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ARTICLE

Effect of NaCl, Myoglobin, Fe(II), and Fe(III) on Lipid Oxidation of Raw and Cooked Chicken Breast and Beef Loin

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Chicken breast and beef loin were ground, and no, NaCl, NaCl + myoglobin, NaCl + Fe(II), or NaCl + Fe(III) additions were made; patties were then prepared. Half of the patties were packaged in oxygen-permeable bags and stored at 4 °C for 10 days, and the other half were cooked in a 95 °C water bath to an internal temperature of 75 °C, packaged in oxygen-permeable zipper bags, and stored at 4 °C for 7 days. The oxidative stability of raw and cooked chicken breast and beef loin were determined during storage. Chicken breast was more resistant to various exogenous oxidative factors than beef loin: addition of NaCl did not increase TBARS values and nonheme content of raw chicken breast, but significantly increased those of raw beef loin patties during storage. Addition of NaCl + Mb did not affect lipid oxidation in raw chicken breast patties, but decreased the TBARS of beef loin during storage. Addition of NaCl + Fe(III) or NaCl + Fe(II) increased the TBARS values of both raw chicken breast and beef loin during storage, but the increase was greater in beef loin than in chicken breast. The TBARS values of all cooked chicken breast and beef loin increased during 7 days of storage, but the increases in cooked chicken patties were significantly smaller than those of cooked beef loin patties with the same treatments. Addition of NaCl and cooking caused severe degradation of myoglobin, leading to a significant increase in free ionic iron content in beef loin. It is suggested that free ionic iron is the major catalyst for lipid oxidation, and the low "storage-stable and heat-stable" ferric ion reducing capacity in chicken breast were responsible for the high oxidative stability for raw and cooked chicken breast compared with beef loin under prooxidants, cooking, and storage conditions.

KEYWORDS: Lipid oxidation; myoglobin; ionic iron; chicken breast; beef loin

INTRODUCTION

Lipid oxidation is a major factor that determines the sensory, functional, and nutritional quality of processed meat products. Secondary byproducts generated by lipid oxidation such as aldehydes have cytotoxic and genotoxic properties due to their high reactivity (*1*, *2*). Therefore, repeated consumptions of highly oxidized meat can be a great threat to human health.

Myoglobin (Mb) has been recognized as a major catalyst for lipid oxidation in meat, but its mode of action for catalyzing lipid oxidation in meat is controversial. It is suggested that the interaction of Mb with hydrogen peroxide (H_2O_2) or lipid hydroperoxides (LOOH) resulted in the formation of ferrylmyoglobin, which initiated free radical chain reaction (*3*, *4*). Myoglobin is also suggested to be a source of free ionic iron and hematin, which can catalyze lipid oxidation in meat (*5*−*8*).

Cooked meat oxidizes more quickly than raw meat (*9*, *10*). Heating of meat can influence various factors associated with lipid oxidation: disruption of muscle cell structure, inactivation of antioxidant enzymes, and release of oxygen and iron from Mb (*11*). The disrupted membrane allows easy access of oxygen, which accelerates lipid oxidation (*12*). Studies have suggested that the inactivation of catalase and glutathione peroxidase (GSH-Px) by heating could be partially responsible for the rapid development of lipid oxidation in cooked meat (*13*, *14*). Igene et al. (*15*) reported that cooking significantly increased the level of free nonheme iron by releasing iron from heme pigments, resulting in the increased rate of lipid oxidation. Our previous study (*16*) indicated that ferric ion-reducing capacity present in cooked meat could be a rate-determining factor of lipid oxidation in cooked meat.

Sodium chloride (NaCl) has a prooxidant effect in meat and meat products depending on its concentration (*17*). The possible prooxidant mechanism of NaCl is attributed to its capability to (1) disrupt the structural integrity of cell membrane, which enables catalysts easy access to lipid substrates (*18*); (2) release free ionic iron from iron-containing molecules such as heme proteins (*17*, *19*); and (3) inhibit the activities of antioxidant enzymes such as catalase, glutathione peroxidase, and superoxide dismutase (*20*, *21*).

