

Effects of Various Cooking and Re-heating Methods on Cholesterol Oxidation Products of Beef Loin

S. O. Lee, D. G. Lim, K. H. Seol, Y. Erwanto¹ and M. Lee*

School of Agricultural Biotechnology, College of Agriculture and Life Science, Seoul National University, Korea

ABSTRACT : The objective of this study was to define the effects of various cooking and re-heating methods (pan roasting, steaming, oven grilling and micro-waving) on the cholesterol and formation of cholesterol oxidation products in beef loin during storage at 4°C. Raw samples showed lower total cholesterol content than cooked products sampled during storage for 6 d. The following cholesterol oxidation products (COPs) were separated by gas chromatography: 7 β -hydroxy cholesterol, 20 α -hydroxy cholesterol, 25-hydroxy cholesterol, cholestane-3 β , 5 α , 6 β triol (triol), α -epoxide and 7-ketocholesterol. Total amounts of COPs/cholesterol at 0 d were 0.74, 0.63, 0.76, 1.23 and 0.83% for the raw sample, pan roasting, steaming, oven grilling and micro waving methods, respectively. After 6 d storage almost of the samples had higher content of total COPs than at 0 and 3 d; the lowest (0.55%) COPs was found in the steaming cooking and re-heating method. The highest (5.96%) of COPs was found in the pan roasting cooking and re-heating method after 6 d storage. In conclusion, the concentration of total cholesterol and cholesterol oxidation of beef loin were increased as a consequence of cooking and re-heating methods. Steaming and micro-waving methods showed the lowest of cholesterol oxidation products under refrigerated storage for 6 d. However, each cooking and re-heating method had its own distinctive cooking effects. (*Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 5 : 756-762*)

Key Words : Beef Loin, Cooking and Re-heating Methods, Cholesterol Oxidation Products

INTRODUCTION

One of the main dietary aspects to be considered in relation to the risk of cardiovascular diseases is the composition of lipid fraction. Animal origin foods are, with edible fats and oils, the main source of lipid compounds. Fats of animal origin are considered in general not very healthy because of their high level of saturated fatty acid (SFA) and cholesterol (Concillo et al., 2004). As known that seven of dietary factors implicated in the development of coronary heart diseases of which at least 5 are related to the lipid fraction: cholesterol raising SFA, thrombogenic SFA, n-6 polyunsaturated fatty acids (PUFA), n-3 PUFA, monounsaturated fatty acid (MUFA), dietary fiber and antioxidants (Ulbricht and Southgate, 1991). Generally, meat contains 125 mg cholesterol per 100 g on an average. Many chemical reactions happen in meat which contains high level of lipid and protein during cooking and processing. Cholesterol oxidation products (COPs) are generated as result of the reaction during cooking or storage. COPs produced from cholesterol in meat were oxidated by the mechanism similar to lipid oxidation (Peng and Morin, 1992). Jung et al. (2005) studied reduction of cholesterol and its implication on blood cholesterol. Cholesterol oxides particular, are considered as atherogenic agent and appear to

have mutagenic, carcinogenic and cytotoxic properties (Kubow, 1990; Guardiola et al., 1996).

Besides leading to reduced consumption of potentially harm-full doses of lipid and cholesterol, the selecting and preparing methods of meat makes it possible to provide a non-negligible amount of polyunsaturated fatty acid (PUFA) of the ω 3 series, a useful contribution, especially in case the intake of marine lipids is low. The research to redress the balance between ω 6 and ω 3 PUFA and to attain low density lipoprotein cholesterol lowering effects, as often experimentally shown (Badiani et al., 2002). COPs most frequently observed in foods are 7-derivatives (7-ketocholesterol, 7 α -hydroxylcholesterol, 7 β -hydroxy cholesterol), 5-6-epoxides (5,6 α -epoxycholesterol, 5,6 β -epoxycholesterol), one triol (3 β ,5 α , 6 β -trihydroxycholesterol or cholestanetriol) and two molecules deriving from side chain oxidation (25-hydroxycholesterol and 20 α -hydroxycholesterol).

The various researches conducted on the effect of cooking and storage on the fatty acid profile of chicken (Conchillo et al., 2004), lipid composition and oxidation in fresh and completely trimmed beef muscle (Badiani et al., 2002), and lipid oxidation and formation of cholesterol oxidation products during frozen storage of raw and cooked chicken (Conchillo et al., 2005). However, data on lipid content changes, cholesterol content and cholesterol oxidation during cooking and re-cooking methods were very little. The research on the characteristic aspects of the cooking methods which are being used and the detection of representative parameter of lipid oxidation after re-heating

* Corresponding Author: M. Lee. Tel: +82-2-880-4804, Fax: +82-2-873-4804, E-mail: moohalee@snu.ac.kr

¹ Department of Animal Products Technology, Faculty of Animal Science, Gadjah Mada University, Jl. Agro, Karangmalang, Yogyakarta 55281, Indonesia.

