Lipid oxidation induced by heating in chicken meat and the relationship with oxidants and antioxidant enzymes activities

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ABSTRACT The aim of the current research was to examine lipid oxidation in chicken meat heated to different temperatures followed by refrigerator storage and the factors contributing to lipid oxidation. It showed that lipid oxidation was significantly promoted when meat was heated up to 70°C and stored for 2 and 4 D as measured by thiobarbituric acid reactive substance. The monounsaturated fatty acids and polyunsaturated fatty acids also decreased significantly ($P < 0.05$) with the increase of heating temperature. The liberation of nonheme iron and increase of hydroxyl radical were observed in heated chicken meat, and the activities of antioxidant enzymes was decreased considerably at higher temperatures. The changes of these prooxidants and antioxidants might constitute a possible mechanism for the stronger lipid oxidation in heated meat.

Key words: lipid oxidation, heating, antioxidant enzymes

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INTRODUCTION

Thermal treatment of meat products is essential to achieve a palatable and safe product. The chicken meat products have become increasingly popular worldwide over the past few years because of their high nutritional quality and low cost, and chicken lipids display a high level of unsaturated fatty acids, which are considered as healthy by the consumers (Bonoli et al., 2007). Chicken meat products are available as either fresh or precooked products, which are usually stored under refrigeration. The demand for high quality precooked or ready-to-eat products is increasing, and it is mainly related to their rapid and easy cooking (Ferreira et al., 2017). However, thermal treatment could accelerate the development of lipid oxidation through generating free radicals and blunting the intrinsic antioxidant defense system (Serpen et al., 2012). Lipid oxidation results in rancid odor, off-flavor development, discoloration, loss of nutritional values, and decrease in storage shelf-life and generates compounds that may pose risks to human health (Min and Ahn, 2005; Cao et al., 2018).

Lipid oxidation in meat is dependent on the content of natural antioxidants, oxidants, and the polyunsaturation degree of fatty acids. Meat contains a combination of initiators, catalysts, and intermediates. Iron is probably the major catalyst for the initiation of lipid peroxidation by generation of hydroxyl radicals (Buettner and Jurkiewicz, 1996). Several studies have shown that heating produces an increase of nonheme iron and a decrease of heme iron, and the release of iron from iron containing proteins might play a role in lipid oxidation (Kristensen and Purslow, 2001). Meat also contains a number of endogenous antioxidant systems including the antioxidant enzymes, peptides, and proteins, functioning as metal ion chelators or free radical scavengers (Zou et al., 2019). However, heating could significantly affect these antioxidant defense systems, and the information on the activity of these antioxidants in meat is very limited. Catalase (**CAT**), superoxide dismutase (**SOD**), and glutathione peroxidase (**GSHPx**) are among the most important antioxidant enzymes against lipid oxidation. Muhlisin et al. (2016) have reported that CAT activity was inactivated, and GSHPx and SOD activity was decreased after cooking. Cooking methods as well as cooking condition such as cooking time and temperature could result in the changes of these oxidants and antioxidants and finally different degree of lipid oxidation (Domínguez et al., 2014). When different

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cooking methods were compared, roasting, which uses high temperatures, produces an increased lipid oxidation compared with other methods (Hernández et al., 1999).

Chicken meat is particularly susceptible to oxidative damage owing to its high degree of lipid unsaturation, and there is little information about the effect of heating temperature on the lipid oxidation and the factors contributing to oxidation. The aim of the present work was to gain more information on the lipid oxidation and physicochemical changes in chicken muscles as influenced by the heating temperature and to better understand the mechanisms driving heat-induced lipid change as the integrated functions of endogenous prooxidants and antioxidants in meat.

MATERIALS AND METHODS

Sample Preparation

The white feather broiler chickens were obtained from a commercial processing plant (Jiangsu Lihua Animal Husbandry Co., Ltd., Nanjing, China). Two skinless, deboned breast fillets (Pectoralis major) were taken immediately from each carcass and placed into a plastic bag on ice.

The meat was trimmed from visible fat and connective tissue and minced by grinding through a plate with 6 mm holes. Samples were mixed thoroughly and packaged in the plastic bag (12 × 17 cm) with 10 mm thickness. A total of 35 breast samples were utilized in this study. Five samples were randomly selected in each treatment group, and heat treatment of the samples was performed in thermostatted circulating water baths at the temperature of 50, 60, 70, 80, 90, and 100°C for 10 min, respectively. The control group was the unheated meat. After treatment, the samples were cooled to room temperature, a portion of samples were stored at −20°C, and the remaining were stored at 4°C for 2 and 4 D respectively followed by storage at −20°C for further analysis.

Analysis of Thiobarbituric Acid Reactive Substances

Lipid oxidation of all samples was measured by the 2-thiobarbituric method according to Sorensen and Jorgensen (1996). Ten grams of sample was homogenized with 30 mL of a 7.5% trichloroacetic acid solution containing 0.1% propylgallate and 0.1% ethylenediaminetetraacetic acid, disodium salt for 30 s in an Ultra Turrax blender (T25, IKA, Labortechnik, Staufen, Germany) at 9,500 rpm and filtered through a Whatman filter No. 42. Equal 5 mL volumes of filtrate and 0.02 mol 2-thiobarbituric solution were mixed with glass stopped tubes and incubated in a water bath at 100°C for 40 min before cooling to room temperature under running cold tap water. The absorbance was measured at 532 nm using spectrophotometer. thiobarbituric acid reactive substances (**TBARS**) was calculated from a standard curve of malondialdehyde (**MDA**), freshly prepared by acidification of 1,1,3,3-tetraethoxypropane in the range from 0.02 μg/mL to 0.3 μg/mL, and expressed as mg of MDA per kg sample.