Our previous study (*22*) suggested that the high oxidative stability of chicken breast meat was due to high total antioxidant capacity (TAC) and very low Mb content in chicken breast compared with beef loin. Ferrylmyoglobin and hematin are the major catalysts in raw beef loin. Both chicken breast and beef loin

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had storage-stable ferric ion 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 a meat system. Myoglobin or ferric or ferrous ion was added to raw and cooked chicken breast and beef loin, and their effects on lipid oxidation 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. Myoglobin (from equine skeletal muscle), ferrous ammonium sulfate, ferric chloride, linoleic acid, 2-thiobarbituric acid (TBA), ferrozine [3-(2-pyridyl)-5,6-bis(4-phenylsulfonic 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. Deionized distilled water (DDW) produced by a Nanopure infinity ultrapure water system equipped with an ultraviolet (UV) lamp (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 Patties. Beef loins from four individual animals and chicken breast muscles from 16 birds were purchased from local packing plants. 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 (90 g) was mixed with NaCl (1.5 g) for 2 min in a bowl mixer (model KSM90; KitchenAid Inc., St. Joseph, MI) ,and then 10 mL of solution containing Mb (5 mg/g of meat as a final concentration), ferrous ammonium sulfate (5 μg of Fe/g of meat), or ferric chloride (5 μg of Fe/g of meat) was added and mixed again for 3 min. Ground meat without NaCl was used as a control. Streptomycin (200 ppm) was added as an antimicrobial agent. The mixture was manually formed into two patties (50 g each), which were then individually packaged in oxygen-permeable zipper bags (polyethylene, 10 cm × 15 cm, 2 mil; Associate Bag Co. Milwaukee, WI). Half of the packaged patties were used for raw meat study, and the other half were cooked in a 95 °C water bath to an internal temperature of 75 °C, followed by cooling for 2 h at 4 °C. After meat juices had been drained from the bag, the patties were repackaged in oxygen-permeable zipper bags. Raw and cooked patties were stored at 4 °C until analyses. Lipid oxidation, nonheme iron, metmyoglobin percentage, and lipoxygenase-like activity of meat samples were determined at 0, 5, and 10 days of storage for raw patties. Lipid oxidation and nonheme iron were determined at 0, 3, and 7 days of storage for cooked patties.

Chemical Analyses of Raw and Cooked Meat Patties. Lipid oxidation was determined using the method of Min and Ahn (*22*). Briefly, a meat sample (5 g) was homogenized with 15 mL of DDW and 100 μL of BHT (6% in 100% ethanol) using a high-speed Brinkman Polytron (model PT 10/35, Switzerland) for 15 s at speed setting 7. The meat homogenate (1 mL) was mixed with 2 mL of 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 vortex-mixed and centrifuged at 3000*g* for 15 min. The absorbance of supernatant was determined at 531 nm against blank (1 mL of DDW and 2 mL of TBA/TCA solution). The amount of 2-thiobarbituric acid reactive substances (TBARS) was expressed as milligrams of malondialdehyde (MDA) per kilogram meat.

Nonheme iron content was determined according to the Ferrozine method of Carter (*23*) with some modifications. In brief, meat sample (4 g) was weighed into a polyethylene bag (model 80, Seward, London, U.K.) and then homogenized with 12 mL of DDW by a Stomacher (Stomacher 80, Seward, U.K.) for 5 min at high speed. The homogenate (1.5 mL) and 0.5 mL of 1% ascorbic acid in 0.2 N HCl (w/v) were transferred to a test tube, thoroughly mixed with 11.3% TCA solution (w/v, 1 mL), and incubated at room temperature for 5 min. The whole mixture was centrifuged at 3000*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 micrograms of nonheme iron per gram of meat.

Meat (5 g) was homogenized with 20 mL of DDW using a Brinkman Polytron for 60 s at speed setting 7 and centrifuged at 20000*g* for 60 min at 4 °C. The supernatant was used for myoglobin content measurement. The Mb and metMb contents (percent) were calculated on the basis of following equations and expressed as milligrams of Mb per gram of meat; the molecular weight of Mb was taken as 17000 Da (*24*).

$$\text{myoglobin concentration (mg/g of meat)} = (-0.166R_1 + 0.086R_2 + 0.088R_3 + 0.099) \times A^{525} \times 0.0175 \times \text{dilution factor}$$

$$\text{metmyoglobin content (\%)} = (-2.514R_1 + 0.777R_2 + 0.800R_3 + 1.098) \times 100$$

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}.

