농학박사 학위논문

Risk assessment of polycyclic aromatic hydrocarbons in edible oils and teas and their reduction during grilling meats

식용유지 및 다류 중 다환방향족탄화수소의 위해성 평가 및 숯불구이 중 저감화

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이 준 구

Abstract

Risk assessment of polycyclic aromatic hydrocarbons in edible oils and teas and their reduction during grilling meats

Lee, Joon-Goo

Department of Agricultural Biotechnology

The Graduate School

Seoul National University

Polycyclic aromatic hydrocarbons (PAHs) are carcinogenic and genotoxic chemicals naturally derived from food during heat processing. Edible oil is one of the most frequently contaminated foods. Many researches were recently conducted to determine the contents of PAHs and assess their risks, but there have been no studies characterising risks of PAHs by calculating Margin of Exposure (MOE) of total PAHs instead of toxic equivalency factors (TEFs) concept in Korea. And tea is one of the most frequently consumed drinks due to its favourite taste and the health benefit. Tea is produced by several processes and drying is important step to develop

the flavour and destroys the enzymes in tea. However, during drying tea, polycyclic aromatic hydrocarbons, some of which are carcinogen and genotoxin, are naturally produced. To analyze the 4 PAHs simultaneously, gas chromatography combined by mass spectrometry was optimized. 303 edible oils and 468 tea products were investigated and contaminated by 4 PAHs up to 12.91 ng g^{-1} and up to 4.63 ng g^{-1}, respectively. The risks of PAHs by drinking tea and consuming edible oils were characterized by determining contents of 4 PAHs in tea and edible oils.

The MOEs were estimated by PAHs contents, daily consumption, and were over 10,000. The risks of PAHs of tea and edible oils in Korea were of low concern. Furthermore, the MOEs of the estimated equivalent BaP calculated by TEFs of other 3 PAHs were higher than those of mixed PAHs, which would be overestimated. The Margin of Exposures (MOEs) calculated by the concentration of BaA, CHR, BbF and BaP and consumption amount of tea and edible oils were higher than 10,000, and the risk of PAHs in tea and edible oils were low concern to public health.

Furthermore, polycyclic aromatic hydrocarbons (PAHs) are formed when muscle meat is cooked using high-temperature methods, such as grilling directly over an open flame. I investigated the effects of grilling procedures on the level of 4 PAHs; benzo[a]anthracene (B[a]A), chrysene (Chr),

benzo[b]fluoranthene (B[b]F), and benzo[a]pyrene (B[a]P). PAHs were extracted and determined by gas chromatography with mass detection (GC-MS). With regard to barbecuing successive meat samples with the same batch of burning charcoal, it was observed that stable combustion contribute to reduction of PAHs. Significant reductions in the sum of the four PAHs were observed through treatments which removed meat drippings and smoke with alternative grilling apparatus. The sums of 4 PAHs were reduced 48-89 % without dripping and 41-74 % without the smoke in grilled pork and beef meats comparing to conventional grilling. We investigated the components of meats drippings. The major constituent of meat dripping was fat. The most important factor contributing to the production of PAHs in grilling was smoke resulting from incomplete combustion of fat dripped onto the fire.

Keywords: food safety, food processing, processing contaminants, polycyclic aromatic hydrocarbons (PAHs), risk assessment, analytical method development, edible oils, teas, grilled meat, reduction of PAHs

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Contents

Abstract.....I

Contents.....IV

List of Figures.....IX

List of Tables.....XI

Chapter I. General Introduction.....1

Chapter II. Risk Characterization of Polycyclic Aromatic Hydrocarbons in Edible Oils.....10

II-1. Introduction.....11

II-2. Materials and Methods.....15

Chemicals and materials.....15

Pretreatment of Sample.....16

Determination of PAHs using GC-MS.....17

Quality control.....18

Exposure estimation and risk characterization.….....19

II-3. Results and Discussion.....23

Quality control.....23

Occurrence of 4 PAHs in edible oils…….....25

Consumption of edible oils…….....31

Exposure estimation.…….....33

Risk characterization and Uncertainty.....37

Statistical analysis.....39

II-4. Conclusion.....40

Chapter III. Risk Characterization of Polycyclic Aromatic Hydrocarbons in Teas.....41

III-1. Introduction.....42

III-2. Materials and Methods.....50

Chemicals and materials.....50

Sampling.....51

Sample preparation

Leached tea and solid tea (powdered leached tea).....51

Solid tea (processed) and liquid tea (preserved by sugar).....53

Liquid tea.....54

Determination of PAHs using GC-MS.....55

Quality control.....58

Exposure estimation and risk characterization.….....59

Statistical analysis.....60

III-3. Results and Discussions.....61

Quality control.....61

Occurrence of 4 PAHs in teas…….....65

Consumption of teas…….....70

Exposure estimation…….....….....71

Risk characterization and Uncertainty.....80

Statistical analysis.....80

III-4. Conclusion.....82

Chapter IV. Reduction of Occurrence of Polycyclic Aromatic Hydrocarbons in Grilled Meats……....83

IV-1. Introduction.....84

IV-2. Materials and Methods.....87

Chemicals and materials.....87

Sample preparation.....87

Charcoal grilling method.....89
Conditions of grilling temperature and time.....91
Conditions of removing meat drippings.....93
Conditions of removing smoke from flame of charcoals....95
Determination of PAHs using GC-MS.....97
Quality contro.....99
Analysis of meat droppings.....100
Statistical analysis.....100
IV-3. Results and Discussion.....101
Quality control.....101
The levels of the 4 PAHs according to different conditions of grilling temperature and time.....104
Occurrence of PAHs in different fat contents.....106
Analysis of meat dripping 108
Reduction of occurrence of PAHs by removing meat dripping.....110
Reduction of occurrence of PAHs by removing smoke from flame of charcolas.....112
IV-4. Conclusion.....116

References.....119

국문 초록.....143

List of Figures

Fig. II-1. Relative contribution of 4 PAHs in 5 edible oil categories.....28

Fig. II -2. Correlations between 4 PAHs and BaP in 5 edible oil categories; (a)Sesame oil, (b)perilla oil, (c)olive oil, (d)red pepper seasoning oil, and (e)red pepper seeds oil.....30

Fig. III-1. Structures of 16 priority PAHs classified by the US EPA.....45

Fig. III-2. The Chromatograms of GC-MS of 4 PAHs and deuterated 2 PAHs with selected ion monitoring (SIM) mode at m/z of 228, 252, 240 and 264 (A) leached tea, (B) solid tea, (C) liquid tea.....62

Fig. IV-1. The designs of outdoor barbecue griller (① meat, ② grill, ③ thermometer, ④ charcoal 90

Fig. IV-2. The change of grilling temperature according to the temp...92

Fig. IV-3. The designs of outdoor barbecue griller for removing the meat drippings (① meat, ② grill, ③ thermometer, ④ charcoal, ⑤ aluminum tube 94

Fig. IV-4. The designs of outdoor barbecue griller for removing the meat drippings (① meat, ② grill, ③ thermometer, ④ charcoal, ⑤ aluminum duct, ⑥ vacuum pump.....96

Fig. IV-5. The Chromatograms of GC-MS of 4 PAHs and deuterated 2 PAHs with selected ion monitoring (SIM) mode at m/z of 228 (A) , m/z of 252 (B), m/z 240 (C) and m/z 264 (D).....103

Fig. IV-6. The amount of fat, moisture and others in dripping of meat during grilling (n=3) 109

Fig. IV-7. The reduction of occurrence of PAHs in grilled meat with different grilling conditions: (A) control, (B) removing fat drippings. The bars are the standard deviations (n=6).....111

Fig. IV-8. The reduction of occurrence of PAHs in grilled meat with different grilling conditions: (A) control, (B) removing smoke. The bars are the standard deviations (n=6).....113

List of Tables

Table I-1. Polycyclic aromatic hydrocarbons selected as the priority targets to manage the safety.....3

Table II-1. Performance parameters of the method in optimum condition.....24

Table II-2. PAHs concentrations in edible oils.....26

Table II-3. Consumption amounts of edible oils and estimated concentrations of each 4 PAH according to the proportion of not-detected samples. 32

Table II-4. Dietary exposures to each PAH and total 4 PAHs by consuming edible oils.....35

Table II-5. MOEs of total 4 PAHs by sum of each PAH and TEQ_{BaP} as BaP concentration estimated by TEFs.....38

Table III-1. Retention time and m/z value of quantitative and qualitative ions of 4 PAHs and 2 deuterated PAHs.....57

Table III-2. The values of validation parameters (linearity and detection limits).....63

Table III-3. The values of validation parameters (recovery and

repeatability).....64

Table III-4. Levels of 4 PAHs in different teas determined by several studies in some countries.....66

Table III-5. The amount of teas consumed by whole population and drinker only.....72

Table III-6. Estimated exposure to 4 PAHs by drinking teas for whole population.....73

Table III-7. Estimated exposure to 4 PAHs by drinking teas for drinker only.....75

Table III-8. Estimated exposure to 4 PAHs by drinkg teas for whole population and drinker only.....78

Table III-9. Margins of Exposures (MOE)of total 4 PAHs.....79

Table IV-1. Retention time and m/z value of quantitative and qualitative ions of 4 PAHs and 2 deuterated PAHs.....98

Table IV-2. The values of validation parameters (linearity, detection limits, and accuracy).....102

Table IV-3. The concentrations of the total and each of 4 PAHs in different conditions of grilling temperature and time (n=6).....105

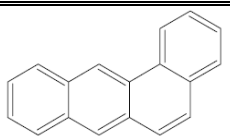
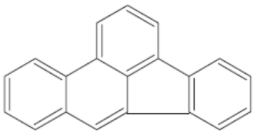
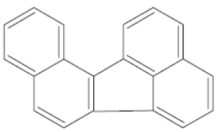
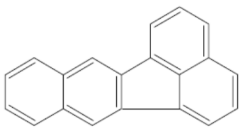
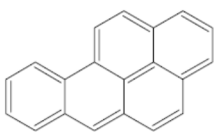
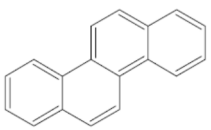
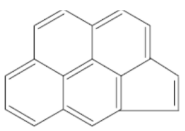
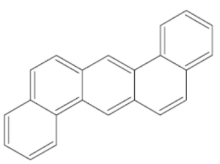
Chapter I.

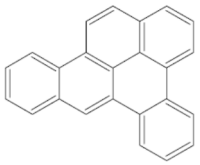
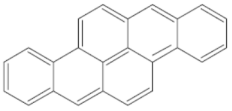
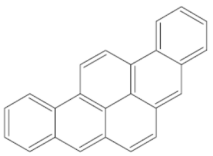
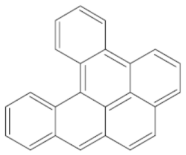
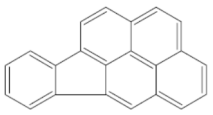
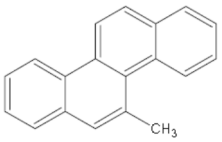
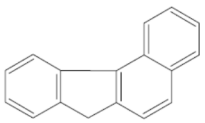
General Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of chemical substances comprised of two or more benzene rings (IARC, 2010). They are occurred from the incomplete combustion of fossil fuels, organism and geochemical process. Some PAHs have been confirmed to have carcinogenic and genotoxic property by binding to deoxyribonucleic acid (DNA) (IARC, 2010). Sixteen PAHs are recently selected as priority targets to manage by Environmental Protection Agency (EPA) on the basis of their occurrence rate and carcinogenic risk (Zelinkova et al., 2015) (Table I-1). The European Union chose benzo(a)pyrene (BaP) as a mark for total PAHs in foods and set maximum levels in different food groups. However, scientists in European Food Safety Authority (EFSA) recently figured out that BaP is improper as an indicator for total PAHs, and 4 PAHs such as Benz(a)anthracene (BaA), Chrysene (CHR), Benzo(b)fluoranthene (BbF), and Benzo(a)pyrene (BaP) would be more suitable as indicators (EFSA, 2008). The European Union has added new maximum levels for sum of 4 PAHs in different food groups (EC, 2011).

The contamination of PAHs in foods is originated from environmental pollutants, manufacturing foods and cooking processes, and PAHs sometimes show up from contaminated packaging material in the low amounts (Lee et al., 2016a, Simko et al., 1995, Speer et al., 1990).

Table I-1. 16 **Polycyclic aromatic hydrocarbons selected as the priority targets to manage the safety**

Compound	Abbr.	MW	Structure
Benz(a)anthracene	BaA	228.3	
Benzo(b)Fluoranthene	BbFA	252.3	
Benzo(j)fluoranthene	BjFA	252.3	
Benzo(k)fluoranthene	BkFA	252.3	
Benzo(g,h,i)perylene	BghiP	276.3	
Benzo(a)pyrene	BaP	252.3	
Chrysene	CHR	228.3	
Cyclopenta(cd)pyrene	CPP	226.3	
Dibenz(a,h)anthracene	DBahA	278.3	

Dibenzo(a,e)pyrene	DBaeP	302.3	
Debenzo(a,h)pyrene	DBahP	302.3	
Debenzo(a,i)pyrene	DBaiP	302.3	
Dibenzo(a,l)pyrene	DBalP	302.3	
Indeno(1,2,3-cd)pyrene	IP	276.3	
5-methylchrysene	MCH	242.3	
Benzo(c)fluorene	BcFL	216.3	

Some studies have been performed to determine PAHs in food samples, and determination of contaminations of PAHs in various food stuffs has been published (EFSA, 2008, Lee et al., 2018). Fats and oils are easily contaminated to PAHs because of their powerful lipophilic characteristics (Balenovic et al., 1995). There are some reasons of PAHs contamination in seed oils. Drying seeds by direct heating with fire or indirect heating with hot air can be a key cause of PAHs contamination in some seed oils. Another possible factor of contamination of PAHs in vegetable oils would be originated from contaminated environment such as soil, water, air or packaging materials (Moret et al., 2000). The direct consumption or production of food with fats and oils as ingredients is one of the most important concerns that PAHs in oils have to be managed (Moret et al., 2005, Teixeira et al., 2007). Furthermore, the absorption of PAHs through intestines is another important reason for managing and controlling the PAHs in fats and oils (Starvic et al., 1994). When crude edible oils are purified and dewaxed through refining processes, the amounts of PAHs can be drastically decreased according to the refining conditions (Moret et al., 2000). Therefore, the management of pressure-extracting oils without refinement is more significant rather than controlling refined oils.

Teas are also easily contaminated by PAHs. Teas are dried to decrease the amount of moisture and destroy the enzymes to develop the tea flavor and taste.

How to dry and how many times dry the tea are regarded to the most important steps. (Özcan et al., 2005, Venskutonis, 1997, Vieira et al., 2010). Tea is traditionally dried by frying tea several times, but recently using smoke, steam, sun-light or flames are easily used for rapidly drying tea. However, these drying processes develop not only quality of tea but also some hazard chemicals in tea. Polycyclic aromatic hydrocarbons (PAHs) are formed during drying tea with high temperature (Adisa et al., 2015, Pincemaille et al., 2014, Vieira et al., 2010). Especially, green tea and black tea imported from China were highly contaminated by PAHs and consumers concerned about drinking teas. Therefore, it is necessary to assess the risks of PAHs in teas.

Some research have assessed the risk of consuming edible oils and drinking teas according to exposure to PAHs to monitor the safety of edible oils and tea, and most of them have used the Toxic Equivalency Quotient (TEQ) to evaluate the risk of exposure to 4 PAHs, since this approach was accepted by the US EPA (Nisbet et al., 1992). TEQ approach is the concept that changes the levels of other PAHs to BAP contents with toxic relations between other PAHs and BaP. The levels of BaP which are converted from those of other PAHs are called BaP equivalent contents (TEQ_{BaP}) and TEQ_{BaP} were calculated by multiplying toxic equivalency factors (TEFs) and each PAHs contents (Jiang et al., 2015, Zhao et al., 2014, Alomirah et al., 2010, Martí-Cid et al., 2008). The exposure to PAHs

was finally shown by the exposure to BaP.