Received October 11, 2005; Accepted January 13, 2006

methods is even less stressed. The objective of this study was, therefore, to determine how different cooking and re-heating practices could affect the intramuscular lipid, cholesterol content and cholesterol oxidation of beef loin during storage.

MATERIALS AND METHODS

Reagent

Cholesterol, cholesterol oxide standard (>90% purity) including 7 α -hydroxycholesterol, 7 β -hydroxycholesterol, α -epoxide, 20 α -hydroxycholesterol, 25-hydroxycholesterol, 5 α -cholestane (internal standard), cholestanetriol, 7-ketocholesterol, and butylated hydroxytoluene (BHT) were purchased from Sigma Chemical Co. (St. Louis, MO). Bis-(trimethylsilyl)-trifluoroacetamide (BSTFA)+1% trimethylchlorosilane (TMCS) was obtained from Supelco (Bellefonte, USA). Pyridine was also purchased from Sigma Chemical Co. Cellite 545 and calcium phosphate (CaHPO₄·2H₂O) was purchased from Fisher Scientific Co. (Malvern, PA) and silicic acid (100 mesh) from Aldrich Chemical Co. (Milwaukee, WI). Hexane, ethylacetate, acetone, methanol and chloroform were HPLC grade and also obtained from Fisher Scientific Co. (Malvern, PA).

Sample preparation

Beef loin was purchased from local market (Korean native cattle, 24 months of age, 300-350 kg in hot carcasses weight). Carcasses were aged 2-3 days at 2-3°C prior to being fabricated into sub primal cuts. Loin were trimmed for surface adipose tissue and sliced to approximately 2 cm. The total fat content and cholesterol level were analyzed in the pre experiment and there was no different of cholesterol level. Cooking methods used were pan roasting (PR), micro waving (MW), steaming (ST) and oven grilling (OG). In PR method, conventional roasting was performed in baking pan (TEFAL, France). The initial surface temperature of samples was 4°C and then placed in a preheated forced air convection oven (GR-643HT, Dong Yang magic, Korea) set at 170°C during 20 min. Samples were introduced and held at this temperature for 10 min on each side. Internal temperature during roasting was 70°C±2°C. MW means that meats were cooked using a microwave generator (M-M270TC, LG, Korea) at 2,450 MHz for 10 min. In ST, meats were heated using an electric steamer (Steam cuisine 900 turbo Diffusion[®], TEFAL, France). OG means meat was heated in a conventional oven at 150°C for 1 h. The internal temperature of samples was measured with a digital thermometer (Fisher Scientific, USA). Meat and oven temperature were controlled with iron-constantin thermocouples, inserted respectively into the center of the cut and placed adjacent to it. In all cooking methods meats were heated until the internal temperature was reached

70°C±2°C. Re-heating methods were done on the 3 and 6 d of storage, cooked samples were re-heated by PR, MW, ST, and OG for 5 min, 1 min, 10 min and 10 min, respectively. All of samples were aerobically packaged in oxygen-permeable bags. The raw and cooked meats were stored at 4°C for 6 d and the amount of cholesterol oxides were determined at 0, 3 and 6 d of storage.

Determination of cholesterol in meat and meat products

To analyze the cholesterol, 0.5 g of loin surface fat evaporated was diluted in 10 ml of freshly methalonic potassium hydroxide solution (1.0 mol/L) and added 1 g of sea-sand. The aliquot was then heated for 25 min. The supernatant solution was transferred into 25 ml volumetric flask with a pipette. The residue with portions of 6 ml isopropanol was boiled under reflux condenser for 5 min. The collecting solutions were cooled at ambient temperature and diluted to the mark with isopropanol. The turbid solutions were filtered through a whatman No. 1 filter paper (Whatman Inc., Clifton, NJ). The clear aliquot was used for cholesterol assay. Cholesterol was analyzed using kit (Cat. No 139050, Bohringer Mannheim, Germany). Blank sample was 0.4 ml of sample solution and 5 ml solution 4 (cholesterol reagent mixture), and sample was mixture of 2.5 ml sample and 0.02 ml, solution 3. The blank sample and sample were mixed, and incubated in a drying oven at 37-40°C for 60 min after sealing by paraffin film. Absorbance of blank (A1) and the sample (A2) were determined using UV spectrophotometer (UV1601, Shimadzu Co, Japan) at 405 nm. Cholesterol content (mg/100 g) was calculated using the equation as followed:

$$\text{Cholesterol (mg/100 g)} = \frac{0.711 \times \Delta A}{\text{Weight of sample (g)}} \times 100 \times 25$$

