Formation of Polycyclic Aromatic Hydrocarbons (PAHs) in Lamb during Roasting

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Formation of polycyclic aromatic hydrocarbons (PAHs) in lamb grilled over charcoal and changes of PAH content with roasting time are described. Two hundred grams of lamb meat containing 65.2% moisture was grilled over charcoal for 0, 3, 5, 10, 15 and 20 min at 500~600°C. The roasted meat was homogenized with 600 ml of chloroform-methanol (2:1, v/v) and extracted three times. After filtration and clean-up on a silica gel column and by thin-layer chromatography, PAHs were analyzed by high performance liquid chromatography and gas liquid chromatography-mass spectrometry. The lipid content decreased with increasing heating time. The maximum concentrations of PAHs were 49.0 ppb for pyrene, 40.9 ppb for chrysene, 36.0 ppb for fluoranthene, 28.0 ppb for 9-methylanthracene, 17.1 ppb for anthracene, and 8.9 ppb for phenanthrene at 3 min roasting, and 17.4 ppb for benzo[a]pyrene at 5 min roasting. The mutagenic activity of each PAH decreased with increasing heating time. PAH formation was well correlated with lipid contents in lamb meat ($r=0.926$, $p<0.05$).

Key words: polycyclic aromatic hydrocarbons; charcoal; lamb meat; high performance liquid chromatography (HPLC); gas liquid chromatography-mass spectrometry (GC/MS); mutagenic activity

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are known to be widespread in the environment. They are present in smoke from burning plants and exhaust gas from diesel engines, and are formed during the combustion and carbonization of many fossil fuels.^{1)~3)} Similarly, carbohydrates, proteins and lipids of organic materials produce PAHs during combustion at high temperature. It is accordingly possible that PAHs are distributed not only in the atmosphere, aquatic sphere and soil but also in cooked foods, such as mutton, chicken, fish and vegetables.^{4)~7)} It has been reported that nitro-PAHs exist in smoked foods, such as smoked chicken.⁸⁾ These reports suggested that many PAHs are produced by cooking. However, the formation of PAHs during cooking on charcoal fires has not been reported so far. Charcoal grilling of lamb meat is a widespread cooking habit, in Hokkaido particularly.

This paper describes the composition of PAHs

found in lamb meat grilled over charcoal, and the changes of PAH content with roasting time.

Materials and Methods

Materials

All organic solvents were used after distillation. Anthracene, chrysene, benzo[a]pyrene (B[a]P) and dibenzo[a,h]anthracene were obtained from Tokyo Kasei Industries Co.; phenanthrene, pyrene and 9-methylanthracene from Wako Pure Chem. Industries; fluoranthene from Eastman Kodak Co.; and silicic acid (100 mesh AR) from Mallinckrodt Co. Lamb meat was obtained from a commercial source.

Extraction and clean-up of PAH

Two hundred grams of lamb meat containing 65.2% moisture was directly grilled over charcoal for 0, 3, 5, 10, 15 and 20 min at 500~600°C. The temperature was measured at the charcoal surface using a Yamato solid-state power controller connected to a thermo-detector and was confirmed to remain in the range of 500 to 600°C

during roasting. Extraction and clean-up of PAH were carried out according to the method of Negishi *et al.*⁸⁾ The roasted lamb was homogenized in 600 ml of chloroform-methanol (2 : 1, v/v) and extracted three times. The extracts were combined and concentrated on a rotary evaporator *in vacuo* to obtain the lipid. One gram of the lipid was taken up in cyclohexane and subjected to silica gel (30 g) column chromatography. The PAHs were eluted successively with 200 ml of cyclohexane, 200 ml of cyclohexane-benzene (1 : 1, v/v), 200 ml of benzene, 200 ml of chloroform-methanol 95 : 5, v/v, 200 ml of chloroform-methanol (9 : 1, v/v) and 200 ml of chloroform-methanol (2 : 1, v/v). All fractions were subjected to thin-layer chromatography (TLC) using Merck precoated silica gel 60 plates and cyclohexane-chloroform (1 : 1, v/v) as a solvent system. The spots in the cyclohexane-benzene (1 : 1, v/v) fraction corresponding to the reference PAH standard were scraped off and the compounds absorbed on silica gel were eluted with chloroform-methanol (2 : 1, v/v) using a sonicator (45 kHz). The eluate was filtered.