However, Scientists recently figured out that the TEQ concept is not suitable for PAHs because each PAHs has different toxicological mechanism. Food Safety Authority (EFSA) studied toxicological values of mixture of PAHs and suggested $BMDL_{10}$ values for 2 PAHs, 4 PAHs and 8 PAHs in food (EFSA, 2008). $BMDL_{10}$ is the benchmark dose of lower confidence limit to increase the amount of animals bearing tumor by 10%, and it is used for genotoxic and carcinogenic chemicals which do not have shreshoulds in toxicological dose-response relationship and health guidance values such as PAHs.

A Margin of Exposure (MOE) has been used for risk characterization for PAHs with BMDL10 since the Joint FAO/WHO Expert committee on Food Additives (JECFA) recommended us to use it in 2005 (JECFA, 2005, Lee et al., 2016b). Genotoxic and carcinogenic substances do not have health guidance values and the exposure to those chemicals should be minimized as low as reasonably achievable (ALARA). However, risk managers get no information from ALARA to make policies for food safety. MOE can give information for risk managers to establish priority lists for hazardous substances. They compare the MOE values to appropriate reference points. When the MOE values of substances are higher than 10,000, they would be of low concern to public health and risk managers should focus on other substances (Benford et al., 2010a,

Benford et al., 2010b).

There are a few studies assessing the risks of PAHs in food with MOE. Especially edible oils and teas are highly consumed in Korea even though they are easily contaminated by PAHs due to heat processing to dry them. Therefore, I determined levels of 4 PAHs in edible oils and tea highly consumed in the Korean market with a validated analytical method and characterized their risks with MOE values. Furthermore, I compared the risks of PAHs in tea assessed by the traditional TEQ concept and the MOE.

The consumption of grilled, smoked and roasted meats has been recently increased not only at home but also in restaurants. And high intake of those foods has been elevating the public health risk comparing to other foods which is processed by other processing methods (Kao, Chen, Huang, Chen, & Chen, 2014; Sundararajan, Ndife, Basel, & Green, 1999).

The generation of PAHs in grilled and smoked foods is not precisely (Farhadian, Jinap, Fa understood but PAHs may be formed from pyrolysis of organic matter including fat and their recombination in high cooking temperature (ridah, & Zaidul, 2010). PAHs in smoke from incomplete combustion of charcoal or wood can attached to the surface of grilled and smoked foods (Rey-Salgueiro, Garcia-Falcon, Martinez-Carballo, & Simal-Gandara, 2008; Bartle, 1991; Knize, Salmon, Pais, & Felton, 1999). However, no research has

explained which grilling and smoking processes influence on the level of PAHs

Therefore, this study determined what procedures in grilling meats affect the occurrence of PAHs. For this, the levels of PAHs generated during grilling meats with different kinds of meat, cooking temperatures, cooking time and cooking apparatus were compared. This study eventually showed the best cooking practices for reducing the risk of exposure to PAHs by consuming the grilled meats.

Chapter II.

Risk Characterization of Polycyclic Aromatic Hydrocarbons in Edible Oils[1]

[1] This chapter has been published : Joon-Goo Lee, Jung-Hyuk Suh and Hae-Jung Yoon, 2019, Occurrence and risk characterization of polycyclic aromatic hydrocarbons of edible oils by the Margin of Exposure (MOE) approach. Appl Biol Chem, 62:51

II-1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of chemical substances comprised of two or more benzene rings (IARC, 2010). They are occurred from the incomplete combustion of fossil fuels, organism and geochemical process. Some PAHs have been confirmed to have carcinogenic and genotoxic property by binding to deoxyribonucleic acid (DNA) (IARC, 2010). Sixteen PAHs are recently selected as priority targets to manage by Environmental Protection Agency (EPA) on the basis of their occurrence rate and carcinogenic risk (Zelinkova et al., 2015). The European Union chose benzo(a)pyrene (BaP) as a mark for total PAHs in foods and set maximum levels in different food groups. However, scientists in European Food Safety Authority (EFSA) recently figured out that BaP is improper as an indicator for total PAHs, and 4 PAHs such as Benz(a)anthracene (BaA), Chrysene (CHR), Benzo(b)fluoranthene (BbF), and Benzo(a)pyrene (BaP) would be more suitable as indicators (EFSA, 2008). The European Union has added new maximum levels for sum of 4 PAHs in different food groups (EC, 2011).

The contamination of PAHs in foods is originated from environmental pollutants, manufacturing foods and cooking processes, and PAHs sometimes show up from contaminated packaging material in the low amounts (Lee et al., 2016a, Simko et al., 1995, Speer et al., 1990). Some studies have been performed to determine

PAHs in food samples, and determination of contaminations of PAHs in various food stuffs has been published (EFSA, 2008, Lee et al., 2018). Fats and oils are easily contaminated to PAHs because of their powerful lipophilic characteristics (Balenovic et al., 1995). There are some reasons of PAHs contamination in seed oils. Drying seeds by direct heating with fire or indirect heating with hot air can be a key cause of PAHs contamination in some seed oils. Another possible factor of contamination of PAHs in vegetable oils would be originated from contaminated environment such as soil, water, air or packaging materials (Moret et al., 2000). The direct consumption or production of food with fats and oils as ingredients is one of the most important concerns that PAHs in oils have to be managed (Moret et al., 2005, Teixeira et al., 2007). Furthermore, the absorption of PAHs through intestines is another important reason for managing and controlling the PAHs in fats and oils (Starvic et al., 1994). When crude edible oils are purified and dewaxed through refining processes, the amounts of PAHs can be drastically decreased according to the refining conditions (Moret et al., 2000). Therefore, the management of pressure-extracting oils without refinement is more significant rather than controlling refined oils. Several research measured degree of exposure to PAHs by dietary intakes of edible oils, since US EPA adopted the toxic equivalency Quotient (TEQ) concept to estimate exposure of PAHs (Nisbet et al., 1992). BaP equivalent concentrations (TEQ_{BaP})

were calculated by multiplying each PAH concentration with its toxic equivalency factor (TEFs) (Nisbet et al., 1992, Jiang et al., 2015, Zhao et al., 2014). However, scientists in panel of EFSA recently published that the TEQ approach should be used only for the chemicals which showed the similar toxic mechanism including polychlorinated dibenzo-p-dioxins and –dibenzofurans (Dioxins). Although some PAHs are inducing carcinogenicity by binding to DNA and leading to mutated DNA, each PAHs cause cancers in different mechanisms (EFSA, 2008). Therefore, health guidance values of toxicity should be induced from mixture of PAHs and be used to characterize the risks of PAHs. Unfortunately, not many research has been published to determine the risks of PAHs by using new toxicological values induced from the mixture of PAHs, not a TEQ approached-value. To assess risks of exposure to PAHs to the public health by dietary intake of edible oils, Margin of Exposures (MOEs) were calculated by the benchmark dose lower confidence limit for a 10% increase in the number of tumour-bearing animals in contrast with control animals((BMDL$_{10}$) (Benford et al., 2010, JECFA, 2005). Hazardous chemicals which are not only genotoxic but also carcinogenic may have limitation of detecting a dose-response correlationship in a bioassay, and health-based guidance values cannot be derived without a possible threshold (Benford et al., 2010). Therefore, it is necessary to decrease their risks to As Low As

Reasonably Achievable (ALARA) levels (O'brien et al., 2006). The MOEs have become the better approach and been used for providing priority of risks to policy makers (Lee et al., 2016b). I determined simultaneously the levels of BaA, CHR, BbF, and BaP in edible oils with the validated analytical method and evaluated their risks of exposure to PAHs to the public health by using MOEs. Furthermore, I compared the risks of exposure to PAHs estimated from new toxicological values induced from the mixture of 4 PAHs to those from traditional toxic values calculated from equivalency factor (TEF) values.

II-2. Materials and Methods

Chemicals and materials. I purchased Benzo[a]anthracene, Chrysene, Benzo[b]fluoranthene and Benzo[a]pyrene as standards and deutrated Chrysene and deutrated Benzo[a]pyrene as internal standards from Supelco (Bellefonte, PA, USA). Stock standards solutions of 100 μg/mL were made by dissolving standards and internal standards in methylene chloride, and working standards solutions of 400 μg/L were prepared by diluting stock standards with methylene chloride. 5 calibration standards were diluted to 0.2, 0.5, 1, 2, 5 μg/L with working standards in methylene chloride for leached and solid teas, and 0.1, 0.2, 0.4, 1 and 2 μg/L for liquid tea. Calibration standards contained 2 internal standards of 4 μg/L. Potassium hydroxide (Wako, Osake, Japan), sodium sulfate (Wako, Osake, Japan) were prepared, and ethyl alcohol (Merck, Darmstadt, Hesse, Germany), N,N-dimethylformamide (Merck, Darmstadt, Hesse, Germany), ethyl acetate (Merck, Darmstadt, Hesse, Germany), n-hexane (Merck, Darmstadt, Hesse, Germany), Methylee chloride (Burdick & Jackson, Muskegon, MI, USA) were HPLC grade. Deionized water was filtered by a Milli-Q system (Bedford, MA, USA), and SPE cartridges packed with silica of 50 mg in plastic syringe of 3cc (Supelco, Bellefonte, PA, USA) were used. I used a rotary evaporator

of Eyela (evaporate solutions, a rotary evaporator (Eyela, Tokyo) and a nitrogen evaporator of Organomation Associates. Inc (Oa-SYS Heating Device 5085, USA) to evaporate and dry solutions.

Pretreatment of sample. A oil sample of 10 g was weighed and moved into a separatory funnel, and then it was shaken with *N,N*-DMF-DW (9:1, v/v) of 50 mL and *n*-hexane of 100 mL in the presence of the 2 deuterated internal standards (4 ng g^{-1}). The *N,N*-DMF-DW (9:1, v/v) was transferred to a new separatory funnel and hexane layer solution extracted twice with 25 mL of *N,N*-DMF-DW (9:1, v/v) by shaking and equilibrating it. A sodium sulfate solution (1 %) of 100 mL aliquot and *n*-hexane of 50 mL were added to the *N,N*-DMF-DW layer and shaken, and the *n*-hexane layer was moved to another separatory funnel. The extraction with n-hexane was repeated twice. The extracted hexane were washed with 40 mL of DW 3 times, and then, anhydrous Na_2SO_4 (15 g) was added to the hexane extract to remove DW remained. The extract was evaporated to approximately 2 mL by a rotary evaporator (Eyela, Tokyo Rikakikai Co. Ltd., Japan) to be purified with SPE catrideges (MFDS, 2013). The enriched extract was purified with the silica cartridge activated with dichloromethane (10mL) and n-hexane (20mL), and then the cartridge was washed with *n*-hexane (5mL) and eluted with *n*-hexane-dichloromethane (3:1,

v/v) (15mL). The eluate was concentrated to approximately 2 mL using a rotary evaporator. The concentrate was purified with a SPE-PAHs cartridge previously activated with n-hexane (1mL), and the cartridge was eluted with 0.5 mL and 1 mL of *n*-hexane followed by 3 mL of ethylacetate. The eluate was dried with a nitrogen evaporator (Oa-SYS Heating Device 5085, Organomation Associates. Inc., USA) at 20 psi stream of nitrogen (40 °C). The analyte was finally prepared by dissolving the dryness in 200 μL of dichloromethane for GC-MS analysis.

Determination of PAHs using GC-MS. Determination PAHs was determined and measured by using a GC chromatography, CP-3800 of Varian (CA, USA) with MS spectrometry, 1200L of Varian (CA, USA). An auto-sampler, Combi-PAL of CTC Analytics (Zwingen, Switzerland) was used based on the Korean Food Code method. A DB-5ms GC capillary column from Agilent technologies (CA, USA) with length of 30 m, inner diameter of 25 μm and film thickness of 0.25 μm was equipped. The oven was heated at a rate of 4 ℃/min to 245 ℃ following being at 80 ℃ for 1 min, and it was finally increased to 270 ℃ at 30 ℃/min followed by being held for 10 min. Helium carrier gas was flowing at 1.5 mL/min. A injector was at the temperature of 320 ℃ and 1 μL of samples were injected with splitless mode. Mass spectrometry was operated with source temperature of 250 ℃ and electron ionization (EI) of 70

eV. Signals are acquired by selective ion monitoring (SIM) mode with dwell time of 0.1 s. The 4 PAHs were quantified by comparison of retention times and ion masses of selected ions to those of the 4 PAHs in standards and qualified by calculating their levels with calibration curve. The BaA and CHR ions were m/z 228, m/z 229, m/z 226, and the quantitative analysis target ion was m/z 228. BbF and BaP were m/z 252, m/z 253, m/z 250, and the quantitative analysis target ion was m/z 252. CHR-d12 was m/z 240, m/z 241, m/z 236, and the quantitative analysis target ion was m/z 240. BaP-d12 was m/z 264, m/z 265, m/z 260, and the quantitative analysis target ion was m/z 264. When the difference of the ratios of other two qualifying ions in sample and standard were within 10 %, the peaks of PAHs in sample were accepted (MFDS, 2013).

Quality control. The method was validated to ensure the quality of analytical results. Performance parameters: specificity, limit of detection (LOD), limit of quantification (LOQ), linearity, recovery, and precision were obtained to validate the method based on guidelines recommended by the International research group (Eurachem, 1998). Specificity was obtained by checking the isolation of PAHs peaks from noise peaks in samples fortified with PAHs. LOD was statistically calculated by multiplying 3 to a standard deviation obtained in repeated analysis of the lowest control 7 samples of 1.0 ng g^{-1}. LOQ was

estimated by multiplying 9 to the same standard deviation. Linearity of calibration curve was obtained by calculating the correlation coefficient (R^2). Six working standards of 1.0, 5.0, 10.0, 50.0, 100.0 and 500.0 μg/L were plotted and calibration curve was obtained by **regression** an equation of 6 plots. The relative recovery of accuracy was evaluated by analyzing five samples fortified with standards of 2.0 ng g^{-1} and 10 ng g^{-1} and deuterated internal standards of 4.0 ng g^{-1} and calculating the average percentage of determined concentration via fortified amount. The repeatability of accuracy was evaluated by calculating the relative standard deviation (RSD^r) obtained in the recovery experiments. The reproducibility of accuracy was evaluated by calculating the relative standard deviation (RSD^R) in experiments conducted by 4 different labs.

Exposure estimation and risk characterization. Exposure to PAHs was estimated by combining PAHs contamination levels and edible oil consuming amounts. PAHs concentration and edible oil consuming data were obtained by this study and KNHANES, respectively. Consumption data of edible oils for total population and consumers were originated from KNHANES IV and V. KNHANES IV was conducted from 2007 to 2009 and KNHANES V was carried out from 2010 to 2012. The second and third programmes of KNHANES IV in 2008 and 2009 and the first programme of KNHANES V in 2010 were selected

to assess exposure to edible oils. The numbers of samples in 2008, 2009 and 2010 were 9,308, 10,078 and 8,473, respectively (CDC, 2008, CDC, 2009, CDC, 2010). The KNHANES is composed of three surveys: health interview, health examination and nutrition survey and food consumption data is collected by nutrition survey. Nutrition survey is conducted by face to face interview in sample person's home using the 24 hour recall method (Kweon et al., 2014).

The values below LOD were statistically assumed based on the recommendation of GEMS/Food. When the proportion of data below LOD was zero, the concentration of PAHs was not statistically modified. Meanwhile, when the proportion was between 60% and 80% and more than 25 samples were detected, or when the proportion of not-detected samples was higher than 80%, the PAHs concentration was assumed to zero for lower-bound (LB) and to value of LOD for upper-bound (UB). When not-detected sample was between 0% and 60%, the concentration of PAHs was replaced to half of LOD value (GEMS/Food, 1995). To calculate total PAHs concentration, the concentrations of each PAH were combined to use new toxicological values. Meanwhile, to use TEQ concept, BaA, CHR and BbF were estimated as BaP equivalent concentrations (TEQ_{BaP}) by multiplying each PAH concentration with its TEF. TEFs of BaA, CHR and BbF were 0.1, 0.01, and 0.1, respectively (Moret et al., 2005).

