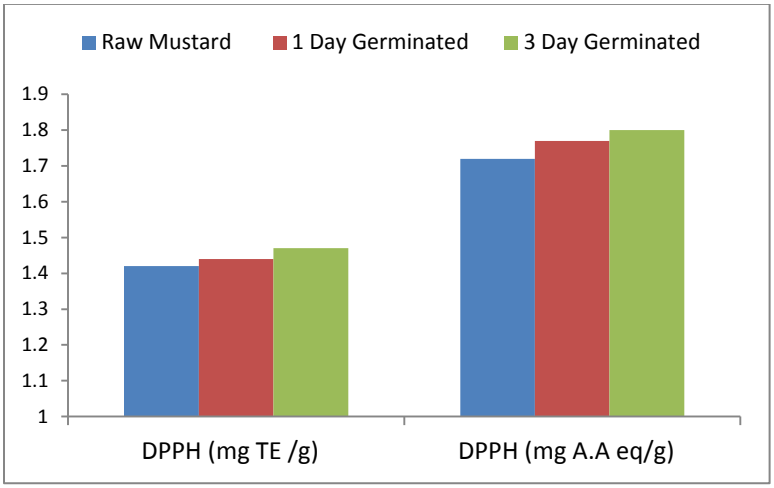


Figure 4.41 DPPH values of germinated mustard seeds

The antioxidant activity of 3days and 1day germinated mustard seeds was significantly higher ($p<0.05$) than the raw mustard seeds in all antioxidant activity assays. Among 3days and 1day germinated mustard seeds, the antioxidant activity of 3days germinated mustard seeds was

higher than1day germinated mustard seeds in all antioxidant activity assays, but statistically not significant ($p>0.05$) difference between them.

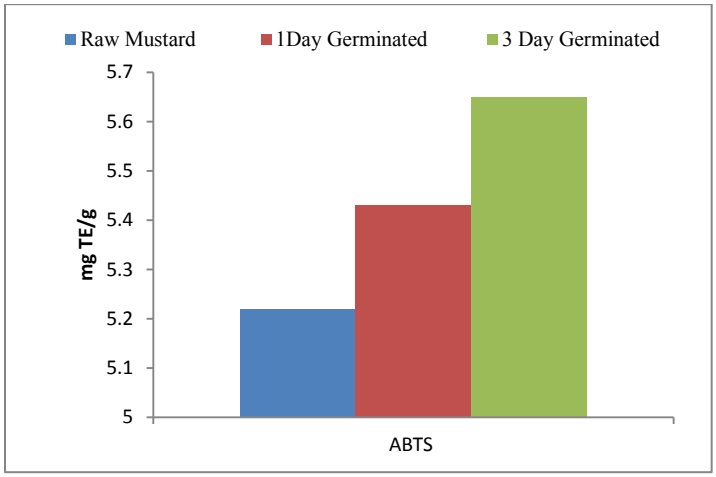


Figure 4.42 TEAC valur of germinated mustard seed

Based upon the results of antioxidant showed in Figure 4.41 - 4.43, the order of antioxidant activity of all assays on germinated mustard seeds can be seen as 3days germinated > 1day germinated > raw mustard seeds. This indicates that phenolic compounds with a higher number of reactive hydroxyl groups have been synthesized during water imbibition and germination. In addition a higher antioxidant activity on the germinated seeds could suggest during water imbibition and germination that the first steps the seed response takes after breaking dormancy is to synthesize phenolic compounds with higher than normal antioxidant activity so as to protect hypocotyl growth against oxidative reactions triggered by environmental factors. This study shows that germinated mustard seeds are an excellent source of dietary phenolic antioxidants.

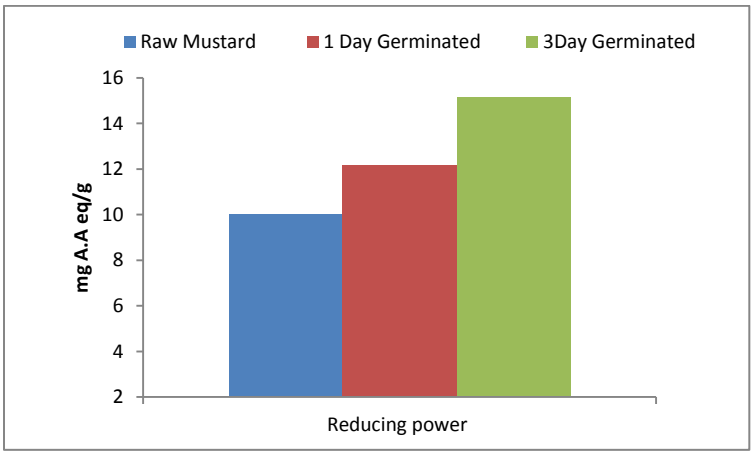


Figure 4.43 Reducing power of germinated mustard seed

Table 4.13 Total phenolic and total flavonoid contents of germinated mustard seed extracts

Samples	**Total phenol (mg GAE/g)**	**Increment**	**Total flavonoid (mg catichin eq./g)**	**Increment**
Raw Mustard Seed	10.03±0.52		0.36±0.26	
1 Day Germinated	11.62±0.06	16(%)	2.16±0.50	500(%)
3 Day Germinated	12.39±0.91	24(%)	3.05±1.10	747(%)

The total phenol content of the 3days germinated mustard seed was increased by 24% and 1day germinated mustard seed was also increased by 16%. The total flavonoid content of the 3days germinated mustard seed was drastically increased by 747% and 1day germinated mustard seed was also increased by 500% (Table 4.13). This indicated germination process is one of effective method for accumulation of flavonoid in mustard seed. The most widespread and diverse group of polyphenols in *Brassica* species are the flavonoids (mainly flavonols but also anthocyanins) and the hydroxycinnamic acids (Cartea *et al*., 2011). The antioxidant ability of flavonoids and phenolic acids is related to the number and position of hydroxyl groups in the molecule; an increase in the number of hydroxyl groups leads to a higher antioxidant activity. Compounds with three hydroxyl groups on the phenyl ring of phenolic acids or the B ring of flavonoids have a high antioxidant activity. The loss of one hydroxyl group decreases activity slightly, whereas the loss of two hydroxyl groups significantly decreases the activity (Cartea *et al*., 2011).

The values of total phenol, total flavonoid and antioxidant activity for a raw or dormant seed would indicate the amount of phenolic antioxidants synthesized while the seed was attached to the parent plant, while for germinated mustard seed, the total phenol, total flavonoid and antioxidant activity values indicate synthesis of phenolic antioxidants after dormancy during water imbibition and germination.

