

## Correlation between Antioxidant Enzyme Activity, Free Iron Content and Lipid Oxidation in Four Lines of Korean Native Chicken Meat

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### Abstract

This study was conducted to observe the association between antioxidant enzyme activity, free iron content and lipid oxidation of Korean native chicken (KNC) meat during refrigerated storage. Four lines of KNC (*Yeonsan ogye*, *Hyunin black*, *Hoengseong yakdak* and *Hwangbong*) were raised under similar conditions. A total of 16 roosters were randomly sampled and slaughtered at the age of 12 mon. The breast and thigh meats were stored aerobically for 10 d at 4°C. Although thigh meat had higher antioxidant enzyme activity, it was more susceptible to lipid oxidation and released more iron during storage than breast meat. Aerobic refrigerated storage for 10 d significantly decreased the activity of antioxidant enzymes and increased the amount of free iron and malondialdehyde. The activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were negatively correlated with lipid oxidation, whereas that of catalase was not. The amount of free iron was positively associated with lipid oxidation. We concluded that chicken line did not affect strongly on antioxidant enzyme activity and lipid oxidation in breast meat of KNC. However, the thigh meat of *Hwangbong* and *Hyunin black* had higher SOD and GSH-Px activity, respectively, and lower malondialdehyde contents than that of other chickens. SOD, GSH-Px and free iron play significant roles in meat lipid oxidation during refrigerated storage.

**Keywords:** antioxidant enzyme, free iron, Korean native chicken, lipid oxidation

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### Introduction

The oxidation of lipids and myoglobin generates quality deterioration in muscle foods during refrigerated storage, such as off-flavor formation and color changes. Furthermore, the oxidation causes some nutrition loss such as polyunsaturated fatty acids and the generation of free radicals (Morrissey *et al.*, 1998). Antioxidant enzyme activity and iron are certain endogenous factors that affect the oxidation of lipids and protein in meat. Meat antioxidant enzymes, such as catalase, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), are naturally developed to inhibit the potential oxidation from endogenous

pro-oxidants, which are mostly derived from reactive oxygen species (ROS), such as superoxide anion radical ( $O_2^-$ ) and hydrogen peroxide ( $H_2O_2$ ), through complex chain mechanisms. These enzymes are considered major antioxidant enzymes found in muscle (Chan *et al.*, 1994).

Iron is the most probable catalyst for the initiation of lipid oxidation by catalyzing the generation of most hydroxyl radicals ( $\bullet OH$ ) (Buettner and Jurkiewicz, 1996). Approximately two-thirds of the iron in the body is found in hemoglobin, with smaller amounts found in myoglobin, iron-containing enzymes and transferrin. However, not all iron contained in meat promotes lipid oxidation. Free ionic iron released from heme pigments and ferritin is considered one of the major catalysts in lipid oxidation (Min and Ahn, 2005).

Oxidation also varies depending on the animal species, in addition to the effects of the level of antioxidant enzyme activity and free iron content. Min *et al.* (2008) confirmed this finding by comparing meat from different spe-

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cies, such as beef, chicken and pork. Little is known regarding the effects of animal breeds or lines within similar species on lipid oxidation and the level of its endogenous factors, especially in chicken. Moreover, the correlation between lipid oxidation and several endogenous factors may differ among animal species. Additional studies on species of native animals from specific locations may add new information to meat antioxidant capacity-related study.

Recently, KNC has gained popularity among Korean meat consumers. Their meats are exclusively served and are believed to have special flavor and texture. Ahn and Park (2002) reported that KNC has more desirable taste compounds than broiler. *Yeonsan ogye*, *Hyunin* black, *Hoengseong yakdak* and *Hwangbong* have been selected as candidates for developing a new chicken line by the Korean governmental institute. Because lipid oxidation is one of the meat quality parameters, the objectives of this study were to observe the antioxidant enzyme activity of four lines of KNC meat and confirm whether lipid oxidation correlates with free iron content and the activities of SOD, catalase and GSH-Px in meat from native animals.