The daily intakes of 4 PAHs and TEQ_{BaP} were calculated by using equation (1) (Yoon et al., 2007).

$$\text{Daily exposure}\left(\frac{\text{ng}}{\text{kg b.w. day}}\right) = \frac{\text{concentration of PAHs (or TEQ}_{\text{BaP}}\text{)}\left(\frac{\text{ng}}{\text{g}}\right) \times \text{daily edible oil intake}(\frac{\text{g}}{\text{day}})}{\text{body weight (kg)}} \quad (1)$$

To characterize a risk of PAHs, MOE was estimated by using equation (2). The MOE is used for assessing the risk of substances which does not show a threshold in the dose-response curve because of their genotoxic and carcinogenic properties. Exposure of it should be minimized according to "As Low As Reasonably Achievable (ALARA)" principle. However, Risk manager cannot get any information from ALARA which degree they should reduce which substances. The MOE could be one of the suitable approaches for the risk managers to set a priority list by comparing an appropriate reference point with human intake. The MOE of 10,000 or high in general would be interpreted as low concern to public health (EFSA, 2008).

$$\text{Margin Of Exposure} = \frac{\text{BMDL}_{10}\ (\frac{\text{ng}}{\text{kg b.w. day}})}{\text{The estimated daily exposure}\ (\frac{\text{ng}}{\text{kg b.w. day}})} \quad (2)$$

In equation (2), MOE is calculated by dividing $BMDL_{10}$ value by the estimated daily exposure, and $BMDL_{10}$ value was set by the dose-response analysis for tumor type. $BMDL_{10}$ for BaP and the sum of 4 PAHs ranged from 0.07 to 0.20 mg kg^{-1} b.w. day^{-1} and from 0.34 to 0.93 mg kg^{-1} b.w. day^{-1} based on total tumour-bearing animals, respectively. Therefore, 0.07 and 0.34 mg kg^{-1} b.w. day^{-1} were conservatively adopted for $BMDL_{10}$ of the BaP and the 4 PAHs (EFSA, 2008).

II-3. Results and discussions

Quality control. The specificity of the estimating method was guaranteed by comparing retention times with reference materials in blank samples and monitoring fragment ions for each target compound. The calibration plots based on the linear regression analysis revealed good correlations between peak area and concentrations over the ranges 1–500 µg/L with correlation coefficients over 0.99 . The LOD and LOQ ranged from 0.02 to 0.13 ng g^{-1} and from 0.06 to 0.44 ng g^{-1} at four types of oil samples, respectively. The relative recoveries of 4 PAHs were from 70.7 to 110.4 % at 2 ng g^{-1} and from 79.9 to 112.6 % at 10 ng g^{-1}.The RSD^r for repeatability at a level of 2 ng g^{-1} were from 0.3 to 6.9 %, and from 1.4 to 8.8 % at a level of 10 ng g^{-1}. The RSD^R for reproducibility was from 2.1 to 17.2 % at a level of 2 ng g^{-1} and from 0.9 to 12.7 % at a level of 10 ng g^{-1}. All values of performances are shown in table II-1, and they are satisfying the criteria proposed by Association of Official Agricultural Chemists (AOAC) (Table 1) (Taverniers et al., 2004).

Table II-1. Performance parameters of the method in optimum condition

Oil type	PAHs	LOD [a] ($ng\ g^{-1}$)	LOQ [b] ($ng\ g^{-1}$)	Recovery		RSD [r c] (%)		RSD [R d] (%)	
				2 $ng\ g^{-1}$	10 $ng\ g^{-1}$	2 $ng\ g^{-1}$	10 $ng\ g^{-1}$	2 $ng\ g^{-1}$	10 $ng\ g^{-1}$
Sesame oil	BaA	0.04	0.14	87.0	112..6	5.4	4.9	9.8	6.9
	CHR	0.05	0.18	93.1	103.6	3.3	5.8	17.2	5.5
	BbF	0.04	0.14	79.0	96.9	3.1	2.6	9.6	9.8
	BaP	0.02	0.08	78.3	99.5	0.3	2.0	5.5	8.9
Perilla oil	BaA	0.08	0.26	106.6	94.5	5.3	5.9	4.9	3.9
	CHR	0.05	0.16	99.1	96.5	3.0	7.1	7.7	0.9
	BbF	0.08	0.25	70.7	95.9	1.1	3.0	12.0	9.8
	BaP	0.07	0.23	83.4	100.5	0.3	6.1	7.4	8.1
Pepper seeds oil	BaA	0.09	0.30	107.7	112.1	3.2	1.7	9..7	8.9
	CHR	0.13	0.44	100.5	112.6	2.4	3.0	2.1	9.4
	BbF	0.02	0.06	73.5	99.7	2.2	2.6	11.5	8.4
	BaP	0.02	0.06	87.6	111.6	1.3	5.4	4.7	12.5
Olive oil	BaA	0.04	0.12	110.4	79.9	5.0	8.0	6.9	10.3
	CHR	0.10	0.32	97.0	94.5	6.9	1.4	7.7	6.7
	BbF	0.03	0.11	71.4	90.2	1.3	8.8	14.4	12.7
	BaP	0.02	0.08	83.0	84.9	1.3	1.9	6.2	5.3

a) LOD: Limit of Detection
b) LOQ: Limit of Quantification
c) RSD^r: Relative standard deviation of repeatability in single-lab.
d) RSD^R: Relative standard deviation of reproducibility in multi-lab.

Occurrence of 4 PAHs in edible oils. Table II-2 shows means and ranges for BaA, CHR, BbF, BaP and the sum of PAHs from edible oils analyzed in this study. A value below the LOQ was assigned to ND (not detected). The perilla oils and sesame oils were highly contaminated with PAHs. A maximum limit value of 2 ng g^{-1} for BaP was established in edible oils in Korea and EU (EC, 2011). The mean concentration of PAHs in 129 sesame oil samples analyzed was 0.41 ng g^{-1} for BaA, 0.41 ng g^{-1} for CHR, 0.35 ng g^{-1} for BbF, 0.18 ng g^{-1} for BaP and 1.35 ng g^{-1} for the sum of 4 PAHs, respectively. The contents of BaP in sesame oils were lower than maximum limit. The mean concentration of BaA (0.54 ng ng^{-1}), CHR (0.97 ng ng^{-1}), BbF (0.61 ng ng^{-1}) and BaP (0.20 ng ng^{-1}) for 71 perilla oil samples were analyzed and BaP were contaminated lower than the established maximum limit. Table II-2 shows PAHs content of edible oils in other countries. Red pepper seeds oil contained the highest 4 PAHs content in edible oils in Korea. However, there were no researches to determine PAHs content in red pepper seed oils in other countries. Meanwhile, PAHs contents in sesame oil and olive oil in Korea were similar with or lower than those in other countries. The relative contributions of each of the 4 PAHs to the total content of 4 PAHs in five edible oil categories were shown in figure 1. CHR had the highest average contributions of 30.8–46.5% in edible oils, and BaA, BbF

Table II-2. PAHs concentrations in edible oils

Country	Sample type	No.	BaA (ng g^{-1})		CHR (ng g^{-1})		BbF (ng g^{-1})		BaP (ng g^{-1})		Total (ng g^{-1})		Reference (origins)
			Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	
(This study)	sesame oil	129	0.41	ND–3.91	0.41	ND–2.52	0.35	ND–2.07	0.18	ND–1.11	1.35	ND–7.46	(Korea)
	perilla oil	71	0.54	ND–2.78	0.97	ND-8.64	0.61	ND–3.66	0.20	ND–1.60	2.33	ND–12.91	(Korea)
	red pepper seeds oil	16	0.88	ND–1.93	1.08	ND–2.68	0.75	ND–1.53	0.47	ND–1.19	3.18	ND–6.26	(unkown)
	olive oil	53	0.51	0.17–1.00	0.95	ND–1.77	0.37	ND–1.05	0.22	ND–0.48	2.05	0.42–4.07	(unkown)
	red pepper seasoning oil	34	0.61	ND–2.52	0.95	ND–2.92	0.64	0.12–1.89	0.32	ND–1.15	2.53	0.12–6.88	(unkwon)
Germany	sesame oil	1	<0.1		0.6 [a)]		0.1 [b)]		N.D				[7]
	olive oil	7	1.6	1.0-1.9	4.6 [a)]	3.7-6.9 [a)]	2.4 [b)]	1.0-2.8 [b)]	0.7	0.2-1.2			
Spain	virgin olive oil	6							0.6	0.3-1.2			[33]
	Olive-pomace oil	10							19.2	0.5-49.3			
Denmark	sesame oil	1							0.2				[34]
	extra virgin olive oil	46							0.15	<0.2-0.4			
	olive oil	6							0.12	<0.2-0.2			
Australia	extra	2	<LO	<LOQ	<LO	<LOQ	<LO	<LOQ [b)]	<LO	<LOQ	<L	<LOQ	[35]

	virgin olive oil		Q		Q		Q [b)]		Q		OQ		
	olive oil	3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ [b)]	<LOQ [b)]	<LOQ	<LOQ	<LOQ	<LOQ	
Italy	olive-pomace oil	3	12.4	6.0-25.0	60.9	18.6-133.2	11.3 [b)]	1.1-31.4 [b)]	5.9	0.9-15.4	90.5	26.8-205.0	
Portugal	virgin olive oil	2	0.7	0.5-0.9	0.3	0.1-0.6	0.4	0.3-0.4	0.2	0.1-0.3	1.6	1.0-2.2	[12]
Kuwait [c)]	sesame oil	9							2.05	0.28-11.05			[14]
	extra virgin olive oil	21							0.53	0-6.77			
	virgin olive oil	7							0.90	0-6.31			
	olive oil	7							N.D.				
	pomace olive oil	6							2.06	0-3.62			

a) Sum of Chrysene and triphenylene

b) Sum of Benzo[b]Fluoranthene, Benzo[j]Fluoranthene and Benzo[k]Fluoranthene

c) Samples purchased from Kuwait markets originated from various countries. The original countries of sesame oils were SA, KW, IN, TH and SG, and those of extra virgin olive oils were from TR, SY, IT, ES, PS, TN, LB, CA and SA. The Virgin olive oils were made from TR, SY, PS and IT, and the pomace olive oils were from ES. Abbreviations of countries: TR; Turkey, SY; Syria, IT; Italy, ES; Spain, PS; Palestine, TN; Tunisia, LB; Lebanon, CA; Canada, SA; Saudi Arabia, SG; Singapore, KW; Kuwait, IN; India, TH; Thailand

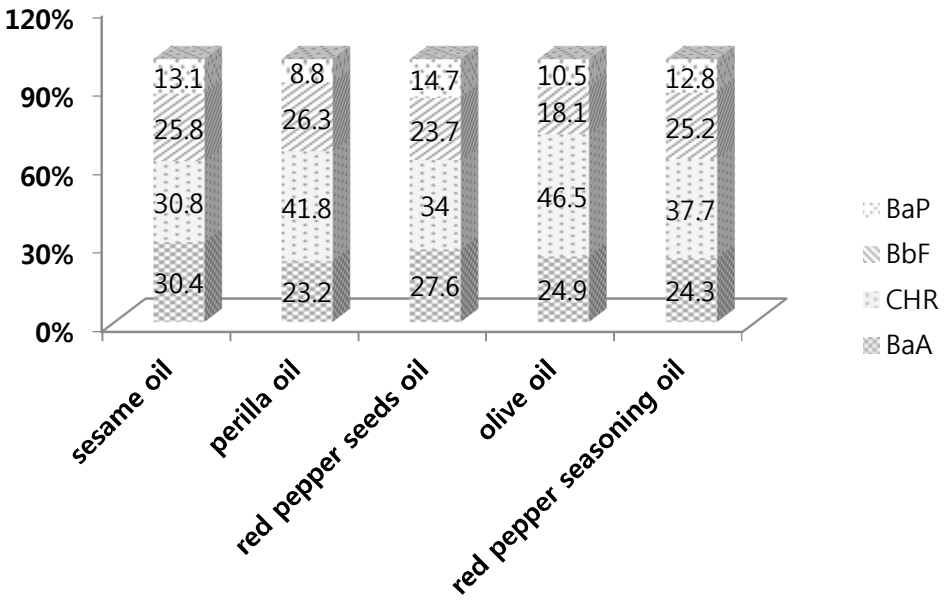


Fig. II-1. Relative contribution of 4 PAHs in 5 edible oil categories.

and BaP had the average contributions of 23.2–30.4%, 18.1–26.3% and 8.8–14.7%, respectively. Figure 2 shows the correlation of BaP and 4 PAHs in 5 edible oils. BaP has strong correlations with 4 PAHs in sesame oil, red pepper seasoning oil and red pepper seeds oil with correlation coefficients higher than 0.8. BaP has also correlations with 4 PAHs in other edible oils. However, some sesame oils and perilla oils contain 4 PAHs when BaP is not detected. Therefore, BaP is not good to represent 4 PAHs, even though BaP has correlations with 4 PAHs. Alomirah also figured out that Bap was not detected in some olive oils while eight genetoxic PAHs were detected and BaP is not good as the indicator for PAHs (Alomirah et al., 2010).

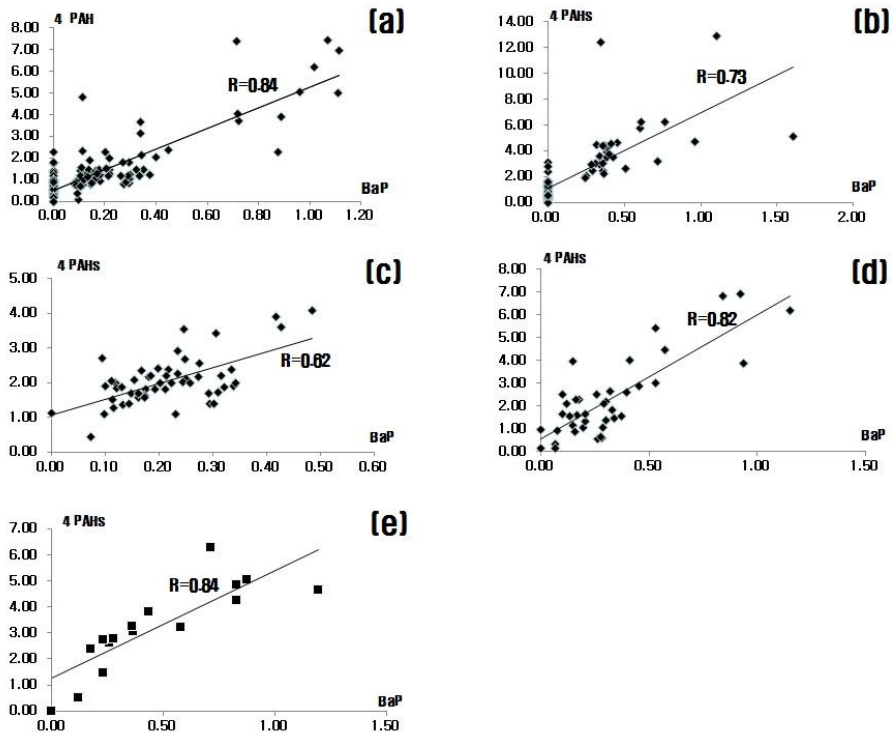


Fig. II-2. Correlations between 4 PAHs and BaP in 5 edible oil categories; (a) Sesame oil, (b) perilla oil, (c) olive oil, (d) red pepper seasoning oil, and (e) red pepper seeds oil.

Consumption of edible oils. The consumption of sesame oils, perilla oils and olive oils were reported in KNHANES, whereas the consumptions of red pepper seeds oils and red pepper seasoning oils did not appear in KNHANES. Therefore, the amounts of red pepper seeds oils and red pepper seasoning oils intake were substituted by the consumption data of hot sauces. The mean daily intake and the consumption at the 95th percentile of edible oils for total population and consumers are shown in Table II-3. The zero consumption data at 95th percentile means that the Korean consumer intake edible oils a lot whenever they eat, but not too frequently.

