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Effects of Processing and Packaging Modifications on the Quality and Shelf Life in Meats

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EFFECTS OF PROCESSING AND PACKAGING MODIFICATIONS ON THE QUALITY AND SHELF LIFE IN MEATS

by

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ABSTRACT

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Shelf life of meat and meat products depends on the quality of meat and packaging stability. Meat color change, lipid oxidation, pH, and microbial contamination are the main causes that affect the nutritional, sensory, and physical characteristics by forming toxic compounds, off flavors, off odors, and undesirable color. The first objective of this study was to examine the effect of newly developed CO_2 generating absorbent pads on beef, tuna and chicken meats. The second objective was to compare the effect of Type I (radical quenching) antioxidants eugenol and rosmarinic acid (RA) to that of Type II (metal chelating) antioxidants milk mineral (MM), phytate, and sodium tri-polyphosphate (STPP) in raw ground chicken patties. In the first study samples of beef semitendinosus muscle, boneless and skinless chicken breast, and yellowfin tuna loins were chosen and packaged with four different pad types. Red meats and chicken exhibited trends as would normally be expected during storage. Specifically, color became less appealing (beef and tuna color changed from red to brown, and chicken changed from yellow–pink to grey); pH increased, microbial counts increased, and lipid oxidation (rancidity) increased. Lightness (L*) values for chicken were within 46 – 54,

for tuna L* value range was 21-26. Redness/greenness (a*) is an indicator of freshness in red meats, and for beef steaks an average value of 12.32 was noted on day 8, indicative of fresh red color. Recorded average lipid oxidation (TBA values in mg/kg of meat) for chicken (< 0.19 mg/kg) and tuna were low (< 0.16 mg/kg) and for beef (1.06 mg/kg). Even though there was a significant effect of storage time *(P < 0.05)* as expected, no significant *(P >0.05)* differences were observed between the four tested pad types over the 8-day testing period.

In study 2, (Chapter 4) a significant antioxidant effect ($P < 0.05$) was observed in L* values of ground chicken color between treatments. Milk mineral effectively preserved fresh color and slowed lipid oxidation in chicken patties by day 10. Inverse correlations ($P < 0.001$) were observed between pH (increasing), and lightness, redness values (decreasing) from day 1 through 10. Increased pH levels from slightly acidic to basic or physiological pH resulted in high numbers for total aerobic plate-counts. There was a significant antioxidant effect on lipid oxidation values by day 10; eugenol and MM were more effective ($P < 0.05$) than STPP in controlling lipid oxidation measured as thiobarbituric acid reactive substances (0.198 mg/kg, 0.198 mg/kg, and 0.268 mg/kg, respectively). In conclusion, CO_2 generating absorbent pads did not have a positive effect on meat quality, while the antioxidant milk mineral efficiently maintained color, and pH, and controlled lipid oxidation and limited the growth of aerobic bacteria when compared to other antioxidants.

(131 pages)

PUBLIC ABSTRACT

Effects of Processing and Packaging Modifications on the Quality and Shelf Life in Meats

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Shelf life of foods, mainly meat and meat products, is affected by its physical and chemical properties like color, pH, water holding capacity etc. In developed countries food losses occur at production, retail and consumer levels with meat commodities accounting for 41% of the total loss in US during 2008. These losses are because of spoilage due to inappropriate packaging, improper storage conditions, food wastage, and lack of consumer awareness. By focusing on shelf life and quality issues at the production level, we can overcome some of the major problems faced by the food manufacturers.

Active packaging is an innovative technology that has been the focus of research over the recent years. In currently available meat packaging techniques (MAP, VP, CAP), contents of package are filled with varying percentage of gases (N_2, O_2, CO_2) to maintain the quality of the product. In active packaging external agents such as O_2, CO_2 or moisture scavengers/emitters, antimicrobials and antioxidants are added to control the environment within the package. To have minimal effect of cross contamination active agents are added into sachets or pads so they do not come in direct contact with the product. In this study a newly developed CO_2 generating pad (trona mineral) was included in the package, which was wrapped with a single layer of polyvinylchloride to mimic retail setup over an 8-day testing period. Trona mineral contains sodium carbonate and sodium bicarbonate, and reaction between water and sodium bicarbonate releases carbon dioxide. Carbon dioxide has an antimicrobial effect on aerobic flora. With increasing amounts of CO_2, less O_2 is available inside the package, resulting in decreased lipid oxidation and less color change. However, due to the small amount of trona mineral in the tested pads, there was no significant effect on packaged meat due to CO_2 generating absorbent pads. Low TBA values, higher L* values and lower aerobic plate counts may indicate freshness of the meats, though further investigation with increased amounts of trona is needed.

Five different antioxidants classified into two types (Type I - radical quenching and type II-metal chelating) were tested for their effect on ground poultry meat in controlling the changes in color, lipid oxidation, and microbial growth. Type I antioxidants examined include eugenol and rosmarinic acid, and the Type II antioxidants milk mineral, phytate, and STPP. A significant effect was observed in lightness (L*) values ($P < 0.05$) of meat color between treatments due to the type of antioxidant. Aerobic plate counts increased over the entire testing period while values for lightness, redness, yellowness and chroma decreased, indicating an increase in pH favored microbial spoilage of the meat. By day 10, eugenol and MM were more effective and significantly different ($P < 0.05$) than STPP in controlling lipid oxidation measured as thiobarbituric acid reactive substances (0.198 mg/kg, 0.198 mg/kg, and 0.268 mg/kg, respectively) and effectively preserved fresh color.

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Abdulla Khan

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LIST OF SYMBOLS, NOTATION, DEFINITIONS

	Abbreviation key
a*	Redness
ANOVA	Analysis of variance
AP	Active packaging
b*	Yellowness
BHT	Butylated hydroxytoluene
CAP	Controlled atmosphere packaging
COMb	Carboxymyoglobin
CFU	Colony forming units
CIE	Commission on Illumination
CO	Carbon monoxide
CO_2	Carbon dioxide
DMb	Deoxymyoglobin
EDTA	Ethylenediamine tetraacetic acid
Fe^{2+}	Ferrous iron
Fe^{3+}	Ferric iron
HO•	Hydroxyl radical
HPP	High pressure processing
IP	Intelligent packaging
L*	Lightness
MAP	Modified atmosphere packaging
Mb	Myoglobin
O_2^-•	Superoxide anion radical

OMb	Oxymyoglobin
MDA	Malondialdehyde
MMb	Metmyoglobin
MM	Milk mineral
N_2	Nitrogen
NADH	Nicotinamide adenine dinucleotide
O_2	Oxygen
PPI	Paper Pak Industries
ppm	Parts per million
PVC	Polyvinylchloride
R•	Radical
RA	Rosmarinic acid
Rep	Replicate
RFIT	Radio frequency identification technology
RO•	Alkoxyl radical
ROO•	Peroxyl radical
SEM	Standard error of the mean
STPP	Sodium tri-polyphosphate
TAC	Total aerobic plate count
TBARS	Thiobarbituric acid reactive substances
Trt	Treatment
trt*day	Interaction effect of treatment and day
VP	Vacuum Packaging

CHAPTER 1

INTRODUCTION AND OBJECTIVES

1. Introduction

About one-third of the total food produced every year for human consumption is either wasted or lost according to the Food and Agriculture Organization (FAO) of the United Nations (Buzby & Hyman, 2012). In developing nations food losses are mainly due to lack of good infrastructure and storage technologies while in developed countries losses occur at the farm or at production, retail and consumer levels (Godfray et al., 2010). Studies (Buzby, Wells, Axtman, & Mickey, 2009; Buzby & Hyman, 2012) report a 10% loss at retail level, and consumer level losses constitute 19%. Total value for food losses in the United States was $165.6 billion in 2008 at the retail and consumer levels, with meat, poultry and fish commodities accounting for 41% of this total loss (Buzby & Hyman, 2012). According to the Environmental Protection Agency, the single largest component of municipal solid waste is food waste (14%), totaling approximately 31 million metric tons (Buzby & Hyman, 2012; Kantor, Lipton, Manchester, & Oliveira, 1997). Of this, only 3% is recovered and recycled while the remaining waste finds its place in landfills and incinerators. Landfills cause 34% of all human related methane emissions in the United States (Gustavsson, Cederberg, Sonesson, van Otterdijk, & Meybeck, 2011; US EPA, 2012). Adverse effects of food wastes on the environment include air, water pollution, emission of greenhouse gases, and etc. Hence, even a minimal reduction of 2 or 3% food loss will be a substantial benefit to the consumers and food industry.

Retail and consumer level losses in the meat industry occur for many reasons, such as spoilage due to unsuitable packaging, improper storage conditions, food wastage, and lack of consumer awareness on storage. Overcoming these factors and producing meats with specific quality that can meet consumer expectations requires some focus on the shelf life, and quality of meats. Consumers often try to judge meat quality based on three sensory attributes: appearance, texture, and flavor (Allen & Cornforth, 2010; Sánchez-Escalante, Djenane, Torrescano, Beltrán, & Roncalés, 2001). Visual appearance of the product has the most influence on consumers' purchase decision and any objectionable change in color of the meat may result in rejection (Gray, Gomaa, & Buckley, 1996; Issanchou, 1996). Color of meat and meat products depends on gases bound to the iron contained within the heme ring, which is stabilized within the protein myoglobin, as well as the oxidation state of the iron. Deoxymyoglobin (DMb; purplish red), oxymyoglobin (MbO_2; bright cherry red), and metmyoglobin (MetMb; brown) are of special interest in fresh meat systems (Mancini & Hunt, 2005).

Lipid oxidation is a major concern in foods and notably in muscle foods because of its negative effects on flavor, color, and texture. Lipid oxidation can result in off flavor development (rancidity) and lower nutritional quality in meat products (Gray et al., 1996; Ladikos & Lougovois, 1990). Lipid oxidation in meats is known to accelerate the oxidation of myoglobin due to intermediate products, followed by color loss and release of iron from the protective protein shell. "Free" iron stimulates color loss indirectly by catalyzing the lipid oxidation and directly through Fe-O-O-Fe intermediate formation (Allen & Cornforth, 2006; Kanner, 1994). By minimizing lipid and myoglobin oxidation we can improve the shelf life stability of muscle foods.

To increase the shelf life of fresh meats various techniques have been proposed. A general approach is to use modified atmosphere packaging (MAP). In MAP, the environment within the meat package is replaced using gas mixtures of oxygen (O_2), nitrogen (N_2), and carbon dioxide (CO_2). Due to the active biological systems after the slaughter, atmosphere inside the meat package continually changes during metabolic reactions that use up headspace gases and generate other gases like CO_2 and moisture. Therefore, composition of gases and packaging material (oxygen permeable polyvinyl chloride wrap; PVC) that can interact with the internal gas environment play a major role in extending shelf life and maintaining the quality of meats (Ohlsson & Bengtsson, 2002). Vacuum packaging and controlled atmosphere packaging (CAP) are mainly used in transport and storage of meat products. Absorbent pads can also be used to improve moisture retention and control microbial growth to limit spoilage. A mixture of citric acid and sodium bicarbonate has been used in absorbent liner pads to provide a controlled release of CO_2 to prevent meat spoilage organisms in fish packaging (Benedict, Strange, Palumbo, & Swift, 1975; Hansen, Mørkøre, Rudi, Olsen, & Eie, 2007).

Little information is available on using absorbent pads with trona mineral in retail meat packaging; some private companies claim to have had success using trona but no independent studies have been published. In addition to MAP, antioxidants can be used in meat packaging to reduce the extent of lipid and myoglobin oxidation (Sánchez-Escalante et al., 2001). In general antioxidants are classified as Type I and Type II. Type I antioxidants are radical quenchers (e^- or H^+ donors) that can stabilize their own radical qualities, interrupt the propagation step of the oxidation cycle and prevent the formation of lipid radicals, and other oxidized molecules. Type II antioxidants inhibit oxidation by

binding and inactivating the metal (Allen & Cornforth, 2010). The problem of maintaining quality and improving the shelf life of meats can be addressed using active packaging and antioxidants. There have been several studies that indicate the use of antioxidants or active packaging in foods can be beneficial, but most of this work is confined to comparison of only one or two antioxidants. Hence, a comprehensive study involving six different antioxidants mixed with ground poultry meat was conducted to understand their effectiveness in limiting color changes, lipid oxidation and aerobic counts.

2. Hypothesis

Shelf life of raw meats can be extended through the use of active packaging or incorporation of antioxidants. Specifically, incorporating CO_2 generating absorbent liner pads in retail packaging can extend the shelf life in fresh meats; and antioxidants can affect the action of non-heme/free iron on shelf life of ground chicken.

3. Objectives

1. To evaluate the effect of CO_2 generating absorbent pads on retail display shelf life of fresh meats by monitoring color change, lipid oxidation, and microbial counts in beef, chicken, and tuna.
2. To evaluate the effect of type I (radical quenching) and type II (metal chelating) antioxidants on the surface color, oxymyoglobin content, microbial load, and lipid oxidation in ground poultry meat.

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CHAPTER 2

LITERATURE REVIEW

1. Meat color

Consumers often distinguish and purchase fresh meat based on its color (Kropf, 1980). This intrinsic quality has an effect on how consumers evaluate their quality expectations and quality experience before consumption (Grunert, Bredahl, & Brunsø, 2004; Issanchou, 1996). Muscle color is affected by several factors such as feeding level, diet composition, age, sex and physical activity of the animal, antioxidant accumulation, glycogen storage, genetic variability, pre-harvest environment, pre-slaughter handling and stunning methods, post-harvest spraying, and packaging techniques, resulting in structural changes in myoglobin molecule (Allen & Cornforth, 2010; Channon, Payne, & Warner, 2000; Mancini & Hunt, 2005). According to (Bekhit & Faustman, 2005), consumer perception of desirable meat color is dependent on species: greyish pink color is considered normal for chicken, turkey, and pork where as bright red color in lamb and beef is recognized as fresh. Color measurement in meats can be evaluated visually, instrumentally, and digitally using computer vision. Digital images are believed to have advantages over traditional evaluation (O'Sullivan et al., 2003). Instruments such as colorimeters and spectrometers are available, however, selecting an appropriate method for color measurements is project specific and is related to experimental objectives (Mancini & Hunt, 2005). Instrumental measure of a* (redness - greenness), b* (yellowness - blueness), and L* (white - black) using a Hunter colorimeter, then calculating Chroma (color saturation), or hue angle (true redness) are commonly used methods to evaluate color stability in different meats (Hunt, 1991).

2. Myoglobin chemistry

Myoglobin (Mb) is a major intracellular protein found in skeletal and cardiac muscles that plays an important role in meat color (Bekhit & Faustman, 2005; Giddings, 1977; Livingston & Brown, 1981) It is a water-soluble single polypeptide globin, which binds oxygen (O_2) reversibly and promotes its diffusion in to mitochondria due to the differences in partial pressure (Wittenberg & Wittenberg, 1989). Mb has a prosthetic group heme (Fig 2.1.) with the central iron atom having six coordination sites: four bonded to nitrogen atoms within the porphyrin ring and the fifth site to the proximal histidine. The sixth coordination site is accessible for bonding with different ligands. The type of ligand at this site and the valence state of the iron atom will affect the meat color (Bekhit & Faustman, 2005; Mancini & Hunt, 2005).

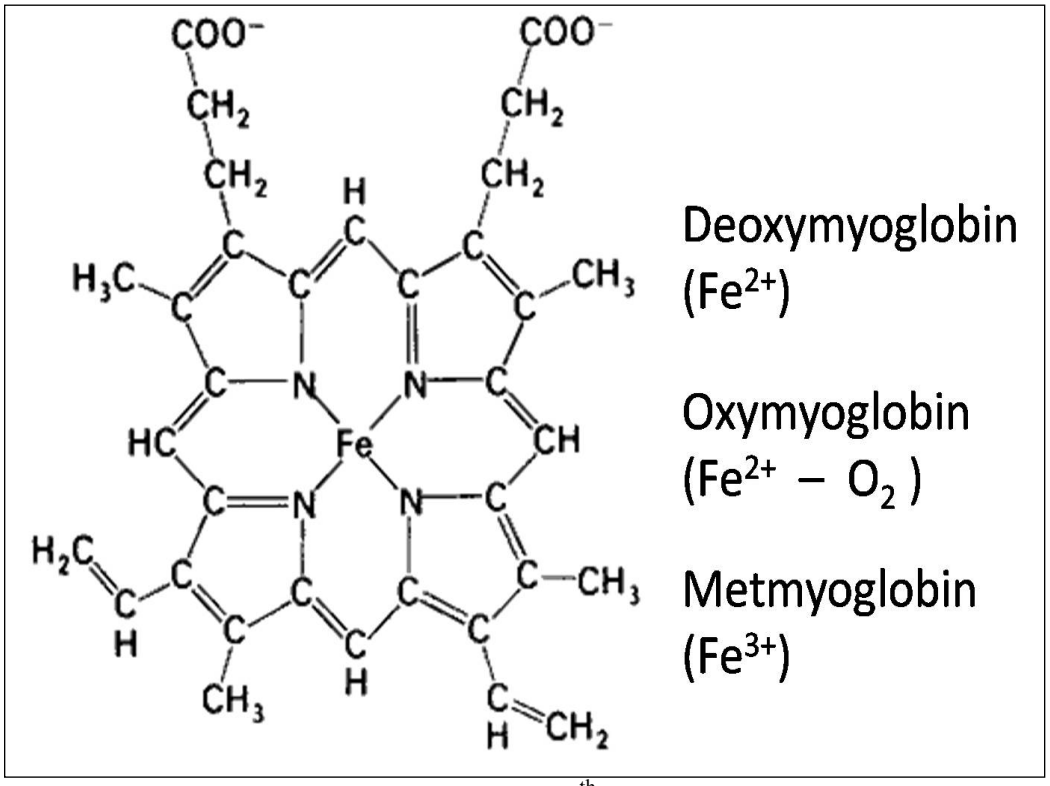

Fig 2-1. Structure of myoglobin heme showing 6th coordination site bound to oxygen (Allen, 2009)

Meat surface color is the result of the inter-conversion of Mb proteins into four major chemical forms oxymyoglobin (OMb), deoxymyoglobin (DMb), metmyoglobin (MMb), and carboxymyoglobin (COMb) as shown in Fig 2.2. Inter-conversion is influenced by many factors such as temperature, pH, O_2 partial pressure, and reducing activity of muscle enzymes. When heme iron is in a ferrous (Fe^{2+}) state and no ligand is bound, it results in DMb, which is purplish red in color. When Mb is exposed to O_2, it turns bright cherry red due to binding of O_2 to the 6^{th} coordination site forming OMb. Oxidation of DMb results in discoloration (brown color) by forming MMb where ferrous iron changes to ferric (Fe^{3+}; Wallace, Houtchens, Maxwell, & Caughey, 1982). With low levels of O_2 partial pressure OMb converts to MMb, which on further reduction due to muscle's reducing capacity forms DMb. Conversion of DMb into COMb is mainly a result of new packaging techniques requiring carbon monoxide gas in lower concentrations. In this whole process distal histidine-64 interacts with the bound ligand at 6^{th} coordination site thereby affecting Mb stability and function (Bekhit & Faustman, 2005; Mancini & Hunt, 2005; Suman & Joseph, 2013). Formation of MMb is the principal reason for meat discoloration, but it is not believed to accumulate in muscles of living animals in higher concentrations. This gave rise to a concept of MMb reducing systems, which can be potential approach in reducing MMb accumulation (Bekhit & Faustman, 2005). It is suggested that the amount of NADH present and MMb reducing activity play an important role in meat color stability (Mancini & Hunt, 2005).

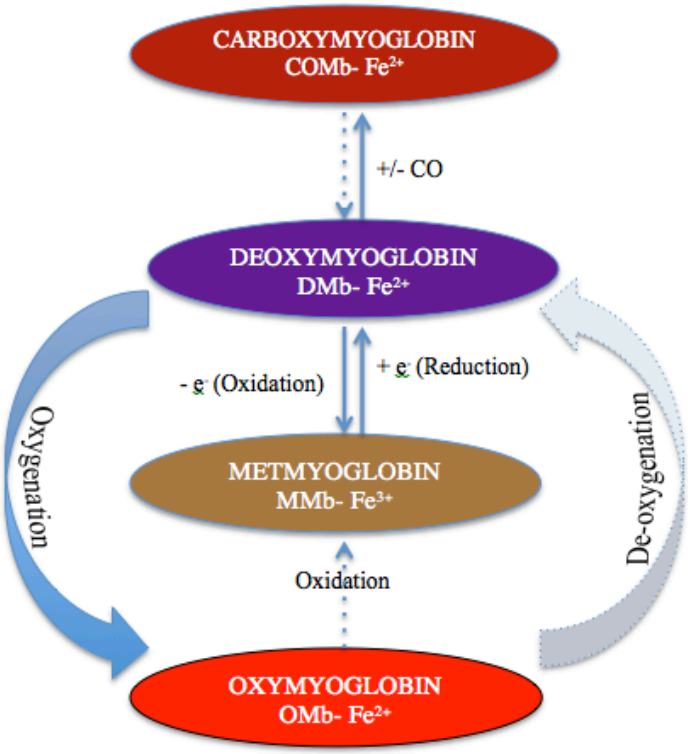

Fig 2-2. Simplified presentation of myoglobin inter-conversion due to redox reactions on meat surface. (Courtesy: Melvin & Andy, 2012)

3. Peroxidation

Peroxidation reactions involving primary oxidative changes in food lipids mainly lead to oxidative and non-oxidative secondary reactions through a free radical chain mechanism involving three phases initiation, propagation and termination (Fernández, Pérez-Álvarez, & Fernández-López, 1997; Gray, 1978; Ranken, 1994; Wassef, 1996) Below are the simplified step-by-step reactions of lipid oxidation.

Initiation:

$$RH + O_2 \longrightarrow R\bullet + \bullet OOH \qquad (a)$$

Propagation:

$$R\bullet + O_2 \longrightarrow ROO\bullet \qquad (b)$$

$$RH + ROO\bullet \longrightarrow ROOH + R\bullet \quad (c)$$

$$ROOH \longrightarrow RO\bullet + \bullet OH \quad (d)$$

Termination:

$$R\bullet + R\bullet \longrightarrow R\text{-}R \quad (e)$$

$$R\bullet + ROO \longrightarrow ROOR \quad (f)$$

$$ROO\bullet + ROO\bullet \longrightarrow ROOR + O_2 \quad (g)$$

In the above reactions RH represents fatty acid, O_2 is diatomic oxygen, R• is a lipid free radical, H• is hydrogen free radical, ROO• is a peroxide free radical, ROOH is a lipid hydroperoxide, R-R is lipid dimer and ROOR is lipid peroxide. Kanner (1994) found that oxidation of unsaturated lipids and cholesterol generates potentially toxic compounds. Free radicals formed by lipid peroxidation co-oxidize cholesterol that results in formation of oxysterols (cholestanetriol and 25-hydroxy-cholesterol) and induce atherogenicity. Changes in carcass temperature can affect the muscle by disrupting the cell structure or inactivating the antioxidant enzymes or by releasing O_2 and iron from the Mb resulting in catalysis of lipid oxidation. Therefore, addition of antioxidants to the raw meat can result in lowering the extent of lipid oxidation. However, oxidation of meats depends on various factors such as muscle type, presence of prooxidants, type of fat in diet, pH, enzymes, carcass temperature, ionic strength, and disruption of muscle membrane integrity (Chaijan, 2008; Gray, 1978; Gray, Gomaa, & Buckley, 1996).

4. Lipid & myoglobin oxidation in meat

Oxidation of unsaturated fatty acids in phospholipids and triacylglycerols is known as lipid oxidation or peroxidation (Faustman, Sun, Mancini, & Suman, 2010).