## Materials and Methods

### Chemicals

HPLC-grade chloroform was purchased from Daejung Chemical and Metals Co. Ltd. (Korea). Distilled water (DW) was purified with a glass still (Mega-Pure™ System MP-11A, Corning Inc., USA). All other reagents were purchased from Sigma-Aldrich Co., LLC. (USA).

### Sample and storage conditions

Four lines of KNC (*Yeonsan ogye*, *Hyunin* black, *Hoengseong yakdak* and *Hwangbong*) were used as samples in this study and raised at Seoul National University animal farm (Korea) under similar condition. Animals were allowed to free access to feed and water. All the experimental procedures were approved by the Institute of Laboratory Animal Resources, Seoul National University (SNU-070823-5). A total of 16 roosters were randomly sampled and transferred to a chicken slaughter house (Well-cock Hyunjin Co. Ltd, Korea) within group cages for 2 h. After 2 h resting with free access to water, animals were mechanically slaughtered at the age of 12 mon ( $n=4$  birds) using a standard procedure. The breast and thigh meat of each animal were then obtained and separately packed within oxygen-permeable bags (low-density polyethylene, 25 × 30 cm; Clean-wrap, Korea). The samples were kept chilled in an icebox and were trans-

ported to the laboratory. The samples were then stored at  $4\pm 1^\circ\text{C}$  for 10 d for oxidation studies. Analyses were performed in triplicate using samples on the initial, middle, and final days.

### Lipid oxidation

Lipid oxidation was measured using the 2-thiobarbituric acid reactive substances (TBARS) method by Sinnhuber and Yu (1977) with slight modification. A total of 0.5 g of sample was vortex-mixed with 0.1 g of antioxidant mixture (consisting of propylene glycol, Tween 20, butylated hydroxytoluene, and butylated hydroxyanisole), 3 mL of 1% TBA in 0.3% NaOH, and 17 mL of 2.5% trichloroacetic acid in 36 mM HCl. The sample was heated in a water bath (BW-20G, Biotechnical Services, Inc., US) at  $100^\circ\text{C}$  for 30 min and then immersed in ice water for 15 min. Five milliliters of aqueous sample was then mixed with 3 mL of chloroform. The optical density (OD) of the upper layer was recorded at 532 nm against blank after centrifugation at 2,400 g for 30 min at  $4^\circ\text{C}$  (1248R, Labogene, Denmark). The results were expressed in mg of malondialdehyde (MDA) per kg of meat. Each sample was analyzed in triplicate.

### Antioxidant enzyme activity measurement

SOD, catalase and GSH-Px were measured in aqueous meat extract dissolved in 50 mM phosphate buffer (pH 7.0 at  $25^\circ\text{C}$ ) according to a previous study by Renner *et al.* (1996). Catalase activity was measured using a method described by Aebi (1984). First, 100  $\mu\text{L}$  of filtered supernatant was mixed with 2.9 mL of 30 mM  $\text{H}_2\text{O}_2$  within a crystal cuvette (light path: 1 cm). The decrease in absorbance at 240 nm was recorded every 10 s for 2 min. Catalase activity was expressed as units/g sample. SOD activity was determined according to the pyrogallol autoxidation method described by Marklund and Marklund (1974) with modification. The optical density of a mixture consisting of 50  $\mu\text{L}$  of filtered supernatant, 3.025 mL of 50 mM Tris-cacodylate-DTPA buffer (pH 8.2 at  $25^\circ\text{C}$ ) and 50  $\mu\text{L}$  of 24.8 mM pyrogallol was recorded at 420 nm every 15 s for 2 min against a blank. One unit of SOD represents the amount of SOD required for the auto oxidation of 50% of the pyrogallol in the meat extract at pH 8.2 and  $25^\circ\text{C}$ . The GSH-Px activity measurement was performed according to the enzymatic protocol by Flohé and Günzler (1984) with slight modification. A total of 100  $\mu\text{L}$  of filtered supernatant was mixed with 0.5 mL of phosphate buffer-0.001 M EDTA-0.1 M  $\text{NaN}_3$ , 100  $\mu\text{L}$  of the assay mixture containing 5 units  $\text{mL}^{-1}$  glutathione reductase in