Table II-3. Consumption amounts of edible oils and estimated concentrations of each 4 PAH according to the proportion of not-detected samples

Type	Daily Intake (g day^{-1})				Estimated Concentration (ng g^{-1})					
	Total population		Consumer only		BaA	CHR	BbF	BaP	4 PAHs [b] (sum)	TEQ_{BaP} [c] (TEFs)
	mean	P95 [a]	mean	P95						
sesame oil	1.60	6.52	2.30	7.60	0.43	0.43	0.36	0.19	1.41	0.27
perilla oil	0.14	0.51	1.70	6.10	0.58	0.99	0.64	0.26	2.47	0.39
red pepper seeds oil	0.01	0	2.00	5.00	0.90	1.12	0.76	0.47	3.23	0.30
olive oil	0.10	0	2.50	9.80	0.51	0.96	0.37	0.22	2.05	0.11
red pepper seasoning oil	0.01	0	2.00	5.00	0.65	0.98	0.64	0.33	2.59	0.46

a) Daily intake of edible oils at the 95th percentile

b) The Sum of BaA, CHR, BbF and BaP contents

c) The estimated equivalent concentrations of BaP calculated by TEFs of BaA, CHR and BbF

Exposure estimation. The proportions of samples contaminated with PAHs below the LOD were below 60% and the values below the LOD was replaced to half of LOD. The estimated mean concentrations of 4 PAHs in edible oils were from 0.19 ng g^{-1} to 1.12 ng g^{-1} (Table II-2). The total contents of 4 PAHs and the total BaP equivalent values were from 1.41 to 3.23 and from 0.11 to 0.46 (Table 3). The average bodyweight of Korean was 58.3 kg (CDC, 2008, CDC, 2009, CDC, 2010). Table 4 shows the average and 95th percentile daily intakes of 4 PAHs for total populations and consumers. The daily exposures to high consumers in the 95th percentile were from three times to five times greater than that of mean daily consumers. Furthermore, the mean and high exposures to total 4 PAHs for total populations and consumers only were shown in Table II-4, and they were compared with the exposures to estimated equivalent BaP. Korean people were the most highly exposed to PAHs from sesame oils among edible oils according to the estimations by both sum of each PAH and TEQ_{BaP}. This highest intake of PAHs by consumption of sesame oils arises because Korean people consume sesame oil over 10 times more than other edible oils although the sesame oils are not highly contaminated with PAHs compared to others. Meanwhile, Korean consuming edible oils was exposed to PAHs mostly by olive oil due to the highest contamination of PAHs. In Brazil, people exposure to 4 PAHs of 7.3 ng kg^{-1} b.w. day^{-1} via edible oils

(soybean oils) (Camargo et al., 2011), and in Australia, People consume canola oil of 0.3 g day^{-1} and it contains BaA of below 0.06 ng g^{-1}, Chr of below 0.1 ng g^{-1}, BaP of below 0.08 ng g^{-1}. People in Australia exposure to BaA of 0.018 ng kg^{-1} day^{-1}, Chr of 0.03 ng kg^{-1} day^{-1} and BaP of 0.024 ng kg^{-1} day^{-1} at most (FSANZ, 2004). In Europe, the exposure to BaP and 4 PAHs via fats (vegetable and animal) are 26 ng day^{-1} and 177 ng day^{-1} (EFSA, 2008). Korean is not highly exposed to PAHs by eating edible oils comparing to other countries' people.

Table II-4. Dietary exposures to each PAH and total 4 PAHs by consuming edible oils

Type	Population	Dietary Exposure (ng kg^{-1} b.w. day^{-1})											
		BaA		CHR		BbF		BaP		4 PAHs [a]		TEQ_{BaP} [b]	
		Mean	P95 [c]	mean	P95	mean	P95	mean	P95	mean	P95	mean	P95
sesame oil	Total	1.17×10^{-2}	4.76×10^{-2}	1.19×10^{-2}	4.83×10^{-2}	9.88×10^{-3}	4.03×10^{-2}	5.22×10^{-3}	2.13×10^{-2}	3.87×10^{-2}	1.58×10^{-1}	7.50×10^{-3}	3.06×10^{-2}
	Consumer only	1.68×10^{-2}	5.55×10^{-2}	1.71×10^{-2}	5.63×10^{-2}	1.42×10^{-2}	4.69×10^{-2}	7.50×10^{-3}	2.48×10^{-2}	5.56×10^{-2}	1.84×10^{-1}	1.08×10^{-2}	3.56×10^{-2}
perilla oil	Total	1.39×10^{-3}	5.07×10^{-3}	2.38×10^{-3}	8.66×10^{-3}	1.54×10^{-3}	5.60×10^{-3}	6.18×10^{-4}	2.25×10^{-3}	5.92×10^{-3}	2.16×10^{-2}	9.34×10^{-4}	3.40×10^{-3}
	Consumer only	1.69×10^{-2}	6.06×10^{-2}	2.89×10^{-2}	1.04×10^{-1}	1.87×10^{-2}	6.70×10^{-2}	7.51×10^{-3}	2.69×10^{-2}	7.19×10^{-2}	2.58×10^{-1}	1.13×10^{-2}	4.07×10^{-2}
red pepper seeds oil	Total	1.54×10^{-4}	-	1.91×10^{-4}	-	1.30×10^{-4}	-	0.80×10^{-4}	-	5.55×10^{-4}		5.17×10^{-5}	
	Consumer only	3.07×10^{-2}	7.68×10^{-2}	3.83×10^{-2}	9.57×10^{-2}	2.59×10^{-2}	6.48×10^{-2}	1.60×10^{-2}	4.00×10^{-2}	1.11×10^{-1}	2.77×10^{-1}	1.03×10^{-2}	2.58×10^{-2}
olive oil	Total	8.74×10^{-4}	-	1.64×10^{-3}	-	6.40×10^{-4}	-	3.71×10^{-4}	-	3.52×10^{-3}		1.83×10^{-4}	

	Consumer only	2.19×10^{-2}	8.57×10^{-2}	4.10×10^{-2}	1.61×10^{-1}	1.60×10^{-2}	6.27×10^{-2}	9.28×10^{-3}	3.64×10^{-2}	8.81×10^{-2}	3.45×10^{-1}	4.58×10^{-3}	1.80×10^{-2}
red pepper seasoning oil	**Total**	1.12×10^{-4}	-	1.68×10^{-4}	-	1.09×10^{-4}	-	0.56×10^{-4}	-	4.45×10^{-4}		8.00×10^{-5}	
	Consumer only	2.25×10^{-2}	5.61×10^{-2}	3.35×10^{-2}	8.38×10^{-2}	2.18×10^{-2}	5.45×10^{-2}	1.11×10^{-2}	2.79×10^{-2}	8.89×10^{-2}	2.22×10^{-1}	1.59×10^{-2}	3.98×10^{-2}

a) The Sum of BaA, CHR, BbF and BaP contents

b) The estimated equivalent concentrations of BaP calculated by TEFs of BaA, CHR and BbF

c) Dietary exposure to PAHs at the 95^{th} percentile

Risk characterization and uncertainty. The risk of PAHs by dietary intake of edible oils was characterised by calculating MOEs (Table II-5). With regard to average consumption, the MOE of 4 PAHs for total population was 6,919,104 and that of consumers only was 818,291. In the case of high consumption in the 95th percentile, the MOEs were 1,893,096 and 264,386 for total population and consumers, respectively. All MOEs were over 1.0 x 10^4 and it was found that the risk of 4 PAHs in edible oils is "low concern from a public health point of view" (EFSA, 2008). According to the French total diet study, edible oils are the main contributors (16.2%) to PAHs exposure via foods (Veyrand et al., 2013). If Korean people are exposed to PAHs by edible oils with contribution of about 20%, MOEs will be between 66,096 and 1,729776. Therefore, exposure to PAHs by consuming food does not still represent a food safety issue to Korean people.

Furthermore, the MOEs of the estimated equivalent BaP calculated by TEFs of other 3 PAHs were from 2,058,824 to 8,000,634 for total population and 437,774 to 1,323,752 for consumers only. These MOEs are from 1.1 to 1.7 times higher than those of total of 4 PAHs. Therefore the risk of 4 PAHs estimated by the TEFs can be over-estimated comparing to the risk of total 4 PAHs. Many studies assessing the risk of PAHs by TEFs need to be re-assess the risk of PAHs by using total PAHs concentration and toxicological value from mixture of PAHs.

Table II-5. MOEs of total 4 PAHs by sum of each PAH and TEQ_{BaP} as BaP concentration estimated by TEFs

Type	MOEs							
	Total Population				Consumer Only			
	4 PAHs [a]		TEQ_{BaP} [b]		4 PAHs		TEQ_{BaP}	
	mean	P95 [c]	mean	P95	mean	P95	mean	P95
sesame oil	8,785,530	2,151,899	9,333,333	2,287,582	6,115,108	1,847,826	6,481,481	1,966,292
perilla oil	57,432,432	15,740,741	74,916,474	20,588,235	4,728,790	1,317,829	6,194,690	1,719,902
red pepper seeds oil	613,076,828	-	1,346,153,846	-	3,063,063	1,227,437	6,796,117	2,713,178
olive oil	96,590,909	-	381,758,653	-	3,859,251	985,507	15,283,843	3,888,889
red pepper seasoning oil	764,501,697	-	879,715,456	-	3,824,522	1,531,532	4,402,516	1,758,794
total	6,919,104	1,893,096	8,000,634	2,058,824	818,291	264,386	1,323,752	437,774

a) The Sum of BaA, CHR, BbF and BaP contents

b) The estimated equivalent concentrations of BaP calculated by TEFs of BaA, CHR and BbF

c) Dietary exposure to PAHs at the 95th percentile

Statistical analysis. The percentages of ND sample of BaA, CHR, BbF and BaP in all leached teas were less than 60%, therefore, the values of ND samples were substituted with half of the LOD. Meanwhile, CHR for green, barley and black tea in the liquid tea variety were not detected at all, and were replaced with 0 for LB and LOD for UB. The percentage of sample containing BaA, BbF and BaP were less than 60% and replaced with half of the LOD. BaA for preserved fruit tea was changed to 0 for LB and the LOD for UB, and half of the LOD was used for the other 3 PAHs for preserved fruit tea. For solid tea, BaP for black, herbal and ginger were substituted with 0 for LB and the LOD for UB, and half of the LOD was used for the other 3 PAHs. All samples of green tea contained all 4 PAHs.

II-4. Conclusion

To characterize the risk of PAHs in Korea by consuming the edible oils, an analytical method to detect 4 PAHs simultaneously was validated. The perilla oils and sesame oils were highly contaminated with 4 PAHs, and this might be because perilla and sesame seeds were fried at high temperature before being extracted. The levels of BaP in edible oils were suitable with regard to the Korean BaP maximum levels. According to the MOEs of 4 PAHs, the risk of PAHs in edible oils was of low concern.

Chapter III.

Risk Assessment of Polycyclic Aromatic Hydrocarbons in Teas[2]

[2] This chapter has been published: Joon-Goo Lee, Taesuk Lim, Sheen-Hee Kim, Dong-Hyun Kang and Hae-Jung Yoon, 2018, Determination and risk characterization of polycyclic aromatic hydrocarbons of tea by using the Margin of Exposure (MOE) approach, Food sci Biotehchnol, 27(6): 1843-1856

III-1. Introduction

Tea is a kind of drink which made of tea plant, even though tea is also referred as drinks made of vegetable substances including tea plant. Teas have several types of teas regarding to production procedures like fermentation and heating (Abd EL-Aty et al., 2014). Tea traditionally are made from the tea plant, camellia sinesis, refers to green or black tea (Wang et al., 2011). Teas have been consumed because of its taste, but many health benefits of teas have recently been figured out and made consumers to prefer to drink teas. Green teas contain fruitful catechins, a group of polyphenols, and especially (-)-epigallocatechin-3-gallage (EGCG), (-)-epigallocatecin (EGC), (-)-epicatechin (EC), and (-)-epicatechin gallate (ECG) of catechins showed the antiporliferative, antimutagenic, antioxidant, antibacterial, antiviral, and chemopreventive functions in some research (Banerjee et al., 2010, Butt et al., 2009).

Teas are classified into three categories in Republic of Korea by food code according to the tea production methods. Leached tea refers to tea made from plant materials like plants sprout, leaves, or flowers. Leached tea is normally produced by drying plant materials to reduce the amount of moisture and sometimes destroy the enzymes in plant materials. Leached tea is dipped and filtered in hot water and people drinks the water. Liquid tea is a

tea which is already dripped and filtered, and consumers don't need to dip and filter it by themselves. Solid tea is a plant material itself or other processed form such as powder. People can melt the solid tea in water and drink the water (MFDS 2016).

Leached tea and some teas are dried to decrease the amount of moisture and destroy the enzymes to develop the tea flavor and taste. How to dry and how many times dry the tea are regarded to the most important steps. (Özcan et al., 2005, Venskutonis, 1997, Vieira et al., 2010). Tea is traditionally dried by frying tea several times, but recently using smoke, steam, sun-light or flames are easily used for rapidly drying tea. However, these drying processes develop not only quality of tea but also some hazard chemicals in tea. Polycyclic aromatic hydrocarbons (PAHs) are formed during drying tea with high temperature (Adisa et al., 2015, Pincemaille et al., 2014, Vieira et al., 2010).

PAHs are one of the well-known hazard chemicals formed with more than two aromatic rings. PAHs are produced by being combined with pyrolyzed organic matter in free-radical polymerization processes during incompletely combustion in high temperature (Longwell 1982, Richter and Howard, 2000).

The environment is widely contaminated with PAHs by different

reasons including bush fire, cars, and plants, and PAHs in the environment can contaminate food too. But Foods also produce the PAHs during cooking and processing them. Increasing the temperature of foods such as heating, grilling or smoking etc. accelerates formation of the PAHs (Boffetta et al., 1997, Lijinsky 1991, Singh et al., 2016). Food is the main source of consumers' exposing to PAHs other than smoking cigarette (Zelinkova and Wenzi, 2015).

PAHs induce mutations, cancers and genotoxicity by interacting with DNA. PAHs are metabolized to active intermediates in liver by cytochrome P450 and the intermediates are chemically attached to DNA (IARC, 2010, Lagerqvist et al., 2011, Ramesh et al., 2015, Szeliga and Dipple, 1998). But not all PAHs show the mutagenic and carcinogenic risks. Some of PAHs in a number of PAHs are mutagens and carcinogens (Kao et al., 2014). 16 priority PAHs such as Naphthalane, Acenaphthene, Acenaphthylene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo[a]anthracene, Chrysene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[a]pyrene, Dibenzo[a,h]anthracene, Benzo[g,h,i]perylene and Indeno[1,2,3,cd]pyrene were selected by the US Environmental Protection Agency (EPA) (1983) based on their carcinogenicity and toxicity for management the risks of PAHs (Fig. III-1). Meanwhile, 4 PAHs:

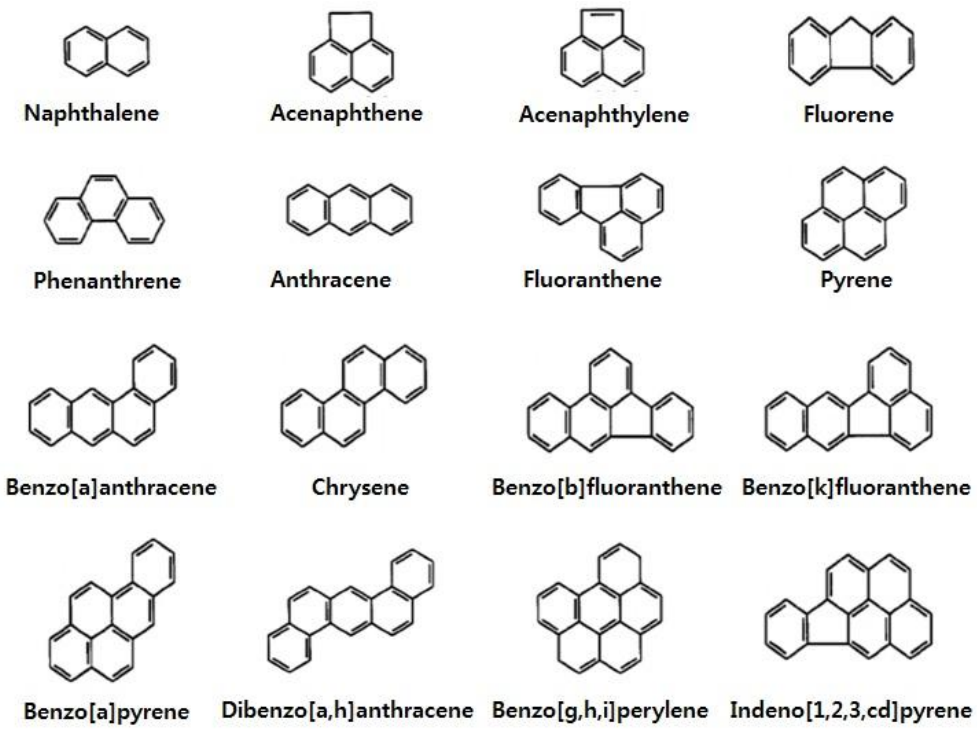


Fig. III-1. Structures of 16 priority PAHs classified by the US EPA.

