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## Influence of Cooking, Storage Period, and Re-heating on Production of Cholesterol Oxides in Chicken Meat

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**Abstract** The objective of present study was to investigate the effect of cooking and their combinations with re-heating methods on the formation of cholesterol oxidation products (COPs) in stored chicken thigh meat. Pan roasting, steaming, oven grilling, charcoal grilling, and microwaving were used for cooking. Re-heating of samples was done using the same cooking methods or microwaving after 3 and 6 d of refrigerated storage. Cooking and re-heating resulted in reduction of crude fat and cholesterol contents of chicken thigh meat depending on storage period before re-heating. Cooking and storage period had no influence on the total amount of COPs. The highest total amount of COPs was observed in meat samples cooked by steaming and reheated by microwaving after 6 d of storage, which showed similar value to raw chicken meat stored for 6 days. However, different re-heating methods formed different types of COPs depending on storage period before re-heating. The high amount ( $p < 0.05$ ) of 25-hydroxycholesterol or  $\alpha$ -epoxide was detected in meat samples reheated by steaming or microwaving at 3 or 6 d of storage after steamed cooking, respectively. As a result, the combination of steaming and re-heating with microwaving could increase the total amount of COPs in chicken thigh meat and different cooking/re-heating methods could form different types of COPs, even though no significant difference in the total amount of COPs depending on storage period.

**Keywords** re-heating, chicken meat, storage period, total cholesterol, cholesterol oxides

## Introduction

Food safety and health have become major issues of consumers and they tend to pay more attention on them. The major cause of food deterioration during storage is lipid

oxidation which affects flavor, appearance, nutritional value, and safety of the foods (Waraho et al., 2011; Zamora and Hidalgo, 2016). In general, fats of animal origin are considered not healthy because of their high level of saturated fatty acid and cholesterol (Conchillo et al., 2005). Poultry meat is preferred over other meats due to its higher polyunsaturated fatty acids (PUFAs) while it is more prone to oxidation (Luna et al., 2010). The balance of pro- and anti-oxidants, PUFAs, cholesterol, proteins, and pigments play a major role in determining the oxidative stability of meat and meat products (Bertelsen et al., 2001). The excessive oxidation of meat lipids leads to high production of potential precursors with reactive aldehydes in tissues and foods becoming a source of oxidative stress (Bou et al., 2009; Luna et al., 2010). These aldehydes can be major contributing factor in several pathological conditions such as atherosclerosis, inflammation, aging, arthritis, Alzheimer's and Parkinson's disease (Rossin et al., 2017). Cholesterol, monounsaturated lipid, is a widely distributed in animal origin foods and can be oxidized under light and oxygen exposure and storage as increasing the formation of cholesterol oxidation products (COPs) (Paniangvait et al., 1995). Cholesterol is a compound with biological important, but its oxidation products (COPs) have been proven to cytotoxic, mutagenic, and carcinogenic (Ryan et al., 2005) and to be a primary factor for triggering atherosclerosis (Garcia-Cruset et al., 2002).

Processing including cooking, deep frying, and dehydrating is one of the main factors causing cholesterol oxidation in animal origin foods (Asmaa and Tajul, 2017). The extent of cholesterol oxidation in food is affected by food matrix composition, presence of pro- and anti-oxidants, food processing including cooking and storage conditions (Garcia-Cruset et al., 2002). Sarantinos et al. (1993) reported increase in COPs content of fried and boiled eggs by prolonging the cooking time. Khan et al. (2015) studied the effects of different cooking and re-heating methods on the cholesterol and COPs in various processed meat products during refrigerated storage. They reported that the highest COPs formation was found in grilling and microwaving as cooking and re-heating method. However, the fresh chicken meat would not be the same response as processed meat, the current study was performed with objective to investigate the effect of various cooking and re-heating methods and storage period after cooking on total cholesterol and COPs formation (type and amount) in chicken thigh meat during storage at 4°C.