Lipid peroxidation is a major degradation reaction, which often results in a significant loss of meat product quality and development of oxidative rancidity (Ladikos & Lougovois, 1990). Lipid oxidation primarily results in alkyl, alkoxyl and peroxy radicals which later result in secondary oxidation products such as aldehydes, ketones, alcohols, hydrocarbons, esters, furans, epoxides, and cyclic peroxides (Faustman et al., 2010). Catalysis of lipid oxidation in meat systems is carried out by several mechanisms like photo-oxidation, reduced O_2 species such as hydrogen peroxide (H_2O_2), superoxide anion radical ($O_2^{-}\bullet$), hydroxyl radical (HO•), O_2 centered radicals of organic compounds (peroxyl, ROO• and alkoxyl, RO•), and active oxygen-iron complexes (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). Non-ruminant meats like fish and poultry have higher concentrations of poly-unsaturated fatty acids that might contribute to more rapid lipid oxidation (Enser, Hallett, Hewitt, Fursey, & Wood, 1996; Tichivangana & Morrissey, 1985). Faustman et al. (2010) found a high degree of lipid oxidation in ground meat in comparison to whole cuts, speculated to be due to increased surface area in contact with O_2. Lipid oxidation has a positive correlation with Mb oxidation, and it has been observed that oxidation of Mb forms chemical species that can accelerate the lipid oxidation and vice versa (Gray, 1978). Joseph et al. (2011) determined the amino acid sequence of turkey myoglobin and compared it to chicken myoglobin that revealed a 100% sequence similarity whereas 92.5% resemblance to ostrich myoglobin and 76.5% homogeneity with pig and 73% with ruminant myoglobin respectively. The differences were based on the total number of amino acids and histidine residues in proximal and distal positions. Transition metals, such as iron and copper generate species that can extract a proton from unsaturated fatty acids. The forms of iron (free or protein bound,

heme or non-heme, oxidized or reduced) are of great importance to the lipid peroxidation of meats. Kanner (1994), Kanner, Harel, and Granit (1992), and Chaijan (2008) reported that oxidation of OMb produces MMb and H_2O_2 that can induce lipid oxidation. Furthermore, end products of lipid oxidation, such as aldehydes, promote oxidation of OMb. Thus, understanding the relationship between lipid and Mb oxidation processes in meats can help increase meat quality and acceptability.

Several techniques are utilized for the measurement of lipid oxidation in meat products. Chemical methods include: peroxide value (AOAC, 1984), Kreis test (Watts & Major, 1946), total and volatile carbonyl compounds, thiobarbituric acid reactive substances test (Buege & Aust, 1978); and physical methods include: infrared spectroscopy, refractometry, polarography etc. (Barriuso, Astiasarán, & Ansorena, 2013; Moore & Roberts, 1998). Measures of lipid oxidation utilize indicator substrates, such as peroxide, hexanal, and malondialdehyde (Fernández et al., 1997). Commonly, lipid oxidation in-vitro is measured as TBA value or TBARS in mg of malondialdehyde (MDA) per kg of meat, where MDA is an intermediate formed by the oxidation of unsaturated fatty acids (Gray & Monahan, 1992). As MDA and other reactive substances are unstable under certain conditions, the TBA test may produce inconsistent outcomes (Fernández et al., 1997). Appropriateness of the method depends on the type of product, processing and storage method, and the degree of correlation of the particular method with sensory analysis (Igene, King, Pearson, & Gray, 1979; Ladikos & Lougovois, 1990). Secondary oxidation of primary carbonyl compounds results in MDA; similarly, endoperoxides, iron dependent oxidative degradation of amino acids, and complex carbohydrates that are TBA-reactive substances can also react with TBA because these

complexes absorb at a different wavelength than MDA-TBA, they can have a varying effect on the calculated TBARS value. Typically, these inferring compounds are removed (for example, by distillation) before adding the TBA.

5. Microbiology of meats

Meat is a highly perishable commodity. The biological composition of meat supports the growth of microorganisms, such as mold and bacteria, with bacteria predominating. During the process of animal harvest, varying levels of bacteria are present (Davies & Board, 1998). Conditions around the meat and treatments determine the level and type of bacteria that survive. Some of the commonly found gram negative spoilage bacteria associated with meat include psychrotropic strains of *Pseudomonas spp., Moraxella, Acinetobacter, Aeromonas, Enterobacteriaceae,* and gram-positive are *Lactobacillus spp., Brochothrix thermosphacta, Carnobacterium spp., Leuconostoc spp.,* along with facultative *Microbacterium thermosphactum,* and *Shewanella putrefaciens* (Borch, Kant-Muermans, & Blixt, 1996; Lambert, Smith, & Dodds, 1991). Some of the mesophilic bacteria in meat that can pose a health risk are *Escherichia coli, Salmonella spp., Staphylococcus aureus, Yersina enterocolitica, Clostridium spp., Campylobacter,* and *Listeria monocytogenes.* Most of the *Vibrio sp.*, bacteria are halophilic which easily spoil sea fish and cured meats because of their high salt and water content (Huis in't Veld, 1996).

Growth rates of aerobic spoilage flora *(Pseudomonads* and *Acinetobacter spp.)* in fresh meat and meat products is maximum when they utilize amino acids from the meat surface as observed by (Gill & Newton, 1977). *Pseudomonas* grow faster than competing

species because of greater affinity to O_2 and insensitivity to the presence of other strains (Gram et al., 2002). *Microbacterium spp.* are aerobic, that utilize glutamate from the meat surface after depletion of glucose, conversely, studies showed that anaerobic *Enterobacter sp*. could only utilize glucose and glucose -6-phosphate.

Defects due to spoilage bacteria in refrigerated meat products are off flavors, discoloration, gas production, slime production, and change in pH (McMillin, 2008). Antimicrobials are added to foods mainly to inhibit the growth of microorganisms and extend the shelf life, but they can affect quality attributes in foods (Mancini & Hunt, 2005). Reports from several studies (Pohlman, Stivarius, McElyea, Johnson, & Johnson, 2002; Stivarius, Pohlman, McElyea, & Apple, 2002) show that 1% ozonated water and 5% acetic acid used as antimicrobial agents decreased redness, whereas 200 ppm chlorine dioxide followed by 10% trisodium phosphate spraying influenced color of beef due to variation in pH. The presence of carbon dioxide (CO_2) as an antimicrobial resulted in reduction of aerobic growth rate of meat spoilage flora by 25-30% (Gill & Tan, 1980). In a study by (Vasavada, Carpenter, Cornforth, & Ghorpade, 2003) the addition of trisodium phosphate, as an antimicrobial, elevated the pH of meat resulting in improved color due to increase in water binding capacity. Antioxidants eugenol and rosmarinic acid act as antimicrobials that can inhibit the microbial growth and increase the shelf life in raw meats (Hernández, Ponce, Jaramillo, & Guerrero, 2009; Lee & Shibamoto, 2001). Some of the antimicrobials eugenol, thymol, anethole and menthol tested for their antibacterial activity showed eugenol to be effective against some of the *Salmonella* and *Vibrio Spp*. (Karapinar & Esen Aktuğ, 1987).

Spoilage due to microbial degradation may result in rejection of the product. Thermal pasteurization, high pressure processing (HPP) and irradiation of packaged meats have found to be promising methods to decrease the microbial activity (Nassu, Juarez, Uttaro, & Aalhus, 2010). Irradiation primarily improves the safety of the products by lowering the number of spoilage and pathogenic organisms, but irradiation of meat results in off flavors and odors (McMillin, 2008).

6. Meat packaging

Packaging is one approach of extending the color shelf life in foods. Packaging of foods was introduced to contain the product, protect it from external effects, provide convenience to consumers, and as a marketing tool (Yam, Takhistov, & Miltz, 2005). In the early 1950s, store cutting and display in refrigerated meat cases replaced butcher cutting and wrapping of meat in paper or waxed paper due to consumer demand (McMillin, 2008). The chemical industry has supplied the required plastic and various polymeric materials to meet the requirements of short term and long term storage packaging materials for the meat industry (McMillin, 2008). Fresh raw meat used to be packed in polystyrene trays and wrapped over with air permeable and moisture barrier PVC film, but this was later replaced by case-ready and centralized packaging. Centralized packaging has its own advantages like space and resource management, and improved quality that can increase profits (Jakobsen & Bertelsen, 2002).

Vacuum packaging (VP) is a type of MAP in which gases are removed by vacuum flushing. In the U.S., 90% of beef is shipped from meat packers to retailers and foodservice operations in VP primal cuts. In many cases, VP is believed to be a cost

effective strategy (Eilert, 2005), but the dark purple color of VP meats is not well received by the consumer. For commercial purposes, master bag packaging and modified atmospheric packaging (MAP) with high CO_2 or O_2 is used (Brody, 2007). Modified atmosphere packaging was developed, giving rise to fresh and minimally processed food preservation technology (Singh, Wani, Saengerlaub, & Langowski, 2011). It maintains the environmental conditions within a package using a mixture of atmospheric gases, such as O_2, CO_2, and nitrogen (N_2), along with trace amounts of carbon monoxide (CO), argon (Ar), or helium (He). High partial pressure of O_2 prevents Mb oxidation and maintains OMb but accelerates lipid oxidation, CO_2 inhibits the growth of spoilage and pathogenic bacteria, and N_2 acts as a filler gas (Ščetar, Kurek, & Galić, 2010). In controlled atmosphere packaging (CAP), environment around the product is continuously monitored using atmospheric gases and conditions like temperature, humidity etc., (Brody, 2005). Typically, fresh meat and poultry is displayed at retail stores in expanded polystyrene tray wrapped with O_2 permeable PVC or polyolefin film stretched and heat shrunk over the tray with product. Georgala and Davidson (1970) reported that meat color in beef can be maintained using a gas mixture of 80% O_2 and 20% CO_2. But rancid flavor develops within 6-10 days, even though high O_2 levels can maintain redness by delaying surface metmyoglobin formation (Jayasingh, Cornforth, Brennand, Carpenter, & Whittier, 2002). Jakobsen & Bertelsen (2000) reported that O_2 levels of 20% or more enhanced meat color, but an O_2 level over 55% did not show any further color stability. Huffman, Davis, Marple, and McGuire, (1975) and Gill and Tan (1980) suggested that CO_2 levels should not exceed 20% in MAP of meats because higher concentrations resulted in adverse effects on color. Jakobsen and Bertelsen (2002) reported that high

levels of CO_2 can cause discoloration of beef due to lowering pH, (which is caused by the absorption of dissociated carbonic acid by the meat tissue). However, the same authors reported data from many studies showing evidence of no discoloration in beef, pork or lamb packaging with 100% CO_2, followed by blooming on exposure to air and O_2. CO has been added to packaging because of its high affinity for myoglobin but CO usage has raised concerns among consumers that it masks the premature browning, oxidation and microbial spoilage in meats, whereas in Norway CO has been used in packaging for over two decades without any incidents (Nassu et al., 2010). An optimum use of 0.4 -1.0% CO in MAP, showed a desirable flavor and color (Sørheim, Aune, & Nesbakken, 1997). The United States FDA approved the use of CO in meat packaging in 2004 (Tarantino, 2009), though it is not used widely due to consumer perception.

7. Active packaging and current trends

Current consumer demands and marketing trends have led to the introduction of active packaging concept. Active packaging is defined as "a type of packaging that changes the condition of the packaging to extend shelf-life or improve safety or sensory properties while maintaining the quality of the food" (Vermeiren, Devlieghere, van Beest, de Kruijf, & Debevere, 1999). In active packaging several drip absorbent pads are used that have a superabsorbent polymer and two plastic films on either side that are permeable to water. This innovative food-packaging concept involves functions such as scavenging of O_2, release of CO_2, moisture, ethylene, or release of ethanol and flavors to promote antimicrobial activity. Antimicrobial agents that are used in packaging films are released over an extended period and control microbial contamination during storage,

transportation, and handling (Quintavalla & Vicini, 2002). A controlled release to generate antimicrobial activity without any migration of chemicals into the food can eliminate the risk and ensure safety to consumers. Some of the compounds that have antimicrobial activity when incorporated in food packaging are ethanol, sorbate, propionate, benzoate and their anhydrides (Han & Floros, 1997). In red meats, fish, and poultry, high levels of CO_2 (10-80%) are desirable where O_2 absorbers/CO_2 emitters are used in the form of sachets in packaging to decrease surface microbial growth and increase shelf life. Sachets contain either ferrous carbonate or a mixture of ascorbic acid and sodium bicarbonate (Vermeiren et al., 1999).

Desiccants and humectants that can regulate moisture/water content are used in meat systems to control surface water activity (a_w). Compounds such as polyacrylate salts and graft copolymers of starch are used to absorb water, thereby reducing the growth of spoilage bacteria. Propylene glycol, NaCl are examples of humectants, and desiccants like silica gel, molecular sieves, calcium oxide (CaO) are also commonly used in sachets (Vermeiren et al., 1999). The food industry's main concern regarding active packaging concept is fear of consumer resistance, lack of knowledge among consumers about its effectiveness, economic and environmental benefits (Vermeiren et al., 1999). Awareness among the consumer populations and acceptance can increase its usage, leading to development of this technology for the future.

8. Antioxidants

To improve oxidative stability of lipids in muscle foods and to extend their shelf life various antioxidants are used. Antioxidants are compounds that can slow down the rate of oxidation by scavenging free radicals like lipid alkyl/peroxy radicals, quench singlet O_2, and inactivate sensitizers. Antioxidants can decrease localized O_2 concentration, decompose peroxides, act as metal chelating agents, prevent chain initiation and propagation reactions (Halliwell, 1990; Sacchetti, Di Mattia, Pittia, & Martino, 2008). They can convert free radicals to more stable non-radicals by donating hydrogen atoms (Choe & Min, 2006). Antioxidants are classified into two categories, Type I (radical quenching) and Type II (metal chelating) antioxidants. Type I antioxidants like butylated hydroxytoluene (BHT) obstruct the oxidation cycle at the propagation step, thereby inhibiting the formation of additional lipid radicals, while Type II antioxidants such as ethylenediamine tetraacetic acid (EDTA) restrict the initiation step in lipid oxidation by binding metals such as iron and copper (Choe & Min, 2006). In a study by (Allen & Cornforth, 2009) Type II antioxidants were observed to be more effective inhibitors of Mb oxidation in beef when compared to Type I antioxidants in lipid free model system containing iron. Allen and Cornforth (2010) investigated the effectiveness of Type I (rosmarinic acid and eugenol) and Type II antioxidants (sodium tri-polyphosphate, phytate, and milk mineral) in fresh ground beef and found that even though metal chelating antioxidants (Type II) effectively preserved the color and prevented lipid oxidation they were not able to control the microbial growth as effectively as radical quenching antioxidants (Type I).

Herbs and spices have been traditionally used in foods as ingredients, to preserve flavor, and, for their antimicrobial properties. Compounds derived from plant products like rosemary, oregano, spices (e.g.: clove, cinnamon, and garlic), tea catechins (Karre, Lopez, & Getty, 2013), and fruit and plant extracts (e.g.: plum, grape seed extract, cranberry, pomegranate, bearberry, and pine bark extract) have been examined for their activity as Type I antioxidants. These natural antioxidants act through various mechanisms to inhibit lipid oxidation (Karre et al., 2013). Rosmarinic acid is an ester of caffeic acid commonly found in plants that have several biological activities, e.g. antiviral, antimicrobial, anti-inflammatory and antioxidant. It is synthesized from two amino acids L-phenylalanine and L-tyrosine (Petersen & Simmonds, 2003). Eugenol is a phenylpropene used in essential oils, flavorings and in medicine as an antiseptic and anesthetic and considered as a natural antibacterial and antioxidant; it is extracted from clove oil, nutmeg, cinnamon, basil etc. (Karapinar & Esen Aktuğ, 1987; Lee & Shibamoto, 2001). In a study by Djenane, Sanchez-Escalante, Beltran, and Roncalés (2003), absence of UV-radiation and treatment with antioxidants rosemary and vitamin C extended meat display life from 10 to 20 days. In recent years natural antioxidants have been extensively studied and used in foods due to consumer demand for “natural” or “organic” foods. Special focus on natural antioxidants and their re-emergence is mainly because of possible carcinogenic effects that can result from synthetic antioxidant use (Hernández et al., 2009).

Studies by Ladikos and Lougovois (1990) revealed that reducing sugars in the form of milk or whey products can improve color and act as antioxidants in red meats. Rosemary spice extract, sage, pepper, black pepper, ginger from ginger rhizome, rose

petals and allspice exhibited antioxidant activity and lowered TBA values in fresh, frozen and precooked pork patties and in fresh beef homogenates (Ladikos & Lougovois, 1990). Early work by Marusich et al. (1975) and Wood and Enser (1997) showed that Vitamin E, α-tocopherol is a major antioxidant found in animal tissues that delayed oxidation of meat. Animal intake of dietary vitamin E extended shelf life and prevented dripping due to decreased membrane phospholipase activity in chicken, turkey, beef, and pork by deposition of vitamin E in muscle. Animals exhibit different mechanisms through which they can control their exposure to reactive O_2 species. Enzymes like superoxide dismutase, catalase, aglutathione peroxidase, and peroxiredoxins work together to convert $O_2^{-\bullet}$ from H_2O_2 to H_2O and lower the production of $HO^{\bullet}$ (Yu, 1994). Some of the dietary factors that can contribute to the antioxidant defense system and can effect the pro- and anti-oxidant in animals are Vitamins A, C, E, B_{12}, riboflavin, niacin, folate, iron, copper, zinc, selenium, manganese, magnesium, carotenoids, and flavonoids (Morrissey et al., 1998).

In a study on rosemary (*R. officinalis* L.) and oregano leaf (*Origanum vulgare* L.) extracts' antioxidant activity (Hernández et al. 2009), ethanol oregano extract contained high concentrations of phenols, and rosemary extract showed more efficient antioxidant activity compared to oregano extract. However, greater phenol concentrations did not correlate with greater antioxidant activity and their effect on lipid and Mb oxidation was related to the extraction method employed, structural variations in antioxidant compound, storage temperature, and initial oxidation state of the meat samples. Four different natural antioxidants ascorbic acid, taurine, carnosine, rosemary extract and their combinations with ascorbic acid were evaluated for inhibition of oxidative changes. Rosemary was

effective against lipid and myoglobin oxidation and maintained desirable sensory characteristics for 20 days in beef while taurine did not have any antioxidant effect. Studies by Bekhit, Geesink, Ilian, Morton, and Bickerstaffe (2003) indicated that resveratrol slowed the oxidative process effectively when compared to quercetin, rutin and carnosine, however its effect was dependent on concentration and method of application. The USDA permits the application of ascorbic acid, iso-ascorbic acid, citric acid, sodium ascorbate, and sodium citrate as antioxidant treatments to slow down color changes in fresh beef, lamb and pork cuts (Allen & Cornforth, 2010).

In the United States, the Meat and Poultry Inspection Acts, and other state laws regulate the use of antioxidants in meats. In spite of extensive research the exact mechanism by which these antioxidants act under different processing conditions is yet to be understood.

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CHAPTER 3

EVALUATING THE EFFECT OF CO_2 GENERATING PADS ON QUALITY AND SHELF LIFE OF MEATS

Abstract

Shelf life of meat and meat products is related to lipid oxidation, pH, and microbial growth. These are the main factors that affect the nutritional, sensory and physical characteristics through the formation of toxic compounds, off flavors, off odors, undesirable color and gas production. The objective of this study was to compare the effect of newly developed CO_2 generating absorbent pad type to three other commercially available pads on the quality and shelf life of different meats. Samples of beef semitendinosus muscles, boneless and skinless chicken breast (pectoral muscle), and yellowfin tuna pectoral fin loins were obtained and packaged with four different pad types. Samples were stored at 4 ^{o}C in a walk-in cooler and analyzed daily for pH, surface color, extent of lipid oxidation, and microbial loads. Data was collected over a period of eight days. In all three meats, color, pH and lipid oxidation exhibited the same trend developing at a similar rate as typically expected during storage ($P < 0.05$). However, no differences ($P > 0.05$) were observed between the four tested pad types over the 8-day testing period.

1. Introduction

Consumers desire fresh and minimally processed foods. Maintaining the fresh meat quality during extended display and storage is a challenge. Various food packaging techniques have been employed to meet consumer demands but with only limited

success. Traditional packaging in meats concentrated mainly on maintaining a physical barrier around the product, while newer technologies are designed to influence the surrounding environments and preserve the quality. Traditional methods for preserving foods include thermal processing, drying, irradiation, and addition of antimicrobial agents or salt during processing steps; some of these methods cannot be applied to fresh meats, which led to the development of active packaging for meats (Quintavalla & Vicini, 2002). The idea of active packaging was developed to delay lipid oxidation, control moisture migration, and limit microbial growth using physical, chemical or biological action. Examples include the use of antimicrobial packaging technologies, carbon dioxide and oxygen emitters or scavengers, and moisture control agents (Pavelková & Flimelová, 2012).

Active ingredients can be included in the packaging materials in the form of sachets or pads. Slow migration of agents from the packaging material to the product or packaging headspace over an extended period can limit microbial growth, thus extending the shelf life (Quintavalla & Vicini, 2002). For example, silver substituted zeolite in the form of sachets has been used as a broad spectrum antimicrobial due to its effect on metabolic enzymes (Rahman, 2007). Antimicrobial films made with low density polyethylene and potassium sorbate (3% w/w) showed positive results in inhibiting bacterial and mold growth in packaged meats (Han & Floros, 1997). Oxygen scavengers used in absorbent pads can chemically absorb free oxygen, forming a stable oxide and extending shelf life of fresh meats (Benson & Payne, 2012). For frozen and fresh meats, grafts, copolymers, and polyacrylate salts can be incorporated in absorbent pads as desiccants to absorb the water inside the package and lower the surface water activity

(a_w), reducing spoilage microbes and extending shelf life without altering the organoleptic and nutritional properties of meat (Vermeiren, Devlieghere, van Beest, de Kruijf, & Debevere, 1999).

A desired effect can be produced in the package, rather than merely creating inert conditions, using intelligent packaging. Intelligent packaging systems can monitor changes inside package using bio sensors, and detect levels of oxygen, pathogens and other changes in metabolism (Nassu, Juarez, Uttaro, & Aalhus, 2010; Yam, Takhistov, & Miltz, 2005). These concepts of intelligent packaging and time temperature sensors along with radio frequency identification technology (RFIT) have been successful at the bench top prototype level. However, very few studies have been carried out to compare the effect of active packaging used at retail level in different meats. The aim of this study was to evaluate and compare the effectiveness of a newly developed CO_2 generating absorbent pad to three other commercially available absorbent liner pads in different meats (beef, chicken and tuna), packaged in foam trays and wrapped with a single layer of polyvinylchloride film to mimic retail packaging, in their ability to promote longer shelf life by limiting lipid oxidation, pH, color changes and inhibiting the growth of microorganisms.

2. **Methods and materials**

2.1. *Sample preparation*

To test the effect of CO_2 generating absorbent pads on beef, semitendinosus muscles (eye of round; IMPS #171C) were obtained from four different animals harvested in the Utah State University meat lab (Logan, UT) within 5 days of harvest. Beef muscles were split longitudinally then cut into 2.54 cm thick steaks. To examine the effect on chicken, pectoralis major muscles (bulk layer-packed boneless skinless whole breast with rib meat, without tenderloins, ice packed; US Trade Description #70603-22-1132111) were obtained from KOCH Foods (Chicago, IL) within 5 days of harvest. Chicken breasts from four different harvest lots were split and trimmed of rib ("dark") meat and excess fat. To examine the effect on fish, four individual yellowfin tuna loins (*Thunnus albacares*; skin-on; vacuum packaged on ice) were obtained from a local grocery store supplier within 36 h of harvest and were skinned, split longitudinally, trimmed of red muscle (deep muscle closest to the spine and ribs), and cut into 1.91 cm thick steaks.

Steaks cut from one individual loin (beef and tuna) were considered as one replicate, while the chicken breasts from one individual harvest lot were treated as one replicate. All steaks and breasts were individually packaged in a completely randomized manner using one the following pad types: (A) Sealed Air standard pad (control; Sealed Air Corp., Elmwood Park, NJ); (B) Sealed Air CO_2 generating pad (developed; Sealed Air Corp., Elmwood Park, NJ); (C) PPI Extendapak pad (Paper Pak Industries, La Verne, CA); and (D) International prime meat pad (Pactiv LLC, Lake Forest, IL). For each

replicate, seven loins or breast pieces were assigned to each pad type. Each of the four pad types were primarily designed to actively absorb drip from the meat and lock moisture inside the package while the CO_2 generating pad is expected to release gas when the drip from meat comes in contact with the trona mineral inside the absorbent pad.

2.2. *Packaging and storage*

Loin/breast pieces were packaged in Cryovac 4L Supermarket Trays, and overwrapped with a single layer of PVC film (O_2 permeability = 8400 cm3/(24 h x m2 x atm.) at 23°C; water vapor transmission = 83 g/(24 h x m2) at 23°C and 50% relative humidity). Steaks were stored in a walk-in cooler maintained at 4℃ (39℉). To simulate retail display conditions, steaks (beef & fish) and breast pieces were arranged in a single layer on racks equipped with fluorescent lighting (3500K/75CRI) per the *American Meat Science Association* meat color measurement guidelines (2012), with a distance of 14 inches between steak samples and light source. Lighting was switched on for the entire duration of the testing period. Steak/breast pieces from each pad type were randomly selected on days 0 through 7 for measuring and analyzing pH, color, lipid oxidation, and microbial load. Four replicates of each meat type were prepared and tested.