Analysis of Fatty Acids

Lipids were extracted from muscle samples according to the method of Folch et al. (1957) with small modifications. Briefly, 3.0 g of muscle sample was homogenized with 60 mL of chloroform/methanol (2/1, V/V) solution at 1,500 rpm using an Ultra Turrax (T25, IKA, Labortechnik). The homogenate was allowed to stand for 1 h and then pass through a layer of filter. After that, 0.2-fold its volume of a solution containing 7.3 g/L NaCl, and 0.5 g/L CaCl2 was added to the filtrate. The mixture was centrifuged for 15 min at 3,000 rpm (Allegra 64R; Beckman Coulter, Brea, CA), and the lower phase was dried under vacuum on a rotary evaporator (RE-85C; Yarong, Shanghai, China) in a 44°C water bath.

One hundred microliters of free fatty acids elute was evaporated to remove the solvent. The residue was mixed with 2.0 mL of 14 g/100 g of BF_3/methanol. One hundred microgram per milliliter of heptadecanoic acid was added to the mixture as an internal standard. The mixture was methylated at 60°C for 30 min. Thereafter, 2 drops of 2,2-dimethoxypropane was added to remove the water produced during methylation. After cooling, 1.0 mL of water and 1.0 mL of *n*-hexane were added and shaken for several minutes. The resulting mixture was allowed to stand for 1 h, and the upper organic phase was dried by rotary evaporation under N_2. The residue was dissolved in 0.4 mL of hexane for GC analysis.

The methylated fatty acids were analyzed with a gas chromatograph (GC-14B; Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a split injector. One and a half microliters of the sample was injected onto a capillary column (CP-Sil 88 for Fame, 50 m × 0.25 mm × 0.20 μm, Varian) containing a nonpolar stationary phase (5% phenylmethyl/95% siloxane). The oven temperature increased from 160°C to 220°C at 6°C/min and maintained for 30 min at 220°C. The detector temperature was maintained at 280°C. The carrier gas was N2, and its pressure was maintained at 80 kPa. The peaks were identified by comparing their retention times with those of the standards. The relative content of fatty acids were determined by the peak areas (Gandemer, 2002).

Antioxidant Enzymes Activity Measurement

Two grams of muscle sample was homogenized with 10 mL Tris-HCl buffer (100 mmol/L, pH 8.0) at 12,000 rpm using an Ultra Turrax (T25, IKA). The homogenate was then centrifuged at 4°C for 20 min at 12,000 × *g* (Allegra 64R, Beckman Coulter), and supernatant was collected and used to determine antioxidant enzyme activities. The protein concentration was determined with the Bradford Protein Assay Kit (A045-2, Jiancheng, Nanjing, China). The superoxide dismutase (**SOD**) activity in the supernatant of the homogenized

meat was measured using the SOD Assay Kit (A001-3, Jiancheng). The CAT activity and GSHPx activity of the samples was determined with the CAT Assay Kit and Total GSHPx Assay Kit (S0051 and S0058, Beyotime Biotechnology, Shanghai, China) according to the manufacturer's instructions.

Iron Determination

Total Iron Samples were digested in nitric acid (GR, Merck, Germany) using a Multiwave 3000 microwave digestion system (Anton-Paar, Courtaboeuf, France) according to Ataro et al. (2008) with modifications. An aliquot of 0.2 g sample was accurately weighed and added with 5 mL nitric acid. The following program was employed: 10 min ramp, 40 min hold, maximum pressure 500 psi, and maximum temperature 180°C. A blank solution was prepared by digesting deionized water and nitric acid using the same digestion procedure. Total iron was determined with the iCAP Q ICP-MS (Thermo Fisher Scientific, UK) under optimum instrument operation conditions (power, 1.5 kW; plasma flow, 13.80 L/min; auxiliary flow, 0.79 L/min; nebulizer flow, 0.98 L/min; sampling depth, 15 mm).

Heme Iron Heme iron was determined using the acidified acetone extraction method of Hornsey (1956). Five grams of sample was mixed with 10 mL of acidified acetone (95.7% acetone, 2.4% HCl). The suspension was homogenized for 30 s at 10,000 rpm. Then, the homogenates were incubated in dark conditions for 1 h before centrifugation at 5,000 rpm for 10 min. The supernatant was filtered through a Whatman paper, and the absorbance was measured at 640 nm against a reagent blank using a spectrophotometer (Shimadzu). The heme iron content was calculated using a molar extinction coefficient of 4,800 mol^{-1} cm^{-1}.

Hydroxyl Radical Production Assay

Hydroxyl radical in the supernatant of the homogenized meat was assessed by colorimetric measurement at 550 nm by Griess Reagent Kit (Jiancheng) according to the manufacturer's protocol.

Statistical Analysis

Statistical analysis of the differences between each group was evaluated by one-way ANOVA using the SPSS 18.0. Differences were regarded as significant at $P < 0.05$. All data were expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Lipid Oxidation

The formation of TBARS is an indicator of lipid oxidation. The TBARS concentration increased with increasing heating temperature and the length of the storage time (Figure 1). This is in agreement with previous study in chicken and duck that formation of TBARS is dependent on both temperature and storage time (Hoac et al., 2006). The formation of TBARS in unheated chicken meat was not significantly different with those heated at 50°C and 60°C from day 0 to day 4 ($P > 0.05$), but it rapidly increased when the chicken meat was heated at 70°C and higher temperatures at day 0 and stored for 2 and 4 D ($P < 0.05$). And after 4 D of storage, the TBARS in unheated, 50°C, and 60°C heated meat only had less than 2-fold increase, whereas it had more than 10-fold increase when heated to 70°C and higher, indicating the heating temperature had great influence on lipid oxidation. It was suggested that a TBARS value ranging from 0.202 to 0.664 mg MDA/kg could be defined as fresh pork (Hasty et al., 2002), and the pork had low level of lipid oxidation when stored at 4°C for 9 D (Zhang et al., 2016).