## Materials and Methods

### Chemicals and reagents

Cholesterol, linoleic acid, oleic acid, COPs standards [7-ketocholesterol (7-keto), 6-ketocholesterol (6-keto), 7 $\alpha$ -hydroxycholesterol (7 $\alpha$ -OH), 7 $\beta$ -hydroxycholesterol (7 $\beta$ -OH), 5,6 $\alpha$ -epoxycholesterol (5,6 $\alpha$ -EP), 5,6 $\beta$ -epoxycholesterol (5,6 $\beta$ -EP), 25-hydroxycholesterol (25-OH), 20-hydroxycholesterol (20-OH), and cholestanetriol (triol)], butylated hydroxytoluene (BHT), pyridine, and silicic acid (100 mesh) were purchased from Sigma-Aldrich Co., LLC (Korea). *Bis*-[trimethylsilyl]-trifluoroacetamide (BSTFA)+1% trimethylchlorosilane (TMCS) was obtained from Supelco (USA). Hexane, ethyl acetate, acetone, methanol and chloroform of HPLC grades, celite545, and calcium phosphate (CaHPO<sub>4</sub>·2H<sub>2</sub>O) were purchased from Fisher Scientific Co. (USA).

### Sample preparation and cooking

Chicken thighs were purchased from a slaughterhouse at about 6 h postmortem and stored at 4±1°C. Thigh meat was trimmed for surface adipose tissue and sliced to approximately 1.5 cm thick. The five cooking methods used for comparison were pan roasting (PR), steaming (ST), oven grilling (OG), charcoal grilling (CG), and microwaving (MW). The initial

surface temperature of the meat samples was 4°C and cooking was done when all the samples had reached an internal temperature of 70±2°C through PR using an electric pan (Excel 10 Electric grill, Tefal, France; 170°C during 20 min), ST using electric steamer (steam cuisine 900 turbo Diffusion®, Tefal, France), OG using a convention oven (GR-643HT, Tong Yang Magic, Korea; 150°C for 1 h), CG (240°C for 5 min) and MW using a microwave oven (M-M270TC, LG Electronics, Korea; 700 W power and 2,450 MHz for 10 min). The internal temperature was checked at a depth of 3 cm from side surface in the core of chicken thigh using thermometer with K-type sensor (TM-747DU, Tenmars, Taiwan). The cooked samples were stored at 4°C for 6 d under aerobic conditions and re-heating was done after 3 and 6 d using the same cooking method or MW. The time of re-heating varied depending on different cooking methods as PR (5 min), ST (10 min), OG (10 min), CG (2 min) and MW (1 min). The raw and cooked samples were analyzed for the fat and cholesterol content and amount of COPs at 0, 3, and 6 d of storage.

### Determination of crude fat content

Crude fat content of meat samples was determined using the Soxhlet method with a solvent extraction system (Soxtec® 2300 Analyzer Unit, Foss Analytical AB, Sweden).

### Determination of total cholesterol content

Total cholesterol content of chicken thigh meat was determined by following the method described by Lee et al. (2006). Briefly, 0.5 g of fat was diluted in 10 mL freshly prepared 1 M methalonic potassium hydroxide solution and 1 g of sea-sand was added to solution. The aliquot was heated at 85°C for 25 min and supernatant was transferred into 25-mL volumetric flask with a pipette. The residues were boiled with 6 mL isopropanol under reflux condenser for 5 min, collected the solutions, cooled and diluted to the mark with isopropanol. The turbid solutions were filtered through a filter paper (Whatman No. 1, Whatman Inc., USA). The clear aliquot was used for cholesterol assay by using kit (Cat. No 139050, Bohringer Mannheim, Germany). Blank sample solution was prepared by mixing 0.4 mL of the extracted sample solution and 5 mL of solution 4 (cholesterol reagent mixture). Sample solution was a mixture of 2.5 mL of the extracted sample solution and 0.02 mL of solution 3 (enzyme mixture). The prepared blank and sample solutions were sealed with paraffin film and incubated at 37–40°C for 60 min. Absorbance of blank (A1) and the sample (A2) were determined using UV spectrophotometer (UV1601, Shimadzu Co., Japan) at 405 nm. Cholesterol contents (mg/100 g) were calculated using the equation as:

$$\text{Cholesterol content (mg/100 g)} = [0.711 \times (A2 - A1) / \text{sample weight (g)}] \times 100 \times 25$$

