Mechanisms of lipid peroxidation in meats from different animal species

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Mechanisms of lipid peroxidation in meats from different animal species

by

Byungrok Min

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Co-Majors: Meat Science; Food Science and Technology

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2006

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# TABLE OF CONTENTS

ABSTRACT ............................................................................................................................................... v

CHAPTER 1. GENERAL INTRODUCTION ........................................................................................ 1
Introduction ........................................................................................................................................ 1
Dissertation organization ..................................................................................................................... 3
Literature Review ............................................................................................................................... 3
References ........................................................................................................................................ 25

CHAPTER 2. FACTORS AFFECTING OXIDATIVE STABILITY OF MEATS FROM DIFFERENT ANIMAL SPECIES ................................................................. 41
Abstract ........................................................................................................................................ 41
Introduction ....................................................................................................................................... 42
Materials and Methods ..................................................................................................................... 43
Results and Discussion .................................................................................................................... 46
Conclusion ....................................................................................................................................... 53
References ....................................................................................................................................... 54

CHAPTER 3. FACTORS AFFECTING OXIDATIVE STABILITY OF VARIOUS FRACTIONS FROM RAW CHICKEN BREAST AND BEEF LOIN ........................................... 70
Abstract ........................................................................................................................................ 70
Introduction ....................................................................................................................................... 71
Materials and Methods ..................................................................................................................... 72
Results and Discussion .................................................................................................................... 77
Conclusion ....................................................................................................................................... 86
References ....................................................................................................................................... 86

CHAPTER 4. PREDICTION OF THE MECHANISM OF METMYOGLOBIN ON LIPID PEROXIDATION IN PHOSPHOLIPID LIPOSOME MODEL SYSTEM ........................................ 104
Abstract ......................................................................................................................................... 104
ABSTRACT

The objectives of this study were 1) to determine the elements that make differences in the oxidative stability of meats from different animal species and 2) to determine the major catalysts of lipid peroxidation in meat and meat products. The most important difference in the susceptibility of meat from different animal species to lipid peroxidation was the balance between anti- and pro-oxidative factors in meat. Raw and cooked ground beef were the most susceptible to lipid peroxidation, followed by chicken thigh meat, pork, and chicken breast meat. The high storage stability of chicken breast meat was attributed to a very low concentration of myoglobin and high total antioxidant capacity (TAC), which prevented the release of free ionic iron from myoglobin and inhibited free radical chain reactions. The high susceptibility of beef to lipid peroxidation was due to the imbalance of pro- and anti-oxidant factors caused by relatively low TAC and high amount of myoglobin, which served as a major source of catalysts such as ferrylmyoglobin, hematin, and/or free ionic iron. The level of lipid peroxidation catalyzed by diethylenetriamine pentaacetic acid (DTPA)-chelatable iron released from myoglobin in the water-soluble high molecular weight (HMW) fraction from beef significantly decreased with the addition of water-soluble low molecular weight (LMW) and water-insoluble (P) fractions, indicating that most of the free ionic iron released from myoglobin were chelated by LMW and P fractions. Therefore, it is suggested that ferrylmyoglobin and/or hematin, rather than free ionic iron, are the major catalysts of lipid peroxidation in raw beef during “normal” storage conditions. Beef showed a significantly higher level of “stable” ferric iron reducing capacity (FRC) than did chicken breast under conditions that can induce the generation of sufficient amounts of free ionic iron, indicating that reduced iron also played an important role in lipid peroxidation of beef.
CHAPTER 1. GENERAL INTRODUCTION

Introduction

Consumer concerns on the quality of meat and meat products have greatly increased during past decades. A recent survey in pan-EU indicated that the most important factors influencing consumer attitudes in purchasing foods were ‘quality or freshness’, ‘price’, ‘taste’, ‘healthfulness’, and ‘family preferences’ (Lennernas et al., 1997). The quality attributes of meat include appearance/color, texture such as tenderness and juiciness, flavor/taste, firmness, cohesiveness, functional properties such as water-holding capacity and emulsifying ability, microbial quality, additives, residues, and nutritive values (Gray, Gomaa, & Buckley, 1996). Among these, however, three sensory properties—appearance/color, texture, and flavor—are the main factors which consumers use to judge meat quality (Liu, Lanari, & Schaefer, 1995). Faustman & Cassens (1990) and Risvik (1994) reported that consumer’s purchase decision for meat is more strongly affected by changes in appearance/color and texture than those in flavors (when off-flavors are not present) because visual appearance is more important factor especially at the market.

Lipid peroxidation is a major cause of quality deterioration in meat and meat products (National Research Council, 1988). Lipid peroxidation causes off-flavor and off-odor development, discoloration, texture change, and drip loss. Lipid peroxidation also causes loss of nutritional value and functionality, and generates compounds that can be detrimental to human health (Addis, 1986; Kanner, 1994; Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). The susceptibility of muscle lipids to oxidation is dependent upon the degree of unsaturation in fatty acids (Igene, Pearson, Dugan, & Price, 1980; Hsieh & Kinsella, 1989; Song & Miyazawa, 2001). Most polyunsaturated fatty acids (PUFA) are associated with phospholipids which are a major component of cell membranes (Sweeten, Cross, Smith, & Smith, 1990; Stanley, 1991). Phospholipids are reported to play more important roles in rancidity and warmed-over flavor (WOF) development in raw and cooked meat than neutral lipids (Wilson, Pearson, & Shorland, 1976; Pikul, Leszczynski, & Kummerow, 1984; Asghar, Gray, Buckley, Pearson, & Booren, 1988).

Lipid peroxidation primarily occurs through a free radical chain reaction. Free radical is
defined as any species that has one or more unpaired electrons which can exist independently and is believed to play critical roles as initiators, catalysts, and intermediates in lipid peroxidation processes (Halliwell & Gutteridge, 1990). Oxygen is the most important factor in the development of lipid peroxidation in meat (Ahn, Wolfe, Sim, & Kim, 1992; Ahn, Ajuyah, Wolfe, & Sim, 1993a). Theoretically, oxygen molecule and polyunsaturated fatty acid (PUFA) cannot interact with each other because of thermodynamic constraints. Ground state oxygen does not have strong reactivity, but can be converted to reactive oxygen species (ROS) such as hydroxyl radical (•OH), superoxide anion (O2⁻), hydrogen peroxide (H₂O₂), hydroperoxyl radical (HO₂⁺), lipid peroxyl radical (LOO•), alkoxyl radical (LO•), iron-oxygen complexes (ferryl and perferryl radical) and singlet oxygen (¹O₂), some of which are highly reactive to initiate lipid peroxidation (Halliwell & Gutteridge, 1999). In addition, numerous agents such as enzymes and transition metals can directly or indirectly catalyze these oxidative processes through enzymic and nonenzymic mechanisms. Especially, iron plays a critical role in the lipid peroxidation process as a major catalyst.

In order to prevent or retard lipid peroxidation in meat effectively, the mechanism of lipid peroxidation should be comprehensively understood. Especially, the control of catalyst is very important because catalyst can rapidly amplify free radical chain reactions. Much attention has been paid to the role of iron, which can directly and/or indirectly catalyze the initiation of lipid peroxidation. Many researchers have tried to elucidate which iron type and how iron is involved in lipid peroxidation in meat as well as what is the “real” initiator to be able to abstract hydrogen atom from lipid molecule at the beginning. Yet, these areas are still debatable even though tremendous research have been done and continues. In addition, the relationship of various factors involved in pre- and post-slaughtering and further processing to lipid peroxidation should be clarified, and development of the prevention strategies using the information from mechanism studies deserves more attention.

The goals of this study were to elucidate critical endogenous factors affecting lipid peroxidation in meats from different animal species and to determine “true” iron catalysts involved in lipid peroxidation of meat and meat products.
Dissertation organization

This dissertation is composed of a general introduction (Chapter 1), 5 individual papers (Chapters 2-6), and a general conclusion (Chapter 7). The general introduction (Chapter 1) contains the introduction of this study and literature review. The literature review contains overview of the mechanism of lipid peroxidation, proposed roles of reactive oxygen species in lipid peroxidation, possible sources of iron in meat, proposed roles of iron in lipid peroxidation, and the effect of various endogenous and exogenous factors on lipid peroxidation of meat and meat products. Chapter 2 shows the analysis of endogenous anti- and pro-oxidative factors affecting oxidative stability of meat from different animal species. Chapter 3 describes determining factors affecting different oxidative stability of chicken breast meat and beef loin. Chapter 4 elucidates the mechanism of metmyoglobin on lipid peroxidation in phospholipid model system. Chapter 5 shows the effect of various iron compounds such as metmyoglobin, ferric and ferrous ion, and iron chelators on the lipid peroxidation potential of fractions from chicken breast meat and beef. Chapter 6 is about the effect of metmyoglobin, ferric and ferrous ion on lipid peroxidation of raw and cooked chicken breast meat and beef. Finally, Chapter 7 contains general conclusion of this dissertation.

Literature Review

Mechanism of lipid peroxidation

Lipid peroxidation is a free radical chain reaction that is comprised of three primary steps: initiation, propagation, and termination. Initiation of lipid peroxidation takes place by attack of any species that has sufficient reactivity to abstract a labile hydrogen atom from a methylene group in lipid molecules (LH) to form lipid radicals (L\(^\ast\)) (Equation 1).

\[
\text{LH} + \text{Initiator}^\ast \rightarrow \text{L}^\ast + \text{Initiator H (reduced form)} \quad \quad \text{(Equation 1)}
\]

Wagner, Buettner, & Burns (1994) reported that the amount of lipid radical generated increased with the total number of bis-allylic carbons, and suggested that the number of bis-
allylic carbons in lipid molecules determines their susceptibility to lipid peroxidation. More importantly, the rate of lipid peroxidation exponentially increased with the number of bis-allylic carbons although lipid chain length had no relationship with the rate of radical formation. The differences in the initiation rate of lipid peroxidation are closely related to the dissociation energies of various carbon-hydrogen (C-H) bonds in fatty acid chains. The weakest C-H bond is at bis-allylic position, whose bond energy is 75-80 kcal/mol, and those at allylic position and alkyl C-H bond are ≈ 88 kcal/mol and ≈ 101 kcal/mol, respectively (Koppenol, 1990; Buettner, 1993). Consequently, the C-H bond at the bis-allylic position is the most reactive site for hydrogen abstraction. The well-known species capable of abstracting a hydrogen atom are ROS, especially *OH. Koppenol (1990) estimated that the reduction potential of PUFA radical/PUFA couple was +0.60 V at neutral pH, suggesting that PUFA could be readily oxidized by *OH (+2.31 V) as well as other ROS.

The abstraction of hydrogen atom (H*) from lipid chain leaves unpaired electron on the carbon of the chain (L*) because H* has only one electron. This carbon radical tends to be stabilized by a molecular rearrangement to form a conjugated diene. The conjugated diene formed can go through various reactions, depending on O2 concentration in biological system. Under aerobic conditions, the most likely fate of conjugated dienes is to react with oxygen molecules (O2) to form a lipid peroxyl radical (LOO*) (Equation 2):

\[ L^* + O_2 \rightarrow LOO^* \]  \hspace{2cm} (Equation 2)

On the other hand, under very low O2 conditions, a conjugated diene can react to each other within the membranes or other membrane components such as protein and cholesterol (Frank, Thiel, & MacLeod, 1989). The formation of conjugated dienes is accompanied by the configuration changes of the double bond from \textit{cis} to \textit{trans} form, which may allow unsaturated fatty acids to pack more tightly, leading to the creation of more rigid domains within bilayer of oxidized lipid (Coolbear & Keough, 1983). This abnormal conjugated diene can be one of the most important markers for lipid peroxidation in various meat systems.

In the propagation step (Equation 3), LOO* formed is able to abstract H* from another lipid molecule such as neighboring or surrounding fatty acids to form lipid hydroperoxide
(LOOH):

\[ \text{LOO}^* + \text{LH} \rightarrow \text{LOOH} + \text{L}^* \]  \hspace{1cm} \text{(Equation 3)}

Hydrogen abstraction from a bis-allylic position on the fatty acid chain by LOO* is favorable with Gibbs' free energy of $-9 \text{ kcal/mol}$ (Koppenol, 1990). In addition, because LOO* has a higher standard reduction potential (+1.0V) than lipid molecule itself (+0.60 V) does, it can oxidize favorably an adjacent PUFA. Newly formed L* can form another LOO* by reacting with $\text{O}_2$, so the free radical chain reaction can continue. LOOH is a prominent non-radical intermediate of lipid peroxidation whose identification often provides valuable information on the related mechanisms. Because LOOHs are more polar molecules than normal fatty acids, LOOHs can disrupt the integral structure and function of the membrane, resulting in deleterious effect to cells and tissues. LOOHs may undergo various reactions, depending on environments in cell or tissue. Four principal fates of LOOH include: 1) spontaneous or protein-facilitated transfer between intracellular and extracellular membranes, 2) enzymatic de-esterification or trans-esterification for subsequent disposal or short-term safe storage, 3) nonenzymatic one-electron reduction to oxyradicals, leading to free radical chain reaction and enhanced cytotoxicity, and 4) enzymatic two-electron reduction to alcohol, resulting in diminished toxicity (Girotti, 2002). Under low hydrogen-donating conditions, however, LOOH tends to undergo further reactions such as combination, intermolecular addition, intramolecular rearrangement, and further reactions with additional $\text{O}_2$ molecule, resulting in the formation of numerous secondary derivatives such as cyclic peroxides, prostaglandin-like bicycloendoperoxides, multi-hydroperoxyl derivatives, etc., double bond isomerization, and formation of dimers and oligomers (Gardner, 1989; Porter, Caldwell, & Mills, 1995). In addition, another complexity of LOOH derivatives formed is caused by the fact that hydrogen abstraction from PUFA can take place at different points on the fatty acid (Gardner, 1989). Especially, hydroperoxyl cyclic peroxides and bicycloendoperoxides can be precursors of malonaldehyde, 2-thiobarbituric acid reactive substance (TBARS). The formation of various LOOHs and their derivatives possibly produced from primary PUFA has been reviewed by others (Hsieh & Kinsella, 1989; Porter et al., 1995; Girotti, 1998).
The last step of lipid peroxidation is termination process in which the LOO•s reacts with each other and/or self-destruction to form non-radical products. Although LOOH is stable at physiological temperature, it can be decomposed by heating at high temperature or by exposure to transitional metal ions (Halliwell & Gutteridge, 1990). Numerous secondary derivatives of hydroperoxides can be decomposed via homolytic and heterolytic β-scission catalyzed by transitional metal ions to generate a huge range of volatile and nonvolatile compounds such as carbonyls (e.g. ketones and aldehydes), alcohols, hydrocarbons (e.g. alkane, alkene), and furans that contribute to the flavor deterioration in many foods (Frankel, 1987). For instance, the homolysis at O–O bonds on the sixth carbon from the methyl end of n-6 fatty acid produces pentane radical by subsequent β-scission (Halliwell & Gutteridge, 1999). The pentane radical abstracts a hydrogen atom from another fatty acid to form pentane gas. Therefore, pentane gas is primarily produced from n-6 fatty acids such as linoleic acid and arachidonic acid. Ethane and ethylene gas can be produced by similar reaction from n-3 fatty acids such as linolenic acid. Kostrucha & Kappus (1986) reported that the formation of ethane and n-pentane in rat liver microsomes increased as the concentration of oxygen decreased, whereas malonaldehyde concentrations were proportional to oxygen concentration. This suggested that ethane or n-pentane and malonaldehyde were derived from two different reaction sequences of microsomal lipid peroxidation with different oxygen sensitivity. Hexanal, 1-octen-3-ol, 2-nonenal, and 4-hydroxy-2-trans-nonenal (HNE) are reported to be originated from n-6 fatty acids and propanal, 4-heptenal, 2,4-heptadienal, 2-hexenal, 2,4,7-decatrienal, 1,5-octadien-3-ol, 2,5-octadien-1-ol, 1,5-octadien-3-one, and 2,6-nonadienal are from n-3 fatty acids (Hsieh & Kinsella, 1989, Frankel, 1993). Among these volatiles, aldehydes are one of most abundant, and are highly reactive and regarded as second toxic messengers that disseminate and augment initial free radical reactions (Esterbauer, Schaur, & Zollner, 1991). Aldehydes generated from lipid peroxidation were reported to be capable of reacting with protein to form adducts which may be related to the deterioration of protein stability and functionality (Lynch, Faustman, Silbart, Rood, & Furr, 2001). Also, Lynch & Faustman (2000) suggested that aldehydes increase oxymyoglobin oxidation and prooxidant activity of metmyoglobin and decrease the enzymatic reduction of metmyoglobin, which is directly related to the deterioration of meat color and flavor. The primary aldehydes
generated during lipid peroxidation in stored beef are propanal, pentenal, hexanal, and 4-hydroxynonenal (HNE) (Lynch et al., 2001). Hexanal among aldehydes is the most prevailing volatile generated from cooked meat. It has been suggested as an index of meat flavor deterioration (MFD) during early storage stages of cooked meat because its concentration increased more quickly than any other aldehydes (Shahidi & Pegg, 1994). In addition, HNE is known to have cytotoxic properties for human and animals by binding to proteins to inhibit their functions (Okada, Wangpoengtrakul, Osawa, Toyokuni, Tanaka, & Uchida, 1999).

Reactive oxygen species (ROS) in lipid peroxidation

Although oxygen is essential for life, it can cause damage to various cells. The toxicity of oxygen is caused not by oxygen itself, but by the increased production of ROS. ROS can be produced under normal physiological conditions, but the amounts do not exceed the capacity of natural defense systems in body. The reduction of oxygen molecule by way of one-electron reduction processes produces short-lived but highly reactive oxygen products such as hydroxyl radical (·OH), superoxide anion (O_2^·), hydrogen peroxide (H_2O_2), hydroperoxyl radical (HO_2^·), and iron-oxygen complexes (ferryl and perferryl radical) (Halliwell & Gutteridge, 1999), all of which may directly or indirectly participate in lipid peroxidation processes in meat and meat products.

1) Superoxide anion radical (O_2^·) and hydroperoxyl radical (HO_2^·)

Superoxide anion radical (O_2^·) is produced by one-electron reduction of oxygen, which acts as an intermediate in a number of biochemical reactions in body. Under physiological conditions, O_2^· could be generated by numerous ways in muscle tissues. One of major sources of O_2^· in muscle tissues are various components of electron transport chain in mitochondria such as NADPH-dependent dehydrogenase and ubiquinone which may leak electrons onto O_2 (Turrens & Boveris, 1980; Thomas, 1995). The autoxidation of heme proteins (Balagopalakrishna, Manoharan, Abugo, & Rifkind, 1996; Baron & Andersen, 2002) and enzymes associated with metabolism such as xanthine oxidase (Sanders-Stephen,
Eisenthal, & Harrison, 1997) are other major sources. Activation of several leukocytes present in the vasculature of muscle tissue by the internalization of bacteria causes production of \( \text{O}_2^- \) because \( \text{O}_2^- \) is one of the major bactericides (Thomas, Learn, Jefferson, & Weatherred, 1988).

Superoxide anion radical (\( \text{O}_2^- \)) is a poorly reactive radical in aqueous solution although it is highly reactive in hydrophobic environments. Hydroperoxyl radical (\( \text{HO}_2^- \)), the protonated form of \( \text{O}_2^- \), is a more reactive than \( \text{O}_2^- \) itself (Equation 4).

\[
\text{HO}_2^- \rightleftharpoons \text{O}_2^- + \text{H}^+ \quad \text{(Equation 4)}
\]

The pK_a of this reaction is approximately pH 4.8 in aqueous solution. Therefore, less than 1\% of \( \text{O}_2^- \) produced exists in this protonated form under physiological conditions (pH 7.4). The negative charges of membrane surface due to phosphatidyl moiety of phospholipid may cause pH drop around the membrane, resulting in the increase of \( \text{O}_2^- \) concentration at the membrane surface (Barber, 1980). The pH in muscle tissue after post-rigor also decreases from around 7 to 5.5-6.0, so the amount of \( \text{HO}_2^- \) could be 10-20\% of total \( \text{O}_2^- \). The poor reactivity and relatively long half-life of \( \text{O}_2^- \) in cytosol allows it to diffuse more effectively from its generation site to targets such as membrane lipid bilayers than \( \text{HO}_2^- \) or other reactive species. Furthermore, much of \( \text{O}_2^- \) generated in cell may be produced near membrane by membrane-bound systems such as electron transport system of mitochondria (Turrens & Boveris, 1980; Thomas, 1995). However, \( \text{O}_2^- \) was suggested not to be able to permeate deep into liposomal bilayer (Frimer, Strul, Buch, & Gottlieb, 1996). Subsequently, some part of \( \text{O}_2^- \) present near membrane could exist as \( \text{HO}_2^- \). Uncharged conditions of \( \text{HO}_2^- \), unlike \( \text{O}_2^- \), allow it to permeate into membrane lipid bilayer, where it could initiate lipid peroxidation process by abstracting hydrogen atom from bis-allylic position of PUFA in phospholipids (Gebicki & Bielski, 1981; Bielski, Arudi, & Sutherland, 1983). Aikens & Dix (1991 & 1992) indicated that the effectiveness of \( \text{HO}_2^- \) for the initiation is directly related to the initial concentration of \( \text{LOO}^- \) in lipids because \( \text{LOO}^- \) generated by either direct or indirect hydrogen atom transfer between \( \text{HO}_2^- \) and \( \text{LOO}^- \) can initiate lipid peroxidation more efficiently than \( \text{HO}_2^- \) itself. However, the evidence for the ability of \( \text{HO}_2^- \) to mediate directly
the initiation of free radical chain reaction has not been proven yet.

A major toxicity of O$_2^-$ in lipid peroxidation is attributed to its ability to reduce ionic irons which are re-oxidized by H$_2$O$_2$ to produce *OH (Equations 5 and 6) - the most reactive oxygen species that can abstract hydrogen atom from bis-allylic position of PUFA chains and initiates lipid peroxidation (Liochev & Fridovich, 1994):

Fe (III)-complex + O$_2^-$ $\rightarrow$ Fe (II)-complex + O$_2$ \hspace{1cm} (Equation 5)
Fe (II)-complex + H$_2$O$_2$ $\rightarrow$ Fe (III)-complex + OH$^-$ + *OH \hspace{1cm} (Equation 6)

In addition, Liochev & Fridovich (1994) and Fridovich (1995) suggested that O$_2^-$ \textit{in vivo} oxidizes the [4Fe-4S] clusters of dehydratases such as mammalian aconitase causing inactivation of enzyme and release of Fe(II) ion. Also, O$_2^-$ is suggested to decrease the activity of antioxidant defense enzymes such as catalase and glutathione peroxidase (Halliwell & Gutteridge, 1999). Meanwhile, O$_2^-$ is indicated to serve not only as a reducing agent for Fe (III), but also as an oxidant for Fe(II) depending on the ligands or chelators of iron. Ahn, Wolfe, & Sim (1993b) and Ahn & Kim (1998a) in their mechanism study with O$_2^-$-generating systems associated with various iron sources indicated that O$_2^-$ is a strong oxidant rather than a reducing agent and the antioxidant effect of O$_2^-$-generating systems, especially xanthine oxidase system, in oil emulsion is due to the oxidation of Fe(II) to Fe(III) by O$_2^-$ and/or H$_2$O$_2$.

2) Hydrogen peroxide (H$_2$O$_2$)

Low concentrations of hydrogen peroxide (H$_2$O$_2$) are present in aerobic cells as a metabolite under physiological conditions. An O$_2^-$-generating system would be expected to yield H$_2$O$_2$ by non-enzymatic or superoxide dismutase (SOD)-catalyzed dismutation. The generation of H$_2$O$_2$ has been easily detected in mitochondria, microsomes, peroxisomes, and phagocytic cells. Also, several enzymes, including aldehyde oxidase, xanthine oxidase, urate oxidase, glucose oxidase, glycolate oxidase, and D-amino acid oxidase, etc., can directly produce H$_2$O$_2$ (Kanner, German, & Kinsella, 1987; Halliwell & Gutteridge, 1990). Giulivi, Hochstein, & Davies (1994) reported that H$_2$O$_2$ is generated at a rate of approximately 3.9 x
$10^{-9}$ M/h x g hemoglobin and the concentration of H$_2$O$_2$ in the steady-state red blood cells is around $2 \times 10^{-10}$ M, indicating that the main source of H$_2$O$_2$ in red blood cells is probably oxyhemoglobin autoxidation. Chan, Faustman, Yin, & Decker (1997) suggested that H$_2$O$_2$ generated during oxymyoglobin oxidation plays an important role in lipid peroxidation. Harel & Kanner (1985) reported that turkey muscle tissues stored at 37°C for 30 minutes generated almost 14.0 nmol H$_2$O$_2$ per gram of fresh weight and its generation increased with storage at 4°C.

H$_2$O$_2$ is not a radical because it has no unpaired electron, and has limited reactivity and permeability to biological membrane unlike the charged O$_2$\(^{\cdot-}\) (Halliwell & Gutteridge, 1985). The poor reactivity and relatively long half-life of H$_2$O$_2$ like O$_2$\(^{\cdot-}\) enables it to diffuse more effectively from its generation site to targets such as membrane lipid bilayers. However, H$_2$O$_2$ can perform most of its damaging effects by generation of more reactive species such as *OH by catalysis of Fe(II) (Lamba, Borchman, & Garner, 1994). In addition, H$_2$O$_2$ can denature heme proteins to release free irons and heme group or convert heme protein to ferryl or perferryl radical, depending on its concentration (Baron & Andersen, 2002).

3) Hydroxyl radical (*OH)

Hydroxyl radical (*OH) is the most reactive oxygen radical with high positive reduction potential (Halliwell & Gutteridge, 1990). Usually, the steady-state concentration of *OH is effectively zero in vivo because it would react at or close to its site of formation with every molecule in living cell, such as DNA, protein, phospholipid, amino acid, sugar, etc. as soon as *OH is produced. Pryor (1988) hypothesized that the high reactivity of *OH is due to a rare combination of three characteristics: high electrophilicity, high thermochemical reactivity, and a mode of production that can occur near target molecules.

Bannister & Thornalley (1983) presented a direct evidence for the generation of *OH by adriamycin in intact red blood cells using the electron paramagnetic resonance (EPR) technique of spin trapping. Most of the *OH generated in vivo or in situ came from Fe(II)-catalyzed decomposition of H$_2$O$_2$ (Halliwell & Gutteridge, 1990). In addition, *OH can also be generated by various sources: sunlight (Joseph & Aravindakumar, 2000), ultraviolet radiation (Floyd, West, Eneff, Hogsett, & Tingey, 1988), ionizing irradiation (Swallow, 1984),
the reaction of hypochlorous acid with O$_2^-$ (Folkes, Candeias, & Wardman, 1995), and sonolysis of water (ultrasound) (Riesz, Kondo, & Krishna, 1990; Henglein & Kormann, 1985).

The reaction of $^\cdot$OH can be inhibited by $^\cdot$OH scavengers such as mannitol, formate, thiourea, dimethylthiourea, methanol, ethanol, 1-butanol, glucose, tris-buffer, or sorbitol (Halliwell & Gutteridge, 1990). Although $^\cdot$OH scavengers usually inhibit the reaction of $^\cdot$OH with other molecules including lipid molecules, sometimes they do not act effectively. There are a couple of reasons that should be considered: 1) the reaction of $^\cdot$OH with a scavenger may generate scavenger radicals that might react with other molecules in the system. The production and reactivity of secondary radicals may sometimes be responsible for the unsuccessful protection by one scavenger (Miller & Raleigh, 1983). 2) More attention has been paid on the possibility of the metal-mediated site-specific mechanism (Lamba et al., 1994; Chevion, 2003): $^\cdot$OH generated in vivo by the reaction of H$_2$O$_2$ with metal ions bound to macromolecules in cells can reacts with the metal-binding molecules or the nearest molecules immediately after production before the scavengers give access to them. Auroma, Grootveld, & Halliwell (1987) demonstrated the evidence for the formation of complex of Fe(II) ion and 2-deoxyrebose, suggesting that Fe(II) ion bound to DNA reacts with H$_2$O$_2$ to form $^\cdot$OH that immediately damages DNA. Baysal, Sullivan, & Stern (1989) suggested that Fe(III) ion binds to membrane and then generates free radicals at the binding site. Although the specific binding site of iron to membrane was not found, they suggested that carboxyl groups of sialic acids, sulfate group of glycolipids and glycoproteins, and sulfin and sulfon group together with phosphate head group of the phospholipids are considered as the major binding sites on erythrocyte membrane. Gutteridge (1984) indicated that $^\cdot$OH scavengers inhibited $^\cdot$OH generation effectively in the presence of EDTA because EDTA may allow Fe(II) ions to be removed from these binding sites. Therefore, the actual toxicity of O$_2^-$ and H$_2$O$_2$ may be dependent on the availability and distribution of metal ion catalysts to generate $^\cdot$OH in cells.

4) Iron-oxygen complexes (ferryl and perferryl radical)

The ferryl [Fe(IV)] and perferryl [Fe(V)] radicals are catalytically active in numerous
biological processes, and these ferryl/perferryl moiety, whether as components of enzyme or simple iron complex, can be very powerful oxidants capable of abstracting hydrogen atom in lipid peroxidation (Bielski, 1991). It has been indicated that an oxidizing intermediate generated in Fe(II)-EDTA-H2O2 system does not undergo the characteristic reactions of \( \cdot \cdot \cdot \cdot \)OH but shows a pattern of reaction more associated with ferryl complex (Koppenol, 1985; Yin, Lingnert, Ekstrand, & Brunk, 1992). Also, it has been suggested that the reaction of metmyoglobin and methemoglobin with low concentration H2O2 generates a short-lived ferryl- and perferrylmyoglobin that contain one oxidizing equivalent on heme and another one on globin, and is not affected by all efficient \( \cdot \cdot \cdot \cdot \)OH scavengers (Gibson, Ingram, & Nicholls, 1958; Harel & Kanner, 1988; Davies, 1989; Egawa, Shimada, & Ishimura, 2000). Xu, Asghar, Gran, Pearson, Haug, & Grulke (1990) reported that free radicals generated by H2O2 activation of metmyoglobin by electron spin resonance (ESR) techniques may be a ferrylmyoglobin.

**Iron in lipid peroxidation**

Iron is the most abundant transitional metal in biological systems. Although iron has the possibility of various oxidation states (from –II to +VI), the forms of Fe(II) and Fe(III) are dominant in biological systems. The ability of iron with various oxidation state, reduction potential, and electron spin configuration depending upon different ligand environments allows it to serve in multifunctional roles as a protein cofactor (Welch, Zane Davis, Van Eden, & Aust, 2002). Metal-binding proteins in biological system are usually classified by the functional role of metal ion: structural, metal-ion storage and transport, electron transport, dioxygen binding, and catalytic protein (Crichton, 2001). However, the versatile potential of iron allows it to catalyze the detrimental oxidation of biomolecules such as DNA, protein, lipid, etc. Therefore, iron metabolism *in vivo* should be tightly regulated by iron-binding proteins in order to ensure the absence of free forms of iron.

1) Iron distribution in tissue

Iron is distributed in five distinct pools, including transport, storage, oxygen-carrying, functional, and low molecular weight irons, represented by transferrin, ferritin, hemoglobin/
myoglobin, iron-dependent enzymes, and small transit pool of iron chelates, respectively (Crichton, 2001). About two-thirds of body iron is found in hemoglobin, with smaller amounts of myoglobin, various iron-containing enzymes, and transferrin. The rest not used for these is stored in intracellular storage protein, ferritin and hemosiderin. Intracellular concentration of free ionic iron seems to be extremely small.

The concentrations of myoglobin and hemoglobin in muscle tissue are dependent on animal species, muscle type, and anatomical location of muscle (Schricker, Miller, & Stouffer, 1982). Both myoglobin and hemoglobin-bound iron accounted for 73.3, 47.0, and 28.5% of total iron concentration in beef, pork, and chicken thigh meat, respectively (Hazell, 1982). Myoglobin (70%) is the predominant iron compound in beef. Myoglobin accounts for most of heme iron in beef and pork, but the level of myoglobin in chicken breast and thigh muscle is very low. Meanwhile, Schricker & Miller (1983) suggested that the heating or addition of H₂O₂ caused the release of heme iron due to oxidative cleavage of porphyrin ring of heme. Han et al (1993) reported that the iron content in water-soluble fractions of heated beef and chicken thigh muscle decreased due to the reduction of heme iron content, but that in water insoluble fractions increased because the denatured heme protein was also included in insoluble fraction.