The phenols synthesized during seed germination could help to obtain enhanced levels of phenols and antioxidant activity. The polyphenols synthesized throughout the germination process could serve as protection against environmental factors and for structure-giving (Bolívar *et al*., 2010).

In general, accumulated total phenol and flavonoid content on the germinated seeds showed a consistent trend as shown in (Table 4.13), where 3 days germinated > 1day germinated > raw mustard seeds. These increases in phenolic content and antioxidant activity show potentially important roles of phenolics during seed germination. These results also confirm that phenolic compounds are responsible for most of the antioxidant properties of the sample extracts, thus reinforcing the validity of expressing antioxidant activity on a phenolic basis. Therefore, the phenols synthesized during mustard germination could help to obtain enhanced levels of phenols and antioxidant activity resulting in their improved nutraceutical properties.

4.9 Determination of the aroma properties of the spices by SPME - GC-MS

The aroma properties of the mustard seed (*brassica nigra*) powder was determined by head space solid phase micro extraction method combined with gas chromatographic-mass spectrometric (GC-MS) techniques in laboratory of aroma research for food industry in KU Leuven, technology campus Ghent, Belgium. The most relevant peaks were identified by comparison of their retention indices with literature data and on the basis of their total mass spectra, by comparison with mass spectra databases (NIST mass spectral library). The chromatograph of the mustard seed powder presented in Figure 4. 56 as well as the identified aroma properties or chemical compounds of mustard seed (*brassica nigra*) powder, retention time and their percentage were presented in Table 4.14.

In fact, dry seeds of *brassica nigra* do not have the pungent flavor. The pungent flavor is only developed when the seeds are ground and macerated in water under specific conditions (Jimmy *et al*., 2001). This is because there are glucosinolate in the seeds of *brassica nigra*, which are the precursors of the essential oil of *brassica nigra*. Glucosinolates are the main secondary metabolites found in mustard seed (*brassica nigra*). Their presence is made known to us whenever we eat mustard as they degrade immediately upon tissue damage to release isothiocyanates or other sulfur-containing compounds, of which isothiocyanates are the most well-known (Mithen R., 2006).

In this experiment, as expected most of the identified chemical compounds were the major sources of sulphur-containing plant compounds, those derived from the glucosinolate–myrosinase (substrate–enzyme) system found in *cruciferous* crops (Figur 4.45) such as *brassica nigra* and *brassica oleracea* (Mithen R., 2006). The differences in mustard flavor or aroma are

due to the structural changes of the released isothiocyanates (Mithen R. 2006; Miyazawa, M. & Jyunichi, K. 2006). For example, oriental and brown mustard (*brassica nigra*) release a volatile compound, ally isothiocyanate, which produces a sharp taste sensation and pungent aroma (Jimmy *et al*., 2001). Yellow mustard releases a non-volatile compound, 4-hydroxybenzyl isothiocyanate, which elicits a hot mouth feel in condiments. Diversity of product formation in the glucosinolate-myrosinase system is mainly due to structural variation of the glucosinolate substrate as shown in Figur 4.45.

The identified compoundes which have relatively high contents are isothiocyanate derivatives and 1-Cyano-2,3-Epithiopropane similarly the results of the glucosinolate–myrosinase (substrate–enzyme) system shown in Figur 4.45. The analysis (Figure 4. 44) revealed that mustard seed powder contained a major aromatic component (peak) at the retention time of 17.69 min for allyl isothiocyanate (71.13%), 20.53 min for 1-Propene, 3-isothiocyanato- (12.48%) and 31.51 min for 1-Cyano-2,3-Epithiopropane (9.54%), this was similar with a result reported by Jimmy *et al*., (2001) and a literature stated in Mithen R. (2006). Vaughn F. & Boydston R. (1997) also reported the isolation of benzyl isothiocyanate volatile compound from mustard seeds by means of solid phase micro-extraction (SPME)

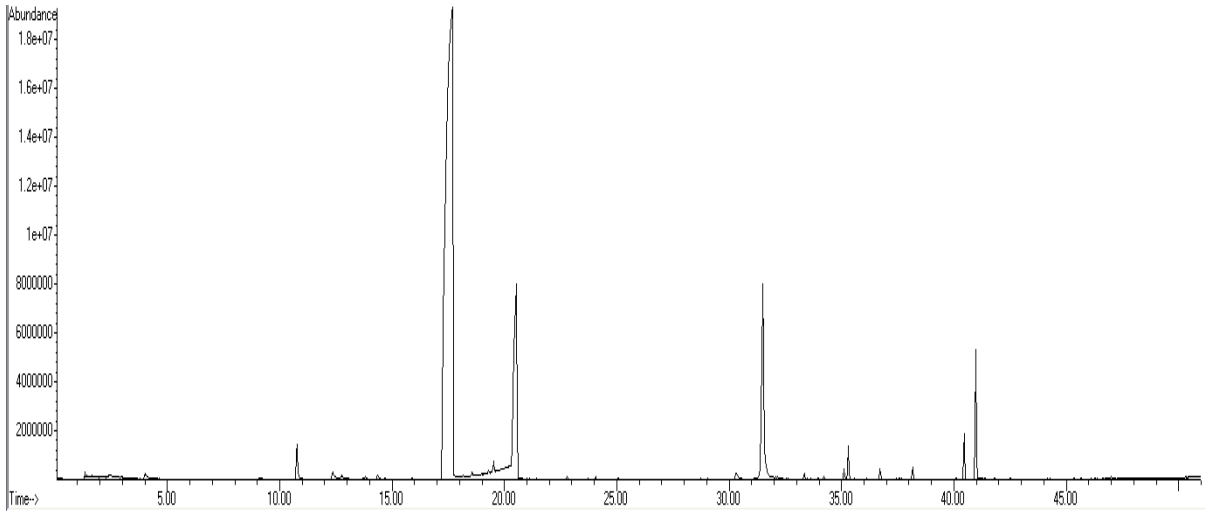


Figure 4.44 GC chromatograph for the aroma of mustard seeds powder

Table 4.14 The identified chemical compounds in mustard seed (*brassica nigra*) powder