### Determination of cholesterol oxidation products (COPs)

The COPs were analyzed according to the method of Lee et al. (1996). A solid-phase column was prepared for the separation of cholesterol oxides (Zubillaga and Maerker, 1991) by mixing silicic acid, celite 545 and CaHPO<sub>4</sub>·2H<sub>2</sub>O (10:9:1, w/w/w) with 30 mL chloroform and packed in a glass column (12 mm×30 cm). The prepared column was repeatedly pre-washed with 5 mL hexane before the application of samples. Total lipids were extracted by following the method of Folch et al. (1957). Briefly, 0.2 g lipid sample was dissolved in 2 mL hexane:ethyl acetate (100:2, v/v) and applied to a prewashed column. The sample container was washed twice with the 2 mL mixture (hexane:ethyl acetate=100:2) and the wash solvent was applied to the column again. Neutral lipid and cholesterol (phospholipids) were removed by adding 50 mL solvent I

(CHCl<sub>3</sub>:CH<sub>3</sub>OH=2:1, v/v) and 60 mL solvent II (hexane:ethyl acetate=4:1, v/v). Forty milliliter of solvent III (acetone:ethylacetate:methanol=50:50:5, v/v/v) was used at 1 mL/min to elute COPs. The collected solutions were dried on a 50°C hot plate with nitrogen gas flushing. The dried extracts were derivatized by heating at 80°C for 1 h in the presence of 200 µL pyridine and 100 µL sylon-bis (trimethylsilyl) trifluoroacetamide+1% trimethylchlorosilane. The COPs were analyzed with a gas chromatograph (HP 5890 plus, Hewlett-Packard, USA) equipped with an on-column capillary injector and a flame ionization detector. Identification of cholesterol and its oxides was performed by comparison of the retention times of the samples with those of the standards and the characteristics of the absorption spectra.

### Statistical analysis

Statistical analysis was performed by one-way Analysis of Variance (ANOVA), and significant differences were detected by Duncan's multiple range test using SAS software (SAS, Release 8.01, SAS Institute Inc., USA). The values are expressed as means±standard error.

## Results and Discussion

### Effect of cooking, re-heating methods, and storage period on crude fat content

Cooking of raw chicken meat samples significantly decreased (19.0–27.4%) the crude fat content at 0 d of storage regardless of cooking methods (Table 1). The reduction in fat content of chicken meat might be due to its open structure which can lose its fat during cooking (Gerber et al., 2009). Changes in crude fat content by re-heating depended on storage period before re-heating. There were no significant differences in crude fat content between raw (or cooked) and re-heated meat samples after 3 d and 6 d of storage. However, after 6 d of storage, the combination of CG cooking and MW re-heating exhibited a significant reduction in crude fat of thigh meat.

### Effect of cooking, re-heating methods, and storage period on total cholesterol content

Cooking by PR, ST, and MW induced lower values ( $p<0.05$ ) and grilled meat samples (both OG and CG) showed similar

**Table 1.** Effect of cooking, re-heating method, and storage period before re-heating on the crude fat content (g/100 g meat) of chicken thigh during storage at 4 °C

Cooking method <sup>1)</sup>	0 d	Re-heating method <sup>1)</sup>	3 d	Re-heating method	6 d
Raw	7.58±0.75 <sup>A</sup>		6.89±1.00		6.90±1.02 <sup>A</sup>
PR	5.60±0.69 <sup>B</sup>	PR	4.70±0.41	PR	5.46±1.05 <sup>CDE</sup>
	5.60±0.69 <sup>B</sup>	MW	5.20±0.77	MW	5.05±0.13 <sup>E</sup>
ST	6.14±0.13 <sup>B</sup>	ST	6.13±1.00	ST	5.87±0.07 <sup>BCD</sup>
	6.14±0.13 <sup>B</sup>	MW	5.17±1.00	MW	6.03±0.11 <sup>BC</sup>
OG	6.44±0.43 <sup>B</sup>	OG	6.56±1.03	OG	6.75±0.10 <sup>A</sup>
	6.44±0.43 <sup>B</sup>	MW	6.88±1.02	MW	6.15±0.13 <sup>AB</sup>
CG	5.50±0.91 <sup>B</sup>	CG	4.47±1.01	CG	4.28±0.11 <sup>F</sup>
	<sup>a</sup> 5.50±0.91 <sup>B</sup>	MW	<sup>a</sup> 5.45±0.73	MW	<sup>b</sup> 4.16±0.13 <sup>F</sup>
MW	5.66±0.06 <sup>B</sup>	MW	5.27±1.05	MW	5.28±0.08 <sup>DE</sup>