50 mM phosphate buffer, 100  $\mu$ L of 10 mM L-glutathione reduced, 100  $\mu$ L of 1.5 mM NADPH in  $\text{NaHCO}_3$ , and 100  $\mu$ L of 1.5 mM  $\text{H}_2\text{O}_2$  within a 10-mm precision cell (104-QS, Hellma Analytics, Germany) and incubated for 5 min. GSH-Px activity was measured by recording the decrease in absorbance of the incubated mixture at 340 nm over 2 min. GSH-Px activity was expressed as units  $\text{g}^{-1}$  sample. One unit represents the amount of meat extract needed to alter 1  $\mu$ mol of NADPH with a mM extinction coefficient of  $6.3 \text{ mM}^{-1} \text{ cm}^{-1}$  at 340 nm.

### Free iron content measurement

The amount of free iron in the meat was measured based on a modified version of the ferrozine method (Ahn *et al.*, 1993; Carter, 1971). A 5 g sample was homogenized with 45 mL of citrate phosphate buffer (pH 5.5) at 13,500 rpm for 30 s using a homogenizer (Ultra-Turrax T25 basic, Ika Werke GmbH and Co., Germany). The homogenate was centrifuged at 3,200 g for 30 min at 4°C (1248R, Labogene, Denmark). Next, 1 mL of the supernatant was mixed with 0.5 mL of freshly prepared 2% ascorbic acid in 0.2 N HCl and incubated at room temperature for 5 min. Then, the mixture was mixed thoroughly with 1.5 mL of 11.3% trichloroacetic acid followed by centrifugation at 10,000 g in an Eppendorf Micro 17R+ centrifuge (Hanil Science Industrial, Korea) for 10 min. Finally, 1 mL of supernatant was mixed with 0.4 mL of 10% ammonium acetate and 0.1 mL of ferriin indicator followed by 5 min incubation. The absorbance was read at 562 nm against a blank. The free iron value was expressed as  $\mu\text{g/g}$  meat.

### Statistical analysis

The one-way analysis of variance (ANOVA) was used for the effect of different meat parts. Thigh meat was observed to have higher TBARS value, antioxidant enz-

yme activity and iron content than breast meat. A  $4 \times 3$  factorial design with three replicates each meat part was employed with chicken lines and storage times as the main effects, using two-way ANOVA. No significant interactions were found. The statistical significance of the differences between the means from different lines and storage times was determined by Duncan's multiple range test. *P* values of less than 0.05 were regarded as significant. The Pearson's correlation coefficients and linear regression between TBARS values and other parameters were measured. Data used for regression analysis were pooled according to chicken lines, meat parts and storage times ( $N=24$ ). Analyses were performed using R-version 3.2.0 with the "Agricolae" library (The R-foundation for Statistical Computing, Austria).

## Results

### Lipid oxidation

The degree of lipid oxidation is represented by the increasing amount of malondialdehyde content during storage. Refrigerated storage significantly increased the production of malondialdehyde for both the breast and thigh meat of each KNC ( $p<0.001$ ) line. The highest malondialdehyde contents were observed on the final day of storage, ranging from 0.40 to 0.45 mg/kg meat for breast meat and 0.53 to 0.79 mg/kg meat for thigh meat (Table 1). Among KNC, the highest TBARS values were found in the thigh meat of *Yeonsan ogye* and *Hoengseong yakdak* ( $p<0.05$ ). However, no significant chicken lines effects were found in breast meat.