Benzo[a]anthracene (BaA), Chrysene (CHR), Benzo[b]fluoranthene (BbF) and Benzo[a]pyrene (BaP) were selected as the indicators of PAHs by the Europe Commission in 2011 after the European Commission had chosen Benzo[a]pyrene as an indicator of PAHs in 20005 because recent study from the European Food Safety Authority (EFSA) concluded that BaP could not represent PAHs in food and managing more than 4 PAHs did not have more advantages than managing only 4 PAHs. The European Commission added the maximum levels of sum of 4 PAHs to the maximum levels of BaP to several food categories (European Commission, 2006; Commission Union, 2011; European Food Safety Agency, 2008). In Korea, the Ministry of Food and Drug Safety (MFDS) has been set the maximum limits of BaP in different food categories such as edible oils, smoked fish, smoked meat etc.

In order to determine the PAHs levels in different kinds of food, several analytical methods with procedures of extraction and chromatography have been developed and optimized for different food matrix. PAHs have been extracted and isolated out of various foods by liquid-liquid extraction (LLE), solid-phase extraction (SPE) or saponification according to their marices. And isolated PAHs are quantified andqualified by chromatography such as liquid chromatography or gas chromatography with several detectors, fluorescence, ultraviolet-visible or mass spectrometry (Jira et al., 2008,

Plaza-Bolaños et al., 2010, Veyrand et al., 2007, Yu et al., 2012).

Several researches have been found that PAHs are occurred in various kinds of foods including edible oil and grilled meat etc. Edible oils are often containing high levels of PAHs because PAHs is easily carried over to edible oils from the seeds due to their being lipophilic when oils are extracted (Gertz and Kogelheide, 1994, Balenovic et al., 1995). Grilled and barbecued meats are also one of the most easily contaminated foods by PAHs because high levels of PAHs are produced when meat drips fall down to flame of charcoal and they are incompletely combusted. The meat drops are mainly composed of water, fats and proteins (Lee et al., 2016a).

Some research have assessed the risk of drinking teas according to exposure to PAHs to monitor the safety of tea, and most of them have used the Toxic Equivalency Quotient (TEQ) to evaluate the risk of exposure to 4 PAHs, since this approach was accepted by the US EPA (Nisbet et al., 1992). TEQ approach is the concept that changes the levels of other PAHs to BAP contents with toxic relations between other PAHs and BaP. The levels of BaP which are converted from those of other PAHs are called BaP equivalent contents (TEQ_{BaP}) and TEQ_{BaP} were calculated by multiplying toxic equivalency factors (TEFs) and each PAHs contents (Jiang et al., 2015, Zhao et al., 2014, Alomirah et al., 2010, Martí-Cid et al., 2008). The

exposure to PAHs was finally shown by the exposure to BaP.

However, Scientists recently figured out that the TEQ concept is not suitable for PAHs because each PAHs has different toxicological mechanism. Food Safety Authority (EFSA) studied toxicological values of mixture of PAHs and suggested $BMDL_{10}$ values for 2 PAHs, 4 PAHs and 8 PAHs in food (EFSA, 2008). $BMDL_{10}$ is the benchmark dose of lower confidence limit to increase the amount of animals bearing tumor by 10%, and it is used for genotoxic and carcinogenic chemicals which do not have shreshoulds in toxicological dose-response relationship and health guidance values such as PAHs.

A Margin of Exposure (MOE) has been used for risk characterization for PAHs with BMDL10 since the Joint FAO/WHO Expert committee on Food Additives (JECFA) recommended us to use it in 2005 (JECFA, 2005, Lee et al., 2016b). Genotoxic and carcinogenic substances do not have health guidance values and the exposure to those chemicals should be minimized as low as reasonably achievable (ALARA). However, risk managers get no information from ALARA to make policies for food safety. MOE can give information for risk managers to establish priority lists for hazardous substances. They compare the MOE values to appropriate reference points. When the MOE values of substances are higher than 10,000, they would be

of low concern to public health and risk managers should focus on other substances (Benford et al., 2010a, Benford et al., 2010b).

There are a few studies assessing the risks of PAHs in food with MOE. Especially teas are highly consumed in Korea even though they are easily contaminated by PAHs due to heat processing to dry them. Therefore, I determined levels of 4 PAHs in tea highly consumed in the Korean market with a validated analytical method and characterized their risks with MOE values. Furthermore, I compared the risks of PAHs in tea assessed by the traditional TEQ concept and the MOE.

III-2. Materials and Methods

Chemicals and materials. I purchased Benzo[a]anthracene, Chrysene, Benzo[b]fluoranthene and Benzo[a]pyrene as standards and deutrated Chrysene and deutrated Benzo[a]pyrene as internal standards from Supelco (Bellefonte, PA, USA). Stock standards solutions of 100 μg/mL were made by dissolving standards and internal standards in methylene chloride, and working standards solutions of 400 μg/L were prepared by diluting stock standards with methylene chloride. 5 calibration standards were diluted to 0.2, 0.5, 1, 2, 5 μg/L with working standards in methylene chloride for leached and solid teas, and 0.1, 0.2, 0.4, 1 and 2 μg/L for liquid tea. Calibration standards contained 2 internal standards of 4 μg/L. Potassium hydroxide (Wako, Osake, Japan), sodium sulfate (Wako, Osake, Japan) were prepared, and ethyl alcohol (Merck, Darmstadt, Hesse, Germany), N,N-dimethylformamide (Merck, Darmstadt, Hesse, Germany), ethyl acetate (Merck, Darmstadt, Hesse, Germany), n-hexane (Merck, Darmstadt, Hesse, Germany), Methylee chloride (Burdick & Jackson, Muskegon, MI, USA) were HPLC grade. Deionized water was filtered by a Milli-Q system (Bedford, MA, USA), and SPE cartridges packed with silica of 50 mg in plastic syringe of 3cc (Supelco, Bellefonte, PA, USA)

were used. I used a rotary evaporator of Eyela (evaporate solutions, a rotary evaporator (Eyela, Tokyo) and a nitrogen evaporator of Organomation Associates. Inc (Oa-SYS Heating Device 5085, USA) to evaporate and dry solutions.

Sampling. I bought 174 leached teas, 155 liquid teas and 139 solid teas on markets in the 6 biggest cities in Korea. Tea products which are frequently consumed and easily contaminated by PAHs were selected based on the consumption data obtained by Korea National Health and Nutrition Examination Survey (KNHANES) and monitoring data from other research.

Sample preparation.

Leached tea and solid tea (powdered leached tea). Leached teas and solid tea powdered from leached teas were homogenized with a blender (Mix-h03, Tongyang magic, China), and blended samples of 5 g and the internal standards of 200 μL were taken into a round-shaped flask of 500 mL. 1 M potassium hydroxide in ethanol of 100 mL was added to the flask, and the mixtures of samples were saponified under reflux at 80 ℃ for 3 h.

After the mixture was cooled down at room temperature, 50 mL of *n*-hexane was added through the reflux condenser to wash them. The mixture in flask was moved to a separatory funnel, and the flask was washed with 50 mL of solvent mixed with ethanol and *n*-hexane (1:1, v/v). The rinsed solution was collected to the separatory funnel. The separatory funnel was shaken and the hexane layer from the separatory funnel was isolated and transferred to another separatory funnel. The remainder of the mixture was extracted with 50 mL of n-hexane 2 times and the extracted hexane layers were combined. Afterwards, deionized water of 50 mL was added to the combined hexane layer to wash it and the layer was washed 2 times more. The washed layer was concentrated to 2mL with a vacuumed rotary evaporator, Eyela of Tokyo Rikakikai Co. Ltd. (Tokyo, Japan) following being dehydrated through anhydrous Na_2SO_4. The concentrated sample was loaded on the silica-SPE cartridge which was washed and activated with 10 mL of dichloromethane and 20 mL of n-hexane in a row by gravity flow. The cartridge was cleaned with 10 mL of n-hexane and eluted with 20 mL of a mixed solvent of n-hexane and dichloromethane (3:1, v/v). The eluted solution was collected in a test tube and evaporated to dryness at 40 ℃ by nitrogen of 20 psi in a TurboVap of Zymark (MA, USA). The sample for GC-MS analysis was prepared by adding 200 μL of dichloromethane to the

dried concentrate and filtering it with a 0.45 μm membrane filter. And a blank sample was prepared with same procedures without meat sample.

Solid tea (processed) and liquid tea (preserved by sugar). Sample was homogenized and 5 g of sample with 200 μl of internal standards were weighed in a separatory funnel. 45 mL of *N,N*-DMF, 5 mL of water and 100 mL of *n*-hexane were added in the separatory funnel. The separatory funnel was shaken and left until it was equilibrated. A lower layer of *N,N*-DMF and water was transferred to another separatory funnel and 25 mL of the *N,N*-DMF and water solution (9:1, v/v) was added to the upper layer of hexane and collected to the separatory funnel containing *N,N*-DMF and water solution. 100 mL of 1% sodium sulfate solution and 50 mL of n-hexane were added to the separatory funnel of *N,N*-DMF and water solution and it was shaken. After equilibration, hexane layer was collected to another separatory funnel and 50 mL of n-hexane was added to the left lower layer of *N,N*-DMF and water solution again. After shaking, the n-hexane layer was separated and combined to the previous separatory funnel with hexane. *N,N*-DMF and water solution was extracted again with n-hexane. The separated hexane was washed with 40 mL of ionized water three times, and left water in hexane was removed with anhydrous sodium sulfate. The hexane was concentrated

to 2 mL with a rotary evaporator. The concentrated sample was loaded on the silica-SPE cartridge which was washed and activated with 10 mL of dichloromethane and 20 mL of n-hexane in a row by gravity flow. The cartridge was cleaned with 10 mL of n-hexane and eluted with 20 mL of a mixed solvent of n-hexane and dichloromethane (3:1, v/v). The eluted solution was collected in a test tube and evaporated to dryness at 40 ℃ by nitrogen of 20 psi in a TurboVap of Zymark (MA, USA). The sample for GC-MS analysis was prepared by adding 200 μL of dichloromethane to the dried concentrate and filtering it with a 0.45 μm membrane filter. And a blank sample was prepared with same procedures without meat sample.

Liquid tea. Sample was homogenized and 100 g of sample with 200 μl of internal standards were weighed in a separatory funnel. 100 mL of n-hexane or 50 mL of n-hexane was added to the separatory funnel to dilute sample, and it was washed with 50 mL of deionized water three times. It was concentrated to 2 mL with a rotary evaporator. The concentrated sample was loaded on the silica-SPE cartridge which was washed and activated with 10 mL of dichloromethane and 20 mL of n-hexane in a row by gravity flow. The cartridge was cleaned with 10 mL of n-hexane and eluted with 20 mL of a mixed solvent of n-hexane and dichloromethane (3:1, v/v). The eluted

solution was collected in a test tube and evaporated to dryness at 40 ℃ by nitrogen of 20 psi in a TurboVap of Zymark (MA, USA). The sample for GC-MS analysis was prepared by adding 200 μL of dichloromethane to the dried concentrate and filtering it with a 0.45 μm membrane filter. And a blank sample was prepared with same procedures without meat sample.

Determination of PAHs using GC-MS. PAHs was determined and measured by using a GC chromatography, CP-3800 of Varian (CA, USA) with MS spectrometry, 1200L of Varian (CA, USA). An auto-sampler, Combi-PAL of CTC Analytics (Zwingen, Switzerland) was used based on the Korean Food Code method. A DB-5ms GC capillary column from Agilent technologies (CA, USA) with length of 30 m, inner diameter of 25 μm and film thickness of 0.25 μm was equipped. The oven was heated at a rate of 4 ℃/min to 245 ℃ following being at 80 ℃ for 1 min, and it was finally increased to 270 ℃ at 30 ℃/min followed by being held for 10 min. Helium carrier gas was flowing at 1.5 mL/min. A injector was at the temperature of 320 ℃ and 1 μL of samples were injected with splitless mode. Mass spectrometry was operated with source temperature of 250 ℃ and electron ionization (EI) of 70 eV. Signals are acquired by selective ion

monitoring (SIM) mode with dwell time of 0.1 s. The 4 PAHs were quantified by comparison of retention times and ion masses of selected ions to those of the 4 PAHs in standards and qualified by calculating their levels with calibration curve (Table III-1).

Table III-1. Retention time and m/z value of quantitative and qualitative ions of 4 PAHs and 2 deuterated PAHs

Compound	Retention time	Ions(m/z)	
		Quantifying Ions	Qualifying Ions
BaA	40.63	228	229, 226
CHR	40.81		
BbF	45.22	252	253, 250
BaP	46.51		
d12-CHR	40.67	240	241, 236
d12-BaP	46.41	264	265, 260

Quality control. Analytical method was validated to assure a quality of data according to guidelines recommended by the Eurachem working group (1998). I selected green tea, mixed tea and herbal tea as representative tea matrix to validate leached tea, liquid tea and solid tea, respectively. Specificity, detection limit, quantification limit, linearity, and precision and recovery were estimated as the performance parameters. Specificity was estimated by separating standard and internal standard peaks from noise peaks in samples. Limit of detection (LOD) was calculated by analyzing 7 independent samples of 1 ng/L of leached, solid, and liquid tea (preserved by sugar) and samples of 0.4 ng/L of liquid tea and multiplying standard deviation of data by 3. Limit of Quantification (LOQ) was simply calculated by multiplying the LOD by 3. Linearity was estimated by calculating correlation coefficient (R2) of the calibration curve which was calculated by regression equation of levels of PAHs and analytical results. The levels of PAHs were from 10 to 250 μg/L for leached, solid and liquid tea (preserved by sugar) and from 50 to 1000 μg/L for liquid tea. Recovery was calculated by analyzing 5 samples spiked with standards and internal standards and comparing the levels of PAHs and the measured levels of PAHs. Precision was estimated by calculating relative standard deviation (RSD) of 5 samples spiked with standards and internal standards.

Exposure estimation and risk characterization. The PAHs levels of teas and tea dietary intake amounts were used for exposure estimation of PAHs. Tea consumption amount were obtained from the the Korea National Health and Nutrition Examination Survey (KNHANES). Total amount of 4 PAHs and total amount of TEQ_{BaP} of 4 PAHs were calculated by summing 4 PAHs concentrations and summing estimated concentrations of TEQ_{BaP} of 4 PAHs which were converted by multiplying levels of BaA, CHR and BbF with their TEF of 0.1, 0.01, and 0.1, respectively (Nisbet et al., 1992). The daily dietary exposure to 4 PAHs was estimated by multiplying concentrations of total amount of 4 PAHs with daily dietary food intake amount of tea and by dividing it with average body weight (Equation (1)).

$$\text{Daily dietary exposure}(\text{ng kg}^{-1}\ \text{b.w.day}^{-1}) = \frac{\text{total concentration of 4 PAHs (or } TEQ_{BaP}) \left(\frac{\text{ng}}{\text{g}}\right) \times \text{daily consumption}(\frac{\text{g}}{\text{day}})}{\text{average body weight (kg)}} \quad (1)$$

Equation (2) shows how the MOE is calculated.