Cholesterol oxides products analysis

Cholesterol oxides products were analyzed according to Lee et al. (1996). A solid-phase column preparation for the separation of cholesterol oxides (Park and Addis, 1985; Zubillage and Maerker, 1991) was made by mixing silicic acid, celite 545 and CaHPO₄·2H₂O (10:9:1, wt/wt/wt) with 30 ml chloroform and then packing in a glass column (12 mm×30 cm). The prepared column was prewashed with 5 ml hexane consecutively, just before sample application. Total lipids were extracted by the method of Folch et al. (1957). The 0.2 g lipid sample was dissolved in 2 ml hexane:ethyl acetate (100:2, v/v), and then the sample solution was applied to the prewashed column. The sample container was washed twice with 2 ml hexane:ethyl acetate, and the wash solvent was applied to the column. Neutral lipid and cholesterol (phospholipids) were removed by adding 50 ml solvent I (CHCl₃:CH₃OH=2:1, v/v) and 60 ml solvent II (hexane: ethyl acetate = 4:1, v/v). Forty milliliter

Table 1. Changes of cholesterol content in raw, cooked and re-cooked beef loin during storage (mg/100 g)

Cooking method	Days of storage		
	0	3***	6***
Raw**	65.14±11.17 ^a	53.19±10.96 ^{a,C}	19.13±3.06 ^{b,B}
PP	71.74±3.69	71.24±2.60 ^A	61.15±6.98 ^A
PM	71.74±3.69	55.10±3.97 ^{BC}	60.67±10.86 ^A
SS	69.12±8.62	73.39±14.84 ^A	63.51±5.79 ^A
SM	69.12±8.62	76.42±10.19 ^A	70.66±1.93 ^A
OO***	64.20±3.22 ^a	33.91±1.72 ^{b,D}	58.66±7.24 ^{a,A}
OM	64.20±3.22	67.75±1.51 ^{AB}	69.48±2.38 ^A
MM**	86.98±14.88 ^a	37.58±5.57 ^{c,D}	64.54±9.48 ^{b,A}

** p<0.01, *** p<0.001.

^{a, b, c} Means±SE with different superscript in the same row differ significantly.

^{A, B, C, D} Means±SE with different superscript in the same column differ significantly.

PP: Pan roasting (cooking method at 0 day)+pan roasting (re-cooking method at 3 and 6 days).

PM: Pan roasting (cooking method at 0 day)+microwaving (re-cooking method at 3 and 6 days)

SS: Steaming (cooking method at 0 day)+steaming (re-cooking method at 3 and 6 days).

SM: Steaming (cooking method at 0 day)+microwaving (re-cooking method at 3 and 6 days).

OO: Oven grilling (cooking method at 0 day)+oven grilling (re-cooking method at 3 and 6 days).

OM: Oven grilling (cooking method at 0 day)+microwaving (re-cooking method at 3 and 6 days).

MM: Microwaving (cooking method at 0 day)+microwaving (re-cooking method at 3 and 6 days).

of solvent III (acetone:ethylacetate:methanol = 50:50:5, v/v/v) was used to elute at 1 ml/min and the collect cholesterol oxides. The collected solution was 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.

Cholesterol oxides were analyzed with a gas chromatograph (HP 5890 plus) equipped with an on-column capillary injector and a flame ionization detector. We used a gas chromatography column of 0.32 mm i.d.×30 m with 0.32 µm film thickness (Supelcowax-10 column). A splitless inlet was used to inject sample (0.5 µl) onto the capillary column, and a ramped oven temperature was used. The initial oven temperature of 70°C was held for 0.5 min and then increased to 275°C at 40°C/min and held at 275°C for 0.5 min. The temperature increase again to 280°C at 2°C/min. Temperatures of the inlet and detector were 260 and 300°C, respectively. Helium was the carrier gas at constant pressure of 14.0 psi. Flame ionization detector air, H₂, and make-up gas (He) flows were 300 ml/min, 30 ml/min, and 28 ml/min, respectively. The area of each peak (Pa·s) was integrated with the ChemStation software, and the amounts of cholesterol oxides were calculated using an internal standard (5α-cholestane).

Statistical analysis

The values are expressed as means±SE. Statistical analysis was performed by one way analysis of variation (ANOVA), and significant differences were detected by Duncan's multiple range test using SAS software (SAS, Release 8.01, SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Cholesterol content of beef loin in various cooking methods

The content of cholesterol of raw and cooked beef loins were shown in Table 1. The content of cholesterol was relatively higher in cooked or re-heated beef loin than that in raw one. Cholesterol content of the raw sample significantly decreased with the increasing of storage time.