Transferrin and ferritin are major iron transport and storage proteins, which are capable of binding two and ~2500 Fe(III) ion at a time, respectively. The structure, function, physiological role, and relationship to oxidative processes of them are well reviewed by Crichton & Charloiteaux-Wauters (1987), Reif (1992), and Welch et al (2002). However, the role of transferrin as a catalyst for lipid peroxidation has not been yet demonstrated in muscle tissues although released irons from transferrin were suggested to participate in lipid peroxidation process in other tissues (Balagopalakrishna, Pak, Pillarisetti, & Goldberg, 1999). Meanwhile, the concentrations of ferritin-bound iron in beef, pork, and chicken thigh muscles were reported to be 1.2%, 4.6%, and 11.1% of total iron concentration in each species, respectively (Hazell, 1982). Ferritin has been suggested to be involved in oxidative deterioration by releasing Fe(II) in the presence of reducing compound such as O₂⁻ and ascorbate (Boyer & Mc Cleary, 1987; Seman, Decker, & Crum, 1991), heating, or refrigerated storage (Decker & Welch, 1990; Kanner & Doll, 1991; Han et al., 1993). Hemosiderin is an
insoluble complex of iron, other metals and proteins, and is thought to be a ferritin
decomposition or polymerization products (Decker & Hultin, 1992). In pork and chicken
muscles over a half of total iron (58%) was detected in insoluble fraction (Hazell, 1982).
Although the amount and precise nature of the insoluble fraction in muscle is unknown, some
of them may be from hemosiderin. Hemosiderin may release its bound iron in the presence of
reducing agents, resulting in the acceleration of \*OH generation, but is far less effective than
ferritin. Therefore, it was proposed that the conversion of ferritin to hemosiderin in vivo is
biologically favorable because it reduces the availability of iron to promote lipid peroxidation
(O’Connell, Halliwell, Moorhouse, Aruoma, Baum, & Peter, 1986). The amount of
hemosiderin in muscle tissues has not been known yet.

An extremely small amount of nonprotein-bound iron is present in cells. This cytosol iron
pool is often considered as the transit pool because it is related to iron in transit between
transferrin and ferritin (Crichton, 1987). Both ferritin and heme protein also provide or draw
iron to or from the low molecular weight iron pool (LaCross & Linder, 1980). Although very
low solubility of iron under physiological conditions causes the precipitation of iron with
anions such as hydroxyl ion (OH\(^ -\)), various chelators can increase its solubility significantly
(Graf, Mahoney, Bryant, & Eaton, 1984). Thus, the intermediate pool of iron is composed of
iron associated with low molecular weight chelators such as organic phosphate esters (e.g.
NAD(P)H, AMP, ADP, and ATP), inorganic phosphates, amino acids, and organic acids (e.g.
citrate), which is called as low molecular weight iron (Halliwell & Gutteridge, 1990; Decker
& Hultin, 1992). The existence of low molecular weight iron pool has been identified in
various tissues (Mulligan, Althaus, & Linder, 1986; Voogd, Sluiter, Van Eijk, & Koster, 1992;
Kozlov, Yegorov, Vladimirov, & Azizova, 1992) as well as in mitochondria for heme
synthesis (Tangeras, 1985). In muscle tissues, the content of low molecular weight iron
accounts for 2.4~3.9% of total iron, depending on animal species and muscle types (Hazell,
1982), and the concentration can be influenced by heating (Buchowski, Mahoney, Carpenter,
& Cornforth, 1988; Han et al., 1993), the presence of ascorbic acid and H\(_2\)O\(_2\) (Schricker &
Miller, 1983) and storage (Kanner, Hazan, & Doll, 1988a). Neither the size nor the chemical
nature of intermediate pool of iron has been clearly identified.
2) Importance of iron as a catalyst for initiation of lipid peroxidation

Nonenzymic catalysis: the initiation mechanism of lipid peroxidation is still an area of controversy. Iron is the most probable catalyst for the initiation of lipid peroxidation by catalyzing the generation of most \( \cdot \text{OH in vivo or in vitro} \) via Fenton reaction (Equation 6) and is cycled by reducing agents such as \( \text{O}_2^- \) and ascorbic acid (Halliwell & Gutteridge, 1990; Liochev & Fridovich, 1994; Buettner & Jurkiewicz, 1996). In general, ascorbic acid can serve as both an antioxidant and a prooxidant, depending on relative concentrations of ascorbate and iron present (Decker & Hultin, 1992). Ascorbate at low concentration is most likely to promote lipid peroxidation in muscle tissue by the reduction of iron, whereas at high concentration it tends to reduce some of \( \text{LOO}^* \) directly to \( \text{LOOH} \), resulting in breaking the free radical chain reactions and also regenerates \( \alpha \)-tocopherol in biological membrane. In turkey muscle, reducing compounds were observed at the level of \( \sim 3 \text{ mg ascorbate equivalent per 100 g of fresh weight} \), 80% of which would be ascorbic acid (Kanner, Salan, Harel, & Shegalovich, 1991a).

Many studies have tried to determine which form of iron is responsible for the catalysis of the lipid peroxidation in meat and meat products. In the beginning, the involvement of heme proteins as catalyzing agents of lipid peroxidation was first described by Robinson in 1924 (Kanner, 1994). Many studies have indicated that heme pigments in meat play a pivotal role in catalysis of lipid peroxidation in the model systems with various meat species such as beef and fish (Johns, Birkinshaw, & Ledward, 1989; Monahan, Crackel, Gray, Buckley, & Morrissey, 1993; Han, McMillin, Godber, Bidner, Younathan, & Hart, 1995; Richards, Modra, & Li, 2002). Rhee & Ziprin (1987) showed that the levels of lipid peroxidation were dependent on animal species and muscle type in raw meat, and beef was more susceptible to lipid peroxidation than pork and chicken muscle. They proposed that total pigment and myoglobin concentrations are well related to the differences in lipid peroxidation of stored, raw muscles in three species.

Alternatively, Kanner et al. (1988a) and Kanner, Shegalovich, Harel, & Hazan (1988b) suggested that lipid peroxidation in minced turkey muscle is primarily affected by “free” ionic iron and this suggestion was confirmed by the chelating ability of ceruloplasmin to low molecular weigh iron (Kanner, Sofer, Harel, & Doll, 1988c). Ascorbate and \( \text{H}_2\text{O}_2 \) presented in
the cytosol of muscle cells can release free iron from heme pigments and ferritin, respectively, which can catalyze lipid peroxidation in meat and meat products (Harel & Kanner, 1985a; Boyer & McCleary, 1987). Also, Ahn, Wolfe, & Sim (1993c) and Ahn & Kim (1998b) suggested that free ionic iron played an important role in the catalysis of lipid peroxidation, but neither transferrin- and ferritin-bound irons nor heme pigments had any catalytic effect in raw turkey muscle. They suggested that the status of ionic iron is more important than the amount of iron. Ahn & Kim (1998b) in their study with oil emulsion systems indicated that Fe(III) catalyzed lipid peroxidation only in the presence of ascorbic acid while Fe(II) did it alone. In addition, studies using cooked meat model systems containing water-extracted muscle fractions indicated that nonheme iron released from heme pigments and ferritin by heating is the principal catalyst rather than myoglobin (Decker & Welch, 1990; Kanner & Doll, 1991; Apte & Morrisssey, 1987). Therefore, these studies indicated that free ionic iron released from heme pigments and ferritin may be considered as the major catalyst for lipid peroxidation in raw and cooked meat.

Kanner & Harel (1985a) and Harel & Kanner (1985b)suggested that ferryl species generated by the interaction of H2O2 with metmyoglobin or methemoglobin can initiate lipid peroxidation in muscle tissue. They showed that H2O2 alone or metmyoglobin alone could not stimulate oxidation reaction in sarcosomal fraction of turkey dark muscle whereas the membranal lipid peroxidation was readily promoted in the presence of H2O2 and metmyoglobin. H2O2- or cumene hydroperoxide-activated metmyoglobin was shown to catalyze lipid peroxidation in model systems containing PUFA (Rao, Wilks, Hamberg, & Ortiz de Montellano, 1994; Hamberg, 1997). Also, H2O2- activated metmyoglobin was reported to be able to oxidize a series of compounds such as phenols, β-carotene, methional, reducing agents, and uric acid (Kanner & Harel, 1985b; Harel & Kanner, 1988; Kanner, 1992). The ratio of H2O2 to metmyoglobin seemed to be important for the generation of the ferryl species. Rhee, Ziprin, & Ordonez (1987) demonstrated that the catalytic activity of metmyoglobin-H2O2 treatment was the highest at the molar ratio of ~1:0.25 in raw microsomal system of beef and at the molar ratio of 1:1.5 or 1:2 in the cooked system although metmyoglobin alone had little catalytic activity. Moreover, nonheme iron concentration in this system was shown to increase as the added concentration of H2O2
increased. Hence, they suggested that the catalytic effect of metmyoglobin-H$_2$O$_2$ treatment is most likely to be attributed to both H$_2$O$_2$-activated metmyoglobin and nonheme iron released from metmyoglobin. Also, H$_2$O$_2$, the essential element for the activation of metmyoglobin, was shown to be endogenously generated in ground turkey muscle tissue (Harel & Kanner, 1985a). Meanwhile, Baron, Skibsted, & Andersen (1997) reported that metmyoglobin can be activated to ferryl species in the presence of lipid hydroperoxide and suggested that the presence of lipid hydroperoxide is a crucial factor for heme protein-catalyzed lipid peroxidation. In addition to lipid hydroperoxide, the prooxidant activity of metmyoglobin is dependent on a linoleate-to-heme ratio (Baron, Skibsted, & Andersen, 2000 & 2002; Baron & Anderson, 2002). They indicated that at a low linoleate-to-heme ratio (1:100), metmyoglobin did not show prooxidant activity because it was converted to hemichrome by binding of fatty acid which is known to be a poor prooxidant. However, at higher ratio (1:200 and 300), metmyoglobin showed prooxidant activity because it was denatured by the high concentration of fatty acids, resulting in exposure or release of heme group to lipids, leading to the initiation of lipid peroxidation. Also, it was indicated that ferryl species produced in the presence of H$_2$O$_2$ were likely to attack other heme “edge” molecule to produce porphyrin radical, resulting in the release of ionic iron (Harel, Salan, & Kanner, 1988). Prasad, Engelman, Jones, & Das (1989) suggested that hematin, i.e. released heme ring, is released from myoglobin before the release of free ionic iron in the presence of H$_2$O$_2$. Hematin can catalyze lipid peroxidation more efficiently than ionic iron because hematin is more reactive than hemoproteins and ferrous ion (Kaschnitz & Hatefi, 1975; Chiu, van den Berg, Kuypers, Hung, Wei, & Liu, 1996), and its hydrophobicity allows it to permeate into membrane easily (Schmitt, Frezzatti, & Schreier, 1993). However, the ratio of hematin to lipids was the determining factor for its prooxidant activity. Schmitt et al. (1993) suggested that hematin can form either dimer at low hematin to lipids ratio or aggregated at high hematin to lipids ratio in aqueous solution. The hematin dimer is less effective than a monomer for lipid peroxidation but could permeate to membrane where it is degraded to monomers. Hematin aggregates are inactive. The interaction of hematin monomers with LOOH within membrane can generate lipid alkoxy radical (LO$^*$)and hematin with hypervalent iron (Fe(IV)=O) both of which were regarded as initiators and catalysts for the hematin-catalyzed lipid
peroxidation (Dix & Marnett, 1985; Kim & Sevanian, 1991). Therefore, all of ferryl species, exposed or released heme, and ionic irons may be responsible for myoglobin-mediated lipid peroxidation, depending on the environment.

In addition to the role of iron as the catalyst for the initiation of lipid peroxidation, iron plays another role in lipid peroxidation process. Pure LOOH can be decomposed by heating or in the presence of transitional metal ions although it is pretty stable at physiological temperature (Halliwell & Gutteridge, 1990). Davies & Slater (1987) indicated that reduced iron complexes react with LOOHs to produce LO* by one-electron reduction. A Fe(II) complex causes the fission of O–O bonds to form LO* in a similar way to their reaction with H₂O₂, and the reactions of Fe(II)-complexes with LOOHs are much faster than their reactions with H₂O₂ (the rate constant (k₂) for Fe(II) + ROOH is ~ 1.5 x 10³ M⁻¹s⁻¹; that for Fe(II)+ H₂O₂ is about 76 M⁻¹s⁻¹) (Halliwell & Gutteridge, 1999; Gamier-Suillerot, Tosi, & Paniago, 1984) (Equation 7). Gamier-Suillerot et al. (1984) proposed that the mechanism of LOOH decomposition by Fe(II) in their small unilamellar vesicle with phospholipids consists of two steps; the fixation of Fe(II) to membrane as a first step, and then the decomposition of LOOH to form LO*. In addition, ferrylmyoglobin as well as metmyoglobin can degrade LOOH to free radicals such as LO* and LOO* (Reeder & Wilson, 1998 & 2001).

\[
\text{LOOH + Fe(II)-complex} \rightarrow \text{Fe(III)-complex} + \text{OH}^- + \text{LO*} \quad \text{(Equation 7)}
\]

Subsequently, this LO* reacts with another lipid molecules (L*H) and/or L*OOH to produce both L** and/or L*OO*, respectively, depending on the concentration of reactants (Davies & Slater, 1987). The ability of LO* to abstract a hydrogen atom from PUFA and LOOH was demonstrated on the basis of the reduction potential of LO* (+1.6V) and the Gibbs free energies changes (ΔG°) in the reaction of LO* with hydrogen at bis-allylic carbon in propene (-23 kcal/mol) and LOOH (-14 kcal/mol) (Koppenol, 1990) (Equation 8 and 9). Fe(III)-complexes can also decompose LOOH to LOO*. Davies (1989) showed that the reaction of metmyoglobin and methemoglobin with t-butyl hydroperoxide generated its LOO* (Equation 10).
\[ \text{LO}^* + \text{L}^*\text{H} \rightarrow \text{LOH} + \text{L}^* \quad \text{(Equation 8)} \]
\[ \text{LO}^* + \text{L}^*\text{OOH} \rightarrow \text{LOH} + \text{L}^*\text{OO}^* \quad \text{(Equation 9)} \]
\[ \text{LOOH} + \text{Fe(III)}\text{-complex} \rightarrow \text{Fe(II)}\text{-complex} + \text{H}^* + \text{LOO}^* \quad \text{(Equation 10)} \]

The \text{LOO}^* formed in equations 9 and 10 subsequently is involved in the propagation step of lipid peroxidation process. However, the reaction rate of \text{Fe(III)} with \text{LOOH} (equation 10) is much slower than that of \text{Fe(II)} (equation 7) (Halliwell & Gutteridge, 1999). Moreover, \text{LO}^* is more reactive for the abstraction reaction than \text{LOO}^* (equation 10). In usual, the micelles or liposomes from commercially available lipid and microsome isolated from disrupted cell are contaminated with a trace amount of \text{LOOH}. Therefore, when iron is added, \text{LOOH} present can react with iron via equations 7 and 10 to form \text{LO}^* and \text{LOO}^* that can participate in the initiation and propagation of lipid peroxidation.

Recently, some researchers suggested iron-catalyzed \text{LOOH}-dependent lipid peroxidation as an initiation mechanism of lipid peroxidation (Fujii, Hiramoto, Terao, & Fukuzawa, 1991; Tadolini, Cabrini, Menna, Pinna, & Hakim, 1997; Tang, Zhang, Qian, & Shen, 2000). Tadolini et al. (1997) also showed that added \text{Fe(III)} to their model system did not affect \text{LOOH}-independent but affected \text{LOOH}-dependent lipid peroxidation, which suggested that \text{Fe(III)} played in an important role in the control of \text{LOOH}-dependent lipid peroxidation. Tang et al. (2000) reported that the removal of pre-existing \text{LOOH} in liposome prevented \text{Fe(II)} from initiating lipid peroxidation, but re-addition of \text{LOOH} promoted lipid peroxidation after short latent period. They suggested that pre-existing \text{LOOH} is required for the “\text{Fe (II)}-initiated” lipid peroxidation. Also, they showed that scavenging \text{LOO}^* inhibited the initiation of lipid peroxidation. They proposed that \text{Fe(II)} initiated lipid peroxidation by decomposing \text{LOOH}, resulted in the formation of \text{LOO}^*, which may be the real initiator of “\text{Fe (II)}-initiated” lipid peroxidation. However, according to equation 7, \text{Fe(II)} reduces \text{LOOH} to form \text{LO}^*, which is more reactive for H atom abstraction than \text{LOO}^* (Koppenol, 1990). They also suggested that the latent period before initiating lipid peroxidation is due to the suppression of \text{LOO}^* and \text{LO}^* activity via reduction to \text{LOOH} and \text{LOH} by \text{Fe(II)} (Equation 11 and 12) and lipid peroxidation is initiated below certain concentration of \text{Fe(II)}. 
Fe(II)-complex + LOO\(^\bullet\) + H\(^+\) \rightarrow Fe(III)-complex + LOOH \quad (11)

Fe(II)-complex + LO\(^\bullet\) + H\(^+\) \rightarrow Fe(III)-complex + LOH \quad (12)

Enzymic catalysis: Isolated microsomes from animal tissues undergo lipid peroxidation when incubated with NADPH or NADH and Fe(II) or Fe(III) salt. Sevanian, Nordenbrand, Kim, Ernster, & Hochstein (1990) observed that both NADPH-cytochrome P450 reductase and cytochrome P450 contained in microsomes were involved in NADPH- and ADP-Fe(III)-dependent lipid peroxidation. The enzyme may generate O\(_2\)^\(-\) that dismutate H\(_2\)O\(_2\) and reduce Fe(III)-chelates to Fe(II)-chelates to form \(^*\)OH, which stimulate microsomal lipid peroxidation (Persson, Terelius, & Ingelman-Sundberg, 1990; Paller & Jacob, 1994).

However, it has been proposed that NADPH-dependent lipid peroxidation in liver microsome in the presence of Fe(III) chelates is initiated by the reduction of Fe(III), followed by addition of O\(_2\) to perferryl species which then stimulates the abstraction of an hydrogen atom from PUFA chain (Bast & Steeghs, 1986; Sevanian et al., 1990).

The presence of enzyme systems in microsomal fractions from beef, pork, and turkey muscle that catalyze lipid peroxidation of microsomal lipids has been reported (Rhee, Dutson, & Smith, 1984; Rhee & Ziprin, 1987; Kanner, Harel, & Hazan, 1986). Rhee (1988) suggested that enzymic lipid peroxidation in skeletal muscle microsomes is dependent on NADH or NADPH and requires ADP and Fe(II) or Fe(III) for maximum rate. The reaction rate is higher with NADH for the microsomal systems of fish muscles than NADPH for those of poultry and red-meat muscles. The reaction rate is also higher with Fe(II) than Fe(III).

The role of lipoxygenase in fish tissues as the enzymic initiator of lipid peroxidation has been actively investigated. German & Kinsella (1985) found that 12-hydroxyeicosatetraenoic acid was observed as a major monohydroxy product from arachidonic acids, indicating a 12-lipoxygenase activity in fish skin. They suggested that endogenous skin 12-lipoxygenase released post-mortem may be a major source for the initiation of lipid peroxidation in fish tissues. Saeed & Howell (2001) demonstrated the presence of 12-lipoxygenase in Atlantic mackerel, which has the possibility of enzymic initiation of lipid peroxidation in chilled and frozen stored fillets of mackerel. Meanwhile, lipoxygenase has
been found in various mammalian species such as human, rat, mouse, pig, cattle, chicken, etc. (Yamamoto, 1992). Kühn & Borchert (2002) reported that among currently known mammalian lipoxygenase isoforms only 12/15-lipoxygenases can directly oxygenate lipid esters to generate lipid hydroperoxy esters even when lipids are bound to membranes and lipoproteins.

Factors affecting lipid peroxidation in meat and meat products

The lipid peroxidation process probably starts immediately after slaughter and during post-slaughter events. The biochemical changes during the conversion of muscle to meat such as post mortem aging cause the destruction of the balance between prooxidant and antioxidant factors. The rate and extent of lipid peroxidation in muscle tissues appears to be dependent on degree of muscle tissue damage during pre-slaughter events such as stress and physical damage and post-slaughter events such as early post mortem, pH, carcass temperature, shortening, and tenderizing techniques such as electrical stimulation (Morrissey et al., 1998). In addition, various processing factors can influence the rate of lipid peroxidation in meat and meat products: composition of raw meat, aging time, cooking or heating, size reduction processes such as grinding, flaking, and emulsification, deboning, especially mechanical deboning, additives such as salt, nitrite, spices, and antioxidants, temperature abuse during handling and distribution, oxygen availability, and prolonged storage (Rhee, 1988; Kanner, 1994).

Differences in total lipid content and fatty acid composition in meat are dependent on animal species, muscle type, and anatomical location of muscle (Love & Pearson, 1971; Wilson et al., 1976; Allen & Foegeding, 1981). Several studies have demonstrated that phospholipids play a critical role in the development of lipid peroxidation in raw and cooked meat. Pikul et al. (1984) suggested that the phospholipid fraction contributed about 90% of the malonaldehyde measured in total fat from chicken meat. Also, the PUFA content of phospholipids was positively related to the development of rancidity (Igene et al., 1980). Yin & Faustman (1994) indicated that the level of lipid peroxidation is more strongly influenced by oxidative stability of membrane components rather than that of cytosolic components. Sasaki, Mitumoto, & Kawabata (2001) indicated that the extent of lipid peroxidation was
correlated with phospholipid peroxidation in the initial period of storage, but was directly correlated with total lipid content in a later period. The content, composition, and quality of dietary fat in feed and the tendency of animal species to store fatty acids into membrane phospholipids can affect the fatty acid composition of membrane and its susceptibility to lipid peroxidation (Ahn, Wolfe & Sim, 1995; Sarraga & Garcia Regueiro, 1999; Song & Miyazawa, 2001).

The susceptibility of meat to lipid peroxidation is dependent on animal species, muscle type, and anatomical location (Rhee & Ziprin, 1987; Rhee, Anderson, & Sams, 1996). They reported that frozen raw beef and pork muscle had higher TBARS value than frozen raw chicken muscle as was heme iron content, but cooked chicken meat was more susceptible to lipid peroxidation than cooked beef and pork. Thus, they concluded that heme pigment content in conjunction with catalase activity determines lipid peroxidation potential of raw meat and the content of PUFA is the major determinant for lipid peroxidation in cooked meats. Salih, Price, Smith, & Dawson (1989) reported that turkey thigh meat was more susceptible to oxidation than turkey breast meat. Also, Kim, Nam, & Ahn (2002) reported that beef showed higher susceptibility to lipid peroxidation than pork and turkey breast muscle.

Oxygen availability is one of the most important factors for the development of lipid peroxidation in raw and cooked meat. Any process causing disruption of the membranes such as size reducing processes (grinding, flaking, mincing, etc), deboning, and cooking results in exposure of the phospholipids to oxygen, and, therefore, accelerates development of oxidative rancidity (Ladikos & Lougovois, 1990). The level of oxygen content in modified atmosphere and vacuum packaged raw and cooked beef was proportional to that of lipid peroxidation (O'Grady, Monahan, Burke, & Allen, 2000; Smiddy, Fitzgerald, Kerry, Papkovsky, O'Sullivan, & Guilbault, 2002). Ahn et al. (1992 & 1993a) reported that vacuum-packaged meat immediately after cooking while the meat is still hot ("hot-packaging") developed significantly lower TBARS during storage than the one with vacuum packaged after chilling, which suggested that the 3-hr chilling provided enough time for oxygen to stimulate lipid peroxidation in cooked meat. They also showed that when oxygen is not present, prooxidants such as ionic iron, hemoglobin, NaCl, fat content, and fatty acid
composition had little effect on the oxidation of cooked meat during storage. In addition, the combination of “hot” packaging and antioxidants such as reducing agents and free radical terminators provided cooked turkey meat patties with better protection from lipid peroxidation than either treatment alone mainly because antioxidants protected meat from oxidation during raw meat preparation and brief exposure to air (Ahn, Wolfe, & Sim, 1993d). Andersson & Lingnert (1999) indicated that the production of volatiles showed different patterns depending on oxygen concentration and some compounds were produced in larger amounts at lower oxygen concentration than at higher one.

The term “warmed-over flavor” was first introduced by Tims and Watts in 1958 to describe the rancidity in cooked meat during refrigerated storage (Asghar et al., 1988). Rancid flavors are readily detectable after 2 days in cooked meat, in contrast to much more slowly developing rancidity in raw meat (Asghar et al., 1988). Generally, heating increases the level of lipid peroxidation such as TBARS and volatile productions. Heating can promote lipid peroxidation by disruption of muscle cell structure, inactivation of antioxidant enzymes, and release of oxygen and iron from myoglobin. The disrupted membranes by heating are exposed to and readily accessed by oxygen, followed by rapid lipid peroxidation (Igene, Yamauchi, Pearson, Gray, & Aust, 1985). Mei, Crum, & Decker (1994) and Lee, Mei, & Decker (1996) suggested that the inactivation of catalase and glutathione peroxidase (GSH-Px) by heating could be partially responsible for the rapid development of lipid peroxidation in cooked meat. Meanwhile, Chen, Pearson, Gray, Fooladi, & Ku (1984) showed that heating rate and final temperature affected the release of non-heme iron from heme pigment. Slow heating increased the release of non-heme iron more rapidly than fast heating. Also, high temperature provides reduced activation energy for oxidation and breaks down preformed hydroperoxide into free radicals, which stimulates further lipid peroxidation processes and off-flavor development (Kanner, 1994). On the other hand, freezing slows down lipid peroxidation, but cannot stop the process. Eun, Boyle, & Hearsberger (1994) demonstrated that freezing retarded the development of NADH-dependent lipid peroxidation in channel catfish muscle microsomes by inactivating enzymes, but thawing resulted in reactivation of peroxidase system. LOO* is soluble in oil fraction and is more stable at low temperature, which they can diffuse to longer distances and spread the reaction potential during freezing.
Sodium chloride is one of the most important additives in meat industry for enhancing preservation, flavor, tenderness, water holding capacity, binding ability, and juiciness (Rhee, 1999). It has been known that sodium chloride has a prooxidant effect in meat and meat products, depending on its concentration. Rhee, Smith, & Terrell (1983) showed that the level of lipid peroxidation increased with the sodium chloride up to 2% while over 3% of sodium chloride had little or no prooxidant effect, and proposed that the prooxidant effect of sodium chloride is decreased and/or inhibited over a certain high concentration. Although the mechanism by which sodium chloride promotes muscle lipid peroxidation has not been clearly understood, but one of the possible explanations is that sodium chloride may disrupt the structural integrity of the membrane to enable catalysts to easily access to lipid substrates (Rhee, 1999). Kanner, Harel, & Jaffe (1991) showed that the prooxidant effect of sodium chloride was inhibited by EDTA and ceruloplasmin, and suggested that sodium chloride enhances the activity of ionic iron for lipid peroxidation. Rhee & Ziprin (2001) also reported that non-heme iron content in ground beef and chicken breast muscle increased with sodium chloride concentration. This effect of sodium chloride may be in part related to its capability to release ionic iron from iron-containing molecules such as heme proteins. In addition, sodium chloride can promote the formation of metmyoglobin, which reacts with \( H_2O_2 \) to form ferrylmyoglobin to catalyze lipid peroxidation (Rhee, 1999). Meanwhile, Lee, Mei, & Decker (1997) showed that sodium chloride lowered the activity of antioxidant enzymes, catalase, glutathione peroxidase, and superoxide dismutase by 8%, 32%, and 27%, respectively, and suggested that the capability of sodium chloride to decrease the activity of those antioxidant enzymes could be partially responsible for the acceleration of lipid peroxidation in muscle tissue. Hernandez, Park, & Rhee (2002) drew similar conclusions, but they suggested that the accelerated lipid peroxidation in salted pork may be partly related to the reduction of glutathione peroxidase activity rather than that of catalase activity. Rhee et al. (1983) reported that sodium chloride and magnesium chloride increased rancidity of raw and cooked ground pork but potassium chloride did not increase in cooked samples, and suggested that replacement of sodium chloride with potassium chloride is effective for decreasing rancidity in processed meat. Potassium chloride, however, has a limitation for
using in the industry because of its bitter taste (Rhee, 1988).

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CHAPTER 2. FACTORS AFFECTING OXIDATIVE STABILITY OF MEATS FROM DIFFERENT ANIMAL SPECIES

A paper submitted to the Journal of Food Science

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Abstract

The susceptibility of meats from different animal species to lipid peroxidation was studied. 2-Thiobarbituric acid reactive substances (TBARS) values of raw pork loin, and chicken breast and thigh meats did not change during 7-d storage \((P < 0.05)\). Low free iron content and high ferric ion reducing capacity (FRC) were responsible for the low TBARS values in those meats during storage. TBARS values of raw beef loin, however, significantly increased during 7-d storage \((P < 0.05)\) because of high free iron content and high lipoxygenase-like activities by ferrylmyoglobin. This suggested that the amounts of free iron, myoglobin, and FRC were the major determinants for the different susceptibility of raw meat to lipid peroxidation. The TBARS values of cooked meat increased significantly with storage. Heat-stable FRC was detected in all cooked meat and was responsible for the increase of TBARS in cooked meat during storage. The rate of TBARS increases and the amounts of nonheme iron and heat-stable FRC in cooked beef loin were higher \((P < 0.05)\) than those in cooked pork loin and chicken breast, suggesting that nonheme iron content and heat-stable FRC were the major determinants of lipid peroxidation in cooked meat. In spite of lower amounts of nonheme iron and heat-stable FRC, cooked chicken thigh showed similar levels of TBARS to cooked beef loin after 7-d storage because of its high PUFA content. The total amount of PUFA in meat, most of which were present in triglycerides, influenced the development of lipid peroxidation only in the presence of sufficient amounts of free irons in cooked meat.

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Introduction

Lipid peroxidation is one of the primary causes of quality deterioration in meat and meat products, and generates compounds that may be detrimental to human health (Addis 1986; Kanner 1994; Morrissey and others 1998). The conversion of muscle to meat after slaughter destroys the balance between prooxidative and antioxidative factors, resulting in initiation and propagation of lipid peroxidation (Morrissey and others 1998). The rate of lipid peroxidation in meat depends upon the balance between endogenous and exogenous factors of the meat (Kanner 1994; Decker and Xu 1998). The endogenous factors include total lipid content, fatty acid composition of fat, types and amounts of iron present, reducing compounds (e.g., ascorbic acid), natural antioxidants (carnosine, anserine and α-tocopherol), and antioxidant enzymes (catalase, superoxide dismutase, etc.) (Chan and Decker 1994; Min and Ahn 2005). The exogenous factors include oxygen, heating, addition of salts, temperature abuse during handling and distribution, and prolonged storage, etc. (Min and Ahn 2005)

The susceptibility of meat to lipid peroxidation varies among meats from different animal species and muscles from the same animal (Rhee and Ziprin 1987; Salih and others 1989; Rhee and others 1996). Among meats, beef is the most susceptible to lipid peroxidation (Kim and others 2002). Rhee and Ziprin (1987) and Rhee and others (1996) suggested that differences in heme pigment content and catalase activity determine the rate of lipid peroxidation in raw meat. They hypothesized that meats with higher heme pigment content (beef) produce more hydrogen peroxide (H₂O₂) during oxymyoglobin autoxidation than that with less heme pigments. Hydrogen peroxide can react with metmyoglobin to generate ferrylmyoglobin, which can initiate lipid peroxidation (Kanner and Harel 1985; Egawa and others 2000; Baron and Anderson 2002). Therefore, catalase activity can be an important determining factor for lipid peroxidation in meat (Rhee and others 1996; Pradhan and others 2000). In addition to various iron catalysts, differences in fat content, fatty acid composition (Allen and Foegeding 1981; Rhee and others 1996), endogenous antioxidants such as carnosine and related dipeptides (Chan and Decker 1994), and antioxidant enzymes (Mei and others 1994) may also play important roles for oxidative stability of meat. Reducing compounds such as ascorbic acid can serve as an electron donor in free radical-mediated
oxidative processes (Buettner and Jurkiewicz 1996) and plays a critical role in the progress of lipid peroxidation. Ascorbic acid can serve either as an antioxidant or a prooxidant, depending on relative concentrations of ascorbic acid and the amount of iron present (Decker and Hultin 1992). The role of lipoxygenase in fish tissues as an enzymic initiator of lipid peroxidation has been actively investigated (Saeed and Howell 2001). Lipoxygenase activities are found in various mammalian tissues (Grossman and others 1988; Yamamoto 1992; Gata and others 1996) and can be an important determinant for the oxidative susceptibility of muscle tissues.

Heating accelerates lipid peroxidation and volatile production in meat (Han and others 1995; Byrne and others 2002; Beltran and others 2003) by disrupting muscle cell structure, inactivating antioxidant enzymes and other antioxidant compounds, and releasing iron from heme pigments (Kanner 1994; Mei and others 1994). High temperature causes reduction of activation energy for lipid peroxidation and decomposes preformed hydroperoxides to free radicals, which stimulates autoxidation process and off-flavor development further (Min and Ahn 2005).

Although many suggestions have been made to explain the differences in oxidative susceptibility among meats from different animal species, no attempt has been made to compare various prooxidant and antioxidant factors among meats from different animal species. The objective of this research was to determine factors influencing oxidative stability of meats from different animal species. Effect of heat treatments on the pro- and antioxidant factors in meats was also examined.