Margin Of Exposure

$$= \frac{\mathrm{BMDL_{10}}\ (\frac{\mathrm{ng}}{\mathrm{kg\ b.w.\ day}})}{\mathrm{The\ estimated\ daily\ dietary\ exposure}\ (\frac{\mathrm{ng}}{\mathrm{kg\ b.w.\ day}})} \quad (2)$$

The MOE was calculated with the $BMDL_{10}$ and the daily dietary exposure. The $BMDL_{10}$ was obtained by calculating the 95 % lower confidence limit of a dose showing a 10 % incidence response collectively. $BMDL_{10}$ of 70,000 ng kg^{-1} b.w.$^{-1}$ day^{-1} for BaP and 340,000 ng kg^{-1} b.w.$^{-1}$ day^{-1} for 4 PAHs were conservatively used (EFSA, 2008).

Statistical analysis. The statistical approach recommended by GEMS/Food was applied to assume the concentration of not detected (ND) samples (GEMS/Food-EURO, 1995). When the proportion of ND samples was less than 60 %, the values of the ND samples were assumed to be half of LOD, and when the proportion of ND samples were between 60 % and 80 % with over 25 quantified data or higher than 80 %, the values are substituted to be zero and the value of LOD as lower-bounder (LB) and upper-bounder (UB), respectively.

III-3. Results and discussions

Quality control. Isolation of peaks of analytes and peaks of internal standards of monitoring fragment ions from background noises in representative samples. The isolation was enough to separate the peaks of standards and internal standards in representative samples, leached, solid and liquid teas (Figure III-1). The LOD was 0.01 ng g^{-1} to 0.32 ng g^{-1} and LOQ was 0.03 ng g^{-1} to 0.99 ng g^{-1}. Linearity was enough to calibrate standards with a good correlation coefficient values of over 0.99 (Table III-2). The BaA showed recoveries of 74.7–113.0 % and CHR had recoveries 82.2–109.0 %, BbF had recoveries of 71.7–106.5 % and BaP had recoveries of 73.3–100.0%. RSDs for BaA, CHR, BbF and BaP were 0.00 to 4.06 %, 0.26 to 13.21 %, from 0.00 to 6.29 % and 0.00 to 14.36 % respectively. (Table III-3). The association of Official Agricultural Chemistry (AOAC) recommended criteria of 40% to 120% for recovery and RSDs of 15% at 10 ng g^{-1}, 20% at 2 ng g^{-1}, 22% at 1ng g^{-1}, 25% at 0.4 ng g^{-1} for repeatability (Taverniers et al., 2004). The results of validation parameters of this study satisfied the criteria to control the quality of analytical results.

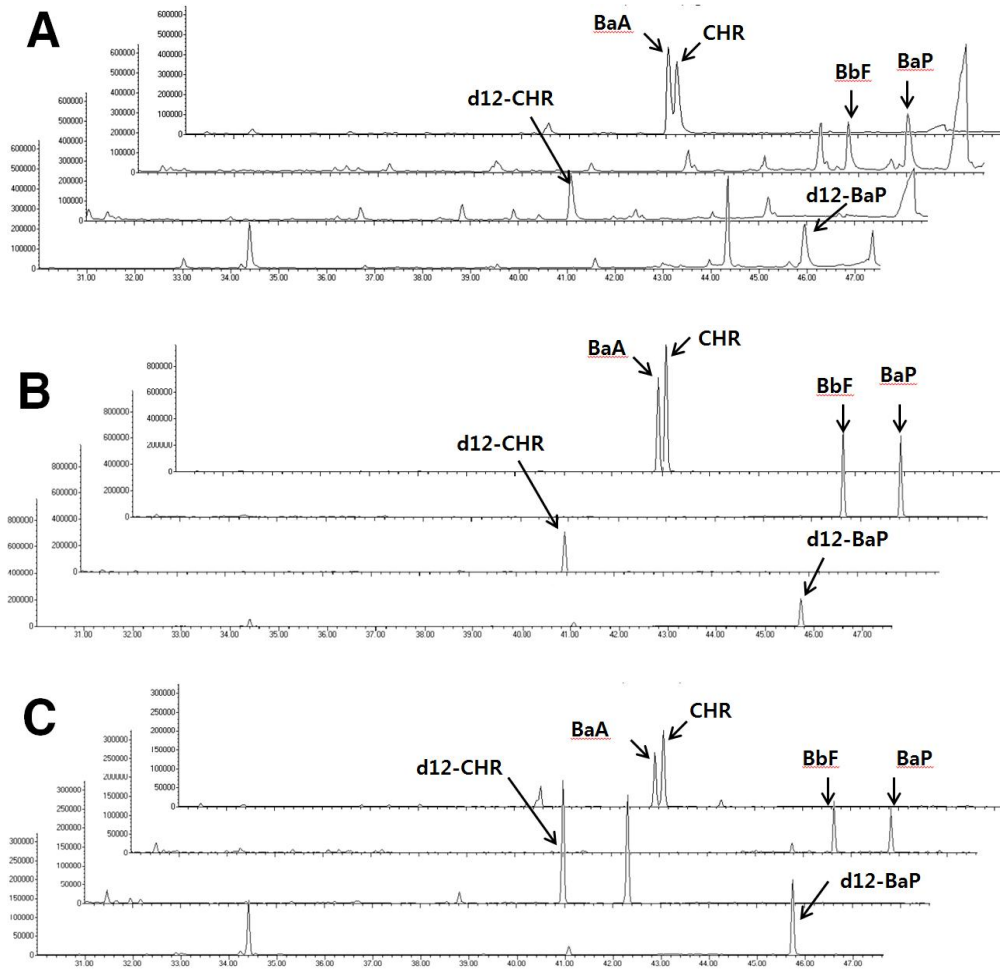


Fig. III-2. The Chromatograms of GC-MS of 4 PAHs and deuterated 2 PAHs with selected ion monitoring (SIM) mode at m/z of 228, 252, 240 and 264 (A) leached tea, (B) solid tea, (C) liquid tea.

Table III-2. The values of validation parameters (linearity and detection limits)

PAHs	Linear equation	Leached tea		Liquid tea		Solid tea	
		LOD (ng g^{-1})	LOQ (ng g^{-1})	LOD (ng g^{-1})	LOQ (ng g^{-1})	LOD (ng g^{-1})	LOQ (ng g^{-1})
BaA	$y = 0.0057x - 0.0618$ [a] (R^2=0.9997)	0.32	0.96	0.01	0.03	0.07	0.21
	$y = 0.0077x - 0.0396$ [b] (R^2=0.9996)						
CHR	$y = 0.0066x - 0.0322$ [a] (R^2=0.9999)	0.30	0.90	0.01	0.03	0.14	0.42
	$y = 0.0120x + 0.2220$ [b] (R^2=0.9979)						
BbF	$y = 0.0038x - 0.0048$ [a] (R^2=0.9999)	0.33	0.99	0.01	0.03	0.20	0.60
	$y = 0.0076x + 0.0872$ [b] (R^2=0.9993)						
BaP	$y = 0.0060x - 0.0030$ [a] (R^2=0.9999)	0.32	0.96	0.01	0.03	0.25	0.75
	$y = 0.0100x + 0.0120$ [b] (R^2=0.9997)						

d) Calibration curve for leached tea and solid tea

e) Calibration curve for liquid tea

Table III-3. The values of validation parameters (recovery and repeatability)

PAHs	Conc. ($ng\ g^{-1}$)	Leached tea		Liquid tea		Solid tea	
		Recovery (%)	RSD ^{r} (%)	Recovery (%)	RSD ^{r} (%)	Recovery (%)	RSD ^{r} (%)
BaA	1 [a] (0.4) [b]	105.0	1.78	102.5	0.00	100.8	0.44
	2(1)	74.7	4.06	108.4	1.24	99.8	0.98
	10(2)	113.0	3.47	110.4	1.41	111.9	0.56
CHR	1(0.4)	109.0	3.24	108.0	1.04	94.4	0.95
	2(1)	82.2	13.21	90.2	1.21	84.4	0.26
	10(2)	95.7	0.58	87.0	0.70	94.5	0.97
BbF	1(0.4)	75.4	0.73	106.5	1.29	87.8	1.25
	2(1)	93.5	5.28	74.0	0.00	73.5	3.47
	10(2)	97.2	6.29	71.7	2.98	76.3	2.02
BaP	1(0.4)	99.2	1.80	100.0	0.00	96.4	2.02
	2(1)	82.8	14.36	73.4	0.75	76.4	1.17
	10(2)	92.7	2.34	73.3	0.61	87.4	0.81

a) Concentration of 4 PAHs fortified in leached tea and solid tea

b) Concentration of 4 PAHs fortified in liquid tea

Occurrence of 4 PAHs in teas. Table 4 shows the results of analyzing BaA, CHR, BbF, BaP in teas. NDs were the results lower than the LOQ. The levels of PAHs in leached teas were higher than those in liquid and solid teas except solid green tea. Solid green tea contained high levels of PAHs. The average levels of BaA, CHR, BbF, BaP and sum of 4 PAHs in teas were 0.47 ng g^{-1}, 1.18 ng g^{-1}, 1.56 ng g^{-1}, 1.42 ng g^{-1} and 4.63 ng g^{-1} respectively. The leached tea, liquid tea and solid tea contained the average levels of BaP of 11.36 ng g^{-1}, 0.11 ng g^{-1} and 1.25 ng g^{-1} and the average sum of 4 PAHs of 3.69 ng g^{-1}, 0.03 ng g^{-1} and 0.14 ng g^{-1}. Mate teas of leached teas were contaminated with the highest level of sum of 4 PAHs of 33.45 ng g^{-1} and it was about 7.5 fold higher than the mean values in teas. Solomon's seal teas contained the second most highly levels of sum of PAHs of 17.34 ng g^{-1} followed by chrysanthemum and dandelion teas. The liquid and solid teas were not highly contaminated with PAHs with average level of 0.65 ng g^{-1} except for solid green tea because of different processing procedures for manufacturing those teas. Liquid teas and solid teas are extracted with water from the leached tea and solid teas are futher processed to dry and pulverize the extract. PAHs might be removed when the liquid teas and solid teas are extracted with water because PAHs are lipophilic and were not extracted from the leached teas with water. In other words, solid green teas were just

Table III-4. Levels of 4 PAHs in different teas determined by several studies in some countries.

Type	Teas	No.	BaA (ng g^{-1})		CHR (ng g^{-1})		BbF (ng g^{-1})		BaP (ng g^{-1})		Total (ng g^{-1})		Country (Reference)
			Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	
Leached tea	Green	49	0.40	ND-4.74	2.52	ND-8.58	3.80	ND-9.88	4.12	ND-9.98	10.83	2.25-23.00	Korea (this study)
	Solomon's seal	20	1.83	ND-5.33	3.90	ND-8.27	6.22	1.37-14.90	5.39	1.17-9.16	17.34	5.32-28.60	
	Cassia seed	13	0.18	ND-1.24	0.94	ND-4.28	1.43	ND-5.31	1.99	ND-4.98	4.54	ND-14.14	
	Black	21	1.65	ND-6.75	3.04	0.94-7.78	2.89	ND-7.66	1.53	ND-4.49	9.11	3.31-20.08	
	Chrysanthemum	13	0.42	ND-1.90	2.19	ND-4.75	4.96	1.36-9.56	5.02	1.90-9.68	12.59	3.37-22.03	
	Mate	10	6.51	1.71-8.91	9.35	2.66-15.22	8.19	4.17-12.39	9.40	3.76-12.45	33.45	12.30-44.25	
	Corn	9	0.69	ND-5.23	1.24	ND-3.57	3.67	ND-8.68	3.04	1.17-8.43	8.65	1.52-18.96	
	Buckwheat	9	0.39	ND-1.82	1.83	ND-3.67	3.52	ND-8.28	3.31	ND-8.14	9.05	ND-20.99	
	Burdock	8	0.29	ND-1.18	1.31	ND-2.83	3.93	ND-8.19	2.44	ND-3.76	7.97	2.83-12.60	
	Matrimony vine	7	ND	ND	0.72	ND-1.81	ND	ND	0.36	ND-1.27	1.07	ND-2.89	
	Dandelion	5	0.60	ND-1.77	4.45	2.56-6.96	3.54	1.25-9.22	3.88	ND-9.35	12.46	7.26-22.06	
	Brown rice	5	0.64	ND-1.73	2.19	ND-4.23	1.54	ND-3.55	3.12	1.87-4.51	7.49	3.93-9.65	
	Mulberry	5	ND	ND	1.20	ND-	2.68	1.97-	0.23	ND-	4.12	1.97-	

	leaves					2.19		3.77		1.17		7.14	
Liquid tea	Green	13	0.07	0.06-0.13	ND	ND	0.01	ND-0.06	0.01	ND-0.10	0.08	0.06-0.30	
	Barley	37	0.07	0.06-0.13	ND	ND	0.03	ND-0.07	0.01	ND-0.11	0.11	0.06-0.30	
	Black	26	0.08	0.06-0.14	ND	ND	0.01	ND-0.06	0.01	ND-0.11	0.10	0.06-0.31	
	Preserved fruit	40	ND	ND	0.03	ND-0.74	0.04	ND-0.29	0.05	ND-0.34	0.12	ND-0.74	
	Ginger	11	ND	ND	ND	ND	ND	ND	0.03	ND-0.29	0.03	ND-0.29	
	Korean raisin	10	0.11	0.07-0.13	ND	ND	0.05	0.04-0.08	0.05	ND-0.11	0.22	0.13-0.31	
	Corn	7	0.11	0.08-0.13	ND	ND	0.06	0.04-0.07	0.08	ND-0.10	0.24	0.19-0.30	
	Jujube	6	ND	ND	ND	ND	0.04	ND-0.26	ND	ND	0.04	ND-0.26	
	Burdock	5	0.07	0.06-0.07	ND	ND	ND	ND	0.01	ND-0.04	0.08	0.06-0.11	
Solid tea	Adlay	22	0.19	ND-0.64	0.16	ND-0.86	0.16	ND-1.59	ND	ND	0.51	ND-2.47	
	Black	22	0.10	ND-0.59	0.06	ND-0.73	0.03	ND-0.72	ND	ND	0.19	ND-1.32	
	Ginger	22	0.23	ND-0.62	0.21	ND-0.71	0.03	ND-0.72	ND	ND	0.48	ND-1.32	
	Herbal	60	0.24	ND-0.95	0.24	ND-1.50	0.13	ND-1.89	ND	ND	0.65	ND-5.48	
	Green	13	0.25	ND-1.78	3.44	1.01-5.11	3.26	ND-5.94	1.47	ND-3.04	8.42	4.57-12.70	
Leached tea	Earl	5	5.40	1-11	135.80	95-240	3.80	1-8	7.60	2-14	152.60	104-273	USA (Adisa et al., 2015)
	Fine	3	5.00	5-5	127.67	50-214	3.33	1-5	6.33	3-10	140.67	54-234	

	Black	6	5.80	2-18	136.83	57-365	4.20	1-14	7.33	2-29	152.50	63-426	
	Chinnamom, Cardamom & Nutmeg	5	1.25	1-2	40.20	27-55	1.33	1-2	2.20	2-3	44.20	30-59	
	Green	9	3.00	1-6	94.11	17-240	2.83	1-5	6.50	2-12	103.78	17.263	
Leached tea	Green	11	12.31	1.8-40.4	25.84	6.7-61.5	11.45	2.2-33.4	9.12	1.6-32.6	58.72	12.3-167.9	Germany (Ziegenhals et al., 2008)
	Mate	8	147.05	38.4-374.6	276.20	81.9-746.3	105.21	34.1-258.1	106.55	24.8-236.5	635.01	1846-1615.5	
	Black	11	5.36	1.3-13.1	10.08	3.4-18.1	4.27	1.5-8.1	4.94	0.8-14.1	.24.65	9.0-44.6	
	Herbal/fruit	7	2.44	1.2-3.5	7.57	4-11.6	2.43	1.3-4.5	1.67	0.8-3.1	14.11	7.8-21.5	
	White	3	38.23	14.1-80.1	49.50	19-95.1	25.93	14.5-44.6	15.17	11.4-19	128.83	59.0-238.8	
Leached tea	Black	26	30.60	1.1-360	24.08	2-220	23.80	1.2-240	25.62	0.6-330	105.95	4.9-1200	Several countries (Schulz et al., 2015)
	Green	15	22.33	0-130	22.96	0-120	19.49	0-85	16.78	0-97	81.73	0-430	
	Oolong	6	18.20	5.7-30	16.70	6.6-28	13.83	4.4-23	10.47	2.7-19	59.17	21-99	
	Pu erh	3	57.67	43-72	55.33	46-73	31.33	26-36	30.33	25-37	176.67	140-220	
	White	3	16.03	5.1-23	14.50	8.5-20	11.67	7-16	5.20	2.4-6.8	47.33	23-65	
	Lapsang souchong	3	466.67	240-620	546.67	270-700	111.33	64-150	346.67	280-460	1433.33	1000-1700	
Leached tea	Green	2	8.60	6.99-10.2	19.65	18.2-21.1					28.25	25.5-31.3	China (Lin et al., 2005)
	Oolong	1	4.43		5.96				5.10		15.49		
	Black	1	175.00		241.00		37.60		39.70		493.3		
	Puerh	1	13.40		23.00		8.03		7.84		52.27		