<sup>1)</sup> Cooking and re-heating method: PR, pan roasting; ST, steaming; OG, oven grilling; CG, charcoal grilling; MW, microwaving.

<sup>A-F</sup> Means±SE with different superscript in the same column differ significantly ( $p<0.05$ ).

<sup>a,b</sup> Means±SE with different superscript in the same row differ significantly ( $p<0.05$ ).

values ( $p>0.05$ ) in total cholesterol content compared to raw meat samples at 0 d (Table 2). The difference in cholesterol content might be caused by loss of cholesterol during cooking process by different cooking methods (Rodriguez-Estrada et al., 1997). The chicken thigh meat re-heated after 3 and 6 d of storage showed significantly lower total cholesterol content compared to raw meat samples regardless of cooking and re-heating methods. With increasing storage period before re-heating, the reheated samples by ST/ST, OG/MW, CG/CG, and CG/MW (cooking method/re-heating method) showed significant decrease in total cholesterol content. Similarly, Saldanha et al. (2008) observed a decrease in total cholesterol and polyunsaturated fatty acid during storage and grilling of sardine. Additionally, Baggio and Bragagnolo (2006) found the lipids and cholesterol content of grilled pork sausages and grilled chester hamburger were significantly lower than those of the raw samples, relatively. Generally, the re-heating combinations of ST, OG and CG in thigh meat after 3 and 6 d of storage showed a significant change in total cholesterol content. The highest reduction (80%) in cholesterol content of thigh meat was observed for CG/MW and CG/CG after 6 d of storage. Then higher reduction was followed by ST/ST and PR/PR after 6 d of storage. The relative reduction of total cholesterol might be caused by an increase in COPs formation in meat samples during storage and/or cooking or re-heating process (Freitas et al., 2015).

### Effect of cooking, re-heating methods, and storage period on cholesterol oxidation products (COPs) and ratio of COPs/ cholesterol

As most predominant free radical-mediated COPs, 7-keto,  $20\alpha$ -OH, 25-OH,  $7\alpha$ -OH,  $7\beta$ -OH, 5,6 $\alpha$ -epoxide, 5,6 $\beta$ -epoxide, and triol were detected in foods (Medina-Meza et al., 2014). In this study, the raw chicken thigh contained  $20\alpha$ -OH, triol, and  $\alpha$ -epoxide (Table 3). Previous studies observed 25-OH in chicken meat (Conchillo et al., 2005),  $\alpha$ -epoxide in raw turkey meat (Nam et al., 2001), and  $20\alpha$ -OH, 25-OH, and triol in raw pork (Min et al., 2016). The cooking method did not affect the type and total amounts of COPs/cholesterol of COPs production in chicken meat except for 25-OH (Tables 3 and 6). The chicken meat sample cooked by CG showed the highest values in 25-OH ( $p<0.05$ ) among the treatments.

