EFFECT OF OIL TYPES ON THE FORMATION OF CARCINOGENIC POLYCYCLIC AROMATIC HYDROCAEBONS IN GRILLED CHICKEN

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Abstract – The study was conducted to investigate the effect of marinated oils on the formation of four major polycyclic aromatic hydrocarbons (PAHs) in grilled chicken breast. Three marinade treatments containing basic marinade (without vegetable oil), palm-oil marinade and sunflower-oil marinade were applied on meat samples before charcoal grilling. Formation of PAHs in grilled chicken with different marination was evaluated after charcoal-grilled at 3 min/side. Compared with the basic marinade, the addition of commercial palm oil (7.4% radical scavenging activity) and sunflower-oil (18.4% radical scavenging activity) in marinade led to significant increase of PAHs in grilled chicken from 26.27 µg/kg to 140.37 µg/kg and 133.29 µg/kg, respectively. Results of this study suggest that addition of oil in marinade can be important factor in increasing the levels of PAHs in grilled meat products.

Key Words – Polycyclic aromatic hydrocarbons (PAHs), Marinade, Oil, Grilled chicken

I. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are produced through incomplete combustion or pyrolysis of organic matter and geological processes [1]. PAHs containing two to four fused benzene rings are called light PAHs and those containing more than four benzene rings are known as heavy PAHs that more stable and more toxic than light ones [2]. A number of PAHs have been reported genotoxic and mutagenic, while other PAHs are defined as synergists which help the mutagenic PAHs. PAHs themselves are not toxic, but they are activated by the attempt of the organism to eliminate these xenobiotics by increasing the polarity through the addition of polar groups [3]. PAH levels in foods have been regulated via European Union (EU) Regulation No 835/2011. Sum of PAHs (PAH4: benzo[a]pyrene (BaP), benz[a]anthracene (BaA), benzo[b]fluoranthene (BbF) and chrysene (CHR)) has been indicated to be a suitable indicator of carcinogenic PAHs in food [4]. A maximum levels for benzo[a]pyrene in smoked meats and smoked meat products was 2.0 µg/kg wet weight and a maximum levels for the sum of PAH4 in these foods was set at 12.0 µg/kg wet weight.

In meat products, the PAHs are formed during processing at high temperature such as smoking, drying, roasting or grilling. Grilling is a one type of heat-cooking processes that use the high temperature of heating source to destroy pathogenic and spoilage microorganism, to contribute the quality of food (color, flavor and texture) and to preserve as a way to extend the shelf-life. Some PAHs may be formed and released during the incomplete combustion or pyrolysis of fat in meat due to fat is the major precursor of PAHs [5]. The effect of fat on the formation of PAHs includes the fat in raw materials [6], [7] and the addition of fat/oil in foods [8], [9] to contribute the juiciness and tenderness of foods

Since the information on the effects of oil (i.e. source, fatty acid composition) on PAH formation are very limited, therefore this study was conducted to investigate the effect of oil marinade treatments including palm oil (rich in saturated fatty acids) and sunflower oil (rich in unsaturated fatty acids) [10] on PAHs formation during charcoal grilling in grilled breast meat.

II. MATERIALS AND METHODS

Sample preparation

Chicken breast samples were cut into small pieces (about 3 x 4 x 0.5 cm), and kept in the freezer at -20°C until used.

Marinade ingredients

All ingredients of the marinade were obtained from a supermarket. The composition of marinade treatments in all parts were presented in Table 1.

Table 1 Marinade treatments and their ingredients composition (for 1 kg of meat)

Marinade treatments	Ingredients	Amount (g)
Basic marinade	Water	50
	Sugar	50
	Oyster sauce	20
	Salt	10
	Garlic powder	5
	Black pepper powder	2.5
Palm-oil marinade	Basic marinade	137.5
	Palm oil	50
Sunflower-oil marinade	Basic marinade	137.5
	Sunflower oil	50

Charcoal grilling process

Approximately 500 g of charcoal and 60 g of wood were placed in the bottom of the grill. The meat samples were grilled 3 min/side. After cooling to room temperature, all samples were packed in foil bags and stored at -20°C until use for PAHs analyses.

Extraction and clean up PAHs in meat sample

The extraction and purification methods were modified from Gomes *et al.* [11]. A sample of 10 g was saponified under reflux in 100 mL of a mixture of methanol and water (80:20, v/v) and extracted with 50 mL of hexane 4 times. The resulting fractions containing PAHs were combined and evaporated to dryness in a parallel evaporator under reduced pressure. The residue was dissolved in 3 mL of acetonitrile, cleaned up by solid-phase extraction (SPE, Sep-Pak Florisil cartridge) which was previously activated with 10 mL of dichloromethane and 20 mL of hexane with a flow rate of 2.0 mL/min and then elute with 18 mL of a mixture of hexane and dichloromethane (3:1, v:v). The eluate was purged N_2 gas to dryness, dissolved in 400 µL acetonitrile and filtered through a 0.45 µm membrane filter prior to HPLC-DAD analysis.

HPLC analysis

PAHs analysis was carried out using an HPLC instrument with photodiode array detector. A ZORBAX Hypersil ODS column of 250 mm x 4.6 mm, 5 µm particle size was used. The gradient system of acetonitrile and water, modified from Chen *et al.* [12] was used to separate PAH4 (BaP, BaA, BbF and CHR) at a flow rate of 1.5 mL/min. The mobile phase started with 60% acetonitrile for 5 min, was then programmed to 100% acetonitrile within 25 min, and was maintained for 15 min. Finally, decrease acetonitrile to 60% within 1 min and maintained for 14 min. For photodiode array detector, scanned wavelength from 200 nm to 600 nm and showed chromatogram at 254 nm (Fig. 1). The quantification of PAHs was performed by the use of an external calibration curve method.