Materials and Methods

Sample preparation

Beef and pork loin muscles from four different animals were purchased from a local packing plant. Loins from each animal were used as a replication. Chicken breast and thigh muscles were obtained from broilers raised in the Poultry Farm at Iowa State University. A total of 16 broilers (6-week-old) were slaughtered. Breast and thigh muscles from 4 birds were pooled and used as a replication. Muscles for each replication were ground separately,
and patties (60-g) were prepared after grinding them twice through an 8-mm plate. The patties were individually packaged in oxygen-permeable zipper bags (polyethylene, 4 x 6, 2 mil.; Associate Bag Co. Milwaukee, WI, U.S.A.). One half of the packaged patties were cooked in bag in a 95 °C water bath to an internal temperature of 75 °C followed by cooling for 2 h at 4 °C. After draining meat juices from the bag, the patties were repackaged. Raw and cooked patties were stored at 4 °C until used. Lipid peroxidation, nonheme iron, reducing compounds, free radical scavenging ability, and lipoxygenase activity of meat samples were determined at 0, 3, and 7 days of storage. Total iron content and fatty acid compositions of total fat, triacylglycerol (TG), phosphatidylcholine (PC), and phosphatidylethanolamine (PE) were determined at Day 0. The percent proportions of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and unsaturated fatty acids (UFA = MUFA + PUFA) were calculated.

**Chemical analyses**

Lipid peroxidation was determined by the method of Ahn and others (1998) and the amounts of 2-thiobarbituric acid reactive substances (TBARS) were expressed as mg malondialdehyde (MDA) per kg meat. Total fat content and fatty acid composition of total fat and lipid classes (TG, PC, and PE) were determined by the method of Ahn and others (1995). Nonheme iron content was determined by the ferrozine iron analysis method (Carter 1971; Ahn and others 1993b) and expressed as μg nonheme iron per g meat. Total iron content was measured by the wet-ashing method of Carpenter and Clark (1995) and the ferrozine method of Ahn and others (1993b) with minor modifications. Meat sample (~2 g) was accurately weighed into a 125 ml Erlenmeyer flask and mixed with 20 ml concentrated nitric acid. The mixture was placed at room temperature overnight for predigestion. The predigested solution was heated on a hot plate (150 °C) until dry. Three to four milliliter of Caro’s acid (50% hydrogen peroxide: concentrated sulfuric acid = 4: 1; Hatch and others 1985) containing peroxymonosulfuric acid was added to the flask and left on the hot plate until all peroxides were evaporated. After cooling, the digest was transferred to a 10-ml volumetric flask using 0.01 N HCl as a rinse. The digest solution (0.5 ml) and 0.01 N HCl (1 ml) were added to a disposable test tube, mixed with 0.5 ml of 1% ascorbic acid in 0.2 N HCl
(w/v), and left at room temperature for 5 min. To the test tube, 0.8 ml of 30% ammonium acetate (w/v) and 0.2 ml of ferrozine color reagent were added and thoroughly mixed. After 10 min, the absorbance of the mixture was determined at 562 nm against a blank. Total iron content was expressed as μg total iron per g meat.

Ferric ion reducing capacity (FRC) was determined using the method of Kanner and others (1991). Briefly, the meat homogenate (1 g) was mixed with 1 mM ferric chloride solution (1 ml) and stirred for 2 min at room temperature. After adding 11.3% TCA solution (1 ml), the mixture was centrifuged at 10,000 × g for 10 min. The supernatant (2 ml) was reacted with 0.8 ml 10% ammonium acetate and 0.2 ml ferrozine color reagent for 10 min. The absorbance of the mixture was read at 562 nm against a blank. Ferric ion reducing capacity (FRC) was expressed as μg ascorbic acid equivalent per g meat.

Free radical scavenging activities of meat were determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (Saiga and others 2003). Ground meat (5 g) was homogenized with 15 ml of 75% ethanol solution (v/v) using a Polytron (Type PT 10/35; Brinkman Instrument Inc., Westbury, N.Y., U.S.A.) for 10 s at top speed, and centrifuged at 3,000 × g for 15 min. After filtering through a Whatman No. 1 filter paper, the filtrate was mixed with 1 mM DPPH radical in 100% ethanol at 4:1 ratio (v/v). The mixture was thoroughly mixed and placed at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm. Sesamol (Sigma-Aldrich, St. Louis, Mo., U.S.A.) was used as a reference antioxidant. The residual DPPH radical was calculated by the following equation:

\[
\text{Residual radicals (\%) = 100 - \frac{(DPPH \ blank + \ control \ sample) - DPPH \ sample}{DPPH \ blank} \times 100}
\]

where, DPPH blank is the absorbance of 75% ethanol solution (4 ml)/ml of DPPH solution, DPPH sample is the absorbance of sample solution (4 ml)/ml of DPPH solution, and control sample is the absorbance of sample solution (4 ml)/ml of 100% ethanol.

Lipoxygenase activities of meat were measured by a modified method of Gata and others (1996). The substrate solution was prepared by mixing 70 mg pure linoleic acid, 70 mg
Tween-20, and 4 ml deionized distilled water. The solution was clarified by adding 0.5 ml of 1 N NaOH, and then diluted with deionized distilled water (DDW) to 25 ml. The substrate solution was flushed with nitrogen gas and kept under nitrogen. Minced meat (5 g) was homogenized with 15 ml of 50 mM acetate buffer (pH 5.8) using a Polytron for 10 s at top speed, and then centrifuged at 12,000 × g at 4 °C for 15 min. The supernatant was filtered through a Whatman No. 1 filter paper and the filtrate was used to determine lipoxygenase activities in meat. Lipoxygenase activity was assessed at 27 °C by the increase of absorbance at 234 nm produced by the formation of conjugated diene from linoleic acid. The reaction mixture was composed of 80 μl sodium linoleate solution (10 mM), 80 μl enzyme solution, and 50 mM acetate buffer (pH 5.8) to a final volume of 1 ml. The results were expressed as units of activity (U) per g meat, and calculated from the molar extinction coefficient of hydroperoxyl linoleic acid (ε = 25,000 M⁻¹cm⁻¹). One unit of lipoxygenase activity was defined as the amount of enzyme catalyzing the formation of 1 μmol of hydroperoxide per minute.

**Statistical analysis**

Data were analyzed using the Statistical Analysis System (SAS) and reported as means and standard error of means (SEM). Tukey’s method (P < 0.05) was used to compare the mean values among treatments (Kuehl 2000).

**Results and Discussion**

**Lipid peroxidation**

The initial (Day 0) TBARS values for all raw meats from different animal species were not different from each other. At Day 3 and Day 7, however, the TBARS values of beef loin were significantly higher than those of pork loin, chicken breast, and chicken thigh, and the rate of TBARS increase among raw meat during storage was the highest in beef loin. The TBARS values of raw pork loin, chicken breast, and chicken thigh did not increase significantly during storage (Figure 1A). The pattern of TBARS change during storage
appears to be consistent with other studies (Rhee and Ziprin 1987; Rhee and others 1996; Kim and others 2002), which reported that beef was the most susceptible to lipid peroxidation among meats. Rhee and others (1996) suggested that the difference in heme pigment content, which is associated with catalase activity, could be responsible for the varying oxidative stability of meats from different animal species.

In cooked meat, the TBARS values of all meats gradually increased with storage time (Figure 1B). The TBARS values of cooked beef loin and chicken thigh were approximately 2 times greater than those of pork loin and chicken breast after 7 days of storage. Our TBARS results of cooked meats were different from those of Rhee and others (1996) who reported the TBARS of cooked chicken thigh was two-fold higher than that of beef, pork, and chicken breast. They indicated that the amount of PUFA played a critical role in accelerated lipid peroxidation in cooked meat.

**Total fat content and fatty acid composition**

Beef loin and chicken thigh had higher total fat content than pork loin and chicken breast in both raw and cooked meat (Table 1) as shown in previous studies (Allen and Foegeding 1981; Rhee and others 1996). The composition of fat is more important than the amount of fat in meat because the susceptibility of muscle lipid to lipid peroxidation depends upon the degree of polyunsaturation in fatty acids. Wagner and others (1994) demonstrated that the number of bis-allylic positions in lipid molecules determines their susceptibility to lipid peroxidation, and the rate of lipid peroxidation increased exponentially with the number of bis-allylic positions although lipid chain length had no relationship with the rate of radical formation. The proportion of PUFA in total fat of chicken breast and thigh was significantly higher than that of pork and beef loin (Table 1). TG is the most abundant lipid class (Ahn and others 1993a) and most TG is stored in adipose tissues where the amounts of prooxidants such as myoglobin are low. The PUFA content (%) in TG and total fat from chicken breast and thigh were significantly higher than those from pork and beef loin (Table 2). Igene and Pearson (1979) and Pikul and others (1984) suggested that PL is primarily responsible for rancidity and warmed-over flavor (WOF) development in raw and cooked meat because the high susceptibility of PL to oxidative changes is attributed not only to its high PUFA content.
but also its omnipresence in cell membrane where prooxidants in cell matrix can have easy access. Pikul and others (1984) suggested that PL fraction contributed about 90% of the malonaldehyde measured in total fat from chicken meat. Therefore, differences in the amount of total fat and PUFA content may not be proportionately related to the different rates of lipid peroxidation in raw meats. However, they could be important factors affecting the rate of lipid peroxidation in cooked meat because heat disrupts cell membrane structure (Min and Ahn 2005) to increase the accessibility of prooxidants to PUFA in TG although prooxidants still have easier access to PUFA in cell membrane than that of TG in adipose tissues. Rhee and others (1996) suggested that the amount of PUFA could be responsible for the differences in lipid peroxidation rates among cooked meats from different animal species.

Fatty acid compositions of PC and PE of raw and cooked meat showed that PE was more polyunsaturated than PC. Thus, PE would be more susceptible to lipid peroxidation than PC (Table 3 and 4). Yin and Faustman (1993) reported that phosphatidylserine (PS) was the most susceptible to lipid peroxidation followed by PE and PC, and the oxidation rates increased with the chain length in fatty acid moiety. Also, PE was suggested to be the major contributor to the development of warmed-over flavor in cooked meat (Igene and Pearson 1979; Igene and others 1981). Although statistical differences in the amounts of PUFA in PC and PE of raw and cooked meat from different animal species were observed, those differences did not influence TBARS values (Figure 1). The PUFA contents in PE and PC of raw and cooked beef loin were similar to those of pork loin, chicken breast, and chicken thigh although beef loin contained significantly lower PUFA contents in total fat than chicken breast, chicken thigh and pork loin. Therefore, prooxidant and antioxidant factors other than fatty acid composition should be involved in high TBARS values of raw and cooked beef loin compared with other meats.

**Total and nonheme iron**

Iron is the most abundant transitional metal in biological systems and can change its oxidation states, reduction potential, and electronic configurations (Halliwell and Gutteridge 1999). Iron has been suggested to play important roles in lipid peroxidation as a catalyst and an initiator. Iron can catalyze the production of hydroxyl radical (\(\cdot{\text{OH}}\)) \textit{in vivo} and \textit{in vitro}
via the Fenton reaction (Buettner and Jurkiewicz 1996). Also, ferrylmyoglobin formed by the interaction of \( \text{H}_2\text{O}_2 \) with metmyoglobin can abstract a hydrogen atom from bisallylic position of PUFA to initiate lipid peroxidation (Rao and others 1994; Egawa and others 2000; Baron and Andersen 2002). Iron is distributed in five distinct pools in biological systems: transferrin, ferritin, heme pigments, iron-dependent enzymes, and small transit pools of iron chelates (so called “free” iron), respectively (Crichton, 2001). It has been suggested that “free” iron (Apte and Morrissey 1987; Kanner and others 1988; Ahn and others 1993b; Ahn and Kim 1998) and/or ferrylmyoglobin (Harel and Kanner 1985; Rhee and others 1987; Chan and others 1997) are primarily responsible for lipid peroxidation in raw and cooked meat.

As expected, total iron content in raw and cooked meats was the highest for beef loin, followed by chicken thigh, chicken breast, and pork loin (Table 5). The content of nonheme iron in chicken thigh was higher than that of other meats at Day 0, and changes of nonheme iron content in pork loin, chicken breast and thigh during storage were not observed (Table 6). However, nonheme iron content in raw beef loin significantly increased with storage time, and the amount at Day 7 was approximately 2 times as high as that at Day 0. The increase of nonheme iron in raw beef loin during storage could be caused by the release of iron from heme pigments, especially myoglobin by \( \text{H}_2\text{O}_2 \) (Harel and Kanner, 1985), and related to the increase of TBARS values during storage in Figure 1A. Heating increases nonheme iron content in meats from all animal species as previously reported (Apte and Morrissey 1987; Rhee and others 1996). Cooked beef loin and chicken thigh had higher nonheme iron content than cooked pork loin and chicken breast, but nonheme iron content did not change during storage in cooked pork loin, chicken breast and chicken thigh as in raw meat. Cooked beef loin showed an increase of nonheme iron with storage time, indicating that nonheme iron in cooked beef loin might be strongly related to the rate of lipid peroxidation in cooked beef loin although beef loin has low PUFA in total fat.

**Ferric ion reducing capacity (FRC)**

The ability of antioxidant compounds to reduce ferric ion to ferrous ion such as ferric-reducing antioxidant power (FRAP) assay had been used to evaluate the antioxidant activity in meat (Benzie and Strain 1999). Various reducing compounds including ascorbic acid,
NAD(P)H, and thiol compounds such as glutathione (GSH) are present in biological cells and may be primarily responsible for ferric ion reducing capacity (FRC) of meat. The amount of reducing compounds in turkey muscles were ~3 mg ascorbic acid equivalent /100 g fresh meat, and 80% of the reducing compounds were ascorbic acid (Kanner and others 1991). Ascorbic acid is an important biological reducing agent, which is able to serve as an electron donor in free radical-mediated oxidative processes (Buettner and Jurkiewicz 1996). In general, ascorbic acid can serve as both an antioxidant and a prooxidant, depending on the concentration of ascorbic acid present. It has been suggested that ascorbic acid at low concentration is most likely to promote lipid peroxidation in muscle tissues by reducing ionic iron (Decker and Hultin 1992), whereas, at high concentration, it tends to inhibit lipid peroxidation by regenerating antioxidants such as α-tocopherol in cell membrane, directly reducing some of the lipid peroxyl radicals to hydroperoxides (Halliwell and Gutteridge 1999), and reducing ferrylmyoglobin (Giulivi and Cadenas 1993; Kroger-Ohlsen and Skibsted 1997). The concentration effect of ascorbic acid on lipid peroxidation also varies, depending upon the concentration of iron present (Decker and Hultin 1992).

In raw meat, FRCs of chicken breast and thigh were significantly higher than those of pork and beef loin (Table 7). The FRCs of raw chicken breast and thigh decreased with storage time while those of raw beef and pork loin did not change. This suggested that significant amounts of FRCs of raw chicken breast and thigh were storage-unstable but most of FRCs in raw beef and pork loin were storage-stable. Also, it is assumed that high FRC and low concentration of nonheme iron are responsible for low TBARS values in raw chicken breast and thigh during storage.

Heating reduced the FRCs of chicken breast and thigh more than those of pork and beef loin. Generally, ascorbic acid is readily decomposed by heating, and, thus, most of FRCs present in cooked meat may not be ascorbic acid. Also, the changes in the amounts of FRCs in pork and beef loin before and after cooking were small, indicating that raw pork and beef loin contained only small amounts of unstable reducing agents such as ascorbic acid.

Heat-stable FRC was detected in all cooked meat. Ferric ion can hardly catalyze lipid peroxidation without reducing compounds to reduce it to ferrous iron, which is a major catalyst form of free ionic iron for lipid peroxidation (Ahn and Kim 1998). Reducing agents
can act as prooxidants when their amounts are relatively low. Cooking decreased the FRC but increased nonheme iron of meat, resulting in comparatively lower FRC in cooked meat than raw meat. Therefore, heat-stable FRC may be primarily responsible for the regeneration of ferrous ion to increase TBARS in cooked meat during storage. The heat-stable FRC of beef loin was the highest and very stable during storage, indicating that high “heat-stable” FRC and nonheme iron are responsible for the rapid TBARS increase in cooked beef loin during storage. Although heat-stable FRC in cooked chicken thigh was lower than that in cooked beef loin, their TBARS after 7 days of storage were similar. This was attributed to high amounts of PUFA in cooked chicken compared with beef loin. Heating disrupts membrane barriers in meat and facilitates the access of prooxidants to PUFA in adipose tissues (Min and Ahn 2005).

**Free radical scavenging activities**

As the concentration of sesamol, the reference for free radical scavenging activities in meat, increased to 500 ppm, the contents of residual DPPH decreased exponentially to approximately 28% of the initial amount, but slightly increased at over 500 ppm of sesamol (Figure 2). This phenomenon could be caused by the absence of reducing compounds, a regenerator of sesamol from sesamol radical. As the concentration of sesamol increases, more sesamol radicals can be generated and accumulated by scavenging one electron from DPPH radical unless they are regenerated to sesamol by reducing compounds. Consequently, the accumulated sesamol radical could compete with residual DPPH radical to react with remaining sesamol, resulting in the reduction of DPPH radical scavenging rate. Therefore, the presence of reducing compounds may be necessary for maximizing the free radical scavenging ability of antioxidants. It is suggested that α-tocopherol radical can react with PUFA without reducing compounds although the rate constant of α-tocopherol radical with PUFA is much smaller than that of reactive oxygen species (ROS) (Halliwell and Gutteridge, 1999).

In raw meat, DPPH radical scavenging activities of chicken breast (11.57% and 20.98%) and thigh (13.82% and 28.62%) meat at Day 0 and Day 3 were significantly higher than those in pork and beef loin, and were similar to or greater than those of 500 ppm sesamol.
(28.02%) (Table 8). Therefore, high amounts of reducing compounds in chicken breast and thigh (Table 7) could be responsible for the regeneration of antioxidant present. In addition, chicken meats are reported to contain more histidine-containing dipeptides such as carnosine and anserine, which have antioxidant activities than beef and pork (Chan and Decker 1994). The scavenging activity of raw chicken thigh decreased faster than that of raw chicken breast because of higher total and nonheme iron content, higher PUFA content in chicken thigh than in breast. The initial scavenging activities of raw and cooked beef loin were much lower than those of chicken breast and thigh because beef loin contained low concentrations of reducing compounds and low amounts of carnosine and anserine. Raw beef loin showed no consumption of radical scavenging activities during storage although TBARS values and nonheme iron content in raw beef loin increased with storage, indicating that the reactive compounds such as ferrylmyoglobin rather than free radicals such as *OH are more important in raw beef loin than raw chicken and pork loin. In addition, beef loin showed lower levels of reducing compounds, especially ascorbic acid (Table 7), which can reduce the ferrylmyoglobin (Giulivi and Cadenas 1993; Kroger-Ohlsen and Skibsted 1997). The level and the increase of nonheme iron in cooked beef loin during storage were much greater than those in raw beef loin, indicating that ionic iron is responsible for the development of lipid peroxidation in cooked beef loin. Although the radical scavenging activities of raw pork loin at Day 0 were similar to that during storage, TBARS did not increase during storage. It seemed that the amount of antioxidants in raw pork loin were large enough to prevent lipid peroxidation. The production of free radicals and ferrylmyoglobin in pork loin were lower than that in beef loin because of low concentrations of nonheme and heme iron (Table 6). In addition, pork has significantly higher catalase activities than beef and chicken (Rhee and others 1996), which can decrease the production of ferrylmyoglobin.

**Lipoxygenase-like activities**

Lipoxygenase activity is essential for the biosynthesis of eicosanoids from arachidonic acids in cell membrane (Garrett and Grisham 1999). Lipoxygenase has been identified in various mammalian tissues including skeletal muscles (Grossman and others 1988; Gata and others 1996; Yamamoto 1992). Lipoxygenase is capable of direct oxygenation of PUFA even
in PL bound to membrane to generate lipid hydroperoxides. Therefore, lipoxygenase can be involved in the initiation of lipid peroxidation of meat.

Raw beef loin and chicken thigh showed higher lipoxygenase-like activities than chicken breast and pork loin. These trends appeared to be highly related to total iron contents, especially heme iron content in meat (heme iron content = total iron content – nonheme iron content). Lower heme iron content in chicken breast and pork loin may be responsible for their lower lipoxygenase-like activities. The initial lipoxygenase-like activities in raw chicken thigh were similar to those in raw beef loin, but decreased during storage. The lipoxygenase-like activities in beef loin increased considerably during storage. The differences in the changes of lipoxygenase-like activities during storage between chicken thigh and beef loin could be attributed to the difference in the concentration of reducing compounds, especially ascorbic acid (Table 7). Because ascorbic acid can reduce ferrylmyoglobin (Giulivi and Cadenas 1993; Kroger-Ohlsen and Skibsted 1997), relatively high lipoxygenase-like activity in beef loin should be attributed to high concentration of myoglobin and low concentration of reducing compounds.

The lipoxygenase-like activities in meat decreased dramatically after cooking, indicating that the enzymes are heat-labile. Lipoxygenase activities measured in this study were greater than those of other reports (Grossman and others 1988; Gata and others 1996), and part of the activities shown in Table 9 could be lipoxygenase-like activities rather than true lipoxygenase activities. Ferrylmyoglobin generated by the interaction of metmyoglobin and $\text{H}_2\text{O}_2$ and hydroxyl radical by the Fenton reaction can directly abstract a hydrogen atom from PUFA and generate lipid hydroperoxide in a similar manner to lipoxygenase (Rao et al. 1994). However, most of these activities were not from $^\bullet\text{OH}$ generated from the Fenton reaction. Therefore, ferrylmyoglobin should be primarily responsible for the lipoxygenase-like activities measured in this study.

**Conclusion**

Raw ground beef loin was the most susceptible to lipid peroxidation among meats from different animal species during storage. The content of free ionic iron, myoglobin, and ferric ion reducing capacity (FRC) were the primary determinants for the different susceptibility of
raw meats to lipid peroxidation. Cooked pork loin and chicken breast were less susceptible to lipid peroxidation than cooked beef loin and chicken thigh although the TBARS values increased during storage in all meat species. The contents of free ionic iron and heat-stable FRC played a key role on the development of lipid peroxidation in cooked meat and the amount of PUFA in cooked meat was also related to lipid peroxidation in cooked meat when sufficient amount of free iron was present.

References


Table 1. Total fat content (% of meat) and fatty acid composition of raw and cooked chicken breast, chicken thigh, pork loin, and beef loin

<table>
<thead>
<tr>
<th></th>
<th>Chicken Breast</th>
<th>Chicken Thigh</th>
<th>Pork Loin</th>
<th>Beef Loin</th>
<th>SEM</th>
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<tr>
<td>Total fat (%)</td>
<td>1.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.42</td>
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<td>27.92&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>43.71&lt;sup&gt;ay&lt;/sup&gt;</td>
<td>1.06</td>
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<td>33.44&lt;sup&gt;y&lt;/sup&gt;</td>
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<td>72.08&lt;sup&gt;aw&lt;/sup&gt;</td>
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<td>Cooked meat Total fat (%)</td>
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<td>29.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>36.21&lt;sup&gt;by&lt;/sup&gt;</td>
<td>42.42&lt;sup&gt;ay&lt;/sup&gt;</td>
<td>1.13</td>
</tr>
<tr>
<td>Cooked meat MUFA</td>
<td>28.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>30.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.55&lt;sup&gt;by&lt;/sup&gt;</td>
<td>43.88&lt;sup&gt;ay&lt;/sup&gt;</td>
<td>1.41</td>
</tr>
<tr>
<td>Cooked meat PUFA</td>
<td>39.58&lt;sup&gt;ay&lt;/sup&gt;</td>
<td>39.99&lt;sup&gt;gy&lt;/sup&gt;</td>
<td>28.24&lt;sup&gt;bz&lt;/sup&gt;</td>
<td>13.70&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.99</td>
</tr>
<tr>
<td>Cooked meat Total UFA</td>
<td>67.78&lt;sup&gt;aw&lt;/sup&gt;</td>
<td>70.83&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>63.79&lt;sup&gt;bx&lt;/sup&gt;</td>
<td>57.58&lt;sup&gt;cx&lt;/sup&gt;</td>
<td>1.13</td>
</tr>
<tr>
<td>Cooked meat SEM</td>
<td>0.66</td>
<td>1.01</td>
<td>1.19</td>
<td>2.38</td>
<td></td>
</tr>
</tbody>
</table>

Means with different letters (a-d) within the same row are significantly different (P < 0.05).
Means with different letters (w-z) within the same column are significantly different (P < 0.05). SEM = Standard error of the means. n = 4.

Abbreviations: SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; UFA, unsaturated fatty acid (MUFA+PUFA).
Table 2. Fatty acid composition of triglycerides (TG) from raw and cooked chicken breast, chicken thigh, pork loin, and beef loin

<table>
<thead>
<tr>
<th></th>
<th>Chicken Breast</th>
<th>Chicken Thigh</th>
<th>Pork Loin</th>
<th>Beef Loin</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFA % of total</td>
<td>30.21&lt;sup&gt;cz&lt;/sup&gt;</td>
<td>27.07&lt;sup&gt;cz&lt;/sup&gt;</td>
<td>44.35&lt;sup&gt;by&lt;/sup&gt;</td>
<td>51.58&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>1.35</td>
</tr>
<tr>
<td>MUFA % of total</td>
<td>41.21&lt;sup&gt;by&lt;/sup&gt;</td>
<td>40.80&lt;sup&gt;bx&lt;/sup&gt;</td>
<td>49.93&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>46.92&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>1.32</td>
</tr>
<tr>
<td>PUFA % of total</td>
<td>28.58&lt;sup&gt;bz&lt;/sup&gt;</td>
<td>32.12&lt;sup&gt;ay&lt;/sup&gt;</td>
<td>5.72&lt;sup&gt;zz&lt;/sup&gt;</td>
<td>1.50&lt;sup&gt;dy&lt;/sup&gt;</td>
<td>0.76</td>
</tr>
<tr>
<td>Total UFA % of</td>
<td>69.79&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>72.93&lt;sup&gt;aw&lt;/sup&gt;</td>
<td>55.65&lt;sup&gt;bw&lt;/sup&gt;</td>
<td>48.42&lt;sup&gt;cx&lt;/sup&gt;</td>
<td>1.35</td>
</tr>
<tr>
<td>SEM</td>
<td>0.56</td>
<td>0.71</td>
<td>1.00</td>
<td>2.04</td>
<td></td>
</tr>
</tbody>
</table>

Means with different letters (a-d) within the same row are significantly different (P < 0.05). Means with different letters (w-z) within the same column are significantly different (P < 0.05). SEM = Standard error of the means. n = 4.

Abbreviations: SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; UFA, unsaturated fatty acid.
Table 3. Fatty acid composition of phosphatidylcholine (PC) from raw and cooked chicken breast, chicken thigh, pork loin, and beef loin

<table>
<thead>
<tr>
<th></th>
<th>Chicken Breast</th>
<th>Chicken Thigh</th>
<th>Pork Loin</th>
<th>Beef Loin</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of phosphatidylcholine</td>
<td>Raw meat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SFA</td>
<td>48.34&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>48.77&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>44.88&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>41.23&lt;sup&gt;by&lt;/sup&gt;</td>
<td>0.99</td>
</tr>
<tr>
<td>MUFA</td>
<td>21.73&lt;sup&gt;bz&lt;/sup&gt;</td>
<td>18.78&lt;sup&gt;bz&lt;/sup&gt;</td>
<td>20.78&lt;sup&gt;bz&lt;/sup&gt;</td>
<td>31.17&lt;sup&gt;az&lt;/sup&gt;</td>
<td>0.96</td>
</tr>
<tr>
<td>PUFA</td>
<td>29.93&lt;sup&gt;bxyz&lt;/sup&gt;</td>
<td>32.45&lt;sup&gt;aby&lt;/sup&gt;</td>
<td>34.34&lt;sup&gt;ay&lt;/sup&gt;</td>
<td>27.60&lt;sup&gt;cxy&lt;/sup&gt;</td>
<td>0.94</td>
</tr>
<tr>
<td>Total UFA</td>
<td>51.66&lt;sup&gt;bwy&lt;/sup&gt;</td>
<td>51.23&lt;sup&gt;bxy&lt;/sup&gt;</td>
<td>55.12&lt;sup&gt;abw&lt;/sup&gt;</td>
<td>58.77&lt;sup&gt;axy&lt;/sup&gt;</td>
<td>0.99</td>
</tr>
<tr>
<td>SEM</td>
<td>0.54</td>
<td>1.07</td>
<td>0.82</td>
<td>1.30</td>
<td></td>
</tr>
</tbody>
</table>

|              | Cooked meat    |               |           |           |     |
| % of phosphatidylcholine |               |               |           |           |     |
| SFA          | 49.21<sup>ax</sup> | 46.72<sup>ab</sup> | 43.49<sup>b</sup> | 37.57<sup>c</sup> | 0.80 |
| MUFA         | 22.36<sup>bz</sup> | 19.05<sup>c</sup> | 21.20<sup>b</sup> | 33.90<sup> ay</sup> | 0.77 |
| PUFA         | 28.43<sup>by</sup> | 34.23<sup>ay</sup> | 35.31<sup>ay</sup> | 28.53<sup>bz</sup> | 0.45 |
| Total UFA    | 50.79<sup>c</sup> | 53.28<sup>bcw</sup> | 56.51<sup>abw</sup> | 62.43<sup>aw</sup> | 0.80 |
| SEM          | 0.46           | 0.23          | 0.31      | 0.49      |     |

Means with different letters (a-c) within the same row are significantly different (P < 0.05). Means with different letters (w-z) within the same column are significantly different (P < 0.05). SEM = Standard error of the means. n = 4.

Abbreviations: SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; UFA, unsaturated fatty acid.
Table 4. Fatty acid composition of phosphatidylethanolamine (PE) from raw and cooked chicken breast, chicken thigh, pork loin, and beef loin

<table>
<thead>
<tr>
<th></th>
<th>Chicken Breast</th>
<th>Chicken Thigh</th>
<th>Pork Loin</th>
<th>Beef Loin</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SFA</td>
<td>MUFA</td>
<td>PUFA</td>
<td>UFA</td>
<td></td>
</tr>
<tr>
<td>Raw meat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SFA</td>
<td>37.16&lt;sup&gt;ay&lt;/sup&gt;</td>
<td>35.54&lt;sup&gt;aby&lt;/sup&gt;</td>
<td>30.66&lt;sup&gt;bz&lt;/sup&gt;</td>
<td>36.10&lt;sup&gt;aby&lt;/sup&gt;</td>
<td>1.51</td>
</tr>
<tr>
<td>MUFA</td>
<td>22.96&lt;sup&gt;az&lt;/sup&gt;</td>
<td>16.49&lt;sup&gt;bz&lt;/sup&gt;</td>
<td>15.84&lt;sup&gt;bz&lt;/sup&gt;</td>
<td>16.73&lt;sup&gt;bz&lt;/sup&gt;</td>
<td>0.96</td>
</tr>
<tr>
<td>PUFA</td>
<td>39.88&lt;sup&gt;by&lt;/sup&gt;</td>
<td>47.97&lt;sup&gt;abx&lt;/sup&gt;</td>
<td>53.50&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>47.16&lt;sup&gt;abx&lt;/sup&gt;</td>
<td>2.04</td>
</tr>
<tr>
<td>Total UFA</td>
<td>62.84&lt;sup&gt;bx&lt;/sup&gt;</td>
<td>64.46&lt;sup&gt;abw&lt;/sup&gt;</td>
<td>69.34&lt;sup&gt;aw&lt;/sup&gt;</td>
<td>63.90&lt;sup&gt;abw&lt;/sup&gt;</td>
<td>1.51</td>
</tr>
<tr>
<td>SEM</td>
<td>1.93</td>
<td>0.30</td>
<td>1.51</td>
<td>1.89</td>
<td></td>
</tr>
</tbody>
</table>

|          |            |            |           |           |     |
| Cooked meat | SFA        | MUFA       | PUFA      | UFA       |     |
| SFA      | 35.85<sup>ay</sup> | 34.30<sup>ay</sup> | 27.81<sup>cxy</sup> | 31.07<sup>by</sup> | 0.65 |
| MUFA     | 22.13<sup>az</sup> | 15.96<sup>cz</sup> | 14.59<sup>cxy</sup> | 18.80<sup>bz</sup> | 0.44 |
| PUFA     | 42.02<sup>cx</sup> | 49.74<sup>bx</sup> | 57.60<sup>ax</sup> | 50.13<sup>bx</sup> | 0.77 |
| Total UFA| 64.15<sup>cw</sup> | 65.70<sup>aw</sup> | 72.19<sup>aw</sup> | 68.93<sup>bw</sup> | 0.65 |
| SEM      | 0.41         | 0.17         | 0.94      | 0.74      |     |

Means with different letters (a-c) within the same row are significantly different (P < 0.05).
Means with different letters (w-z) within the same column are significantly different (P < 0.05). SEM = Standard error of the means. n = 4.