### Changes in antioxidant enzyme activity

The decline of antioxidant enzyme activity in KNC meat as affected by refrigerated storage is shown in Table

**Table 1. Changes in TBARS values (mg MDA/kg meat) of Koran native chickens during refrigerated storage**

Meat part	Storage (d)	<i>Yeonsan ogye</i>	<i>Hyunin black</i>	<i>Hoengseong yakdak</i>	<i>Hwangbong</i>	SEM
Breast	0	0.08y	0.09z	0.09z	0.12y	0.01
	5	0.21y	0.25y	0.28y	0.36x	0.02
	10	0.41x	0.45x	0.43x	0.40x	0.01
	Mean	0.23	0.26	0.26	0.28	
	SEM	0.08	0.08	0.08	0.08	
Thigh	0	0.28y	0.25y	0.26y	0.23y	0.01
	5	0.20y	0.30y	0.34y	0.25y	0.01
	10	0.79x	0.62x	0.64x	0.53x	0.02
	Mean	0.42a	0.39ab	0.42a	0.34b	
	SEM	0.14	0.09	0.09	0.07	

Means in the same column (x, y, z) and row (a, b) followed by different letters are significantly different ( $p<0.05$ ). SEM, standard error for the means.

**Table 2. Antioxidant enzyme activity (units/g meat) of Korean native chickens during refrigerated storage**

Enzyme	Meat part	Storage time (d)	<i>Yeosan ogye</i>	<i>Hyunin black</i>	<i>Hoengseong yakdak</i>	<i>Hwangbong</i>	SEM
Catalase	Breast	0	72.04x	70.38x	73.77x	69.14x	0.45
		5	51.59y	55.61y	49.68y	57.96y	0.84
		10	41.40y	48.55y	49.01y	36.29y	1.37
		Mean	55.01	58.18	57.49	54.46	
		SEM	6.98	4.98	6.31	7.47	
	Thigh	0	118.07x	133.07x	131.11x	125.70x	1.50
		5	83.84y	101.06y	101.97y	115.73y	2.92
		10	77.52y	81.97y	69.02y	50.97y	3.06
		Mean	93.14	105.37	100.70	97.47	
		SEM	9.76	11.55	13.89	18.14	
SOD	Breast	0	148.00x	137.33x	124.00x	148.00x	2.55
		5	101.33y	77.33y	80.00y	98.67y	2.78
		10	68.57y	61.71y	73.14y	68.57y	1.05
		Mean	105.97	92.13	92.38	105.08	
		SEM	17.85	17.85	12.34	17.93	
	Thigh	0	157.33x	146.67x	144.00x	172.00x	2.84
		5	114.67y	104.00y	112.00y	112.00y	1.03
		10	70.86y	68.57y	91.43y	98.29y	3.31
		Mean	114.29b	106.41b	115.81b	127.43a	
		SEM	19.34	17.49	11.85	17.53	
GSH-Px	Breast	0	0.30x	0.32x	0.27x	0.37x	0.01
		5	0.19y	0.19y	0.23x	0.21y	0.004
		10	0.12y	0.13y	0.13y	0.11y	0.002
		Mean	0.20	0.21	0.21	0.23	
		SEM	0.04	0.04	0.03	0.06	
	Thigh	0	0.62x	0.74x	0.54x	0.59x	0.02
		5	0.39y	0.45y	0.23y	0.31y	0.02
		10	0.13z	0.14z	0.12y	0.13z	0.003
		Mean	0.38ab	0.45a	0.30b	0.34b	
		SEM	0.11	0.13	0.10	0.10	

Means in the same column (x, y, z) and row (a, b) followed by different letters are significantly different ( $p < 0.05$ ). SEM, standard error for the means.