Lipoxygenase-like activities of meat samples were determined using the method of Gata et al. (*25*) with some modifications. Meat sample (5 g) was homogenized with 15 mL of 50 mM acetate buffer (pH 5.6) using a high-speed homogenizer (Brinkman Polytron, model PT 10/35) for 10 s at top speed and then centrifuged at 12000*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 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 gram of meat, calculated from the molar extinction coefficient of hydroperoxyl linoleic acid (ε = 25000 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. A factorial design (2 meats × 5 treatments × 3 storage times) was used in this study. Data were analyzed using JMP software (version 5.1.1; SAS Institute Inc., Cary, NC) and reported as mean and standard error of the means (SEM). Differences among means were assessed by Tukey's method ($P < 0.05$).

RESULTS AND DISCUSSION

The initial (day 0) TBARS value of control raw chicken breast was significantly lower than that of the control raw beef loin (**Table 1**). The TBARS values of control raw chicken breast did not increase during storage, but those of control raw beef continued to increase during storage. This indicates that chicken breast is highly resistant to oxidative changes by mechanical stresses (grinding and mixing) and storage. Our previous study (*22*) indicated that chicken breast had high total antioxidant capacity (TAC), which was well-maintained during 10 days of storage at 4 °C, but it had lower free ionic iron and myoblogin contents than beef loin. TAC was determined by using the cupric reducing antioxidant capacity (CUPRAC) method and was expressed as micrograms of ascorbic acid equivalent per gram of meat. The concentrations of nonheme iron (**Table 2**) and Mb (**Table 3**) in all raw chicken breast patties were lower than those of raw beef loin patties, and their contents 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 influenced the development of lipid oxidation in meat (*26*).

Table 1. TBARS Values of Raw and Cooked Chicken Breast and Beef Loin Patties Treated with Different Prooxidants during Storage at 4 °C[a]

	TBARS value (mg of MDA/kg of meat)										
	chicken breast					beef loin					
storage (days)	control	NaCl	Mb + NaCl	Fe(III) + NaCl	Fe(II) + NaCl	control	NaCl	Mb + NaCl	Fe(III) + NaCl	Fe(II) + NaCl	SEM
Raw Meat											
0	0.22h	0.23h	0.21h	0.74gz	0.93fz	1.46dz	2.06cz	1.23ez	2.66bz	2.99az	0.03
5	0.18g	0.21g	0.19g	1.25fy	1.55fy	3.07dy	6.31cy	2.34ey	7.63by	8.58ay	0.11
10	0.19f	0.22f	0.21f	1.54ex	2.00ex	3.80dx	15.45bx	4.85cx	16.72ax	17.04ax	0.20
SEM	0.01	0.01	0.01	0.02	0.04	0.14	0.27	0.04	0.19	0.21	
Cooked Meat											
0	0.92gz	1.21efz	1.13fgz	1.82bcz	1.73cz	1.38dez	1.94bcz	1.51dz	1.98bz	2.29az	0.05
3	2.14ey	2.52ey	3.47dy	3.73cdy	3.77cdy	3.30dy	4.94aby	4.32bcy	4.83aby	5.11ay	0.13
7	2.81cx	3.12cx	7.59bx	7.06bx	7.04bx	7.76bx	11.14bx	10.04ax	10.39ax	10.58ax	0.24
SEM	0.05	0.05	0.17	0.10	0.08	0.14	0.14	0.24	0.20	0.26	

[a] 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 ($P < 0.05$). $n = 4$. Abbreviations: Mb, myoglobin (5 mg/g of meat); Fe(III), ferric chloride (5 μg of ferric ion/g of meat); Fe(II), ferrous ammonium sulfate (5 μg of ferric ion/g of meat); SEM, standard error of the means.

Table 2. Nonheme Iron Content of Raw and Cooked Chicken Breast and Beef Loin Patties Treated with Different Prooxidants during Storage at 4 °C[a]

	μg of nonheme iron/g of meat										
	chicken breast					beef loin					
storage (days)	control	NaCl	Mb + NaCl	Fe(III) + NaCl	Fe(II) + NaCl	control	NaCl	Mb + NaCl	Fe(III) + NaCl	Fe(II) + NaCl	SEM
Raw Meat											
0	1.20d	1.19d	1.19d	5.32b	5.12b	2.38cz	2.69cz	2.69cz	7.50az	7.60az	0.08
5	1.37e	1.33e	1.33e	5.75c	5.58c	3.41dy	5.11cy	3.63dy	9.87by	11.07ay	0.19
10	1.41e	1.49e	1.47e	5.69d	5.84d	5.11dx	9.61bx	8.14cx	13.78ax	14.72ax	0.26
SEM	0.12	0.09	0.09	0.14	0.10	0.21	0.28	0.11	0.26	0.33	
Cooked Meat											
0	1.72f	1.58f	2.41ez	6.06b	6.09b	4.40dz	4.65dz	5.36cz	9.43ay	9.78ay	0.13
3	1.69e	1.75e	3.32dy	6.21c	6.24c	5.92cy	6.40cy	7.45by	9.89ay	10.23ay	0.21
7	1.75e	1.85e	4.63ex	6.30d	6.28d	8.29cx	8.77bcx	9.63bx	12.18ax	12.65ax	0.27
SEM	0.03	0.12	0.09	0.10	0.15	0.21	0.21	0.28	0.26	0.39	