The free total fatty acids contents in chicken meat heated to different temperatures are presented in Table 1. C16:0, C18:1, and C18:2 were the most abundant fatty acids in chicken meat, and the unsaturated fatty acids accounted for around 60% of the total fatty acids in chicken meat. In general, the saturated fatty acids were increased, whereas the monounsaturated fatty acids and polyunsaturated fatty acids decreased significantly ($P < 0.05$) with the elevation of heating temperature. The results were in accordance with that obtained in TBARS measurement. The unsaturated fatty acids are the main targets of oxidants and most of the factors triggering lipid oxidation are located in the water phase, but iron can be released from high-molecular-weight proteins at the point where iron bonds to the proteins surface and to the lecithoid phosphoric site, which accelerates lipid oxidation (Przybylski and Eskin, 1991; Marco et al., 2004).

Iron Content

As shown in Figure 2, the heme iron content gradually decreased from unheated to 100°C heated meat, but in

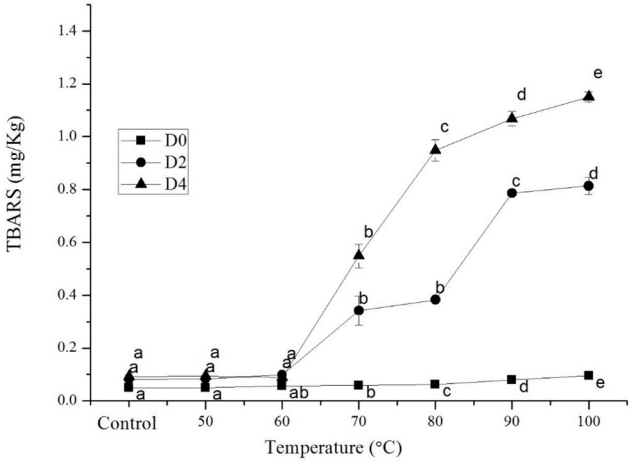

Figure 1. Formation of TBARS in chicken meat after heating to different temperatures and subsequent storage for 0, 2, 4 D at 4°C. Bars indicate standard deviation. For each day, different superscripts indicate significant difference ($P < 0.05$).

Table 1. Free fatty acids in chicken meat after heating to different temperatures and subsequent storage for 0, 2, and 4 D at 4°C (mg/g lipids).

