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Effect of Different Cooking Methods on Formation of Cholesterol Oxidation Products in Pork and Beef

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Abstract: Cooking process can lead to the formation of cholesterol oxidation products (COPs) which can give negative biological effects to human. The objective of this work was to study the effect of different cooking methods (grilled and roasted) on formation of COPs in beef and pork. The analysis involved four major steps; saponification, extraction, derivatisation and quantification by GC/MS-QQQ. Five common COPs (5-α-cholestane, 7-ketocholesterol, α-epoxycholesterol, β-epoxycholesterol and 25-hydroxycholesterol) that are generally reported in foods were analyzed to study the differences of their content between raw, grilled and roasted meat. Besides cholesterol, the most abundant compound in both types of samples that can be detected was β-epoxycholesterol. Grilling process for both samples was observed to contain the highest cholesterol and total COPs level. Beef samples contain higher total cholesterol and COPs compared to pork. It implies that consume beef regularly gives bad effect to health. It is recommended to do analysis on the collected drip loss during the cooking methods as the cholesterol and COPs might be lost during heat treatment and more reference standards of COPs need to be used in this study.

Key words: Cholesterol · Cholesterol oxidation products · Beef · Pork · Grilling · Roasting

INTRODUCTION

The terms Halal and *Haram* are applied to many facets of life; and one of the most common uses of these terms is in reference to meat products. In Islam, there are many things that are clearly Halal or *Haram*. Halal means permissible. *Haram* means forbidden. In Islam, forbidden items include pork and all its products and derivatives [1]. Often *Halal* is associated with another term: *Halalan-Toyyiban*. *Halalan-Toyyiban* merely means permissible, wholesome and safe for consumption in relate to Syari'ah law. Besides fulfilling the Syari'ah law, which is a must for Muslim, the food safety factor plays a significant contributor in determining*Toyyiban* (safe, clean, nutritious, quality) aspect of the food [1]. In processed meats, the occurrence of lipid oxidation is influenced not only by lipid composition, pro-oxidant and antioxidant content, but also by processing conditions.

COPs determination in cooked meat is very important since many studies have demonstrated that these compounds are more dangerous for arterial cells than cholesterol and they are directly related to atherosclerosis, coronary diseases and mutagenic activity [2-4]. More recently, interest in the possible toxicological effects of lipid oxidation products, particularly COPs, has increased.

Foods of animal origin can develop these derivatives by the action of oxygen, heat, polyunsaturated fatty acids, water, pH, radiation and inadequate packaging and storage, affecting their quality and exposing consumers to potential health problems [5]. Thus, the aim of this study

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is to evaluate one of the factors that influence the formation of COPs i.e. heat. The objectives of this research are to evaluate the effect of the different cooking methods on formation of cholesterol oxides in pork and beef, to determine the amount of COPs in pork and beef and to compare the formation of cholesterol oxides in pork and beef.

MATERIALS AND METHODS

Samples: The sirloin part of pork and beef were purchased at a local market. The beef and pork were cut into slices into square cuts randomly divided into three groups. Raw treatments represented control samples. Grilled (230°C during 20 minutes) and roasted (190°C during 25 min) represented the different cooking methods of beef and pork samples, respectively.

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

Direct Saponification and Extraction: Cholesterol and cholesterol oxides were extracted simultaneously by direct saponification [6]. A total of 2 g of minced pork and beef (raw and cooked) was weighed into a 100 ml of conical flask respectively. A total of 10 ml of 1 M potassium hydroxide (KOH) in 95 % ethanol was added to the sample at room temperature for 22 hrs in the dark. A total of 5 ml of distilled water and 10 ml of hexane were added to the samples and the mixture was vortexed. The upper layer (hexane fraction) was collected and transferred into capped universal tube. The non saponifiable matter was extracted three times with 10 ml of hexane. The collected hexane was dried by nitrogen flushing at ambient temperature.

Derivatisation of Cholesterol Oxides: A total of 100 ìl of TMSI reagent was added to the dried COPs samples and then incubated at 80°C for one hour. The reagent was then evaporated under a stream of nitrogen gas and stored and TMS-ether derivatives were dissolved in 1 ml of hexane (GC grade). The tubes were sonicated in an ultrasonic bath for 1 min and were centrifuged for 30 min at 13,000 rpm and were then preceded for subsequent analysis by GC/MS-QQQ [7].

Determination of Cholesterol Oxides by GC/MS-QQQ: Analysis was performed on a gas chromatography combined with triple quadrupole 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 Quantitative version. A capillary column DB5: MS UI 30 m × 0.25 mm × 0.25 µm was used to separate the COPs. The column temperature was set at 250°C and risen to the constant temperature at 280°C for 5 mins. The temperature was raised to 300°C at 50°C/min. The detector was set at 320°C and gas flow was 1.2 ml/min. Volume of injection was 1 ìl and splitless mode was used. GC/MS-QQQ was set-up with the characteristic ions. The temperature source was 230°C, (Electron Ionization), while Mass Spectrometer 1 and Mass Spectrometer 2 temperature was set at 150°C.