Abbreviations: SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; UFA, unsaturated fatty acid.
Table 5. Total iron content of raw and cooked chicken breast, chicken thigh, pork loin, and beef loin

<table>
<thead>
<tr>
<th></th>
<th>Chicken Breast</th>
<th>Chicken Thigh</th>
<th>Pork Loin</th>
<th>Beef Loin</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw meat</td>
<td>6.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.53&lt;sup&gt;c&lt;/sup&gt;</td>
<td>19.51&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.73</td>
</tr>
<tr>
<td>Cooked meat</td>
<td>6.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.14&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>0.47</td>
</tr>
<tr>
<td>SEM</td>
<td>0.36</td>
<td>0.10</td>
<td>0.31</td>
<td>1.12</td>
<td></td>
</tr>
</tbody>
</table>

Means with different letters (a-c) within the same row are significantly different (P < 0.05).
Means with different letters (x-y) within the same column are significantly different (P < 0.05) determined by Student’s t-test. SEM = Standard error of the means. n = 4.
Table 6. Nonheme iron content of raw and cooked chicken breast, chicken thigh, pork loin, and beef loin during storage at 4 °C

<table>
<thead>
<tr>
<th>Storage</th>
<th>Chicken Breast</th>
<th>Chicken Thigh</th>
<th>Pork Loin</th>
<th>Beef Loin</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw meat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 d</td>
<td>1.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.93&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.13&lt;sup&gt;b,c,y&lt;/sup&gt;</td>
<td>0.07</td>
</tr>
<tr>
<td>3 d</td>
<td>1.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.35&lt;sup&gt;a,b,y&lt;/sup&gt;</td>
<td>0.09</td>
</tr>
<tr>
<td>7 d</td>
<td>1.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.13&lt;sup&gt;a,x&lt;/sup&gt;</td>
<td>0.10</td>
</tr>
<tr>
<td>SEM</td>
<td>0.10</td>
<td>0.10</td>
<td>0.07</td>
<td>0.10</td>
<td></td>
</tr>
</tbody>
</table>

| Cooked meat |                |               |           |          |     |
| 0 d         | 1.75<sup>b</sup> | 2.88<sup>a</sup> | 1.33<sup>b</sup> | 2.41<sup>a,y</sup> | 0.03 |
| 3 d         | 1.35<sup>c</sup> | 2.66<sup>b</sup> | 1.89<sup>c</sup> | 3.70<sup>a,x</sup> | 0.18 |
| 7 d         | 1.50<sup>c</sup> | 2.77<sup>b</sup> | 1.89<sup>c</sup> | 4.82<sup>a,x</sup> | 0.36 |
| SEM         | 0.11           | 0.10          | 0.10      | 0.31     |     |

Means with different letters (a-c) within the same row are significantly different (P < 0.05).
Means with different letters (x-z) within the same column are significantly different (P < 0.05). SEM = Standard error of the means. n = 4.
Table 7. Ferric ion reducing capacity of raw and cooked chicken breast, chicken thigh, pork loin, and beef loin during storage at 4 °C

<table>
<thead>
<tr>
<th>Storage</th>
<th>Chicken Breast</th>
<th>Chicken Thigh</th>
<th>Pork Loin</th>
<th>Beef Loin</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw meat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 d</td>
<td>22.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.56</td>
</tr>
<tr>
<td>3 d</td>
<td>13.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.66</td>
</tr>
<tr>
<td>7 d</td>
<td>11.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.43&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.37</td>
</tr>
<tr>
<td>SEM</td>
<td>0.74</td>
<td>0.60</td>
<td>0.43</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>Cooked meat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 d</td>
<td>3.22&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.96&lt;sup&gt;cx&lt;/sup&gt;</td>
<td>6.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.10</td>
</tr>
<tr>
<td>3 d</td>
<td>2.71&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.89&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.44&lt;sup&gt;bxy&lt;/sup&gt;</td>
<td>5.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.08</td>
</tr>
<tr>
<td>7 d</td>
<td>2.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.31</td>
</tr>
<tr>
<td>SEM</td>
<td>0.08</td>
<td>0.12</td>
<td>0.17</td>
<td>0.32</td>
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</tr>
</tbody>
</table>

Means with different letters (a-c) within the same row are significantly different (P < 0.05).
Means with different letters (x-z) within the same column are significantly different (P < 0.05). SEM = Standard error of the means. n = 4.
Table 8. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of raw and cooked chicken breast, chicken thigh, pork loin, and beef loin during storage at 4 °C

<table>
<thead>
<tr>
<th>Storage</th>
<th>Chicken Breast</th>
<th>Chicken Thigh</th>
<th>Pork Loin</th>
<th>Beef Loin</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw meat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 d</td>
<td>11.57&lt;sup&gt;bz&lt;/sup&gt;</td>
<td>13.82&lt;sup&gt;bz&lt;/sup&gt;</td>
<td>30.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.21</td>
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<tr>
<td>3 d</td>
<td>20.98&lt;sup&gt;by&lt;/sup&gt;</td>
<td>28.62&lt;sup&gt;aby&lt;/sup&gt;</td>
<td>36.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.30</td>
</tr>
<tr>
<td>7 d</td>
<td>46.79&lt;sup&gt;abx&lt;/sup&gt;</td>
<td>56.49&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>39.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.36</td>
</tr>
<tr>
<td>SEM</td>
<td>1.50</td>
<td>1.35</td>
<td>2.53</td>
<td>3.24</td>
<td></td>
</tr>
</tbody>
</table>

| Cooked meat |                |               |           |           |     |
| 0 d         | 16.85<sup>bz</sup> | 15.57<sup>bz</sup> | 40.13<sup>ay</sup> | 32.56<sup>az</sup> | 2.58 |
| 3 d         | 37.27<sup>by</sup> | 34.42<sup>by</sup> | 54.11<sup>ax</sup> | 43.02<sup>by</sup> | 2.07 |
| 7 d         | 45.14<sup>bx</sup> | 43.56<sup>bx</sup> | 57.85<sup>ax</sup> | 55.12<sup>ax</sup> | 2.09 |
| SEM         | 1.95           | 1.05          | 3.05      | 2.48      |     |

Means with different letters (a-c) within the same row are significantly different (P < 0.05). Means with different letters (x-z) within the same column are significantly different (P < 0.05). SEM = Standard error of the means. n = 4.
Table 9. Lipoxygenase-like activity of raw and cooked chicken breast, chicken thigh, pork loin, and beef loin during storage at 4 °C

<table>
<thead>
<tr>
<th>Storage</th>
<th>Chicken Breast</th>
<th>Chicken Thigh</th>
<th>Pork Loin</th>
<th>Beef Loin</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw meat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 d</td>
<td>1.64&lt;sup&gt;b&lt;/sup&gt;&lt;sub&gt;x&lt;/sub&gt;</td>
<td>4.56&lt;sup&gt;a&lt;/sup&gt;&lt;sub&gt;x&lt;/sub&gt;</td>
<td>0.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.66&lt;sup&gt;az&lt;/sup&gt;</td>
<td>0.31</td>
</tr>
<tr>
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<td>1.47&lt;sup&gt;c&lt;/sup&gt;&lt;sub&gt;x&lt;/sub&gt;</td>
<td>3.89&lt;sup&gt;by&lt;/sup&gt;</td>
<td>0.62&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.69&lt;sup&gt;ay&lt;/sup&gt;</td>
<td>0.17</td>
</tr>
<tr>
<td>7 d</td>
<td>0.76&lt;sup&gt;c&lt;/sup&gt;&lt;sub&gt;y&lt;/sub&gt;</td>
<td>2.27&lt;sup&gt;bz&lt;/sup&gt;</td>
<td>0.59&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.89&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>0.17</td>
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<td>SEM</td>
<td>0.09</td>
<td>0.12</td>
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<td>Cooked meat</td>
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<td>0 d</td>
<td>0.21&lt;sup&gt;b&lt;/sup&gt;&lt;sub&gt;x&lt;/sub&gt;</td>
<td>0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.14&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>3 d</td>
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<td>0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.31&lt;sup&gt;a&lt;/sup&gt;</td>
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Means with different letters (a-c) within the same row are significantly different (P < 0.05).
Means with different letters (x-z) within the same column are significantly different (P < 0.05). SEM = standard error of the means. n = 4.

Unit / g meat is defined as the amount of enzyme per g meat catalyzing the formation of 1 μmol of hydroperoxide per minute.
Figure 1. TBARS values of raw (A) and cooked (B) chicken breast, chicken thigh, pork loin, and beef loin during storage at 4 °C (mg MDA / kg meat). Means with different letters (a-d) within meats from different animal species are significantly different (P < 0.05). Means with different letters (x-z) within a storage period are significantly different (P < 0.05). n = 4.
Figure 2. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of sesamol as a standard. The numbers over dots in this figure represent the concentration of sesamol used in the experiment. Means with different letters (a-h) within concentrations of sesamol are significantly different (P < 0.05). n = 4.
CHAPTER 3. FACTORS AFFECTING OXIDATIVE STABILITY OF VARIOUS FRACTIONS FROM RAW CHICKEN BREAST AND BEEF LOIN

A paper which will be submitted to Meat Science

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Abstract

Endogenous factors influencing different susceptibility of raw chicken breast and beef loin to lipid peroxidation was determined by analyzing anti- and pro-oxidant factors in the fractions of meat extracts. The fractions used in this study were homogenate, precipitate and supernatant after centrifugation, and high and low molecular weight fractions from the supernatant. Chicken breast showed greater oxidative stability than beef loin during 10-d storage ($P < 0.05$). All fractions from chicken breast showed lower amounts of free ionic iron and myoglobin and higher total antioxidant capacity (TAC) than those from beef loin during storage. This suggested that the oxidative stability of chicken breast was ascribed to low concentration of myoglobin and high total antioxidant capacity that prevented the release of free ionic iron from myoglobin or other iron sources. The water-soluble high molecular weight fraction, which contained myoglobin, was responsible for the high lipoxygenase-like activity and lipid peroxidation potential in beef loin. The concentration of ionic iron increased in all fractions from beef loin due to degradation of myoglobin during storage. In addition, TAC in all fractions from beef loin decreased during storage. Myoglobin solution (0.74 mg / ml in 50 mM acetate buffer, pH 5.6) showed very high lipoxygenase-like activity and lipid peroxidation potential. These results suggested that the high susceptibility of beef loin to lipid peroxidation is due to the imbalance of pro- and anti-oxidant factors caused by relatively low TAC and high amount of myoglobin, which served as a major source of catalysts, ferrylmyoglobin, hematin and/or free ionic iron, for lipid peroxidation.

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Introduction

Lipid peroxidation is a major cause of quality deterioration in meat. In addition, lipid peroxidation causes loss of nutritional values and generates and accumulates compounds that may pose continual risks to human health. Muscle has a large number of endogenous prooxidants such as myoglobin and ionic irons, which are essential elements for life. These are tightly regulated by various endogenous antioxidant factors such as reducing compounds (e.g., ascorbic acid), natural antioxidants (carnosine, anserine and α-tocopherol), and antioxidant enzymes (catalase, superoxide dismutase, etc) in muscle (Chan & Decker, 1994; Min & Ahn, 2005). The capability of muscle to regulate these prooxidants, however, may be decreased rapidly during the conversion of muscle to meat (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998).

Among the meats from different animal species, beef is the most susceptible to lipid peroxidation (Rhee & Ziprin, 1987; Rhee, Anderson, & Sams, 1996; Kim, Nam, & Ahn, 2002; Min, Nam, Cordray, & Ahn, 2006). It has been suggested that the amounts of endogenous catalysts such as myoglobin and free ionic iron, reducing compounds, antioxidants (e.g., carnosine and related dipeptides), and catalase are important factors that determine the rate of lipid peroxidation in meat (Chan & Decker, 1994; Rhee et al., 1996; Pradhan, Rhee, & Hernandez, 2000; Min et al., 2006). Myoglobin can catalyze lipid peroxidation in meat via various ways: metmyoglobin can react with hydrogen peroxide or lipid hydroperoxides to produce ferrylmyoglobin and/or release hematin or free ionic iron both of which can catalyze initiation and propagation of lipid peroxidation (Kanner & Harel, 1985; Harel & Kanner, 1985; Dix & Marnett, 1985; Rhee, Ziprin, & Ordonez, 1987; Kanner, Hazan, & Doll, 1988; Kim & Sevanian, 1991; Reeder & Wilson, 1998; Egawa, Shimada, & Ishimura, 2000; Baron, Skibsted, & Andersen, 2002; Baron & Anderson, 2002). Therefore, the extent of catalase activity can be a determining factor for the different rates of lipid peroxidation in meat (Rhee et al., 1996; Pradhan et al., 2000). Reducing compounds such as ascorbic acid can serve as either an antioxidant or a prooxidant, depending on the relative concentration of ascorbic acid to the amount of iron present (Giulivi & Cadenas, 1993; Kroger-Ohlsen & Skibsted, 1997; Halliwell & Gutteridge, 1999; Min & Ahn, 2005). Min et al. (2006) showed that the amounts of reducing compounds in meats from different animal
species vary significantly. However, differences in the amount of total fat and polyunsaturated fatty acid (PUFA) had little effect on the oxidative stability of raw meat because most lipids are stored in adipose tissues where the amounts of prooxidants are low (Min & Ahn, 2005).

Although several research studied the effect of cytosol fractions from meat or fish on lipid peroxidation in model systems, no attempt has been made to compare various antioxidant and prooxidant factors in various fractions of meat homogenates from different animal species. The objective of this research was to determine causes of different oxidative susceptibility between chicken breast and beef loin by evaluating antioxidant and prooxidant factors and lipid oxidation potential of fractions of meat homogenate from chicken breast and beef loin.

**Materials and Methods**

**Chemicals and Reagents**

Metmyoglobin (from equine skeletal muscle), linoleic acid, 2-thiobarbituric acid (TBA), ferrozine (3-(2-pyridyl)-5,6-bis (4-phenyl sulfonic acid)-1,2,4-triazine), neocuproine (2,9-dimethyl-1,10-phenanthroline), ferric chloride, chelex-100 chelating resin (50-100 dry mesh, sodium form), butylated hydroxytoluene (BHT), and Tween 20 were purchased from Sigma (St. Louis, MO). Ascorbic acid, cupric chloride, and hydrogen peroxide were obtained from Fisher (Pittsburgh, PA). All other chemicals and reagents used were of reagent grade.

Deionized distilled water (DDW) by Nanopure infinity™ ultrapure water system with ultraviolet (UV) (Barnstead, Dubuque, IA) was used for the preparation of all reagents and buffers. All DDW and buffers were treated with the Chelex-100 chelating resin to remove any free metal ion before use.

**Sample Preparation**

Beef loins from four individual animals and chicken breast muscles from 16 birds were purchased from local stores. Loin from each animal was used as a replication. Breast muscles from 4 birds randomly selected were pooled and used as a replication. Muscles for each
replication were ground separately. After grinding of muscles through an 8-mm plate twice, eighty-gram patties were prepared and individually packaged in oxygen-permeable zipper bags (polyethylene, 4 x 6, 2 mil.; Associate Bag Co. Milwaukee, WI). The patties were stored at 4 °C until used. Lipid oxidation was determined at 0, 5, and 10 days of storage. Total iron content was measured at only Day 0.

Meat homogenate (H) was prepared by homogenizing the ground meat with three volumes of 50 mM acetate buffer (pH 5.6) using a Brinkman Polytron (Type PT 10/35; Brinkman Instrument Inc., Westbury, N.Y.) for 15s at speed setting 7. A portion of the homogenate was stored at 4 °C for subsequent analyses and the rest were centrifuged at 15,000 × g for 30 min at 4 °C. After centrifugation, the precipitate was resuspended in three volumes of 50 mM acetate buffer (pH 5.6) and centrifuged to remove water-soluble remnants. After washing twice, the precipitate was suspended with three volumes of the buffer and used as a precipitate (P) fraction. The supernatant was filtered through a Whatman paper No. 1 twice and used as a supernatant (S) fraction. The high molecular weight (HMW) fraction was obtained by dialyzing (MW cut-off of 12,000; Sigma, St. Louis, MO) the supernatant at 4 °C against 50 volumes of 50 mM acetate buffer (pH 5.6) with changes of the buffer every 5 h. After dialysis, the retentate was recovered and centrifuged at 3,000 × g for 40 min at 4 °C. The supernatant was collected and used as a HMW fraction. The low molecular weight (LMW) fraction was obtained by ultrafiltration of the supernatant using a Centricon Plus-20 centrifugal filter (MW cutoff of 10,000; Millipore, Billerica, MA). The homogenate (H), precipitate (P), supernatant (S), HMW fraction, and LMW fraction from meat were prepared at 0, 5, and 10 days of storage (Figure 1). All fractions were stored at 4 °C until use and used within 2 days. Nonheme iron, myoglobin, metmyoglobin percentage, total antioxidant capacity index, ferric iron reducing capacity, lipoxygenase-like activity, and lipid oxidation potentials for each fraction were determined. The H fraction represented raw meat because the first step of all the analyses for raw meat is homogenization of raw meat.

**Lipid oxidation and total iron content in raw meat**

Lipid oxidation was determined by the method of Ahn, Olson, Lee, Jo, Wu, & Chen (1998). Briefly, five grams of ground meat was homogenized with 15 ml DDW and 100 μl
BHT solution (6% in 100% ethanol) using a polytron for 15s at speed 7. The meat homogenate (1 ml) was mixed with 2 ml TBA / TCA solution (15 mM TBA / 15% trichloroacetic acid (TCA; w/v)). The mixture was incubated in boiling water bath for 15 min. After cooling, the mixture was centrifuged at 3000 x g for 15 min. The absorbance of the supernatant was determined at 531 nm against a reagent blank. The amount of 2-thiobarbituric acid reactive substances (TBARS) was expressed as mg malondialdehyde (MDA) per kg meat.

Total iron content was measured by the combination of the wet-ashing method (Carpenter & Clark, 1995) and the ferrozine iron analysis method (Ahn, Wolfe, & Sim, 1993) with slight modification. Ground meat (≈2 g) was accurately weighed into a 125 ml Erlenmeyer flask and mixed with 15 ml concentrated nitric acid. The mixture was placed at room temperature overnight for predigestion. The predigested solution was heated on a hot plate (150 °C) until dry. Three to four milliliter of Caro’s acid (50% hydrogen peroxide: concentrated sulfuric acid = 4:1) containing peroxymonsulfuric acid was added to the flask left on a hot plate until all peroxide was evaporated. After cooling, the digest was quantitatively transferred to a 10-ml volumetric flask using 0.01 N HCl as a rinse. The diluted sample (0.5 ml) and 0.01 N HCl (1 ml) were mixed with 0.5 ml of 1% ascorbic acid in 0.2 N HCl (w/v) and left at room temperature for 5 min. 0.8 ml of 30% ammonium acetate (w/v) and 0.2 ml of ferrozine color reagent (6.1 mM ferrozine and 14.4 mM neocuproine in 0.14 N HCl solution) were added to the test tube and thoroughly mixed. After 10 min, the absorbance of the mixture was determined at 562 nm against a reagent blank. The total iron content was expressed as μg total iron per g meat.

**Chemical analyses**

Nonheme iron content was determined by the ferrozine method of Ahn et al. (1993). In brief, sample (1.5 ml) and ascorbic acid (0.5 ml, 1% in 0.2 N HCl, w/v) were thoroughly mixed with 11.3% TCA solution (w/v, 1 ml). After 5 min at room temperature, the mixture was centrifuged at 3,000 x g for 15 min at 20 °C. The supernatant (2 ml) was mixed with 0.8 ml of 10% ammonium acetate (w/v) and 0.2 ml of the ferrozine color reagent. After 10 min of color development, the absorbance of the mixture was determined at 562 nm against a
reagent blank. The nonheme iron content in each fraction was expressed as μg nonheme iron per g meat.

Myoglobin and metmyoglobin percentage were determined by the method of Krzywicki (1982) with slight modification. The fractions were centrifuged at 15,000 × g for 1 min at 4 °C. The absorbance of the supernatant was read at 525, 545, 565, 572, and 730 nm. The content of myoglobin and metmyoglobin were calculated on the basis of following equations and expressed as mg myoglobin per g meat.

Myoglobin concentration (mg / g meat)  
\[ = (-0.166R_1 + 0.086R_2 + 0.088R_3 + 0.099) \times A_{525} \times 0.0175 \times \text{dilution factor} \]

Metmyoglobin content (%)  
\[ = (-2.514R_1 + 0.777R_2 + 0.800R_3 + 1.098) \times 100 \]

where \( A_{525} \) is absorbance at 525 nm, \( R_1 \) is \( A_{572} / A_{525} \), \( R_2 \) is \( A_{565} / A_{525} \), and \( R_3 \) is \( A_{545} / A_{525} \).

Total antioxidant capacity (TAC) was determined by the cupric reducing antioxidant capacity (CUPRAC) method of Apak, Güçlü, Özyürek, & Karademir (2004). Sample (1.1 ml) was thoroughly mixed with the mixture of 10 mM copper(II) chloride (1 ml), 1.0 M ammonium acetate (1 ml), and 7.5 mM neocuproine in 96% ethanol (1 ml). After standing at room temperature for 1 hr, the absorbance at 450 nm was read against a reagent blank. The total antioxidant capacity (TAC) was expressed as μg ascorbic acid equivalent per g meat.

Ferric ion reducing capacity (FRC) in each fraction was measured by the method of Kanner, Salan, Harel, & Shegalovich (1991). Sample (1 ml) was mixed with 1 mM ferric chloride solution and placed for 10 min at room temperature. After the addition of 11.3% TCA solution, the mixture was centrifuged at 10,000 × g for 10 min. The supernatant (2 ml) was reacted with 0.8 ml 10% ammonium acetate and 0.2 ml the ferrozine color reagent for 10 min. The absorbance was read at 562 nm against a reagent blank. The ferric ion reducing capacity (FRC) was expressed as μg ascorbic acid equivalent per g meat.

Lipoxygenase-like activities of S, HMW, and LMW fractions were measured by the modification of Gata, Pinto, & Macias (1996). Linoleic acid (10 mM) in 0.02 N NaOH solution was used as a substrate solution, which was flushed with and kept under nitrogen.
Tween 20 was used as an emulsifier. The reaction mixture was composed of 80 μl of the substrate solution, 80 μl of each fraction as an enzyme solution, and 50 mM acetate buffer (pH 5.6) to a final volume of 1 ml. Lipoxygenase-like activity was assessed by the increase of absorbance at 234 nm by the generation of conjugated dienes from linoleic acid at 27 °C. The results were expressed as units of activity (U) per g meat, calculated from the molar extinction coefficient of hydroperoxyl linoleic acid (ε = 25,000 M⁻¹cm⁻¹). One unit of lipoxygenase-like activity was defined as the amount of enzyme catalyzing the formation of 1 μmol of hydroperoxide per minute.

**Lipid peroxidation potential**

Lipid peroxidation potential (LPP) of each fraction was determined using a liposome model system prepared with egg phospholipids. The fatty acid composition of the phospholipids (Table 1) was determined by the method of Ahn, Wolfe, & Sim (1995). An aliquot of phospholipids dissolved in chloroform were transferred to a scintillation vial and evaporated under nitrogen gas to make thin film on the wall. Each fraction was added to the phospholipid-coated vial and then the vial was shaken vigorously for 2 min to make fraction-liposome solution with final concentration of 3 mg phospholipids per ml fraction. The fraction-liposomes were incubated at 37 °C for 90 min to accelerate lipid oxidation. Lipid oxidation in the fraction-liposomes was determined at 0, 15, 30, 60, and 90 min. After addition of BHT solution (10 μl) to stop the reaction, an aliquot (0.5 ml) was mixed with 1 ml of TBA / TCA solution, and, subsequently, was incubated in boiling water bath for 15 min. After cooling, the mixture was centrifuged at 15,000 × g for 10 min. The absorbance of the supernatant was determined at 531 nm against a reagent blank. The extent of lipid oxidation in the fraction-liposome was expressed as mmol MDA per kg phospholipids, calculated from the molar extinction coefficient of 1.56 × 10⁵ M⁻¹cm⁻¹. The phospholipid liposome model system with 50 mM acetate buffer (pH 5.6) was used as a control.

**Preparation and analyses of metmyoglobin solution**

Metmyoglobin was used as a reference material. Metmyoglobin dissolved in the same buffer for ground meat by the ratio of 1 mg per 1 ml was centrifuged at 3,000 × g at 4 °C for
60 min to remove undissolved impurities. The concentration of myoglobin and percentages of metmyoglobin in the supernatant were measured by the method of Krzywicki (1982). The concentration of myoglobin and percentages of metmyoglobin were 0.74 mg/ml and 100.73%, respectively. The lipoxygenase-like activity and lipid oxidation potential of metmyoglobin solution was determined to compare with those of each fraction. The metmyoglobin solution was treated with Chelex-100 chelating resin to remove free ionic ions prior to the analyses.

**Statistical analysis**

Four replications were used for all sample analyses. Data were analyzed using the JMP software (version 5.1.1; SAS Institute Inc., Cary, NC) and reported as means and standard error of means (SEM). Differences among means were assessed by Tukey’s method \( P < 0.05 \) (Kuehl, 2000). In addition, Student’s t-test was used to compare the difference between the same fractions from chicken breast and beef loin during storage.

**Results and Discussion**

**Lipid peroxidation and total iron content in meat**

TBARS values of beef loin were significantly higher than those of chicken breast (Figure 2A). TBARS value of beef loin significantly increased during storage, but that of chicken breast did not change. This result confirmed previous studies (Rhee et al., 1996; Kim et al., 2002; Min et al., 2006), which indicated that chicken breast was less susceptible to oxidative stresses than beef. The concentrations of heme pigments, free ionic iron, antioxidative enzymes such as catalase, and reducing compounds are suggested to be the major factors influencing different susceptibility of beef and chicken breast to lipid peroxidation (Rhee et al., 1996; Min et al., 2006). The extent of polyunsaturation in total fat was not a determining factor for the different oxidative stability between raw beef and chicken breast (Min et al., 2006). In addition, total iron content in beef loin was much higher than that in chicken breast as expected (Figure 2B).
Myoglobin and nonheme iron in fractions

As expected, the myoglobin concentration in beef loin was greater than that in chicken breast (Table 2). The myoglobin concentrations in both of LMW fractions from chicken breast and beef loin were not shown because those were undetectable. The concentrations of myoglobin and metmyoglobin percentage in all fractions from chicken breast did not change during storage (Table 3). The concentration of myoglobin in all fractions from chicken breast in our study may have been overestimated due to the turbidity of extracts (Kranen, van Kuppevelt, Goedhart, Veerkamp, Lambooy, & Veerkamp, 1999).

The myoglobin concentrations in H, S, and HMW fractions extracted from beef loin significantly decreased during storage, but increased in precipitate (P) fraction due probably to the deposition of denatured myoglobin. In addition, metmyoglobin percentage significantly increased in H, S, and HMW fractions from beef loin, but did not change in P fraction due to the presence of mitochondria and microsomes where many enzymes including metmyoglobin-reducing enzyme system (Rhee, 1988; Thomas, 1995; Arihara, Cassens, Greaser, Luchansky, & Mozdziak, 1995). The metmyoglobin percentage in H fraction from beef loin at Day 10 was significantly lower than those in S and HMW fraction because of P fraction in H fraction. It has been well known that the oxidation of oxymyoglobin is involved in the development of lipid peroxidation (Lin & Hultin; 1977; Yin & Faustman, 1993; Gorelik & Kanner, 2001). Oxymyoglobin can be oxidized by autoxidation and interaction with \( \text{H}_2\text{O}_2 \), lipid hydroperoxides, lipid radicals (e.g., peroxyl and alkoxyl radical), and secondary products from lipid peroxidation such as aldehydes (Brantley, Smerdson, Wilkinson, Singleton, & Otson, 1993; Lynch & Faustman, 2000). The autoxidation of myoglobin results in the formation of metmyoglobin and superoxide anion (\( \text{O}_2^- \)), which is subsequently dismutated to produce \( \text{H}_2\text{O}_2 \) (Baron & Andersen, 2002). \( \text{H}_2\text{O}_2 \) can interact with metmyoglobin to produce ferrylmyoglobin, a major catalyst for lipid peroxidation, break down myoglobin to release hematin and/or free ionic iron, and react with ferrous ion to produce hydroxyl radical (\( ^*\text{OH} \)), the most powerful initiator for lipid peroxidation (Fenton reaction) (Min & Ahn, 2005). Then, ferric ion generated from the Fenton reaction can be reduced to ferrous ion by \( \text{O}_2^- \). Therefore, the autoxidation of myoglobin may be one of the major sources of catalysts and initiators for the development of lipid peroxidation in meat.
The metmyoglobin percentage in HMW fraction changed rapidly after removing LMW fraction. The changes in the metmyoglobin percentage in S fraction were slower than those in HMW fraction, but the metmyoglobin percentage in S and HMW fractions at Day 10 were not different. Therefore, the reducing power present in P and LMW fractions seemed to be responsible for the retardation of myoglobin oxidation in beef loin and the reducing compounds in LMW fraction appeared to be degraded or not regenerated during storage. It was shown that LMW fractions of cytosol from fish and turkey have inhibitory effect on hemoglobin and myoglobin-catalyzed lipid peroxidation due to reducing compounds and/or antioxidants in LMW fraction (Kanner et al., 1991; Underland, Hultin, & Richard, 2003).

Initial (Day 0) nonheme iron contents in H, P, S, and HMW fractions from chicken breast was significantly lower than their counterparts from beef loin during storage (Table 4). However, nonheme contents in LMW fractions from both meats were undetectable at Day 0 and 5. Nonheme iron was equally distributed to water-soluble (S) and water-insoluble (P) fractions from chicken breast, and the contents in H and P fraction from chicken breast increased significantly during storage. It is assumed that most of nonheme iron generated in chicken breast during storage may be stored in water-insoluble fraction, especially hemosiderin, and thus is not activated for lipid peroxidation. Hemosiderin is a water-insoluble complex of iron, other metals and proteins, and considered as a ferritin decomposition or polymerization products (Decker & Hultin, 1992).

Nonheme iron content in all fractions from beef loin significantly increased during storage, and the rates of increase in all fractions from beef loin were greatly higher than those from chicken breast. Nonheme iron contents in H and P fractions increased linearly during storage. However, most of the increase (~80%) in S fraction was observed from Day 5 to Day 10. This phenomenon was attributed to the increase of nonheme iron content in LMW fraction from Day 5 to Day 10. Therefore, it is assumed that released nonheme irons in the beginning of storage may be captured in ferritin, which is subsequently converted to hemosiderin. However, the amount of nonheme iron not bound to proteins should have increased in S and LWM fraction because the amount of ferritin in beef is very limited (Hazell, 1982). The major source of nonheme iron in beef is myoglobin and accounts for over 90% of heme proteins present in beef (Hazell, 1982). The interaction of metmyoglobin
with H$_2$O$_2$ or lipid hydroperoxide results in the release of free ionic iron (Harel, Salan & Kanner, 1988). Free ionic iron can serve as a catalyst in the production of *OH from H$_2$O$_2$ as well as in the degradation of lipid hydroperoxides to produce peroxyl and alkoxyl radicals, which can initiate lipid peroxidation and/or be self-degraded to the secondary products of lipid peroxidation (Min & Ahn, 2005).

**Total antioxidant capacity (TAC) and ferric ion reducing capacity (FRC)**

Total antioxidant capacity (TAC) in H, S, HMW, and LMW fractions from chicken breast during storage were significantly higher than their counterparts from beef loin, but those in P fraction from both meats were not different, except for at Day 10 (Table 5). TAC in all fractions from chicken breast did not change during storage, but TAC in those from beef loin decreased significantly. We assumed that chicken breast has higher initial TAC and stronger endogenous capability to maintain the TAC, which inhibit the development of lipid peroxidation better than beef loin. In addition, water-insoluble (P) fraction showed significantly higher TAC than water-soluble (S) fraction in both chicken breast and beef loin during storage. HMW fraction had significantly higher TAC than LMW fraction in both meats during storage.

As shown in Table 6, the initial (Day 0) ferric ion reducing capacity (FRC) in H fraction from chicken breast was twice as high as that from beef loin, but rapidly decreased to the same level as beef loin from Day 0 to Day 5. However, FRC in H fraction from chicken breast did not change from Day 5 to Day 10 and those from beef loin did not change during whole storage period. The initial FRC in P fraction from chicken breast was not different from that from beef loin, and did not change during storage. The S and LMW fractions from chicken breast showed much higher FRCs than those from beef loin at Day 0, but rapidly decreased during storage, indicating that the rapid decrease of FRC in S and H fractions from chicken was attributed to that in LMW fraction. The FRC in HMW fraction from chicken breast was lower than that from beef loin and did not change during storage. These observations indicated that chicken breast seems to have large amounts of unstable low molecular weight reducing compounds such as ascorbic acid, which appears to be negligible in beef loin although both chicken breast and beef loin have the same level of stable systems.
to maintain the FRC during storage. We assumed that the stability of FRC in chicken breast is due mostly to the water-insoluble fraction containing reducing enzyme systems in mitochondria and microsomes, and partly to the stable compounds in water-soluble fraction. Reducing compounds observed in turkey muscle was at ~3 mg ascorbate equivalent / 100 g of fresh weight, and 80% of which were ascorbic acid (Kanner et al., 1991). In addition, other reducing agents such as NAD(P)H, glutathione, and thiol compounds are present in meat (Kanner, 1994).

The FRCs in P, S, HMW, and LMW from beef loin decreased gradually during storage, and were almost the same level as those from chicken breast at Day 10, except for lower in P and higher in LMW fraction. The FRC in P and HMW fractions from beef loin decreased during storage due probably to oxidative damage in enzyme systems and sulfhydryls components of proteins. It is assumed that the stronger interaction among various reducing components such as enzymes, proteins, and low molecular weight reducing compounds in P, HMW, and LMW fractions from beef loin might be contributed to the stability of FRC in H fraction.