	Compounds	Retention time	Percentage
1	Urea	1.336	0.04
2	Carbondisulfide	1.658	0.01
3	Cyclotrisiloxane, Hexamethyl	2.368	0.01
4	N-Ethil-1,3-Dithioisindoline	2.425	0.02
5	Diazoethane	3.995	0.07
6	2-Butenenitril	10.757	0.94
7	Isothiazol, 3-Methyl	12.361	0.24
8	Gamma-Terpinen	12.771	0.08
9	Para Cymene	13.813	0.05
10	Isobutyl Isothiocyanate	15.871	0.03
11	Allyl Isothiocyanate	17.693	71.13
12	2-Methyl-3,4-Dihydro-2h-Thiopyran	19.509	0.19
13	1-Propene, 3-isothiocyanato-	20.533	12.48
14	1h-Indene,Octahydro-2,2,4,4,7,7-Hexamethyl-Trans	22.802	0.05
15	1h-Purin-6-Amine,[(2-Fluorophenyl)Methyl]	24.06	0.03
16	Methyl Nonyl Ketone	25.052	0.02
17	1-Cyano-2,3-Epithiopropane	31.511	9.54
18	1h-Purin-6-Amine,[(2-Fluorophenyl)Methyl]	33.35	0.10
19	Benzeneacetonitrile	33.966	Trace
20	Benzenepropanenitrile	36.713	0.22
21	Octanoic Acid	37.565	0.01
22	Benzyl Isothiocyanate	38.162	0.29
23	Phnol,5-Methyl-2-(1-Methylethyl)	40.449	1.01
24	Phenethylisothiocyanate	40.973	3.31
25	Decanoic Acid	42.5	Trace

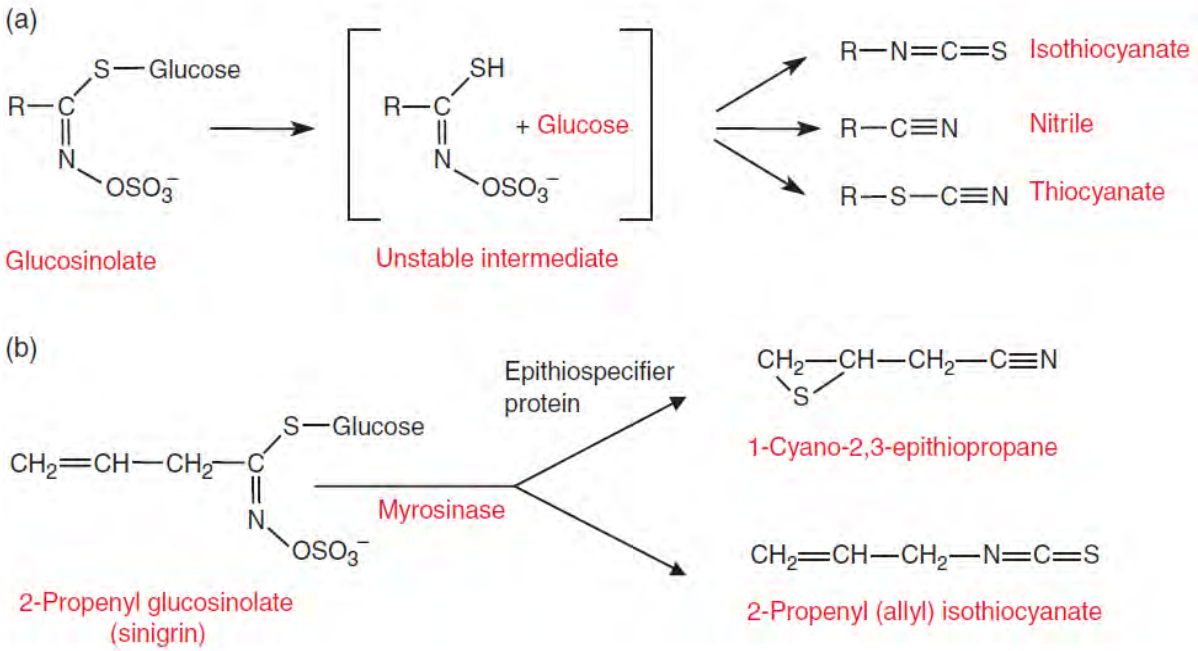


Figure 4.45 The glucosinolate–myrosinase (substrate–enzyme) system found in *cruciferous* crops
a) Structure of glucosinolates and its enzymic degradation to an unstable intermediate and subsequent conversion to isothiocyanate, nitrile and, more rarely, a thionitrile. (b) Glucosinolates with alkenyl side chains may degrade to either isothiocyanates or, in the presence of the ESP protein, epithionitriles.
Source: Mithen R., (2006)

CHAPTER 5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusion

This study had evaluated the effect of high power incandescent light on open window of display booth and Ethiopian spices (*capsicum annuum*, *capsicum frutescens* & *brassica nigra*) on the color and oxidation stability of raw beef for the first time.

It was confirmed that high power (more than 400 watt) incandescent display light had a detrimental effect on color and oxidation stability of displayed raw beef samples. Raw beef slices exposed under incandescent light continuously at 6280±210 lux showed higher TBARS value, ΔE, hue angle & MMb%, and lower L*, a*, b*, Chroma & OMb% value than the raw beef slices kept in the dark at room temperature as the light exposure time increased. These results are a clear evidence to reach to a conclusion for the influence of high power incandescent display light and the light exposure time on color and oxidation stability of raw beef. Moreover, the yellowness (b*) value of raw beef sample has greater value than the redness (a*) value of covered displayed beef samples compared to opened displayed beef samples. Overall, the covered displayed raw beef samples have more color stability than the opened displayed raw beef samples. However, those the covered raw beef samples exposed to incandescent display light exhibited significantly lower oxidation stability and higher temperature on meat surface compared to open displayed raw beef samples.

The color stability of displayed raw beef is highly related to the muscle type. Among the selected three beef muscle types we found that the *semitendinosus* (ST) muscle type has good color stability than the *longissimus dorsi* (LD) and *triceps brachii* (BT) muscle types; the *triceps brachii* muscle types has the lowest the color stability. The color of raw beef from ST is generally lighter, redder and more yellow than that from the LD and the BT.

The impact of short wavelengths and light intensity on color change stability of displayed raw beef was also observed. The display light sources with higher ratio in the shorter wavelengths like the most frequently used fluorescent lamps have a higher discoloration on raw beef. The display light sources with a higher radiation in the medium and long wavelength range of the visible spectra have reduced the discoloration of displayed raw meat. Generally, use of high

intensity lighting for meat display may result in more and faster meat sales, but if sales do not keep pace with discoloration of meat, loss of profit may result.

The chili pepper, African bird´s eye and mustard seeds extracts were found to exhibit effective lipid antioxidant activity on the spices treated raw beef samples which stored in dark refrigerator for two weeks. As a result of this, all these spices extract enhanced color stability in raw beef especially until one week storage period in dark refrigerator. In addition, all spices extract performed well in preventing the growth of aerobic bacteria on the surface of spices treated raw beef samples during two weeks storage period and they were able to preserve fresh color as compared to the controls which were not treated by the spices. However, the dosage of spices extracts had not significant difference on color and oxidation stability of raw beef samples which treated by different dose of spices extracts.