[a] 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 ($P < 0.05$). $n = 4$. Abbreviations: Mb, myoglobin (5 mg/g of meat); Fe(III), ferric chloride (5 μg of ferric ion/g of meat); Fe(II), ferrous ammonium sulfate (5 μg of ferric ion/g of meat); SEM, standard error of the means.

Table 3. Myoglobin Content and Percent of metMb in Raw Chicken Breast and Beef Loin Patties Treated with Different Prooxidants during Storage at 4 °C[a]

	chicken breast					beef loin					
storage (days)	control	NaCl	Mb + NaCl	Fe(III) + NaCl	Fe(II) + NaCl	control	NaCl	Mb + NaCl	Fe(III) + NaCl	Fe(II) + NaCl	SEM
Milligrams of Mb per Gram of Meat											
0	0.65ex	0.62e	5.07dx	0.60e	0.62e	5.45cx	5.83bx	9.59ax	5.89bx	5.83bx	0.02
5	0.55fy	0.62f	4.81bcy	0.63f	0.62f	4.93by	4.74cdy	8.04ay	4.62dey	4.49ey	0.03
10	0.52fy	0.66f	4.87by	0.62f	0.61f	4.26cz	2.89dz	5.90az	2.80dez	2.54ez	0.06
SEM	0.01	0.01	0.03	0.01	0.02	0.02	0.05	0.04	0.05	0.08	
Percent metMb											
0	59.45ey	58.82ex	81.41bx	60.15ey	60.65ey	73.46cy	76.51cz	65.28dy	92.47az	93.95ay	0.77
5	63.76bx	57.63cy	51.81dy	63.18bx	63.72bx	101.96ax	100.84ax	98.22ax	100.54ax	100.34ax	1.03
10	62.81dx	54.83ez	26.87fz	61.36dy	61.45dy	101.74ax	94.61cy	98.81bx	93.72cy	92.92cy	0.46
SEM	0.69	0.28	1.78	0.40	0.37	1.05	0.76	0.54	0.29	0.36	

[a] 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 ($P < 0.05$). $n = 4$. Abbreviations: Mb, myoglobin (5 mg/g of meat); Fe(III), ferric chloride (5 μg of ferric ion/g of meat); Fe(II), ferrous ammonium sulfate (5 μg of ferric ion/g of meat); SEM, standard error of the means.

Addition of NaCl did not induce lipid oxidation and increase TBARS values of raw chicken breast patties, but significantly increased the TBARS values of raw beef loin patties at day 0 and during storage. This indicated that beef loin was more susceptible to NaCl-induced lipid oxidation than chicken breast. The addition of NaCl also significantly increased the concentration of nonheme iron and decreased Mb content in raw beef loin during storage (**Tables 2** and **3**). All of the raw beef loin patties with

Table 4. Lipoxygenase-like Activity of Raw Chicken Breast and Beef Loin Patties Treated with Different Prooxidants during Storage at 4 °C[a]

	lipoxygenase-like activity (units/g of meat)										
	chicken breast					beef loin					
storage (days)	control	NaCl	Mb + NaCl	Fe(III) + NaCl	Fe(II) + NaCl	control	NaCl	Mb + NaCl	Fe(III) + NaCl	Fe(II) + NaCl	SEM
0	0.97f	1.75f	14.64ex	1.58fx	1.81fx	28.33cx	26.51dx	33.25ax	30.99bx	30.84bx	0.37
5	0.85f	1.52f	9.86ey	1.22fy	1.09fy	31.05abx	28.48bcx	34.12ax	23.67dy	25.34cdy	0.11
10	0.90d	1.60d	6.92cz	1.14dy	1.13dy	24.80ay	14.15by	27.12ay	13.82bz	12.32bz	0.54
SEM	0.04	0.09	0.36	0.05	0.06	0.79	1.26	0.48	0.63	0.57	