2.3. *Color measurement*

L^*, a^* and b^* values were measured using a HunterLab Miniscan portable colorimeter (Reston, VA, USA) with a 5 mm diameter aperture, set to use illuminant D-65. The colorimeter was standardized through a single layer of PVC film against both white and black standard tiles. Three color measurements were taken per sample. Hue

angle (true redness) was calculated as [arctangent (b^*/a^*)], and chroma (color saturation) was calculated as [$\sqrt{(a^{*2} + b^{*2})}$] (Hunt, 1991)

2.4. *pH*

A portable pH meter (HANNA Instruments HI99161, Ann Arbor, MI, USA) fitted with a semi-solid food probe was used to measure pH. The pH probe was inserted directly into the meat and held until pH reading was constant. For each sample three readings were taken.

2.5. *Total aerobic plate count (TAC)*

Microbial counts on the surface of meats were determined using a swabbing procedure. A 5 cm x 5 cm area on the top surface of steaks/breasts was swabbed with a 3M Swab-Sampler kit containing swab and 10mL Buffered Peptone Water Broth (3M Corporation, St. Paul, MN, USA) in three different directions. The swab was placed in the peptone water and vortexed for 1 min. Peptone water for dilutions was prepared by dissolving 15 g of dehydrated peptone water culture media (Neogen, Lansing, MI, USA) in 1 L of distilled water, and autoclaved for 15 min at 121°C. Serial dilutions were prepared and appropriate concentrations were plated on Petrifilm aerobic count plates (3M Corporation, St. Paul, MN, USA) in duplicate. Petrifilms were incubated at 32°C for 48 h. and the number of colonies were counted according to manufacturer guidelines and expressed as CFU/cm^2.

2.6. *Thiobarbituric acid reactive substances*

The thiobarbituric acid reactive substances (TBARS) assay was performed as described by (Buege & Aust, 1978). In brief, 1.0 g cores of meat were mixed with 3.0 ml of stock solution containing 0.375% thiobarbituric acid, 15% trichloroacetic acid, and 0.25 N HCl. The mixture was heated for 10 min in a boiling water bath (95^0C) to develop a pink color, then cooled under tap water and centrifuged (3148 X g; Beckman Coulter, Inc. Indianapolis, IN). The absorbance of the supernatant was measured spectrophotometrically at 532 nm. TBA values (mg MDA/kg of meat) were calculated using an extinction coefficient of 156,000 M^{-1} cm^{-1}as follows:

$$\text{TBA (mg/kg)} = A_{532} \times \frac{1\text{ M Chromagen}}{156{,}000} \times \frac{1\text{ mol/L}}{\text{M}} \times \frac{0.003\text{ L}}{1.0\text{ gmeat}} \times \frac{72.07\text{ gMDA}}{\text{mole}} \times \frac{1000\text{ g}}{\text{kg}}$$

2.7. *Statistical analysis*

Analysis of variance was performed using the proc mixed function in SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA). TBA values, chroma, hue angle, and L*, a*, b* values were evaluated as a split-plot design, with absorbent pad type as whole-plot factor and time as sub-plot factor. Proc mixed function was used with treatments, time and their interaction as fixed factors and replicate as random factor. A 95% statistical significance level (α =0.05) was used. Posthoc mean comparisons were made using the Tukey-Kramer adjustment. Pearson product moment correlations were used to look at the linear correlation between different characteristics (pH, TBA, color etc.).

Table 3-1 shows a comparison of results for all three meats (beef, tuna, and chicken) examined. No effect of treatment was seen in any of the meats. TBA and pH values were with in the normal range over the storage time of 7 days. A detailed discussion of each attribute in case of each meat follows.

Table 3-1.
Comparison of beef, tuna, and chicken meats based on the physical, chemical, and microbial testing parameters over 7 days: representing the effect of the treatments.

Category	Beef	Tuna	Chicken
Treatment Effect	No significance	No significance	No significance
Color	$17 < a^* < 13$ Normal range	$6 < a^* < 2$ Unacceptable by day 7	$48 < L^* < 51$ Normal range
TBARS	0.5 – 1.5 mg/kg of meat	0.1 – 0.3 mg/kg of meat	0.1 – 0.5 mg/kg of meat
Lipid Oxidation	Acceptable on day 7	Acceptable on day 7	Acceptable on day 7
pH	5.2 – 5.6	5.4 – 6.0	5.5 – 5.9
Microbial counts	< 3 log cfu/cm^2	> 7 log cfu/cm^2	> 7 log cfu/cm^2
pH vs. TAC	Particular trend due to differences in growth conditions		
Off-odors	Comparatively low in beef and tuna		Strong off-odor

3. Results and discussion

3.1. Beef

3.1.1. *Color*

Effect due to day ($P < 0.05$) was seen in beef samples for color where as treatment effect and interaction effect were not observed (Table 3-1). Changes in color (CIE L*, a*, and b*) values were seen in beef for all four treatments by the end of 7th day, as shown in (Table 3-2). L* decreased significantly ($P < 0.05$) by day 2 for CO_2 generating and PPI Extendapak pads. A similar trend ($P > 0.05$) was seen for all treatments (Fig 3-1). a* value is a common indicator of freshness in red meats, with higher values corresponding to a desirable 'red' or 'bright cherry red' color (Larraín, 2007). PPI Extendapak pad and international meat pad redness values declined ($P < 0.05$) by day 2 (Table 3-2), while CO_2 generating and control pads did not show significant changes in redness throughout the course of the study ($P > 0.05$). Overall, a* values had a tendency to decline (Fig 3-2) for all treatments, though this change was not always statistically significant. Yellowness intensity for beef loins decreased ($P < 0.05$) for all treatments by day 2, except for CO_2 generating pad which maintained b* values for 3 days (Fig 3-3).

3.1.2. *Thiobarbituric acid reactive substances*

The TBA values for beef samples are shown below in (Table 3-3). There was a significant ($P < 0.001$) effect on the beef steaks due to the day, but no treatment ($P > 0.999$) or treatment x day interaction effect ($P > 0.999$) was seen. In all four treatments TBA values had a tendency ($P > 0.05$) to increase through day 4, with significantly higher TBA values ($P < 0.05$) seen by days 5 and 6 (Fig 3-4).

Table 3-2.
(CIE L*, a*, b*) average color values by storage time (day 1& 7) of beef steaks stored at 4°C for 7 days.

Treatment	A	Lightness (L*)		A	Redness (a*)		A	Yellowness (b*)	
		Day 1[B]	Day 7[B]		Day 1[B]	Day 7[B]		Day 1[B]	Day 7[B]
Control pad	**ns**	42.66 ± 1.81 a	40.53 ± 1.55 a	**ns**	13.78 ± 2.39 a	12.35 ± 1.61 a	**2**	17.13 ± 1.33 a	15.43 ± 0.60 a
CO_2 pad	**2**	42.18 ± 0.98 a	40.35 ± 1.56 a	**ns**	14.06 ± 2.17 a	12.52 ± 1.21 a	**3**	17.22 ± 1.45 a	15.43 ± 0.49 a
PPI pad	**2**	40.87 ± 2.23 a	37.82 ± 3.79 a	**2**	15.57 ± 1.97 a	12.69 ± 2.91 a	**2**	18.15 ± 1.28 a	15.61 ± 1.59 a
International pad	**ns**	42.44 ± 1.77 a	39.44 ± 3.56 a	**2**	14.61 ± 2.89 a	11.75 ± 1.98 a	**2**	18.29 ± 1.70 a	14.46 ± 0.92 a

Control = Sealed Air standard pad; CO_2 pad = Sealed Air CO_2 generating pad; PPI pad = PPI Extendapak pad; International pad = International prime meat pad.
A: Time point where first significant difference ($P < 0.05$) occurs as compared to initial reading for each meat system.
B: Values represent mean ± standard deviation at a given time point.
Values sharing the same letters within a column are not significantly different ($P > 0.05$).

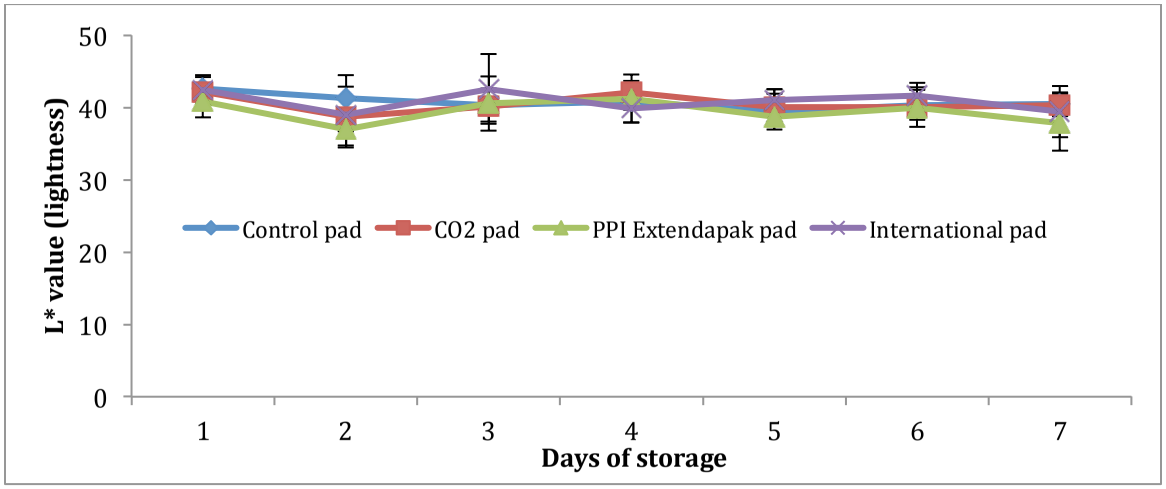

Fig. 3-1. HunterLab Miniscan CIE L* lightness values in beef steaks treated with four different treatments stored at 4 ^{0}C for 7 days. Error bars represent standard deviation of the mean at a given time point. Effect of day was seen ($P = 0.0174$)

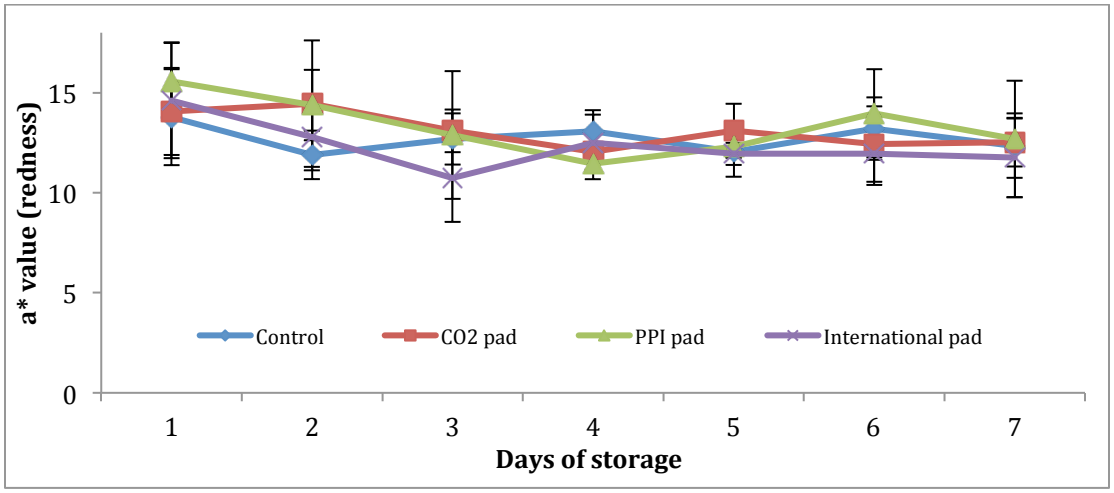

Fig. 3-2. HunterLab Miniscan CIE a* redness values in beef steaks treated with four different treatments stored at 4 ^{0}C for 7 days. Error bars represent standard deviation of the mean at a given time point. Effect of day was seen (P = 0.0061)

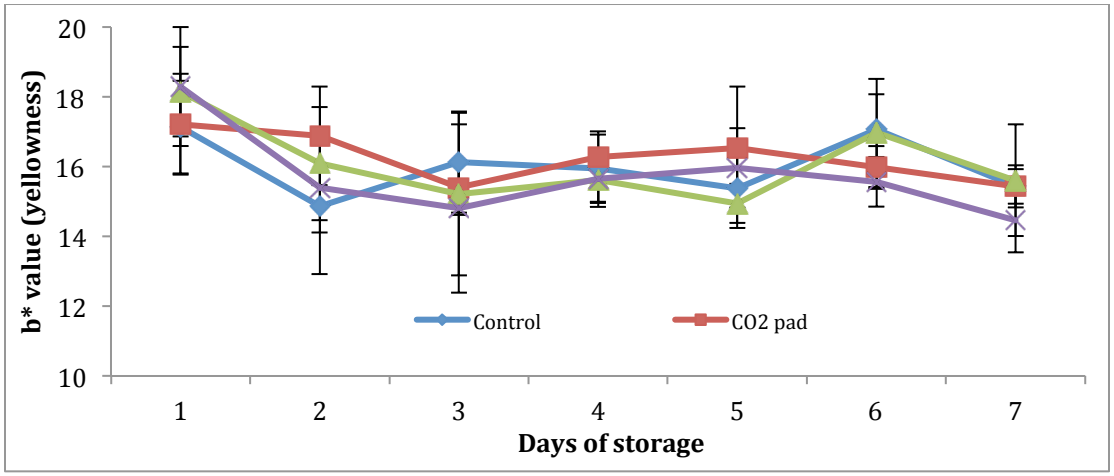

Fig. 3-3. HunterLab Miniscan CIE b* yellowness values in beef steaks treated with four different treatments stored at 4 ^{0}C for 7 days. Error bars represent standard deviation of the mean at a given time point. Effect of day was seen (P < 0.0001)

Table 3-3.
TBARS value for beef: time to first significant difference as compared to day 1 and means at day 1 and day 7

Treatment	Days to first significant difference[A]	TBARS	
		Day 1[B]	Day 7[B]
Control	5	0.28 ± 0.19 a	0.97 ± 0.85 a
CO_2 pad	5	0.27 ± 0.25 a	1.02 ± 0.91 a
PPI pad	6	0.27 ± 0.20 a	1.08 ± 0.85 a
International pad	5	0.28 ± 0.23 a	1.17 ± 1.11 a

Control = Sealed Air standard pad; CO_2 pad = Sealed Air CO_2 generating pad;
PPI pad = PPI Extendapak pad; International pad = International prime meat pad.
A: Time point where first significant difference ($P < 0.05$) occurs as compared to initial reading for each meat system formulation.
B: Values represent mean ± standard deviation at a given time point.
Values sharing the same letters within a column are not significantly different ($P > 0.05$).

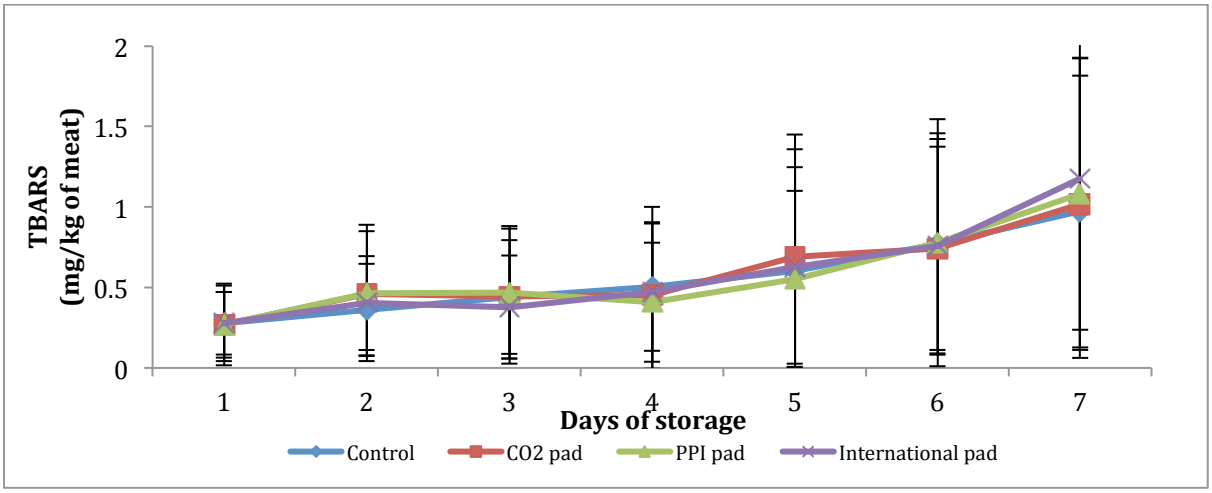

Fig. 3-4. TBARS values in beef steaks treated with four different treatments, and stored at 4 ^{0}C for 7 days. Error bars represent standard deviation of the mean at a given time point. Effect of day was seen ($P < 0.0001$)

3.1.3. *Total Aerobic Plate Count (TAC)*

Microbial growth in beef for all four treatments numerically increased ($P > 0.05$) through day 2. On day 2 microbial counts were highest in all the samples, but this peak was followed by a dramatic decrease ($P < 0.05$) on day 3. The low microbial counts remained constant ($P > 0.05$) throughout the remainder of the study (Fig 3-5).

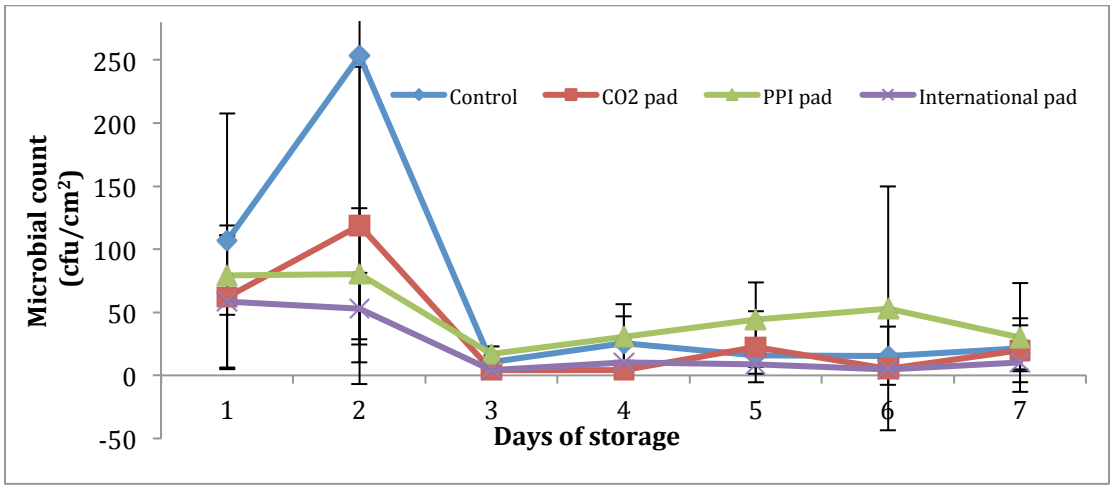

Fig. 3-5. TAC values in beef steaks treated with four different treatments, and stored at 4 ^{0}C for 7 days. Error bars represent standard deviation of the mean at a given time point. Effect of day and treatment were seen ($P < 0.0001$; $P = 0.0378$)

3.1.4. *pH measurement*

Differences ($P < 0.001$) were seen in beef pH values over the 7 days but no effect due to treatment and interaction effect of treatment and day were seen for any of the four treatments. All pH values recorded were between 5.21 – 5.42. On day 2 and day 5, higher ($P < 0.05$) pH values were seen in sealed air CO_2 pad type and international pad type samples as compared to day 1 (Fig 3-6), though values were within the accepted "normal" pH range for fresh beef (pH < 5.7; Tarrant & Sherington, 1980).

3.2.Tuna

3.2.1. *Color*

L* values increased ($P < 0.05$) by day 5 for control samples, but no changes ($P > 0.05$) were seen for other package types (Table 3-4). Tuna a* values tended ($P > 0.05$) to decrease over the course of the experiment, with significant ($P < 0.05$) decreases by day 2 for international pad type, day 3 for PPI Extendapak pad type, and day 7 for CO_2 pad

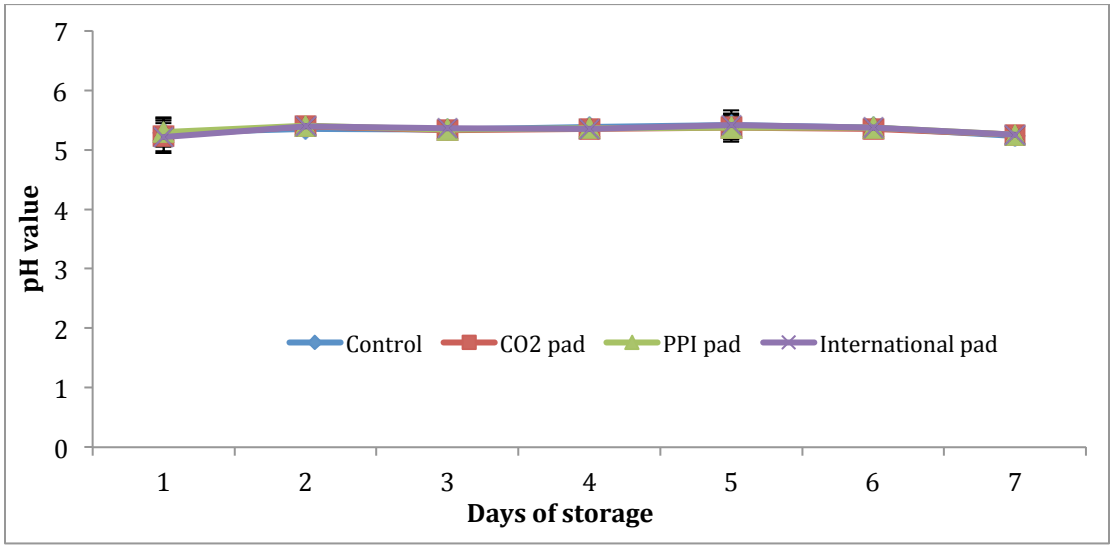

Fig. 3-6. pH values in beef steak samples treated with four different treatments, and stored at 4 ^{0}C for 7 days. Error bars represent standard deviation of the mean at a given time point. Effect of day was seen (P = 0.0002)

type (Fig 3-7). Tuna b* values tended (P > 0.05) to decrease over the course of the study (Fig 3-9).

3.2.2. *Thiobarbituric acid reactive substances*

Lipid oxidation values were low over the entire 7-day testing period in tuna samples (Table 3-5). Lipid oxidation tended to increase (P > 0.2238) through day 3 in all four treatments, but by day 4 lipid oxidation values decreased significantly (P < 0.0012) except in PPI Extendapak pad type compared to previous days. By day 7, TBA values increased in all the samples (P < 0.05; Fig 3-10).

Table 3-4.

(CIE L*, a*, b*) average color values by storage time (day 1& 7) of tuna steaks stored at 4°C for 7 days.

Treatment	A	Lightness (L*)		A	Redness (a*)		A	Yellowness (b*)	
		Day 1B	Day 7B		Day 1B	Day 7B		Day 1B	Day 7B
Control pad	**6**	21.34 ± 1.81 a	25.51 ± 3.40 a	**ns**	3.31 ± 1.10 a	2.40 ± 1.33 a	**5**	4.40 ± 0.71 a	3.71 ± 1.64 a
CO_2 pad	**ns**	25.08 ± 3.05 a	22.99 ± 1.08 a	**7**	2.99 ± 1.14 a	1.41 ± 0.58 a	**6**	4.57 ± 0.35 a	3.31 ± 0.59 a
PPI pad	**ns**	23.29 ± 0.80 a	23.47 ± 3.99 a	**3**	4.30 ± 1.70 a	2.01 ± 0.24 a	**6**	4.97 ± 1.23 a	3.51 ± 0.21 a
International pad	**ns**	23.98 ± 5.61 a	22.88 ± 4.76 a	**2**	2.19 ± 0.90 a	2.43 ± 0.59 a	**ns**	3.56 ± 0.65 a	3.30 ± 1.14 a

Control = Sealed Air standard pad; CO_2 pad = Sealed Air CO_2 generating pad; PPI pad = PPI Extendapak pad; International pad = International prime meat pad.

A: Time point where first significant difference ($P < 0.05$) occurs as compared to initial reading for each meat system.

B: Values represent mean ± standard deviation at a given time point.

Values sharing the same letters within a column are not significantly different ($P > 0.05$).