Analysis of PAH

A Hitachi Model 638 high performance liquid chromatograph (HPLC) fitted with an ERC-ODS-1171 column (6.0 × 200 mm) was used. The composition of the mobile phase was acetonitrile-water (7 : 3, v/v) and the effluent was monitored at 254 nm. The flow rate was 1.0 ml/min at 150 kg/cm² and the column oven temperature was

30°C.

Gas liquid chromatography-mass spectrometry (EI, GC/MS) was performed on a Shimadzu QP-1000 using a capillary fused silica column (0.33 mm × 25 m) coated with CBP-1. Operating conditions were as follows: injection temperature, 300°C; column temperature, 180~270°C at 5°C/min; flow rate of the carrier gas (helium), 30 ml/min; ion source and molecular separator temperature, 250°C and ionization voltage, 70 eV.

Recovery test of PAH

Recovery tests of anthracene, phenanthrene, fluoranthene, pyrene, chrysene, B[a]P and di benzo[a, h]anthracene added at levels of 100 µg, respectively, to 100 g of lamb meat were done by GC/MS.

Statistical analysis

The statistical significance of differences between values was analyzed by using the Pearson correlation coefficient.

Table 2. Yields of Lipids from Roasted Lamb

Roasting time (min)	Lamb weight (g)	Yield (g)	Content (%)
0	200.0	37.97	18.99
3	200.0	28.91	14.46
5	200.0	21.71	10.86
10	200.0	10.14	5.07
15	200.0	5.00	2.50
20	200.0	2.20	1.10

Table 1. Recovery of PAHs Added to Lamb Meat

Added PAH	Average recovery (%) (two runs)
Anthracene + phenanthrene	84.5
Fluoranthene	95.0
Pyrene	87.6
Chrysene	88.0
Benzo[a]pyrene	85.0
Dibenzo[a, h]anthracene	84.9

The peak corresponding to anthracene + phenanthrene in GC/MS was calculated with respect to total contents of added anthracene and phenanthrene standards.

Fig. 1. TIC and mass chromatograms of polycyclic aromatic hydrocarbons in roasted lamb

Fig. 2. Mass spectra of polycyclic aromatic hydrocarbons in roasted lamb

Results and Discussion

The recoveries of PAHs added to lamb meat are shown in Table 1. The recoveries were in the range of 84.5~95.0% and are quite sufficient for the present purpose. The lipids in the chloroform-methanol (2:1, v/v) extract from each roasted lamb sample are shown in Table 2. The amounts decreased with increasing grilling time and the content ranged from 1.1 to 19.0%. This decrease was considered to reflect the loss of fat by dripping onto the heat source. The amounts in the eluate from the silica gel column chromatography with cyclohexane-benzene (1:1, v/v) ranged from 154.4 to 3807.3 mg for 3 and 20 min, respectively and also decreased with increasing heating time.

PAHs in the eluates were purified by TLC and were identified as anthracene, phenanthrene, 9-methylanthracene, pyrene, chrysene, B[a]P and dibenzo[a, h]anthracene by GC/MS (Figs. 1 and 2). PAHs were quantified by GC/MS and HPLC. Anthracene and phenanthrene, which were not separated by GC/MS, were separated by HPLC