Cholesterol contents showed the significance differences among storage time on the raw (p<0.01), OO cooking method (p<0.001) and MM cooking method (p<0.01), while there were no differences on PP, PM, SS, SM and OM cooking methods. At the 0 day, cholesterol contents were similar between raw and cooked samples, while at 3 and 6 days after storage showed significant differences. After 6 days of storage the cholesterol contents of raw meat was lower (19.13%) than other cooking methods, while cholesterol contents were 58.66% to 70.66% for all the cooking and re-cooking methods on the same day of storage. Steaming followed by micro waving (SM method) showed the highest cholesterol contents after storage. The different results were reported by Rodriguez-Estrada et al. (1997) that the cholesterol of raw beef was significantly higher than that found in the cooked ones, which may be due to the loss of fat during cooking. Our result indicated the higher cholesterol in the cooked product, it might be the cooking method cause decreasing of the moisture content during cooking but there was no significant loss of fat including cholesterol. As described in previous research that the increase of cholesterol content correlated with the observed loss in weight due to water evaporation during cooking (Kowale et al., 1996). Pan roasting method followed by the same method at 3 and 6 d

Table 2. Cholesterol oxidation products (COPs) contents in raw, pan roasting, steaming, oven grilling, and microwaving samples of beef loins in various cooking methods at 0 day

Treatment ¹	Cholesterol oxides ² (µg/100 g)						Total amount of COPs/cholesterol (%)
	7β-OH	20α-OH***	25-OH	Triol***	α-Epoxy***	7-keto	
Raw	n.d. ²	n.d. ^C	n.d. ^B	300.09±148.05	179.66±107.47 ^B	n.d.	0.74±0.35
PR	n.d.	n.d. ^C	36.06±9.08 ^A	371.85±199.57	n.d. ^C	n.d.	0.63±0.29
ST	n.d.	55.38±1.81 ^A	n.d. ^B	433.33±53.70	n.d. ^C	n.d.	0.76±0.14
OG	n.d.	n.d. ^C	n.d. ^B	326.35±79.37	464.84±98.31 ^A	n.d.	1.23±0.29
MW	n.d.	25.91±6.69 ^B	n.d. ^B	506.03±110.86	n.d. ^C	n.d.	0.83±0.19

*** p<0.001.

^{A, B, C} Means±SE with different superscript in the same row differ significantly.

¹ 7β-OH (7β-hydroxycholesterol), 20α-OH (20α-hydroxycholesterol), 25-OH (25-hydroxycholesterol), triol (cholestane-3β,5α,6β-triol), α-epoxy (cholesterol-5α,6α-epoxy), 7-keto (7-ketocholesterol).

² n.d. = Not detected.

³ PR: Pan roasting, ST: steaming, OG: oven grilling, MW: microwaving.

showed the stable content of cholesterol, the same results also were shown in PM, SS, SM and OM. The decreasing of the moisture content during cooking will lead to the higher percentage of others component. These results are coincided with Badiani et al. (2002) that the cholesterol contents were higher on the boiling, broiling, oven roasting and micro waving of beef meat than that the raw one. Ono et al. (1984) has also shown the data on the percentage of cholesterol content of lamb and they found that percentage values were higher into 101, 104, and 105 after cooking. The higher content of cholesterol into more than 100% after cooking was predicted as a consequently of the cooking. As the former fraction in increasingly represented when tissues are considered in that order, and as it is more likely to migrate from adipose to muscle tissue by means of cooking, this could represent a reliable explanation for the higher value of cholesterol after cooking (Badiani et al., 2002).

Effect of various cooking on the formation of COPs

Cholesterol is an unsaturated lipid susceptible to oxidation, giving rise to COPs. Some COP are considered as transport forms of cholesterol in catabolic pathways and many toxicological effects of COPs have been reported (Kim and Nawar, 1991; Nawar et al., 1991; Guardiola et al., 1996; Conchillo et al., 2005). The formation of cholesterol oxidation product was shown in Table 2. Cholestane-3β, 5α, 6β-triol was the most abundant COPs in raw sample and in cooked sample. Conchillo et al. (2005) also found cholestanetriol and 25-hydroxycholesterol in raw and cooked chicken. Cholestanetriol and 25-hydroxycholesterol are considered most cytotoxic, were the least abundant COPs in cooked meat. All cooking methods caused the formation of triol and the highest level of triol was found in MW cooking method. Pan roasting cooking method (PR) induced the presence of 25-hydroxycholesterol, while ST and MW cooking methods induced formation of 20-α-hydroxycholesterol. The physical state of cholesterol greatly influences the type of oxidation products. Formation

of the 20α-hydroxycholesterol and 25-hydroxycholesterol were produced by the oxidation of the lateral chain of cholesterol (Lee et al., 1977). The 25-hydroxycholesterol was found in the pan roasting methods at 0 d in a low level, while cholesterol 5,6α-epoxy was detected in raw and oven grilling cooked sample. The resultant of epoxides are typically unstable in aqueous environment and chemically reactive (Fretland and Omienchinski, 2000). Another possibility is the reaction of molecular oxygen free radicals directly with the double bond, forming a 5, 6-epoxycholesterol by addition, which by further dehydration can be transformed into a triol (cholestane- 3β, 5α, 6β-triol) (Valenzuela et al., 2003). Lee (2001) reported that the kinds of cholesterol oxides products found in cooked meat were basically the same as that founding raw chicken meat. Monahan (1992) found that 7β-hydroxy cholesterol was the major cholesterol oxide in cooked meat.