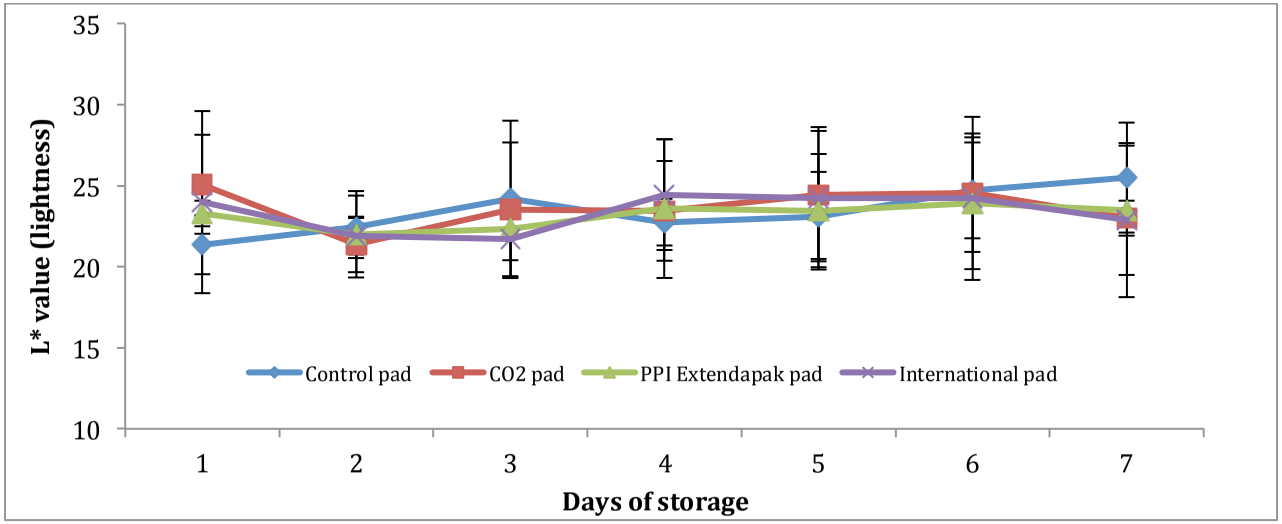

Fig. 3-7. HunterLab Miniscan CIE L* lightness values in tuna steaks treated with four different treatments stored at 4 °C for 7 days. Error bars represent standard deviation of the mean at a given time point. Effect of day was not seen ($P = 0.0853$)

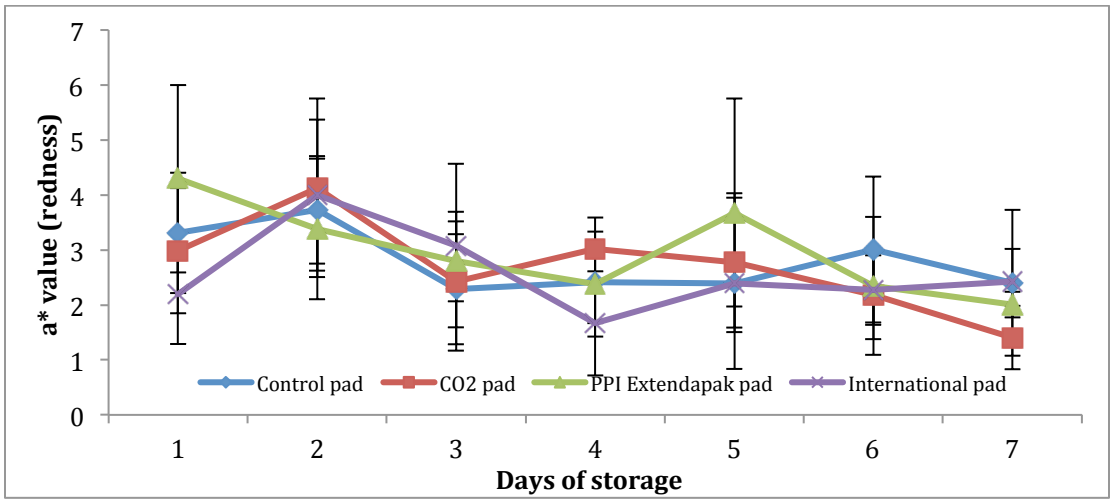

Fig. 3-8. HunterLab Miniscan CIE a* redness values in tuna steaks treated with four different treatments stored at 4 ^{o}C for 7 days. Error bars represent standard deviation of the mean at a given time point. Effect of day was seen ($P < 0.0001$)

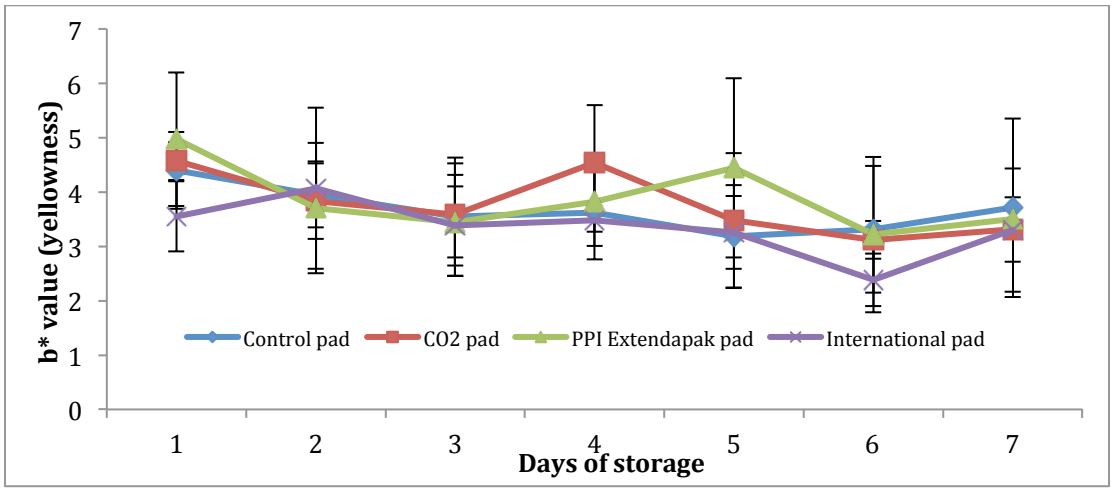

Fig. 3-9. HunterLab Miniscan CIE b* yellowness values in tuna steaks treated with four different treatments stored at 4 ^{o}C for 7 days. Error bars represent standard deviation of the mean at a given time point. Effect of day was seen ($P = 0.001$)

Table 3-5.
TBARS value for tuna: time to first significant difference as compared to day 1 and means at day 1 and day 7

Treatment	Days to first significant difference[A]	TBARS	
		Day 1[B]	Day 7[B]
Control pad	6	0.14 ± 0.04 a	0.19 ± 0.08 a
CO_2 pad	7	0.13 ± 0.04 a	0.21 ± 0.13 a
PPI pad	6	0.11 ± 0.01 a	0.20 ± 0.12 a
International pad	6	0.13 ± 0.03 a	0.24 ± 0.05 a

Control = Sealed Air standard pad; CO_2 pad = Sealed Air CO_2 generating pad;
PPI pad = PPI Extendapak pad; International pad = International prime meat pad.
A: Time point where first significant difference ($P < 0.05$) occurs as compared to initial reading for each meat system formulation.
B: Values represent mean ± standard deviation at a given time point. Values sharing the same letters within a column are not significantly different ($P > 0.05$).

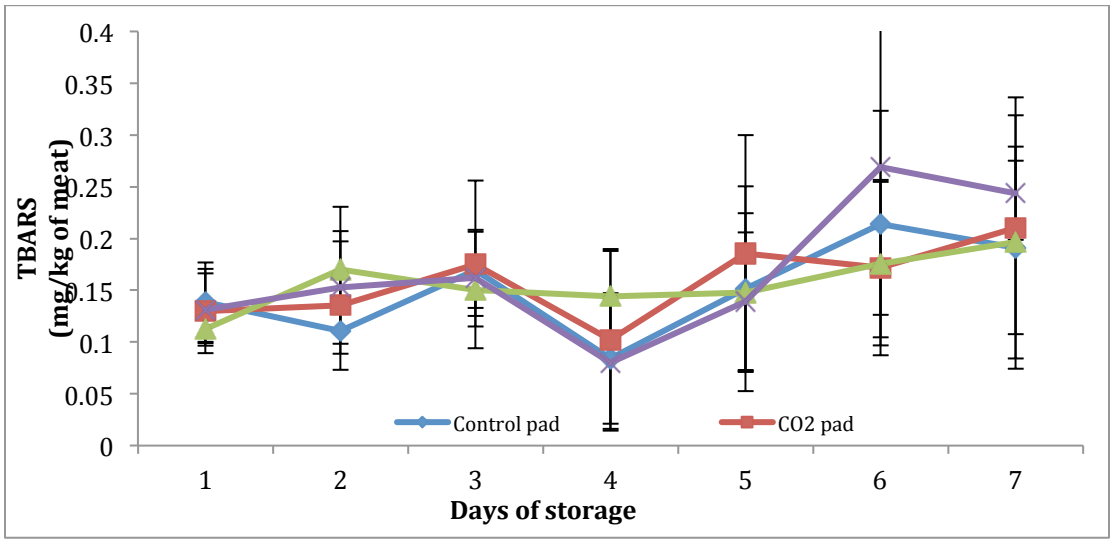

Fig. 3-10. TBARS values in tuna steaks treated with four different treatments, and stored at 4 °C for 7 days. Error bars represent standard deviation of the mean at a given time point. Effect of day was seen ($P < 0.0001$)

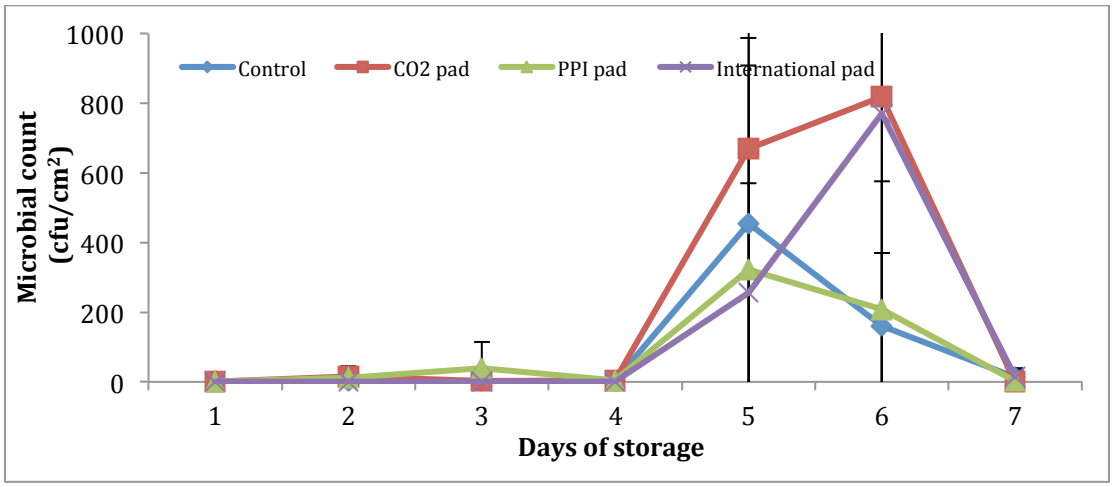

Fig. 3-11. TAC values in tuna steaks treated with four different treatments, and stored at 4 ^{o}C for 7 days. Error bars represent standard deviation of the mean at a given time point. Effect of day was seen (P = 0.0001).

3.2.3. *Total Aerobic Plate Count (TAC)*

TAC in tuna remained stable during first 4 days of testing period in all four treatments. From day 4 to day 5 there was a significant increase ($P < 0.05$) in the number of colonies, which further increased through day 6 for sealed air CO_2 pad and international pad types. By day 7 microbial counts decreased in all treatments (Fig 3-11).

3.2.4. *pH measurement*

For tuna samples with sealed air control pad type, pH values increased significantly ($P < 0.05$) through day 2 then remained constant through day 6. For the other three treatments, pH values remained relatively stable until day 5, followed by a significant decrease ($P < 0.05$) in pH values on day 6. pH increased significantly ($P < 0.05$) on day 7 for all four treatments (Fig 3-12, Table 3-6). In all cases, pH was within the normally accepted range for tuna of 5.2 – 6.1 (US-FDA, 2012).

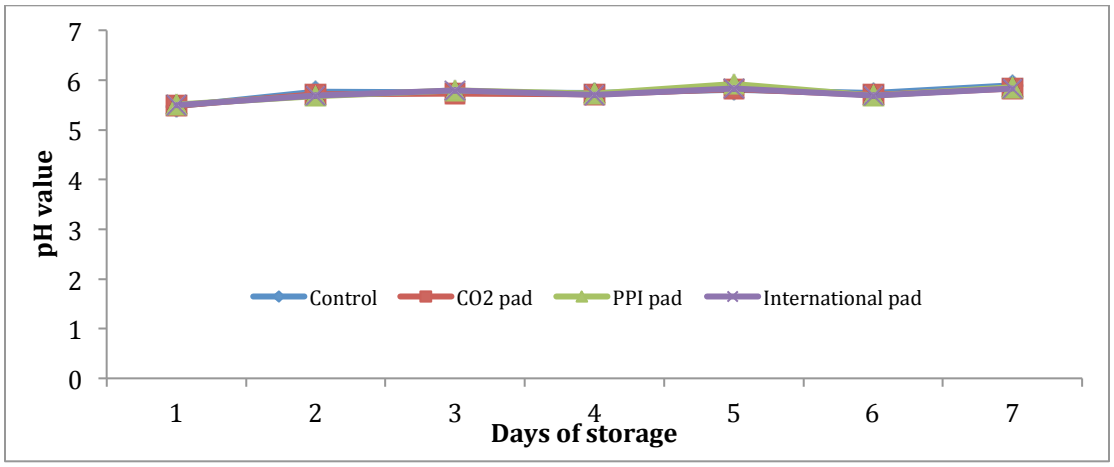

Fig. 3-12. pH values in tuna steaks treated with four different treatments, and stored at 4 ^{o}C for 7 days. Error bars represent standard deviation of the mean at a given time point. Effect of day was seen ($P < 0.0001$).

Table 3-6. The pH values for tuna: time to first significant difference as compared to day 1 and means at day 1 and day 7

Treatment	Days to first significant difference[A]	pH	
		Day 1[B]	Day 7[B]
Control pad	2	5.48 ± 0.08 a	5.89 ± 0.07 a
CO_2 pad	2	5.48 ± 0.05 a	5.83 ± 0.01 a
PPI Extendapak pad	2	5.51 ± 0.10 a	5.86 ± 0.03 a
International pad	2	5.50 ± 0.08 a	5.83 ± 0.02 a

Control = Sealed Air standard pad; CO_2 pad = Sealed Air CO_2 generating pad;
PPI pad = PPI Extendapak pad; International pad = International prime meat pad.
A: Time point where first significant difference ($P < 0.05$) occurs as compared to initial reading for each meat system formulation. B: Values represent mean ± standard deviation at a given time point. Values sharing the same letters within a column are not significantly different ($P > 0.05$).

3.3. Chicken

3.3.1. *Color*

Chicken L* values increased ($P < 0.05$) on day 2 in samples with sealed air control pad type, but remained constant for other treatments (Table 3-7, Fig 3-13). Similarly, in the case of redness, values tended to increase ($P > 0.05$) gradually through

day 7 (Fig 3-14). Conversely, b* values decreased significantly ($P < 0.05$) by day 3 for the international pad type treatment (Table 3-7; Fig 3-15). No other differences in b* value were seen.

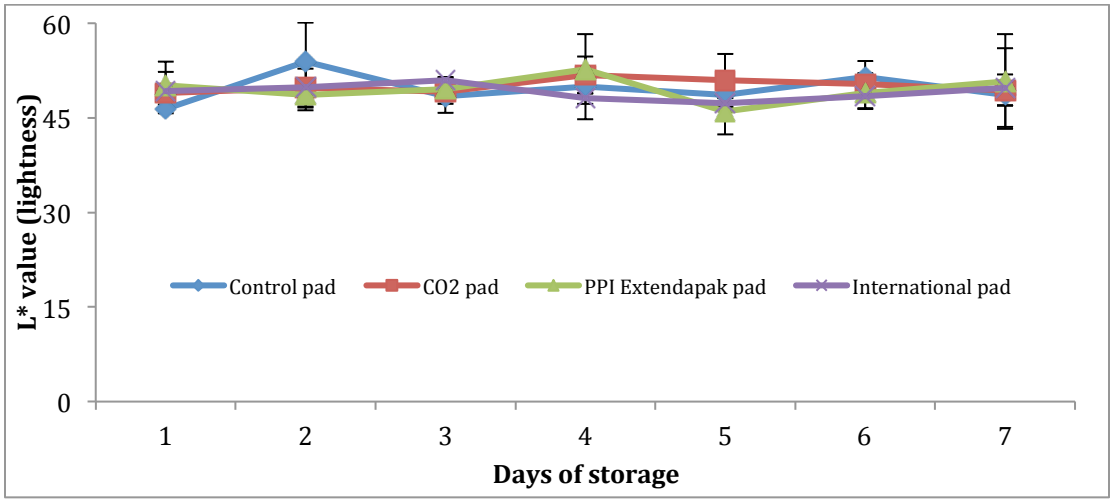

Fig. 3-13. HunterLab Miniscan CIE L* lightness values in chicken (pectoralis muscle) treated with four treatments and stored at 4 °C for 7 days. Error bars represent standard deviation of the mean at a given time point.

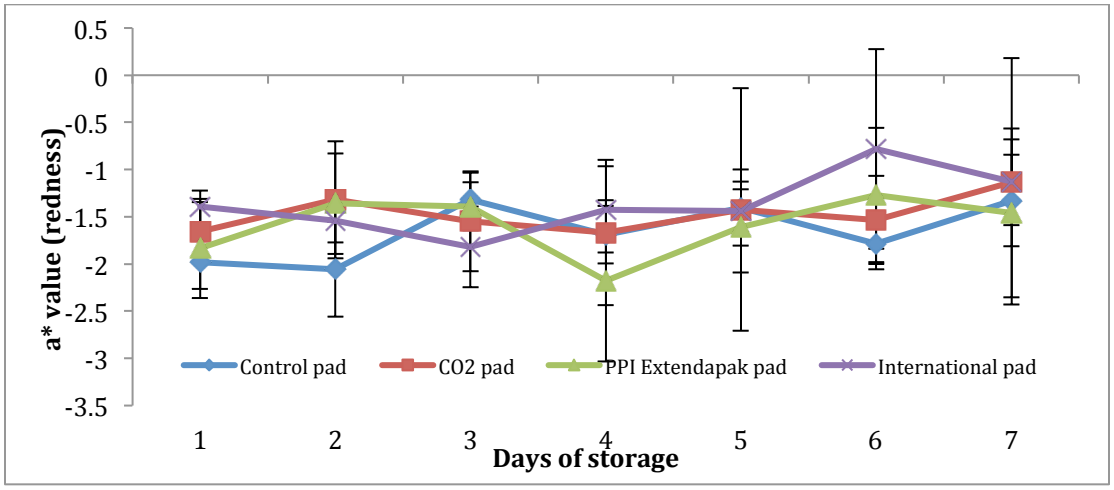

Fig. 3-14. HunterLab Miniscan CIE a* redness values in chicken (pectoralis muscle) treated with four different treatments, and stored at 4 °C for 7 days. Error bars represent standard deviation of the mean at a given time point.

Table 3-7.

(CIE L*, a*, b*) average color values by storage time (day 1& 7) of chicken samples stored at 4°C for 7 days.

Treatment	A	Lightness (L*)		A	Redness (a*)		A	Yellowness (b*)	
		Day 1B	Day 7B		Day 1B	Day 7B		Day 1B	Day 7B
Control pad	**2**	46.40 ± 0.69 a	48.59 ± 1.59 a	**ns**	-1.98 ± 0.28 a	-1.33 ± 0.48 a	**ns**	2.44 ± 1.15 a	3.48 ± 0.51 a
CO_2 pad	**ns**	48.91 ± 1.35 a	49.38 ± 2.47 a	**ns**	-1.66 ± 0.32 a	-1.14 ± 0.45 a	**ns**	3.32 ± 0.97 a	2.68 ± 2.04 a
PPI pad	**ns**	50.18 ± 3.70 a	50.77 ± 7.52 a	**ns**	-1.84 ± 0.53 a	-1.46 ± 0.89 a	**ns**	3.15 ± 1.90 a	3.11 ± 1.06 a
International pad	**ns**	49.28 ± 3.04 a	49.75 ± 6.23 a	**ns**	-1.39 ± 0.17 a	-1.13 ± 1.30 a	**3**	4.66 ± 0.35 a	2.89 ± 1.99 a

Control = Sealed Air standard pad; CO_2 pad = Sealed Air CO_2 generating pad; PPI pad = PPI Extendapak pad; International pad = International prime meat pad.

A: Time point where first significant difference ($P < 0.05$) occurs as compared to initial reading for each meat system.

B: Values represent mean ± standard deviation at a given time point. Values sharing the same letters within a column are not significantly different ($P > 0.05$).

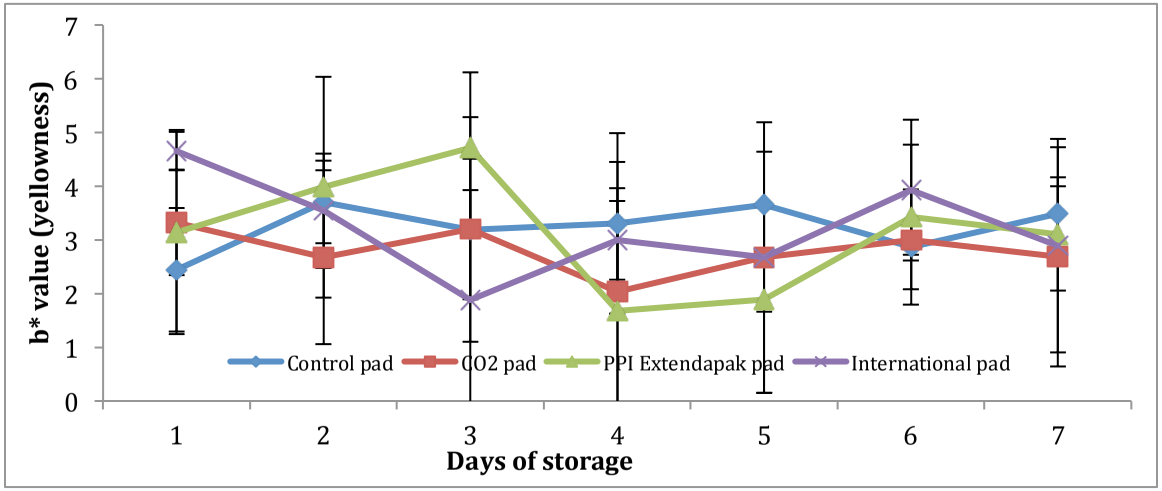

Fig. 3-15. HunterLab Miniscan CIE b* yellowness values in chicken (pectoralis muscle) treated with four treatments, and stored at 4 °C for 7 days. Error bars represent standard deviation of the mean at a given time point.

3.3.2. *Thiobarbituric acid reactive substances*

In chicken samples with control pad type, TBA values remained constant ($P > 0.05$) through day 4, and then increased ($P < 0.05$) through day 7. In case of CO_2 generating pad type, lipid oxidation values remained stable for 2 days then increased ($P < 0.05$) until day 7. For PPI Extendapak pad type, TBA values tended to increase ($P > 0.05$) over storage time, with significant increases ($P < 0.05$) seen by day 7 (Fig 3-16; Table 3-8).

Table 3-8.TRABS values for chicken: time to first significant difference as compared to day 1 and means at day 1 and day 7

Treatment	Days to first significant difference[A]	TBARS	
		Day 1[B]	Day 7[B]
Control	4	0.05 ± 0.02 a	0.19 ± 0.01 a
CO_2 pad	3	0.06 ± 0.02 a	0.31 ± 0.10 a
PPI pad	6	0.11 ± 0.07 a	0.27 ± 0.08 a
International pad	7	0.19 ± 0.10 a	0.33 ± 0.14 a

Control = Sealed Air standard pad; CO_2 pad = Sealed Air CO_2 generating pad;
PPI pad = PPI Extendapak pad; International pad = International prime meat pad.
A: Time point where first significant difference ($P < 0.05$) occurs as compared to initial reading for each meat system formulation. B: Values represent mean ± standard deviation at a given time point. Values sharing the same letters within a column are not significantly different ($P > 0.05$).