It was also determined that the Ethiopian spices (chili pepper, African bird´s eye and mustard seed) are rich in phenolic compound and demonstrated good antioxidant activity as measured by three different methods. The antioxidant activity of the mixture of three spices revealed synergetic effect. Moreover, we noticed germination of seeds is one of the processing methods for increasing antioxidant activity and polyphenol content. The germinated mustard seeds have more antioxidant activity, and total phenol and flavonoids contents than the raw/dry mustard seeds. In addition, there is a strong correlation between the antioxidant activities of the germinated mustard seeds with their total phenolic content. Hence, people eating them with raw beef might be somewhat beneficial to protect themselves against oxidative damage.

In the analysis of aroma compounds of mustard seeds, several sulfur contained compounds had been identified by SPME-GC-MS. Within the sulfur contained compounds which were identified from mustard seeds the ally isothiocyanate was the most dominant. This ally isothiocyanate has beneficial effects on antimicrobial profiles of mustard (*brassica nigra*) and it is the responsible compound for the pungent aroma of mustard seeds.

5.2 Recommendation

- The use of the display light sources with a reduced radiation in the short wavelength range is recommended for displaying of beef carcasses.

- The incandescent light source is considered to be a full-spectrum source. It produces a fairly balanced emission at all the visible wavelengths and has good color rendering. Even if it has high heat emission, it is preferable for displaying of meat. Therefore, special incandescent lamp (the tungsten filament lamp with integral reflector) is advisable to be used over red meat displays to bring out the fresh appearance of the meat. This light source is compact and can be focused and directed on the displayed meat surface. Its reflector is dichroic, reflecting light forward but allowing heat to pass through to the back of the luminaire. Dichroic lamps are therefore well suited to display lighting where heat could damage the meat on displaying like Ethiopian butcher shop case.
- Another alternative recommendation for displaying of carcasses, especially for Ethiopian butcher shops is the use of flat mirrors in three sides of the wall of butcher shops to increase illumination flux that fall on the surface of the displayed carcasses instead of using many lamps and increase the distances of light source from the displayed carcasses.
- The Ethiopian spices which selected in this study have good antioxidant activity and phenolic compound content; which are also abundantly available in Ethiopia, hence, can be used as a source of antioxidant for production of shelf stable food products and also as a source of healthy compounds for the development of dietary supplements via advance research and development activities. Moreover, these spices may be used not only for raw beef consumption but also can be used for preservation of raw meat.
- Three days germinated mustard seed powder is recommended for diets especially for Ethiopian spices shops and butcheries who mostly prepare mustard seed powder for consumtion of raw meat.
- Although a comprehensive study was conducted in this research, information about the qualitative and quantitative analysis of major individual polyphenols in the spices was unexploited. Hence, further work is required to isolate and identify the polyphenol compounds that may have contributed to the antioxidant activities in c*apsicum and brassica nigra* samples.

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Appendix A. General Linear Model-statistical results

Multiple Comparisons for types of spices extract used for treatment of raw beef steaks

Tukey HSD

Dependent Variable	(I) types of spices for treatment	(J) types of spices for treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
L*white	control by water	pepper	1.1392*	.30268	.004	.3191	1.9592
		African birdseye	1.3267*	.30268	.001	.5066	2.1467
		mustard	2.4725*	.30268	.000	1.6524	3.2926
	pepper	control by water	-1.1392*	.30268	.004	-1.9592	-.3191
		African birdseye	.1875	.30268	.925	-.6326	1.0076
		mustard	1.3333*	.30268	.001	.5133	2.1534
		control by water	-1.3267*	.30268	.001	-2.1467	-.5066
	African birdseye	pepper	-.1875	.30268	.925	-1.0076	.6326
		mustard	1.1458*	.30268	.003	.3258	1.9659
		control by water	-2.4725*	.30268	.000	-3.2926	-1.6524
	mustard	pepper	-1.3333*	.30268	.001	-2.1534	-.5133
		African birdseye	-1.1458*	.30268	.003	-1.9659	-.3258
a*-red	control by water	pepper	1.6983*	.30650	.000	.8679	2.5288
		African birdseye	.2800	.30650	.798	-.5504	1.1104
		mustard	-.5392	.30650	.311	-1.3696	.2913
	pepper	control by water	-1.6983*	.30650	.000	-2.5288	-.8679
		African birdseye	-1.4183*	.30650	.000	-2.2488	-.5879
		mustard	-2.2375*	.30650	.000	-3.0679	-1.4071
	African birdseye	control by water	-.2800	.30650	.798	-1.1104	.5504
		pepper	1.4183*	.30650	.000	.5879	2.2488
		mustard	-.8192	.30650	.054	-1.6496	.0113
	mustard	control by water	.5392	.30650	.311	-.2913	1.3696
		pepper	2.2375*	.30650	.000	1.4071	3.0679
		African birdseye	.8192	.30650	.054	-.0113	1.6496
b*-yellow	control by water	pepper	1.3350*	.27770	.000	.5826	2.0874
		African birdseye	.0108	.27770	1.000	-.7415	.7632
		mustard	-1.1108*	.27770	.002	-1.8632	-.3585
	pepper	control by water	-1.3350*	.27770	.000	-2.0874	-.5826
		African birdseye	-1.3242*	.27770	.000	-2.0765	-.5718
		mustard	-2.4458*	.27770	.000	-3.1982	-1.6935