Fatty acids	Days	Control	50°C	60°C	70°C	80°C	90°C	100°C
c4:0	D0	0.11 ± 0.01^{a}	0.12 ± 0.01^{a}	0.12 ± 0.01^{a}	0.16 ± 0.00^{b}	0.18 ± 0.00^{c}	0.27 ± 0.00^{d}	0.50 ± 0.00^{e}
	D2	0.11 ± 0.00^{a}	0.12 ± 0.01^{a,b}	0.13 ± 0.01^{b}	0.16 ± 0.01^{c}	0.19 ± 0.01^{d}	0.28 ± 0.01^{e}	0.70 ± 0.06^{f}
	D4	0.09 ± 0.07^{a}	0.18 ± 0.01^{b}	0.18 ± 0.01^{b}	0.17 ± 0.00^{b}	0.19 ± 0.01^{b,c}	0.25 ± 0.01^{d}	0.26 ± 0.01^{d}
c14:0	D0	0.21 ± 0.02^{a}	0.44 ± 0.04^{c}	0.47 ± 0.03^{c,d}	0.59 ± 0.00^{e}	0.41 ± 0.02^{c}	0.36 ± 0.03^{b}	0.47 ± 0.01^{c,d}
	D2	0.36 ± 0.00^{a}	0.35 ± 0.02^{a}	0.39 ± 0.00^{b}	0.41 ± 0.01^{c}	0.44 ± 0.01^{d}	0.57 ± 0.01^{e}	0.59 ± 0.01^{e}
	D4	0.35 ± 0.01^{a}	0.37 ± 0.01^{a}	0.4 ± 0.01^{b}	0.35 ± 0.00^{a}	0.39 ± 0.01^{b}	0.36 ± 0.01^{a}	0.38 ± 0.01^{a,b}
c16:0	D0	20.02 ± 0.41^{a}	20.52 ± 0.23^{b}	21.87 ± 0.03^{c}	22.48 ± 0.55^{b}	22.84 ± 0.36^{c}	24.03 ± 0.00^{e}	23.34 ± 0.35^{d}
	D2	20.82 ± 0.03^{a}	20.66 ± 0.06^{a}	21.38 ± 0.08^{b}	22.93 ± 0.06^{c}	23.61 ± 0.02^{e}	23.04 ± 0.64^{d}	23.41 ± 0.42^{e}
	D4	20.64 ± 0.06^{a}	21.25 ± 0.09^{b}	22.59 ± 0.05^{c}	22.90 ± 0.13^{c}	24.29 ± 0.61^{d}	25.41 ± 0.46^{e}	24.61 ± 0.54^{d}
c18:0	D0	8.59 ± 0.52^{a}	8.68 ± 0.54^{a}	9.34 ± 0.13^{b}	10.64 ± 0.58^{c}	12.52 ± 0.51^{e}	11.98 ± 0.34^{d}	12.45 ± 0.46^{e}
	D2	8.95 ± 0.02^{a}	9.14 ± 0.08^{a}	9.38 ± 0.11^{a,b}	10.25 ± 0.64^{c}	10.52 ± 0.54^{c}	10.98 ± 1.00^{d}	11.69 ± 0.59^{e}
	D4	8.83 ± 0.10^{a}	8.61 ± 0.07^{a}	9.47 ± 0.22^{b}	9.92 ± 0.08^{b}	10.70 ± 0.07^{c}	11.91 ± 1.01^{d}	11.51 ± 0.52^{d}
c20:0	D0	0.07 ± 0.02^{a}	0.07 ± 0.04^{a}	0.07 ± 0.01^{a}	0.08 ± 0.01^{a}	0.07 ± 0.01^{a}	0.07 ± 0.03^{a}	0.07 ± 0.02^{a}
	D2	0.07 ± 0.01^{a}	0.07 ± 0.00^{a}	0.08 ± 0.10^{a}	0.08 ± 0.00^{a}	0.09 ± 0.01^{a,b}	0.09 ± 0.02^{a,b}	0.10 ± 0.04^{b}
	D4	0.07 ± 0.00^{a}	0.09 ± 0.01^{a,b}	0.09 ± 0.00^{a,b}	0.10 ± 0.03^{b}	0.10 ± 0.00^{b}	0.17 ± 0.01^{c}	0.10 ± 0.00^{b}
c22:0	D0	0.18 ± 0.01^{a}	0.27 ± 0.01^{b}	0.28 ± 0.02^{b}	0.39 ± 0.01^{d}	0.33 ± 0.00^{c}	0.43 ± 0.00^{e}	0.47 ± 0.04^{f}
	D2	0.25 ± 0.01^{a}	0.27 ± 0.00^{a,b}	0.29 ± 0.01^{b}	0.34 ± 0.02^{c}	0.36 ± 0.01^{c}	0.34 ± 0.01^{c}	0.42 ± 0.01^{d}
	D4	0.20 ± 0.00^{a}	0.25 ± 0.00^{b}	0.28 ± 0.01^{c}	0.27 ± 0.00^{b,c}	0.25 ± 0.00^{b}	0.29 ± 0.01^{c}	0.29 ± 0.01^{c}
c24:0	D0	0.09 ± 0.00^{a}	0.10 ± 0.01^{a}	0.15 ± 0.00^{b}	0.07 ± 0.01^{a}	0.08 ± 0.00^{a}	0.09 ± 0.01^{a}	0.10 ± 0.01^{a}
	D2	0.09 ± 0.00^{a}	0.09 ± 0.01^{a}	0.11 ± 0.02^{a}	0.08 ± 0.00^{a}	0.09 ± 0.02^{a}	0.09 ± 0.03^{a}	0.10 ± 0.01^{a}
	D4	0.08 ± 0.01^{a}	0.11 ± 0.01^{a}	0.10 ± 0.03^{a}	0.10 ± 0.06^{a}	0.09 ± 0.01^{a}	0.11 ± 0.01^{a}	0.09 ± 0.00^{a}
SFA	D0	29.83 ± 0.00^{a}	30.55 ± 0.19^{b}	32.58 ± 0.24^{c}	35.66 ± 0.33^{d}	37.75 ± 1.10^{e}	38.23 ± 0.31^{f}	38.43 ± 0.75^{f}
	D2	30.8 ± 0.04^{a}	30.91 ± 0.14^{a}	32.01 ± 0.20^{b}	34.97 ± 0.59^{c}	37.02 ± 0.60^{d}	37.53 ± 0.37^{e}	39.35 ± 0.98^{f}