The  $\alpha$ -epoxide, a secondary oxidation product, was observed in chicken thigh meat after cooking by all methods and storage period except for OG/MW after 6 d of storage (Tables 3, 4, and 5). The 7-keto and  $20\alpha$ -OH were not detected in all

**Table 2.** Effect of cooking, re-heating method, and storage period before re-heating on the cholesterol content (mg/100 g meat) of chicken thigh during storage at 4 °C

Cooking method <sup>1)</sup>	0 d	Re-heating method <sup>1)</sup>	3 d	Re-heating method	6 d
Raw	153.6±19.41 <sup>A</sup>		153.8±18.92 <sup>A</sup>		156.0±12.34 <sup>A</sup>
PR	99.6±17.64 <sup>CD</sup>	PR	74.1±6.94 <sup>I</sup>	PR	69.2±11.41 <sup>G</sup>
	99.6±17.64 <sup>CD</sup>	MW	76.4±12.77 <sup>H</sup>	MW	79.4±2.98 <sup>F</sup>
ST	<sup>ab</sup> 81.3±23.67 <sup>D</sup>	ST	<sup>a</sup> 128.3±19.79 <sup>C</sup>	ST	<sup>b</sup> 58.9±3.10 <sup>H</sup>
	<sup>b</sup> 81.3±23.67 <sup>D</sup>	MW	<sup>a</sup> 123.9±24.07 <sup>D</sup>	MW	<sup>a</sup> 130.3±6.30 <sup>C</sup>
OG	<sup>a</sup> 122.7±9.99 <sup>ABC</sup>	OG	<sup>b</sup> 80.2±14.81 <sup>G</sup>	OG	<sup>a</sup> 139.7±7.11 <sup>B</sup>
	<sup>a</sup> 122.7±9.99 <sup>ABC</sup>	MW	<sup>a</sup> 135.2±21.13 <sup>B</sup>	MW	<sup>b</sup> 93.9±2.24 <sup>E</sup>
CG	<sup>a</sup> 142.7±21.53 <sup>AB</sup>	CG	<sup>b</sup> 51.1±9.90 <sup>J</sup>	CG	<sup>b</sup> 47.3±1.54 <sup>I</sup>
	<sup>a</sup> 142.7±21.53 <sup>AB</sup>	MW	<sup>a</sup> 101.4±13.86 <sup>F</sup>	MW	<sup>b</sup> 31.5±2.01 <sup>J</sup>
MW	108.7±17.29 <sup>BCD</sup>	MW	103.9±16.91 <sup>E</sup>	MW	94.0±4.68 <sup>D</sup>

<sup>1)</sup> Cooking and re-heating method: PR, pan roasting; ST, steaming; OG, oven grilling; CG, charcoal grilling; MW, microwaving.

<sup>A-H</sup> Means±SE with different superscript in the same column differ significantly ( $p<0.05$ ).

<sup>a,b</sup> Means±SE with different superscript in the same row differ significantly ( $p<0.05$ ).

**Table 3. Effect of cooking method on the cholesterol oxidation products (COPs) and ratio of COPs/cholesterol in chicken thigh at day 0**

Cooking method <sup>1)</sup>	COPs ( $\mu\text{g}/100\text{ g}$ )						Total amount of COPs /cholesterol (%)
	7 $\beta$ -OH <sup>2)</sup>	7-keto	20 $\alpha$ -OH	25-OH	Triol	$\alpha$ -epoxide	
Raw	nd <sup>3)</sup>	nd	3.7 $\pm$ 16.87	nd <sup>B</sup>	3.2 $\pm$ 0.06	79.3 $\pm$ 207.31	0.20 $\pm$ 0.09
PR	nd	nd	nd	nd <sup>B</sup>	nd	172.5 $\pm$ 150.23	0.17 $\pm$ 0.15
ST	nd	nd	4.4 $\pm$ 7.56	nd <sup>B</sup>	24.8 $\pm$ 32.58	176.6 $\pm$ 152.94	0.13 $\pm$ 0.08
OG	7.0 $\pm$ 12.04	nd	3.8 $\pm$ 6.50	nd <sup>B</sup>	nd	169.5 $\pm$ 147.00	0.12 $\pm$ 0.10
CG	nd	nd	nd	65.1 $\pm$ 8.13 <sup>A</sup>	nd	78.8 $\pm$ 136.48	0.09 $\pm$ 0.07
MW	nd	nd	nd	nd <sup>B</sup>	nd	131.3 $\pm$ 125.96	0.09 $\pm$ 0.09

<sup>1)</sup> Cooking method: PR, pan roasting; ST, steaming; OG, oven grilling; CG, charcoal grilling; MW, microwaving.

