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Effect of Grilling and Roasting on Formation of Cholesterol Oxidation Products (COPs) in Chicken and Mutton

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Abstract: The objective of this paper is to determine the effect of grilling and roasting on cholesterol oxidation products (COPs) in mutton and chicken. Four steps of analysis have been conducted: saponification, extraction, derivatisation and quantification by GCMS-QQQ. The temperature and time used for grilling was 230°C (20 minutes) while roasting was 190°C (25 minutes) using microwave. This study showed that there was no significant difference between raw mutton and raw chicken in the amount of cholesterol. Raw mutton have higher amount of cholesterol than raw chicken due to the high content of SFA and cholesterol. In both of the cooking treatments, there was no significant difference in the amount for most of COPs, but for the grilling process, in β-epoxide were significantly higher. In conclusion, the roasting treatment is better to be applied in meat compared to grilling in term of COPs. It is suggested in the future works that the drip loss during the cooking being analyzed as the cholesterol and COPs might be lost during heat treatment and more reference standards of COPs need to be used.

Key word: Grilling · Roasting · Cholesterol Oxidation Products · Chicken · Mutton

INTRODUCTION

Cholesterol is a type of eukaryotic sterols which is found in animals [1]. Cholesterol is more sensitive to free radical oxidation by the diatomic molecular oxygen (O_2) in air. It is the main reaction involved in oxidative deterioration. Oxidation of lipid and sterols (cholesterol) follows the same oxidation pattern which is hydroperoxide produced from autoxidation, photoxidation and enzymatic oxidation.

The oxidation of cholesterol occurs easily in various foods, including meat and poultry and cholesterol oxidation products occur through a chemical process similar to that of unsaturated fatty acid oxidation [2]. Hydroperoxide derived from oxidation of unsaturated fatty acids play a significant role to facilitate cholesterol oxidation at the double bond, which is more sensitive to oxidation [3]. Besides decreasing quality of the food, lipid oxidation also affects the fatty acid composition and cholesterol, which may be harmful to human health such as cholesterol oxides [4]. The purpose of this study was to identify the effect of grilling and roasting as different cooking methods on the amount of COPs in chicken and mutton by comparing with COPs in raw chicken and mutton as control sample.

MATERIALS AND METHODS

Samples: The shoulder part of mutton and thigh of chicken were purchased from a local market in Nilai, Negeri Sembilan, Malaysia. Samples were cut and divided into three groups: raw meat as a control sample (R), grilling (G) at 230°C for 20 minutes and roasting (RO) at 190°C for 25 minutes, for the mutton and chicken samples, respectively. The samples were blended after cooking.

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Chemicals: Cholesterol and cholesterol oxidized standards such as 5α-cholestane, 25-hydroxycholesterol (25-HC), 7-ketocholesterol (7-keto), α-epoxycholesterol, β-epoxycholesterol and cholesterol. The purities of the standards ranged from 95% to 99%.

Gas chromatography, analytical grade chemicals and solvent was purchased from Merck (M) Sdn Bhd and Sigma Aldrich (M) Sdn Bhd.

Saponification and Extraction of COPs: A total of 2 g of blended meats (raw and cooked) were weighed into a 100 ml of conical flask. Then, 10 ml of 1M potassium hydroxide (KOH) in 95% ethanol was added to the sample and kept at room temperature for 22 hrs in the dark condition. The mixtures of 5 ml of distilled water and 10 ml of hexane were added to the sample and the mixture was vortexed for 10 to 30 sec. The upper layer (hexane fraction) was collected separated and transferred into a capped universal tube. The lower layer (non saponifiable matter) was extracted three times with 10 ml of hexane. The collected hexane extract was dried [6].

Derivatisation of Cholesterol Oxides: A 100 µl of TMSI reagent was added to the dried COPs samples and then incubated at 80 °C for one hour. After that, the reagent was evaporated under a stream of nitrogen gas and TMS-ether derivative was dissolved in 1 ml of chromatography grade hexane. The tube was sonicated for 1 min and centrifuged for 3 mins at 13,000 rpm and then stored at -20 °C for subsequent analysis by GCMS-QQQ within 1 week after derivatisation [7].

Determination of Cholesterol Oxides by Gas Chromatography (GC/MS-QQQ): Analysis was performed on gas chromatography system combined with a prototype Triple Quadrupole GC/MS system (GC/MS-QQQ). The analysis was operated with multiple reactions monitoring (MRM) mode. The data was obtained as analyzed by Mass Hunter Work Station Software using a Quantitative Version B 03.01/Build 3.1.170.0. A capillary column DB5: MS UI 30 m x 0.25 mm x 0.25 µm was used to separate the COPs. The column were set at 250°C and raised to the constant temperature at 280 °C in 5 mins. The temperature was raised to 300°C at 50°C/min. The detector was set at 320°C and the gas flow was 1.2 ml/minute, volume of injection was 1 µl and splitless mode was used. The GC/MS-QQQ source of temperature was 230°C using Electron Ionization, while Mass Spectrometer 1 and Mass Spectrometer 2 temperature was at 150°C.