The free radical scavenging activity of antioxidants such as α-tocopherol is achieved by one electron reduction of free radicals, resulting in breaking down of lipid peroxidation chain reaction. The TAC and FRC used in this study represented the reducing capacity of antioxidants to reduce cupric (Cu(II)) and ferric (Fe(III)) ions to cuprous (Cu(I)) and ferrous (Fe(II)) ions in each fraction, respectively, and expressed as μg ascorbic acid equivalent / g meat. Huge differences between TAC and FRC for every fraction from chicken breast and beef loin were observed (Table 5 and 6), and those gaps should be primarily attributed to the differences in one electron reduction potential of copper ion and iron ion and their reactivity in redox reactions (Buettner, 1993; Lynch & Frei, 1995; Burkitt, 2001; Apak et al., 2004).

The one electron reduction potential of Cu(II) / Cu(I) couple (0.615 volt at pH 7 in the presence of neocuproine; Burkitt, 2001) is higher than that of Fe(III) / Fe(II) couple (0.11 V; Buettner, 1993). Therefore, the substances with their reduction potentials (E°') between 0.11 and 0.65 V are not thermodynamically feasible to reduce Fe(III) to Fe(II), but Cu(II) to Cu(I). Because the reduction potentials of the major free radicals involved in free radical chain reaction of lipid peroxidation such as hydroxyl radical (2.31 V), alkoxyl radical (RO• / ROH,
1.6 V), and peroxyl radical (ROO* / ROH, 1.0 V) (Koppenol, 1990) are higher than that of copper ion (0.65 V), those are thermodynamically able to scavenge free radicals to terminate lipid peroxidation processes. Furthermore, copper ions are likely to be chemically more reactive than iron ions, resulting in faster kinetics of copper ions in redox reactions (Lynch & Frei, 1995; Apak et al., 2004). For example, the rate constant in Fenton reaction catalyzed by the copper ion would be 61.8 times greater than that by the iron ion (Halliwell & Gutteridge, 1999). In addition, Mira, Fernandez, Santos, Rocha, & Jennings (2002) reported that most of the flavonoids assessed in their study showed higher reducing capacity for copper ions than iron ions. Consequently, the large part of TAC in each fraction may not participate in the reduction of Fe(III) to Fe(II) although the FRC is likely to be a part of TAC.

Reducing compounds may act as both antioxidants and prooxidants (Min & Ahn, 2005). Ascorbic acid, an important biological reducing agent, can serve as an antioxidant by regenerating primary antioxidants such as α-tocopherol in cell membrane and the direct reduction of free radicals such as alkoxyl and peroxyl radicals to break down the free radical chain reaction of lipid peroxidation. On the other hand, ascorbic acid can play a role in a prooxidant by catalyzing the formation of •OH via ascorbic acid-assisted Fenton reaction. It has been suggested that the role of ascorbic acid in lipid peroxidation processes may be dependent upon its relative concentration to free ionic iron content; an antioxidant in higher concentration but a prooxidant in lower concentration (Decker & Hultin, 1992; Gorelik & Kanner, 2001). Therefore, we assumed that the FRC observed can be related to an antioxidant or prooxidant activity in each fraction, depending on the concentration of free ionic iron. The FRC in fractions from chicken breast may act as an antioxidant due possibly to low free iron concentration in LMW fraction (Table 4).

Lipoxygenase-like activity

Rao, Wilks, Hamberg, & Ortiz de Monellano (1994) suggested that myoglobin has lipoxygenase (LOX)-like activity by forming ferrylmyoglobin after the interaction of metmyoglobin with H2O2. In addition, many researches indicated that ferrylmyoglobin has an ability to initiate lipid peroxidation by abstracting hydrogen atom from bisallylic carbon on fatty acid chain to produce lipid hydroperoxides (Kanner & Harel, 1985; Rhee et al., 1987;
Chan, Faustman, Yin, & Decker, 1997; Baron & Anderson, 2002). The LOX-like activity of myoglobin in linoleic acid emulsion system was 8.39 Units per mg myoglobin.

S and HMW fractions from beef loin showed extremely high LOX-like activities than LMW fraction due to the presence of myoglobin in S and HMW fractions (Table 7). LOX-like activities in S and HMW fractions increased during storage. However, the change patterns of LOX-like activities in S and HMW fractions during storage appeared to be different from each other and were highly correlated to those of metmyoglobin percentage ($r = 0.90$ and 0.94, respectively) and TAC ($r = -0.83$ and -0.88, respectively) in both fractions. LOX-like activities in S and HMW fractions from chicken breast were much lower than those from beef loin during storage because of very lower concentration of myoglobin and high TAC in the fractions from chicken breast. Reducing compounds such as ascorbic acid are able to reduce ferrylmyoglobin to metmyoglobin (Giulivi & Cadenas, 1993; Kroger-Ohlsen & Skibsted, 1997). Therefore, much higher LOX-like activity in beef loin may be attributed to the high concentration of myoglobin and low concentration and high decreasing rate of TAC in beef loin.

**Lipid peroxidation potential of fractions**

Lipid peroxidation potential (LPP) is the ability to increase lipid peroxidation in phospholipid liposome model system during incubation. LPP of each fraction can be determined by the interaction of prooxidative and antioxidative factors in each fraction. Differences in LPP among fractions could be derived from the differences in the balance of prooxidant and antioxidant factors. Myoglobin showed very high LPP in phospholipid liposome model system (Figure 3). Huge differences in LPP between H fraction from chicken breast and beef loin during storage were observed (Figure 4), indicating difference in the oxidative stability between chicken breast and beef loin.

At Day 0, the LPP of HMW fraction from chicken breast during incubation was significantly lower than that of other fractions but was slightly raised during storage. On the other hand, lipid peroxidation by LMW fraction from chicken breast at Day 0 rapidly increased at beginning of incubation but not changed after 30 min. The LPP of LMW from chicken breast at both Day 5 and 10 was very low. Reducing compounds such as ascorbic
acid as a prooxidant has been reported to reduce ferric ion to ferrous ion, which catalyzes 
H₂O₂ to form *OH, the initiator of lipid peroxidation (Min & Ahn, 2005). The FRC of LMW 
fraction including unstable low molecular weight reducing compounds was high at Day 0 but 
rapidly decreased during storage (Table 6). Thus, the FRC of LMW fraction from chicken 
breast may be responsible for the changes in LPP of LMW fraction during storage. The lipid 
peroxidation by S fraction from chicken breast at Day 0 also increased during incubation due 
to the effect of LMW fraction, but its LPP was lower than that of LMW fraction due to 
antioxidant activity of HWP fraction. Kanner et al. (1991) indicated that HMW fraction from 
turkey meat shows an antioxidant effect in model system with ascorbic acid and ferric ion 
although LMW fraction acts as a prooxidant in the same system. The LPP of P fraction from 
chicken breast at Day 0 was lower than that of H, P, and LMW fractions, but higher than 
HMW fraction. The increase of lipid peroxidation by H fraction from chicken breast at Day 0 
was observed, but its LPP was lower than that of S fraction. It is suggested that the high LPP 
of H fractions from chicken breast at Day 0 was caused by the catalytic activity of free ionic 
iron reduced by FRC in LMW fraction, and assumed that the decreases of prooxidative 
activity induced by LMW fraction in H fraction may be attributed to the antioxidant activities 
in P and HMW fractions. Meanwhile, the LPP of S and H fractions from chicken breast 
decreased significantly during storage as in LMW fraction. At Day 10, the LPP of H fraction 
from chicken breast was significantly lower than that of S, P, and HWF factions, and was 
almost the same as that of control. This low LPP of H fraction was caused by the interaction 
of antioxidant factors in HWP and P fractions. Although some of reducing agents such as 
ascorbic acid can act as a prooxidant, the interaction of antioxidant capacity between water-
soluble (S), especially HMW fraction, and water-insoluble (P) fraction seemed to suppress 
the prooxidant activities in chicken breast.

At Day 0, high LPP of HMW fraction from beef loin was observed (Figure 4), and its 
LPP was raised during storage although lower than that of myoglobin solution (Figure 3). 
Myoglobin may be responsible for the high LPP of HMW fraction. The LPP of HMW 
fraction may be related to the LOX-like activity of HMW fraction and myoglobin solution 
(Table 7). Myoglobin can catalyze and accelerate lipid peroxidation via the formation of 
ferrylmyoglobin or the release of hematin and free ionic iron in the presence of H₂O₂ or lipid
hydroperoxides (Kanner & Harel, 1985; Harel et al., 1988; Kim & Sevanian, 1991; Baron et al., 2002; Min & Ahn, 2005). Ferrylmyoglobin can initiate and propagate the free radical chain reaction of lipid peroxidation (Harel & Kanner, 1985; Reeder & Wilson, 1998; Baron & Andersen, 2002). The hematin released from myoglobin can react with lipid hydroperoxides or H2O2 to form high oxidation state (Fe(IV)) of hematin, which can catalyze initiation and propagation of lipid peroxidation (Dix & Marnett, 1985; Kim & Sevanian, 1991). The hematin may be more dangerous because it is more reactive than metmyoglobin and has a hydrophobic characteristic, which enables it to permeate into cell membrane to catalyze lipid peroxidation (Kaschnitz & Hatefi, 1975; Schmitt, Frezzatti, & Schreier, 1993; Baron et al., 2002). Therefore, both ferrylmyoglobin and hematin can be contributed to the LOX-like activity of HMW fraction. In addition, free ionic irons released from myoglobin can be a catalyst for lipid peroxidation via the Fenton reaction in the presence of reducing agents (Harel et al., 1988; Ahn & Kim, 1998; Min & Ahn, 2005). The free ionic irons can be responsible for the LPP of HMW fraction from beef loin because of the FRC of HMW fraction. Therefore, it can be suggested that myoglobin can be a major oxidative factor in beef loin. However, it is difficult to know which mechanism is dominant for the high LPP of HMW fraction from beef loin.

Lipid peroxidation by LMW fraction from beef loin at Day 0 progressed was rapidly at the beginning, slowed down after 15 min, but did not change after Days 5 and 10. This changing pattern of lipid peroxidation by the LMW fraction from beef loin during storage was similar to that from chicken breast, but the LPP of LMW fraction from beef loin at Day 0 was small because of its low concentration of unstable reducing compounds (Table 6). The initial TBARS values of fractions from beef loin increased during storage due to both rapid conversion of ferric iron to ferrous iron and the pre-existing TBARS in beef loin loin. The LPP of S fraction from beef loin at Day 0 was significantly lower than that of HMW fraction due to the effect of TAC of LMW fraction. However, the LPP of S fraction increased significantly during storage because of decrease of its TAC (Table 5). Myoglobin can be stabilized by reducing agents, which can reduce ferrylmyoglobin to metmyoglobin and prevent disruption of myoglobin to release hematin and/or free ionic iron (Giulivi & Cadenas, 1993; Kroger-Ohlsen & Skibsted, 1997). The LPP of P fraction from beef loin was very low.
at Day 0, but increased slightly during storage because the TAC in P fraction decreased during storage. Lipid peroxidation by H fraction from beef loin at Day 0 increased during incubation, and the LPP of H fraction slightly increased during storage. Consequently, the increase of lipid peroxidation in beef loin during storage was caused by the prooxidant factors, probably myoglobin, in HMW fraction. Although the antioxidant activities in P and S (HMW + LMW) fractions lowered the LPP of HMW fraction, the antioxidant activities in beef loin may be not enough to attenuate the prooxidative effect of HMW fraction from beef loin.

**Conclusion**

Raw chicken breast was more resistant to oxidative changes than raw beef loin. High TAC and its regenerating ability, extremely low concentration of free ionic iron, and the chelating ability of water-insoluble (P) fraction contributed to the stability of chicken breast. High myoglobin content (especially degraded myoglobin to hematin and free ionic iron) and relatively low TAC were responsible for the imbalance of endogenous anti- and pro-oxidant factors, leading to the high susceptibility of beef loin to lipid peroxidation. Myoglobin in HMW fraction appeared to be responsible for LPP in beef loin. However, it is uncertain which catalytic compound from myoglobin (i.e. ferrylmyoglobin, hematin or free ionic iron), is predominant factor for the initiation and propagation of lipid peroxidation in beef loin.

**References**


Table 1. Fatty acid composition of phospholipids used for the liposome model system

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic acid</td>
<td>0.19 ± 0.03</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>28.70 ± 0.22</td>
</tr>
<tr>
<td>Palmitoleic acid</td>
<td>1.28 ± 0.18</td>
</tr>
<tr>
<td>Margaric acid</td>
<td>0.28 ± 0.01</td>
</tr>
<tr>
<td>Margaroleic acid</td>
<td>0.12 ± 0.03</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>16.25 ± 0.14</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>27.01 ± 0.20</td>
</tr>
<tr>
<td><em>trans</em>-Vaccenic acid</td>
<td>1.59 ± 0.15</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>15.38 ± 0.14</td>
</tr>
<tr>
<td>γ-Linolenic acid</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>Gondoic acid</td>
<td>0.21 ± 0.03</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>6.68 ± 0.09</td>
</tr>
<tr>
<td>DTA</td>
<td>0.41 ± 0.08</td>
</tr>
<tr>
<td>DPA</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>DHA</td>
<td>1.59 ± 0.04</td>
</tr>
</tbody>
</table>

Means was expressed with standard deviation. n = 4.

Abbreviations: DTA, all *cis*-7, 10, 13, 16-docosatetraenoic acid; DPA, all-*cis*-7, 10, 13, 16, 19-docosapentaenoic acid, DHA, all *cis*-4, 7, 10, 13, 16, 19-docosahexaenoic acid.
Table 2. Amount of myoglobin in fractions collected from chicken breast and beef loin during storage at 4 °C

| Storage | Chicken breast | | | | | | Beef loin | | | | | | |
|---------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|         | H   | P    | S    | HMW  | LMW  | H   | P    | S    | HMW  | LMW  | SEM |
| 0 d     | 1.16^c | 0.35^de | 0.56^d | 0.25^dey | 0.01^e | 5.97^bx | 0.39^dey | 3.05^bx | 2.93^bx | 0.02^cx | 0.09 |
| 5 d     | 1.11^c | 0.35^e | 0.58^de | 0.42^dex | 0.00^f | 5.14^by | 0.71^dx | 2.89^bx | 2.67^bx | 0.00^fy | 0.07 |
| 10d     | 1.16^c | 0.40^d | 0.62^d | 0.44^dx | 0.00^e | 4.50^iz | 1.00^cx | 2.43^by | 2.26^by | 0.01^cy | 0.06 |
| SEM     | 0.04 | 0.02 | 0.03 | 0.04 | 0.00 | 0.18 | 0.08 | 0.08 | 0.08 | 0.00 |

Means with different letters (a-f) within the same row are significantly different (P < 0.05).

Means with different letters (x-z) within the same column are significantly different (P < 0.05).

SEM = Standard error of the means. n = 4.

Abbreviations: H, homogenate fraction; P, precipitate fraction; S, supernatant fraction; HMW, high molecular weight fraction from supernatant fraction; LMW, low molecular weight fraction from supernatant fraction.
Table 3. Percent (%) metmyoglobin present in fractions collected from chicken breast and beef loin during storage at 4 °C

<table>
<thead>
<tr>
<th>Storage</th>
<th>H</th>
<th>P</th>
<th>S</th>
<th>HMW</th>
<th>H</th>
<th>P</th>
<th>S</th>
<th>HMW</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 d</td>
<td>64.60[^b]</td>
<td>65.48[^bc]</td>
<td>64.44[^b]</td>
<td>60.79[^bx]</td>
<td>68.39[^bxy]</td>
<td>66.39[^b]</td>
<td>67.86[^by]</td>
<td>95.75[^ax]</td>
<td>1.78</td>
</tr>
<tr>
<td>SEM</td>
<td>0.47</td>
<td>1.00</td>
<td>0.86</td>
<td>2.14</td>
<td>3.63</td>
<td>0.54</td>
<td>3.70</td>
<td>3.34</td>
<td></td>
</tr>
</tbody>
</table>

Means with different letters (a-d) within the same row are significantly different (P < 0.05).

Means with different letters (x-z) within the same column are significantly different (P < 0.05).

SEM = Standard error of the means. n = 4.

% metmyoglobin: percentage of total myoglobin

Abbreviations: H, homogenate fraction; P, precipitate fraction; S, supernatant fraction; HMW, high molecular weight fraction from supernatant fraction.
Table 4. Nonheme iron content of fractions collected from chicken breast and beef loin during storage at 4 °C

<table>
<thead>
<tr>
<th>Storage</th>
<th>H</th>
<th>P</th>
<th>S</th>
<th>HMW</th>
<th>LMW</th>
<th>H</th>
<th>P</th>
<th>S</th>
<th>HMW</th>
<th>LMW</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 d</td>
<td>1.02&lt;sup&gt;by&lt;/sup&gt;</td>
<td>0.42&lt;sup&gt;cdz&lt;/sup&gt;</td>
<td>0.32&lt;sup&gt;ed&lt;/sup&gt;</td>
<td>0.27&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.06&lt;sup&gt;ex&lt;/sup&gt;</td>
<td>0.54&lt;sup&gt;ez&lt;/sup&gt;</td>
<td>0.52&lt;sup&gt;cdy&lt;/sup&gt;</td>
<td>0.50&lt;sup&gt;cdy&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;ey&lt;/sup&gt;</td>
<td>0.06</td>
</tr>
<tr>
<td>5 d</td>
<td>1.30&lt;sup&gt;by&lt;/sup&gt;</td>
<td>0.65&lt;sup&gt;cdy&lt;/sup&gt;</td>
<td>0.34&lt;sup&gt;de&lt;/sup&gt;</td>
<td>0.23&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;f&lt;/sup&gt;</td>
<td>3.03&lt;sup&gt;ay&lt;/sup&gt;</td>
<td>1.24&lt;sup&gt;by&lt;/sup&gt;</td>
<td>0.73&lt;sup&gt;cxy&lt;/sup&gt;</td>
<td>0.62&lt;sup&gt;cdy&lt;/sup&gt;</td>
<td>0.00&lt;sup&gt;efy&lt;/sup&gt;</td>
<td>0.08</td>
</tr>
<tr>
<td>10 d</td>
<td>1.73&lt;sup&gt;bx&lt;/sup&gt;</td>
<td>0.81&lt;sup&gt;cex&lt;/sup&gt;</td>
<td>0.59&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.35&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.34&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>1.87&lt;sup&gt;bx&lt;/sup&gt;</td>
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<td>0.24</td>
<td>0.12</td>
<td>0.11</td>
<td>0.06</td>
<td>0.04</td>
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Means with different letters (a-f) within the same row are significantly different (P < 0.05).

Means with different letters (x-z) within the same column are significantly different (P < 0.05).

SEM = Standard error of the means. n = 4.

Abbreviations: H, homogenate fraction; P, precipitate fraction; S, supernatant fraction; HMW, high molecular weight fraction from supernatant fraction; LMW, low molecular weight fraction from supernatant fraction.
Table 5. Total antioxidant capacity (TAC) of fractions collected from chicken breast and beef loin determined by the cupric reducing antioxidant capacity (CUPRAC) method during storage at 4 °C

<table>
<thead>
<tr>
<th>Storage</th>
<th>Chicken breast</th>
<th></th>
<th></th>
<th></th>
<th>Beef loin</th>
<th></th>
<th></th>
<th></th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
<td>P</td>
<td>S</td>
<td>HMW</td>
<td>LMW</td>
<td>H</td>
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<td>S</td>
<td>HMW</td>
</tr>
<tr>
<td>0 d</td>
<td>2943&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1307&lt;sup&gt;c&lt;/sup&gt;</td>
<td>651&lt;sup&gt;d&lt;/sup&gt;</td>
<td>469&lt;sup&gt;e&lt;/sup&gt;</td>
<td>109&lt;sup&gt;sh&lt;/sup&gt;</td>
<td>2605&lt;sup&gt;bx&lt;/sup&gt;</td>
<td>1336&lt;sup&gt;cx&lt;/sup&gt;</td>
<td>369&lt;sup&gt;efx&lt;/sup&gt;</td>
<td>278&lt;sup&gt;fgx&lt;/sup&gt;</td>
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<tr>
<td>5 d</td>
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<td>1308&lt;sup&gt;c&lt;/sup&gt;</td>
<td>663&lt;sup&gt;d&lt;/sup&gt;</td>
<td>483&lt;sup&gt;e&lt;/sup&gt;</td>
<td>107&lt;sup&gt;fh&lt;/sup&gt;</td>
<td>2151&lt;sup&gt;by&lt;/sup&gt;</td>
<td>1340&lt;sup&gt;cx&lt;/sup&gt;</td>
<td>298&lt;sup&gt;fby&lt;/sup&gt;</td>
<td>235&lt;sup&gt;fgy&lt;/sup&gt;</td>
</tr>
<tr>
<td>10 d</td>
<td>2763&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1301&lt;sup&gt;c&lt;/sup&gt;</td>
<td>676&lt;sup&gt;d&lt;/sup&gt;</td>
<td>477&lt;sup&gt;dec&lt;/sup&gt;</td>
<td>104&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1912&lt;sup&gt;by&lt;/sup&gt;</td>
<td>1160&lt;sup&gt;cy&lt;/sup&gt;</td>
<td>272&lt;sup&gt;efy&lt;/sup&gt;</td>
<td>214&lt;sup&gt;efy&lt;/sup&gt;</td>
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<td>3</td>
<td>71</td>
<td>23</td>
<td>9</td>
<td>6</td>
</tr>
</tbody>
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Means with different letters (a-i) within the same row are significantly different (P < 0.05).

Means with different letters (x-z) within the same column are significantly different (P < 0.05).

SEM = Standard error of the means. n = 4.

Abbreviations: H, homogenate fraction; P, precipitate fraction; S, supernatant fraction; HMW, high molecular weight fraction from supernatant fraction; LMW, low molecular weight fraction from supernatant fraction.
Table 6. Ferric ion reducing capacity (FRC) of fractions collected from chicken breast and beef loin during storage at 4 °C

<table>
<thead>
<tr>
<th>Storage</th>
<th>Chicken breast</th>
<th>Beef loin</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
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<td>S</td>
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<td>0 d</td>
<td>24.72&lt;sup&gt;ax&lt;/sup&gt;</td>
<td>6.93&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.33&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5 d</td>
<td>13.21&lt;sup&gt;xy&lt;/sup&gt;</td>
<td>7.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.38&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>10 d</td>
<td>13.90&lt;sup&gt;xy&lt;/sup&gt;</td>
<td>6.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.63&lt;sup&gt;cdz&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td>1.17</td>
<td>0.65</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Means with different letters (a-e) within the same row are significantly different (P < 0.05).

Means with different letters (x-z) within the same column are significantly different (P < 0.05).

SEM = Standard error of the means. n = 4.

Abbreviations: H, homogenate fraction; P, precipitate fraction; S, supernatant fraction; HMW, high molecular weight fraction from supernatant fraction; LMW, low molecular weight fraction from supernatant fraction.
Table 7. Lipoxygenase-like activities of fractions collected from chicken breast and beef loin during storage at 4 °C

| Storage | Chicken breast | | | Beef loin | | | 
|---------|----------------|---------|-----------------|-----------------|---------|-----------------|---------|-----------------|---------|-----------------|---------|-----------------|---------|-----------------|
|         | S  | HMW  | LMW  | S  | HMW  | LMW   | SEM |                   |         |                   |         |                   |         |                   |
| 0 d     | 1.20<sup>c</sup> | 0.19<sup>cy</sup> | 0.04<sup>cy</sup> | 16.20<sup>bz</sup> | 21.26<sup>ay</sup> | 0.13<sup>ex</sup> | 0.51 |                   |         |                   |         |                   |         |                   |
| 5 d     | 1.30<sup>c</sup> | 0.40<sup>cx</sup> | 0.09<sup>cxy</sup> | 31.74<sup>by</sup> | 34.43<sup>ax</sup> | 0.03<sup>cy</sup> | 0.41 |                   |         |                   |         |                   |         |                   |
| 10 d    | 1.20<sup>b</sup> | 0.33<sup>bx</sup> | 0.14<sup>bx</sup> | 37.38<sup>ax</sup> | 35.44<sup>ax</sup> | 0.03<sup>by</sup> | 1.00 |                   |         |                   |         |                   |         |                   |
| SEM     | 0.06 | 0.02 | 0.02 | 1.34 | 1.02 | 0.01 |       |                   |         |                   |         |                   |         |                   |

Means with different letters (a-c) within the same row are significantly different (P < 0.05).

Means with different letters (x-z) within the same column are significantly different (P < 0.05). SEM = Standard error of the means. n = 4.

Unit/g meat is defined as the amount of enzyme per g meat catalyzing the formation of 1 μmol of hydroperoxide per minute.

Abbreviations: S, supernatant fraction; HMW, high molecular weight fraction from supernatant fraction; LMW, low molecular weight fraction from supernatant fraction.
Figure 1. Flow diagram of fraction preparation from raw chicken breast and beef loin.
Abbreviations: H, homogenate fraction; P, precipitate fraction; S, supernatant fraction; HMW, high molecular weight fraction from supernatant fraction; LMW, low molecular weight fraction from supernatant fraction; MW, molecular weight.
Figure 2. TBARS values (A; mg malondialdehyde (MDA) / kg meat) and total iron content (B; µg iron / g meat) in raw chicken breast and beef loin during storage at 4 °C. Total iron content was measured only at 0 day. Means with different letters (a-b) between chicken breast and beef loin are significantly different (P < 0.05). Means with different letters (x-z) within a storage period are significantly different (P < 0.05). n = 4.
Figure 3. Lipid peroxidation potential of metmyoglobin in the phospholipid liposome model system during the incubation at 37 °C for 90 min (expressed as TBARS values, mmol malondialdehyde (MDA) / kg phospholipid). The myoglobin solution contained 0.74 mg myoglobin per ml 50 mM acetate buffer (pH 5.6) with 100.73 % of metmyoglobin. Means with the standard deviation were expressed. n = 4.
Figure 4. Lipid peroxidation potential of fractions collected from chicken breast (A1-3) and beef loin (B1-3) in the phospholipid liposome model system during storage 4 °C during the incubation at 37 °C for 90 min (expressed as TBARS value, mmol malondialdehyde (MDA) / kg phospholipid). The phospholipid liposome model system with 50 mM acetate buffer (pH 5.6) was used as a control (PL). Means with the standard deviation were indicated. n = 4. Abbreviation: H, homogenate fraction; P, precipitate fraction; S, supernatant fraction; HMW, high molecular weight fraction from supernatant fraction; LMW, low molecular weight fraction from supernatant fraction.
CHAPTER 4. PREDICTION OF THE MECHANISM OF METMYOGLOBIN ON LIPID PEROXIDATION IN PHOSPHOLIPID LIPOSONE MODEL SYSTEM

A paper which will be submitted to Meat Science

B. Min\textsuperscript{1}, and D.U. Ahn\textsuperscript{2}

Abstract

The objective of this study was to determine the mode of action of metmyoglobin on lipid peroxidation in a phospholipid liposome model system: the formation of ferrylmyoglobin or the source of hematin or free ionic iron as a major catalyst. Metmyoglobin catalyzed the development of lipid peroxidation in the liposome system. As the concentration of metmyoglobin in the liposome solution increased, the rate of lipid peroxidation decreased. Metmyoglobin released free ionic iron during incubation by the interaction of metmyoglobin with lipid hydroperoxides (LOOH), suggesting that at least the same amount of hematin as the free ionic iron may be released during incubation. The amount of free ionic iron was higher in the liposome solution with higher concentration of metmyoglobin, but the degradation rate was higher in the liposome solution with lower metmyoglobin concentration. However, the released free iron was not involved in lipid peroxidation in this model system. The antioxidant effect of desferrioxamine (DFO) and diethylenetriamine pentaacetic acid (DTPA) was observed, but DFO was more efficient than DTPA because of multifunctional antioxidant ability of DFO as an iron and hematin chelator and an electron donor. The antioxidant activity of DTPA in a liposome solution with lower metmyoglobin concentration was two times as great as that with a higher concentration due probably to the increased prooxidative activity of DTPA-chelatable compounds, probably hematin. Consequently, it is suggested that the ferrylmyoglobin and DTPA-chelatable compounds, probably hematin, generated from the interaction of metmyoglobin with LOOH may be the

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major catalysts in metmyoglobin-induced lipid peroxidation in phospholipid liposome model system, rather than released free ionic irons.

**Introduction**

Myoglobin has been recognized as a major catalyst for the development of lipid peroxidation in meat, but its mode of action for catalyzing lipid peroxidation has been controversial. It has been suggested that the interaction of metmyoglobin with hydrogen peroxide (H$_2$O$_2$) or lipid hydroperoxides (LOOH) results in the formation of ferrylmyoglobin, which can initiate the free radical chain reaction (Kanner & Harel, 1985; Davies, 1990; Rao, Wilks, Hamberg, & Ortiz de Montellano, 1994; Chan, Faustman, Yin, & Decker, 1997; Egawa, Shimada, & Ishimura, 2000; Baron & Anderson, 2002; Min & Ahn, 2005). In addition, ferrylmyoglobin as well as metmyoglobin can degrade LOOH to free radicals such as alkoxyl and peroxyl radicals (Reeder & Wilson, 1998 & 2001), which can initiate and/or catalyze a series of propagation and termination step in the free radical chain reaction of lipid peroxidation (Frankel, 1987; Halliwell & Gutteridge, 1990). However, others limited the role of myoglobin as only a source for free ionic iron or hematin (Gutteridge, 1986; Puppo & Halliwell, 1988; Harel, Salan, & Kanner, 1988; Kanner, Shegalovich, Harel, & Hazan, 1988; Prasad, Engelman, Jones, & Das, 1989; Ahn & Kim, 1998; Baron, Skibsted, & Andersen, 2002). They indicated that free ionic iron and/or hematin released from myoglobin in the presence of H$_2$O$_2$ or lipid hydroperoxide, rather than ferrylmyoglobin, may be major catalysts for the lipid peroxidation in meat. The ratio of peroxides to metmyoglobin may be a determining factor for the formation of ferrylmyoglobin or the release of free ionic iron or hematin (Rhee, Ziprin, & Ordonez, 1987). Hematin appears to be released from myoglobin in the presence of H$_2$O$_2$, followed by the liberation of free ionic iron from hematin (Prasad et al., 1989). Hematin may react with H$_2$O$_2$ or lipid hydroperoxide to form hematin with higher oxidation state of hematin iron (Ferrylhematin, Fe(IV=O)), which can initiate and propagate lipid peroxidation, and its catalytic activity is dependent upon the concentration of peroxides (Dix & Marnett, 1985; Kim & Sevanian, 1991). They indicated that LOOH such as linoleic acid hydroperoxide are more efficient for the development of the hematin-catalyzed lipid peroxidation, compared to H$_2$O$_2$ and both ferrylhematin and alkoxyl radical (LO•) generated
from the interaction of hematin with LOOH are responsible for the hematin-catalyzed lipid peroxidation. Also, they suggested that hematin can be destroyed during the reaction with LOOH to release free ionic iron. Hematin can be easily intercalated into membrane to catalyze lipid peroxidation more effectively due to its hydrophobicity (Schmitt, Frezzatti, & Schreier, 1993).

On the other hand, the inhibiting activity of metmyoglobin for lipid peroxidation, depending on the concentration of metmyoglobin, the ratio of the concentration of fatty acid anions or LOOH to myoglobin, and the presence and concentration of reducing agents, has been reported (Harel & Kanner, 1989; Kanner, Salan, Harel, & Shegalovich, 1991; Gorelik & Kanner, 2001; Baron et al., 2002; Lapidot, Granit, & Kanner, 2005). The concentration of metmyoglobin may be a determining factor for its prooxidative activity in the presence of fatty acid or LOOH (Baron et al. 2002; Lapidot et al., 2005). In addition, myoglobin may serve as a pseudo-hydroperoxidase activity in the presence of reducing agents such as ascorbic acid and phenolic antioxidants as the electron donor to remove lipid hydroperoxides (Harel & Kanner, 1989; Gorelik & Kanner, 2001; Lapidot et al., 2005).

Iron chelators such as diethylenetriamine pentaacetic acid (DTPA) and desferrioxamine (DFO) have been widely used to elucidate the mechanism of iron compounds on lipid peroxidation (Kanner & Harel, 1987; Harel et al., 1988; Harel & Kanner, 1988; Ahn, Wolfe, & Sim, 1993). DFO has been known as an excellent chelating agent for ferric ion and DTPA for ferrous and ferric ion (Kanner & Harel, 1987; Rahhal & Richter, 1989). Both DFO and DTPA have chelating ability to hematin (Baysal, Monteiro, Sullivan, & Stern, 1990; Radi, Turrens, & Freeman, 1991). DFO can also act as an electron donor to ferrylmyoglobin to suppress the prooxidant activity of ferrylmyoglobin and release of free ionic iron from metmyoglobin as well as to the free radicals to break down the free radical chain reaction of lipid peroxidation (Kanner & Harel, 1987; Rice-Evans, Okunade, & Khan, 1989).