	D4	31.07 ± 0.21^{a}	31.62 ± 0.03^{b}	33.82 ± 0.24^{c}	34.60 ± 0.27^{d}	37.03 ± 0.64^{e}	39.58 ± 1.10^{g}	38.29 ± 0.18^{f}
c16:1	D0	2.41 ± 0.07^{a}	2.28 ± 0.05^{b}	2.11 ± 0.07^{c}	1.75 ± 0.07^{d}	1.25 ± 0.35^{e}	1.06 ± 0.10^{f}	0.58 ± 0.49^{g}
	D2	2.75 ± 0.17^{a}	2.52 ± 0.00^{c}	2.45 ± 0.23^{c}	2.59 ± 0.04^{c}	2.66 ± 0.15^{b}	1.95 ± 0.41^{e}	2.25 ± 0.14^{d}
	D4	3.13 ± 0.01^{a}	2.85 ± 0.15^{b}	2.49 ± 0.24^{c}	2.55 ± 0.03^{c}	2.55 ± 0.02^{c}	2.12 ± 0.05^{e}	2.33 ± 0.18^{d}
c20:1	D0	3.16 ± 0.01^{a}	3.36 ± 0.27^{d}	3.27 ± 0.10^{b}	3.32 ± 0.02^{c}	3.32 ± 0.01^{c}	3.38 ± 0.04^{d}	3.92 ± 0.02^{e}
	D2	3.32 ± 0.02^{d}	3.33 ± 0.01^{d}	3.16 ± 0.02^{b}	3.21 ± 0.1^{c}	3.47 ± 0.06^{e}	3.19 ± 0.05^{c}	3.03 ± 0.00^{a}
	D4	3.16 ± 0.0^{b}	3.08 ± 0.01^{a}	3.06 ± 0.00^{a}	3.15 ± 0.12^{b}	3.05 ± 0.03^{a}	3.61 ± 0.08^{c}	3.15 ± 0.04^{b}
c18:1n9t	D0	0.12 ± 0.00^{a}	0.13 ± 0.00^{a}	0.15 ± 0.01^{a}	0.14 ± 0.00^{a}	0.14 ± 0.00^{a}	0.14 ± 0.00^{a}	0.15 ± 0.00^{a}
	D2	0.13 ± 0.00^{b}	0.13 ± 0.01^{a}	0.12 ± 0.00^{a}	0.14 ± 0.00^{a}	0.19 ± 0.00^{b}	0.20 ± 0.00^{b}	0.20 ± 0.00^{b}
	D4	0.14 ± 0.00^{a}	0.13 ± 0.00^{a}	0.13 ± 0.00^{a}	0.13 ± 0.00^{a}	0.13 ± 0.00^{a}	0.15 ± 0.00^{a,b}	0.17 ± 0.01^{b}
c18:1n9c	D0	24.31 ± 0.62^{a}	24.04 ± 0.12^{a}	23.54 ± 0.11^{a}	21.82 ± 0.59^{e}	22.15 ± 0.09^{d}	20.81 ± 0.42^{f}	21.00 ± 0.58^{f}
	D2	22.18 ± 0.08^{a}	22.04 ± 0.03^{a}	22.12 ± 0.17^{a}	21.06 ± 0.02^{c}	20.71 ± 0.50^{e}	20.93 ± 0.07^{d}	20.32 ± 0.57^{f}
	D4	22.56 ± 0.06^{b}	22.05 ± 0.02^{c}	23.19 ± 0.13^{a}	22.94 ± 0.01^{a,b}	21.25 ± 0.02^{d}	20.71 ± 0.23^{e}	19.97 ± 0.56^{f}
MUFA	D0	30.79 ± 0.6^{a}	29.80 ± 0.12^{b}	29.84 ± 0.28^{b}	27.83 ± 0.61^{c}	27.65 ± 0.32^{c}	26.03 ± 0.51^{e}	26.45 ± 0.28^{d}
	D2	29.02 ± 0.16^{a}	28.73 ± 0.04^{b}	28.56 ± 0.39^{b}	27.85 ± 0.09^{c}	27.95 ± 0.68^{c}	27.22 ± 0.39^{d}	26.78 ± 0.69^{e}
	D4	29.60 ± 0.06^{a}	28.99 ± 0.93^{b}	28.80 ± 0.34^{b}	27.90 ± 0.14^{c}	27.82 ± 0.08^{c}	27.44 ± 0.29^{d}	26.46 ± 0.68^{e}
c18:2n6c	D0	20.39 ± 0.01^{a}	19.81 ± 0.58^{b}	18.87 ± 0.53^{c}	17.61 ± 0.03^{d}	17.02 ± 0.55^{e}	16.93 ± 0.03^{e}	17.19 ± 0.39^{e}
	D2	20.26 ± 0.01^{a}	19.43 ± 0.38^{b}	18.99 ± 0.61^{c}	18.76 ± 0.58^{c}	18.75 ± 0.58^{c}	18.73 ± 0.61^{c}	17.67 ± 0.58^{d}
	D4	21.39 ± 0.53^{a}	20.38 ± 0.08^{b}	19.39 ± 0.01^{c}	18.27 ± 0.56^{d}	17.06 ± 0.05^{e}	17.08 ± 0.04^{e}	16.81 ± 0.50^{e}
c18:3n3	D0	0.65 ± 0.01^{a}	0.61 ± 0.00^{a}	0.74 ± 0.02^{b}	0.79 ± 0.00^{b}	0.76 ± 0.00^{b}	0.84 ± 0.00^{c}	0.91 ± 0.01^{d}
	D2	0.79 ± 0.12^{b}	0.66 ± 0.00^{a}	0.92 ± 0.00^{c}	0.76 ± 0.00^{b}	0.77 ± 0.00^{b}	0.69 ± 0.00^{a}	0.91 ± 0.00^{c}
	D4	0.86 ± 0.00^{b}	0.75 ± 0.00^{a}	0.86 ± 0.00^{b}	0.88 ± 0.00^{c}	0.94 ± 0.00^{d}	0.82 ± 0.00^{b}	0.95 ± 0.04^{d}
c18:3n6	D0	0.16 ± 0.00^{a}	0.14 ± 0.01^{a}	0.15 ± 0.00^{a}	0.15 ± 0.15^{a}	0.13 ± 0.13^{a}	0.13 ± 0.00^{a}	0.15 ± 0.01^{a}