2. The SOD activity on the initial day of storage ranged from 124 to 148 units/g meat for breast and 144 to 172 units/g meat for thigh. This value then decreased on the final day of storage, ranging from 61.71 to 73.14 units/g meat and 68.57 to 98.29 units/g meat for breast and thigh, respectively. At day 0, the activity of catalase ranged from 69.14 to 73.77 units/g meat for breast and 118.07 to 133.07 units/g meat for thigh. At day 10, this value decreased to 36.29-48.55 units/g meat and 50.97-81.97 units/g meat for breast and thigh, respectively. The activity of GSH-Px decreased as well, ranging from 0.27 to 0.37 units/g meat for breast and from 0.54 to 0.62 units/g meat for thigh at day 0, dropping to 0.11-0.13 units/g meat and 0.12-0.14 units/g meat at day 10 for breast and thigh, respectively. Chicken lines affected the activity of SOD and GSH-Px in thigh meat ( $p < 0.05$ ), whereas no effects were observed on antioxidant enzyme activity in breast meat.

The highest activity of SOD was found in thigh meat of *Hwangbong* and *Hyunin black* had the highest GSH-Px activity in its thigh meat.

#### Free iron content during storage

The amount of iron released during refrigerated storage from KNC meat is shown in Table 3. At day 0, the amount of released iron ranged from 0.30 to 0.39  $\mu\text{g/g}$  meat for breast and from 0.76 to 1.15  $\mu\text{g/g}$  meat for thigh. After 10 d of storage, this value elevated to 0.41-0.49  $\mu\text{g/g}$  meat and 1.21-1.25  $\mu\text{g/g}$  meat for breast and thigh, respectively. No significant differences were found among chicken lines.

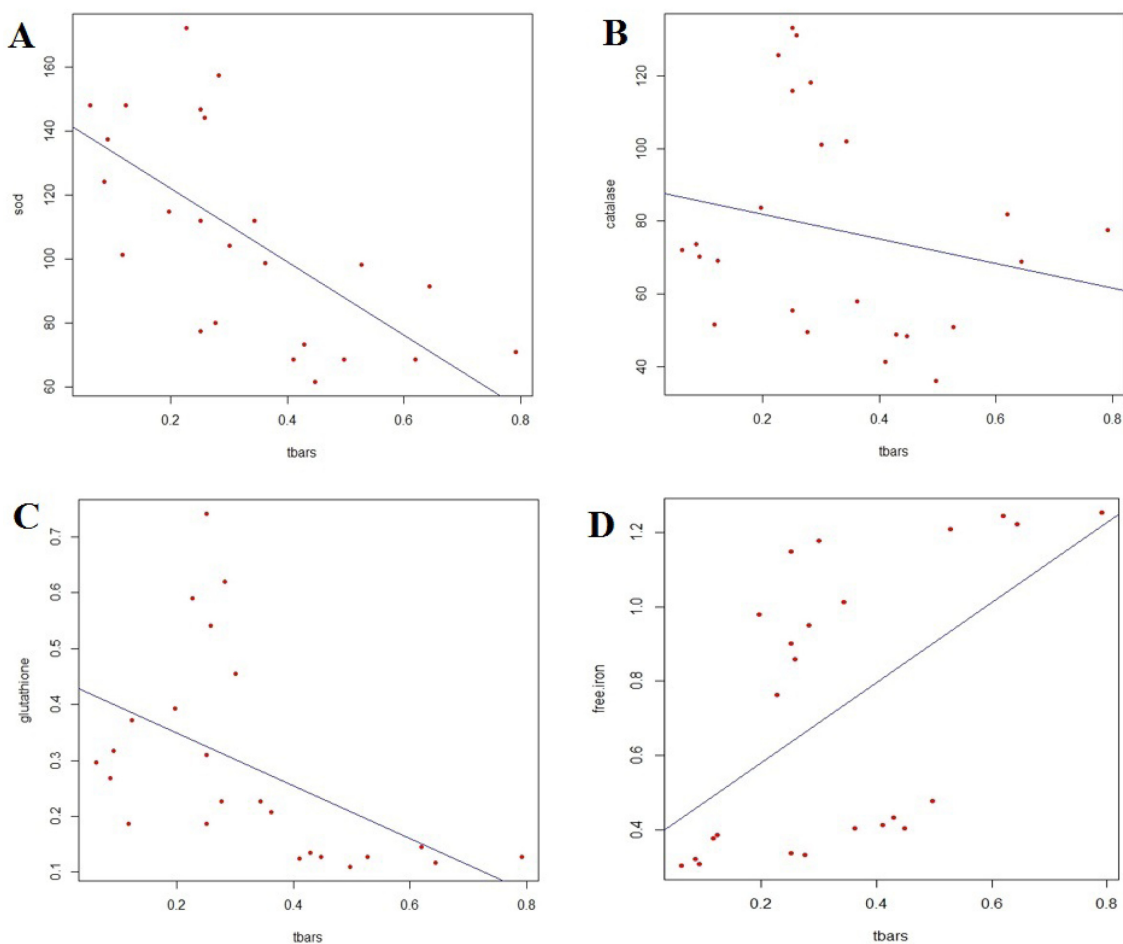
#### Correlation between TBARS values and antioxidant enzyme activity