Statistical Analysis: The data from GC/MS-QQQ was evaluated by One-Way Analysis of Variance (ANOVA). The statistical analysis significance of each factor considered was calculated at $\alpha = 0.05$ level using the F-test. Duncan's Multiple Range Test (DMRT) was carried out to resolve the difference among the treatment means. A value of $p<0.05$ was used to indicate significance difference. All the statistical evaluations of the result were performed using the Statistical Analysis Software 4.3 (SAS).

RESULTS AND DISCUSSION

Effect of Different Cooking Methods in Beef and Pork on Formation of COPs: In this study, there were no significant differences among cooking methods on COPs values ($p>0.05$) (Table 1). However, a trend was observed, the raw and roasted groups having lower total cholesterol and COPs compared to the grilled group of treatments. Grilled group showed the highest total cholesterol and COPs level (beef: 4.2×10^4 ppb, pork: 1.2×10^4 ppb). It seems that a shorter time and higher temperature of cooking affect oxidation processes more than a longer time and lower temperature.

According to several studies, such as those of Chien *et al*. [8], Kim and Nawar [9] the conversion of cholesterol to COPs depends on the temperature. However, in the study by Vicente and Torres [10], samples (hamburgers) which were fried on a hot plate, cooked on a stove or cooked in a microwave oven with roasting capabilities for

Table 1: Amount of cholesterol and derivatised cholesterols in beef and pork with different treatments.

	Beef		x10^{-9}	Pork		
COP (ppb)	Raw	Grilled	Roasted	Raw	Grilled	Roasted
5-α cholestane	6.5±4.9^{a}	8.4±4.5^{a}	6.7±2.1^{a}	3.0±4.25^{a}	2.3±1.04^{a}	2.0± 1.16^{a}
7-keto	$6.8\times10^{-2}\pm 1.0\times10^{-1a}$	$3.1\times10^{-1}\pm 4.3\times10^{-1a}$	$4.2\times10^{-1}\pm 1.0^{a}$	$2.1\times10^{-2}\pm 6.0\times10^{-2b}$	$9.5\times10^{-2}\pm 1.3\times10^{-1a}$	Nd
α -epoxide	$6.9\times10^{2}\pm 1.4\times10^{3a}$	$3.8\times10^{2}\pm 1.1\times10^{3a}$	$4.3\times10^{2}\pm 8.0\times10^{2a}$	$3.4\times10^{2}\pm 4.9\times10^{2a}$	Nd	Nd
β-epoxide	$6.1\times10^{2}\pm 8.2\times10^{2a}$	$1.2\times10^{3}\pm 1.2\times10^{3a}$	$7.4\times10^{2}\pm 8.6\times10^{2a}$	$6.4\times10^{2}\pm 4.6\times10^{2a}$	$9.5\times10^{2}\pm 4.8\times10^{2a}$	$1.1\times10^{3}\pm 4.2\times10^{2a}$
25-HC	2.3±2.36^{a}	2.3±2.67^{a}	2.9± 3.7^{a}	$2.2\times10^{-1}\pm 6.3\times10^{-1a}$	1.3±2.1^{a}	1.2 ± 1.6^{a}
cholesterol	$2.9\times10^{4}\pm 2.6\times10^{4a}$	$4.0\times10^{4}\pm 2.9\times10^{4a}$	$3.1\times10^{4}\pm 2.3\times10^{4a}$	$6.0\times10^{3}\pm 3.8\times10^{3b}$	$1.1\times10^{4}\pm 3.6\times10^{3a}$	$9.2\times10^{3}\pm 3.5\times10^{3b}$
Total cholesterol and COPs	3.0×10^{4}	4.2×10^{4}	3.2×10^{4}	7.0×10^{3}	1.2×10^{4}	1.0×10^{4}

Results are expressed as mean ± standard deviation. $^{a-b}$ Averages with different letters in the same row (within samples) indicate significant differences. There is a significant differences ($p<0.05$). ND: Not detected

Table 2: Comparison concentration of cholesterol and COPs in the different treatment.