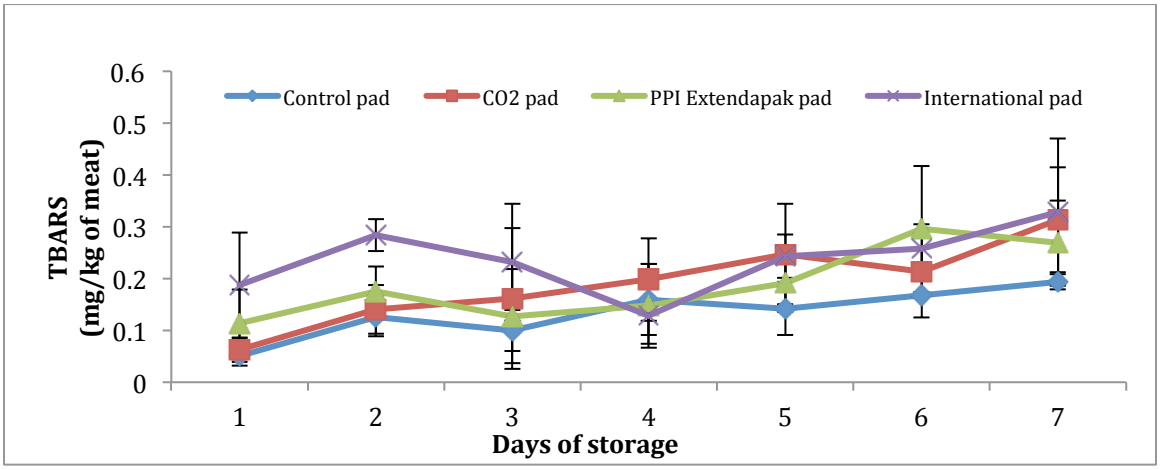

Fig. 3-16. TBARS values in chicken breast treated with four different treatments, and stored at 4 °C for 7 days. Error bars represent standard deviation of the mean at a given time point. Effect of day was seen ($P < 0.0001$)

3.3.3. *Microbial growth and pH*

Microbial growth curve for chicken samples over the seven-day testing period is shown below (Fig 3-17). TAC increased ($P < 0.05$) from day 1 to day 3 for sealed air CO_2 pad, PPI Extendapak pad and international pad types. After day 3 growths declined in the samples and were relatively constant until day 7. The pH values in all chicken samples increased ($P < 0.05$) by day 4, then decreased ($P < 0.05$) through day 6 (Fig 3-18). Average pH values for chicken samples were between 5.43 – 5.83, falling below the normal range of 6.7 (Davies & Board, 1998).

3.4. Correlations

In all the three meats (beef, chicken, and tuna) a* and b* had a strong positive correlation (*$r = 0.69, P < 0.0001; r = 0.58, P < 0.0001; r = 0.71, P < 0.0001,$ respectively*), indicating that as samples became darker/less red and more yellow. This is consistent with visual observations (data not shown) made by the researchers on day 6 and 7. Off odors in beef might be due to lipid oxidation, whereas deterioration of chicken and tuna samples may be the result of high microbial growth. Total aerobic plate count was higher for tuna (Fig 3.15), which could have resulted in increased ($P < 0.05$) pH by day 7, as some of the previous researchers (Allen, Fletcher, Northcutt, & Russell, 1998; Du et al., 2001; Jay, 1995) have reported the similar effect. High correlation between redness and chroma values for tuna was (*$r = 0.90, P < 0.0001$*) and for beef was (*$r = 0.92, P < 0.0001$*), which is most likely due to higher myoglobin content of red meats when compared to white meats (Sánchez-Zapata, 2011).

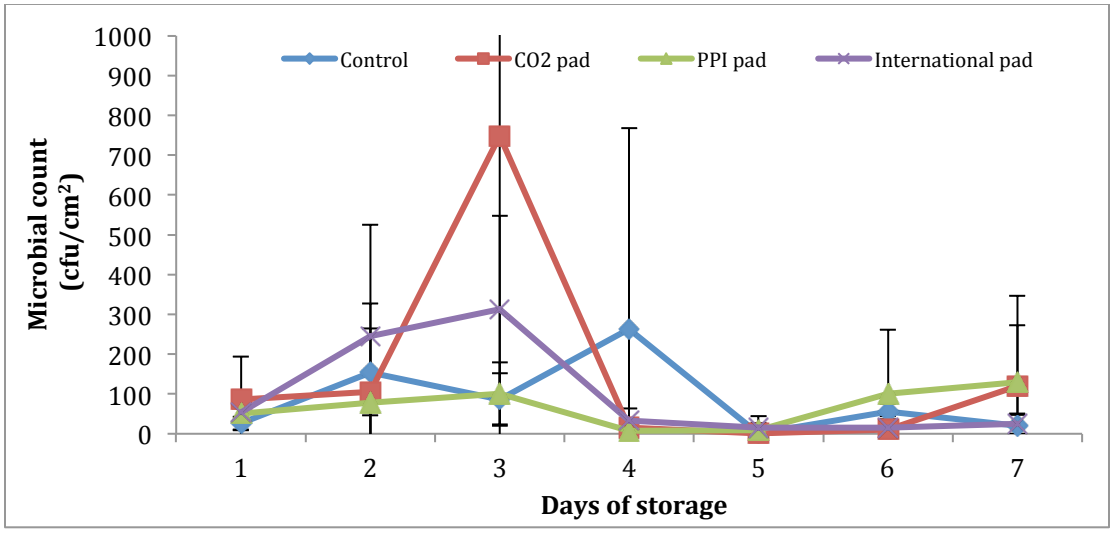

Fig. 3-17. TAC values in chicken samples treated with four different treatments, and stored at 4 ^{o}C for 7 days. Error bars represent standard deviation of the mean at a given time point.

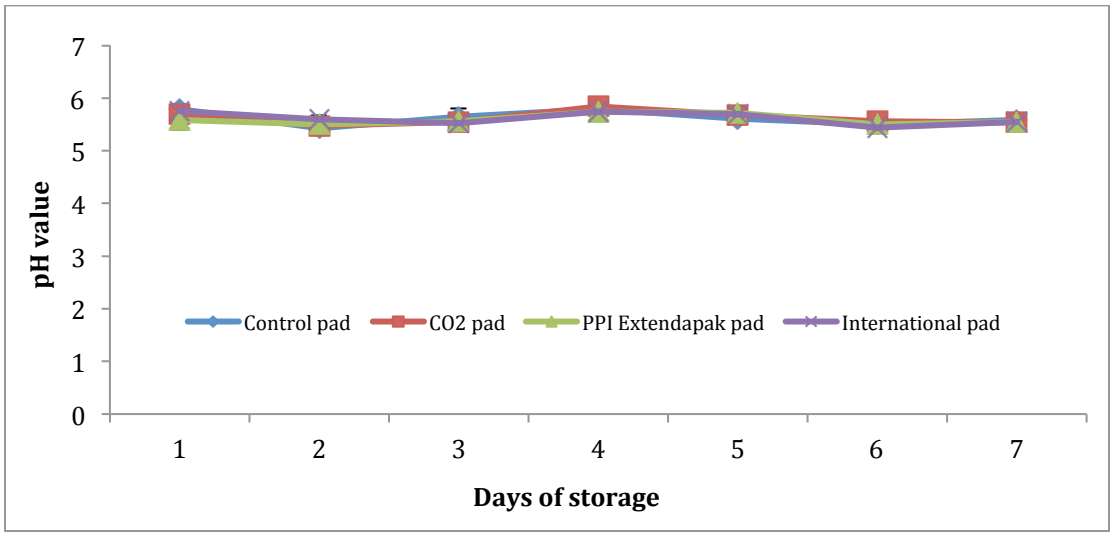

Fig. 3-18. pH values in chicken samples treated with four different treatments, and stored at 4 ^{o}C for 7 days. Error bars represent standard deviation of the mean at a given time point. Effect of day was seen ($P < 0.0001$)

3.5. Overall Comparisons

Excessive level of color change was not identified in any of the four treatments (Table 3-1). In beef steaks color values decreased, which is common in retail packaging due to other quality and chemical factors like pH, and lipid oxidation etc. (Karamucki, Gardzielewska, Rybarczyk, Jakubowska, & Natalczyk, 2010). A similar decreasing trend was seen in most of the tuna samples over the testing period, but in the case of chicken, lightness and redness values increased ($P > 0.05$). For tuna, lightness values for control pad and PPI Extendapak pad types increased ($P > 0.05$) by day 7. Previous studies suggest this is likely due to the water holding capacity of those samples (Sánchez-Zapata, 2011). Similar observations were seen in broiler breast chicken by (Allen, Russell, & Fletcher, 1997).

Beef samples were more susceptible to lipid oxidation changes, and by day 7 TBA values in beef reached above 1 mg MDA/kg of meat. This is consistent with informal observations made during sample testing, indicating a small degree of rancidity and off odors. Even though none of the treatments prevented lipid oxidation, TBA values remained lower than 2 mg MDA/kg, which is identified as the minimum value for strong off odors and high rancidity development, and the threshold for consumer acceptability (Greene & Cumuze, 1982). Several studies reported that susceptibility of muscle to undergo lipid oxidation depends on the species, presence of mono/poly unsaturated fatty acids and the positioning of double bonds (Rhee, 1999; Wood, Enser, Richardson, & Whittington, 2007). In this study TBA values for chicken were lower compared to the values for beef, which is consistent with previous studies under similar conditions that

found raw chicken was more resistant to oxidative changes than red meats (Min & Ahn, 2009; Rhee & Ziprin, 1987; Siu & Draper, 1978).

In beef samples, sealed air CO_2 generating pad and international prime meat pad had lower ($P < 0.05$) microbial counts than control pad and PPI Extendapak pad types. In comparison to chicken (> 7 log10 cfu/cm^2) and tuna (> 7 log10 cfu/cm^2), aerobic counts were low for beef (< 3 log10 cfu/cm^2). For beef and chicken samples, onset of log phase was during the first 3 days, while for tuna it started from day 4. This delayed growth was due to the species specific spoilage microorganisms in chicken (*S. aureus, Pseudomonas* spp.*, B. thermosphacta, Lactic acid bacteria, Salmonella*) and fish (*Vibrionaceae* spp.*, Photobacterium phosphoreum, Shewanella* spp., etc.,) which was previously explained in (Gram & Dalgaard, 2002; Gram et al., 2002) studies to affect the microbial growth pattern.

H+ ions concentration and undissociated acids influence the growth of bacteria in meat. In general pH in beef ranges from 5.1 – 6.2, for chicken 5.7 – 6.8, and for tuna 5.2 – 6.1 (Davies & Board, 1998; US-FDA, 2012). For all the three meats pH values were within acceptable ranges over the entire testing period.

4. Conclusion

In this study inclusion of CO_2 generating absorbent pads and other commercially available meat absorbent pads were used to observe the quality changes by modification of packaging materials in different meats. Even though there were several differences seen in color, microbial count, and lipid oxidation values due to the day effect, there was no ($P > 0.05$) effect due to the pad type on meat. The following reasons can be accounted

to the in-effectiveness of absorbent pads. Newly developed sealed air CO_2 generating absorbent pads might have not generated enough CO_2 to maintain the shelf life as there was not enough chemical source [Trona material/sodium carbonate (Na_2CO_3) and sodium bicarbonate ($NaHCO_3$)], the low availability of water for the raw trona mineral to react and generate CO_2, and the permeability of the PVC overwrap used in packaging. CO_2 permeability through PVC is believed to be higher than O_2, so CO_2 likely diffused very shortly after being generated (Siracusa, 2012; Woolley, 1967). Additionally, factors such as temperature, pH, and headspace to meat volume ratio can limit the effectiveness of headspace CO_2 as a shelf life extension agent (Jakobsen & Bertelsen, 2002). A combination of increased amounts of trona mineral and modified atmosphere packaging type or polyvinylchloride films coated with a thin layer of metal or selective polymer material such as plant leaf waxes, cellulosies etc., that can control the environment inside the package may help to extend the shelf life and maintain the quality of packaged meats. Additional studies to optimize packaging conditions, while still remaining economically feasible, are needed.

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CHAPTER 4

EFFECT OF DIFFERENT ANTIOXIDANTS ON OXIDATIVE STABILITY, MICROBIAL GROWTH, PH, AND COLOR IN RAW POULTRY MEAT

Abstract

Meat color is an intrinsic property that plays a major role in consumer's perception. Lipid oxidation by-products as well as free iron can adversely affect meat color. This study was to compare the effect of Type I (radical quenching) antioxidants eugenol and rosmarinic acid (RA) to that of Type II (metal chelating) antioxidants milk mineral (MM), phytate, and sodium tri-polyphosphate (STPP) in raw ground chicken patties packed with a single layer of polyvinylchloride (PVC) overwrap. Packaged patties were stored at 4 ^{o}C and analyzed on 0, 1, 4, 7, and 10 days for pH, surface color, extent of lipid oxidation, oxymyoglobin content, and microbial load. Color stability was measured using Hunter miniscan calorimeter (*L*, a*, b** values). An effect was observed in L* values ($P < 0.05$) of meat color between treatments due to the type of antioxidant. Lightness values for STPP and phytate were low and differed ($P < 0.05$) from eugenol and rosmarinic acid. Milk mineral effectively preserved fresh color and slowed lipid oxidation in chicken patties by day 10. Inverse correlations were observed between pH (increasing), and lightness ($r = - 0.40$ $P < 0.0001$), redness ($r = - 0.46$, $P < 0.0001$) values decreasing respectively from day 1 through 10. Aerobic plate counts increased over the entire testing period while values for lightness, redness, yellowness and chroma decreased indicating an increase in pH favored microbial spoilage of the meat or vice versa. By day 10, eugenol and MM were more effective and significantly different *(P <*

0.05) than STPP in controlling lipid oxidation measured as thiobarbituric acid reactive substances (0.198 mg/kg, 0.198 mg/kg, and 0.268 mg/kg, respectively). A positive correlation ($r = 0.24$, $P < 0.005$) between lipid oxidation and color saturation were observed indicating that as lipids became oxidized red color was more distinct and the samples turned more dark over the storage period.

1. Introduction

Meat color is a result of the myoglobin pigment. The oxidation state of the oxygen binding molecule myoglobin dictates the color changes in meat. Meat pigments that can influence color are deoxymyoglobin (DMb), oxymyoglobin (OMb), and metmyoglobin (MMb). Poultry meat has low percent myoglobin when compared to other red meats, but it is of equal importance in maintaining color (Blessing & Müller, 1974; Fox, 1966; Han, McMillin, & Godber, 1994). Color ranges from light pink to light red for fresh poultry meat that has OMb, but when it oxidizes to MMb the color changes to light brown (Livingston & Brown, 1981). In some cases myoglobin oxidation occurs rapidly and this may lead to lipid oxidation and color loss. By-products of lipid oxidation can directly interact with myoglobin in poultry meat leading to color change (Naveena et al., 2010). Iron catalyzed lipid oxidation can accelerate color loss in poultry by the same mechanism as that which occurs in beef, essentially due to structural similarity in myoglobin sequence (Naveena et al., 2010).

Ground meat becomes rancid and changes color faster than whole meat cuts due to the grinding process where it is exposed to air, surface microflora coming in contact to previously intact muscle, and the disruption of intra-cellular membranes, resulting in loss

of enzymes and other reductants that can slow the oxidation process (Sánchez-Escalante, Djenane, Torrescano, Beltrán, & Roncalés, 2001). Addition of antioxidants during meat processing can reduce the extent of oxidation and color loss (Bolumar, Andersen, & Orlien, 2011; Hernández, Ponce, Jaramillo, & Guerrero, 2009). Several types of antioxidants ranging from natural to synthetic can be added depending on their mechanism of action and consumer demands. Plant derived polyphenols act as potential antioxidants and antimicrobials (Lee & Shibamoto, 2001). Free radical scavenging activity of type I antioxidants coupled with their reactivity as hydrogen or electron donating agents and their interference in the propagation step of the oxidation cycle are of considerable importance (Choe & Min, 2006). Type II antioxidants act as metal chelating agents by binding to metals like iron and copper, thereby stabilizing a non-redox active form of the metal and inactivating lipid oxidation in the initiation step (Choe & Min, 2006).

The antioxidants used in this study were eugenol, rosmarinic acid, phytate, milk mineral, and sodium tri-polyphosphate (STPP). They can be classified into two types based on how they act: Type I – radical quenching (eugenol, rosmarinic acid), and Type II – metal-chelating (phytate, milk mineral, and sodium tri-polyphosphate) antioxidants. Eugenol, which is the main component in cloves, acts as an antimicrobial and is believed to prevent lipid oxidation (Vasavada, Dwivedi, & Cornforth, 2006). Rosmarinic acid is a spice derived antioxidant used in medicine for its antifungal and antimicrobial activity across the world and it is widely accepted to exhibit highest antioxidant activity (Genena, Hense, Smânia Junior, & Souza, 2008). Phytate, or phytic acid, is a food derived phosphate found in nuts and grains which can act as a chelating agent exhibiting its

antioxidant property (Muraoka & Miura, 2004). Milk mineral, a fine white powder, is a by-product of the production of whey protein concentrates obtained by purifying and drying of ultrafiltration permeate of whey. Major components of milk mineral is mineral fraction with calcium and phosphorus along with trace amounts of proteins and fat (K. Allen & Cornforth, 2007). STPP is a colorless inorganic compound primarily added to foods for its water holding capacity (John & Henry, 1967).

Chemical properties of poultry meat such as breed, age, composition of macronutrients and physical factors affecting meat quality have been extensively studied (Tougan et al., 2013). Similarly, antioxidant properties, and their behavior in beef have been highly researched, but fewer studies exist that compare the effect of the different antioxidant types (Allen & Cornforth, 2010). Based on previous studies, Type I antioxidants were used at a level of 0.05% by weight and similarly Type II antioxidants were used at 0.5% by weight. The aim of this study was to compare the effect of Type I and Type II antioxidants on the extension of quality characteristics (color stability, pH and lipid peroxidation) of raw chicken patties packaged in polyvinylchloride (PVC) overwrap. Susceptibility of the patties to microbial spoilage was also investigated.

2. Materials and methods

2.1. Treatments

STPP was obtained from Fisher Scientific (Fairlawn, NJ, USA). Milk mineral (TruCal D50 Milk Calcium Complex) obtained from Glanbia (Monroe, WI, USA).

Rosmarinic acid (97%), phytic acid (dodecasodium salt hydrate, 90%), and eugenol (99%) were obtained from Sigma Scientific (St. Louis, MO, USA).

2.2. Preparation of ground chicken

Previously frozen USDA Grade-A quality chicken thigh and breast pieces were purchased in retail packaging from local retail grocery store in Logan, UT. They were delivered to the Utah State University meat lab and used in preparation of ground chicken on the same day. Visible fat was trimmed off and equal portions of thigh and breast pieces were prepared by coarsely (0.60 cm plate) then finely (0.32 cm plate) grinding through a Hobart grinder model 4125 (Hobart Mfg. Co., Troy, OH, USA). Two 3-ml spectrophotometer cuvettes were filled completely with the ground chicken and later used to obtain reference reflectance spectra (see following section). Thousand gram portions of ground chicken were mixed separately in a Hobart grinder with either 5 g MM, STPP, or phytate (0.5%) or 0.5 g eugenol or RA (0.05%). Type I antioxidants used were eugenol and RA, and Type II antioxidants were MM, STPP, and Phytate. For this study, all Type II antioxidants were examined at 0.5% to determine whether they can exhibit any antioxidant effects at these lower levels. Chicken alone (control) and ground chicken + antioxidant mixtures (treatment samples) were then re-ground through the fine plate. Five patties were prepared for each treatment by shaping 130 g portions using a circular form from a Hollymatic patty machine (Hollymatic Corp., Park Forest, IL, USA). Patties were then placed on 13 x 13 cm square of Filtrete washable furnace filter (3M Center, St. Paul, MN, USA) that had been wrapped in a single layer of PVC film (Koch, Kansas City, MO, USA; O_2 permeability = 8400 cm3/ (24 h x m^2 x atm.) at 23 °C; water vapor transmission

= 83 g/ (24 h x m^2) at 23 °C and 50% relative humidity). Patties and filters were kept in Cryovac 4L Supermarket Trays and wrapped in an additional layer of PVC film, so both the top and bottom surfaces of the patty were in contact with a single layer of PVC, allowing both surfaces sufficient contact with oxygen to bloom. Patties were held at 4 °C for 10 days and analyzed on 0, 1, 4, 7, or 10 days. Five complete replicates were performed. After samples were taken for day 0 analyses, the remaining ground chicken was packaged in quart-sized Ziploc freezer bags (S.C. Johnson and Son, Inc., Racine, WI, USA) and frozen (-10^{o}C) to hold for determination of iron (Ferrozine assay) on dry ash and fat content using modified Folch method.

2.3. Oxymyoglobin determination

Conversion of OMb to MMb was confirmed spectrophotometrically using a Shimadzu UV-Vis 2600/2700 spectrophotometer with a reflectance attachment (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA). OMb content was determined based on the presence of the characteristic reflectance minima at 545 and 580 nm (Bowen, 1949). For each sample of freshly ground reference chicken, 3-ml cuvettes were prepared by packing in ground chicken tightly to exclude air, and then a spectral scan was performed from 450 to 650 nm. Relative loss of OMb was determined by comparing the ratio of OMb to MMb in each sample to the initial ratio of the freshly ground chicken (Allen & Cornforth, 2009). Reflectance spectra for samples were measured in duplicate.

$$\text{OMb : MMb ratio} = \frac{\left(\frac{1}{R_{545+}\ R_{580}}\right)}{(1/R_{505})} \qquad \%\ \text{OMb remaining} = \frac{\text{Sample OMb : MMb ratio}}{\text{Reference OMb : MMb ratio}} \times 100$$

2.4. Color measurement

L^*, a^* and b^* values were measured using a HunterLab Miniscan portable colorimeter (Reston, VA, USA) with a 5 mm diameter aperture, set to use illuminant D-65. The colorimeter was standardized through a single layer of PVC film using both white and black standard tiles. Three-color measurements were taken for each patty. Hue angle (true redness) was calculated as [arctangent (b^*/a^*)], and chroma (color saturation) was calculated as [$\sqrt{(a^{*2} + b^{*2})}$] (Hunt, 1991).

2.5. pH measurement

A portable pH meter (HANNA Instruments HI99161, Ann Arbor, MI, USA) fitted with a semi-solid food probe was used to measure pH. The pH probe was inserted directly in to the meat patty and held until pH reading was constant. For each sample three readings were taken.

2.6. Thiobarbituric acid reactive substances (TBARS)

The TBARS assay was performed as described by (Buege & Aust, 1978). In brief, 1.0 g ground chicken cores were mixed with 3.0 ml of stock solution containing 0.375% thiobarbituric acid, 15% trichloroacetic acid, and 0.25 N HCl. The mixture was heated for 10 min in a boiling water bath (100 °C) to develop a pink color, which was cooled in tap water and then centrifuged (3148 X g; Beckman Coulter, Inc. Indianapolis, IN). The absorbance of the supernatant was measured spectrophotometrically at 532 nm. TBA values (mg malondialdehyde (MDA)/kg of meat) were calculated using an extinction coefficient of 156,000 M^{-1} cm^{-1}as follows:

$$\text{TBA (mg/kg)} = A_{532} \times \frac{1\text{ M Chromagen}}{156{,}000} \times \frac{1\text{ mol/L}}{\text{M}} \times \frac{0.003\text{ L}}{1.0\text{ gmeat}} \times \frac{72.07\text{ gMDA}}{\text{mole}} \times \frac{1000\text{ g}}{\text{kg}}$$

2.7. Microbial load (TAC)

Total aerobic counts were measured based on AOAC method 990.12. Briefly, 10 g of sample was stomached in 90 ml Butterfield's phosphate diluent, then further diluted and plated (1 ml) on petrifilm aerobic count plates (3M Corporation, St. Paul, MN, USA) according to the manufacturer instructions. Plates were incubated at 32 °C for 48 h then counted and interpreted as per the manufacturer's guidelines and expressed as CFU/g. All samples were plated in duplicate.

2.8. Iron determination

To determine the iron content in samples, samples were dry ashed then analyzed using the Ferrozine assay (Carpenter & Clark, 1995). Briefly, 5 g of dry sample was taken into porcelain crucibles and accurately weighed, then heated on a hot plate until the samples were well charred and stopped smoking. Crucibles were placed in a 550^{o}C muffle furnace (Thermo Fisher Scientific Inc., Waltham, MA, USA) until the ash was white (for 24 h), then the samples were cooled in a desiccator and weighed to determine the ash percentage. Then ash was dissolved in a small amount of 1 N HCl and diluted to 50 ml with 0.1 N HCl for iron determination. 0.5 ml of the diluted sample was taken into 10 ml test tubes and 1.250 ml ascorbic acid (0.02% in 0.2 N HCl) was added and vortexed and set for 10 min. 2 ml of 30% ammonium acetate was added to the test tube vortexed again and 1.250 ml of Ferrozine (1 mM in water) was added and vortexed and set in dark for 15 min for color development. Absorbance of the solution was measured at 562 nm using a Shimadzu UV-Vis 2600/2700 spectrophotometer (Shimadzu Scientific

Instruments, Inc., Columbia, MD, USA). A standard curve (absorbance vs. concentration) was plotted with appropriate concentrations (0.05, 0.1, 0.5, 1, 2, 4, 6, 8 and 10 µg iron /ml) and concentration of samples was determined.