	Brick	1	31.90		40.30		12.90		14.60		99.7		
	Jasmine	1	67.30		45.90		54.00		28.10		195.3		
	Kuding	1	7.70		17.40						25.1		
Leached tea	Sage	5	12.93	0.31-45.19	2.54	0.17-6.02	1.37	0.06-3.12	0.78	0.03-1.38	17.62		Syria (Krajian et al., 2013)
	Mellissa	5	0.45	0.08-0.71	3.22	0.64-8.18	0.81	0.57-1.08	0.29	0.19-0.38	4.77		
	Marjoram	5	1.38	0.1-3.31	7.87	2.12-10.64	1.77	0.76-4.15	0.97	0.41-2.09	11.99		
	Rosmary	4	10.43	2.88-24.55	12.24	1.24-39.1	1.17	0.39-2.03	0.68	0.14-1.52	24.52		
	Wild thyme	5	1.19	0.11-2.19	1.29	0.23-2.46	0.47	0.29-0.73	0.49	0.12-1.76	3.44		
	Mint	5	1.34	0.22-4.24	2.94	0.24-7.7	0.6	0.28-0.98	0.49	0.02-1.59	5.37		
	Hollzhock	4	0.94	0.32-2	2.25	1.01-4.45	2.1	0.29-5.06	0.22	0.02-0.39	5.51		
	Chamomile	5	1.67	0.17-4.04	1.25	0.47-2.25	0.9	0.11-1.83	0.17	0.07-0.32	3.99		
	Damask rose	4	0.34	0.18-0.76	1.16	0.55-2.14	0.65	0.16-1.15	0.41	0.19-0.99	2.56		
	Roselle	5	0.27	0.1-0.62	0.78	0.04-1.54	0.17	0.04-0.28	0.08	0.03-0.13	1.3		
Leached tea	Yerba mate	8	72.83	24.5-99.9	121.44	42.3-169	51.03	13.3-76.3	37.13	8.03-53.3	282.42	88.1-397.7	Brazil (Kamangar et al., 2008)
Leached tea	Yerba mate	50	28.7		67.6		21.9		26.9		145.1		Argentina (Londoño et al., 2014)

grinded and pulverized with leached teas, not extracted with water. Therefore, solid green teas contained high levels of PAHs (Zhu, 2006, Vieira et al., 2010). Table III-4 shows the levels of PAHs in leached teas. The contents of PAHs in Korean leached teas were lower than those in other countries' leached tea. Mate teas consumed in Germany and Brazil contained higher levels of 4 PAHs than other teas (Ziegenhals et al., 2008, Kamangar et al., 2008).

Consumption of teas. I obtained tea consumption data of the whole population and tea drinkers only from the second and third programs of KNHANES IV (2007-2009) surveying 9,308 and 10,078 sample populations and the first program of KNHANES V (2010-2012) surveying 8,473 sample populations (CDC, 2008, CDC, 2009, CDC, 2010). KNHANES has surveyed green tea, solomon's seal tea, chrysanthemum tea and mate tea and I could obtained their the consumption data. However, KNHANES has not been surveying cassia seed teas, black teas, corn teas, buckwheat teas, burdock teas, matrimony vine teas, dandelion teas, brown rice teas and mulberry leaves teas and I could not obtain their consumption data. Therefore, green teas, solomon's seal teas, chrysanthemum teas and mate teas were only used for exposure estimation of PAHs in leached tea variety. KNHANES has surveyed of green teas, barley teas, black teas and preserved fruit teas and I

could obtain their the consumption data. However, KNHANES has not been surveying ginger teas, Korean raisin teas, corn teas, jujube teas and burdock teas and I could not obtain their consumption data. Therefore, green teas, barley teas, black teas and preserved fruit teas were only used for exposure estimation of PAHs in liquid tea variety. Adlay teas, black teas, ginger teas, herbal teas and green teas were surveyed in KNHANES and all monitored solid teas were used for exposure estimation of solid teas. The data of 95th consumption of teas were zero. This is because that Koreans consume an amount of tea in a single time, but not frequently (Table III-5).

Exposure estimation. The exposures to PAHs via drinking teas were estimated by calculating with the contents of 4 PAHs that are analyzed and statistically processed and the tea consumption data obtained from KNHANES. Table III-6 shows the estimated exposure to each of the 4 PAHs for the whole population. The estimated LB and UB exposures to BaA, CHR, BbF and BaP through drinking teas were 2.32×10^{-2} ng kg^{-1} b.w^{-1}. day^{-1} (LB) and 2.38×10^{-2} ng kg^{-1} b.w^{-1}. day^{-1}(UB), 1.32×10^{-2} ng kg^{-1} b.w^{-1}. day^{-1} (LB) and 1.55×10^{-2} ng kg^{-1} b.w^{-1}. day^{-1}(UB), 1.70×10^{-2} ng kg^{-1} b.w^{-1}. day^{-1} (LB, UB) and 2.01×10^{-2} ng kg^{-1} b.w^{-1}. day^{-1} (LB) to 2.04×10^{-2} ng kg^{-1} b.w^{-1}. day^{-1} ng kg^{-1} b.w^{-1}. day^{-1}(UB) respectively. Meanwhile, tea consumers were

Table III- 5. The amount of teas consumed by whole population and drinker only

Type	Teas	Daily consumption (g day^{-1})			
		Whole population		Drinker only	
		mean	P95 [a)]	mean	P95
Leached tea	Green	0.04	-	2.80	7.50
	Solomon's seal	0.02	-	7.60	24.00
	Chrysanthemum	0.04×10^{-2}	-	1.50	3.00
	Mate	0.04	-	2.80	7.50
Liquid tea	Green	11.84	-	357.40	960.00
	Barley	0.87	-	356.00	800.00
	Black	0.66	-	274.90	600.00
	Preserved fruit	0.12	-	41.00	73.50
Solid tea	Adlay	0.09	-	20.00	40.00
	Black	0.03	-	17.30	42.00
	Ginger	0.02	-	10.10	22.10
	Herbal	0.04	-	23.00	31.20
	Green	0.04	-	2.80	7.50

a) Daily intake of teas at the 95th percentile

Table III-6. Estimated exposure to 4 PAHs by drinking teas for whole population

Type	Teas	Estimated Exposure (ng kg^{-1} b.w. day^{-1})															
		BaA				CHR				BbF				BaP			
		Mean		P95 [a]		mean		P95		mean		P95		mean		P95	
		LB [b]	UB [c]	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB
Leached tea	Green	4.80×10^{-4}	4.80×10^{-4}			1.79×10^{-3}	1.79×10^{-3}			2.63×10^{-3}	2.63×10^{-3}			2.83×10^{-3}	2.83×10^{-3}		
	Solomon's seal	6.48×10^{-4}	6.48×10^{-4}			1.36×10^{-3}	1.36×10^{-3}			2.13×10^{-3}	2.13×10^{-3}			1.85×10^{-3}	1.85×10^{-3}		
	Chrysanthemum	0.05×10^{-4}	0.05×10^{-4}		-	0.15×10^{-4}	0.15×10^{-4}		-	0.34×10^{-4}	0.34×10^{-4}			0.34×10^{-4}	0.34×10^{-4}		
	Mate	4.47×10^{-3}	4.47×10^{-3}		-	6.42×10^{-3}	6.42×10^{-3}		-	5.62×10^{-3}	5.62×10^{-3}			6.45×10^{-3}	6.45×10^{-3}		
Liquid tea	Green	1.42×10^{-2}	1.42×10^{-2}		-	-	2.03×10^{-3}		-	2.03×10^{-3}	2.03×10^{-3}			6.09×10^{-3}	6.09×10^{-3}		
	Barley	1.05×10^{-3}	1.05×10^{-3}		-	-	1.49×10^{-4}			5.97×10^{-4}	5.97×10^{-4}			4.48×10^{-4}	4.48×10^{-4}		
	Black	9.06×10^{-4}	9.06×10^{-4}		-	-	1.13×10^{-4}			2.26×10^{-4}	2.26×10^{-4}			3.40×10^{-4}	3.40×10^{-4}		
	Preserved fruit	1.85×10^{-4}	7.41×10^{-4}		-	3.29×10^{-4}	3.29×10^{-4}			4.12×10^{-4}	4.12×10^{-4}			4.73×10^{-4}	4.73×10^{-4}		
Solid tea	Adlay	4.01×10^{-4}	4.01×10^{-4}		-	4.79×10^{-4}	4.79×10^{-4}			5.87×10^{-4}	5.87×10^{-4}			2.47×10^{-4}	2.47×10^{-4}		
	Black	1.03×10^{-4}	1.03×10^{-4}		-	0.87×10^{-4}	0.87×10^{-4}			0.98×10^{-4}	0.98×10^{-4}			0.31×10^{-4}	1.39×10^{-4}		

	Ginger	0.99×10^{-4}	0.99×10^{-4}	-	1.20×10^{-4}	1.20×10^{-4}	0.89×10^{-4}	0.89×10^{-4}	0.14×10^{-4}	0.93×10^{-4}
	Herbal	1.99×10^{-4}	1.99×10^{-4}	-	2.54×10^{-4}	2.54×10^{-4}	2.40×10^{-4}	2.40×10^{-4}	0.96×10^{-4}	2.20×10^{-4}
	Green	4.80×10^{-4}	4.80×10^{-4}	-	2.36×10^{-3}	2.36×10^{-3}	2.29×10^{-3}	2.29×10^{-3}	1.17×10^{-3}	1.17×10^{-3}
Total		2.32×10^{-2}	2.38×10^{-2}		1.32×10^{-2}	1.55×10^{-2}	1.70×10^{-2}	1.70×10^{-2}	2.01×10^{-2}	2.04×10^{-2}

a) Daily intake of teas at the 95^{th} percentile

b) Lower bound : the left censored data are regarded as zero

c) Upper bound : the left censored data are regarded as LOD

Table III-7. Estimated exposure to 4 PAHs by drinking teas for drinker only

Type	Teas	Estimated Exposure (ng kg^{-1} b.w. day^{-1})															
		BaA				CHR				BbF				BaP			
		Mean		P95 [a]		mean		P95		mean		P95		mean		P95	
		LB [b]	UB [c]	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB
Leached tea	Green	3.36 ×10^{-2}	3.36 ×10^{-2}	9.01 ×10^{-2}	9.01 ×10^{-2}	1.25 ×10^{-1}	1.25 ×10^{-1}	3.36 ×10^{-1}	3.36 ×10^{-1}	1.84 ×10^{-1}	1.84 ×10^{-1}	4.93 ×10^{-1}	4.93 ×10^{-1}	1.98 ×10^{-1}	1.98 ×10^{-1}	5.31 ×10^{-1}	5.31 ×10^{-1}
	Solomon's seal	2.46 ×10^{-1}	2.46 ×10^{-1}	7.78 ×10^{-1}	7.78 ×10^{-1}	5.18 ×10^{-1}	5.18 ×10^{-1}	1.63	1.63	8.11 ×10^{-1}	8.11 ×10^{-1}	2.56	2.56	7.03 ×10^{-1}	7.03 ×10^{-1}	2.22	2.22
	Chrysanthemum	1.83 ×10^{-2}	1.83 ×10^{-2}	3.65 ×10^{-2}	3.65 ×10^{-2}	5.69 ×10^{-2}	5.69 ×10^{-2}	1.14 ×10^{-1}	1.14 ×10^{-1}	1.28 ×10^{-1}	1.28 ×10^{-1}	2.55 ×10^{-1}	2.55 ×10^{-1}	1.29 ×10^{-1}	1.29 ×10^{-1}	2.58 ×10^{-1}	2.58 ×10^{-1}
	Mate	3.13 ×10^{-1}	3.13 ×10^{-1}	8.37 ×10^{-1}	8.37 ×10^{-1}	4.49 ×10^{-1}	4.49 ×10^{-1}	1.20	1.20	3.93 ×10^{-1}	3.93 ×10^{-1}	1.05	1.05	4.51 ×10^{-1}	4.51 ×10^{-1}	1.21	1.21
Liquid tea	Green	4.29 ×10^{-1}	4.29 ×10^{-1}	1.15	1.15	-	6.13 ×10^{-2}	-	1.65 ×10^{-1}	6.13 ×10^{-2}	6.13 ×10^{-2}	1.65 ×10^{-1}	1.65 ×10^{-1}	1.84 ×10^{-1}	1.84 ×10^{-1}	4.94 ×10^{-1}	4.94 ×10^{-1}
	Barley	4.27 ×10^{-1}	4.27 ×10^{-1}	9.61 ×10^{-1}	9.61 ×10^{-1}	-	6.11 ×10^{-2}	-	1.37 ×10^{-1}	2.44 ×10^{-1}	2.44 ×10^{-1}	5.49 ×10^{-1}	5.49 ×10^{-1}	1.83 ×10^{-1}	1.83 ×10^{-1}	4.12 ×10^{-1}	4.12 ×10^{-1}
	Black	3.77 ×10^{-1}	3.77 ×10^{-1}	8.23 ×10^{-1}	8.23 ×10^{-1}	-	4.72 ×10^{-2}	-	1.03 ×10^{-1}	9.43 ×10^{-2}	9.43 ×10^{-2}	2.06 ×10^{-1}	2.06 ×10^{-1}	1.41 ×10^{-1}	1.41 ×10^{-1}	3.09 ×10^{-1}	3.09 ×10^{-1}
	Preserved fruit	6.32 ×10^{-2}	2.53 ×10^{-1}	1.13 ×10^{-1}	4.54 ×10^{-1}	1.13 ×10^{-1}	1.13 ×10^{-1}	2.02 ×10^{-1}	2.02 ×10^{-1}	1.41 ×10^{-1}	1.41 ×10^{-1}	2.52 ×10^{-1}	2.52 ×10^{-1}	1.62 ×10^{-1}	1.62 ×10^{-1}	2.90 ×10^{-1}	2.90 ×10^{-1}
Solid tea	Adlay	8.92 ×10^{-2}	8.92 ×10^{-2}	1.78 ×10^{-1}	1.78 ×10^{-1}	1.06 ×10^{-1}	1.06 ×10^{-1}	2.13 ×10^{-1}	2.13 ×10^{-1}	1.30 ×10^{-1}	1.30 ×10^{-1}	2.61 ×10^{-1}	2.61 ×10^{-1}	5.49 ×10^{-2}	5.49 ×10^{-2}	1.10 ×10^{-1}	1.10 ×10^{-1}
	Black	5.93 ×10^{-2}	5.93 ×10^{-2}	1.44 ×10^{-1}	1.44 ×10^{-1}	5.04 ×10^{-2}	5.04 ×10^{-2}	1.22 ×10^{-1}	1.22 ×10^{-1}	5.64 ×10^{-2}	5.64 ×10^{-2}	1.37 ×10^{-1}	1.37 ×10^{-1}	1.78 ×10^{-2}	8.01 ×10^{-2}	4.32 ×10^{-2}	1.95 ×10^{-1}