The objectives of this experiment were to determine the concentration effect of metmyoglobin and the addition effect of ferric ion and chelators such as DFO and DTPA on the metmyoglobin-induced lipid peroxidation in the phospholipid liposome model system in order to predict the mode of action of metmyoglobin in the metmyoglobin-induced lipid
peroxidation. In addition, the effect of chelators and ferric ion on the lipoxygenase-like activity of myoglobin in the linoleic acid emulsion system was determined.

Materials and Methods

Chemicals and Reagents
Metmyoglobin (from equine skeletal muscle), linoleic acid, 2-thiobarbituric acid (TBA), ferrozine (3-(2-pyridyl)-5,6-bis (4-phenyl sulfonic acid)-1,2,4-triazine), neocuproine (2,9-dimethyl-1,10-phenanthroline), ferric chloride, diethylenetriamine pentaacetic acid (DTPA), desferrioxamine (DFO), chelex-100 chelating resin (50-100 dry mesh, sodium form), butylated hydroxytoluene (BHT), and Tween 20 were purchased from Sigma (St. Louis, MO). All other chemicals and reagents used were of reagent grade. Deionized distilled water (DDW) by Nanopure infinity™ ultrapure water system with ultraviolet (UV) (Barnstead, Dubuque, IA) was used for the preparation of all reagents and buffers. All DDW and buffers were treated with the chelex-100 chelating resin to remove any free metal ion before use.

Preparation of metmyoglobin solution
An appropriate amount of metmyoglobin was dissolved in 50 mM acetate buffer (pH 5.6 which is ultimate pH of beef) at 4 °C. The metmyoglobin solution was centrifuged at 3000 x g at 4 °C for 60 min to remove undissolved impurities. The concentration of myoglobin and percentages of metmyoglobin in supernatant were calculated according to Krzywicki (1982). The myoglobin concentration of supernatant was adjusted to 2 mg / ml by diluting with the buffer. Subsequently, myoglobin solution (2 mg / ml) was further diluted to 1, 0.5, and 0.25 mg / ml. The average concentration of myoglobin and percentages of metmyoglobin were 2.02 ± 0.02, 1.02 ± 0.01, 0.05 ± 0.01, and 0.25 ± 0.00 mg / ml and 100.82 ± 0.19, 100.90 ± 0.24, 100.84 ± 0.12, 100.28 ± 0.86 %, respectively. DTPA (2 mM), DFO (2 mM), and ferric chloride (5 μg / ml) were added to metmyoglobin solution (1 and 0.25 mg / ml solution, respectively). The metmyoglobin solution was treated with Chelex-100 chelating resin to remove any free ironic ions prior to analyses.
Lipid peroxidation in metmyoglobin-liposome model system

The metmyoglobin-liposome model system was prepared using egg phospholipids. The fatty acid composition of the phospholipids (Table 1) was determined by the method of Ahn, Wolfe, & Sim (1995). Chloroform containing the phospholipids were placed in a scintillation vial and evaporated under nitrogen gas to make thin film on the wall. The metmyoglobin solutions was added to the phospholipid-coated vial and then the vial was shaken vigorously for 2 min to make metmyoglobin-liposome solution with final concentration of 3mg phospholipids per ml. The solution was incubated at 37 °C for 90 min to accelerate lipid peroxidation. Lipid peroxidation was determined at 0, 15, 30, 60, and 90 min. The aliquot (0.5 ml) was taken and mixed with 10 µl BHT solution (6% BHT in ethanol) to stop the reaction. Then, the mixture was mixed with 1 ml TBA / TCA solution (15 mM TBA / 15% trichloroacetic acid (TCA; w/v)), and, subsequently, incubated in boiling water bath for 15 min. After cooling, the mixture was centrifuged at 15,000 x g for 10 min. The absorbance of the supernatant was determined at 531 nm against a reagent blank. Lipid oxidation was expressed as mmol malondialdehyde (MDA) per kg phospholipids, calculated from the molar extinction coefficient of $1.56 \times 10^5 \text{M}^{-1}\text{cm}^{-1}$. In addition, the generation of nonheme iron during the incubation was measured at 0, 15, 30, 60, and 90 min by a method of Ahn et al. (1993) and expressed as µg iron / ml metmyoglobin-liposome solution.

Lipoxygenase-like activity of metmyoglobin

Lipoxygenase-like (LOX-like) activities of metmyoglobin (1mg / ml) were measured by a method of Gata, Pinto, & Macias (1996) with modification. 10mM Linoleic acid in 0.02 N NaOH solution emulsified with Tween-20 was used as a substrate solution, which was flushed with and kept under nitrogen. The reaction mixture was composed of 80 µl of the substrate solution, 80 µl of each metmyoglobin solution as an enzyme solution, and 50 mM acetate buffer (pH 5.6) to a final volume (1 ml). Lipoxygenase-like activity was assessed by the increase of absorbance at 234 nm by the generation of conjugated dienes from linoleic acid at 27 °C. The results were expressed as units of activity (U) per ml, calculated from the molar extinction coefficient of hydroperoxyl linoleic acid ($\varepsilon = 25,000 \text{M}^{-1}\text{cm}^{-1}$). One unit of
lipoxygenase-like activity was defined as the amount of enzyme catalyzing the formation of 1 µmol of hydroperoxide per minute.

**Statistical analysis**

All the analyses were performed on the samples with four replications. Data were analyzed using the JMP software (version 5.1.1; SAS Institute Inc., Cary, NC). Differences among means were determined by Tukey's method (P < 0.05) (Kuehl, 2000).

**Results and Discussion**

Metmyoglobin at all concentrations induced lipid peroxidation in phospholipid liposome model system during the 90 min-incubation (Figure 1). The lipid peroxidation appeared to increase linearly during the incubation, but the rate of lipid peroxidation decreased with the increase of metmyoglobin concentration. Especially, after 30 min of incubation, the increase of lipid peroxidation at a higher metmyoglobin concentration (2.0 mg/ml) was significantly slower than that at lower concentrations (0.25 and 0.5 mg/ml). TBARS values at the highest metmyoglobin concentration (2 mg/ml) were significantly lower than that at the lowest concentration (0.25 mg/ml) after 60 and 90 min of incubation (8.83% and 16.3%, respectively). The presence of LOOH was detected right after the preparation of the liposome model system (Data not shown) and the generation of trace amount of LOOH during the preparation of liposome solution have been widely recognized (Kim & Sevanian, 1991; Halliwell & Gutteridge, 1990). This result indicates that the concentration of metmyoglobin is a critical factor for determining the prooxidant activity of myoglobin in the presence of LOOH and/or fatty acid: at a low concentration in lipid system, metmyoglobin acts as a prooxidant, but less effectively at high concentrations (Baron et al., 2002; Lapidot et al., 2005).

Release of free ionic iron from metmyoglobin was observed during incubation (Figure 2). The amount of free ionic iron significantly increased during incubation, and was proportional to the concentration of metmyoglobin. The concentrations of free ionic iron after 90 min of incubation were 15.93, 11.82, 11.10, and 7.88 µM at 2.0, 1.0, 0.5, and 0.25 mg metmyoglobin per ml metmyoglobin-liposome solution, respectively, indicating that 13.94,
20.69, 38.85, and 55.14% of metmyoglobin in 2.0, 1.0, 0.5, and 0.25 mg/ml, respectively, were decomposed to liberate free ionic iron. These results agreed with many previous reports (Gutteridge, 1986; Puppo & Halliwell, 1988; Harel et al., 1988; Prasad et al., 1989; Baron et al., 2002; Baron & Andersen, 2002; Lapidot et al., 2005), which suggested that the interaction of \( \text{H}_2\text{O}_2 \) or \( \text{LOOH} \) with metmyoglobin can cause the liberation of free ionic iron as well as hematin. \( \text{LOOH} \) preexisted and generated during the incubation may be the major catalysts to release free ionic iron from metmyoglobin because \( \text{H}_2\text{O}_2 \) was not added in this study.

Prasad et al. (1989) suggested that hematin was released from myoglobin before the release of free ionic iron in the presence of \( \text{H}_2\text{O}_2 \) and the amount of hematin released from metmyoglobin during incubation was greater than that of released free ionic iron. Thus, the amount of hematin produced during incubation was proportional to the concentration of metmyoglobin in the liposome system as did the amount of released free ionic iron. The release of hematin was confirmed by Chiu, Berg, Kuypers, Hung, Wei, & Liu (1996) but it was readily decomposed by \( \text{LOOH} \) to release free ionic iron (Kim & Sevanian, 1991). Hematin catalyzed lipid peroxidation more efficiently than ionic iron because of its hydrophobicity that allowed it to permeate into membrane (Schmitt et al., 1993). Although hematin was more active than other hemeproteins and ferrous ion (Kaschnitz & Hatefi, 1975; Chiu et al., 1996), the ratio of hematin to lipids was the determining factor for its prooxidant activity (Schmitt et al., 1993). They suggested that hematin formed either dimer at low ratio or aggregated at high ratio in aqueous solution: a dimer was less effective than a monomer for lipid peroxidation but could permeate to membrane where it was degraded to monomer, and aggregates were inactive. The hematin monomer within membrane interacted with \( \text{LOOH} \) to form alkoxy radical and hematin-containing hypervalent iron (Fe(IV)=O) both of which were regarded as initiators and catalysts for the hematin-catalyzed lipid peroxidation (Dix & Marnett, 1985; Kim & Sevanian, 1991). Therefore, a high amount of hematin at a high concentration of metmyoglobin in a liposome system (2mg/ml) should be partially responsible for the lower lipid peroxidation rate, compared to that at a lower metmyoglobin concentration (1 mg/ml) in Figure 1.
The addition of ferric ion did not affect myoglobin-catalyzed lipid peroxidation in phospholipid liposome model system (Figure 3), indicating that either ferrylmyoglobin or hematin generated from metmyoglobin rather than free ionic iron were the major catalysts for metmyoglobin-induced lipid peroxidation in this system. It has been suggested that the oxidation state of iron is more important than the amount of iron for the development of lipid peroxidation (Ahn & Kim, 1998). However, the released free ionic iron may play a significant role in the acceleration of lipid peroxidation in meat where the ferric ion-reducing capacity has been detected (Kanner et al., 1991; Min, Nam, Cordray, & Ahn, 2006; Min & Ahn, 2006).

Iron chelators, DTPA and DFO, showed different antioxidant effects in the liposome model system (Figure 3). DFO inhibited myoglobin-catalyzed lipid peroxidation effectively, but DTPA showed only partial inhibition. Both DTPA and DFO are known as strong iron chelators and inhibit free ionic iron-catalyzed lipid peroxidation (Graf, Mahoney, Bryant, & Eaton, 1984). However, DFO showed stronger antioxidant activity than DTPA (Gutteridge, Richmond, & Halliwell, 1979). The antioxidant activity of DTPA depended on the ratio of DTPA to free ionic iron, but DFO was not affected. DFO can act as not only an efficient iron chelator, but also an electron donor or hydrogen donor to ferrylmyoglobin, resulting in the suppression of ferrylmyoglobin-catalyzed lipid peroxidation (Kanner & Harel, 1987; Rice-Evans et al., 1989). Rice-Evans et al. (1989) suggested that DFO can prevent the release of free ionic iron from myoglobin by reducing ferrylmyoglobin and breaking free radical chain reactions. In addition, DFO can interact with hematin via the iron moiety to prevent their catalytic and membrane-intercalating activity for lipid peroxidation (Baysal et al., 1990). On the other hand, DTPA can inhibit iron-catalyzed lipid peroxidation by occupying all six coordination sites of iron. Also, DTPA can inhibit hematin-catalyzed lipid peroxidation (Radi, et al., 1991). Free hematin may have one or two unoccupied or loosely bound coordination site. It is assumed that DTPA or DFO may bind to those coordination sites to inactivate the catalytic activity of hematin, but no evidence is available. DTPA did not inhibit lipid peroxidation catalyzed by ferrylmyoglobin (Harel & Kanner, 1988). Consequently, the high inhibitory effect of DFO was from the synergistic effect of DFO as a chelator for chelatable compounds, probably hematin, and an electron donor to ferrylmyoglobin and free radicals,
whereas the partial effect of DTPA was attributed to its chelating ability, indicating that DTPA-chelatable compounds, probably hematin (Baysal et al., 1990; Radi et al., 1991), was partially responsible for the metmyoglobin-induced lipid peroxidation. The free ionic iron was already ruled out because it did not show any prooxidant effect in this study. The antioxidant activity (95.24 %) of DFO at low myoglobin concentration (0.25 mg / ml) was higher than that (89.43 %) at high myoglobin concentration. Moreover, the antioxidant activity (36.24 %) of DTPA at the liposome model system with low concentration of metmyoglobin (0.25 mg / ml) was twice as high as that (18.08 %) with high concentration of metmyoglobin (1.0 mg / ml) (Figure 3A and 3B), indicating that DTPA-chelatable compounds, probably hematin, were contributed more to the development of lipid peroxidation at lower concentration of metmyoglobin than at higher one.

LOX-like activity is related to the generation of conjugated diene at the initial stage of lipid peroxidation. LOX-like activity of metmyoglobin was not changed by ferric ion in the absence of reducing agents (Figure 4), indicating that free ionic ion released from myoglobin was not involved in the initiation of lipid peroxidation in metmyoglobin-induced lipid peroxidation. The addition of DFO and DTPA to the liposome model system decreased LOX-like activity of metmyoglobin, but DTPA (34.98 %) suppressed it more effectively than DFO (15.57 %). It is assumed that this result may be due to the difference in the model system. However, there is no suggestion available to explain this phenomenon.

**Conclusion**

Lipid peroxidation in phospholipid liposome model system was accelerated in the presence of metmyoglobin. Increases in the concentration of metmyoglobin caused a slight decrease of lipid peroxidation, due probably to the ratio of myoglobin to LOOH or fatty acid and the ratio of hematin to lipid. The concentration of free ionic iron released from metmyoglobin increased during incubation but was not involved in the development of lipid peroxidation. The addition of DFO and DTPA inhibited lipid peroxidation. DFO was more effective than DTPA because DFO can inactivate hematin and reduce ferrylmyoglobin and free radicals whereas DTPA only binds to hematin. The antioxidant activity of DTPA increased with the decrease of metmyoglobin concentration, indicating that the chelatable
compounds, probably hematin, may catalyze lipid oxidation actively at low concentration of metmyoglobin in liposome system. It is suggested that the metmyoglobin-induced lipid peroxidation may be caused by both ferrylmyoglobin and hematin generated from the interaction of metmyoglobin with LOOH in phospholipid liposome system, rather than the released free ionic irons.

References


Table 1. Fatty acid composition of phospholipids used in model study

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic acid</td>
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</tr>
<tr>
<td>Palmitic acid</td>
<td>28.70 ± 0.22</td>
</tr>
<tr>
<td>Palmitoleic acid</td>
<td>1.28 ± 0.18</td>
</tr>
<tr>
<td>Margaric acid</td>
<td>0.28 ± 0.01</td>
</tr>
<tr>
<td>Margaroleic acid</td>
<td>0.12 ± 0.03</td>
</tr>
<tr>
<td>Stearic acid</td>
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</tr>
<tr>
<td>Oleic acid</td>
<td>27.01 ± 0.20</td>
</tr>
<tr>
<td>trans-Vaccenic acid</td>
<td>1.59 ± 0.15</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>15.38 ± 0.14</td>
</tr>
<tr>
<td>γ-Linolenic acid</td>
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</tr>
<tr>
<td>Gondoic acid</td>
<td>0.21 ± 0.03</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>6.68 ± 0.09</td>
</tr>
<tr>
<td>DTA</td>
<td>0.41 ± 0.08</td>
</tr>
<tr>
<td>DPA</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>DHA</td>
<td>1.59 ± 0.04</td>
</tr>
</tbody>
</table>

Means was expressed with the standard deviation. n = 4.

Abbreviations: DTA, all cis-7, 10, 13, 16-docosatetraenoic acid; DPA, all-cis-7, 10, 13, 16, 19-docosapentaenoic acid, DHA, all cis-4, 7, 10, 13, 16, 19-docosahexaenoic acid.
Figure 1. Lipid peroxidation potential of metmyoglobin with various concentration in phospholipid liposome model system during incubation at 37 °C for 90 min (TBARS: mmol malondialdehyde (MDA) / kg phospholipid). The concentrations of metmyoglobin in 50 mM acetate buffer (pH 5.6) were 2 (Mb2.0), 1 (Mb1.0), 0.5 (Mb0.5), and 0.25 (Mb0.25) mg per ml, respectively. Phospholipid liposome model system with buffer alone was used as a control (PL). Means with standard deviation were expressed. n = 4.
Figure 2. Formation of nonheme iron in phospholipid liposome model systems with various concentrations of metmyoglobin during incubation at 37 °C for 90 min (μg nonheme iron / ml metmyoglobin-liposome solution). The concentrations of metmyoglobin in 50 mM acetate buffer (pH 5.6) were 2 (Mb2.0), 1 (Mb1.0), 0.5 (Mb0.5), and 0.25 (Mb0.25) mg per ml, respectively. Phospholipid liposome model system with buffer alone was used as a control (PL). Means with standard deviation were expressed. n = 4.
Figure 3. Lipid peroxidation potential of metmyoglobin treated with desferrioxamine (DFO, 2 mM), diethylenetriamine pentaacetic acid (DTPA, 2 mM), or ferric chloride (Fe(III), 5 ppm) in phospholipid liposome model system during incubation at 37 °C for 90 min (TBARS value, mmol malondialdehyde (MDA) / L phospholipid liposome solution). The concentrations of metmyoglobin in liposome solutions were 0.25 (A) and 1.0 (B) mg per ml, respectively. Phospholipid liposome model systems with metmyoglobin and buffer were used as a control (Mb) and blank control (PL), respectively. Means with standard deviation were expressed. n = 4.
(A) 0.25 mg metmyoglobin / ml

(B) 1.0 mg metmyoglobin / ml
Figure 4. Lipooxygenase-like activity (Unit / ml) of metmyoglobin solution treated with none (control), metmyoglobin (1 mg / ml) desferrioxamine (DFO, 2 mM), diethylenetriamine pentaacetic acid (DTPA, 2 mM), or ferric chloride (Fe(III), 5 ppm) in 50 mM acetate buffer, pH 5.6. Means with different letters (a-c) are significantly different (P < 0.05). n = 4.
CHAPTER 5. EFFECT OF VARIOUS IRON COMPOUNDS AND IRON CHELATORS ON THE LIPID PEROXIDATION OF FRACTIONS FROM RAW CHICKEN BREAST AND BEEF LOIN

A paper which will be submitted to Meat Science

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Abstract

The endogenous anti- and prooxidant factors in chicken breast and beef loin was evaluated. Metmyoglobin, ferrous and ferric ion, or chelators were added to a model system prepared with meat fractions and phospholipids and their effects on lipid peroxidation were determined. All fractions from chicken breast showed stronger antioxidant activities than its counterparts from beef loin in metmyoglobin- and ferrous ion-added model system. Iron chelating ability and the synergistic interactions among antioxidant agents in water-soluble low molecular weight (LMW) and water-insoluble (P) fractions were primarily responsible for the high antioxidant activity in chicken breast. Water-soluble high molecular weight (HMW) fraction from beef loin was primarily responsible for high susceptibility of beef loin to lipid peroxidation because HMW fraction had the lowest antioxidant activity among fractions and high myoglobin, the major source of ferrylmyoglobin, hematin, and/or free ionic iron. Therefore, it is suggested that relatively high total antioxidant capacity and very low myoglobin content were responsible for the high oxidative stability of chicken breast, compared to beef loin. The level of lipid peroxidation catalyzed by diethylenetriamine pentaacetic acid (DTPA)-chelatable free ionic iron in HMW fraction from beef loin decreased significantly by the addition of LMW and P fraction, indicating that most of the free ionic iron released from myoglobin was chelated by those fractions. However, the catalytic activity of DTPA-unchelatable iron compounds, i.e. ferrylmyoglobin and/or hematin, in HMW fraction from beef loin was not changed by addition of those fractions and was

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primarily responsible for the increase of lipid peroxidation in homogenate (H) fraction. Therefore, it is proposed that the major catalysts generated from myoglobin in beef loin are not free ionic iron but ferrylmyoglobin and/or hematin in “normal” storage condition. In addition, fractions from both chicken breast and beef loin increased lipid peroxidation in ferric ion-added model system because of their ferric ion reducing capacity (FRC). Beef loin showed higher FRC than chicken breast and HMW fraction was primarily responsible for the FRC in beef loin. It is suggested that the reduced free ionic iron by FRC can be a major oxidation catalyst of meat under conditions where the amount of free ionic iron can be generated continuously over storage time.

Introduction

Despite extensive research for several decades, the primary catalysts for lipid peroxidation in meat are still controversial. Lapidot, Grant, & Kanner (2005) suggested that metmyoglobin is a silent compound in the absence of hydrogen peroxide (H$_2$O$_2$) and lipid hydroperoxide (LOOH). However, myoglobin appeared to be the center compound in these debates because myoglobin, in the presence of H$_2$O$_2$ or LOOH, can be converted to ferrylmyoglobin and serve as a major source of hematin and free ionic iron, all of which are able to initiate and catalyze propagation of lipid peroxidation (Min & Ahn, 2005). Much research has shown that ferrylmyoglobin generated from the interaction of metmyoglobin with H$_2$O$_2$ or LOOH has capability of abstracting hydrogen atom from bis-allylic carbon on fatty acid chain and is a major initiator of lipid oxidation (Kanner & Harel, 1985; Rao, Wilks, Hamberg, & Ortiz de Montellano, 1994; Hamberg, 1997; Baron, Skibsted, & Andersen, 1997, Baron & Andersen, 2002). Ferrylmyoglobin also has an ability to degrade LOOH to alkoxyl or peroxyl radical, which undergoes chain-propagation step or further decomposed to produce secondary by-products of lipid peroxidation (Reeder & Wilson, 1998 & 2001). Free ionic iron released from heme proteins, iron-containing proteins, or ferritin is also shown to initiate lipid peroxidation in meat via the Fenton reaction in the presence of H$_2$O$_2$ or LOOH and reducing agents such as superoxide anion (O$_2^-$), ascorbic acid, NAD(P)H, thiol, etc. (Kanner, Hazan, & Doll, 1988; Kanner, Shegalovich, Harel, & Hazan, 1988; Decker &

The activities of myoglobin as a major catalyst and/or source of primary catalysts such as free ionic iron in the processes of lipid peroxidation can be affected by various conditions such as the concentration of myoglobin, presence and concentration of \( \text{H}_2\text{O}_2 \), LOOH, fatty acid anions, and reducing compounds, etc. (Rhee, Ziprin, & Ordonez, 1987; Harel & Kanner, 1989; Kanner, Salan, Harel, & Shegalovich, 1991; Gorelik & Kanner, 2001; Baron, Skibsted, & Andersen, 2002; Lapidot et al., 2005). In addition, free ionic iron can serve as a catalyst of lipid oxidation in the presence of reducing compounds and \( \text{O}_2^- \)-generating systems (Turrens & Boveris, 1980; Kanner, Harel, & Hazan, 1986; Rhee, 1988; Kanner, 1994; Min & Ahn, 2005). The status of free ionic iron seems to be more important than the amount of ionic iron for the development of lipid peroxidation (Ahn et al., 1993a; Ahn & Kim, 1998). Water-soluble and water-insoluble components influencing the catalytic activities of myoglobin and free ionic iron are present in cytosol of meat. Therefore, it is assumed that lipid peroxidation potential, the balance between antioxidant and prooxidant activities, of cytosol in muscle tissue is the most important factor for determining the action of myoglobin and free ionic iron in meat. The susceptibility of meats from different animal species to lipid peroxidation is different, and chicken breast is much less susceptible to lipid peroxidation than beef loin (Min, Nam, Cordray, & Ahn, 2006). Chicken breast showed slower increase in free ionic iron concentration and lower myoglobin and lipoxygenase-like activities, but higher concentration of reducing compounds than beef loin (Min et al., 2006).

Iron chelators such as diethylenetriamine pentaacetic acid (DTPA) and desferrioxamine (DFO) are widely used in order to determine the participation of free ionic iron in lipid peroxidation process (Kanner & Harel, 1987; Harel & Kanner, 1988; Ahn, Wolfe, & Sim, 1993b). DTPA is an excellent chelating agent for both ferrous and ferric ion and DFO chelates ferric ion, and inhibit the catalyzing activities for ionic iron (Kanner & Harel, 1987; Rahhal & Richter, 1989). In addition, DFO serves as an electron donor and suppresses the catalytic activity of ferrylmyoglobin and the breakdown of free radical chain reaction of lipid peroxidation (Kanner & Harel, 1987; Rice-Evans, Okunade, & Khan, 1989). Thus, DFO can
be more efficient inhibitor of lipid peroxidation than DTPA (Gutteridge, Richmond, & Halliwell, 1979).

The objective of this study was to evaluate the effect of metmyoglobin, ferrous iron, ferric ion, or chelators (DFO and DTPA) on the development of lipid peroxidation in a liposome model system prepared with meat fractions and phospholipids.

**Materials and Methods**

**Chemicals and Reagents**

Metmyoglobin (from equine skeletal muscle), ferrous ammonium sulfate, ferric chloride, diethylenetriamine pentaacetic acid (DTPA), desferrioxamine (DFO), linoleic acid, 2-thiobarbituric acid (TBA), ferrozine (3-(2-pyridyl)-5,6-bis (4-phenyl sulfonic acid)-1,2,4-triazine), neocuproine (2,9-dimethyl-1,10-phenanthroline), ferric chloride, Chelex-100 chelating resin (50-100 dry mesh, sodium form), butylated hydroxytoluene (BHT), and Tween 20 were purchased from Sigma (St. Louis, MO). All other chemicals were of reagent grade. Double distilled water (DDW) by Nanopure infinity™ ultrapure water system with ultraviolet (UV) (Bamstead, Dubuque, IA) was used for the preparation of all reagents and buffers. All DDW and buffers were treated with the Chelex-100 chelating resin to remove any free metal ion before use.

**Preparation of fractions**

Beef loins from four different animals and chicken breast muscles from 12 birds were purchased from local retail stores. A loin from each animal was used as a replication. Breast muscles from 3 birds randomly selected among 12 birds were pooled and used as a replication. Muscles for each replication were separately ground. After grinding muscles through an 8-mm plate twice, eighty-gram patties were prepared.

Meat homogenate (H) was prepared by homogenizing the ground meat with three volumes of 50 mM acetate buffer (pH 5.6) using a Brinkman Polytron (Type PT 10/35; Brinkman Instrument Inc., Westbury, N.Y.) for 15s at speed setting 7. A portion of the homogenate was stored at 4 °C for subsequent analyses and the rest was centrifuged at
15,000 \times g for 30 min at 4 °C. After centrifugation, the precipitate was re-suspended in three volumes of 50 mM acetate buffer (pH 5.6) and centrifuged to remove remaining water-solubles. After washing twice, the precipitate was suspended in three volumes of 50 mM acetate buffer (pH 5.6) and used as a precipitate (P) fraction. The supernatant was filtered through a Whatman No. 1 filter paper twice and used as a supernatant (S) fraction. A portion of S fraction was ultrafiltered by centrifuging through a Centricon Plus-20 centrifugal filter (MW cut-off of 10,000; Millipore, Billerica, MA). The filtrate was collected as a low-molecular-weight (LMW) fraction and the retentate was recovered with acetate buffer to the initial volume. The recovered retentate was ultrafiltered by centrifugation two more times to remove any low molecular weight substances. After rinsing twice, the high molecular weight (HMW) fraction was acquired. Finally, the homogenate (H), precipitate (P), supernatant (S), HMW fraction, and LMW fraction from meat were collected (Figure 1).

Metmyoglobin, ferrous ammonium sulfate, ferric chloride, DTPA, and DFO solution dissolved in 50 mM acetate buffer (pH 5.6) were mixed with each fraction at the ratio of 1:1 (v/v) just before the analyses, leading the final concentration of each treatment to 1.0 mg/ml, 5 \mu g/ml, 5 \mu g/ml, 2 mM, and 2 mM, respectively. The buffer was mixed with each fraction and each treatment as a fraction-control or a treatment-control, respectively. Lipid peroxidation potential in the phospholipid liposome model system and lipoygenase-like activity were determined for each combination of treatments and fractions.

**Lipid peroxidation potential in phospholipid liposome model system**

The addition effect of metmyoglobin, Fe(II), Fe(III), DFO, and DTPA on lipid peroxidation potential (LPP) of fractions from chicken breast and beef loin in liposome model system prepared with phospholipid and the fractions was determined. Phospholipid was from egg yolk and the fatty acid composition of the phospholipids (Table 1) was determined by the method of Ahn, Wolfe, & Sim (1995). Chloroform containing the phospholipids were placed in a scintillation vial and evaporated under nitrogen gas to make a thin film on the wall. Each meat fraction was added to the phospholipid-coated vial and then the vial was shaken vigorously for 2 min to make fraction-liposome solution with final concentration of 3 mg phospholipids per ml fraction. The fraction-liposome solutions were
incubated at 37 °C for 120 min to accelerate lipid peroxidation. Lipid peroxidation in the fraction-liposome solution was determined at 0, 15, 30, 60, 90, and 120 min. After the addition of 10 μl of 6 % BHT in ethanol to stop the reaction, an aliquot (0.5 ml) of the sample was mixed with 1 ml of TBA/TCA solution (15 mM TBA/15% trichloroacetic acid (TCA; w/v)), and then incubated in boiling water bath for 15 min. After cooling, the mixture was centrifuged at 15,000 x g for 10 min. The absorbance of supernatant was determined at 531 nm against a reagent blank. The extent of lipid peroxidation in the fraction-liposome solution was expressed as mmol malondialdehyde (MDA) per kg phospholipid (PL), calculated from the molar extinction coefficient of 1.56 x 10^5 M^-1 cm^-1. The TEARS values (mg MDA per kg meat) of chicken breast and beef loin used in this study was 0.19 and 0.26, respectively, which were not significantly different from each other (P < 0.05)

**Lipoxygenase-like activity**

Lipoxygenase-like activities of S, HMW, and LMW fractions with treatments were measured by a modification of Gata, Pinto, & Macias (1996). Linoleic acid (10 mM) in 0.02 N NaOH solution emulsified by Tween-20 was used as a substrate solution, and was flushed with nitrogen gas. The reaction mixture was composed of 80 μl of the substrate solution, 80 μl of each fraction as an enzyme solution, and 50 mM acetate buffer (pH 5.6) to a final volume of 1 ml. Lipoxygenase-like activity was determined by the increase of absorbance at 234 nm due to the generation of conjugated dienes from linoleic acid at 27 °C. The results were expressed as units of activity (U) per g meat, calculated from the molar extinction coefficient of hydroperoxyl linoleic acid (ε = 25,000 M^-1 cm^-1). One unit of lipoxygenase-like activity was defined as the amount of enzyme catalyzing the formation of 1 μmol of hydroperoxide per minute.

**Statistical analysis**

Factorial designs (5 fractions x 2 meats x 6 treatments) were used in this study. All the analyses were performed with four replications. Data were analyzed using the JMP software (version 5.1.1; SAS Institute Inc., Cary, NC) and reported as means and standard error of the
means (SEM). Differences among means were assessed by Tukey’s method \((P < 0.05)\) (Kuehl, 2000).

**Results and Discussion**

Lipid peroxidation potential (LPP) was defined as the capability of increasing lipid peroxidation in the phospholipid liposome model system during the incubation and was expressed as the amount of increased TBARS values during incubation, compared to control. Fe(III) showed extremely low LPP (0.68 mmol MDA / kg PL) due to absence of reducing compounds (Figure 2). The LPP of metmyoglobin (22.55 mmol MDA / kg PL) was significantly higher than that of Fe(II) (14.77 mmol MDA / kg PL). Different patterns of TBARS increases by metmyoglobin and Fe(II) were observed. TBARS value increased rapidly right after the addition of Fe(II) (0 min) but the rate slowed down during incubation. This result showed faster catalytic kinetics of lipid peroxidation catalyzed by Fe(II) at the beginning of reaction. The slow increase of TBARS in meat during storage may be due to the accumulation of Fe(III) and absence of reducing agents (Kanner, 1994). Different prooxidant activities between Fe(III) and Fe(II) in this study are consistent with Ahn & Kim (1998). They suggested that the status of free ionic iron is more important than the amount. The presence of reducing agents is critical for the conversion of Fe(III) to Fe(II) and the continuous catalysis of lipid peroxidation. Kanner (1994) called it as the iron redox cycle-dependent lipid peroxidation. Addition of metmyoglobin to model system increased TBARS values linearly during incubation. This result may be related to the production of catalytic compounds such as ferrylmyoglobin or hematin from metmyoglobin.