2.9. Fat analysis

Fat content in chicken samples was determined using modified Folch method using chloroform-methanol extraction (Folch, Lees, & Stanley, 1957; Luna, 2011). Briefly, frozen samples were dried using liquid N_2. Dry powdered 1.0 g sample was taken into 50 ml centrifuge tube (Becton Dickinson Labware, Franklin Lakes, NJ, USA) and 3.2 ml of deionized water was added and vortexed. To this 8 ml of methanol and chloroform each were added and vortexed for 2 min followed by 4 ml addition of deionized water and vortexing for 30 sec. Samples were centrifuged at 3500 rpm for 10 min then 4 ml of chloroform extract was pipetted into 10 ml culture tubes and evaporated on a heating block in fume hood for 15 min. Dry fat residue with small amounts of solvent left in it was kept in 101 ^{o}C oven until completely dry. Dry weight of the fat residue was taken and based on the below formula percent fat was calculated.

$$\% \text{ Fat} = (Weight\ of\ residue\ in\ gms)/(Weight\ of\ wet\ sample)\times 2\times 100$$

2.10. Statistical analysis

Analysis of variance (repeated measures) was performed using the proc mixed function in SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA). TBA values, chroma, hue angle, and L*, a*, b* values were evaluated as a split-plot design, with antioxidant type as whole-plot factor and time as sub-plot factor. Proc mixed function was used with

treatments, time and their interaction as fixed factors and replicate as random factor. 95% statistical significance level (α =0.05) was used. Post hoc mean comparison and Pearson correlation coefficients to see the relationships between different properties were made using the Tukey-Kramer adjustment.

3. Results & discussion

Samples analyzed on day 0 for iron content and percent fat were within normally accepted levels (Jaspreet, David, Janet, & Pamela, 2014; National Chicken Council, 2012). Average ash percentage was 0.92 and average free iron present was 0.89 μg/g of meat. The average fat content for chicken used in this study was 6.76% (see Appendix B for detailed statistics). A summary of the significant differences between treatments based on the average values for each attribute for all six treatments is given in (Table 4-1). A detailed discussion for each characteristic follows.

Table 4-1. Pooled average values over 7 days: for each characteristic property of raw ground chicken patties in all the six treatments. Values not sharing the same letter within a column are significantly different ($P < 0.05$).

Treatment	L*	b*	a*	Hue-angle	TBA	TAC	pH
Control	47.261^{bc}	12.228	0.964^{b}	85.546^{a}	0.279^{c}	4.69	6.29^{a}
0.05% Eugenol	46.886^{bc}	11.810	0.609^{ab}	87.096^{a}	0.198^{a}	4.85	6.29^{a}
0.5% MM	47.578^{c}	11.752	0.638^{ab}	86.846^{a}	0.198^{a}	4.88	6.25^{a}
0.5% Phytate	46.042^{ab}	11.693	0.539^{a}	87.254^{b}	0.210^{ab}	5.13	6.53^{b}
0.05% RA	47.181^{bc}	11.929	0.718^{ab}	86.592^{a}	0.268^{bc}	4.45	6.28^{a}
0.5% STPP	45.176^{a}	11.520	0.694^{ab}	86.492^{a}	0.247^{abc}	4.97	6.48^{b}
SEM	*0.370*	*0.098*	*0.060*	*0.124*	*0.015*	*0.10*	*0.05*
P-values	*0.0001*	*0.1061*	*0.0401*	*0.0191*	*0.0002*	*0.079*	*0.0001*

3.1. Color measurement

A significant effect ($P < 0.05$) of treatment was seen for L* values. Based on the International Commission on Illumination (CIE) lightness standard values (International Commission on Illumination, 1978), all of the samples except 0.5% STPP fell within the "normal" range on day 0 ($48 < L^* < 51$), whereas on day 10 all samples were in the range of "darker than normal" ($L^* < 47$). A significant effect ($P < 0.001$) due to day was observed within the treatment, as expected (Tables 4-1, 4-2). Overall lightness values for Type II antioxidants STPP ($P < 0.05$) and phytate ($P > 0.05$) were lower than samples treated with Type I antioxidants eugenol, and rosmarinic acid. Even though milk mineral was not different ($P > 0.05$) from eugenol, rosmarinic acid, or the control it had the highest average lightness ($L^* = 47.58$) value meaning it was lighter in color (Table 4-1). Hunter a* values decreased from day 0 to day 4 but a significant difference ($P < 0.05$) was seen on day 7 in all treatments (Tables 4-1, 4-3). Average phytate a* values were different ($P < 0.05$) from the control, while STPP, RA, eugenol, and milk mineral average a* values showed no difference (Fig. 4-1). Eugenol, MM, and phytate had higher values for hue angle after 10 days, indicating a loss of true redness over time in these samples (Table 4-4).

Results show that L* and b* values strongly influenced color in all cases, which can be seen from the hue angle data **(**Table 4-4). Based on the CIE color solid and AMSA color evaluation guidelines (Melvin & Andy, 2012) hue angle values ranged between 80^o – 95^o indicating the samples remained in the yellowish region of the spectrum (Table 4-4). Similarly a* values shifted from red (+a*) to green (-a*) over time, which was most likely due to loss of OMb. Hence net effect on patty color was a shift from initial

yellowish-orange tinge to yellowish-green undertone. This change was identified instrumentally, but was less obvious to observe visually compared to the darkening of the samples (Table 4-2).

Table 4-2. L* values for raw ground poultry patties: time to first significant difference as compared to day 0 and means at day 0 and day 10

Treatment	Days to first significant difference[A]	Lightness (L*)	
		Day 0[B]	Day 10[B]
Ground chicken	ns	49.10 ± 1.81 a	45.89 ± 1.34 a
0.05% Eugenol	ns	48.49 ± 1.73 a	45.40 ± 1.75 a
0.5% MM	ns	49.48 ± 1.95 a	46.57 ± 2.66 a
0.5% Phytate	ns	46.24 ± 1.26 a	46.79 ± 2.30 a
0.05% RA	ns	48.74 ± 2.47 a	45.09 ± 1.92 a
0.5% STPP	ns	46.20 ± 2.72 a	44.65 ± 2.12 a

STPP = sodium tri-polyphosphate; RA = rosmarinic acid; MM = milk mineral.
A: Time point where first significant difference ($P < 0.05$) occurs as compared to initial reading for each meat system formulation. B: Values represent mean ± standard deviation at a given time point (n = 5). Values sharing letters within the column are not significantly different ($P > 0.05$).

Table 4-3. Hunter a* value: time to first significant difference as compared to day 0 and means at day 0 and day 10

Treatment	Days to first significant difference[A]	Redness (a*)	
		Day 0[B]	Day 10[B]
Ground chicken	7	1.50 ± 0.66 a	0.20 ± 0.43 b
0.05% Eugenol	7	1.44 ± 0.62 a	-0.48 ± 0.41 b
0.5% MM	7	1.05 ± 0.89 a	-0.23 ± 0.15 b
0.5% Phytate	7	0.99 ± 0.99 a	-0.17 ± 0.50 b
0.05% RA	7	1.13 ± 0.52 a	0.00 ± 0.38 b
0.5% STPP	7	1.18 ± 0.69 a	0.11 ± 0.24 b

STPP = sodium tri-polyphosphate; RA = rosmarinic acid; MM = milk mineral.
A: Time point where first significant difference ($P < 0.05$) occurs as compared to initial reading for each meat system formulation. B: Values represent mean ± standard deviation at a given time point (n = 5). Values sharing letters within the column are not significantly different ($P > 0.05$).

Table 4-4. Hue angle: time to first significant difference as compared to day 0 and means at day 0 and day 10

Treatment	Days to first significant difference[A]	Hue angle in degrees	
		Day 0[B]	Day 10[B]
Ground chicken	ns	83.26 ± 2.94 a	89.02 ± 1.93 a
0.05% Eugenol	10	83.32 ± 2.18 a	92.44 ± 2.10 b
0.5% MM	7	85.12 ± 3.56 a	91.07 ± 0.71 b
0.5% Phytate	10	85.43 ± 4.51 a	92.13 ± 3.20 b
0.05% RA	ns	84.84 ± 3.21 a	90.08 ± 2.03 a
0.5% STPP	ns	84.09 ± 3.31 a	89.47 ± 1.08 a

STPP = sodium tri-polyphosphate; RA = rosmarinic acid; MM = milk mineral.
A: Time point where first significant difference ($P < 0.05$) occurs as compared to initial reading for each meat system formulation. B: Values represent mean ± standard deviation at a given time point (n = 5). Values sharing letters are not significantly different ($P > 0.05$).

Table 4-5. b* values for raw ground poultry patties: time to first significant difference as compared to day 0 and means at day 0 and day 10

Treatment	Days to first significant difference[A]	Yellowness (b*)	
		Day 0[B]	Day 10[B]
Ground chicken	ns	12.66 ± 0.14 a	11.63 ± 1.13 a
0.05% Eugenol	ns	12.10 ± 1.52 a	11.34 ± 1.11 a
0.5% MM	ns	11.72 ± 1.26 a	12.19 ± 1.30 a
0.5% Phytate	ns	11.57 ± 1.09 a	12.15 ± 1.16 a
0.05% RA	ns	11.99 ± 1.16 a	11.51 ± 1.10 a
0.5% STPP	ns	11.44 ± 1.56 a	11.74 ± 1.30 a

STPP = sodium tri-polyphosphate; RA = rosmarinic acid; MM = milk mineral.
A: Time point where first significant difference ($P < 0.05$) occurs as compared to initial reading for each meat system formulation. B: Values represent mean ± standard deviation at a given time point (n = 5). Values sharing letters are not significantly different ($P > 0.05$).

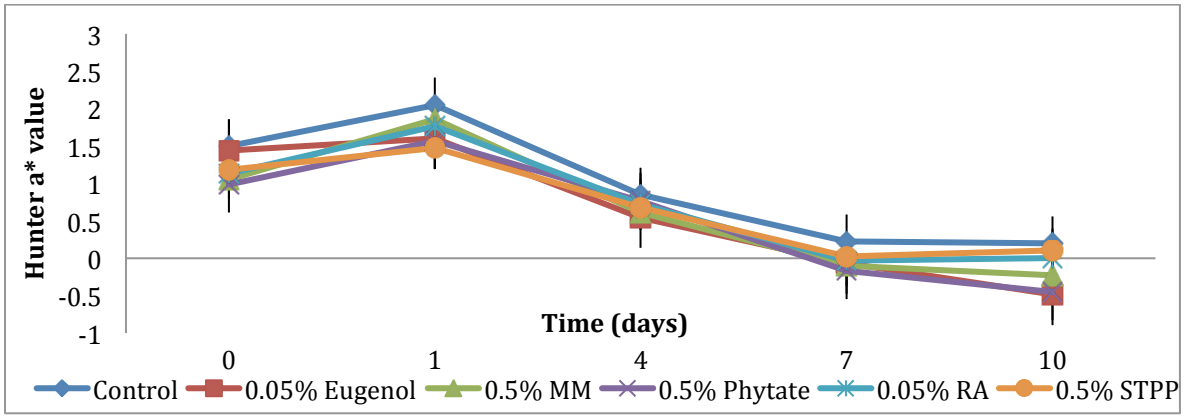

Fig. 4-1. Hunter a* values in ground chicken patties treated with 0.05% eugenol, 0.5% milk mineral, 0.5% phytate, 0.05% rosmarinic acid, 0.5% sodium tripolyphosphate as compared to control. Day effect was seen ($P < 0.0001$); no trt*day effect ($P = 0.945$).

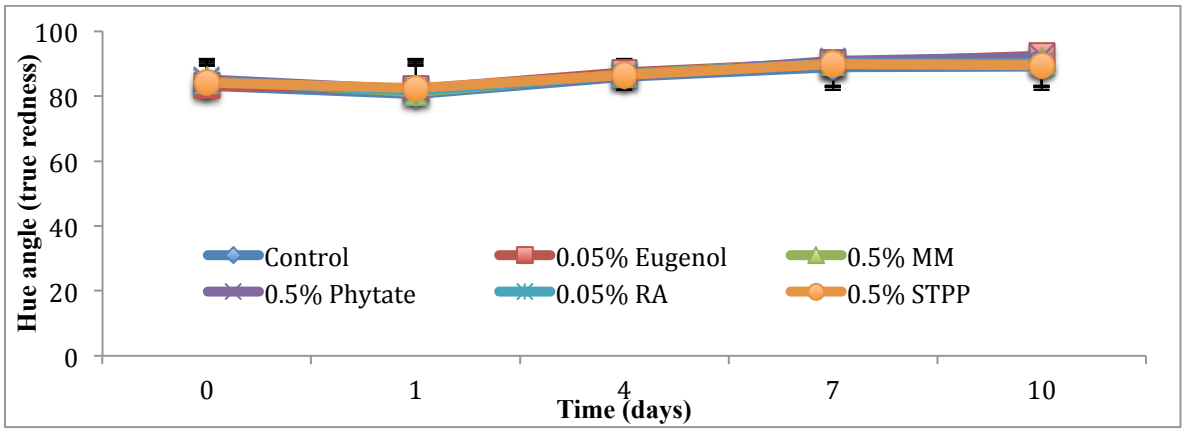

Fig. 4-2. Hue angle values in ground chicken patties treated with 0.05% eugenol, 0.5% milk mineral, 0.5% phytate, 0.05% rosmarinic acid, 0.5% sodium tri-polyphosphate as compared to control. Day effect was seen ($P < 0.0001$); no trt*day effect ($P = 0.299$).

This is consistent with (Gulen Yildiz Turp & Meltem Serdarogu, 2004)**,** who did not find any improvement in a* redness values in chicken patties in the presence of rosemary. First difference ($P < 0.05$) in hue angle (as compared to day 0) was observed on day 7 for MM and on day 10 for eugenol, whereas for all other treatments a noticeable difference was not observed even on day 10 (Fig. 4-2).

3.2. Thiobarbituric acid reactive substances (TBARS)

TBARS values tended to increase for eugenol, MM, phytate, and RA ($P > 0.05$), while significant increases were seen for STPP ($P < 0.05$) during the storage period. However, all the values were under 1 mg MDA/kg of meat, suggesting the samples would still be acceptable from a sensory standpoint (Chouliara, Karatapanis, Savvaidis, & Kontominas, 2007). Antioxidants MM, eugenol and phytate were relatively more effective in preventing lipid oxidation than STPP and rosmarinic acid when compared to control values ($P > 0.05$; Table 4-6; Fig. 4-3.). Because of the spice derived antioxidant nature eugenol and rosmarinic acid have a distinctive aroma, which masked rancidity in

these respective patties. However, a strong spice or herb aroma can be considered a negative sensory aspect from consumer point of view (Vasavada et al., 2006). Milk mineral was the most effective in preventing lipid oxidation (Table 4-1, 4-6). TBA value for STPP was significantly ($P < 0.05$) different from day 0 to day 10 indicating higher oxidation byproducts in the samples.

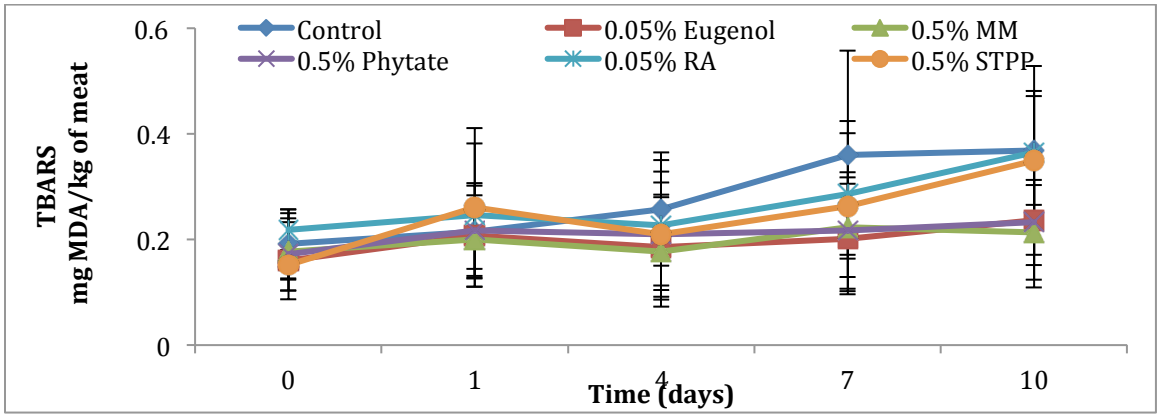

Fig. 4-3. TBA values in ground chicken patties treated with 0.05% eugenol, 0.5% milk mineral, 0.5% phytate, 0.05% rosmarinic acid, 0.5% sodium tri poly phosphate (STPP) as compared to control. Day effect was seen ($P < 0.0001$); no trt*day effect ($P = 0.578$).

Table 4-6. TBARS: time to first significant difference as compared to day 0 and means at day 0 and day 10

Treatment	Days to first significant difference[A]	TBARS	
		Day 0[B]	Day 10[B]
Ground chicken	ns	0.19 ± 0.07 a	0.37 ± 0.10 a
0.05% Eugenol	ns	0.16 ± 0.07 a	0.24 ± 0.13 a
0.5% MM	ns	0.18 ± 0.07 a	0.21 ± 0.09 a
0.5% Phytate	ns	0.17 ± 0.07 a	0.23 ± 0.08 a
0.05% RA	ns	0.22 ± 0.04 a	0.36 ± 0.11 a
0.5% STPP	10	0.15 ± 0.03 a	0.35 ± 0.18 a

STPP = sodium tri-polyphosphate; RA = rosmarinic acid; MM = milk mineral.
A: Time point where first significant difference ($P < 0.05$) occurs as compared to initial reading for each meat system formulation. B: Values represent mean ± standard deviation at a given time point ($n = 5$). Values sharing letters are not significantly different ($P > 0.05$).

Table 4-7.
TAC: time to first significant difference as compared to day 0 and means at day 0 and day 10

Treatment	Days to first significant difference[A]	TAC (log cfu/g)	
		Day 0[B]	Day 10[B]
Ground chicken	10	3.76 ± 0.95 a	5.92 ± 2.05 a
0.05% Eugenol	ns	4.25 ± 0.67 a	5.62 ± 2.04 a
0.5% MM	ns	4.15 ± 0.95 a	5.76 ± 1.83 a
0.5% Phytate	ns	4.18 ± 1.36 a	5.96 ± 1.75 a
0.05% RA	10	3.51 ± 1.21 a	5.68 ± 2.03 a
0.5% STPP	10	4.02 ± 1.04 a	6.13 ± 1.58 a

STPP = sodium tri-polyphosphate; RA = rosmarinic acid; MM = milk mineral.
A: Time point where first significant difference ($P < 0.05$) occurs as compared to initial reading for each meat system formulation. B: Values represent mean ± standard deviation at a given time point (n = 5). Values sharing letters are not significantly different ($P > 0.05$).

3.3.Total Aerobic Plate Count (TAC)

A continuous increase in TAC numbers was observed over the storage time period in all the samples. In patties treated with eugenol, MM, and phytate there were no significant (*P > 0.05*) differences observed during the 10 days, whereas in control and patties treated with rosmarinic acid and STPP a noticeable increase was seen on day 10. Even though some of the values on day 7 and all treatments on day 10 have TAC approaching 8-log cfu/g (Table 4-1, 4-7), their average count was well below the point where fresh poultry exhibits off odors and sliminess due to bacterial spoilage (Jay, 1995).

3.4. pH

Measured pH of all the patties for all treatments was over 6.0 from day 0 (Table 4-8). Eugenol, rosmarinic acid, STPP, and control had increased *(P < 0.05)* pH values by day 10 with respect to day 0 (Fig. 4-4.). For MM and phytate pH did not change notably *(P > 0.05)* from day 0 to day 10, which indicates an effect due to these treatments (Table

4-1, 4-8.). In comparison, low pH values were recorded for MM while phytate and STPP had higher pH values throughout the testing period.

Relative loss of OMb was not significant ($P > 0.05$) for any of the treatment samples, indicating that its concentration did not change during storage. This result is in agreement with those reported by (Min & Ahn, 2009), who found the concentrations of Mb and MMb percentage in chicken breast samples did not change ($P > 0.05$) over 10 days. The differences in pH, lipid oxidation and color seen in the current study can be explained based on the amounts of endogenous catalysts like myoglobin, free ionic iron reducing compounds, antioxidants that determine the extent of lipid oxidation. Min & Ahn (2009) reported raw chicken is more resistant to oxidative changes than other meats, where this stability is influenced by iron chelating ability, the presence of anti- or pro-oxidants, and the relative concentration of ionic iron present (Pradhan, Rhee, & Hernández, 2000).

Table 4-8. pH values for raw ground poultry patties: time to first significant difference as compared to day 0 and means at day 0 and day 10

Treatment	Days to first significant difference[A]	pH	
		Day 0[B]	Day 10[B]
Ground chicken	10	6.18 ± 0.23 a	6.64 ± 0.26 a
0.05% Eugenol	10	6.17 ± 0.16 a	6.62 ± 0.35 a
0.5% MM	ns	6.17 ± 0.14 a	6.50 ± 0.19 a
0.5% Phytate	ns	6.48 ± 0.21 a	6.80 ± 0.84 a
0.05% RA	10	6.10 ± 0.09 a	6.60 ± 0.34 a
0.5% STPP	10	6.37 ± 0.16 a	6.76 ± 0.32 a

STPP = sodium tri-polyphosphate; RA = rosmarinic acid; MM = milk mineral.
A: Time point where first significant difference ($P < 0.05$) occurs as compared to initial reading for each meat system formulation. B: Values represent mean ± standard deviation at a given time point (n = 5). Values sharing letters are not significantly different ($P > 0.05$).

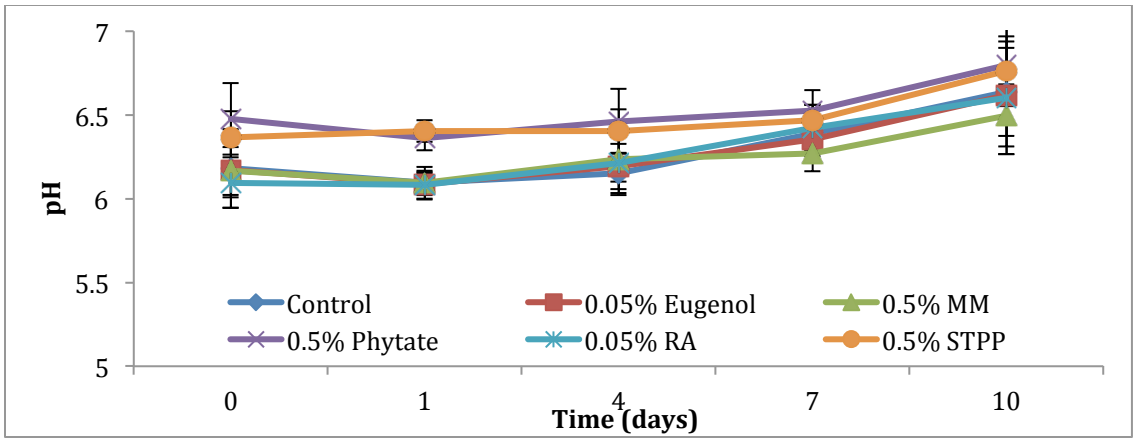

Fig. 4-4. pH values in ground chicken patties treated with 0.05% eugenol, 0.5% milk mineral, 0.5% phytate, 0.05% rosmarinic acid, 0.5% sodium tri poly phosphate (STPP) as compared to control. Day effect was seen ($P < 0.0001$); no trt*day effect ($P = 0.965$).

Table 4-9. Significant Pearson correlation coefficients (r-values) for meat system measurements

	L*	a*	b*	Chroma[A]
TPC (log cfu/g)	-0.28229	-0.46289	-0.41476	-0.43127
	P < 0.005	*P < 0.0001*	*P < 0.0001*	*P < 0.0001*
Lightness (L*)		0.39605	0.42469	0.441
		P < 0.0001	*P < 0.0001*	*P < 0.0001*
TBARS			0.2378	0.23674
			P < 0.005	*P < 0.005*
Yellowness (b*)				0.99821
				P < 0.0001

A: Chroma calculated as (color saturation) was calculated as $\sqrt{}$ ($a^{*2} + b^{*2}$).