<sup>2)</sup> Abbreviation: 7 $\beta$ -OH, 7 $\beta$ -hydroxycholesterol; 7-keto, 7-ketocholesterol; 20 $\alpha$ -OH, 20 $\alpha$ -hydroxycholesterol; 25-OH, 25-hydroxycholesterol; triol, cholestane-3 $\beta$ , 5 $\alpha$ , 6 $\beta$ -triol;  $\alpha$ -epoxide, cholesterol-5 $\alpha$  and 6 $\alpha$ -epoxide.

<sup>3)</sup> nd=not detected.

<sup>A,B</sup> Means $\pm$ SE with different superscript in the same column differ significantly ( $p < 0.05$ ).

combinations of cooking and re-heating methods (Table 4). The application of ST/ST in chicken meat after 3 days of storage led to the highest value ( $p < 0.05$ ) in 25-OH formation. However, there were no significant differences in total amount of COPs/cholesterol among the various combinations of cooking and re-heating (Table 6).

The storage of chicken meat for 6 d induced the formation of different type of COPs by cooking/re-heating methods (Table 5). The chicken meat cooked by ST/MW after 6 d of storage showed the highest values in  $\alpha$ -epoxide ( $p < 0.001$ ) and total amount of COPs ( $p < 0.001$ ) among the treatments (Tables 5 and 6). According to Dominguez et al (2014), microwaving closely associated with COPs formation of foal meat. Thus, the combination of steaming and microwaving could induce an increase in amount of COPs in cooked chicken thigh in this study. Interestingly, raw chicken meat (1.02% of total cholesterol) stored for 6 d showed similar ratio ( $p > 0.05$ ) in amount of COPs compared to cooked by ST/MW (0.81% of total cholesterol) after 6 days of storage. However, the amount of COPs (0.81 or 1.02% of total cholesterol) is normally detected because food could contain frequently 1% of total cholesterol and occasionally 10% COPs (Addis, 1986).

**Table 4. Effect of cooking and re-heating method on the cholesterol oxidation products (COPs) and ratio of COPs/cholesterol in chicken thigh at day 3**

Cooking/re-heating method <sup>1)</sup>	COPs ( $\mu\text{g}/100\text{ g}$ )						Total amount of COPs /cholesterol (%)
	7 $\beta$ -OH <sup>2)</sup>	7-keto	20 $\alpha$ -OH	25-OH	Triol	$\alpha$ -epoxide	
Raw	nd <sup>3)</sup>	nd	nd	nd <sup>B</sup>	nd	nd	nd
PR/PR	2.4 $\pm$ 4.19	nd	nd	nd <sup>B</sup>	33.4 $\pm$ 57.84	103.4 $\pm$ 108.59	0.15 $\pm$ 0.09
PR/MW	nd	nd	nd	12.9 $\pm$ 22.39 <sup>B</sup>	87.9 $\pm$ 152.17	50.6 $\pm$ 87.61	0.16 $\pm$ 0.18
ST/ST	6.0 $\pm$ 5.21	nd	nd	192.9 $\pm$ 167.12 <sup>A</sup>	nd	143.3 $\pm$ 239.18	0.55 $\pm$ 0.69
ST/MW	nd	nd	nd	82.8 $\pm$ 97.33 <sup>AB</sup>	136.7 $\pm$ 236.82	71.1 $\pm$ 123.22	0.42 $\pm$ 0.38
OG/OG	11.0 $\pm$ 19.02	nd	nd	8.3 $\pm$ 14.41 <sup>B</sup>	194.2 $\pm$ 182.33	114.6 $\pm$ 153.59	0.26 $\pm$ 0.07
OG/MW	nd	nd	nd	15.0 $\pm$ 25.97 <sup>B</sup>	160.1 $\pm$ 160.41	120.3 $\pm$ 208.37	0.24 $\pm$ 0.08
CG/CG	nd	nd	nd	65.1 $\pm$ 8.13 <sup>B</sup>	nd	78.8 $\pm$ 136.48	0.07 $\pm$ 0.06
CG/MW	nd	nd	nd	21.0 $\pm$ 36.37 <sup>B</sup>	154.2 $\pm$ 267.10	51.6 $\pm$ 89.45	0.15 $\pm$ 0.16
MW/MW	8.3 $\pm$ 14.44	nd	Nd	13.4 $\pm$ 23.14 <sup>B</sup>	98.2 $\pm$ 170.03	65.6 $\pm$ 87.78	0.16 $\pm$ 0.15

<sup>1)</sup> Cooking and re-heating method: PR, pan roasting; MW, microwaving; ST, steaming; OG, oven grilling; CG, charcoal grilling.