Statistical Analysis: The data from GC/MS-QQQ were evaluated statistically by SAS software (9.2) and a one-way ANOVA was used to evaluate the effect of cooking method (raw, grilling and roasting) on COP in samples of chicken and mutton and the least significance value ($p<0.05$) was reported.

RESULTS AND DISCUSSION

The Effects of Cooking on Cholesterol Oxidation Products (COPs) in Mutton and Chicken: The raw mutton treatment has a high content of cholesterol than raw chicken (Table 1). In De Almeida *et al*. [8] study, they state from the consumer perceived that red meat is unhealthful because it is high in saturated fatty acid (SFA) and cholesterol. In fact, it has been recently demonstrated that replacement of red meat with chicken is associated with a significant decrease in apolipoprotein B and total cholesterol level in microalbuminuric type 2 diabetic patients [9].

Cholesterol is not a product of oxidation. According to Saldanha and Bragagnolo [10], the total lipid, fatty acids and cholesterol contents decreased significantly ($p<0.02$) after thermal treatment, with simultaneous increase of the cholesterol oxides contents. Regarding the previous study by Vicente and Torres, [11], cholesterol level which were analyzed in raw and thermally process samples, showed a consistent reduction of between 15.4% and 24.04% of initial content [11].

The increasing level of cholesterol in thermally processes samples might be caused by the conversion of other substances into cholesterol from food during processing thermal degradation. This contrast with our study and there may have a few things that we need to control such as the diet of chicken and mutton. In this study there were no significant different between raw mutton and raw chicken, $p>0.05$ (Table 2).

In cooked samples, grilling gave the highest amount of COPs especially in chicken. Based on studies by Chien *et al*. [12] and Kim & Nawar [13], the conversions of cholesterol to COPs depend on the temperature. The highest amount of COPs on grilling was due to high temperature with lower time compared to roasting. Furthermore, in Saldanha and Bragagnolo [10] study, they state that the formation of COP is significantly increased after grilling. In this study, results showed significant differences between grilling treatment of chicken and mutton for β-epoxide products ($p<0.05$). Mariutti *et al*. [14] in their investigation stated the 7-ketocholesterol was the

Table 1: Cholesterol and cholesterol oxides content in chicken and mutton with the significance different ($p<0.05$), unit in part per million (ppm)

	Chicken			Mutton		
STD COP	Raw	Grill	Roast	Raw	Grill	Roast
5α-cholestane	$5.9x10^{-3}$ ±$4.3x10^{-3a}$	$10.4x10^{-3}$ ±$4.8x10^{-3a}$	$6.5x10^{-3}$ ±$6.7x10^{-3a}$	$10.6x10^{-3}$±$5.5x10^{-3b}$	$12.0x10^{-3}$ ±$2.4x10^{-3b}$	$18.4x10^{-3}$ ±$2.9x10^{-3a}$
Cholesterol	30.9 ±25.2[b]	74.1±21.5[a]	49.5±18.0[ab]	38.9±25.2[a]	42.9±21.5[a]	43.6±18.0[a]
α-epoxide	ND[a]	1.13±1.35[a]	0.77±1.1[a]	0.71±1.33[a]	1.38±1.99[a]	ND[a]
β-epoxide	1.19±0.68[b]	2.84±1.34[a]	2.14±1.52[ab]	0.38±0.18[c]	0.83±0.45[b]	2.28±0.14[a]
25-HC	ND[b]	$5.1x10^{-3}$ ±$5.0x10^{-3a}$	$2.7x10^{-3}$ ±$2.5x10^{-3ab}$	ND[a]	$2.4x10^{-3}$ ±$3.5x10^{-3a}$	$3.4x10^{-3}$ ±$3.4x10^{-3a}$
7-keto	$0.1x10^{-3}$ ±$0.1x10^{-3a}$	$0.1x10^{-3}$ ±$0.1x10^{-3a}$	$0.1x10^{-3}$ ±$0.1x10^{-3a}$	ND[a]	ND[a]	$0.04x10^{-3}$ ±$0.1x10^{-3a}$

Mean ± standard deviation

The significant difference between treatment in chicken and mutton as shown in the present of different superscript letters

Table 2: Show the significant different between chicken and mutton in raw and cooking treatment ($p<0.05$)

STD COPs	Raw	Grill	Roast
5α-cholestane	0.0512	0.4011	0.0898
Cholesterol	0.9277	0.0568	0.2827
α-epoxide	0.1539	0.6278	0.0703
β-epoxide	0.1023	0.0057	0.6797
25-HC	ND	0.3120	0.5033
7-keto	0.1553	0.1511	0.9932

cholesterol oxide found in higher amount in raw chicken on day 0, while the formation of 7β- and 7α-hydroxycholesterol was verified only from day 30 on. This clearly explained the cooking resulted in increase of total cholesterol oxides.

CONCLUSION

This study showed that there was no significant difference between raw mutton and raw chicken in the amount of cholesterol. In the cooked treatments, there was no significant difference in amount for most of COPs, but only β-epoxide gave significant different for grilling process. Therefore, it is concluded that the roasting cooking method is better to be applied in meat compared to roasting, due to the potential bad effect towards health. It is suggested in the future works that the drip loss during the cooking being analyzed as the cholesterol and COPs might be lost during heat treatment and more reference standards of COPs need to be used.

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