Allen, Fletcher, Northcutt, and Russell (1998) suggested low pH is associated with low water-holding capacity, and also reported samples with an increased shelf life. In the current study, the two Type II metal chelating antioxidants STPP and phytate are believed to have high water holding capacity initially due to an abundance of phosphate groups, which increase pH. This resulted in initial higher L* values, but later the patties treated with STPP and phytate had lower L* values (Appendix B) and higher pH when compared to other antioxidant treatments. Harmayani, Sofos, and Schmidt (1991) found an inverse correlation between use of phosphates and reduction of gram-negative bacteria

fresh meats. This is in line with the strong odors observed informally during testing at day 7 in those samples, most likely the result of microbial growth and lipid oxidation. Type I antioxidants eugenol and rosmarinic acid were more effective in preventing microbial growth (< 6 log cfu/g) and strong off odors. Significant effect ($P < 0.05$) due to the antioxidant type was observed in log cfu/g values, mainly between patties treated with phytate and rosmarinic acid. Patties with rosmarinic acid showed the lowest levels of microbial growth, which is consistent with previous findings that spice derived antioxidants inhibit microbial growth (Genena et al., 2008). Conversely, eugenol was more effective in preventing lipid oxidation than rosmarinic acid, most likely due to accumulation of eugenol in the lipid phase due to its low water solubility (Allen & Cornforth, 2010)**.** MM effectively maintained desirable color and pH in chicken patties and prevented lipid oxidation by directly chelating the free iron (see Appendix B for detailed statistics and data). In this current study STPP was not as effective as phytate and MM in controlling lipid oxidation and maintaining color, which is in agreement with previous results (K. Allen & Cornforth, 2007). They reported that the orthophosphates formed from STPP due to the action of natural phosphatase enzymes present in raw meats are not effective metal chelators.

A significant positive correlation ($P < 0.001$) between L*, a*, and b* was observed in this study (Table 4-9.) suggesting that all the values decreased consistently over time resulting in lighter and more green/brownish samples. A negative correlation between color and microbial growth was observed which indicates a constant increase in log cfu/g values with decreasing color. An inverse linear relationship between pH and color was seen suggesting an increase in pH in the samples may lead to decrease in color.

The positive correlation *(r = 0.2378; P < 0.05)* between TBARS and b* indicates that an increase in lipid oxidation led to conditions that ultimately changed the color of samples to more yellow. This is consistent with previously reported results indicating specific lipid oxidation by-products are capable of forming adducts with Mb, altering the shape of the protein shell and providing increased access of oxidizing molecules to the heme center (Naveena et al., 2010).

4. Conclusion

The current study compared the effects of different antioxidants in raw poultry meat and it was observed that raw chicken is relatively more resistant to oxidative changes when compared to lipid oxidation values of raw beef and pork from other studies. Lower pH and total aerobic counts, consistent MbO_2 to MetMb ratio, and iron chelating ability of MM and other antioxidants contributed to the stability of chicken patties. Milk mineral was most effective in preventing lipid oxidation, minimal changes in pH, and in maintaining the fresh color of chicken, but was not able to control growth of microorganisms. However it is not known if an increased concentration of antioxidants could alter the results or how antioxidants work in cooked chicken and other ready-to-eat meats. Hence, assuming that many other factors can be responsible for major deterioration reaction and other changes in quality of meats this subject require further study.

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CHAPTER 5

OVERALL SUMMARY

Color in meats is considered an important factor by consumers in assessing its quality (Issanchou, 1996; Kropf, 1980). Shelf life in meats mainly depends on product quality, and packaging integrity. To improve shelf life and quality using packaging various factors affecting the product are assessed (Singh, Wani, Saengerlaub, & Langowski, 2011). Different technologies like modified atmosphere packaging, vacuum packaging, and active packaging have been developed that work mainly by the addition of external components to the packaging (Brody, 2007; Gill & Tan, 1980; Gray, Gomaa, & Buckley, 1996; Han & Floros, 1997; Huffman, Davis, Marple, & McGuire, 1975; Kanner, 1994; Vermeiren, Devlieghere, van Beest, de Kruijf, & Debevere, 1999). The focus of present study (Chapter 3) was to mainly look at the effect of CO_2 generating absorbent pads on different meats (beef, chicken and fish) in controlling lipid oxidation, changes in color and their antimicrobial activity. Not many differences were seen due to the treatments but storage condition, time, and type of meat had an effect on some of the quality factors. Increase in pH was within the normal range over the 8-day testing period, whereas microbial loads were lower than expected. This could be attributed to the fact that immediately after the harvest cuts were vacuum packaged, and good practices were followed by the processing facilities. Lipid oxidation in chicken was lower than in other two meats (Table 3-3, Table 3-4.), and it is believed due to the fact that poultry meat is more resistant to oxidative changes (Allen, Fletcher, Northcutt, & Russell, 1998). It was concluded that significant level of CO_2 was not generated because of the amount of trona mineral and higher amounts can be added based on the economic feasibility and further

research developments.

Another study was conducted (Chapter 4) to evaluate different antioxidants on their ability to maintain color and limit the oxidation process in raw poultry meat. Results showed a significant effect on lipid oxidation and color changes due to antioxidant type. Sodium tri-polyphosphate (STPP), a type II antioxidant, was not effective in preventing lipid oxidation and color changes. High water holding capacity of STPP might have initiated lipid oxidation and also favored microbial growth. High lipid oxidation resulted in color loss in patties treated with STPP. Milk mineral was the most effective in preventing microbial growth, controlling lipid oxidation, and maintaining fresh-like color in patties. Eugenol and rosmarinic acid were only successful in preventing microbial growth and color when compared to other antioxidants, but rosmarinic acid was not able to control lipid oxidation and rancidity in the samples. Phytate was able to limit the oxidation of ground chicken patties but it had no effect on color changes and microbial growth. Milk mineral, a type II metal chelating antioxidant, was able to stabilize 'free' non-heme iron and limit oxidation. Mineral fraction contains calcium, phosphate, magnesium and citrate and the inorganic calcium phosphate acts differently compared to phosphate component in phytate or STPP (Allen & Cornforth, 2007). A significant change was observed in pH on day 7 in all the treatments and by day 10, pH > 6.7 for all the samples except for milk mineral (pH = 6.4). Rancidity and off-odors were observed in some of the samples. Similar positive results were seen in beef, pork and turkey studies indicating milk mineral at different concentrations (0.5%, 1.0 % and 1.5%), as a potential food grade antioxidant that can be used at commercial level to increase the shelf life in meats (Allen & Cornforth, 2010; Allen & Cornforth, 2007; Cornforth & West, 2002;

Jayasingh & Cornforth, 2004; Vasavada & Cornforth, 2005; Vissa & Cornforth, 2006).

In developed countries the concept of active and intelligent packaging in meats is still at preliminary stage, addition of other active components such as O_2, CO_2, and moisture scavengers to the packaging or release of antioxidants or antimicrobials instead of adding them to the raw meat packaging during the processing steps is future application of active packaging (Ozdemir & Floros, 2004). Given the fact that not much work was carried out related to shelf life studies on fish meat in a combination with packaging technique and in the presence of antioxidants, this would be an interesting study, and also looking at the effect of these antioxidants on cooked meats.

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APPENDICES

APPENDIX A

STATISTICS FOR CHAPTER 3

Table A1. Chroma values for beef: time to first significant difference as compared to day 1 and means at day 1 and day 7

Treatment	Days to first significant difference[A]	Saturation	
		Day 1[B]	Day 7[B]
Control pad	2	22.01 ± 2.44 a	19.79 ± 1.36 b
CO_2 pad	ns	22.24 ± 2.41 a	19.88 ± 1.06 a
PPI Extendapak pad	3	23.95 ± 1.68 a	20.18 ± 2.69 b
International pad	2	23.45 ± 2.99 a	18.66 ± 1.77 b

Table A2. Hue angle values for beef: time to first significant difference as compared to day 1 and means at day 1 and day 7

Treatment	Days to first significant difference[A]	Hue angle	
		Day 1[B]	Day 7[B]
Control pad	ns	0.89 ± 0.06 a	0.89 ± 0.05 a
CO_2 pad	ns	0.89 ± 0.05 a	0.89 ± 0.04 a
PPI Extendapak pad	4	0.86 ± 0.07 a	0.89 ± 0.09 b
International pad	ns	0.90 ± 0.06 a	0.89 ± 0.06 a

Table A3. TAC values for beef: time to first significant difference as compared to day 1 and means at day 1 and day 7

Treatment	Days to first significant difference[A]	log cfu/cm^2	
		Day 1[B]	Day 7[B]
Control pad	3	1.91 ± 0.36 a	1.11 ± 0.60 b
CO_2 pad	2	1.59 ± 0.52 a	1.08 ± 0.47 b
PPI Extendapak pad	4	1.88 ± 0.16 a	1.09 ± 0.69 b
International pad	3	1.57 ± 0.50 a	0.97 ± 0.27 b

Control = Sealed Air standard pad; CO_2 pad = Sealed Air CO_2 generating pad;

PPI pad = PPI Extendapak pad; International pad = International prime meat pad.

A: Time point where first significant difference ($P < 0.05$) occurs as compared to initial reading for each meat system formulation.

B: Values represent mean ± standard deviation at a given time point (n = 5). Values sharing letters within a column are not significantly different ($P > 0.05$).

Table A4. pH values for beef: time to first significant difference as compared to day 1 and means at day 1 and day 7

Treatment	Days to first significant difference[A]	pH	
		Day 1[B]	Day 7[B]
Control pad	ns	5.28 ± 0.20 a	5.23 ± 0.08 a
CO_2 pad	ns	5.23 ± 0.29 a	5.26 ± 0.02 a
PPI Extendapak pad	ns	5.30 ± 0.25 a	5.25 ± 0.05 a
International pad	ns	5.21 ± 0.24 a	5.26 ± 0.06 a

Table A5. Chroma values for chicken: time to first significant difference as compared to day 1 and means at day 1 and day 7

Treatment	Days to first significant difference[A]	Saturation	
		Day 1[B]	Day 7[B]
Control pad	ns	3.25 ± 0.73 a	3.76 ± 0.38 a
CO_2 pad	ns	3.76 ± 0.74 a	3.30 ± 1.11 a
PPI Extendapak pad	ns	3.83 ± 1.42 a	3.80 ± 0.91 a
International pad	5	4.86 ± 0.38 a	3.60 ± 1.13 b

Table A6. Hue angle values for chicken: time to first significant difference as compared to day 1 and means at day 1 and day 7

Treatment	Days to first significant difference[A]	Hue angle	
		Day 1[B]	Day 7[B]
Control pad	ns	-0.84 ± 0.30 a	-1.19 ± 0.16 a
CO_2 pad	5	-1.08 ± 0.20 a	-0.95 ± 0.74 b
PPI Extendapak pad	ns	-0.94 ± 0.38 a	-1.12 ± 0.25 a
International pad	7	-1.28 ± 0.02 a	-0.27 ± 1.31 b

Table A7. TAC values for chicken: time to first significant difference as compared to day 1 and means at day 1 and day 7

Treatment	Days to first significant difference[A]	Log cfu/cm^2	
		Day 1[B]	Day 7[B]
Control pad	5	1.32 ± 0.39 a	0.77 ± 0.91 b
CO_2 pad	5	1.64 ± 0.61 a	0.99 ± 1.27 b
PPI Extendapak pad	5	1.58 ± 0.40 a	1.93 ± 0.44 b
International pad	5	1.67 ± 0.28 a	1.10 ± 0.76 b

Table A8. pH values for chicken: time to first significant difference as compared to day 1 and means at day 1 and day 7

Treatment	Days to first significant difference[A]	pH	
		Day 1[B]	Day 7[B]
Control pad	2	5.79 ± 0.08 a	5.59 ± 0.10 b
CO_2 pad	2	5.70 ± 0.07 a	5.54 ± 0.08 b
PPI Extendapak pad	4	5.59 ± 0.18 a	5.55 ± 0.12 b
International pad	2	5.75 ± 0.08 a	5.55 ± 0.08 b

Table A9. Chroma values for tuna: time to first significant difference as compared to day 1 and means at day 1 and day 7

Treatment	Days to first significant difference[A]	Saturation	
		Day 1[B]	Day 7[B]
Control pad	ns	5.53 ± 1.16 a	4.49 ± 1.89 a
CO_2 pad	6	5.50 ± 0.91 a	3.64 ± 0.55 b
PPI Extendapak pad	6	6.59 ± 2.00 a	4.04 ± 0.25 b
International pad	ns	4.21 ± 0.92 a	4.11 ± 1.25 a

Table A10. Hue angle values for tuna: time to first significant difference as compared to day 1 and means at day 1 and day 7

Treatment	Days to first significant difference[A]	Hue angle	
		Day 1[B]	Day 7[B]
Control pad	ns	0.94 ± 0.12 a	0.96 ± 0.25 a
CO_2 pad	ns	1.01 ± 0.14 a	1.16 ± 0.17 a
PPI Extendapak pad	ns	0.88 ± 0.11 a	1.05 ± 0.05 a
International pad	ns	1.04 ± 0.17 a	0.92 ± 0.08 a

Table A11. TAC values for tuna: time to first significant difference as compared to day 1 and means at day 1 and day 7

Treatment	Days to first significant difference[A]	Log cfu/cm^2	
		Day 1[B]	Day 7[B]
Control pad	ns	2.35 ± 4.04 a	14.0 ± 25.4 a
CO_2 pad	5	0.50 ± 0.20 a	1.00 ± 0.00 b
PPI Extendapak pad	ns	1.10 ± 1.94 a	3.00 ± 2.00 a
International pad	6	0.45 ± 0.34 a	15.0 ± 19.4 b

Control = Sealed Air standard pad; CO_2 pad = Sealed Air CO_2 generating pad;
PPI pad = PPI Extendapak pad; International pad = International prime meat pad.

A: Time point where first significant difference ($P < 0.05$) occurs as compared to initial reading for each meat system formulation.

B: Values represent mean ± standard deviation at a given time point (n = 5). Values sharing letters within a came column are not significantly different ($P > 0.05$).

Table A12. Type 3 tests fixed effects (ANOVA) for lightness in beef meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	3	81	1.91	0.1339
day	6	81	2.75	0.0174
trt*day	18	81	0.64	0.8589

Table A13. Type 3 tests fixed effects (ANOVA) for redness in beef meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	3	81	1.63	0.1893
day	6	81	3.28	0.0061
trt*day	18	81	0.74	0.7576

Table A14. Type 3 tests fixed effects (ANOVA) for yellowness in beef meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	3	81	0.77	0.5144
day	6	81	6.59	<.0001
trt*day	18	81	1	0.4676

Table A15. Type 3 tests fixed effects (ANOVA) for color saturation in beef meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	3	81	1.4	0.2492
day	6	81	5.49	<.0001
trt*day	18	81	0.94	0.5384

Table A16. Type 3 tests fixed effects (ANOVA) for hue angle in beef meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	3	81	1.23	0.3039
day	6	81	1.15	0.3391
trt*day	18	81	0.6	0.8907

Table A17. Type 3 tests fixed effects (ANOVA) for TBARS in beef meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	3	81	0.01	0.999
day	6	81	16.46	<.0001
trt*day	18	81	0.19	0.9999

Table A18. Type 3 tests fixed effects (ANOVA) for aerobic plate counts in beef meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	3	72	4.33	0.0378
day	6	72	14.29	<.0001
trt*day	18	72	0.52	0.942

Table A19. Type 3 tests fixed effects (ANOVA) for pH in beef meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	3	81	0.1	0.9579
day	6	81	5.01	0.0002
trt*day	18	81	0.2	0.9998

Table A20. Type 3 tests fixed effects (ANOVA) for lightness in chicken meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	3	81	0.3	0.825
day	6	81	1.16	0.3383
trt*day	18	81	1.2	0.2835

Table A21. Type 3 tests fixed effects (ANOVA) for redness in chicken meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	3	81	1.27	0.2901
day	6	81	1.38	0.2325
trt*day	18	81	0.79	0.7083

Table A22. Type 3 tests fixed effects (ANOVA) for yellowness in chicken meat

Effect	Num DF	Den DF	F Value	Pr > F
trt	3	81	0.5	0.6864
day	6	81	0.89	0.5045
trt*day	18	81	1.03	0.4321

Table A23. Type 3 tests fixed effects (ANOVA) for color saturation in chicken meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	3	81	0.59	0.6202
day	6	81	0.72	0.6323
trt*day	18	81	0.96	0.5086

Table A24. Type 3 tests fixed effects (ANOVA) for hue angle in chicken meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	3	81	0.84	0.4774
day	6	81	0.71	0.6406
trt*day	18	81	1.32	0.1986

Table A25. Type 3 tests fixed effects (ANOVA) for aerobic plate counts in chicken meat

Effect	Num DF	Den DF	F Value	Pr > F
trt	3	81	0.65	0.5862
day	6	81	12.55	<.0001
trt*day	18	81	1.27	0.2301

Table A26. Type 3 tests fixed effects (ANOVA) for TBARS in chicken meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	3	81	10.88	0.0009
day	6	81	12.48	<.0001
trt*day	18	81	1.54	0.102

Table A27. Type 3 tests fixed effects (ANOVA) for pH in chicken meat

Effect	Num DF	Den DF	F Value	Pr > F
trt	3	81	0.15	0.93
day	6	81	28.69	<.0001
trt*day	18	81	2.2	0.0098

Table A28. Type 3 tests fixed effects (ANOVA) for lightness in tuna meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	3	81	0.21	0.8877
day	6	81	1.94	0.0853
trt*day	18	81	0.84	0.6449

Table A29. Type 3 tests fixed effects (ANOVA) for redness in tuna meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	3	81	0.92	0.4342
day	6	81	6.15	<.0001
trt*day	18	81	1.38	0.164

Table A30. Type 3 tests fixed effects (ANOVA) for yellowness in tuna meat

Effect	Num DF	Den DF	F Value	Pr > F
trt	3	81	2.07	0.1105
day	6	81	4.18	0.001
trt*day	18	81	0.76	0.7432

Table A31. Type 3 tests fixed effects (ANOVA) for color saturation in tuna meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	3	81	1.46	0.2303
day	6	81	4.57	0.0005
trt*day	18	81	1.03	0.432

Table A32. Type 3 tests fixed effects (ANOVA) for hue angle in tuna meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	3	81	0.6	0.6176
day	6	81	5.85	<.0001
trt*day	18	81	1.4	0.1526

Table A33. Type 3 tests fixed effects (ANOVA) for aerobic plate counts in tuna meat

Effect	Num DF	Den DF	F Value	Pr > F
trt	3	81	0.19	0.9006
day	6	81	8.89	<.0001
trt*day	18	81	0.39	0.9863

Table A34. Type 3 tests fixed effects (ANOVA) for TBARS in tuna meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	3	81	0.53	0.6661
day	6	81	9.46	<.0001
trt*day	18	81	1.09	0.3774

Table A35. Type 3 tests fixed effects (ANOVA) for pH in tuna meat

Effect	Num DF	Den DF	F Value	Pr > F
trt	3	81	2.16	0.0989
day	6	81	78.08	<.0001
trt*day	18	81	1.45	0.1336

Table A36: Pearson correlations for beef meat system measurements

	a*	Chroma	Hue angle	TBARS
Chroma			-0.47	-0.23
			p < 0.0001	*P < 0.05*
Redness (a*)		0.92	-0.77	-0.20
		p < 0.0001	*p < 0.0001*	*P < 0.05*
Lightness (L*)	-0.46	-0.27	0.64	-0.22
	p < 0.0001	*P < 0.005*	*p < 0.0001*	*P < 0.05*
Yellowness (b*)	0.69	0.91		-0.22
	p < 0.0001	*p < 0.0001*		*P < 0.05*

Table A37: Pearson correlations for tuna meat system measurements

	log cfu/cm^2	a*	Chroma	Hue angle	TBARS
Chroma				-0.41	-0.33
				P < 0.0001	*P < 0.005*
Redness (a*)			0.90	-0.73	-0.35
			P < 0.0001	*P < 0.0001*	*P < 0.001*
Yellowness (b*)		0.71	0.93		-0.28
		P < 0.0001	*P < 0.0001*		*P < 0.005*
Lightness (L*)	0.33	-0.49	-0.37	0.58	0.54
	P < 0.005	*P < 0.0001*	*P < 0.005*	*P < 0.0001*	*P < 0.001*

Table A38: Pearson correlations for tuna meat system measurements

	a*	Chroma	Hue angle	pH
Chroma			-0.35	
			P < 0.0001	
Redness (a*)		0.38		
		P < 0.0001		
Lightness (L*)	-0.60			-0.32
	P < 0.0001			*P < 0.005*
Yellowness (b*)	0.58	0.92	-0.50786	
	P < 0.0001	*P < 0.0001*	*P < 0.0001*	

Hue angle (true redness) calculated as [arctangent (b*/a*)].
Chroma calculated as (color saturation) was calculated as $\sqrt{(a^{*2} + b^{*2})}$.
Pearson correlation coefficients (r-values).

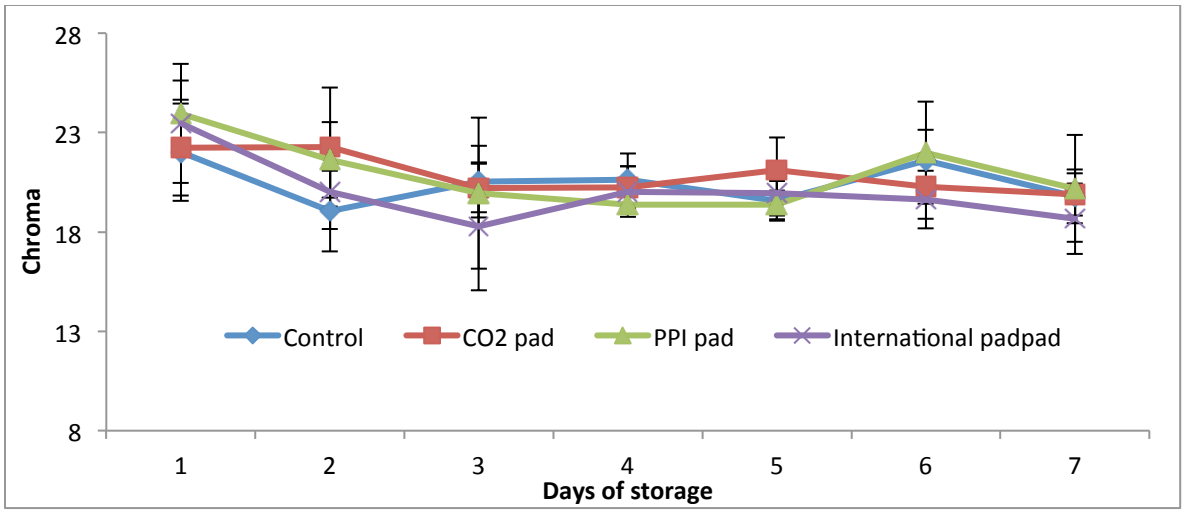

Fig. A1. Chroma values in beef steaks treated with 5 treatments and error bars representing standard deviation

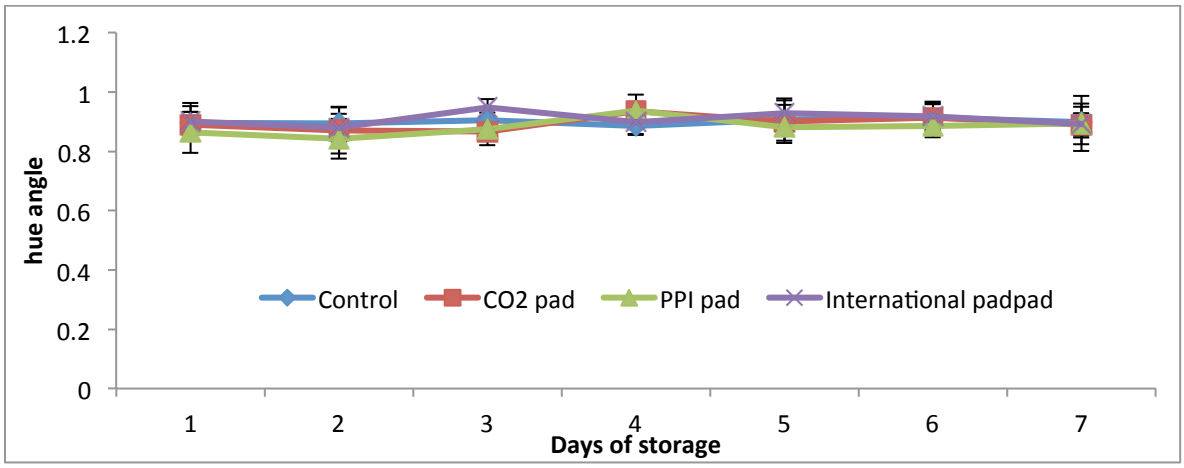

Fig. A2. Hue angle values in beef steaks treated with 5 treatments and error bars representing standard deviation