<sup>2)</sup> Abbreviation: 7 $\beta$ -OH, 7 $\beta$ -hydroxycholesterol; 7-keto, 7-ketocholesterol; 20 $\alpha$ -OH, 20 $\alpha$ -hydroxycholesterol; 25-OH, 25-hydroxycholesterol; triol, cholestane-3 $\beta$ , 5 $\alpha$ , 6 $\beta$ -triol;  $\alpha$ -epoxide, cholesterol-5 $\alpha$  and 6 $\alpha$ -epoxide.

<sup>3)</sup> nd=not detected.

<sup>A,B</sup> Means $\pm$ SE with different superscript in the same column differ significantly ( $p < 0.05$ ).

**Table 5.** Effect of cooking and re-heating method on the cholesterol oxidation products (COPs) and ratio of COPs/cholesterol in chicken thigh at day 6

Cooking/re-heating method <sup>1)</sup>	COPs ( $\mu\text{g}/100\text{ g}$ )						Total amount of COPs /cholesterol (%) <sup>***</sup>
	7 $\beta$ -OH <sup>2)</sup>	7-keto	20 $\alpha$ -OH	25-OH	Triol	$\alpha$ -epoxide <sup>*</sup>	
Raw	nd <sup>3)</sup>	nd	nd	1.5 $\pm$ 0.59	nd	nd <sup>B</sup>	1.02 $\pm$ 0.16 <sup>A</sup>
PR/PR	nd	nd	nd	9.1 $\pm$ 15.72	71.5 $\pm$ 123.81	33.4 $\pm$ 57.84 <sup>B</sup>	0.12 $\pm$ 0.15 <sup>B</sup>
PR/MW	nd	nd	nd	88.6 $\pm$ 138.86	68.7 $\pm$ 119.05	53.9 $\pm$ 93.38 <sup>B</sup>	0.22 $\pm$ 0.07 <sup>B</sup>
ST/ST	nd	nd	nd	10.4 $\pm$ 17.94	43.7 $\pm$ 75.66	33.4 $\pm$ 57.89 <sup>B</sup>	0.12 $\pm$ 0.11 <sup>B</sup>
ST/MW	nd	145.5 $\pm$ 252.05	nd	91.4 $\pm$ 109.68	nd	311.5 $\pm$ 126.29 <sup>A</sup>	0.81 $\pm$ 0.69 <sup>A</sup>
OG/OG	nd	126.1 $\pm$ 218.35	nd	19.5 $\pm$ 33.71	137.8 $\pm$ 148.95	144.2 $\pm$ 185.24 <sup>AB</sup>	0.35 $\pm$ 0.23 <sup>B</sup>
OG/MW	nd	nd	nd	9.7 $\pm$ 16.82	33.7 $\pm$ 58.37	nd <sup>B</sup>	0.03 $\pm$ 0.06 <sup>B</sup>
CG/CG	1.9 $\pm$ 3.23	nd	nd	2.8 $\pm$ 4.79	37.8 $\pm$ 65.50	23.3 $\pm$ 40.11 <sup>B</sup>	0.05 $\pm$ 0.04 <sup>B</sup>
CG/MW	nd	nd	nd	1.8 $\pm$ 3.16	42.5 $\pm$ 73.63	35.2 $\pm$ 30.54 <sup>B</sup>	0.05 $\pm$ 0.06 <sup>B</sup>
MW/MW	nd	nd	nd	17.4 $\pm$ 30.16	92.2 $\pm$ 159.63	174.0 $\pm$ 150.91 <sup>AB</sup>	0.26 $\pm$ 0.02 <sup>B</sup>

<sup>1)</sup> Cooking and re-heating method: PR, pan roasting; ST, steaming; OG, oven grilling; CG, charcoal grilling; MW, microwaving.