Fig. 3. HPLC chromatogram of polycyclic aromatic hydrocarbons in roasted lamb

Table 3. Contents of Polycyclic Aromatic Hydrocarbons (PAHs) in Roasted Lamb

PAH component	Roasting time (min)					
	0	3	5	10	15	20
	(ppb)					
Anthracene	0.7	17.1	14.9	16.5	5.2	9.5
Phenanthrene	0.4	8.9	7.8	8.6	2.7	5.0
9-Methylanthracene	ND	28.0	14.4	12.3	0.7	ND
Fluoranthene	ND	36.4	23.9	32.7	4.1	2.1
Pyrene	ND	49.0	21.2	30.2	3.9	2.0
Chrysene	ND	40.9	23.9	10.2	0.5	7.6
Benzo[a]pyrene	ND	16.9	17.4	6.6	0.9	6.7
Dibenzo[a, h]anthracene	ND	ND	ND	ND	ND	5.8

ND: not detectable (0.1 ppb)

Anthracene and phenanthrene were quantified by HPLC and other PAHs were quantified by GC/MS.

Fig. 4. Relation between mutagenic activity of polycyclic aromatic hydrocarbons on TA 100 and roasting time

Points were calculated from chrysene 1670/10, benzo[*a*]pyrene 2398/5 and dibenzo[*a, h*]anthracene 401/10 revertants per plate (μ g), with *Salmonella typhimurium* TA100 strain

Fig. 5. Relation between lipid content and total polycyclic aromatic hydrocarbons concentration in roasted lamb

(Fig. 3).

The content of each PAH induced by roasting is shown in Table 3. The maximum concentrations of PAH were 49.0 ppb for pyrene, 40.9 ppb for chrysene, 36.0 ppb for fluoranthene, 28.0 ppb

for 9-methylantracene, 17.1 ppb for anthracene, and 8.9 ppb for phenanthrene at 3 min roasting, and 17.4 ppb for B[*a*]P at 5 min roasting. Dibenzo[*a, h*]anthracene (5.8 ppb) was detected only at 20 min. These results conflict with a report that the maximum concentrations of B[*a*]P and chrysene were detected after 25 min.⁷⁾ The difference may be caused by the cooking methods and the cooking temperatures. In fact, PAH levels in lamb meat and the influence of cooking time upon PAH formation were similar to those in sardines, presumably since these samples were grilled directly.⁶⁾ It is general that the formation of PAHs is accelerated at 500~800°C.⁹⁾ Although the lamb meat was grilled at the cooking temperature of 500~600°C in this experiment, Sivaswamy *et al.* fried mutton meat on a pan at a cooking temperature of 220~225°C.⁷⁾

The relation between mutagenic activity of PAHs and the roasting time is shown in Fig. 4; *Salmonella typhimurium* TA 100 strain was used.¹⁰⁾ It was reported that the mutagenicity was increased by longer heating.¹¹⁾ However, in this experiment, it was suggested that the mutagenic activity of PAH was highest at 3 min, when the lamb was sufficiently roasted for eating, and decreased after grilling for 5 min. The correlation coefficient (*r*) between the mutagenic activity and the roasting time was 0.830. It may be considered that the formation rate of PAHs was accelerated, but the PAHs were lost in fat that dripped onto the heat source, since the cooking temperature was higher than that in other reports.^{6), 7), 11)}

PAH concentration in relation to the lipid content is shown in Fig. 5. The PAH concentration correlated positively with the lipid content, the correlation coefficient (*r*) being 0.926 (*p* < 0.05). This is consistent with the relation between lipid content and amount of 3,4-benzopyrene in fish.⁴⁾ It is considered that PAHs are produced from cholesterol, triacylglycerol, fatty acid and carotenoid,^{4), 12)} but in this experiment we did not investigate the composition of lipid in the roasted lamb meat. The relation between the lipid composition and the formation of PAHs by charcoal grilling is now being examined.

It was concluded that PAH concentration in roasted lamb was highest at 3 min in this exper-

iment, when the lamb meat was sufficiently roasted for eating. The barbecue style using charcoal is popular in Hokkaido. It will be necessary to investigate the formation of PAHs in barbecued materials other than lamb meat.

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