		control by water	-.0108	.27770	1.000	-.7632	.7415
	African birdseye	pepper	1.3242*	.27770	.000	.5718	2.0765
		mustard	-1.1217*	.27770	.002	-1.8740	-.3693
		control by water	1.1108*	.27770	.002	.3585	1.8632
	mustard	pepper	2.4458*	.27770	.000	1.6935	3.1982
		African birdseye	1.1217*	.27770	.002	.3693	1.8740
		pepper	2.1708*	.36638	.000	1.1782	3.1635
	control by water	African birdseye	.2242	.36638	.928	-.7685	1.2168
		mustard	1.0058*	.36638	.046	.0132	1.9985
		control by water	-2.1708*	.36638	.000	-3.1635	-1.1782
	pepper	African birdseye	-1.9467*	.36638	.000	-2.9393	-.9540
		mustard	-1.1650*	.36638	.016	-2.1577	-.1723
chroma		control by water	-.2242	.36638	.928	-1.2168	.7685
	African birdseye	pepper	1.9467*	.36638	.000	.9540	2.9393
		mustard	.7817	.36638	.164	-.2110	1.7743
		control by water	-1.0058*	.36638	.046	-1.9985	-.0132
	mustard	pepper	1.1650*	.36638	.016	.1723	2.1577
		African birdseye	-.7817	.36638	.164	-1.7743	.2110
		pepper	.0350	.34580	1.000	-.9019	.9719
	control by water	African birdseye	-.0417	.34580	.999	-.9786	.8952
		mustard	4.5333*	.34580	.000	3.5964	5.4702
		control by water	-.0350	.34580	1.000	-.9719	.9019
	pepper	African birdseye	-.0767	.34580	.996	-1.0136	.8602
		mustard	4.4983*	.34580	.000	3.5614	5.4352
hue		control by water	.0417	.34580	.999	-.8952	.9786
	African birdseye	pepper	.0767	.34580	.996	-.8602	1.0136
		mustard	4.5750*	.34580	.000	3.6381	5.5119
		control by water	-4.5333*	.34580	.000	-5.4702	-3.5964
	mustard	pepper	-4.4983*	.34580	.000	-5.4352	-3.5614
		African birdseye	-4.5750*	.34580	.000	-5.5119	-3.6381
		pepper	-2.2333*	.44245	.000	-3.4321	-1.0346
	control by water	African birdseye	-2.0783*	.44245	.000	-3.2771	-.8796
		mustard	1.4567*	.44245	.012	.2579	2.6554
		control by water	2.2333*	.44245	.000	1.0346	3.4321
	pepper	African birdseye	.1550	.44245	.985	-1.0438	1.3538
oxymyoglobin		mustard	3.6900*	.44245	.000	2.4912	4.8888
		control by water	2.0783*	.44245	.000	.8796	3.2771
	African birdseye	pepper	-.1550	.44245	.985	-1.3538	1.0438
		mustard	3.5350*	.44245	.000	2.3362	4.7338
	mustard	control by water	-1.4567*	.44245	.012	-2.6554	-.2579

		pepper	-3.6900*	.44245	.000	-4.8888	-2.4912
		African birdseye	-3.5350*	.44245	.000	-4.7338	-2.3362
		pepper	-.7525	.39323	.243	-1.8179	.3129
	control by water	African birdseye	.8550	.39323	.152	-.2104	1.9204
		mustard	-.9125	.39323	.114	-1.9779	.1529
		control by water	.7525	.39323	.243	-.3129	1.8179
	pepper	African birdseye	1.6075*	.39323	.001	.5421	2.6729
		mustard	-.1600	.39323	.977	-1.2254	.9054
metmyoglobin		control by water	-.8550	.39323	.152	-1.9204	.2104
	African birdseye	pepper	-1.6075*	.39323	.001	-2.6729	-.5421
		mustard	-1.7675*	.39323	.000	-2.8329	-.7021
		control by water	.9125	.39323	.114	-.1529	1.9779
	mustard	pepper	.1600	.39323	.977	-.9054	1.2254
		African birdseye	1.7675*	.39323	.000	.7021	2.8329
		pepper	.0575*	.00445	.000	.0454	.0696
	control by water	African birdseye	.0650*	.00445	.000	.0529	.0771
		mustard	.0625*	.00445	.000	.0504	.0746
		control by water	-.0575*	.00445	.000	-.0696	-.0454
	pepper	African birdseye	.0075	.00445	.348	-.0046	.0196
		mustard	.0050	.00445	.678	-.0071	.0171
TBARS		control by water	-.0650*	.00445	.000	-.0771	-.0529
	African birdseye	pepper	-.0075	.00445	.348	-.0196	.0046
		mustard	-.0025	.00445	.943	-.0146	.0096
		control by water	-.0625*	.00445	.000	-.0746	-.0504
	mustard	pepper	-.0050	.00445	.678	-.0171	.0071
		African birdseye	.0025	.00445	.943	-.0096	.0146
		pepper	.4200*	.12501	.010	.0813	.7587
	control by water	African birdseye	.3575*	.12501	.035	.0188	.6962
		mustard	.4050*	.12501	.014	.0663	.7437
		control by water	-.4200*	.12501	.010	-.7587	-.0813
	pepper	African birdseye	-.0625	.12501	.958	-.4012	.2762
		mustard	-.0150	.12501	.999	-.3537	.3237
microbial load		control by water	-.3575*	.12501	.035	-.6962	-.0188
	African birdseye	pepper	.0625	.12501	.958	-.2762	.4012
		mustard	.0475	.12501	.981	-.2912	.3862
		control by water	-.4050*	.12501	.014	-.7437	-.0663
	mustard	pepper	.0150	.12501	.999	-.3237	.3537
		African birdseye	-.0475	.12501	.981	-.3862	.2912
		pepper	-1.0750*	.05643	.000	-1.2279	-.9221
Delta E	control by water	African birdseye	-.7600*	.05643	.000	-.9129	-.6071

		mustard	-3.7125*	.05643	.000	-3.8654	-3.5596
		control by water	1.0750*	.05643	.000	.9221	1.2279
	pepper	African birdseye	.3150*	.05643	.000	.1621	.4679
		mustard	-2.6375*	.05643	.000	-2.7904	-2.4846
		control by water	.7600*	.05643	.000	.6071	.9129
	African birdseye	pepper	-.3150*	.05643	.000	-.4679	-.1621
		mustard	-2.9525*	.05643	.000	-3.1054	-2.7996
		control by water	3.7125*	.05643	.000	3.5596	3.8654
	mustard	pepper	2.6375*	.05643	.000	2.4846	2.7904
		African birdseye	2.9525*	.05643	.000	2.7996	3.1054

Based on observed means.

The error term is Mean Square(Error) = .019.

*. The mean difference is significant at the .05 level.