In this study, the 7β-hydroxy cholesterol was not found in raw meat and in cooked meat. The 7-ketocholesterol was not found until 3 d of storage and then appeared after 6 d. Choncillo et al. (2005) found 7β-hydroxy cholesterol and 7-ketocholesterol in raw chicken meat and cooked meat but the level was lower in cooked meat than in the raw one. Wahle et al. (1993) also reported the decreasing of 7-ketocholesterol levels in powdered whole egg stored in frozen sample. There were some differences (p<0.001) among cooking methods on the case of 25-hydroxycholesterol, 20α-hydroxycholesterol and cholesterol 5,6α-epoxy. Lee et al. (2001) reported that the oxidative changes of cholesterol during storage is faster than that of raw meat and they suggested that cooked meat should be vacuum-packaged as soon as possible after cooking to reduce oxidative changes in cholesterol during storage. Monahan (1992) suggested that several sources showed fresh contained none, or undetectable level of COPs. Most oxides were found in food subjected to processing conditions or exposure to heat. Cooking of foods under standard domestic conditions increased production of COPs

Table 3. Cholesterol oxidation products (COPs) contents in raw, pan roasting, steaming, oven grilling and microwaving samples of beef loins after various re-heating at 6 days

Storage time	Treatment ¹	Cholesterol oxides ² (µg/100 g)						Total amount of COPs /cholesterol (%)***
		7β-OH	20α-OH	25-OH***	Triol*	α-Epoxyde	7-keto***	
3 d	Raw	n.d. ³	41.28±22.80 ^{CD}	n.d.	381.12±23.84 ^A	n.d. ^C	n.d.	0.66±0.12 ^{AB}
	PP	n.d.	38.06±1.77 ^{CD}	n.d.	306.70±123.95 ^{AB}	n.d. ^C	n.d.	0.48±0.15 ^B
	PM	n.d.	97.68±41.05 ^{BCD}	n.d.	n.d. ^C	n.d. ^C	n.d.	0.13±0.05 ^C
	SS	n.d.	377.99±189.90 ^A	n.d.	n.d. ^C	273.17±48.23 ^A	n.d.	0.94±0.30 ^A
	SM	n.d.	185.28±97.28 ^{BC}	n.d.	n.d. ^C	n.d. ^C	n.d.	0.27±0.12 ^{BC}
	OO	n.d.	12.39±1.01 ^D	n.d.	240.24±100.06 ^B	170.37±13.15 ^B	n.d.	0.66±0.19 ^{AB}
	OM	n.d.	206.46±80.45 ^B	n.d.	n.d. ^C	196.79±91.25 ^B	n.d.	0.63±0.27 ^B
	MM	n.d.	82.15±20.79 ^{BCD}	n.d.	n.d. ^C	n.d. ^C	n.d.	0.09±0.01 ^C
6 d	Raw	n.d. ³	n.d.	837.09±238.38 ^A	210.26±89.32 ^{BC}	n.d.	n.d. ^C	1.62±0.48 ^{BC}
	PP	n.d.	n.d.	235.52±125.13 ^B	562.49±327.91 ^{AB}	n.d.	3,479.23±368.72 ^A	5.96±0.84 ^A
	PM	n.d.	n.d.	n.d. ^C	354.06±106.69 ^{ABC}	287.72±126.96	826.49±396.95 ^B	2.02±0.77 ^{BC}
	SS	n.d.	n.d.	n.d. ^C	n.d. ^C	1,252.31±216.88	n.d. ^C	0.55±0.15 ^D
	SM	n.d.	n.d.	n.d. ^C	462.78±125.61 ^{AB}	349.27±65.30	863.10±215.52 ^B	2.43±0.52 ^B
	OO	n.d.	n.d.	29.26±22.27 ^C	426.29±98.84 ^{AB}	776.00±122.28	n.d. ^C	1.93±0.41 ^{BC}
	OM	n.d.	n.d.	n.d. ^C	512.74±171.02 ^{AB}	470.56±151.13	n.d. ^C	1.54±0.52 ^{BC}
	MM	n.d.	n.d.	n.d. ^C	647.20±390.92 ^A	455.47±84.76	n.d. ^C	1.23±0.34 ^{CD}

* p<0.05, *** p<0.001.