The correlation between TBARS values and the activity of antioxidant enzyme is shown in Fig. 1A, 1B and 1C

**Table 3. Changes in free iron content ( $\mu\text{g/g}$  meat) of Korean native chickens during refrigerated storage**

Meat part	Storage (d)	<i>Yeonsan ogye</i>	<i>Hyunin</i> black	<i>Hoengseong yakdak</i>	<i>Hwangbong</i>	SEM
Breast	0	0.30y	0.31y	0.32y	0.39y	0.01
	5	0.38x	0.34y	0.33y	0.40y	0.01
	10	0.41x	0.41x	0.43x	0.48x	0.01
	Mean	0.36	0.35	0.36	0.42	
	SEM	0.02	0.02	0.03	0.02	
Thigh	0	0.95y	1.15y	0.86y	0.76y	0.04
	5	0.98y	1.18x	1.01y	0.90y	0.03
	10	1.25x	1.25x	1.22x	1.21x	0.005
	Mean	1.06	1.19	1.03	0.96	
	SEM	0.07	0.02	0.08	0.10	

Means in the same column (x, y) followed by different letters are significantly different ( $p < 0.05$ ). SEM, standard error for the means.



**Fig. 1. Correlation between TBARS and (A) SOD activity ( $r = -0.655$ ,  $p < 0.001$ ,  $y = 144.90 - 114.60x$ ), (B) catalase activity ( $r = -0.215$ ,  $p = 0.3125$ ,  $y = 88.79 - 33.86x$ ), (C) GSH-Px activity ( $r = -0.493$ ,  $p = 0.014$ ,  $y = 0.44 - 0.47x$ ), and (D) free iron content ( $r = 0.552$ ,  $p = 0.005$ ,  $y = 0.36 + 1.08x$ ) in four lines of KNC meat.**

for SOD, catalase and GSH-Px, respectively. The SOD and GSH-Px activities were negatively correlated with TBARS values in the breast and thigh meat of KNC dur-

ing refrigerated storage, whereas catalase activity was not significantly associated with lipid oxidation. The correlation coefficients for SOD and GSH-Px were -0.655 and

-0.493, respectively.

### Correlation between TBARS values and free iron content

The association of TBARS values with the amount of released-iron of KNC meat during refrigerated storage is shown in Fig. 1D. A significant correlation was found between free iron content and lipid oxidation ( $p < 0.01$ ). The free iron content was positively correlated with the increasing amount of malondialdehyde content in KNC meat, with a correlation coefficient of 0.552.

## Discussion

Muscle fibers can be categorized into two different metabolic types, oxidative (red) or glycolytic (white), based on their chemical composition and enzyme activities (Warris, 2000). In this study, the oxidative and glycolytic muscles were represented by thigh and breast meat, respectively. Because oxidative muscle fibers are found relatively higher in thigh meat than breast meat. Our results are in line with Renner *et al.* (1996), in which, oxidative muscle was found to exhibit higher antioxidant enzyme activities than glycolytic muscle. Although previous results support increased antioxidant enzyme expression in the highly oxidative environments of living tissues, it does not substantiate the role for these enzymes in postmortem muscle tissues or so-called meat (Bekhit *et al.*, 2013). Moreover, Lee *et al.* (1996) reported a higher antioxidant enzyme activity in the thigh meat of turkey than in its breast meat.