[a] 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 ($P < 0.05$). $n = 4$. Abbreviations: Mb, myoglobin (5 mg/g of meat); Fe(III), ferric chloride (5 μg of ferric ion/g of meat); Fe(II), ferrous ammonium sulfate (5 μg of ferric ion/g of meat); SEM, standard error of the means.

added NaCl showed similar increases in nonheme iron contents (**Table 2**) and decreases in Mb concentrations during storage (**Table 3**). The lipoxigenase (LOX)-like activities in all raw beef loin with added NaCl (**Table 4**) were highly correlated with their Mb concentrations (r = 0.83−0.99). This indicated that NaCl induced the degradation of Mb to release free ionic irons and resulted in the development of lipid oxidation in raw beef loin patties. Lipid oxidation, and nonheme iron and Mb contents, however, in raw Mb-added chicken breast patties did not change (**Tables 1−3**). This suggested that NaCl stimulated the release of free ionic iron from only Mb, which accelerated the development of lipid oxidation in beef loin. Our previous studies (*22*) suggested that the storage-stable ferric ion-reducing capacity (SFRC) was detected in both raw chicken breast and beef loin. However, the SFRC of raw beef loin was greater than that of chicken breast and acted as a prooxidant in the presence of a sufficient amount of free ionic iron. They also indicated that SFRC was the rate-limiting factor for lipid oxidation of meat in the presence of high free ionic iron content. Ferric ion reducing capacity (FRC) is basically the total reducing power of meat and is expressed as micrograms of ascorbic acid equivalent per gram of meat. SFRC is the ferric iron reducing capacity remaining after storage, whereas HFRC is the ferric iron reducing capacity surviving after cooking. Therefore, the higher TBARS value in NaCl-added raw beef loin than in raw chicken breast was attributed to the higher released ionic iron from Mb and SFRC.

Addition of Mb + NaCl also did not affect lipid oxidation in raw chicken breast patties. Myoglobin did not act as a prooxidant in raw chicken breast patties. The amounts of nonheme iron and Mb in (Mb + NaCl)-added chicken breast patties did not change during storage, but metMb percentage decreased significantly (**Tables 2** and **3**). The decrease of metMb percentage resulted in a gradual increase in reduced Mb percentage from 17.25% at day 0 and to 71.05% at day 10. Addition of Mb increased LOX-like activity in raw chicken breast, although the LOX-like activities of control, NaCl-added, [Fe(II) + NaCl]-added, and [Fe(III) + NaCl]-added raw chicken breast patties were negligible (**Table 4**). The LOX-like activity in the (Mb + NaCl)-added raw chicken breast patties significantly decreased during storage as their metMb percentage decreased (**Table 3**). The LOX-like activities in (Mb + NaCl)-added raw chicken breast during storage were highly correlated with its metMb percentage (r = 0.98). Among Mb species, metMb is responsible for the LOX-like activity in meat (*5*). For the development of lipid oxidation in meat, production or release of reactive compounds such as ferrylmyoglobin, hematin, and free ionic iron from Mb is required (*27*). LOX-like activity in raw beef loin increased as metMb percentage increased (r = 0.90) during 10 days of storage (*22*). Baron et al. (*27*) indicated that oxy-, deoxy-, and metMb can be converted to ferrylmyoglobin by hydrogen peroxide produced during oxymyoglobin autoxidation. The correlation between LOX-like activity and metMb percentage in this study suggested that ferrylmyoglobin formed from metMb in the presence of H_2O_2 could be responsible for LOX-like activity in meat. However, the primary role of oxyMb for lipid oxidation in meat is still unclear.

Studies (*17*, *19*) have reported that NaCl induced the release of free ionic irons from Mb. However, our result showed that NaCl did not induce the release of free ionic irons from Mb or accelerate lipid oxidation in raw (Mb + NaCl)-added chicken breast patties (**Tables 1** and **2**). The LOX-like activity in (Mb + NaCl)-added raw chicken breast was significantly lower than that of the control raw beef loin at day 0, even though the concentrations of Mb in those two meats were similar (5.07 and 5.83 mg/g, respectively). Therefore, the reduction of metMb to ferrous Mb and the maintenance of the pigment in reduced status by high TAC contributed to higher oxidative stability of (Mb + NaCl)-added raw chicken breast relative to beef loin during storage.