A, B, C Means±E with different superscript in the same column and same storage time are significantly different.

¹ PP: Pan roasting (cooking method at 0 day)+pan roasting (re-cooking method at 3 and 6 days), PM: pan roasting (cooking method at 0 day)+microwaving (re-cooking method at 3 and 6 days), SS: steaming (cooking method at 0 day)+steaming (re-cooking method at 3 and 6 days), SM: steaming (cooking method at 0 day)+microwaving (re-cooking method at 3 and 6 days), OO: oven grilling (cooking method at 0 day)+oven grilling (re-cooking method at 3 and 6 days), OM: oven grilling (cooking method at 0 day)+microwaving (re-cooking method at 3 and 6 days), MM: microwaving (cooking method at 0 day)+microwaving (re-cooking method at 3 and 6 days).

² 7β-OH (7β-hydroxycholesterol), 20α-OH (20α-hydroxycholesterol), 25-OH (25-hydroxycholesterol), triol (cholestane-3β,5α,6β-triol), α-epoxyde (cholesterol-5α,6α-epoxyde), 7-keto (7-ketocholesterol).

³ n.d. = Not detected.

(Finocchiaro et al., 1984; Hubbart et al., 1989).

Effect of storage on COPs

The result of the cholesterol oxidation products (COPs) at 0, 3 and 6 d was shown in Table 2 and 3. The samples were stored in aerobic packaging under refrigerate temperature (4°C).

Table 2 showed COPs content of raw and cooked meat at 0 d (µg/100 g). Initially, triol (cholestane-3β, 5α, 6β-triol) and α-epoxyde (cholesterol-5α, 6α-epoxyde) were found in raw beef loin. These levels were slightly affected during cooking (De Vore, 1988). The contents of COPs were increased in the cooked beef loins. Triol was detected as the highest level in meat sample cooked with MW. Total amount of COP per total cholesterol (0.63-1.23) % was higher in the cooked meat than that in raw one except PR method sample. Our result suggested that oxidation of cholesterol had occurred in cooked meats during storage (Cluskey, 1997). According to study of Pie et al. (1991), veal and pork cholesterol were degraded significantly during heating. The production rate of COPs was the highest level in beef loin cooked with OG method at 0 d. 7β-OH (7β-hydroxycholesterol) and 7-keto (7-ketocholesterol) were not detected in all samples at 0 d after cooked (Table 2).

Table 3 shows COPs content of raw and re-heated meat at 3 and 6 d. At 3 d of storage, the 7β-OH, 25-OH (25-hydroxycholesterol) and 7-keto were not detected, whereas 20α-OH (20α-hydroxycholesterol) was found in all meat samples. The total amount of COPs per total cholesterol was lower in meat sample re-heated at 3 d than meat sample cooked at 0 d (Table 2) except meat sample re-heated with SS cooking method. Re-heated meat sample with SS cooking method contained the most rate of COPs production (0.94%).

As revealed through Table 3, the content of COPs in meat sample at 6 d were higher (p<0.001) than those at 0 (Table 2) and 3 d. Changes due to in refrigerate storage on cholesterol oxides were faster than in comparison to frozen storage. Similar result of the increasing of cholesterol oxidation products were reported for refrigerate storage by Park and Addis (1987). They reported that the increase in cholesterol oxidation products during storage might be attributed to the presence of free radical chain reaction initiators, such as hydroperoxides formed by the oxidation of polyunsaturated fatty acids. Total amount of COPs/cholesterol was highest in meat re-cooked by pan roasting method (PP), whereas was the lowest in re-cooked by steaming method (SS). Triol was constantly present in meat during storage. 7β-OH was not found, while 7-keto