LPPs of homogenate (H), supernatant (S), and low molecular weight (LMW) fractions from chicken breast (CH, CS, and CLMW fraction, respectively) in model system increased during incubation. CLMW fraction was primarily responsible for the increase of CH and CS fractions. An increase of TBARS values in CLMW fraction occurred from 0 min to 30 min, and then did not change after 30 min, due probably to the depletion of reducing agents. These results are consistent with our previous study (Min & Ahn, 2006a). These indicated that the increase of TBARS values by the CLMW fraction is due to its unstable ferric ion reducing capacity (UFRC) which is disappeared rapidly. This UFRC may be attributed to unstable low
molecular weight reducing compounds such as ascorbic acid, NAD(P)H, glutathione, thiol, etc. (Min & Ahn, 2005). Our previous study also indicated that the UFRC did not act as a prooxidant in chicken breast. The pattern of TBARS changes by LMW fraction from beef loin (BLMW fraction) was similar to that by CLMW fraction, but LPP of BLMW fraction was smaller than that of CLMW fraction because of low UFRC in BLMW fraction compared with CLMW fraction, which is in agreement with our previous results (Min & Ahn, 2006a). It seems that the UFRC in the BLMW fraction did not act as a prooxidant because the TBARS value of raw beef loin was low (0.26 mg MDA / kg meat) despite of fast kinetics of free ionic iron.

The addition of Fe(III) increased the LPPs of CH, CP, CS, and CHMW fraction by 1.11, 0.82, 0.79, and 0.60 mmol MDA / kg PL, respectively, but not LPP of CLMW fraction (Figure 3 A1-A5). The increase of LPP in CH fraction seemed to be dependent on CP and CHMW fraction. Our previous study (Min & Ahn, 2006a) showed that CP and CHMW fractions had storage-stable ferric iron reducing capacities (SFRC). The SFRC in CP and CHMW fraction may be responsible for an increase of LPP of CH fraction in Fe(III)-added model system. Therefore, it is suggested that SFRC can act as a prooxidant in the presence of high amount of ferric ion.

The addition of Fe(II) increased LPPs of all fractions, CH, CP, CS, CHMW, and CLMW fraction by 2.78, 4.00, 3.39, 4.60, and 2.42 mmol MDA / kg PL, respectively. This result indicated that each fraction showed substantial inhibiting activity for LPP of Fe(II) (14.77 mmol MDA / kg PL in Figure 2) (81.19, 72.95, 77.05, 68.83, and 83.65 %, respectively). Our previous study (Min & Ahn, 2006a) showed that chicken breast has relatively high total antioxidant activity (TAC). The CLMW fraction (83.65 %) showed the highest inhibiting activity for LPP of Fe(II) among fractions, indicating that UFRC in CLMW fraction serves as an active antioxidant. The high concentration of carnosine and anserine in chicken breast (Chan & Decker, 1994) may be partially responsible for the high TAC of CLMW fraction. The addition of CHMW fraction (68.83 %) to CLMW fraction (83.65 %) decreased the inhibiting activity of CLMW fraction, resulting in lower inhibiting activity of CS fraction (77.05 %) than CLMW fraction. This result is consistent with the study of Erickson, Hultin, & Borhan (1990) which showed the inhibition effect of LMW cytosol fraction on lipid
peroxidation decreased by the addition of HMW cytosol fraction. They suggested that the HMW fraction caused attenuation of chelating activities by ADP and inorganic phosphate in the LMW fraction. Therefore, the antioxidant effect of CLMW may be due to its high UFRC, high carnosine and anserine concentration, and high iron chelating ability.

However, the interaction of CS fraction (77.05 %) with CP fraction (72.95 %) increased the inhibiting rate of CH fraction (81.19 %) for the Fe(II)-dependent lipid peroxidation. This result indicated that the synergistic effect of antioxidant factors between CP and CS fractions, especially CLMW fraction, account for the higher antioxidant activity in CH fraction. Our previous study (Min & Ahn, 2006a) indicated that the total antioxidant activity (TAC) in CH fraction is higher than the simple sum of CP and CS fraction. Most of the increased nonheme irons during 10-d storage were accumulated into the CP fraction due probably to hemosiderin which is water-insoluble iron storage protein produced by the denaturation of ferritin and is inactive to lipid peroxidation (Decker & Hultin, 1992). The addition of DTPA and DFO completely inhibited the development of lipid peroxidation by all fractions from chicken breast (Figure 3 A1-A5). Therefore, it is suggested that iron chelating ability and the synergistic interactions among antioxidant agents in CLMW and CP fraction are responsible for TAC in chicken breast.

The addition of metmyoglobin increased the LPPs of all fractions from chicken breast (Figure 3 A1-A5). The increases of LPPs in CH, CP, CS, CHWP, and CLWP fractions were 4.58, 14.56, 5.52, 11.76, and 9.44 mmol MDA / kg PL, respectively. These results indicated that the inhibiting activities of those fractions for LPP of metmyoglobin (22.55 mmol MDA / kg PL) in Figure 2 were 79.68, 35.42, 75.53, 47.83, and 58.11 %, respectively. The inhibiting activity of CH fraction for LPP of metmyoglobin (79.68 %) seemed to be smaller than that of Fe(II) (83.78 %), due to greater LPP of metmyoglobin than Fe(II). The inhibiting activities of CP, CHMW, and CLMW fractions (35.42%, 47.83, and 58.11 %, respectively) for LPP of metmyoglobin were worse than that for LPP of Fe(II) (72.95, 68.83, and 83.65 %). The remarkable synergistic interaction of antioxidant activities among CP, CHMW and CLMW fractions were responsible for the relatively high inhibition effect of CH fraction (79.68 %). In spite of high inhibiting activity of CH fraction for LPP of metmyoglobin, the addition of metmyoglobin caused a significant increase in LPP of CH fraction (4.58 mmol MDA / kg
PL), which is similar to that of H fraction from beef loin (4.29 mmol MDA / kg PL). The concentration of myoglobin was slightly different from each other: 1.13 mg / ml in the metmyoglobin-added CH fraction and 0.88 mg / ml in the BH fraction. Our previous study (Min & Ahn, 2006b) indicated that an increase of metmyoglobin concentration from 0.25 to 2 mg / ml did not increase the level of lipid peroxidation. Therefore, despite of the high TAC of chicken breast, addition of myoglobin to chicken breast developed lipid peroxidation in similar level to beef loin, indicating that low concentration of metmyoglobin in chicken breast is an important contributing factor to its high oxidative stability.

As shown in Figure 3 (B1-B5), addition of Fe(III) increased LPPs of all fractions from beef loin (BH, BP, BS, BHMW, and BLMW fraction) by 3.78, 0.93, 2.99, 2.72, and 1.49 mmol MDA / kg PL, which were higher than those of their counterpart fractions from chicken breast (1.11, 0.82, 0.79, 0.60, and 0.07 mmol MDA / kg PL, respectively). This result indicated that beef loin had higher SFRC than chicken breast. Therefore, beef loin has higher susceptibility to lipid peroxidation than chicken breast under the conditions which can provide high amount of free ionic irons. The increase of LPP in BH fraction (3.78 mmol MDA / kg PL) by the addition of Fe(III) was around the sum of BP (0.93 mmol MDA / kg PL) and BS (2.99 mmol MDA / kg PL) fractions. Our previous study (Min & Ahn, 2006a) indicated that the FRC of BH fraction was the sum of BP and BS fractions, and was very stable during storage. BHMW fraction seemed to be primarily responsible for SFRC in BF and BH fraction. This result and our previous findings (Min & Ahn, 2006a) suggested that the BHMW fraction is the major fraction responsible for the susceptibility of beef loin to lipid peroxidation because of not only myoglobin, but also high SFRC to reduce released free ionic iron released from myoglobin.

The addition of Fe(II) increased LPPs of BH, BP, BS, BHMW, and BLMW fractions by 6.67, 4.89, 7.28, 8.32, and 4.32 mmol MDA / kg PL, respectively. This result indicated that all fractions from beef loin showed lower inhibiting activities for LPP of Fe(II) (54.83, 66.88, 50.71, 43.67, and 70.77 %, respectively) than their counterpart fractions from chicken breast (81.19, 72.95, 77.05, 68.83, and 83.65 %, respectively). This result suggested that lower TAC in beef loin was a critical factor for higher susceptibility to lipid peroxidation for beef loin than chicken breast. The inhibition activity of BLMW and BP fractions seemed to attenuate
prooxidative activities of BHMW fraction, resulting in the relatively high inhibiting activity of BH fraction. Our previous study (Min & Ahn, 2006a) showed that TAC and UFRC in BLMW fraction were significantly lower than that in CLMW fraction. Also, Chan and Decker (1994) indicated that the concentration of carnosine and anserine in chicken breast is more than 3 times higher than those in beef loin. Therefore, both lower antioxidant activity of BLMW fraction and higher prooxidant effect of BHMW fraction were responsible for lower antioxidant activity in BH fraction.

The addition of metmyoglobin increased LPPs of all fractions from beef loin (BH, BP, BS, BHWp, and BLWP fractions) by 3.66, 8.45, 3.92, 2.16, and 11.37 mmol MDA / kg PL, respectively. This result indicated that the inhibition activities of those fractions for LPP of metmyoglobin were 83.78, 62.53, 82.60, 90.42, and 49.54 %, respectively. The inhibiting activity of BH, BF, and BHMW fraction may be overestimated because of myoglobin concentration effect. The BH, BS, and BHMW fractions already contained pre-existing myoglobin (0.88, 0.78, and 0.76 mg / ml fraction-liposome, respectively). The concentrations of myoglobin in metmyoglobin-added BH, BS, BHMW, BP, and BLMW fractions-liposome were 1.85, 1.72, 1.70, 1.02, and 0.99 mg / ml, respectively. Our previous study (Min & Ahn, 2006b) reported that an increase of metmyoglobin concentration from 0.25 to 2 mg / ml did not increase the level of lipid peroxidation. It has been suggested the concentration of metmyoglobin was a critical factor for determining its prooxidative activity in the presence of LOOH and/or fatty acid: at a low concentration in lipid system, metmyoglobin acted as a prooxidant, but not at a high concentration (Baron et al., 2002; Lapidot et al., 2005).

The increases of LPPs of BH, BS, and BHMW fractions after adding metmyoglobin was 3.66, 3.92, and 2.16 mmol MDA / kg PL, respectively, which was similar to that after adding Fe(III) (3.78, 2.99, and 2.72 mmol MDA / kg PL, respectively). Our previous study (Min & Ahn, 2006b) reported that the amount of released free ionic iron increased with the increase of metmyoglobin concentration. Therefore, the increases of LPPs by the addition of metmyoglobin may not be caused by metmyoglobin itself, but by free ionic iron released from metmyoglobin. The high SFRC in BHMW may reduce free ionic iron to catalyze lipid peroxidation.
The inhibiting activity of the BP fraction (62.53 %) for LPP of metmyoglobin was much higher than that of the CP fraction (35.43 %) but similar to that for LPP of Fe(II) (66.88 %). This result indicated that the mode of action of antioxidant activity of BP and CP fractions could be different from each other. It is assumed that the antioxidant activity of BP fraction may be catalyst-independent and involved with the breakdown of free radical chain reaction. But, the antioxidant activity in CP fraction may be equally related to free radical scavenging activity and catalyst-dependent mechanism such as iron-chelating activity. The BLMW and CLMW fraction showed lower inhibiting activity for LPP of metmyoglobin (49.54 and 58.11 %, respectively) than that of Fe(II) (70.77 and 83.65 %, respectively) due to stronger catalytic activity of metmyoglobin and/or difference in major catalyst of both systems. The CLMW fraction showed higher inhibiting activity than BLMW for LPP of both, due to higher TAC in CLMW.

The effects of various fractions from raw chicken breast and beef loin on LPPs of metmyoglobin (metMb), Fe(II), and Fe(III) are summarized in Table 2. The LPP of Fe(III) were increased more considerably by raw beef loin fractions than by counterpart fractions from raw chicken breast because of higher SFRC in raw beef loin than raw chicken breast. The LPP of Fe(II) were inhibited better when fractions from raw chicken breast were used than the counterpart fractions from raw beef loin because of higher TAC in raw chicken breast than beef loin. Although the LPP of metmyoglobin were inhibited better when fractions from raw beef loin than counterpart fractions from raw chicken breast were used, the inhibiting rates by raw beef loin fractions were overestimated due to myoglobin concentration effect. Therefore, lower TAC and higher SFRC in raw beef loin than raw chicken breast may be the major contributors to higher susceptibility of raw beef loin than raw chicken breast to lipid peroxidation.

The addition of DTPA and DFO completely suppressed the LPPs of BP and BC fractions where myoglobin did not exist (Figure 3 B2 and B5). However, DFO was more efficient suppressor in BH, BS, and BHWM fraction than DTPA. This result is in agreement with our previous study (Min & Ahn, 2006b). DFO was not only a very effective iron chelator to inhibit lipid peroxidation, but also an excellent electron donor to ferrylmyoglobin and free radicals (Kanner & Harel, 1987; Rice-Evans et al., 1989). DTPA can act only as a chelator.
The addition of DTPA partially lowered LPPs of BH, BS, and BHMW fraction. This result indicated that DTPA-unchelatable compounds played important role in catalyzing lipid peroxidation. The LPP of DTPA-added BH, BS, and BHWM fractions were similar to each other (3.21, 3.29, and 3.19 mmol MDA / kg PL, respectively). This result indicated that the catalytic activity of DPTA-unchelatable catalysts from myoglobin were stable and not inhibited by the antioxidant activity in BP and BLMW fractions. However, decreases of LPPs of BH, BS, and BHMW fractions by DTPA were 0.83, 3.28, and 9.38 mmol MDA / kg PL, respectively. These gaps generated by DTPA should be originated from the catalytic activity of free ionic iron and high SFRC in BHMW fraction. Our previous study (Min & Ahn, 2006a) and our findings in this study indicated that the BH fraction had the highest SFRC, followed by BS and BHMW fraction. Therefore, the decrease of DTPA effect should be related to the availability of free ionic iron released from myoglobin in the fractions. The addition of BLMW to BHMW fraction and, subsequently, BP to BS fraction may reduce the availability of free ionic iron due to their chelating ability. The organic compounds containing phosphate group such as ADP, ATP, etc. may be the primary chelators in BLMW fraction (Erickson et al., 1990), and the hemosiderin in BP fraction (Decker & Hultin, 1992). Previous our study (Min & Ahn, 2006a) showed that the nonheme iron content in BP fraction significantly increased during 10-d storage. However, the ability of low molecular weight chelators to inhibit the development of lipid peroxidation is dependent on the concentration between chelators and free iron: At high ratio, they can inactivate catalytic activity of free ionic iron by occupying all coordination sites of iron, but not at low ratio (Graf, Mahoney, Bryant, & Eaton, 1984). Therefore, a continuous increase of free ionic iron content not only attenuates the chelating effect of BLMW fraction, but also turns pre-existing iron chelates into prooxidants. This synergistic effect resulted in rapid increase of TBARS values of metmyoglobin-added BH fraction-liposome after 90 min of incubation (Figure 3 B1). Therefore, DTPA-unchelatable catalysts such as ferrylmyoglobin and/or hematin generated from myoglobin seems to be major catalysts in raw beef loin, rather than released free ionic iron because of chelating activity of beef loin. However, the increase of free ionic iron content beyond the chelating capacity may cause an exponential increase of lipid peroxidation in beef loin.
Figure 4 showed the addition effect of catalysts such as metmyoglobin, Fe(II), and Fe(III) and iron chelators such as DTPA and DFO on a lipoxygenase (LOX)-like activity in each fraction from chicken breast and beef loin. The LOX-like activity induced by myoglobin has been detected (Rao et al., 1994, Min et al., 2006, Min & Ahn, 2006a & b). LOX-like activity was attributed to the formation of ferrylmyoglobin by the interaction of metmyoglobin with H$_2$O$_2$ or LOOH (Min & Ahn, 2005). LOX-like activity is related to the reaction rate of free radical chain reaction. LOX-like activities in all fractions from chicken breast and BLMW fraction were negligible due to tiny amount of myoglobin and did not changed by the addition of Fe(II), Fe(III), DTPA, and DFO. The LOX-like activity of metmyoglobin was lowered by CLMW fraction due to the highest antioxidant activity among fractions, but did not change by CS and CHMW fraction. Therefore, the low amount of myoglobin in chicken breast was one of critical contributing factors to its high oxidative stability.

LOX-like activities in metmyoglobin in buffer, BS and BHMW fractions were different from each other because of slight different concentrations of metmyoglobin (1.0, 0.78, and 0.76 mg / ml). The addition of Fe(III) did not change LOX-like activities of both fractions, but the addition of Fe(II) decreased their LOX-like activities. We observed that the formation of deoxy- and oxymyoglobin increased gradually after the addition of Fe(II) (Data not shown) due to the reduction of metmyoglobin by electron transfer from Fe(II). Oxymyoglobin catalyzed lipid peroxidation more slowly than metmyoglobin (Gorelik & Kanner, 2001). In addition, the addition of DFO and DTPA decreased their LOX-like activities, and DTPA was more effective than DFO in contrast to their effect in the phospholipid liposome system. This result is consistent with the findings in our previous study using metmyoglobin (Min & Ahn, 2006b).

**Conclusion**

Higher TAC and low amount of myoglobin as a source of catalysts for lipid peroxidation were responsible for high oxidative stability of chicken breast, compared to beef loin. The high TAC in chicken breast was derived from its iron chelating ability and the synergistic interactions among antioxidant agents in CLMW and CP fraction. The BHMW fraction, which contains myoglobin and high SFRC, was the major contributor to the high
susceptibility of beef loin to lipid peroxidation. Major catalysts for lipid peroxidation in beef loin were DTPA-unchelatable, ferrylmyoglobin and/or hematin in “normal” storage conditions because most free ionic irons released from myoglobin in beef loin were chelated by BLMW and BP fractions. However, free ionic iron reduced by SFRC was the major catalyst under conditions where free ionic iron content exceeds the chelating capacity in beef loin.

References


Table 1. Fatty acid composition of phospholipids extracted from egg yolk by ethanol

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Content (%)</th>
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<tbody>
<tr>
<td>Myristic acid</td>
<td>0.19 ± 0.03</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>28.70 ± 0.22</td>
</tr>
<tr>
<td>Palmitoleic acid</td>
<td>1.28 ± 0.18</td>
</tr>
<tr>
<td>Margaric acid</td>
<td>0.28 ± 0.01</td>
</tr>
<tr>
<td>Margaroleic acid</td>
<td>0.12 ± 0.03</td>
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<tr>
<td>Stearic acid</td>
<td>16.25 ± 0.14</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>27.01 ± 0.20</td>
</tr>
<tr>
<td>trans-Vaccenic acid</td>
<td>1.59 ± 0.15</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>15.38 ± 0.14</td>
</tr>
<tr>
<td>γ-Linolenic acid</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>Gondoic acid</td>
<td>0.21 ± 0.03</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>6.68 ± 0.09</td>
</tr>
<tr>
<td>DTA</td>
<td>0.41 ± 0.08</td>
</tr>
<tr>
<td>DPA</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>DHA</td>
<td>1.59 ± 0.04</td>
</tr>
</tbody>
</table>

Means was expressed with standard deviation. n = 4.

Abbreviations: DTA, all cis-7, 10, 13, 16-docosatetraenoic acid; DPA, all-cis-7, 10, 13, 16, 19-docosapentaenoic acid; DHA, all cis-4, 7, 10, 13, 16, 19-docosahexaenoic acid.
Table 2. Effect of various fractions from raw chicken breast and beef loin on the lipid peroxidation potential (LPP) of metmyoglobin (metMb), Fe(II), and Fe(III)

<table>
<thead>
<tr>
<th>Fraction</th>
<th>metMb</th>
<th>Fe(II)</th>
<th>Fe(III)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chicken/Beef</td>
<td>Chicken/Beef</td>
<td>Chicken/Beef</td>
</tr>
<tr>
<td>H</td>
<td>79.68/83.78</td>
<td>81.19/54.83</td>
<td>164.98/560.40</td>
</tr>
<tr>
<td>P</td>
<td>35.43/62.53</td>
<td>72.95/66.88</td>
<td>122.10/138.00</td>
</tr>
<tr>
<td>S</td>
<td>75.53/82.60</td>
<td>77.05/50.71</td>
<td>116.72/472.04</td>
</tr>
<tr>
<td>HMW</td>
<td>47.83/90.42</td>
<td>68.83/43.67</td>
<td>88.48/429.39</td>
</tr>
<tr>
<td>LMW</td>
<td>58.11/49.54</td>
<td>83.65/70.77</td>
<td>10.25/221.17</td>
</tr>
</tbody>
</table>

Numbers < 100 % indicate inhibition rate and > 100 % increasing rate of LPP of metMb, Fe(II), and Fe(III) by the fractions from raw chicken breast and beef loin.

Abbreviations: H, homogenate fraction; P, precipitate fraction; S, supernatant fraction; HMW, high molecular weight fraction from supernatant fraction; LMW, low molecular weight fraction from supernatant fraction.
Figure 1. Flow diagram of fraction preparation from raw chicken breast and beef loin. Abbreviations: H, homogenate fraction; P, precipitate fraction; S, supernatant fraction; HMW, high molecular weight fraction from supernatant fraction; LMW, low molecular weight fraction from supernatant fraction; MW, molecular weight.
Figure 2. Lipid peroxidation potential of myoglobin (Mb, 1 mg / ml liposome solution) or free ionic irons (ferrous (Fe(II)) and ferric (Fe(III)) ion (5 μg / ml liposome solution) in the phospholipid liposome model system during incubation at 37 °C for 120 min (TBARS values, mmol malondialdehyde (MDA) / L liposome solution). The phospholipid liposome model system with 50 mM acetate buffer (pH 5.6) was used as a control (PL). Means with the standard deviation were indicated. n = 4.
Figure 3. Lipid peroxidation potential of fractions treated with myoglobin (Mb, 1 mg / ml reaction solution), free ionic irons (ferrous (Fe(II)) or ferric (Fe(III)) ion, 5 ppm), or chelates (desferrioxamine (DFO, 2 mM) and diethylenetriamine pentaacetic acid (DTPA, 2 mM)) in phospholipid model system during incubation at 37 °C for 120 min (TBARS values, mmol malondialdehyde (MDA) / L reaction solution). The fractions collected from raw chicken breast (A) and beef loin (B) were homogenate (1), precipitate (2), supernatant (3), high molecular weight (HMW; 4) and low molecular weight (LMW; 5) fractions. The phospholipid liposome model system with each fraction was used as a control (Ctrl) and with 50 mM acetate buffer (pH 5.6) was as a blank control (PL). Means with the standard deviation were indicated. n = 4.
Figure 3. (continued)
Figure 3. (continued)
Figure 3. (continued)
Figure 4. Lipoxygenase-like activity of fractions treated with myoglobin (Mb; 1 mg/ml solution), free ionic iron (ferrous (Fe(II)) or ferric (Fe(III)) ion, 5 ppm), or chelates (desferrioxamine (DFO, 2 mM) and diethylenetriamine pentaacetic acid (DTPA, 2 mM)) (Unit/ml solution). The fractions used were supernatant (S), high molecular weight (HMW), and low molecular weight (LMW) fraction from chicken breast (C) and beef loin (B). The lipoxygenase-like activity of each fraction mixed with 50 mM acetate buffer (pH 5.6) alone was used as a control (Ctrl). The lipoxygenase-like activities of each treatment (Mb, Fe(II), Fe(III), DTPA, and DFO) in 50mM acetate buffer was indicated as “Buffer” in the fraction axis. Means with standard deviation were indicated. n = 4.
CHAPTER 6. EFFECT OF METMYOGLOBIN, FERROUS AND FERRIC ION ON THE LIPID PEROXIDATION OF RAW AND COOKED CHICKEN BREAST AND BEEF LOIN

A paper which will be submitted to Meat Science

B. Min¹ and D.U. Ahn²

Abstract

The effect of metmyoglobin (metMb), ferric iron, and ferrous ion on lipid peroxidation of raw and cooked chicken breast and beef loin were determined. NaCl was added along with the additives to facilitate the accessibility of the additives to phospholipids in cell membrane. Raw and cooked chicken breast with each treatment during storage showed higher oxidative stability than raw and cooked beef loin during 10-d storage. The addition of NaCl did not affect the oxidative stability of chicken breast, but significantly increased the susceptibility of raw beef loin to lipid peroxidation due to the increase in nonheme iron content. The addition of metMb did not have adverse effect on the oxidative stability of raw chicken breast. MetMb in chicken breast was gradually reduced to oxymyoglobin during storage. The addition of ferric iron or ferrous iron to raw chicken breast increased TBARS values during storage, but the increase was lower than that in raw beef loin. This was caused by the stable ferric iron reducing capacity (SFRC), which reduced ferric iron to ferrous ion. SFRC was higher in beef loin than in chicken breast. Therefore, it is suggested that higher total antioxidant capacity (TAC) and lower SFRC may be responsible for the higher oxidative stability of chicken breast than beef loin. Nonheme iron content in metMb-added cooked chicken breast and beef loin increased during storage due to degradation of myoglobin. TBARS values of metMb, ferric iron or ferrous ion-added cooked chicken breast significantly increased during storage. This indicated that “heat-stable” ferric iron reducing capacity (HFRS) was responsible for the conversion of ferric ion to ferrous iron in cooked meat. The rate of lipid peroxidation in raw

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beef loin added with NaCl alone was not different from that with ferrous iron or ferric ion-added one although nonheme iron content in former were much lower than that in latter. Same phenomena were observed in cooked beef loin and chicken (metMb- vs ferric and ferrous iron-added). Therefore, it is suggested that free ionic iron is a major catalyst, and SFRC and HFRC are limiting factors for lipid peroxidation in NaCl-added and/or cooked meat where the amount of free ionic iron increases continuously during storage.

Introduction

Lipid peroxidation is a major factor that determines sensory, functional, and nutritional quality and shelf life of processed meat products. The secondary by-products of lipid peroxidation such as aldehydes generated may have cytotoxic and genetoxic properties due to their high reactivity (Esterbauer, Schaur, & Zollner, 1991; Lynch, Faustman, Silbart, Rood, & Furr, 2001). Therefore, repeated consumptions of highly oxidized meat can be a great threat to human health.

Myoglobin has been recognized as a major source of catalysts for lipid peroxidation in meat, but its mode of action for catalyzing lipid peroxidation in meat is controversial. It is suggested that the interaction of metmyoglobin with hydrogen peroxide (H$_2$O$_2$) or lipid hydroperoxides (LOOH) results in the formation of ferrylmyoglobin, which can initiate free radical chain reaction (Harel & Kanner, 1985; Rao, Wilks, Hamberg, & Ortiz de Montellano, 1994; Baron, Skibsted, & Andersen, 1997 & 2000; Baron & Anderson, 2002). Myoglobin is also suggested to be a source of free ionic iron and hematin and catalyze lipid peroxidation in meat in the presence of H$_2$O$_2$ or lipid hydroperoxide (Gutteridge, 1986; Harel, Salan, & Kanner, 1988; Kanner, Shegalovich, Harel, & Hazan, 1988; Prasad, Engelman, Jones, & Das, 1989; Ahn & Kim, 1998; Baron, Skibsted, & Andersen, 2002).

Cooked meat oxidizes faster than raw meat (Salih, Price, Smith, & Dawson, 1989; Han, McMillin, Godber, Bidner, Younathan, & Hard, 1995; Byrne, Bredie, Mottram, & Martens, 2002; Beltran, Pla, Yuste, & Mor-Mur, 2003). Heating can influence various factors associated with lipid peroxidation: disruption of muscle cell structure, inactivation of antioxidant enzymes, and release of oxygen and iron from myoglobin (Kanner, 1994). The disrupted membrane allows easy access of oxygen, which accelerates lipid oxidation (Igene,
Yamauchi, Pearson, Gray, & Aust, 1985). Mei, Crum, & Decker (1994) and Lee, Mei, & Decker (1996) suggested that the inactivation of catalase and glutathione peroxidase (GSH-Px) by heating could be partially responsible for the rapid development of lipid peroxidation in cooked meat. Igene, King, Pearson, & Gray (1979) demonstrated that cooking significantly increased the level of free non-heme iron content by releasing iron from heme pigments. They indicated that the release of non-heme iron during cooking results in the increased rate of lipid oxidation.

Sodium chloride (NaCl) has a prooxidant effect in meat and meat products depending on its concentration (Rhee & Ziprin, 2001). The possible prooxidant mechanisms of NaCl is attributed to its capability of 1) disrupting structural integrity of cell membrane, which enables catalysts easy access to lipid substrates (Rhee, 1999), 2) releasing free ionic iron from iron-containing molecules such as heme proteins (Kanner, Harel, & Jaffe, 1991; Rhee & Ziprin, 2001), and 3) inhibiting the activities of antioxidant enzymes such as catalase, glutathione peroxidase, and superoxide dismutase (Lee, Mei, & Decker, 1997; Hernandez, Park, & Rhee, 2002).

Our previous study (Min & Ahn, 2006c) suggested that the high oxidative stability of chicken breast meat was due to high total antioxidant capacity (TAC) and very low myoglobin content in chicken breast, compared with beef loin. Ferrylmyoglobin and hematin are the major catalysts in raw beef loin because beef loin has iron chelating capacity. Both chicken breast and beef loin had storage-stable ferric ionic reducing capacity (SFRC), which acted as a prooxidant when free ionic iron content in meat was high. Therefore, free ionic iron can be a major catalyst in the presence of SFRC. The objective of this study was to confirm our previous findings using meat system. Metmyoglobin, ferric iron, or ferrous ion was added to raw and cooked chicken breast and beef loin and their effects on lipid peroxidation and other prooxidant factors were evaluated. NaCl was added along with the catalysts to facilitate their accessibility to cell membrane.

**Materials and Methods**

**Chemicals and Reagents**
Metmyoglobin (from equine skeletal muscle), ferrous ammonium sulfate, ferric chloride, linoleic acid, 2-thiobarbituric acid (TBA), ferrozine (3-(2-pyridyl)-5,6-bis (4-phenyl sulfonic acid)-1,2,4-triazine), neocuproine (2,9-dimethyl-1,10-phenanthroline), ferric chloride, Chelex-100 chelating resin (50-100 dry mesh, sodium form), butylated hydroxytoluene (BHT), and Tween 20 were purchased from Sigma (St. Louis, MO). All other chemicals were of reagent grade. Double distilled water (DDW) by Nanopure infinity™ ultrapure water system with ultraviolet (UV) (Barnstead, Dubuque, IA) was used for the preparation of all reagents and buffers. All DDW and buffers were treated with the Chelex-100 chelating resin to remove any free metal ion before use.

**Preparation of raw and cooked meat**

Beef loins from four individual animals and chicken breast muscles from 16 birds were purchased from local retail stores. A loin from each animal was used as a replication. Breast muscles from 4 birds randomly selected from the 16 birds were pooled and used as a replication. Muscles for each replication were separately ground through an 8-mm plate twice. Ground meat (90g) were mixed with NaCl (1.5 g) for 2 min in a bowl mixer (Model KSM90; KitchenAid Inc., St. Joseph, MI, USA) and then 10 ml of metmyoglobin (5 mg / g meat as a final conc.), ferrous ammonium sulfate (5 µg / g), and ferric chloride (5 µg / g) solution in DDW were added and mixed again for 3 min. Ground meat with only DDW (10 ml) and DDW (10 ml) + NaCl (1.5 g) were used as a control and NaCl-control, respectively. Streptomycin (200 ppm) was added as an antimicrobial agent. The mixture was manually formed into two patties (50 g each), and then were individually packaged in oxygen-permeable zipper bags (polyethylene, 4 x 6, 2 mil.; Associate Bag Co. Milwaukee, Wis., U.S.A.). One of the packaged patties was cooked in a 95 °C water bath to an internal temperature of 75 °C, followed by cooling for 2 h at 4 °C. After draining meat juices from the bag, the patties were repackaged. Raw and cooked patties were stored at 4 °C until used. Lipid peroxidation, nonheme iron, myoglobin, metmyoglobin percentage, and lipoxygenase-like activity of meat samples were determined at 0, 5, and 10 days of storage for raw patties. Lipid peroxidation and nonheme iron were determined at 0, 3, and 7 days of storage for cooked meat.
Chemical analyses of treatment-added raw and cooked meat

Lipid peroxidation was determined by the method of Ahn, Olson, Lee, Jo, Wu, & Chen (1998). Briefly, a meat sample (5 g) was homogenized with 15 ml DDW and 100 μl BHT (6% in 100% ethanol) by the Brinkman Polytron for 15s at speed setting 7. The meat homogenate (1 ml) was mixed with 2 ml TBA / TCA solution (15 mM TBA / 15% trichloroacetic acid (TCA; w/v). The mixture was incubated in a boiling water bath for 15 min. After cooling, the mixture was centrifuged at 3,000 \( \times \) g for 15 min. The absorbance of supernatant was determined at 531 nm against blank (1 ml DDW & 2 ml TBA / TCA solution). The amount of 2-thiobarbituric acid reactive substances (TBARS) was expressed as mg malondialdehyde (MDA) per kg meat.

Nonheme iron content was determined by the ferrozine method of Ahn, Wolfe, & Sim (1993). In brief, Meat sample (4 g) was weighed into polyethylene bag (Model 80, 101 × 152 mm, Seward, London, UK) and then homogenized with 12 ml DDW by Stomacher (Stomacher 80, Seward, London, UK) for 5 min at high speed. The homogenate (1.5 ml) with 0.5 ml of 1% ascorbic acid in 0.2 N HCl (w/v) was thoroughly mixed with 11.3% TCA solution (w/v, 1 ml) after standing at room temperature for 5 min. The whole mixture was centrifuged at 3,000 \( \times \) g for 15 min. The supernatant (2 ml) was mixed with 0.8 ml of 10% ammonium acetate (w/v) and 0.2 ml of the ferrozine color reagent and the mixture was left at room temperature for 10 min. The absorbance of the mixture was determined at 562 nm against a reagent blank. The nonheme iron content was expressed as μg nonheme iron per g meat.

