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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Summary and Implications

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-day 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 high "storage-stable" ferric ionic reducing capacity 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.

Introduction

Lipid oxidation is a major factor that determines the sensory, functional, and nutritional quality of processed meat products. The secondary by-products of lipid oxidation such as aldehydes generated have cytotoxic and genotoxic properties due to their high reactivity. 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. Myoglobin is also suggested to be a source of free ionic iron and hematin, which can catalyze lipid oxidation in meat. Cooked meat oxidizes faster than raw meat. 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. The disrupted membrane allows easy access of oxygen, which accelerates lipid oxidation. Sodium chloride (NaCl) has a prooxidant effect in meat and meat products depending on its concentration. The possible prooxidant mechanisms of NaCl is attributed to its capability to 1) disrupt structural integrity of cell membrane, which enables catalysts easy access to lipid substrates, 2) releasing free ionic iron from iron-containing molecules such as heme proteins, and 3) inhibiting the activities of antioxidant enzymes such as catalase, glutathione peroxidase, and superoxide dismutase. Ferrylmyoglobin and hematin are the major catalysts in raw beef loin. 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.

Materials and Methods

Preparation of raw and cooked meat patties. Muscles ground through an 8-mm plate twice were mixed with NaCl for 2 min in a bowl mixer and then 10 ml of solution containing none, Mb (5 mg/g meat as a final conc.), ferrous ammonium sulfate (5 µg Fe/g meat), or ferric chloride (5 µg Fe/g 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, and then individually packaged in oxygen-permeable zipper bags. 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. 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.

Chemical analyses of raw and cooked meat patties. Lipid oxidation was determined using the TBARS method. Nonheme iron content was determined by the Ferrozine method. Lipoxygenase-like activities of meat samples were assessed by the generation of conjugated dienes from linoleic acid at 27 °C.

Results and Discussion

The TBARS value of control raw chicken breast did not increase during storage. The concentrations of nonheme iron and Mb in all raw chicken breast patties were lower than those of raw beef loin patties and their contents did not change during storage. 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. 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 the raw beef loin patties with added NaCl showed similar increases in nonheme iron contents (Table 2) and decreases in Mb concentrations during storage (Table 3). The lipoxygenase (LOX)-like activities in all raw beef loin added with NaCl were highly correlated with their Mb concentrations (r = 0.83 ~ 0.99). Lipid oxidation, and nonheme iron and Mb content, however, in raw Mb-added chicken breast patties did not change (Tables 1-3).

Addition of Mb+NaCl also did not affect lipid oxidation 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 gradual increase in reduced Mb percentage from 17.25% at Day 0 and to 71.05% at Day 10. Addition of Mb increased lipoxygenase (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 metMb+NaCl-added raw chicken breast patties significantly decreased during storage as their metMb percentage decreased (Table 3). LOX-like activity in raw beef loin increased as metMb percentage increased (r = 0.90) during 10 days of storage. The correlation between LOX-like activity and metMb percentage in this studies suggested that ferrylmyoglobin formed from metMb in the presence of H_2O_2 could be responsible for LOX-like activity in meat. 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, reduction of metMb to ferrous Mb and maintaining the pigment in reduced status by high total antioxidant capacity (TAC) attributed to higher oxidative stability of Mb+NaCl-added raw chicken breast than 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. High concentration of Mb and high Mb to lipid hydroperoxide attenuates the prooxidant activity of Mb. 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. 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). 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.

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 Day 0 and Day 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 MDA/kg meat, respectively) were similar to that of NaCl-added ones (15.45 mg MDA/kg 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 sufficient amount of free ionic irons. TBARS values of all cooked chicken breast and beef loin increased during 7-day 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. Also, 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. High percentage of polyunsaturated fatty acids in 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 the storage were significantly lower than those of the cooked beef loin. Beef loin contains greater 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. This suggested that the raw and cooked chicken breast patties are more resistant to the exogenous oxidative stresses such as addition of NaCl, ionic iron, myoglobin, 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 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 presence of NaCl were responsible for the denaturation of Mb and the release of free ionic irons from Mb. 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 that 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. HFRC was detected in both cooked chicken breast and beef, but higher in beef loin than chicken breast. 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, 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 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 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 indicated that cooked beef loin has significantly a greater amount of HFRC than cooked chicken breast. Therefore, greater levels of 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

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

	Chicken breast					Beef loin				
Storage	Control	NaCl	Mb + NaCl	Fe(III)+ NaCl	Fe(II)+ NaCl	Control	Mb + NaCl	Fe(III)+ NaCl	Fe(II)+ NaCl	NaCl
	TBARS value (mg MDA/kg meat)									
Raw meat										
0 d	0.22^{h}	0.23^{h}	0.21^{h}	0.74^{gz}	0.93^{fz}	1.46^{dz}	2.06^{cz}	1.23^{ez}	2.66^{bz}	2.99^{az}
5 d	0.18^{g}	0.21^{g}	0.19^{g}	1.25^{fy}	1.55^{fy}	3.07^{dy}	6.31^{cy}	2.34^{ey}	7.63^{by}	8.58^{ay}
10 d	0.19^{f}	0.22^{f}	0.21^{f}	1.54^{ex}	2.00^{ex}	3.80^{dx}	15.45^{bx}	4.85^{cx}	16.72^{ax}	17.04^{ax}
Cooked meat										
0 d	0.92^{gz}	1.21^{efz}	1.13^{fgz}	1.82^{bcz}	1.73^{cz}	1.38^{dez}	1.94^{bcz}	1.51^{dz}	1.98^{bz}	2.29^{az}
3 d	2.14^{ey}	2.52^{ey}	3.47^{dy}	3.73^{cdy}	3.77^{cdy}	3.30^{dy}	4.94^{aby}	4.32^{bcy}	4.83^{aby}	5.11^{ay}
7 d	2.81^{cx}	3.12^{cx}	7.59^{bx}	7.06^{bx}	7.04^{bx}	7.76^{bx}	11.14^{bx}	10.04^{ax}	10.39^{ax}	10.58^{ax}

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 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 patties treated with different prooxidants during storage at 4 °C.