	D2	0.13 ± 0.00^{a}	0.14 ± 0.00^{a}	0.13 ± 0.00^{a}	0.12 ± 0.02^{a}	0.13 ± 0.00^{a}	0.12 ± 0.00^{a}	0.13 ± 0.00^{a}
	D4	0.13 ± 0.03^{a}	0.11 ± 0.00^{a}	0.13 ± 0.00^{a}	0.13 ± 0.00^{a}	0.15 ± 0.01^{a,b}	0.16 ± 0.00^{b}	0.13 ± 0.01^{a}
c20:2	D0	0.93 ± 0.01^{a}	0.72 ± 0.01^{b}	0.70 ± 0.01^{b}	0.69 ± 0.00^{b}	0.69 ± 0.00^{b}	0.69 ± 0.01^{b}	0.72 ± 0.01^{b}
	D2	0.67 ± 0.01^{b}	0.73 ± 0.01^{c}	0.62 ± 0.00^{a}	0.65 ± 0.00^{a,b}	0.67 ± 0.00^{b}	0.87 ± 0.00^{d}	0.73 ± 0.00^{c}
	D4	0.61 ± 0.00^{b}	0.75 ± 0.00^{d}	0.55 ± 0.00^{a}	0.69 ± 0.00^{c}	0.97 ± 0.00^{e}	0.70 ± 0.00^{c}	0.62 ± 0.04^{b}
c20:3n3	D0	0.20 ± 0.00^{a}	0.16 ± 0.01^{b}	0.16 ± 0.00^{b}	0.15 ± 0.01^{b}	0.15 ± 0.00^{b}	0.13 ± 0.01^{b}	0.14 ± 0.01^{b}
	D2	0.18 ± 0.00^{a}	0.17 ± 0.00^{a}	0.17 ± 0.00^{a}	0.14 ± 0.00^{a}	0.15 ± 0.00^{a}	0.15 ± 0.00^{a}	0.14 ± 0.00^{a}
	D4	0.19 ± 0.00^{a}	0.17 ± 0.00^{a}	0.17 ± 0.00^{a}	0.19 ± 0.00^{a}	0.19 ± 0.00^{a}	0.18 ± 0.00^{a}	0.20 ± 0.00^{a}
c20:3n6	D0	1.64 ± 0.04^{a}	1.29 ± 0.01^{b}	1.29 ± 0.00^{b}	1.24 ± 0.00^{c}	1.22 ± 0.00^{c}	1.20 ± 0.01^{c}	1.25 ± 0.01^{c}
	D2	1.98 ± 0.02^{a}	1.94 ± 0.00^{a,b}	1.95 ± 0.00^{a,b}	1.72 ± 0.00^{d}	1.93 ± 0.00^{b}	1.84 ± 0.01^{c}	1.83 ± 0.01^{c}
	D4	1.20 ± 0.00^{c,d}	1.23 ± 0.00^{c}	1.30 ± 0.00^{b}	1.54 ± 0.00^{a}	1.17 ± 0.00^{d}	0.88 ± 0.00^{e}	0.76 ± 0.00^{f}
c20:4n6	D0	5.48 ± 0.00^{a}	5.03 ± 0.00^{b}	5.01 ± 0.13^{b}	5.06 ± 0.00^{b}	5.20 ± 0.20^{c}	5.19 ± 0.24^{c}	5.11 ± 0.22^{c}
	D2	5.51 ± 0.01^{a}	4.94 ± 0.00^{b}	4.57 ± 0.00^{c}	4.03 ± 0.58^{e}	4.00 ± 0.58^{e}	4.40 ± 0.50^{d}	4.36 ± 0.02^{d}
	D4	5.71 ± 0.01^{a}	4.43 ± 0.57^{e}	4.26 ± 0.00^{f}	4.58 ± 0.58^{d}	4.94 ± 0.01^{b}	4.70 ± 0.03^{c}	4.62 ± 0.01^{d}
c20:5n3	D0	0.39 ± 0.01^{a}	0.30 ± 0.01^{b}	0.28 ± 0.00^{b}	0.23 ± 0.01^{c}	0.22 ± 0.00^{c}	0.22 ± 0.01^{c}	0.20 ± 0.00^{c}
	D2	0.31 ± 0.00^{b}	0.36 ± 0.00^{a}	0.38 ± 0.00^{a}	0.29 ± 0.00^{b,c}	0.33 ± 0.00^{b}	0.27 ± 0.00^{c}	0.24 ± 0.00^{c}
	D4	0.19 ± 0.00^{b}	0.14 ± 0.01^{a}	0.18 ± 0.00^{b}	0.21 ± 0.00^{b,c}	0.23 ± 0.00^{c}	0.21 ± 0.00^{b,c}	0.52 ± 0.00^{d}
c22:6	D0	0.58 ± 0.01^{a}	0.48 ± 0.00^{b}	0.21 ± 0.13^{d}	0.48 ± 0.00^{b}	0.47 ± 0.00^{b}	0.46 ± 0.13^{b}	0.28 ± 0.00^{c}
	D2	0.57 ± 0.02^{a}	0.47 ± 0.01^{b}	0.46 ± 0.00^{b}	0.41 ± 0.00^{c}	0.44 ± 0.01^{b,c}	0.45 ± 0.00^{b,c}	0.42 ± 0.00^{c}
	D4	0.38 ± 0.00^{c}	0.33 ± 0.00^{b}	0.22 ± 0.00^{a}	0.41 ± 0.00^{c,d}	0.43 ± 0.01^{d}	0.48 ± 0.00^{e}	0.53 ± 0.00^{f}
PUFA	D0	30.42 ± 0.05^{a}	28.55 ± 0.57^{b}	27.42 ± 0.53^{c}	26.43 ± 0.00^{d}	25.85 ± 0.57^{f}	25.80 ± 0.24^{f}	25.94 ± 0.29^{e}
	D2	30.41 ± 0.01^{a}	28.85 ± 0.40^{b}	28.19 ± 0.60^{c}	26.90 ± 1.15^{f}	27.17 ± 1.16^{e}	27.53 ± 1.03^{d}	26.44 ± 0.57^{g}
	D4	30.65 ± 0.00^{a}	28.29 ± 0.53^{b}	27.04 ± 0.01^{c}	26.9 ± 0.61^{d}	26.07 ± 0.05^{e}	25.20 ± 0.62^{f}	25.13 ± 0.53^{f}