The TBARS values of (Mb + NaCl)-added raw beef loin patties were significantly lower than those of NaCl-added ones at all storage times. Many studies (*27*, *28*) have suggested that the prooxidant activity of Mb depends on its concentration and Mb to lipid hydroperoxide. A high concentration of Mb and a high Mb to lipid hydroperoxide ratio attenuate the prooxidant activity of Mb. The Mb concentration in the (Mb + NaCl)-added raw beef loin patties was almost twice that of the control at day 0. Therefore, the concentration effect of Mb may be responsible for the lower TBARS in (Mb + NaCl)-added raw beef loin patties than control at day 0. Many other studies (*28*−*30*) also indicated that Mb inhibited free ionic iron-catalyzed lipid oxidation in the presence of reducing agents via its peroxidase activity. However, metMb concentration of the (Mb + NaCl)-added raw beef loin patties significantly decreased during storage (**Table 3**), whereas its nonheme iron greatly increased (**Table 2**). The decrease of Mb concentration in (Mb + NaCl)-added raw beef loin not only lowered the concentration and peroxidase effects of Mb but also increased the amount of free ionic iron significantly. Therefore, these changes were responsible for the exponential increase of lipid oxidation in (Mb + NaCl)-added raw beef loin after 5 days of storage.

Addition of Fe(II) + NaCl significantly increased the TBARS value of raw chicken breast at day 0 due to high and rapid catalytic activity of Fe(II). Addition of Fe(III) + NaCl to raw chicken breast patties also increased the TBARS value at day 0, but the increase was smaller than that by Fe(II) because Fe(III) does not have catalytic capacity for lipid oxidation unless it is reduced to Fe(II) (*8*). Addition of both Fe(II) and Fe(III) significantly increased the TBARS values of raw chicken breast patties during storage, due probably to SFRC detected in raw chicken breast (*16*, *22*).

The initial TBARS values of [Fe(II) + NaCl]- and [Fe(III) + NaCl]-added raw beef loin were higher than those of the raw control and NaCl-added beef loin because they had greater amounts of free ionic iron (**Table 2**). The TBARS values of [Fe(II) + NaCl]-added raw beef loin were higher than those of

[Fe(III) + NaCl]-added ones at days 0 and 5, but showed no difference 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 nonheme iron contents (**Table 2**) in [Fe(II) + NaCl]- and [Fe(III) + NaCl]-added raw beef loin patties at all storage days were much higher than those of NaCl-added ones. However, the increase of TBARS values in [Fe(II) + NaCl]- and [Fe(III) + NaCl]-added raw beef loin patties (16.72 and 17.04 mg of MDA/kg of meat, respectively) was similar to that of NaCl-added ones (15.45 mg of MDA/kg of meat). This indicated that the SFRC was the major rate-limiting factor for the continuous increase of TBARS values in beef loin during storage in the presence of a sufficient amount of free ionic irons. This agrees with the results of Ahn and Kim (*8*), who suggested that the status of free iron is the most important factor in lipid oxidation of raw meat.

TBARS values of all cooked chicken breast and beef loin increased during 7 days of storage, but the increases in control and catalyst-added cooked chicken patties were significantly smaller than those of cooked beef loin patties with the same treatments (**Table 1**). Nonheme iron contents of control and catalyst-added beef patties increased after cooking and during storage. However, nonheme iron contents of control and catalyst-added cooked chicken breast did not change during storage. Also, the addition of NaCl significantly increased lipid oxidation of cooked chicken breast patties, but not as much as that in Mb + NaCl, Fe(III) + NaCl, and Fe(II) + NaCl treatments. A high percentage of polyunsaturated fatty acids in the triacylglycerol fraction of chicken breast was expected to accelerate lipid oxidation in cooked chicken breast meat, but the TBARS values and lipid oxidation rates of control and NaCl-added cooked chicken breast patties during storage were significantly lower than those of the cooked beef loin. Beef loin contains higher fat content than chicken breast, and fat content as well as fatty acid composition are very important for the lipid oxidation of meat during storage. However, the extent of TBARS changes in beef was much greater than could be explained by fat content or fatty acid composition alone. This suggested that the raw and cooked chicken breast patties are more resistant to exogenous oxidative stresses such as the addition of NaCl, ionic iron, myoglobin, and mechanical stress than raw and cooked beef loin patties because chicken breast has higher TAC and lower concentration of Mb as a source of free ionic irons than beef loin.