	Raw		Grill		Roast	
COP (ppb)	Beef	Pork	Beef	Pork	Beef	Pork
5-α cholestane	6.5±4.9^{a}	3.0±4.25^{a}	8.4 ± 4.5^{a}	2.3±1.04^{b}	6.7 ± 2.1^{a}	2.0±1.16^{b}
7-keto	$6.8\times10^{-2}\pm 1.0\times10^{-1a}$	$2.1\times10^{-2}\pm 6.0\times10^{-2b}$	$3.1\times10^{-1}\pm 4.3\times10^{-1a}$	$9.5\times10^{-2}\pm 1.3\times10^{-1a}$	$4.2\times10^{-1}\pm 1.0^{a}$	ND
α -epoxide	$6.9\times10^{2}\pm 1.4\times10^{3a}$	$3.4\times10^{2}\pm 4.9\times10^{2a}$	$3.8\times10^{2}\pm 1.1\times10^{3a}$	ND	$4.3\times10^{2}\pm 8.0\times10^{2a}$	ND
β-epoxide	$6.1\times10^{2}\pm 8.2\times10^{2a}$	$6.4\times10^{2}\pm 4.6\times10^{2a}$	$1.2\times10^{3}\pm 1.2\times10^{3a}$	$9.5\times10^{2}\pm 4.8\times10^{2a}$	$7.4\times10^{2}\pm 8.6\times10^{2a}$	$1.1\times10^{3}\pm 4.2\times10^{2a}$
25-HC	2.3±2.36^{a}	$2.2\times10^{-1}\pm 6.3\times10^{-1b}$	2.3 ± 2.67^{a}	1.3 ± 2.1^{a}	2.9 ± 3.7^{a}	1.2 ± 1.6^{a}
cholesterol	$2.9\times10^{4}\pm 2.6\times10^{4a}$	$6.0\times10^{3}\pm 3.8\times10^{3b}$	$4.0\times10^{4}\pm 2.9\times10^{4a}$	$1.1\times10^{4}\pm 3.6\times10^{3b}$	$3.1\times10^{4}\pm 2.3\times10^{4a}$	$9.2\times10^{3}\pm 3.5\times10^{3b}$

a-b Averages with different letters in the same row (within samples) indicate significant differences. The comparison is between pork and beef in different treatment. The result is significantly difference when $p<0.05$. ND: Not detected

long enough to reach medium consumption conditions, showing no or low levels of COPs after thermal processing. The absence of COPs in raw samples can be explained by the fact that all of them were fresh and kept in a freezer at -20°C and in the dark storage and testing. Thermally processed samples also showed no or low levels of COPs, because the temperature during thermal processing did not reach the values required to propagate the formation of COPs [10].

The range of cholesterol content in the three different treatments of beef (Table 1) was from $2.9\times10^{4} \pm 2.6\times10^{4}$ ppb/2 g to $4.0\times10^{4} \pm 2.9\times10^{4}$ ppb/2 g (grilled beef) and $3.1\times10^{4} \pm 2.3\times10^{4}$ ppb/2 g (roasted beef). These data was similar to the range reported by Sampaio *et al.* [11]. According to reports by Echarte *et al.* [12] and Echarte *et al.* [13] the variations in oxidation could be attributed to the methods of cooking,

for example grilling or roasting. Different authors support the findings obtained in the present study for cooked pork, showing that cooking methods significantly increase the formation of COPs in different meats [14- 18].

In addition, the denatured protein by cooking, which can lead to the loss of antioxidant enzyme activate the release of catalytically-active iron from metallo-proteins (mainly myoglobin); disruption of cell membranes, which bring polyunsaturated fatty acids into contact with pro-oxidant; transformation of myoglobin from an antioxidant to a pro-oxidant species; and thermal decomposition of hyperoxides to pro-oxidant species, such as alkoxyl hydroxyl radicals. Thus, cooking may lead to significantly increased oxidation, as reflected by COPs values [15].

Based on Table 2, cholesterol and 25-hydroxycholesterol was detected to have a significant difference ($p<0.05$) between raw beef and pork. There was a significant difference significantly between 5-α cholestane, 25-hydroxycholesterol and cholesterol in both grilled samples. There was also a significant difference of 5-α cholestane and cholesterol in roasted beef and pork (5-α cholestane in rosted beef = 6.5 ppb; 5-α cholestane in roasted pork = 3.0 ppb; cholesterol in roasted beef = 3.1×10^{4} ppb; cholesterol in roasted pork = 9.2×10^{3}).

CONCLUSION

Cholesterol was found to be the highest content in both samples, followed by β-epoxycholesterol, α-epoxycholestrol, 25-hydroxycholestrol and the least was 7-ketocholesterol. The total cholesterol and COPs were higher in the grilling treatment compared to roasting treatment. Besides, the beef sample was detected to contain higher of total cholesterol and COPs. It implies that consume beef regularly gives bad effect to health. It is recommended to do analysis on the collected drip loss during the cooking treatments as the cholesterol and COPs might be lost during heating. Meanwhile, a wider range of COPs reference standards must be used (e.g. 7β-hydroxycholesterol and 7α-hydroxycholesterol) instead

of six standards that were used in this experiment, to obtain a more accurate evaluation on the effects of cooking methods towards the formation of COPs.

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