Storage time

Homogeneous Subsets

L* value

storage time	N	Subset			
		1	2	3	4
after 14 day	12	25.8375			
starting time	12		33.6908		
after 7 day	12			36.4225	
after 2 day	12				37.0375
Sig.		1.000	1.000	1.000	1.000

a* value

Duncan

storage time	N	Subset			
		1	2	3	4
after 14day	12	14.9800			
after 7day	12		21.2917		
starting time	12			24.5075	
after 2 day	12				24.5075
Sig.		1.000	1.000	1.000	1.000

b* value

storage time	N	Subset		
		1	2	3
after 14day	12	15.6692		
after 7day	12		20.1417	
starting time	12		20.4267	
after 2day	12			25.7833
Sig.		1.000	.209	1.000

Duncan Chroma

storage time	N	Subset			
		1	2	3	4
after 14day	12	21.7717			
after 7day	12		29.2608		
starting time	12			31.9717	
after 2day	12				41.1292
Sig.		1.000	1.000	1.000	1.000

Hue angle

Duncan

storage time	N	Subset		
		1	2	3
after 2day	12	39.0001		
starting time	12	39.7040		
after 7day	12		42.8314	
after 14day	12			45.8727
Sig.		.094	1.000	1.000

oxymyoglobin

Duncan

storage time	N	Subset	
		1	2
after 2day	12	53.4067	
starting time	12		55.7783
after 7day	12		56.3550
after 14day	12		56.4183
Sig.		1.000	.181

metmyoglobin

Duncan

storage time	N	Subset	
		1	2
after 7day	12	42.5825	
after 14day	12	42.7600	
starting time	12	42.8800	
after 2day	12		45.2675
Sig.		.482	1.000

TBARS

storage time	N	Subset			
		1	2	3	4
starting time	12	.2100			
after 14day	12		.3425		
after 7day	12			.4675	
after 2day	12				.5550
Sig.		1.000	1.000	1.000	1.000

microbial load

Duncan

storage time	N	Subset			
		1	2	3	4
starting time	12	5.4200			
after 2day	12		5.8875		
after 7day	12			8.5175	
after 14day	12				9.7625
Sig.		1.000	1.000	1.000	1.000

Delta E

Duncan

storage time	N	Subset			
		1	2	3	4
starting time	12	.0000			
after 7day	12		4.4267		
after 2day	12			9.6604	
after 14day	12				13.9309
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed. Based on observed means.

The error term is Mean Square(Error) = .502.

a. Uses Harmonic Mean Sample Size = 12.000.

b. The group sizes are unequal. Type I error levels are not guaranteed.

c. Alpha = .05.

Multiple Comparisons for the experiment using optical filters

Tukey HSD

Dependent Variable	(I) types of filters	(J) types of filters	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
L* value	w/o/filter	transparent	.6333	.29481	.281	-.2416	1.5083
		yellow	-1.5200*	.29481	.000	-2.3950	-.6450
		green	-1.9225*	.29481	.000	-2.7975	-1.0475
		blue	-3.0033*	.29481	.000	-3.8783	-2.1284
		red	-1.7217*	.29481	.000	-2.5966	-.8467
	transparent	w/o/filter	-.6333	.29481	.281	-1.5083	.2416
		yellow	-2.1533*	.29481	.000	-3.0283	-1.2784
		green	-2.5558*	.29481	.000	-3.4308	-1.6809
		blue	-3.6367*	.29481	.000	-4.5116	-2.7617
		red	-2.3550*	.29481	.000	-3.2300	-1.4800
	yellow	w/o/filter	1.5200*	.29481	.000	.6450	2.3950
		transparent	2.1533*	.29481	.000	1.2784	3.0283
		green	-.4025	.29481	.747	-1.2775	.4725
		blue	-1.4833*	.29481	.000	-2.3583	-.6084
		red	-.2017	.29481	.983	-1.0766	.6733
	green	w/o/filter	1.9225*	.29481	.000	1.0475	2.7975
		transparent	2.5558*	.29481	.000	1.6809	3.4308
		yellow	.4025	.29481	.747	-.4725	1.2775
		blue	-1.0808*	.29481	.008	-1.9558	-.2059
		red	.2008	.29481	.983	-.6741	1.0758
	blue	w/o/filter	3.0033*	.29481	.000	2.1284	3.8783
		transparent	3.6367*	.29481	.000	2.7617	4.5116
		yellow	1.4833*	.29481	.000	.6084	2.3583
		green	1.0808*	.29481	.008	.2059	1.9558
		red	1.2817*	.29481	.001	.4067	2.1566
	red	w/o/filter	1.7217*	.29481	.000	.8467	2.5966

		transparent	2.3550*	.29481	.000	1.4800	3.2300
		yellow	.2017	.29481	.983	-.6733	1.0766
		green	-.2008	.29481	.983	-1.0758	.6741
		blue	-1.2817*	.29481	.001	-2.1566	-.4067
		transparent	-.3892	.14280	.089	-.8130	.0347
		yellow	-1.6900*	.14280	.000	-2.1138	-1.2662
	w/o/filter	green	-3.2242*	.14280	.000	-3.6480	-2.8003
		blue	-3.5308*	.14280	.000	-3.9547	-3.1070
		red	-3.7083*	.14280	.000	-4.1322	-3.2845
		w/o/filter	.3892	.14280	.089	-.0347	.8130
		yellow	-1.3008*	.14280	.000	-1.7247	-.8770
	transparent	green	-2.8350*	.14280	.000	-3.2588	-2.4112
		blue	-3.1417*	.14280	.000	-3.5655	-2.7178
		red	-3.3192*	.14280	.000	-3.7430	-2.8953
		w/o/filter	1.6900*	.14280	.000	1.2662	2.1138
		transparent	1.3008*	.14280	.000	.8770	1.7247
	yellow	green	-1.5342*	.14280	.000	-1.9580	-1.1103
		blue	-1.8408*	.14280	.000	-2.2647	-1.4170
		red	-2.0183*	.14280	.000	-2.4422	-1.5945
a* value		w/o/filter	3.2242*	.14280	.000	2.8003	3.6480
		transparent	2.8350*	.14280	.000	2.4112	3.2588
	green	yellow	1.5342*	.14280	.000	1.1103	1.9580
		blue	-.3067	.14280	.281	-.7305	.1172
		red	-.4842*	.14280	.017	-.9080	-.0603
		w/o/filter	3.5308*	.14280	.000	3.1070	3.9547
		transparent	3.1417*	.14280	.000	2.7178	3.5655
	blue	yellow	1.8408*	.14280	.000	1.4170	2.2647
		green	.3067	.14280	.281	-.1172	.7305
		red	-.1775	.14280	.813	-.6013	.2463
		w/o/filter	3.7083*	.14280	.000	3.2845	4.1322
		transparent	3.3192*	.14280	.000	2.8953	3.7430
	red	yellow	2.0183*	.14280	.000	1.5945	2.4422
		green	.4842*	.14280	.017	.0603	.9080
		blue	.1775	.14280	.813	-.2463	.6013
		transparent	-.3033	.16719	.467	-.7995	.1929
		yellow	-1.2308*	.16719	.000	-1.7270	-.7346
	w/o/filter	green	-2.9850*	.16719	.000	-3.4812	-2.4888
b* value		blue	-3.8008*	.16719	.000	-4.2970	-3.3046
		red	-3.7850*	.16719	.000	-4.2812	-3.2888
	transparent	w/o/filter	.3033	.16719	.467	-.1929	.7995