The TBARS value of (Mb + NaCl)-added cooked chicken breast at day 0 was not different from that of the NaCl-added but was lower than that of [Fe(II) + NaCl]- and [Fe(III) + NaCl]-added cooked patties. However, the increase in TBARS value of (Mb + NaCl)-added cooked chicken breast during storage was significantly higher than that of NaCl-added ones (**Table 1**). The changes of TBARS values in cooked (Mb + NaCl)-added chicken breast patties were closely correlated to their nonheme iron concentration (r = 0.99) (**Table 2**). Heating and the presence of NaCl were responsible for the denaturation of Mb and the release of free ionic irons from Mb (*11*). The nonheme iron contents in (Mb + NaCl)-added cooked chicken breast patties were significantly lower than those in [Fe(II) + NaCl]- and [Fe(III) + NaCl]-added cooked chicken breast (**Table 2**). However, the increase of TBARS value in (Mb + NaCl)-added cooked chicken breast was not significantly different from those of [Fe(II) + NaCl]- and [Fe(III) + NaCl]-added cooked chicken breast during storage (**Table 1**). These results confirm the previous suggestion that the "heat-stable" ferric ion reducing capacity (HFRC) is the rate-limiting factor for lipid oxidation in the presence of sufficient amount of free ionic iron (*16*). HFRC was detected in both cooked chicken breast and beef, but was higher in beef loin than chicken breast (*16*). We assume that HFRC is a part of SFRC and reduces Fe(III) to Fe(II), which catalyzes lipid oxidation in cooked meat. The TBARS at day 0 and the increases of TBARS values in (Mb + NaCl)-, [Fe(II) + NaCl]-, and [Fe(III) + NaCl]-added cooked chicken breast during storage were slower than those of the cooked beef loin due probably to the lower HFRC in chicken breast than in beef loin.

The TBARS values in control, NaCl-added, and (Mb + NaCl)-, [Fe(II) + NaCl]-, and [Fe(III) + NaCl]-added cooked beef loin patties 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) (**Tables 2** and **3**). The increase in TBARS values of cooked control beef loin was smaller than those of the catalyst-added beef loin patties during storage (**Table 1**) because of the smaller increase of nonheme iron content in control beef loin (**Table 2**). The increases of TBARS values among cooked beef loin patties with different treatments at each storage time were not significantly different from each other (**Table 1**), even though the concentrations of nonheme iron in cooked NaCl-added and (Mb + NaCl)-added beef loin at all storage days were significantly lower than those of [Fe(II) + NaCl]- and [Fe(III) + NaCl]-added cooked beef loins (**Table 2**). This result also confirms that HFRC in beef loin is a critical rate-limiting factor for lipid oxidation in the presence of a sufficient amount of free ionic iron. The increases of TBARS values in cooked beef loin patties were significantly higher than those of the cooked chicken breast patties with the same treatments. Our previous study (*16*) indicated that cooked beef loin has a significantly higher amount of HFRC than cooked chicken breast. Therefore, high, stable HFRC and continuous increase of free ionic iron released from Mb in beef loin during storage may be primarily responsible for the high susceptibility of cooked beef loin to lipid oxidation.

In conclusion, chicken breast was more resistant to various exogenous oxidative factors than beef loin because of its higher TAC and lower SFRC (*16*, *22*). SFRC acted as a prooxidant in the presence of a sufficient amount of free ionic irons in raw meat patties. The addition of NaCl and cooking caused severe degradation of Mb, leading to a significant increase in free ionic iron content in beef loin. Increases of TBARS values in cooked beef loin and catalyst-added chicken breast during storage were caused by HFRC (*16*). The HFRC was responsible for the reduction of ferric to ferrous ion in cooked meat, and the conversion was smaller in cooked chicken breast than in cooked beef loin because of its low HFRC. Compared with [Fe(II) + NaCl]- and [Fe(III) + NaCl]-added treatments, the rate of lipid oxidation in NaCl-added raw beef loin during storage was slow, even though the nonheme iron content in the former was much greater than that in latter. It is suggested that free ionic iron is the major catalyst for lipid oxidation and that the low "storage-stable and heat-stable" ferric ion reducing capacity in chicken breast were responsible for the high oxidative stability for raw and cooked chicken breast compared with beef loin under prooxidant, cooking, and storage conditions.

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