Myoglobin and metmyoglobin percentage were determined by the method of Krzywicki (1982). The myoglobin and metmyoglobin content were calculated on the basis of following equations and expressed as mg myoglobin per g meat where the molecular weight of myoglobin was taken as 17,000 daltons and percentages (%), respectively.

Myoglobin concentration (mg / g meat)

\[
= (-0.166R_1 + 0.086R_2 + 0.088 R_3 + 0.099) \times A^{525} \times 0.0175 \times \text{dilution factor}
\]

Metmyoglobin content (%) = (-2.514 \( R_1 \) + 0.777 \( R_2 \) + 0.800 \( R_3 \) + 1.098) \times 100
Lipoxygenase-like activities of meat samples were determined by a modification of Gata, Pinto, & Macias (1996). Meat sample (5 g) was homogenized with 15 ml of 50 mM acetate buffer (pH 5.6) using a Polytron for 10 s at top speed, and then centrifuged at 12,000 × g at 4 °C for 15 min. The supernatant was filtered through a Whatman No. 1 filter paper and the filtrate was used to determine lipoxygenase activities in meat sample. Linoleic acid (10 mM) in 0.02 N NaOH solution emulsified with Tween-20 was used as a substrate solution, and the emulsion was flushed with nitrogen gas and kept under nitrogen. The reaction mixture was composed of 80 µl of the substrate solution, 80 µl of each fraction as an enzyme solution, and 50 mM acetate buffer (pH 5.6) to a final volume of 1 ml. Lipoxygenase-like activity was assessed by the increase of absorbance at 234 nm by the generation of conjugated dienes from linoleic acid at 27 °C. The results were expressed as units of activity (U) per g meat, calculated from the molar extinction coefficient of hydroperoxyl linoleic acid (ε = 25,000 M⁻¹cm⁻¹). One unit of lipoxygenase-like activity was defined as the amount of enzyme catalyzing the formation of 1 µmol of hydroperoxide per minute.

Statistical analysis

Factorial designs (2 meats × 5 treatments × 3 storage times) were used in this study. Data were analyzed using the JMP software (version 5.1.1; SAS Institute Inc., Cary, NC) and reported as means and standard error of means (SEM). Differences among means were assessed by Tukey’s method (P < 0.05) (Kuehl, 2000).

Results and Discussion

Table 1 showed that the initial (Day 0) TBARS value of raw control chicken breast meat was significantly lower than that of raw beef loin and the TBARS did not increase during storage. This indicated that chicken breast meat is highly resistant to exogenous oxidative stress such as mechanical stress (grinding and mixing) and prolonged storage due to its high TAC, and very low of free ionic iron and myoglobin content, compared with beef loin. The
concentrations of nonheme iron (Table 2) and myoglobin (Table 3) in control and all catalyst-added chicken breast were lower than those in beef loin, and their content did not change during storage. Various processing factors such as size reduction processes (grinding, flaking, and emulsification), deboning, especially mechanical deboning, additives, temperature abuse during handling and distribution, oxygen availability, and prolonged storage influence the rate of lipid peroxidation in meat (Min & Ahn, 2005). The addition of NaCl alone (NaCl) and metmyoglobin + NaCl (metMb+NaCl) did not increase lipid peroxidation of chicken breast meat, but significantly increased TBARS values in beef loin. Metmyoglobin did not act as a prooxidant in chicken breast meat. The concentrations of nonheme iron and myoglobin in metMb+NaCl-added chicken breast hardly changed during storage, but metmyoglobin percentage significantly decreased (Table 2 and 3). The decrease of metmyoglobin percentage led to the gradual increase of oxymyoglobin percentage from 17.25% (Day 0) to 71.05% (Day 10) due to the high total antioxidant capacity (TAC) in chicken breast meat. Oxymyoglobin is not involved in lipid peroxidation under high reducing conditions like in chicken breast meat. Therefore, the oxidative stability of metMb+NaCl-added chicken breast was attributed to the high TAC in chicken breast meat because TAC reduced metmyoglobin to oxymyoglobin and maintained the reduced status during storage. In contrast, our previous study (Min & Ahn, 2006c) showed that the addition of metmyoglobin to chicken breast homogenate significantly increased lipid oxidation in phospholipid liposome system during incubation. This happened because of the catalytic effect of both metmyoglobin and released free ionic irons by the severe experimental conditions such as homogenization and prolonged incubation at high temperature.

The addition of Fe(II)+NaCl significantly increased the initial (Day 0) TBARS of chicken breast meat due to fast kinetics of Fe(II). Addition of Fe(III)+NaCl to chicken breast also increased initial TBARS but the increase was lower than that by Fe(II)+NaCl. The addition of Fe(II)+NaCl and Fe(III)+NaCl to raw chicken breast increased TBARS during storage, but their rate were not different. Nonheme iron content in chicken breast added with Fe(II)+NaCl and Fe(III)+NaCl did not change during storage. Storage-stable ferric ion reducing capacity (SFRC) detected in both chicken breast and beef loin acted as a prooxidant in the presence of sufficient amount of free ionic irons but the SFRC in beef loin is much higher than that in
chicken breast (Min & Ahn, 2006a and c). Therefore, this result should be related to the SFRC present in chicken breast meat. The increase of initial TBARS value and the rate of TBARS value increase by Fe(II)+NaCl and Fe(III)+NaCl in chicken breast meat were considerably lower than that in beef loin because chicken breast had higher TAC and lower SFRC than beef loin.

The initial (Day 0) TBARS value of beef loin was higher than that of chicken breast and TBARS values continued to increase during storage (Table 1). The addition of NaCl to beef loin increased the initial TBARS and greatly increased the TBARS values of beef loin during storage. Tables 2 and 3 showed that the addition of NaCl significantly increased the concentration of nonheme iron and decreased myoglobin content in beef loin during storage. This suggested that myoglobin is the source of nonheme iron. The increase of nonheme iron content (Table 2) and decrease of myoglobin concentration (Table 3) in NaCl-added beef loin during storage were similar to those in metMb+NaCl, Fe(II)+NaCl, and Fe(III)+NaCl-added treatments. This indicated that NaCl, rather than metmyoglobin, Fe(II), or Fe(III), was responsible for the changes. It is assumed that the acceleration of lipid peroxidation by NaCl in meat was achieved by the denaturation of myoglobin, which resulted in the release of free ionic irons. Therefore, the increase of TBARS values in NaCl-added beef loin was attributed to the interaction between the released free ionic iron from myoglobin and SFRC of beef loin.

The initial TBARS values of Fe(II)+NaCl- and Fe(III)+NaCl-added beef loin was higher than that of the control and NaCl-added beef loin because they had greater amounts of free ionic iron (Table 2). The addition of Fe(II)+NaCl and Fe(III)+NaCl also increased the TBARS values of beef loin during storage. The TBARS values of Fe(II)+NaCl-added beef loin were higher than those of Fe(III)+NaCl-added ones at Day 0 and Day 5, but not different at Day 10. This could be caused by the chemical properties of Fe(III), which should be converted to Fe(II) to catalyze lipid oxidation. The TBARS values (Table 1) and nonheme iron contents (Table 2) in Fe(II)+NaCl- and Fe(III)+NaCl-added beef loin at all storage days were higher than those of NaCl-added. However, the rate of TBARS increase during storage in Fe(II)+NaCl- and Fe(III)+NaCl-added beef loin was similar to that in NaCl-added one. This indicated that the SFRC in beef loin is a major limiting factor for the continuous increase of TBARS values during storage in the presence of sufficient amount of free ionic
This assumption is supported by Ahn & Kim (1998) who suggested that the status of free iron is the most important factor in lipid oxidation of raw meat.

The TBARS values of metMb+NaCl-added beef loin were slightly lower than that of the control at Day 0 and 5, but higher at Day 10. Many studies (Gorelik & Kanner, 2001; Baron et al., 2002; Lapidot et al., 2005) suggested that the prooxidant activity of myoglobin depends on its concentration, myoglobin to lipid hydroperoxide or fatty acid ratio: High concentration of myoglobin, and high myoglobin to lipid hydroperoxide or fatty acid ratio attenuates or even inactivates its prooxidant activity. Min & Ahn (2006b) observed that an increase in metmyoglobin concentration decreased the lipid peroxidation in phospholipid liposome system. Myoglobin concentration in metMb+NaCl-added beef loin was almost twice as high as control at Day 0. Therefore, the concentration effect of myoglobin may be responsible for the lower TBARS in metMb-added beef loin than control at Day 0. Furthermore, many studies (Harel & Kanner, 1989; Kanner et al., 1991; Gorelik & Kanner, 2001; Lapidot et al., 2005) also indicated that myoglobin inhibited free ionic iron-catalyzed lipid peroxidation in the presence of reducing agents via its peroxidase activity. However, myoglobin concentration in the metMb+NaCl-added beef loin significantly decreased during storage (Table 3) while that of nonheme iron greatly increased (Table 2). The decrease of myoglobin concentration in metMb+NaCl-added beef loin resulted in not only the attenuation of the concentration effect and peroxidase effect of myoglobin, but also significantly increased the amount of free ionic iron. Therefore, these changes were responsible for the exponential increase of lipid peroxidation in beef loin after Day 5 days of storage.

The TBARS values (Table 1) and nonheme iron content (Table 2) in control and all catalyst-added chicken breast meat increased by cooking, but the nonheme iron content did not change during storage. The TBARS values of cooked control and NaCl-added chicken breast meat increased during storage and their increases were similar to each other. These results indicated that NaCl did not affect lipid peroxidation of chicken breast meat. The high percentage of polyunsaturated fatty acid in the triacylglycerol fraction of chicken breast meat should accelerate lipid oxidation in cooked chicken breast meat (Min, Nam, Cordray, & Ahn, 2006). However, the initial (Day 0) TBARS values and lipid oxidation rates of cooked
control and NaCl-added chicken breast meat during storage were significantly slower than those of the cooked control and NaCl-added beef loin. This result suggested that raw and cooked chicken breast meat is more resistant to the exogenous oxidative stress such as cooking, addition of NaCl, and mechanical stress than beef loin because of high TAC in chicken breast.

The initial TBARS value of cooked metMb+NaCl-added chicken breast meat was not different from that of the control, but lower than Fe(II)- and Fe(III)-added ones. The increase of TBARS value in cooked metMb+NaCl-added chicken breast meat during storage was significantly higher than that of the control. The increase of TBARS values in cooked metMb+NaCl-added chicken breast meat was closely correlated to the concentration of nonheme iron (r = 0.99) (Table 2). Although the nonheme iron contents in cooked metMb+NaCl-added chicken breast meat were significantly lower than that in cooked Fe(II)+NaCl- and Fe(III)+NaCl-added one at Day 0, 5, and 10, the increase of TBARS values in cooked metMb+NaCl-added chicken breast meat were not significantly different from those in the Fe(II)+NaCl- and Fe(III)+NaCl-added one during storage. These results confirmed our previous suggestion that the SFRC may be the limiting factor for lipid peroxidation in the presence of sufficient amount of free ionic iron. “Heat-stable” ferric ion reducing capacity (HFRC) was detected in chicken breast meat and beef loin, but its amount in chicken breast meat was significantly lower than that in beef loin (Min et al., 2006). We assumed that HFRC should be a part of SFRC and acts like SFRC in cooked meat. The initial TBARS (Day 0) and the increases of TBARS values in metMb+NaCl-, Fe(II)+NaCl-, and Fe(III)+NaCl-added chicken breast meat during storage were lower than those in metMb+NaCl-, Fe(II)+NaCl-, and Fe(III)+NaCl-added beef loin due to lower HFRC in chicken breast. Therefore, the increased free ionic iron content by cooking, the presence of HFRC, and high PUFA content are the major determinants for the increase of TBARS values in cooked chicken breast meat.

The TBARS values in cooked control, NaCl-added, metMb+NaCl-, Fe(II)+NaCl-, and Fe(III)+NaCl-added beef loin increased significantly during storage (Table 1), and their increases were closely related to the changes in the concentration of nonheme iron (r = 0.97, 0.98, 0.97, 0.91, and 0.95, respectively) (Table 2 and 3). The increase of TBARS values in
cooked control beef loin during storage was lower than the catalyst-added beef loin because of the smaller increase in nonheme iron content (Table 2). The increases of TBARS values in all cooked catalyst-added beef loin during storage were not significantly different from each other (Table 1) even though the concentrations of nonheme iron in cooked NaCl-added and metMb+NaCl-added beef loin at all storage days were significantly lower than those of cooked Fe(II)+NaCl- and Fe(III)+NaCl-added beef loin (Table 2). These results also confirmed that the high HFRC in beef loin is the most crucial factor for the development of lipid peroxidation in the presence of sufficient amount of free ionic iron. Therefore, high HFRC, high free ionic iron content and the continuous increase of free ionic iron in beef loin during storage should be primarily responsible for the high susceptibility of cooked beef loin to lipid peroxidation.

Lipoxygenase (LOX)-like activities in raw control and NaCl-, Fe(II)+NaCl-, and Fe(III)+NaCl-added chicken breast meat were negligible (Table 4). The addition of metmyoglobin increased the LOX-like activity in chicken breast meat. However, the LOX-like activity in metMb+NaCl-added chicken breast meat significantly decreased during storage even though the myoglobin concentration in chicken breast hardly changed during storage (Table 3). The ability of chicken breast meat to reduce metmyoglobin should be highly related to the oxidative stability of metMb+NaCl-added chicken breast meat (Table 1). Among myoglobin species, metmyoglobin is responsible for LOX-like activity in meat. In addition, the LOX-like activity in metMb+NaCl-added chicken breast meat was significantly lower than that of control beef loin at Day 0 even though the concentrations of metmyoglobin in those two were similar (4.12 and 4.00 mg / g, respectively). These results indicated that chicken breast meat has much higher TAC than beef loin, resulting in higher storage stability in chicken breast meat.

The LOX-like activity of control beef loin was higher than that of NaCl-, Fe(II)+NaCl-, Fe(III)+NaCl-added, but lower than that of metMb+NaCl-added one (Table 4). Although LOX-like activities in control and catalyst-added beef loin significantly decreased during storage, the decrease of LOX-like activity in control was much lower than that of all catalyst+NaCl-added ones. In addition, LOX-like activities in control, NaCl-, metMb+NaCl-, Fe(II)+NaCl-, and Fe(III)+NaCl-added meats were highly correlated to their myoglobin
concentrations ($r = 0.53, 0.83, 0.83, 0.99, \text{ and } 0.99$, respectively). Therefore, significant decrease of LOX-like activity in all catalyst-added beef loin should be caused by the degradation of metmyoglobin by NaCl.

**Conclusion**

Chicken breast meat was more resistant to various exogenous oxidative factors than beef loin because of its higher TAC and lower SFRC. SFRC acted as a prooxidant in the presence of sufficient amount of free ionic irons and chicken breast had lower SFRC than beef loin. The addition of NaCl and cooking caused severe degradation of myoglobin, leading to a significant increase of free ionic iron content in beef loin. Continuous increases of TBARS values in cooked beef loin and catalyst-added chicken breast during storage were observed and the increases were caused by HFRS. HFRS was responsible for the reduction of ferric ion to ferrous iron in cooked meat, and the conversion was smaller in cooked chicken breast than in cooked beef loin. The rate of lipid peroxidation in raw NaCl-added beef loin during storage was not different from that of raw Fe(II)+NaCl and Fe(III)+NaCl-added ones even though nonheme iron content in former was much lower than that in latter. Therefore, it is suggested that free ionic iron is the major oxidation catalyst, and SFRC and HFRC are the limiting factors for lipid peroxidation in raw NaCl-added and cooked meat, respectively.

**References**


Table 1. TBARS values of raw and cooked chicken breast and beef loin treated with different prooxidants during storage at 4 °C.

<table>
<thead>
<tr>
<th>Storage</th>
<th>Control</th>
<th>NaCl</th>
<th>NaCl</th>
<th>NaCl</th>
<th>NaCl</th>
<th>Mb +</th>
<th>Fe(III)+</th>
<th>Fe(II)+</th>
<th>Mb +</th>
<th>Fe(III)+</th>
<th>Fe(II)+</th>
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<td></td>
<td></td>
</tr>
<tr>
<td>Raw meat</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>0 d</td>
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<td>0.23&lt;sup&gt;h&lt;/sup&gt;</td>
<td>0.21&lt;sup&gt;h&lt;/sup&gt;</td>
<td>0.74&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.93&lt;sup&gt;fz&lt;/sup&gt;</td>
<td>1.46&lt;sup&gt;dz&lt;/sup&gt;</td>
<td>2.06&lt;sup&gt;cz&lt;/sup&gt;</td>
<td>1.23&lt;sup&gt;ez&lt;/sup&gt;</td>
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<td>0.19&lt;sup&gt;g&lt;/sup&gt;</td>
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<td>3.07&lt;sup&gt;d&lt;/sup&gt;y</td>
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<td>2.34&lt;sup&gt;e&lt;/sup&gt;y</td>
<td>7.63&lt;sup&gt;b&lt;/sup&gt;y</td>
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<td>0.11</td>
</tr>
<tr>
<td>10 d</td>
<td>0.19&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.22&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.21&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.54&lt;sup&gt;ex&lt;/sup&gt;</td>
<td>2.00&lt;sup&gt;ex&lt;/sup&gt;</td>
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<td>16.72&lt;sup&gt;a&lt;/sup&gt;x</td>
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<td>0.14</td>
<td>0.27</td>
<td>0.04</td>
<td>0.19</td>
<td>0.19</td>
<td>0.21</td>
</tr>
</tbody>
</table>

| Cooked meat|         |      |      |      |      |      |         |         |      |         |         |
| 0 d        | 0.92<sup>gz</sup> | 1.21<sup>efz</sup> | 1.13<sup>fgz</sup> | 1.82<sup>b</sup>c<sup>z</sup> | 1.73<sup>e</sup>z | 1.38<sup>d</sup>ez | 1.94<sup>b</sup>c<sup>z</sup> | 1.51<sup>d</sup>z | 1.98<sup>b</sup>z | 2.29<sup>a</sup>z | 0.05 |
| 3 d        | 2.14<sup>e</sup>y | 2.52<sup>e</sup>y | 3.47<sup>d</sup>y | 3.73<sup>ed</sup>y | 3.77<sup>ed</sup>y | 3.30<sup>d</sup>dy | 4.94<sup>ab</sup>y | 4.32<sup>b</sup>cy | 4.83<sup>ab</sup>y | 5.11<sup>b</sup>y | 0.13 |
| 7 d        | 2.81<sup>cx</sup> | 3.12<sup>cx</sup> | 7.59<sup>bx</sup> | 7.06<sup>bx</sup> | 7.04<sup>bx</sup> | 7.76<sup>bx</sup> | 11.14<sup>bx</sup> | 10.04<sup>ax</sup> | 10.39<sup>ax</sup> | 10.58<sup>ax</sup> | 0.24 |
| SEM        | 0.05    | 0.05  | 0.17  | 0.10  | 0.08  | 0.14  | 0.14    | 0.24    | 0.20  | 0.20    | 0.26    |

Means with different letters (a-h) within the same row are significantly different (P < 0.05). Means with different letters (x-z) within the same column are significantly different (P < 0.05). SEM = Standard error of the means. n = 4.

Control contained only DDW. NaCl contained DDW and NaCl. Raw meat mixed with treatments was cooked in a 95 °C water bath to an internal temperature of 75 °C, followed by cooling for 2 h at 4 °C.

Abbreviations: Mb, metmyoglobin (5 mg / g meat); Fe(III), ferric chloride (5 μg ferric ion / g meat); Fe(II), ferrous ammonium sulfate (5 μg ferric ion / g meat).
Table 2. Nonheme iron content of raw and cooked chicken breast and beef loin treated with different prooxidants during storage at 4 °C.

<table>
<thead>
<tr>
<th>Storage</th>
<th>Chicken breast</th>
<th>Beef</th>
<th>Mb +</th>
<th>Fe(III)+</th>
<th>Fe(II)+</th>
<th>Mb +</th>
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<th>Fe(II)+</th>
</tr>
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<tbody>
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</tr>
<tr>
<td>Raw meat</td>
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<td></td>
</tr>
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<td>1.19&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.19&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.12&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>3.63&lt;sup&gt;dy&lt;/sup&gt;</td>
</tr>
<tr>
<td>10 d</td>
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<td>0.10</td>
<td>0.21</td>
<td>0.28</td>
<td>0.11</td>
</tr>
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</table>

| Cooked meat |         |      |      |      |      |         |      |      |      |      |      |      |
| 0 d     | 1.72<sup>f</sup> | 1.58<sup>f</sup> | 2.41<sup>ez</sup> | 6.06<sup>b</sup> | 6.09<sup>b</sup> | 4.40<sup>dz</sup> | 4.65<sup>dz</sup> | 5.36<sup>cz</sup> | 9.43<sup>ay</sup> | 9.78<sup>ay</sup> | 0.13 |
| 3 d     | 1.69<sup>e</sup> | 1.75<sup>e</sup> | 3.32<sup>dy</sup> | 6.21<sup>c</sup> | 6.24<sup>c</sup> | 5.92<sup>cy</sup> | 6.40<sup>cy</sup> | 7.45<sup>by</sup> | 9.89<sup>by</sup> | 10.23<sup>by</sup> | 0.21 |
| 7 d     | 1.75<sup>e</sup> | 1.85<sup>e</sup> | 4.63<sup>ex</sup> | 6.30<sup>d</sup> | 6.28<sup>d</sup> | 8.29<sup>ax</sup> | 8.77<sup>bxx</sup> | 9.63<sup>bx</sup> | 12.18<sup>ax</sup> | 12.65<sup>ax</sup> | 0.27 |
| SEM     | 0.03 | 0.12 | 0.09 | 0.10 | 0.15 | 0.21 | 0.21 | 0.28 | 0.26 | 0.26 | 0.39 |

Means with different letters (a-f) within the same row are significantly different (P < 0.05). Means with different letters (x-z) within the same column are significantly different (P < 0.05). SEM = Standard error of the means. n = 4.

Control contained only DDW. NaCl contained DDW and NaCl. Raw meat mixed with treatments was cooked in a 95 °C water bath to an internal temperature of 75 °C, followed by cooling for 2 h at 4 °C.

Abbreviations: Mb, metmyoglobin (5 mg / g meat); Fe(III), ferric chloride (5 μg ferric ion / g meat); Fe(II), ferrous ammonium sulfate (5 μg ferric ion / g meat).
Table 3. Percent (%) of myoglobin and metmyoglobin in raw chicken breast and beef loin treated with different prooxidants during storage at 4 °C.

<table>
<thead>
<tr>
<th>Storage</th>
<th>Chicken breast</th>
<th>Beef</th>
<th>Mb+</th>
<th>Fe(III)+</th>
<th>Fe(II)+</th>
<th>Mb+</th>
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<th>Fe(II)+</th>
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<td>Control</td>
<td>NaCl</td>
<td>NaCl</td>
<td>NaCl</td>
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<tr>
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<td>0.62&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>0.62&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.45&lt;sup&gt;ex&lt;/sup&gt;</td>
<td>5.83&lt;sup&gt;b&lt;/sup&gt;</td>
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</tr>
<tr>
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<td>0.62&lt;sup&gt;f&lt;/sup&gt;</td>
<td>4.93&lt;sup&gt;by&lt;/sup&gt;</td>
<td>4.74&lt;sup&gt;cdy&lt;/sup&gt;</td>
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</tr>
<tr>
<td>10 d</td>
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<td>0.66&lt;sup&gt;f&lt;/sup&gt;</td>
<td>4.87&lt;sup&gt;by&lt;/sup&gt;</td>
<td>0.62&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.61&lt;sup&gt;f&lt;/sup&gt;</td>
<td>4.26&lt;sup&gt;cz&lt;/sup&gt;</td>
<td>2.89&lt;sup&gt;dz&lt;/sup&gt;</td>
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<td>0.05</td>
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<table>
<thead>
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<th>% metmyoglobin</th>
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<td>60.15&lt;sup&gt;cy&lt;/sup&gt;</td>
<td>60.65&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>92.47&lt;sup&gt;ax&lt;/sup&gt;</td>
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<tr>
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<td>63.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57.63&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
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<td>0.76</td>
<td>0.54</td>
<td>0.29</td>
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Means with different letters (a-f) within the same row are significantly different (P < 0.05). Means with different letters (x-z) within the same column are significantly different (P < 0.05). SEM = Standard error of the means. n = 4.

Control contained only DDW. NaCl contained DDW and NaCl. Raw meat mixed with treatments was cooked in a 95 °C water bath to an internal temperature of 75 °C, followed by cooling for 2 h at 4 °C.

Abbreviations: Mb, metmyoglobin (5 mg / g meat); Fe(III), ferric chloride (5 μg ferric ion / g meat); Fe(II), ferrous ammonium sulfate (5 μg ferric ion / g meat)
Table 4. Lipoxygenase-like activity of raw chicken breast and beef loin treated with different prooxidants during storage at 4 °C.

<table>
<thead>
<tr>
<th>Storage</th>
<th>Chicken breast</th>
<th>Beef</th>
<th>Units / g meat</th>
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<td>Mb + Fe(III)+</td>
<td>Mb + Fe(III)+</td>
</tr>
<tr>
<td>0 d</td>
<td>0.97&lt;sup&gt;f&lt;/sup&gt; 1.75&lt;sup&gt;f&lt;/sup&gt; 14.64&lt;sup&gt;ex&lt;/sup&gt; 1.58&lt;sup&gt;fx&lt;/sup&gt; 1.81&lt;sup&gt;fx&lt;/sup&gt;</td>
<td>28.33&lt;sup&gt;cx&lt;/sup&gt; 26.51&lt;sup&gt;dx&lt;/sup&gt; 33.25&lt;sup&gt;ax&lt;/sup&gt; 30.99&lt;sup&gt;bx&lt;/sup&gt; 30.84&lt;sup&gt;bx&lt;/sup&gt; 0.37</td>
<td></td>
</tr>
<tr>
<td>5 d</td>
<td>0.85&lt;sup&gt;f&lt;/sup&gt; 1.52&lt;sup&gt;f&lt;/sup&gt; 9.86&lt;sup&gt;ey&lt;/sup&gt; 1.22&lt;sup&gt;fy&lt;/sup&gt; 1.09&lt;sup&gt;fy&lt;/sup&gt;</td>
<td>31.05&lt;sup&gt;abx&lt;/sup&gt; 28.48&lt;sup&gt;bex&lt;/sup&gt; 34.12&lt;sup&gt;ax&lt;/sup&gt; 23.67&lt;sup&gt;dy&lt;/sup&gt; 25.34&lt;sup&gt;cxy&lt;/sup&gt; 0.11</td>
<td></td>
</tr>
<tr>
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<td>0.90&lt;sup&gt;d&lt;/sup&gt; 1.60&lt;sup&gt;d&lt;/sup&gt; 6.92&lt;sup&gt;cxy&lt;/sup&gt; 1.14&lt;sup&gt;dy&lt;/sup&gt; 1.13&lt;sup&gt;dy&lt;/sup&gt;</td>
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<td>0.79 1.26 0.48 0.63 0.57</td>
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</table>

Means with different letters (a-f) within the same row are significantly different (P < 0.05). Means with different letters (x-z) within the same column are significantly different (P < 0.05). SEM = Standard error of the means. n = 4.

Control contained only DDW. NaCl contained DDW and NaCl. Raw meat mixed with treatments was cooked in a 95 °C water bath to an internal temperature of 75 °C, followed by cooling for 2 h at 4 °C.

Abbreviations: Mb, metmyoglobin (5mg / g meat); Fe(III), ferric chloride (5 μg ferric ion / g meat); Fe(II), ferrous ammonium sulfate (5 μg ferric ion / g meat).
CHAPTER 7. GENERAL CONCLUSIONS

Lipid peroxidation is recognized as the primary cause of quality deterioration in meat and meat products, and generates compounds that can be a great threat for human health. In spite of tremendous amount of research for several decades, the major catalyst and initiator for lipid peroxidation in meat are still controversial. The susceptibility of meat from different animal species to lipid peroxidation varies. Finding the causes and mechanisms involved in the different oxidative susceptibility of meat from different animals will be important in developing strategies to minimize quality deterioration in meat and meat products during storage.

The oxidative stability of meat from different animal species was dependent upon the difference in the balance between endogenous anti- and pro-oxidants in meat. Ground beef loin in both raw and cooked form was the most susceptible to lipid peroxidation during storage, followed by chicken thigh meat, pork loin, and chicken breast meat. Chicken breast meat showed a great oxidative stability in spite of its high content of polyunsaturated fatty acid (PUFA). The total amount of PUFA in meat, most of which were present in triglyceride, affected the development of lipid peroxidation of cooked meat but not raw meat. Raw chicken breast meat had low concentration of myoglobin but high total antioxidant capacity (TAC) to provide antioxidant factor-overwhelmed environment, resulting in its oxidative stability during storage. The primary role of TAC was the inhibition of free radical chain reaction and the release of ionic iron from myoglobin and other sources. The synergistic interaction of low molecular weight (LMW) reducing agents in LMW fraction and enzymatic systems in water-insoluble (P) fraction were primarily responsible for the TAC in raw chicken breast meat. The ferric iron reducing capacity (FRC) was detected in both raw chicken breast and beef loin. The FRC can be divided into two types: stable and unstable. The reducing agents in LMW fraction of chicken breast were mainly responsible for the unstable FRC and acted like an antioxidant. However, stable FRC was primarily detected in P fraction and water-soluble high molecular weight (HMW) fraction and acted like a prooxidant in the presence of high amount of free ionic iron. The amount of stable FRC was higher in beef loin than in chicken breast.
Raw beef loin had low TAC, and high “stable FRC” and myoglobin content. Myoglobin in beef loin served as a source of oxidation catalysts such as ferrylmyoglobin, hematin, and/or free ionic iron, and resulted in the imbalance between pro- and antioxidant activities in beef loin. Water-soluble HMW fraction was primarily responsible for the high susceptibility of beef loin to lipid peroxidation. The HMW fraction in beef loin showed high amount of myoglobin and stable FRC. Increases of lipid peroxidation by diethylenetriamine pentaacetic acid (DTPA)-unchelatable iron compounds, i.e., ferrylmyoglobin and/or hematin, in HMW fraction from beef loin was not different from water-soluble (HMW + LMW fractions) and homogenate (HWM + LMW + P fractions) fractions. However, the catalytic activity of DTPA-chelatable iron, i.e., free ionic iron released mostly from myoglobin, in HMW fraction significantly decreased by the addition of LMW and P fractions. Almost all free ionic iron released from myoglobin were chelated by both LMW and P fractions in beef loin. Therefore, it is suggested that ferrylmyoglobin and/or hematin rather than free ionic iron are the major oxidation catalysts in raw beef loin during “normal” storage conditions.

However, severe oxidative stresses such as cooking, addition of NaCl, severe mechanical stress, and temperature abuse caused a rapid increase in the release of free ionic iron from myoglobin. The combination of these oxidative stresses can be easily observed in meat processing. Therefore, the free ionic iron reduced by the “stable FRC” served as a major catalyst in processed meat products. Adding high levels of free ionic iron to meat did not increase the rate of lipid peroxidation. These results indicated that the “stable FRC” was the determining factor for lipid peroxidation in meat in the presence of sufficient amount of free ionic iron. In addition, “heat-stable FRC” were observed both in cooked chicken breast and beef loin, but the amount was much higher in cooked beef loin than in cooked chicken breast. Cooked beef loin was more susceptible to lipid peroxidation than cooked chicken breast because beef loin had higher “heat-stable FRC” and free ionic iron than did chicken breast even though fatty acids of chicken breast contained higher proportion of PUFA than did beef loin.
ACKNOWLEDGEMENTS

My deepest acknowledgements go to Dr. Dong Uk Ahn, my major professor, for his continuous encouragement, guidance, warmth and support throughout the program at Iowa State University. Completing this dissertation would not have been possible without his patience and help.

I am truly grateful to Dr. Pamela J. White for her invaluable advice, incessant encouragement, and kindness and for serving as my co-major professor.

I would like to thank my Ph.D. committee members, Dr. Joseph G. Sebranek, Dr. Dennis G. Olson, Dr. Terri D. Bolyston, and Dr. Donald C. Beitz for their valuable comments and guidance.

I appreciate my lab colleagues who have been nice, helpful, and friendly whenever I need.

To my parents, Kyung-Keun Min and Myoung-Ja Hwang, I thank you for your support and belief throughout my life. I am truly grateful to you for being with me always. To my parents-in-law, Myohee Han and Sunghee Oh, I thank you for your support and continuous encouragement. To my sister, Jung-Won Min, I appreciate you for your continuous help and encouragement.

I would like to give my thanks from the bottom of my heart to my wife, Jung-Ah Han for her lifetime sacrifice, tolerance, support, and cordial advices. I truly love you. Last but not least, to my son Hyungjoon and my daughter Heejoo, you made my life perfect.