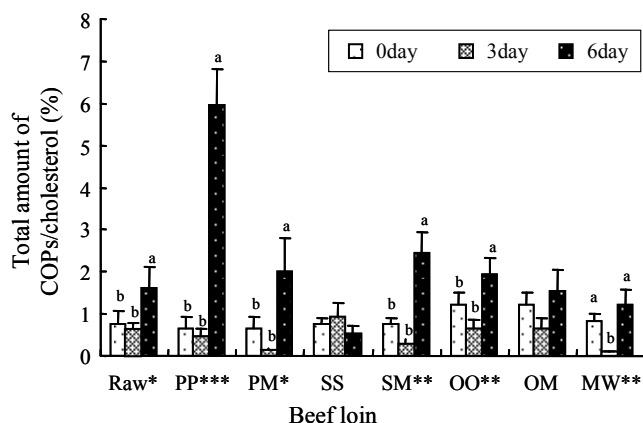


Figure 1. Total amount of COPs/cholesterol (%) in raw and re-heating beef loins with various cooking methods during storage. * p<0.05, ** p<0.01, *** p<0.001. ^{a, b} the different letter on the bar in the same treatment meant that there was a significant different. PP: pan roasting (cooking method at 0 d)+pan roasting (re-cooking method at 3 and 6 d), PM: pan roasting (cooking method at 0 d)+micro waving (re-cooking method at 3 and 6 d), SS: steaming (cooking method at 0 d)+steaming (re-cooking method at 3 and 6 d), SM: steaming (cooking method at 0 d)+micro waving (re-cooking method at 3 and 6 d), OO: oven grilling (cooking method at 0 d)+oven grilling (re-cooking method at 3 and 6 d), OM: oven grilling (cooking method at 0 d)+micro waving (re-cooking method at 3 and 6 d), MM: micro waving (cooking method at 0 d)+micro waving (re-cooking method at 3 and 6 d).

was observed in three samples (PP, PM and SM) (p<0.001). De vore (1988) measured 7-keto cholesterol and also found important amounts of this derivative comprising up to 0.3% of cholesterol in cooked meat that had previously been stored at 4°C. Kowale et al. (1996) reported that a significant increase in the total cholesterol content during heat processing as due to loss of moisture during cooking and was in agreement with the reports of Badiani et al. (2002) in trimmed beef meat and Kesava-Rao et al. (1996) in buffalo meat. The oxidation of cholesterol proceeds as a part of the lipid oxidation process. Variation in the percentage yields of cholesterol oxides was evident in study of cholesterol oxidation on mutton during cooking and storage (Kowale et al., 1996).

Figure 1 showed the difference of total amount of COPs per total cholesterol (%) during storage. The levels of COPs on raw meats were significantly different (p<0.05) during storage. The total amount of COPs or cholesterol (%) was higher at 6 d than that at 0 and 3 d. This result might be explained by oxidative conditions such as prolonged storage or re-heating in the presence of pro-oxidants. Finocchiaro and Richardson (1983) identified 8 common COPs in certain cholesterol-rich foods subjected to oxidative conditions such as elevated temperature, storage period, and processing. Rodriguez-Estrada et al. (1997) reported that the cholesterol of raw beef was significantly higher than

that found in the cooked ones, which may be due to the loss of fat during cooking. Our result indicated the higher COPs and cholesterol in the cooked product (data not shown) were caused by decreasing of the moisture content during cooking. The decreasing of the moisture content will lead to the higher percentage of others component. Badiani et al. (2002) suggested this result that the cholesterol was higher on the oven roasting and boiling of beef meat. Ono et al. (1984) has shown the data on the percentage of cholesterol content of lamb and they found that percentage values increased after cooking.

CONCLUSIONS

In conclusions, the cooking and re-heating increased the level COPs products. Steaming and micro waving showed the lowest COPs product during storage until 6 d. The formation of COPs products were various depend on the cooking and re-heating methods.

REFERENCES

- Badiani, A., S. Stipa, F. Bitossi, P. P. Gatta, G. Vignola and R. Chizzolini. 2002. Lipid composition, retention and oxidation in fresh and completely trimmed beef muscles as affected by common culinary practices. *Meat Sci.* 60:169-186.
- Conchillo, A., D. Ansoreno and I. Astiasaran. 2004. The effect of cooking and storage on the fatty acid profile of chicken breast. *Eur. J. Lipid Sci. Technol.* 106:301-306.
- Conchillo, A., D. Ansoreno and I. Astiasaran. 2005. Intensity of lipid oxidation and formation of cholesterol oxidation products during frozen storage of raw and cooked chicken. *J. Sci. Food Agric.* 85:141-146.
- Carnovale, E. and L. Marletta. 1997. *Tabelle di Composizione degli Alimenti*. Roma, I: Istituto Nazionale della Nutrizione.
- Chan, W., J. Brown, S. M. Lee and D. H. Buss. 1995. Meat, poultry and game-5th supplement to the 5th edition of McCance and Widdowson's. In: *The Composition of Foods*. Cambridge, UK: The Royal Society of Chemistry.
- Cluskey, S. M., J. F. Connolly, R. Devery, B. O'brien, J. Kelly, D. Harrington and C. Stanton. 1997. Lipid and cholesterol oxidation in whole milk powder during processing and storage. *J. Food Sci.* 62(2):331-337.
- De Vore, V. R. 1988. TBA values and 7-ketocholesterol in refrigerated raw and cooked ground beef. *J. Food Sci.* 53:1058-1061.
- Finocchiaro, E. T. and T. Richardson. 1983. Sterol oxides in foodstuffs: A review. *J. Food Prot.* 46:917-925.
- Finocchiaro, E. T., K. Lee and T. Richardson. 1984. Identification and quantification of cholesterol oxides in grated cheese and bleached butter oil. *J. Am. Organic Chem. Soc.* 61:877-883.
- Folch, J., M. Lees and G. H. Sloan-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226:497-507.
- Fretland, A. J. and C. J. Omienchinski. 2000. Epoxide hydrolases: *Biochemistry and Molecular Biology.* 129:41-59.
- Guardiola, F., R. Codony, P. B. Addis, M. Rafecas and J. Boatella.