Catalase and GSH-Px are considered the major peroxide-removing enzymes located in cytosol, whereas SOD plays a role in the protection against damage resulting from superoxide anion radicals (Chan *et al.*, 1994). SOD and catalase are coupled enzymes, in which SOD scavenges superoxide anions by forming hydrogen peroxide, and catalase safely decomposes hydrogen peroxide to water and oxygen; GSH-Px can decompose both hydrogen peroxide and lipoperoxides formed during lipid oxidation (Gatellier *et al.*, 2004; Terevinto *et al.*, 2010). Antioxidant enzyme activity differs in the meat from different animal species (Pradhan *et al.*, 2000). Hernandez *et al.* (2004) and Chen *et al.* (2012) noted that antioxidant enzyme activity also varies in pork from different lines, which agrees with our results, particularly that of thigh meat. However, the antioxidant enzyme activity of breast meat in present study contrast previous results because breast meat is composed of relatively low oxidative muscle fibers compared to

thigh meat.

As expected, the decline of the activity of antioxidant enzyme was affected by the duration of storage. Storage temperature and the presence of oxygen might also be responsible for this decline. Our results contrast with Kang *et al.* (2014), who reported that the activity of GSH-Px in Hanwoo beef increased during refrigerated storage after 7 d of freezing within vacuum-packaging. However, no freezing condition was applied in this study and the samples were stored aerobically. Because these enzymes are protein complexes, they can also be degraded through oxidation processes into carbonyls. Soyer *et al.* (2010) used the increasing amount of carbonyls as a biomarker for protein oxidation in chicken meat. Moreover, the decline of antioxidant enzyme activity may be resulted by the utilization of the enzyme to protect cells from post slaughter auto-oxidation. Thus, their antioxidant ability declined during storage under aerobic conditions. Only the catalase activity was not significantly associated with lipid oxidation as strong as other antioxidant enzymes observed in this study. Previous study by Chen *et al.* (2012) mentioned that the activity of catalase has lower association with lipid oxidation in pork loin than the activity of SOD and GSH-Px. Therefore, we suggest that SOD and GSH-Px significantly influence the inhibition of lipid oxidation in KNC meat.

Lombardi-boccia *et al.* (2002) reported that the oxidative meat (thigh, drumstick and wing) contained more iron than the breast meat of the broiler and turkey because oxidative muscles have higher myoglobin content. Furthermore, Min *et al.* (2008) reported that the heme and non-heme iron contents markedly differed among the meat types; the iron content was higher in chicken legs and wings than that in breast meat. In this study, the amount of free iron content was consistently higher in thigh meat from different lines of KNC.

In contrast to antioxidant enzyme activity, the free iron content increased when lipid oxidation occurred during storage, resulting in a significant correlation. However, our results did not corroborate with those of Min *et al.* (2008), who reconfirmed that ferrylmyoglobin and hematin are the major catalysts particularly during oxidation processes rather than free ionic iron, and found a declining trend of free ionic iron during chilled storage in broiler breast and thigh. The decrease may be attributed to the differences in slaughter age and pre-treatment of the samples.

## Conclusions

The activity of antioxidant enzymes and degree of lipid

oxidation differ significantly in thigh meat of KNC, instead of breast meat. In particular, the thigh meat of *Hwang-bong* and *Hyunin* black exhibited higher SOD and GSH-Px activity, respectively, and were less susceptible to lipid oxidation than that of other KNC. SOD, GSH-Px and free iron play significant roles in inhibiting and accelerating lipid oxidation in KNC meat.

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