Control = Sealed Air standard pad; CO_2 pad = Sealed Air CO_2 generating pad; PPI pad = PPI Extendapak pad; International pad = International prime meat pad) as compared to control

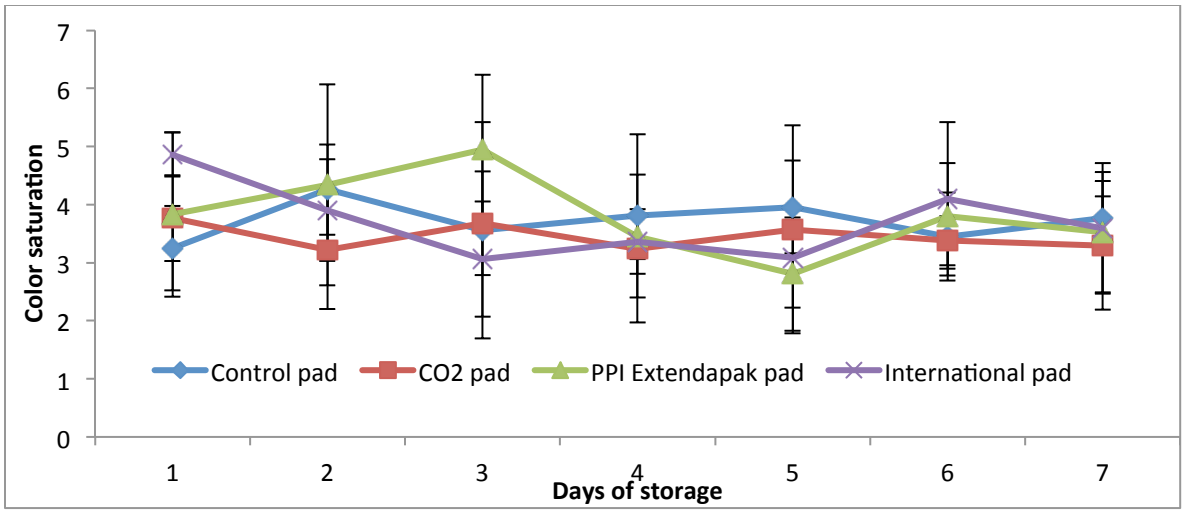

Fig. A3. Chroma values in chicken breast treated with 5 treatments and error bars representing standard deviation

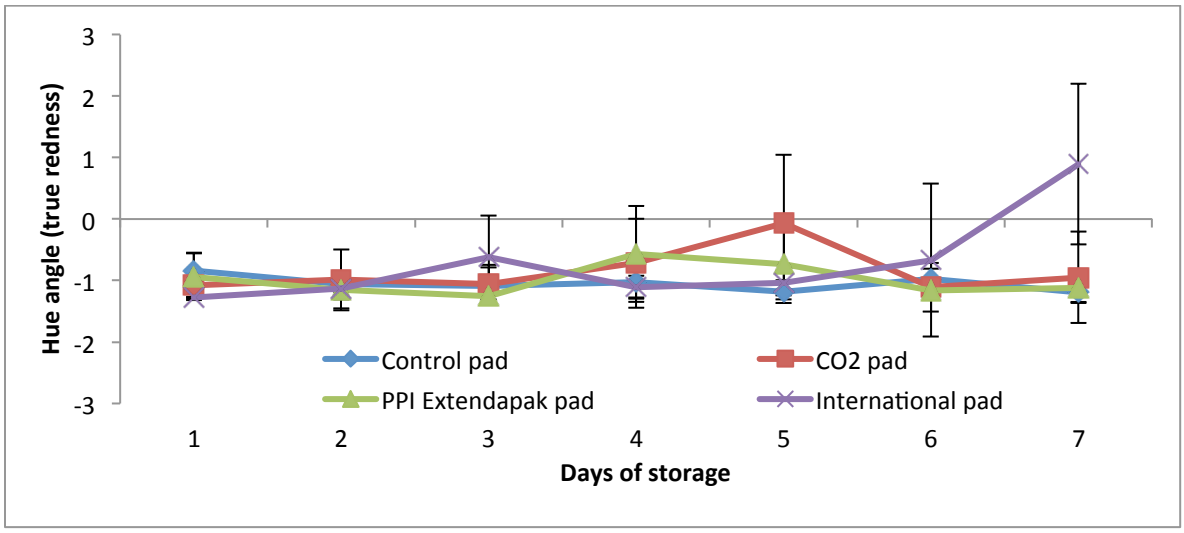

Fig. A4. Hue angle values in chicken breast treated with 5 treatments and error bars representing standard deviation

Control = Sealed Air standard pad; CO_2 pad = Sealed Air CO_2 generating pad; PPI pad = PPI Extendapak pad; International pad = International prime meat pad) as compared to control

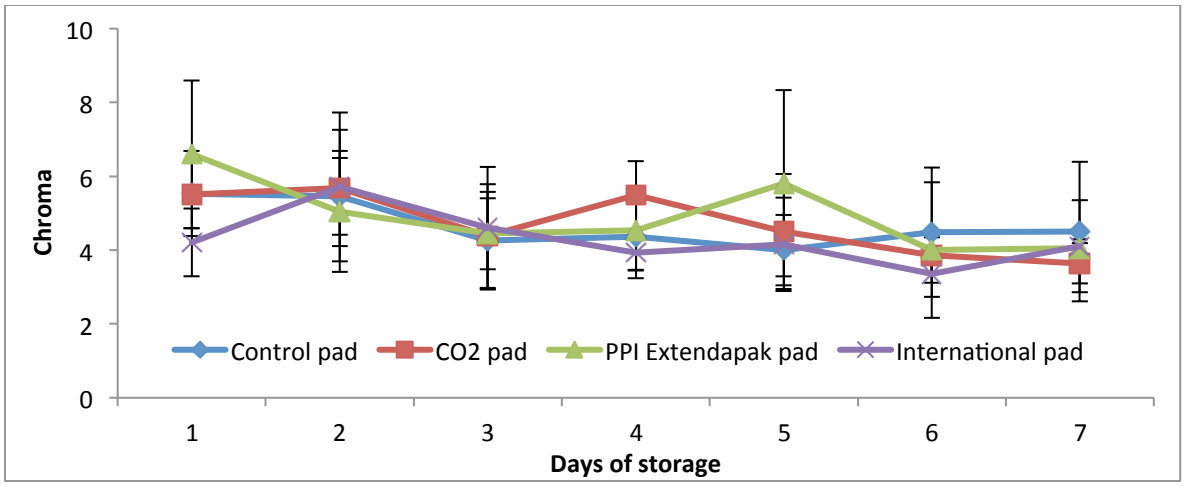

Fig. A5. Chroma values in tuna loins treated with 5 treatments and error bars representing standard deviation

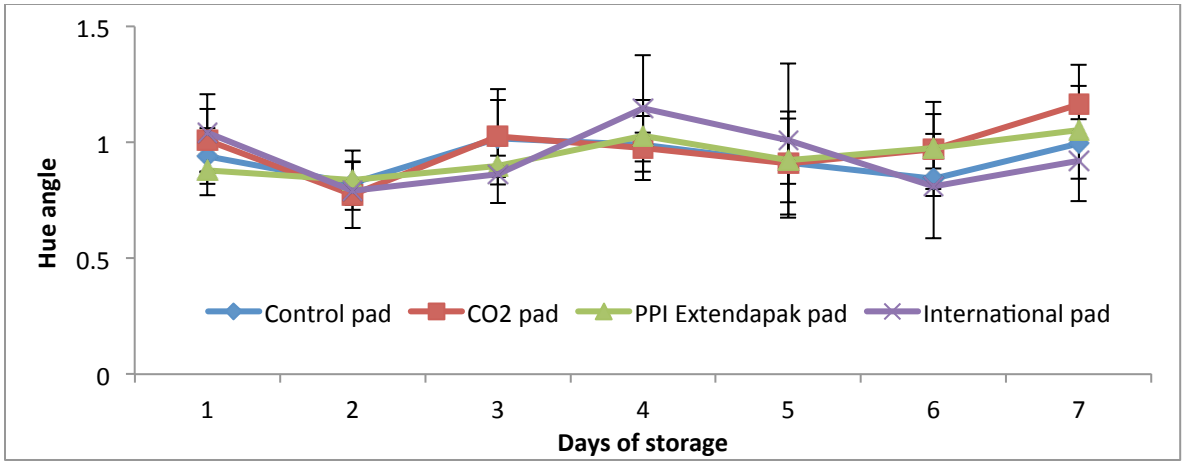

Fig. A6. Hue angle values in tuna loins treated with 5 treatments and error bars representing standard deviation

Control = Sealed Air standard pad; CO_2 pad = Sealed Air CO_2 generating pad; PPI pad = PPI Extendapak pad; International pad = International prime meat pad) as compared to control

APPENDIX B

STATISTICS FOR CHAPTER 4

Table B1. Chroma values for raw ground poultry patties: time to first significant difference as compared to day 0 and means at day 0 and day 10

Treatment	Days to first significant difference[A]	Color Saturation	
		Day 0[B]	Day 10[B]
Ground chicken	ns	1.45 ± 0.05 a	0.93 ± 1.38 a
0.05% Eugenol	ns	1.45 ± 0.04 a	-0.90 ± 1.37 a
0.5% MM	ns	1.49 ± 0.06 a	-0.92 ± 1.39 a
0.5% Phytate	ns	0.86 ± 1.34 a	-0.90 ± 1.38 a
0.05% RA	ns	1.48 ± 0.06 a	0.32 ± 1.69 a
0.5% STPP	ns	1.47 ± 0.06 a	0.93 ± 1.39 a

STPP = sodium tri-polyphosphate; RA = rosmarinic acid; MM = milk mineral.

A: Time point where first significant difference ($P < 0.05$) occurs as compared to initial reading for each meat system formulation. B: Values represent mean ± standard deviation at a given time point (n = 5). Values sharing letters are not significantly different ($P > 0.05$).

Table B2. Type 3 tests fixed effects (ANOVA) for lightness in raw ground poultry meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	5	116	7.96	< 0.0001
day	4	116	16.23	< 0.0001
trt*day	20	116	0.9	0.5917

Table B3. Type 3 tests fixed effects (ANOVA) for redness in raw ground poultry meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	5	116	2.42	0.0401
day	4	116	85.38	< 0.0001
trt*day	20	116	0.54	0.9454

Table B4. Type 3 tests fixed effects (ANOVA) for yellowness in raw ground poultry meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	5	116	2.11	0.1061
day	4	116	2.34	0.061
trt*day	20	116	0.97	0.5025

Table B5. Type 3 tests fixed effects (ANOVA) for color saturation in raw ground poultry meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	5	116	2.2	0.0943
day	4	116	2.06	0.0922
trt*day	20	116	0.95	0.5247

Table B6. Type 3 tests fixed effects (ANOVA) for hue angle in raw ground poultry meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	5	116	2.83	0.0191
day	4	116	21.44	< 0.0001
trt*day	20	116	1.16	0.2986

Table B7. Type 3 tests fixed effects (ANOVA) for TBA in raw ground poultry meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	5	116	5.39	0.0002
day	4	116	9.86	< 0.0001
trt*day	20	116	0.91	0.5779

Table B8. Type 3 tests fixed effects (ANOVA) for aerobic plate counts in raw ground poultry meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	5	116	2.03	0.0790
day	4	116	27.26	<0.0001
trt*day	20	116	0.3	0.9986

Table B9. Type 3 tests fixed effects (ANOVA) for pH in raw ground poultry meat.

Effect	Num DF	Den DF	F Value	Pr > F
trt	5	116	12.55	< 0.0001
day	4	116	37.3	< 0.0001
trt*day	20	116	0.49	0.9653

Table B10. Average fat percent values in raw ground poultry meat measured on Day 0.

Replicate	Percent Fat		Average & Std. dev. % Fat
	sample a	sample b	
Rep 1	5.98141696	5.10123065	5.54 ± 0.62
Rep 2	5.62335253	7.09935287	6.36 ± 1.04
Rep 3	6.68512658	5.84840323	6.27 ± 0.59
Rep 4	7.06304868	7.15279158	7.11 ± 0.06
Rep 5	9.02033998	8.03188839	8.53 ± 0.70

Table B11. Average free iron present in raw ground poultry patties measured on Day 0.

Sample	Absorbance	ppm of iron in dissolved ash (µg iron/ml) (std. curve)	Calculated iron in sample (µg/g)
Rep 1	0.031	0.081	0.797
Rep 1	0.030	0.078	0.766
Rep 2	0.029	0.076	0.761
Rep 2	0.034	0.091	0.908
Rep 3	0.035	0.094	0.923
Rep 3	0.040	0.110	1.087
Rep 4	0.031	0.082	0.824
Rep 4	0.020	0.046	0.462
Rep 5	0.018	0.040	0.397
Rep 5	0.065	0.194	1.937

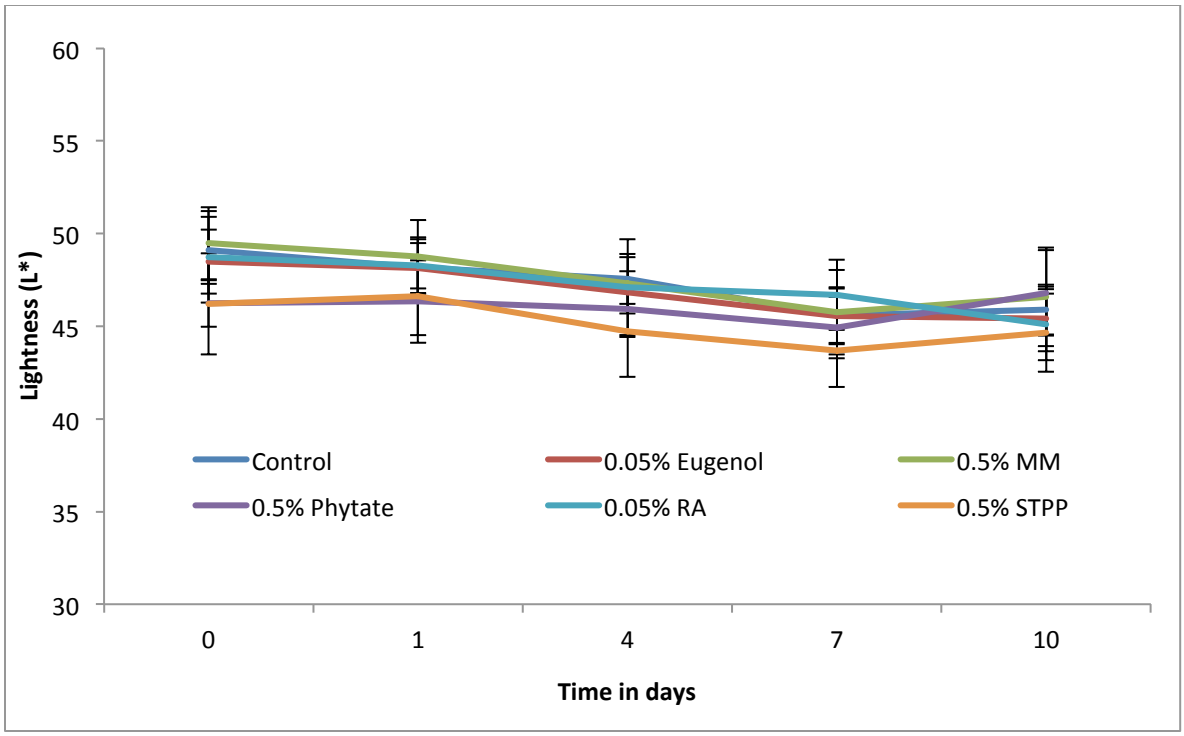

Fig. B1. Hunter L* average values in raw ground chicken patties treated with 0.05% eugenol, 0.5% milk mineral, 0.5% phytate, 0.05% rosmarinic acid, 0.5% sodium tri poly phosphate (STPP) as compared to control.

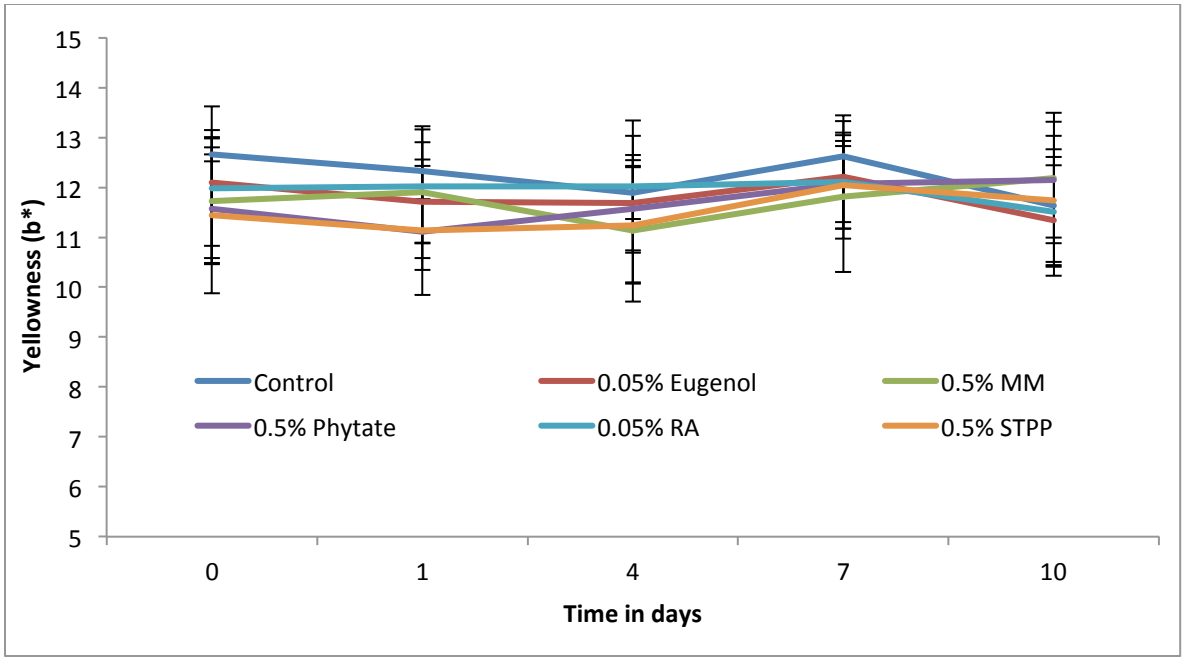

Fig. B2. Hunter b* average values in raw ground chicken patties treated with 0.05% eugenol, 0.5% milk mineral, 0.5% phytate, 0.05% rosmarinic acid, 0.5% sodium tri poly phosphate (STPP) as compared to control.

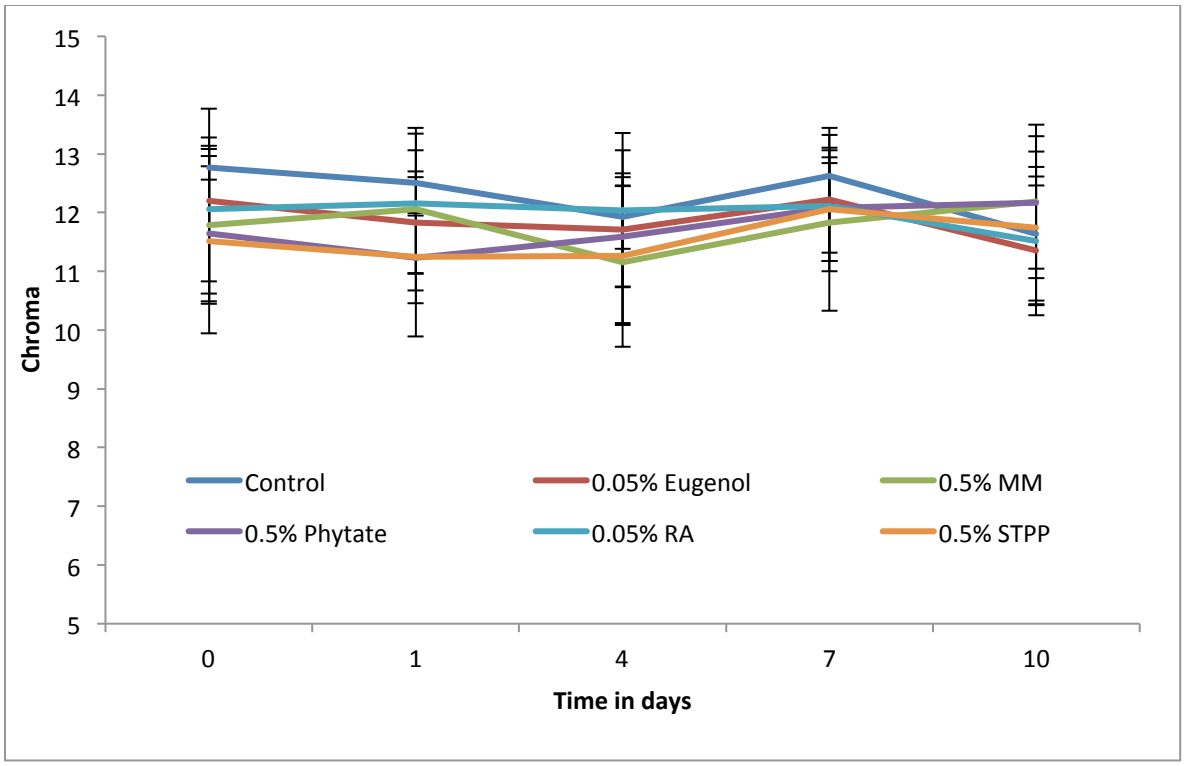

Fig. B3. Color saturation average values in raw ground chicken patties treated with 0.05% eugenol, 0.5% milk mineral, 0.5% phytate, 0.05% rosmarinic acid, 0.5% sodium tri poly phosphate (STPP) as compared to control.

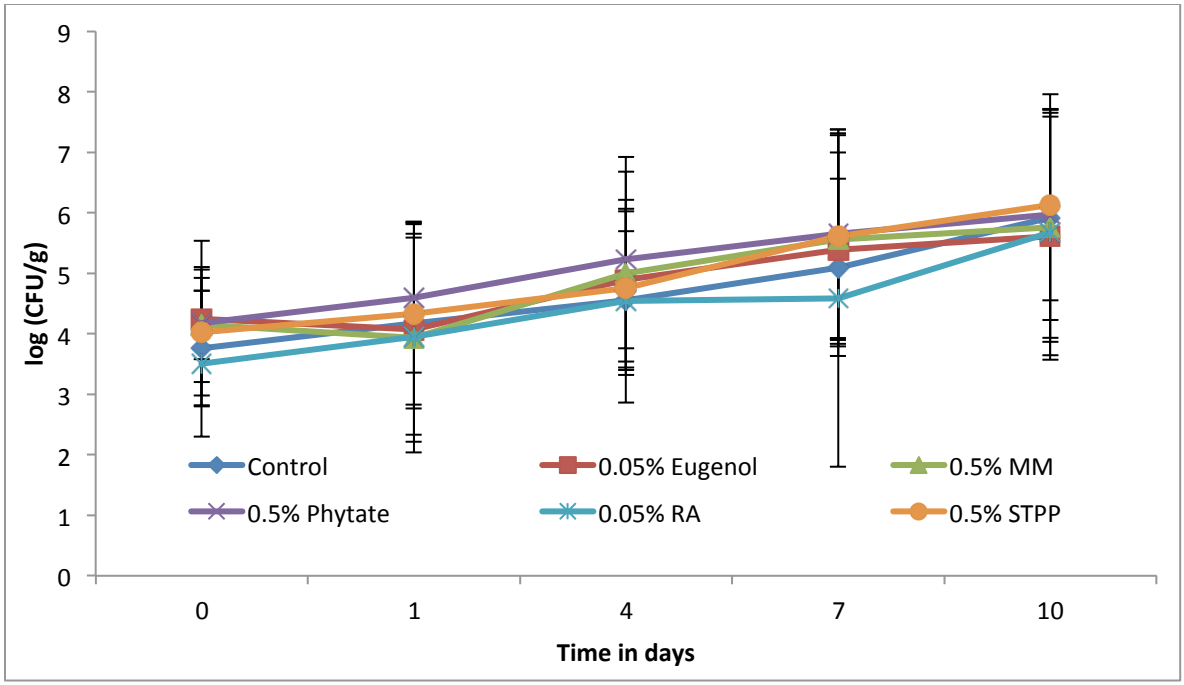

Fig. B4. Aerobic plate count average values in raw ground chicken patties treated with 0.05% eugenol, 0.5% milk mineral, 0.5% phytate, 0.05% rosmarinic acid, 0.5% sodium tri poly phosphate (STPP) as compared to control.