Antioxidant properties

DPPH assay has been modified by Christodouleas *et al.* [13] to evaluate the scavenging activity of oil samples by spectrophotometry at 515 nm. Modified DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in 2- propanol. DPPH· radical will react with antioxidant in sample and the color of sample solution will be change. Then, the radical scavenging activity (%RSA) of diluted samples was calculated by Equation 1:

$$\%RSA = \frac{A0-A}{A0} \times 100$$

Where A and A_0 are the absorbance of sample and blank sample, respectively.

III. RESULTS AND DISCUSSION

PAHs concentrations were determined in grilled chicken breast treated with different marinade treatments (basic marinade, palm-oil marinade and sunflower-oil marinade); the results are summarized in Table 2. The results showed that the lowest of PAH4 concentrations was observed in the basic marinade chicken breast (26.27 µg/kg) whereas the high concentration was found in both of palm-oil marinade and sunflower-oil marinade chicken breast (140.37 µg/kg and 133.29 µg/kg, respectively). The concentration of PAH4 of both palm-oil and sunflower-oil marinated chicken breast samples were significantly ($p \leq 0.05$) higher than that in

the basic marinated chicken breast sample. As presented in Table 1, palm-oil and sunflower-oil marinade treatment contain extra fat due to addition of vegetable oil. Therefore, the high concentration of PAH4 in these two types of marinated chicken breast may be due to the higher fat content of these samples [6], [7]. Saito *et al.* [7] reported that the fat content was the main component that generated PAHs during thermal cooking. Also, according to Farhadian *et al*.[8], PAHs formation during charcoal grilling was dependent upon the fat content of marinade treatment.

Table 2 PAH4 concentration of difference marinated meat samples, P/S index and %RSA of vegetable oils

Marinade treatments	P/S index*	%RSA**	PAH4 (µg/kg)
Basic marinade	-	-	26.27[b]
Palm-oil marinade	0.22	7.41	140.37[a]
Sunflower-oil marinade	5.80	18.49	133.29[a]

* Polyunsaturated to saturated fatty acids ratio
** %Radical scavenging activity
[a,b]Mean values in a column followed by the same letter are not significantly different ($p \leq 0.05$).

The definite mechanism of PAHs formation is not well understood; however, some researchers proposed that they might be formed through free radical reaction, intramolecular addition or polymerization of small molecules [14], [15]. Unsaturated fatty acids is the one type of compound that simpler to form small molecules and/or free radical compounds such as acetylene (C_2H_2), butadiene (C_4H_{10}) and cyclopentadienyl radical ($C_5H_5\cdot$) that can react with other hydrocarbon compounds via complicated mechanism to form more stable molecules for example PAHs than the saturated fatty acids. So the amount of PAHs generated in grilled meat samples was positively correlated with the amount of unsaturated fatty acid. As previously mentioned, sunflower oil that has the higher unsaturated fatty acid (P/S index 5.80) than palm oil (P/S index 0.22) [10] should be affect to occurrence of PAHs in grilled meat samples more than palm oil. On the other hand the results showed that the concentration of PAH4 in the sunflower-oil marinade chicken breasts (133.29 µg/kg) was not significantly difference ($p \leq 0.05$) compared with those marinated with palm oil (140.37 µg/kg). This may be due to the higher antioxidant content such as vitamin E and BHA (butylated hydroxyanisole) in sunflower oil. Modified DPPH assay [13] were used to evaluate the radical scavenging activity (%RSA) and the results showed that sunflower oil has the antioxidant activity (18.49%RSA) more than palm oil (7.41%RSA). These results indicate the positive effects of antioxidant activity in oil on reducing the formation of PAHs in grilled chicken breast samples. The reduction effect of marinating in reducing the PAHs formation may be due to the presence of antioxidant activity of ingredients [16].

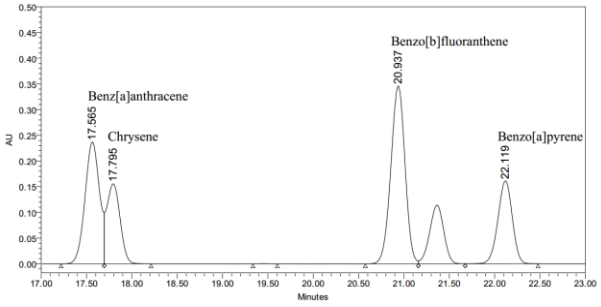

Figure 1. Chromatogram of standard PAH4 at 254 nm

IV. CONCLUSION

Vegetable oils (palm oil and sunflower oil) as ingredients of marinade treatment for grilled chicken breast have contributed to the higher concentration of PAH4 as compared to basic marinade treatment (without vegetable oil). However, marinade treatment using oil with high level of antioxidant activity led to more reduction of PAHs concentrations. The best order of marinade treatment for the grilled chicken breast samples used in the study was basic marinade (without vegetable oil), sunflower-oil marinade or palm-oil marinade, respectively. Therefore, the obtained results from this study provide the basic knowledge for further studies about PAHs and the other carcinogenic compounds such as heterocyclic amines (HCAs), which mare produced during high temperature processes of many foods. Considering the carcinogenic potential of PAHs, it is important to set up the strong measures to increase the awareness and reduce the intake of food contaminated with PAHs.

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