1996. Biological effects of oxysterols. Current status. *Food Chem. Toxicol.* 34:193-211.
- Greenfield, H., Y. L. Kuo and R. B. H. Wills. 1987. Composition of the Australian foods. 34. Beef and Veal. *Food Tech. Aust.* 39:208-215.
- Hubbard, R. W., Y. Ono and A. Sanchez. 1989. Atherogenic effect of oxidized products of cholesterol. *Prog. Food Nutr. Sci.* 13:17-44.
- Jung, T. H., J. J. Kim, S. H. Yu, J. Ahn and H. S. Kwak. 2005. Properties of cholesterol-reduced butter and effect of gamma linolenic acid added butter on blood cholesterol. *Asian-Aust. J. Anim. Sci.* 18(11):1646-1654.
- Kim, S. K. and W. W. Nawar. 1991. Oxidative interactions of cholesterol with triacylglycerols. *J. Am. Oil Chem. Soc.* 68:496-498.
- Kowale, B. N., V. Kesava-Rao, N. P. Babu, N. Sharma and G. S. Bisht. 1996. Lipid Oxidation and cholesterol oxidation in mutton during cooking and storage. *Meat Sci.* 43:195-202.
- Kesava-Rao, V., B. N. Kowale, N. P. Babu and G. S. Bisht. 1996. Effect of cooking and storage on lipid oxidation and development of cholesterol oxidation products in water buffalo meat. *Meat Sci.* 43:179-185.
- Kubow, S. 1990. Toxicity of dietary lipid peroxidation products. *Trends Food Sci. Technol.* 1:67-71.
- Lee, J. H., D. W. Shoeman, S. S. Kim and A. S. Csallany. 1977. The effect of superoxide anion in the production of seven major cholesterol oxidation products in aprotic and protic conditions. *Int. J. Food Sci. Nutr.* 48:151-159.
- Lee, M., J. G. Sebranek, D. G. Olson and J. S. Dickson. 1996. Irradiation and packaging of fresh meat and poultry. *J. Food Prot.* 59:62-72.
- Lee, J. I., S. Kang, D. U. Ahn and M. Lee. 2001. Formation of cholesterol oxides in irradiated raw and cooked chicken meat during storage. *Poult. Sci.* 80:105-108.
- Monahan, F. J., J. I. Gray, A. M. Boreen, E. R. Miller, D. J. Buckley, P. A. Morrissey and E. A. Gomma. 1992. Influence of dietary treatment on lipid and cholesterol oxidation in pork. *J. Agric. Food Chem.* 40:1310-1315.
- Nawar, W. W., S. K. Kim, Y. J. Li and M. Vajdi. 1991. Measurement of oxidative interactions of cholesterol. *J. Am. Oil Chem. Soc.* 68:496-498.
- Ono, K., B. W. Berry, H. K. Johnson, E. Russek, C. F. Parker, V. R. Cahill and P. G. Althous. 1984. Nutrition composition of lamb of two age groups. *J. Food Sci.* 49:1233-1239.
- Park, S. W. and P. B. Addis. 1985. HPLC determination of C-7 oxidized cholesterol derivatives in foods. *J. Food Sci.* 50:1437-1441, 1444.
- Park, S. W. and P. B. Addis. 1987. Cholesterol oxidation products in some muscle food. *J. Food Sci.* 6:1500-1503.
- Peng, S. and R. J. Morin. 1992. Biological Effects of Cholesterol Oxides. CRC Press. Boca Raton.
- Pie, J. E., K. Spahis and C. Seillan. 1991. Cholesterol oxidation in meat products during cooking and frozen storage. *J. Agric. Food Chem.* 39:250-254.
- Rodriguez-Estrada, M. T., G. Penazzi, M. F. Caboni, G. Bertacco and G. Lercker. 1997. Effect of different cooking methods on some lipid and protein components of hamburgers. *Meat Sci.* 45(3):365-375.
- Valenzuela, A., J. Sanhueza and S. Nieto. 2003. Cholesterol oxidation: Health hazard and the role of antioxidants in prevention. *Biol. Res.* 36:291-302.
- Wahle, K. W. J., P. P. Hoppe and G. McIntosh. 1993. Effects of storage and various intrinsic vitamin E concentrations on lipid oxidation in dried egg powders cooked chicken. *J. Sci. Food Agric.* 61:463-469.
- Zubillaga, M. P. and G. Maerker. 1991. Quantification of three cholesterol oxides in raw meat and chicken. *J. Food Sci.* 56:1194-1196.