	Chicken breast					Beef loin				
Storage	Control	NaCl	Mb + NaCl	Fe(III)+ NaCl	Fe(II)+ NaCl	Control	NaCl	Mb + NaCl	Fe(III)+ NaCl	Fe(II)+ NaCl
	µg nonheme iron/g meat									
Raw meat										
0 d	1.20^{d}	1.19^{d}	1.19^{d}	5.32^{b}	5.12^{b}	2.38^{cz}	2.69^{cz}	2.69^{cz}	7.50^{az}	7.60^{az}
5 d	1.37^{e}	1.33^{e}	1.33^{e}	5.75^{c}	5.58^{c}	3.41^{dy}	5.11^{cy}	3.63^{dy}	9.87^{by}	11.07^{ay}
10 d	1.41^{e}	1.49^{e}	1.47^{e}	5.69^{d}	5.84^{d}	5.11^{dx}	9.61^{bx}	8.14^{cx}	13.78^{ax}	14.72^{ax}
Cooked meat										
0 d	1.72^{f}	1.58^{f}	2.41^{ez}	6.06^{b}	6.09^{b}	4.40^{dz}	4.65^{dz}	5.36^{cz}	9.43^{ay}	9.78^{ay}
3 d	1.69^{e}	1.75^{e}	3.32^{dy}	6.21^{c}	6.24^{c}	5.92^{cy}	6.40^{cy}	7.45^{by}	9.89^{ay}	10.23^{ay}
7 d	1.75^{e}	1.85^{e}	4.63^{ex}	6.30^{d}	6.28^{d}	8.29^{cx}	8.77^{bcx}	9.63^{bx}	12.18^{ax}	12.65^{ax}

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 meat); Fe(III), ferric chloride (5 µg ferric ion/g meat); Fe(II), ferrous ammonium sulfate (5 µg ferric ion/g meat).

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.

	Chicken breast					Beef loin				
Storage	Control	NaCl	Mb + NaCl	Fe(III)+ NaCl	Fe(II)+ NaCl	Control	NaCl	Mb + NaCl	Fe(III)+ NaCl	Fe(II)+ NaCl
	mg Mb/g meat									
0 d	0.65^{ex}	0.62^{e}	5.07^{dx}	0.60^{e}	0.62^{e}	5.45^{cx}	5.83^{bx}	9.59^{ax}	5.89^{bx}	5.83^{bx}
5 d	0.55^{fy}	0.62^{f}	4.81^{bcy}	0.63^{f}	0.62^{f}	4.93^{by}	4.74^{cdy}	8.04^{ay}	4.62^{dey}	4.49^{ey}
10 d	0.52^{fy}	0.66^{f}	4.87^{by}	0.62^{f}	0.61^{f}	4.26^{cz}	2.89^{dz}	5.90^{az}	2.80^{dez}	2.54^{ez}
	% metMb									
0 d	59.45^{ey}	58.82^{ex}	81.41^{bx}	60.15^{ey}	60.65^{ey}	73.46^{cy}	76.51^{cz}	65.28^{dy}	92.47^{az}	93.95^{ay}
5 d	63.76^{bx}	57.63^{cy}	51.81^{dy}	63.18^{bx}	63.72^{bx}	101.96^{ax}	100.84^{ax}	98.22^{ax}	100.54^{ax}	100.34^{ax}
10 d	62.81^{dx}	54.83^{ez}	26.87^{fz}	61.36^{dy}	61.45^{dy}	101.74^{ax}	94.61^{cy}	98.81^{bx}	93.72^{cy}	92.92^{cy}

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 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 patties treated with different prooxidants during storage at 4 °C.

	Chicken breast					Beef loin				
Storage	Control	NaCl	Mb + NaCl	Fe(III)+ NaCl	Fe(II)+ NaCl	Control	NaCl	Mb + NaCl	Fe(III)+ NaCl	Fe(II)+ NaCl
	lipoxygenase-like activity (Unit/g meat)									
0 d	0.97^{f}	1.75^{f}	14.64^{ex}	1.58^{fx}	1.81^{fx}	28.33^{cx}	26.51^{dx}	33.25^{ax}	30.99^{bx}	30.84^{bx}
5 d	0.85^{f}	1.52^{f}	9.86^{ey}	1.22^{fy}	1.09^{fy}	31.05^{abx}	28.48^{bcx}	34.12^{ax}	23.67^{dy}	25.34^{cdy}
10 d	0.90^{d}	1.60^{d}	6.92^{cz}	1.14^{dy}	1.13^{dy}	24.80^{ay}	14.15^{by}	27.12^{ay}	13.82^{bz}	12.32^{bz}

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 (5mg/g meat); Fe(III), ferric chloride (5 µg ferric ion/g meat); Fe(II), ferrous ammonium sulfate (5 µg ferric ion/g meat.)