		yellow	-.9275*	.16719	.000	-1.4237	-.4313
		green	-2.6817*	.16719	.000	-3.1779	-2.1855
		blue	-3.4975*	.16719	.000	-3.9937	-3.0013
		red	-3.4817*	.16719	.000	-3.9779	-2.9855
		w/o/filter	1.2308*	.16719	.000	.7346	1.7270
		transparent	.9275*	.16719	.000	.4313	1.4237
	yellow	green	-1.7542*	.16719	.000	-2.2504	-1.2580
		blue	-2.5700*	.16719	.000	-3.0662	-2.0738
		red	-2.5542*	.16719	.000	-3.0504	-2.0580
		w/o/filter	2.9850*	.16719	.000	2.4888	3.4812
		transparent	2.6817*	.16719	.000	2.1855	3.1779
	green	yellow	1.7542*	.16719	.000	1.2580	2.2504
		blue	-.8158*	.16719	.000	-1.3120	-.3196
		red	-.8000*	.16719	.000	-1.2962	-.3038
		w/o/filter	3.8008*	.16719	.000	3.3046	4.2970
		transparent	3.4975*	.16719	.000	3.0013	3.9937
	blue	yellow	2.5700*	.16719	.000	2.0738	3.0662
		green	.8158*	.16719	.000	.3196	1.3120
		red	.0158	.16719	1.000	-.4804	.5120
		w/o/filter	3.7850*	.16719	.000	3.2888	4.2812
		transparent	3.4817*	.16719	.000	2.9855	3.9779
	red	yellow	2.5542*	.16719	.000	2.0580	3.0504
		green	.8000*	.16719	.000	.3038	1.2962
		blue	-.0158	.16719	1.000	-.5120	.4804
		transparent	-.5167*	.17207	.046	-1.0273	-.0060
		yellow	-2.0792*	.17207	.000	-2.5898	-1.5685
	w/o/filter	green	-4.2875*	.17207	.000	-4.7982	-3.7768
		blue	-4.9967*	.17207	.000	-5.5073	-4.4860
		red	-5.1383*	.17207	.000	-5.6490	-4.6277
		w/o/filter	.5167*	.17207	.046	.0060	1.0273
		yellow	-1.5625*	.17207	.000	-2.0732	-1.0518
	transparent	green	-3.7708*	.17207	.000	-4.2815	-3.2602
Chroma		blue	-4.4800*	.17207	.000	-4.9907	-3.9693
		red	-4.6217*	.17207	.000	-5.1323	-4.1110
		w/o/filter	2.0792*	.17207	.000	1.5685	2.5898
		transparent	1.5625*	.17207	.000	1.0518	2.0732
	yellow	green	-2.2083*	.17207	.000	-2.7190	-1.6977
		blue	-2.9175*	.17207	.000	-3.4282	-2.4068
		red	-3.0592*	.17207	.000	-3.5698	-2.5485
	green	w/o/filter	4.2875*	.17207	.000	3.7768	4.7982

		transparent	3.7708*	.17207	.000	3.2602	4.2815
		yellow	2.2083*	.17207	.000	1.6977	2.7190
		blue	-.7092*	.17207	.002	-1.2198	-.1985
		red	-.8508*	.17207	.000	-1.3615	-.3402
		w/o/filter	4.9967*	.17207	.000	4.4860	5.5073
		transparent	4.4800*	.17207	.000	3.9693	4.9907
	blue	yellow	2.9175*	.17207	.000	2.4068	3.4282
		green	.7092*	.17207	.002	.1985	1.2198
		red	-.1417	.17207	.962	-.6523	.3690
		w/o/filter	5.1383*	.17207	.000	4.6277	5.6490
		transparent	4.6217*	.17207	.000	4.1110	5.1323
	red	yellow	3.0592*	.17207	.000	2.5485	3.5698
		green	.8508*	.17207	.000	.3402	1.3615
		blue	.1417	.17207	.962	-.3690	.6523
		transparent	.3633	.54833	.985	-1.2641	1.9907
		yellow	-.6300	.54833	.858	-2.2574	.9974
	w/o/filter	green	-3.6625*	.54833	.000	-5.2899	-2.0351
		blue	-5.2608*	.54833	.000	-6.8882	-3.6334
		red	-4.7425*	.54833	.000	-6.3699	-3.1151
		w/o/filter	-.3633	.54833	.985	-1.9907	1.2641
		yellow	-.9933	.54833	.468	-2.6207	.6341
	transparent	green	-4.0258*	.54833	.000	-5.6532	-2.3984
		blue	-5.6242*	.54833	.000	-7.2516	-3.9968
		red	-5.1058*	.54833	.000	-6.7332	-3.4784
		w/o/filter	.6300	.54833	.858	-.9974	2.2574
		transparent	.9933	.54833	.468	-.6341	2.6207
Hue angle	yellow	green	-3.0325*	.54833	.000	-4.6599	-1.4051
		blue	-4.6308*	.54833	.000	-6.2582	-3.0034
		red	-4.1125*	.54833	.000	-5.7399	-2.4851
		w/o/filter	3.6625*	.54833	.000	2.0351	5.2899
		transparent	4.0258*	.54833	.000	2.3984	5.6532
	green	yellow	3.0325*	.54833	.000	1.4051	4.6599
		blue	-1.5983	.54833	.057	-3.2257	.0291
		red	-1.0800	.54833	.374	-2.7074	.5474
		w/o/filter	5.2608*	.54833	.000	3.6334	6.8882
		transparent	5.6242*	.54833	.000	3.9968	7.2516
	blue	yellow	4.6308*	.54833	.000	3.0034	6.2582
		green	1.5983	.54833	.057	-.0291	3.2257
		red	.5183	.54833	.932	-1.1091	2.1457
	red	w/o/filter	4.7425*	.54833	.000	3.1151	6.3699