<sup>2)</sup> Abbreviation: 7 $\beta$ -OH, 7 $\beta$ -hydroxycholesterol; 7-keto, 7-ketocholesterol; 20 $\alpha$ -OH, 20 $\alpha$ -hydroxycholesterol; 25-OH, 25-hydroxycholesterol; triol, cholestane-3 $\beta$ , 5 $\alpha$ , 6 $\beta$ -triol;  $\alpha$ -epoxide, cholesterol-5 $\alpha$  and 6 $\alpha$ -epoxide.

<sup>3)</sup> nd=not detected.

<sup>A,B</sup> Means $\pm$ SE with different superscript in the same column differ significantly. \*  $p$ <0.05, \*\*\*  $p$ <0.001.

**Table 6.** Effect of cooking, re-heating method, and storage period before re-heating on the ratio of total amount of cholesterol oxidation products (COPs)/ cholesterol (%) in chicken thigh during storage at 4 °C

Cooking method <sup>1)</sup>	0 d	Re-heating method <sup>1)</sup>	3 d	Re-heating method	6 d
Raw	0.20 $\pm$ 0.09		nd <sup>2)</sup>		1.02 $\pm$ 0.16 <sup>A</sup>
PR	0.17 $\pm$ 0.15	PR	0.15 $\pm$ 0.09	PR	0.12 $\pm$ 0.15 <sup>B</sup>
		MW	0.16 $\pm$ 0.18	MW	0.22 $\pm$ 0.07 <sup>B</sup>
ST	0.13 $\pm$ 0.08	ST	0.55 $\pm$ 0.69	ST	0.12 $\pm$ 0.11 <sup>B</sup>
		MW	0.42 $\pm$ 0.38	MW	0.81 $\pm$ 0.69 <sup>A</sup>
OG	0.12 $\pm$ 0.10	OG	0.26 $\pm$ 0.07	OG	0.35 $\pm$ 0.23 <sup>B</sup>
		MW	0.24 $\pm$ 0.08	MW	0.03 $\pm$ 0.06 <sup>B</sup>
CG	0.09 $\pm$ 0.07	CG	0.07 $\pm$ 0.06	CG	0.05 $\pm$ 0.04 <sup>B</sup>
		MW	0.15 $\pm$ 0.16	MW	0.05 $\pm$ 0.06 <sup>B</sup>
MW	0.09 $\pm$ 0.09	MW	0.16 $\pm$ 0.15	MW	0.26 $\pm$ 0.02 <sup>B</sup>

<sup>1)</sup> Cooking and re-heating method: PR, pan roasting; ST, steaming; OG, oven grilling; CG, charcoal grilling; MW, microwaving.

<sup>2)</sup> nd=not detected.

<sup>A,B</sup> Means $\pm$ SE with different superscript in the same column differ significantly ( $p$ <0.05).

The extension of storage did not significantly affect the increase in amount of COPs/cholesterol in chicken thigh regardless of cooking and re-heating methods (Table 6). As mentioned previous section, the reduction in cholesterol content of meat samples by cooking and re-heating generally leads to increase in the amounts of COPs. However, in this study, the increase in amount of COPs did not be observed depending on loss of cholesterol content. It seems likely that the thermally degraded products during heating were not detected or identified (Hur et al., 2007).

## Conclusion

Based on the results of this study, the combination of steaming and microwaving as cooking and re-heating method is not

recommended in chicken thigh meat stored for more than 6 d due to the formation of total COPs. However, the amount of identified total COPs is similar to that found in ordinary foods. In addition, the amount of total COPs in raw chicken thigh was not different from that of cooked and reheated one after 6 d of storage. For this reason, further study on identification of thermal degradation product of cholesterol will be helpful to confirm the effect of re-heating method and storage period on cooked chicken thigh compared to raw meat.

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