$^{a–f}$Means in the same row with different letters differ significantly ($P < 0.05$).

Data expressed as mean ± SD.

Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

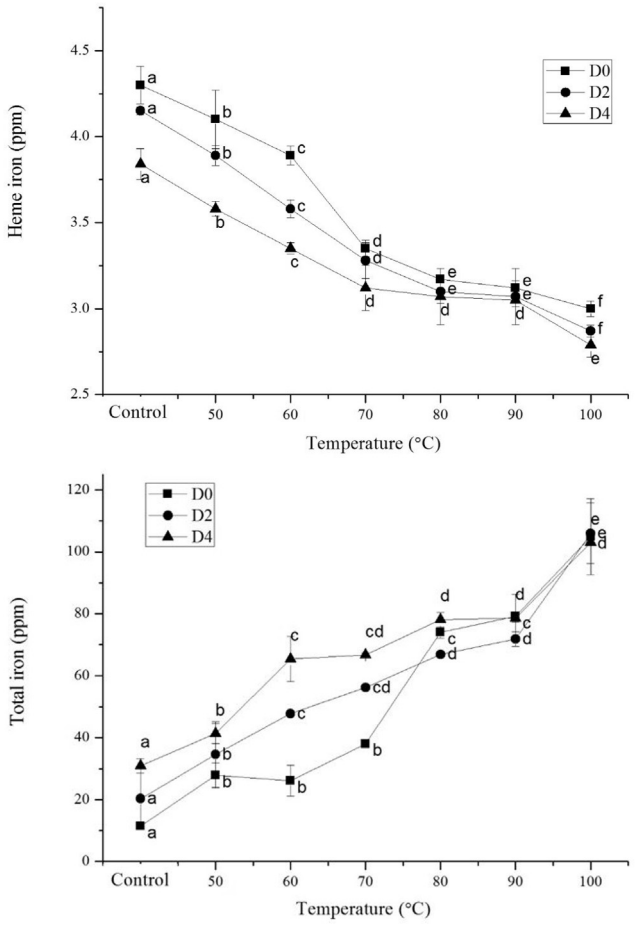

Figure 2. Heme iron and total iron content in chicken meat after heating to different temperatures and subsequent storage for 0, 2, and 4 D at 4°C. Bars indicate standard deviation. For each day, different superscripts indicate significant difference ($P < 0.05$).

contrast, the total iron content increased with the rise of temperature. The storage further decreased the heme iron content and increased the total iron. The inverse relationship between heme iron and total iron is in agreement with previous studies (Kristensen and Purslow 2001; Lombardi-Boccia et al., 2002; Muhlisin et al., 2016). Liberation of iron from iron containing protein and the moisture loss upon heating might account for the increase of the total iron content in meat (Lombardi-Boccia et al., 2002). It has been proposed that the major part of heme iron in meat is located in myoglobin, and the thermal stability of myoglobin is highly dependent on the intact heme iron (Chanthai et al., 1996). Both heme-iron and simpler iron species have been identified as oxidation catalysts in muscle tissue (Carlsen et al., 2005); however, Min et al. (2010) found that lipid oxidation was more pronounced in cooked chicken meat added with free ionic iron than myoglobin, which suggested free ionic iron is the major catalyst for lipid oxidation.

Hydroxyl Radical Production

The hydroxyl radical was not detected in unheated and 50°C to 80°C heated meat on day 0, and a very low level of hydroxyl radical was detected in 90°C and 100°C heated meat (Figure 3). On day 2 and day 4, a large amount of hydroxyl radical was generated, and it showed an upward trend with the increase of heating temperature. The hydroxyl radical increased significantly from unheated to 60°C heated meat ($P < 0.05$) and maintained at the same level at 70°C ($P > 0.05$), and it further increased at 80°C but had no significant difference with those heated to 90°C and 100°C ($P > 0.05$). The reactive oxygen species, the hydroxyl radical, the superoxide anion, and its conjugate acid are proposed to be mainly generated in the Fenton reaction which involves the oxidation of organic compounds in the presence of iron and hydrogen peroxide (Carlsen et al., 2005). Superoxide radicals are not particularly effective at causing hydrogen abstraction, and owing to the negative charge, they are not vey lipophilic, but hydroxyl radicals are much more effective and have a low degree of selectivity in their reactions (Reis and Spickett, 2012).

Antioxidant Enzymes Activities

The susceptibility of muscle to lipid oxidation is influenced by a number of factors, and antioxidant enzymes are considered as important protectors against lipid oxidation. SOD and catalase are coupled enzymes. SOD scavenges superoxide anion by forming hydrogen peroxide, and catalase decomposes hydrogen peroxide to water and superoxide anion. GSHPx can decompose both hydrogen peroxide and lipoperoxides formed during lipid oxidation (Gatellier et al., 2004). The activity of CAT increased significantly when the meat was heated at 50°C ($P < 0.05$) and decreased at 60°C, but it had no significant difference with that of unheated meat ($P > 0.05$) (Figure 4). Heating to 70°C destroyed more than 50% of its initial activity, and more than 80% activity disappeared when heating to 100°C. SOD showed a similar trend that the activity increased initially at

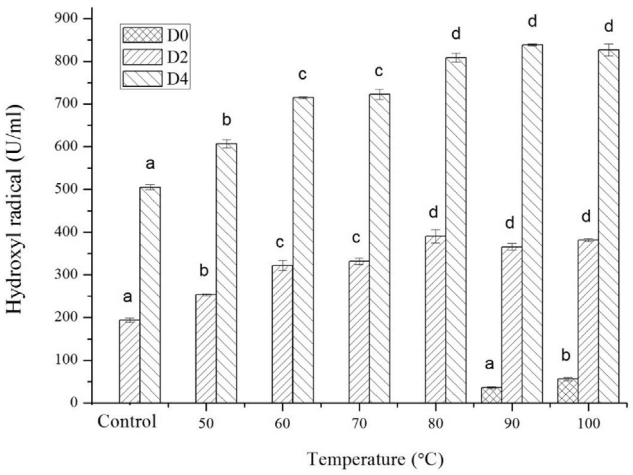

Figure 3. Hydroxyl radical content in chicken meat after heating to different temperatures and subsequent storage for 0, 2, and 4 D at 4°C. Bars indicate standard deviation. For each day, different superscripts indicate significant difference ($P < 0.05$).