		transparent	5.1058*	.54833	.000	3.4784	6.7332
		yellow	4.1125*	.54833	.000	2.4851	5.7399
		green	1.0800	.54833	.374	-.5474	2.7074
		blue	-.5183	.54833	.932	-2.1457	1.1091
		transparent	.8575*	.04457	.000	.7252	.9898
		yellow	1.8575*	.04457	.000	1.7252	1.9898
	w/o/filter	green	2.5150*	.04457	.000	2.3827	2.6473
		blue	.3950*	.04457	.000	.2627	.5273
		red	3.1650*	.04457	.000	3.0327	3.2973
		w/o/filter	-.8575*	.04457	.000	-.9898	-.7252
		yellow	1.0000*	.04457	.000	.8677	1.1323
	transparent	green	1.6575*	.04457	.000	1.5252	1.7898
		blue	-.4625*	.04457	.000	-.5948	-.3302
		red	2.3075*	.04457	.000	2.1752	2.4398
		w/o/filter	-1.8575*	.04457	.000	-1.9898	-1.7252
		transparent	-1.0000*	.04457	.000	-1.1323	-.8677
	yellow	green	.6575*	.04457	.000	.5252	.7898
		blue	-1.4625*	.04457	.000	-1.5948	-1.3302
		red	1.3075*	.04457	.000	1.1752	1.4398
Delta E		w/o/filter	-2.5150*	.04457	.000	-2.6473	-2.3827
		transparent	-1.6575*	.04457	.000	-1.7898	-1.5252
	green	yellow	-.6575*	.04457	.000	-.7898	-.5252
		blue	-2.1200*	.04457	.000	-2.2523	-1.9877
		red	.6500*	.04457	.000	.5177	.7823
		w/o/filter	-.3950*	.04457	.000	-.5273	-.2627
		transparent	.4625*	.04457	.000	.3302	.5948
	blue	yellow	1.4625*	.04457	.000	1.3302	1.5948
		green	2.1200*	.04457	.000	1.9877	2.2523
		red	2.7700*	.04457	.000	2.6377	2.9023
		w/o/filter	-3.1650*	.04457	.000	-3.2973	-3.0327
		transparent	-2.3075*	.04457	.000	-2.4398	-2.1752
	red	yellow	-1.3075*	.04457	.000	-1.4398	-1.1752
		green	-.6500*	.04457	.000	-.7823	-.5177
		blue	-2.7700*	.04457	.000	-2.9023	-2.6377

The error term is Mean Square (Error) = .012.

*. The mean difference is significant at the .05 level.

Appendix B. GC-MS Instrument Control Parameters Information

Wed Nov 26 11:51:09 2014
Control Information
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Sample Inlet : GC
Injection Source : Manual
Mass Spectrometer : Enabled
Injection Location: Rear
No Sample Prep method has been assigned to this method.

==========

6890 GC METHOD

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OVEN
Initial temp: 35 C (On) Maximum temp: 325 C
Initial time: 5.00 min Equilibration time: 0.50 min
Ramps:

#	Rate	Final temp	Final time
1	4.00	183	0.00
2	7.00	246	0.00
3	0 (Off)		

Post temp: 0 C
Post time: 0.00 min
Run time: 51.00 min

FRONT INLET (CIS3)
Mode: Splitless
Initial temp: 0 C (Off)
Pressure: 0 kPa (Off)
Purge flow: 0.0 mL/min
Purge time: 0.00 min
Total flow: 45.0 mL/min
Gas saver: Off
Gas type: Helium

BACK INLET (SPLIT/SPLITLESS)
Mode: Splitless
Initial temp: 250 C (On)
Pressure: 65 kPa (On)
Purge flow: 30.0 mL/min
Purge time: 2.00 min
Total flow: 33.6 mL/min
Gas saver: On
Saver flow: 20.0 mL/min
Saver time: 3.00 min
Gas type: Helium

COLUMN 1
Capillary Column
Model Number:
Description: ZB-WAX
Max temperature: 325 C
Nominal length: 30.0 m
Nominal diameter: 250.00 um
Nominal film thickness: 0.25 um
Mode: constant flow
Initial flow: 0.8 mL/min
Nominal init pressure: 65 kPa
Average velocity: 21 cm/sec
Inlet: Back Inlet
Outlet: MSD
Outlet pressure: ambient

COLUMN 2
(not installed)

FRONT DETECTOR (NO DET) BACK DETECTOR (NO DET)
SIGNAL 2
Save Data: Off
THERMAL AUX 2
Use: MSD Transfer Line Heater
Initial temp: 280 C (On)
POST RUN
Post Time: 0.00 min
TIME TABLE
Time(min) Parameter & Setpoint
Column 1 Inventory Number :
Column 2 Inventory Number :

GERSTEL MAESTRO
SYSTEM SETTINGS
Maestro Runtime : 51.00 min
GC Cool Down Time : 10.00 min
Cryo Timeout : 15.00 min

GERSTEL CIS
CIS : not used - use these parameters if it becomes 'used'
CRYO COOLING
Cryo Cooling : not used
TEMPERATURE PROGRAM
Heater Mode : Standard
Initial Temperature : 60 'C
Equilibration Time : 1.00 min
Initial Time : 1.00 min
Ramp 1
Rate : 10.00 'C/s
End Temp : 240 'C
Hold Time : 1.00 min
Ramp 2
Rate : 0.0 'C/s
GERSTEL MPS PREP
Sample Prep : not used
GERSTEL MPS SPME Injection
SAMPLE PREPARATION
SPME : from Incubator
Incubator : Agitator
Incubation Temperature : 60 'C
Incubation Time : 10.00 min
Agitator On Time : 5 s
Agitator Off Time : 2 s
Agitator Speed : 500 rpm
SAMPLE PARAMETERS
Sample Tray Type : VT32-20
Vial Penetration : 30.00 mm
Extraction Time : 35.00 min
Inj. Penetration : 45.00 mm
Desorption Time : 300 s
FIBER BAKEOUT
Bakeout : used

Bakeout at : NdlHeatr
Bakeout Temp. : 270.0 'C
Pre Bakeout Time : 0.00 min
Post Bakeout Time : 7.00 min
Bakeout Penetration : 43.00 mm
DERIVATISATION
Derivatisation : not used - use these parameters if it becomes 'used'
Deriv. Time : 1.00 min
Deriv. Penetration : 24.00 mm

MS ACQUISITION PARAMETERS

General Information
------- -----------
Tune File : atune.u
Acquistion Mode : Scan

MS Information
-- -----------
Solvent Delay : 0.00 min
EMV Mode : Gain Factor
Gain Factor : 1.00
Resulting EM Voltage : 1988
[Scan Parameters]
Low Mass : 40.0
High Mass : 230.0
Threshold : 50
Sample # : 2 A/D Samples 4
[MSZones]
MS Source : 230 C maximum 250 C
MS Quad : 150 C maximum 200 C

END OF MS ACQUISITION PARAMETERS

END OF INSTRUMENT CONTROL PARAMETERS

DECLARATION

I undersigned declare that this thesis is my original work and has not been presented for any degree in any university and all the source of materials used for the thesis has been duly acknowledged.

Name: Efrem Mengistu ----------

Signature

This thesis has been submitted for examination with our approval as advisors;

1. Prof. Negussie Retta ----------

Signature

2. Dr. Gulelat Desse ----------

Signature

Date and place of submission: Centre for Food Science and Nutrition,

Addis Ababa University

May 2015