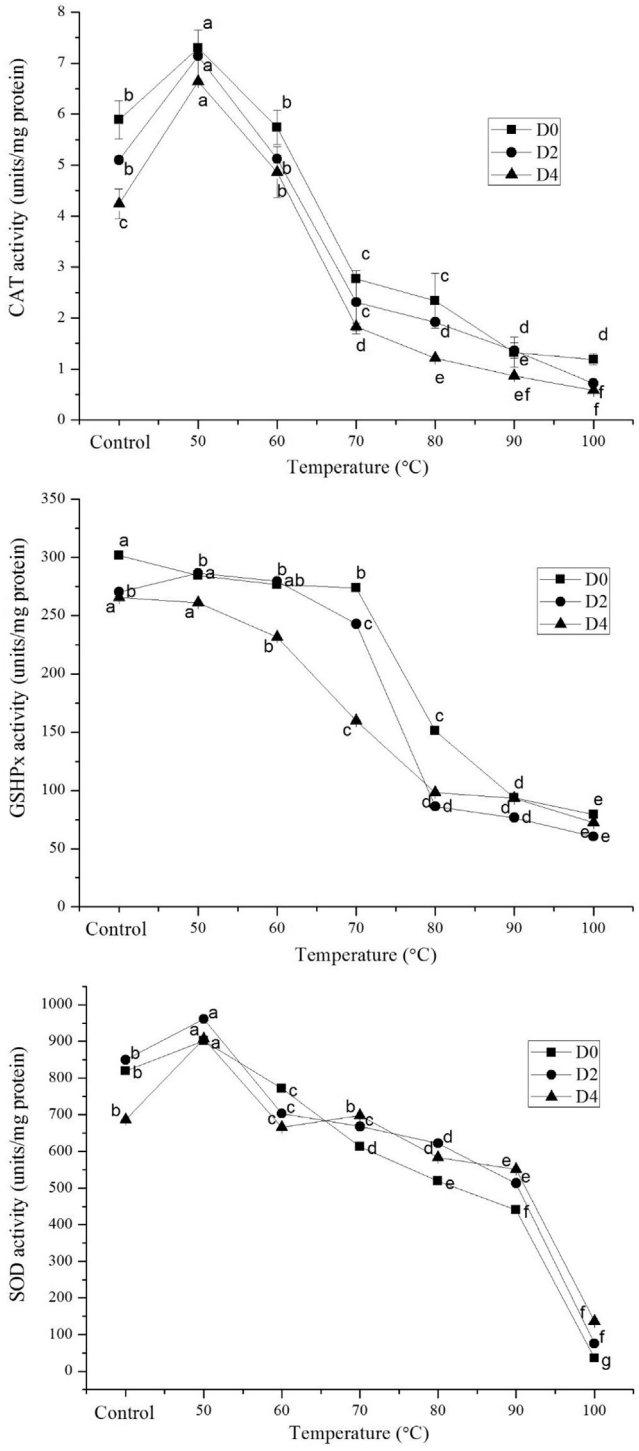

Figure 4. Antioxidant enzymes activities in chicken meat after heating to different temperatures and subsequent storage for 0, 2, and 4 D at 4°C. Bars indicate standard deviation. For each day, different superscripts indicate significant difference ($P < 0.05$). Abbreviations: CAT, catalase; SOD, superoxide dismutase; GSHPx, glutathione peroxidase.

50°C and then significantly decreased after heating to 60°C and higher temperatures ($P < 0.05$). The activity of GSHPx had little change in the range of 50°C to 70°C heating treatment, but it rapidly declined when heated to 80°C and higher ($P < 0.05$) at day 0. After the heat treatment, the activities of these enzymes remained at approximately the same level during the storage.

It has been shown that catalase from camel liver, SOD from sea cucumber, and GSHPx from rat liver had the optimal temperature of 45°C, 40°C, and 32°C, respectively (Shul'Gim et al., 2008; Chafik et al., 2017; Li et al., 2018). GSHPx was found to be stable after heating to 63°C and lose its activity when heated to 80°C–90°C, catalase had a similar sensitivity, whereas SOD had a somewhat higher resistance to heat (Mei et al., 1994; Lindmark-Månsson et al., 2001). With the increase of the heating temperature, the antioxidant enzyme activities may reach their optimum, but further increase of the temperature led to denaturation of these enzymes and consequently gave rise to the rapid lipid oxidation. These antioxidant enzymes have also been reported to resist the hydroxyl radical (Guo et al., 2015; Zeng et al., 2018), and they might play the role in protecting the lipids against high level of hydroxyl radical on day 2 and day 4.

CONCLUSIONS

Our findings show that lipid oxidation was more rapid in chicken meat heated above 70°C, and the unsaturated fatty acids content decreased accordingly with the increased temperature. Heating also led to the increased nonheme iron and hydroxyl radical content. The activity of CAT significantly decreased at 70°C, and SOD and GSHPx lost most of their activities above 70°C of heating, which was corresponding to the increased lipid oxidation level. The increase in nonheme iron and hydroxyl radical along with the decrease in antioxidant enzymes activities might be responsible for the higher lipid oxidation in meat during heating, and more work could be conducted to understand the biochemical changes contributing to lipid oxidation during heating of meat products.

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Conflict of Interest: The authors declare that they have no conflicts of interest regarding the contents of this article.

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