Ginger	5.02×10^{-2}	5.02×10^{-2}	1.10×10^{-1}	1.10×10^{-1}	6.06×10^{-2}	6.06×10^{-2}	1.33×10^{-1}	1.33×10^{-1}	4.50×10^{-2}	4.50×10^{-2}	9.86×10^{-2}	9.86×10^{-2}	6.93×10^{-3}	4.68×10^{-2}	1.52×10^{-2}	1.02×10^{-1}
Herbal	1.14×10^{-1}	1.14×10^{-1}	1.55×10^{-1}	1.55×10^{-1}	1.46×10^{-1}	1.46×10^{-1}	1.98×10^{-1}	1.98×10^{-1}	1.38×10^{-1}	1.38×10^{-1}	1.87×10^{-1}	1.87×10^{-1}	5.52×10^{-2}	1.26×10^{-1}	7.49×10^{-2}	1.71×10^{-1}
Green	3.36×10^{-2}	3.36×10^{-2}	9.01×10^{-2}	9.01×10^{-2}	1.65×10^{-1}	1.65×10^{-1}	4.43×10^{-1}	4.43×10^{-1}	1.60×10^{-1}	1.60×10^{-1}	4.28×10^{-1}	4.28×10^{-1}	8.16×10^{-2}	8.16×10^{-2}	2.19×10^{-1}	2.19×10^{-1}
Total	2.25	2.44	5.47	5.81	1.79	1.96	4.59	5.00	2.59	2.59	6.64	6.64	2.37	2.54	6.19	6.52

a) Daily intake of teas at the 95th percentile

b) Lower bound : the left censored data are regarded as zero

c) Upper bound : the left censored data are regarded as LOD

exposed to BaA, CHR, BbF and BaP 2.25 ng kg^{-1} b.w. day^{-1} (LB) and 2.44 ng kg^{-1} b.w. day^{-1}(UB), 1.79 ng kg^{-1} b.w. day^{-1} (LB) and 1.96 ng kg^{-1} b.w. day^{-1}, 2.59 ng kg^{-1} b.w. day^{-1} and 2.37 ng kg^{-1} b.w. day^{-1} (LB) and 2.54 ng kg^{-1} b.w. day^{-1} (UB) (Table III-7). The exposures to sum of 4 PAHs for whole population and tea drinkers were estimated to 7.35×10^{-3} ng kg^{-1} b.w. day^{-1} (LB) and 7.68×10^{-2} ng kg^{-1} b.w. day^{-1} (UB) and 9.00 ng kg^{-1} b.w. day^{-1} (LB) and 9.54 ng kg^{-1} b.w. day^{-1} (UB) (Table III-8). Korean was mainly exposed to 4 PAHs by drinking teas with mate leached tea (30.0-31.1%) and liquid green tea (30.3-31.8%). Solomon's seal leached tea was the main contributor for exposure to PAHs for tea drinkers only (23.9-25.3%). Mate leached teas and liquid green teas are two main contributors of exposing to PAHs for the whole population. This is because that mate tea was highly contaminated by PAHs and liquid green tea is highly consumed. Meanwhile, solomon's seal tea is highly but occasionally consumed. As the result of exposure estimation, Koreans are mainly exposed to PAHs by drinking liquid teas, because Koreans consume a number of amount of liquid teas. Therefore, liquid teas must be carefully produced and managed in order not to be contaminated with PAHs, even though liquid teas did not contain PAHs much.

Table III-8. Estimated exposure to 4 PAHs by drinkg teas for whole population and drinker only

Type	Teas	Estimated Exposure (ng kg^{-1} b.w. day^{-1})							
		Whole population				Eater only			
		mean		P95 [a]		mean		P95	
		LB [b]	UB [c]	LB	UB	LB	UB	LB	UB
Leached tea	Green	7.74×10^{-3}	7.74×10^{-3}	-	-	5.42×10^{-1}	5.42×10^{-1}	1.45	1.45
	Solomon's seal	5.99×10^{-3}	5.99×10^{-3}	-	-	2.28	2.28	7.19	7.19
	Chrysanthemum	0.88×10^{-4}	0.88×10^{-4}	-	-	3.32×10^{-1}	3.32×10^{-1}	6.63×10^{-1}	6.63×10^{-1}
	Mate	2.30×10^{-2}	2.30×10^{-2}	-	-	1.61	1.61	4.30	4.30
Liquid tea	Green	2.23×10^{-2}	2.44×10^{-2}	-	-	6.74×10^{-1}	7.36×10^{-1}	1.81	1.98
	Barley	2.09×10^{-3}	2.24×10^{-3}	-	-	8.55×10^{-1}	9.16×10^{-1}	1.92	2.06
	Black	1.47×10^{-3}	1.59×10^{-3}	-	-	6.13×10^{-1}	6.60×10^{-1}	1.34	1.44
	Preserved fruit	1.38×10^{-3}	1.96×10^{-3}	-	-	4.71×10^{-1}	6.68×10^{-1}	8.45×10^{-1}	1.20
Solid tea	Adlay	1.73×10^{-3}	1.73×10^{-3}	-	-	3.84×10^{-1}	3.84×10^{-1}	7.68×10^{-1}	7.68×10^{-1}
	Black	3.14×10^{-4}	4.27×10^{-4}	-	-	1.81×10^{-1}	2.46×10^{-1}	4.39×10^{-1}	5.98×10^{-1}
	Ginger	3.22×10^{-4}	4.01×10^{-4}	-	-	1.63×10^{-1}	2.03×10^{-1}	3.56×10^{-1}	4.44×10^{-1}
	Herbal	7.96×10^{-4}	9.13×10^{-4}	-	-	4.58×10^{-1}	5.25×10^{-1}	6.21×10^{-1}	7.12×10^{-1}
	Green	6.29×10^{-3}	6.29×10^{-3}	-	-	4.40×10^{-1}	4.40×10^{-1}	1.18	1.18
Total		7.35×10^{-3}	7.68×10^{-2}			9.00	9.54	22.9	24.0

d) Daily intake of teas at the 95th percentile

e) Lower bound : the left censored data are regarded as zero

f) Upper bound : the left censored data are regarded as LOD

Table III-9. Margin of Exposures (MOE) of total 4 PAHs

Type	Margin of Exposure (MOE)							
	Whole population				Eater only			
	4 PAHs				4 PAHs			
	mean		P95 [a)]		mean		P95	
	LB	UB	LB	UB	LB	UB	LB	UB
Leached tea	9.25×10^6	9.25×10^6	-	-	7.15×10^4	7.15×10^4	2.50×10^4	2.50×10^4
Liquid tea	1.25×10^7	1.13×10^7	-	-	1.30×10^5	1.14×10^5	5.75×10^4	5.10×10^4
Solid tea	3.60×10^7	3.48×10^7	-	-	2.09×10^5	1.89×10^5	1.01×10^5	9.19×10^4
Total	4.63×10^6	4.43×10^6	-	-	3.78×10^4	3.57×10^4	1.49×10^4	1.42×10^4

a) Daily intake of teas at the 95th percentile

Risk characterization and uncertainty. MOEs of sum of 4 PAHs in teas were calculated (Table III-9) with estimated exposure data. The LB and UB MOEs of the sum of 4 PAHs by drinking teas were 4.63×10^6 (LB) and 4.43×10^6 (UB) for the whole Korean and 3.78×104 (LB) and 3.57×104 (UB) for tea consuming Korean. The risk of PAHs by drinking teas were of "low concern from a public health point of view", because the values of MOEs were higher than the criteria of safety, 10,000 (EFSA, 2005). The priority of management of PAHs of teas is low and policy makers should focus on other hazards in food. There were the limits of risk characterization. The uncertainty of analytical data left-censored and consumption data obtained from 24 hours recall survey could cause under- or over-estimation of exposure to PAHs. The risks of PAHs in teas which are characterized to low concern to public health could be estimated more or lower in case of considering specific sensitive group of people and non-dietary sources such as air, water etc. Advantages of drinking teas like consuming bioactive compounds such as catechins should be considered to estimate the risks of drinking teas. It might decrease the risks of PAHs in teas.

Statistical analysis. The percentages of left-censored data of PAHs marked as ND were less than 60% in all leached teas. The values of ND were

calculated as half of the value of LOD. All values of CHR in green teas, barley teas and black teas in the liquid tea variety were not detected and they were statistically used as 0 for LB and the values of LOD for UB. The percentages of left-censored data of BaA, BbF and BaP were between 0 and 60% and they are calculated as half of the values of LODs. The average level of BaA in preserved fruit teas was replaced with 0 for LB and the value of LOD for UB. And half of the value of LOD was used for CHR, BbF and BaP in preserved fruit teas. In case of solid teas, the levels of BaP in black teas, herbal teas and ginger teas were calculated with 0 for LB and the value of LOD for UB. Half of the value of LOD was used for BaA, CHR and BbF. All samples of green teas were detected and the true values of PAHs in teas were used.

III-4. Conclusion

The risks of exposure to PAHs by drinking teas in Korea were characterized by analyzing PAHs in teas. An analytical method to detect 4 PAHs simultaneously was validated. The leached tea showed high contaminations of PAHs, and liquid and solid tea contained low amount of PAHs due to their production process. However, high amount of liquid green tea consumption contributes to exposures to PAHs. As a result of risk assessment by MOE, the risk of PAHs in tea was low concern to public health with MOEs higher than 10,000. Furthermore, the benefits of drinking tea and the consumption patterns of tea might decrease the risk of PAHs in tea.

Chapter IV.

Reduction of occurrence of Polycyclic Aromatic Hydrocarbons in Grilled Meats[3]

[3] This chapter has been published: Joon-Goo Lee, Su-Yeon Kim, Jung-Sik Moon, Sheen-Jee Kim, Dong-Hyun Kang and Hae-Jung Yoon, 2016, Effects of grilling procedures on levels of polycyclic aromatic hydrocarbons in grilled meats, Food Chemistry, 199: 632-638

IV-1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are an organic compounds group which contains more than two aromatic rings. When organic matter such as wood, coal, meat or other organic substances are incompletely combusted, aromatic rings are naturally occurred. Those aromatic rings are combined and derive PAHs (Codex Alimentarius Commission (CX/FAC05/37/34), 2004; Rey-Salgueiro, Martinez-Carballo, Garcia-Falcon, & Simal-Gandara, 2008).

PAHs are unintentionally generated during cooking and processing food, and they have been one of the most risky chemicals in food because of their carcinogenic and genetoxic properties (Jägerstad & Skog, 2005). 15 priority PAHs of a number of PAHs were classified by the EU scientific committee based on their carcinogenicity and B[a]P was selected as a marker for 15 priority PAHs in food (European Commission (EC), 2006; Commission Regulation (EU), 2011; Wenzl, Simon, Anklam, & Kleiner, 2006). But Europe Food Safety Authority (EFSA) has recently figured out that B[a]P is not acceptable as a general indicator for PAHs and EFSA concluded that 4 PAHs (B[a]A, Chr, B[b]F, B[a]P) are better for representing PAHs according to their toxicological data (EFSA, 2008 ; Rose et. al., 2015).

PAHs widely contaminated environment with bush fire, usage of fossil fuel and waste incineration etc. Foodstuffs are also contaminated with PAHs from contaminated environment as well as heat treatment for cooking food (WHO, 1998, 2005). Some researchers have figured out that Grilled and smoked meat products are one of the most contaminated foods since Lijinsky and Shubik (1964) determined the level of PAHs in charcoal broiled beef products (Chung, Yettella, Kim, Kwon, Kim, & Min., 2011; Rose et. al., 2015; Duedahl-Olesen, Aaslyng, Meinert, Christensen, Jensen, & Binderup, 2015; Chen & Lin, 1997).

The consumption of grilled, smoked and roasted meats has been recently increased not only at home but also in restaurants. And high intake of those foods has been elevating the public health risk comparing to other foods which is processed by other processing methods (Kao, Chen, Huang, Chen, & Chen, 2014; Sundararajan, Ndife, Basel, & Green, 1999).

The generation of PAHs in grilled and smoked foods is not precisely (Farhadian, Jinap, Fa understood but PAHs may be formed from pyrolysis of organic matter including fat and their recombination in high cooking temperature (ridah, & Zaidul, 2010). PAHs in smoke from incomplete combustion of charcoal or wood can attached to the surface of grilled and smoked foods (Rey-Salgueiro, Garcia-Falcon, Martinez-Carballo, & Simal-

Gandara, 2008; Bartle, 1991; Knize, Salmon, Pais, & Felton, 1999). However, no research has explained which grilling and smoking processes influence on the level of PAHs

Therefore, this study determined what procedures in grilling meats affect the occurrence of PAHs. For this, the levels of PAHs generated during grilling meats with different kinds of meat, cooking temperatures, cooking time and cooking apparatus were compared. This study eventually showed the best cooking practices for reducing the risk of exposure to PAHs by consuming the grilled meats.

IV-2. Materials and Methods

Chemicals and materials. 4 PAHs external standards and 2 deuterated internal standards were prepared by purchasing B[a]A, Chr, B[b]F, B[a]P, Chr-d12 and B[a]P-d12 from Supelco (Bellefonte, PA, USA). 4 PAHs standards were mixed in dichloromethane and the mixture was diluted to 100 μg/mL with dichloromethane to make a stock standard solution. 2 deuterated PAHs standards were also mixed and diluted with dichloromethane for stock standard solutions. Working standard solutions ranged from 10 to 500 μg/L were made by combining the 4 PAHs and 2 deuterated stock standard solutions and diluting it with dichloromethane.

Dichloromethane from Burdick & Jackson (Muskegon, MI, USA), ethanol and hexane from Merck (Darmstadt, Hesse, Germany) and water was filtered by a Milli-Q system of Millipore Corporation (Bedford, Ma, USA) were used. Silica-SPE cartridges (1g/6cc) were purchased from Waters Corporation (Milford, MA, USA).

Sample preparation. Beef and pork of 6 kg were purchased from markets in Korea. Loin and ribs for beef and neck lean and belly for pork were selected since these kinds of meats were normally consumed by grilling

in Korea and were containing different fat contents. The fat portions of Beef loin, beef ribs, pork neck lean and pork belly were 31.7%, 24.4$, 9.5% and 26.4%, respectively (RDA, 2011). The meat samples were cut into 8.8 cm in diameter with thickness of 1 cm by using petri-dish.

Extraction and purification of samples were conducted by the procedures in Korean Food Code (MFDS, 2014). Grilled samples were homogenized with a mixer, Mix-h03 of Tongyang-magic (Seoul, Korea) and 10 g of homogenized sample and the deuterated internal standard of 4 μg/kg were put in a round flask and saponified with 100 mL of 1 M potassium hydroxide in ethanol in ethanol in a water bath of 80 ℃ for 3 hours. N-hexane of 50 mL was slowly added to the flask through the reflux condenser following cooling the reflux condenser. The saponified sample was transferred to a separatory funnel with 25 mL of ethanol and 25 mL of n-hexane. The hexane layer was isolated from the separatory funnel and the aqueous remainder was extracted two times with 50 mL of n-hexane. The 3 hexane layers separated from the separatory funnel were combined and washed 3 times with 50 mL of filtered water. The washed hexane layer was concentrated to 2 mL with a vacuumed rotary evaporator, Eyela of Tokyo Rikakikai Co. Ltd. (Tokyo, Japan) following being dehydrated through anhydrous Na_2SO_4. The concentrated sample was loaded on the silica-SPE cartridge which was

previously activated with 10 mL of dichloromethane and 20 mL of n-hexane in a row by gravity flow. The cartridge was cleaned with 10 mL of n-hexane and was eluted with 20 mL of solvent of n-hexane and dichloromethane (3:1, v/v). The eluted solution was collected in a test tube and evaporated to dryness at 40 ℃ by nitrogen of 20 psi in a TurboVap of Zymark (MA, USA). The sample for GC-MS analysis was prepared by adding 200 μL of dichloromethane to the dried concentrate and filtering it with a 0.45 μm membrane filter. And a blank sample was prepared with same procedures without meat sample.

Charcoal grilling method. A outdoor barbecue griller which has bottom width of 17 x 17 cm, upper width of 34 x 32 cm and height of 8 cm was prepared with 700 g of wood charcoal. The charcoal was ignited by a propane torch for 2 min and grilling temperature was determined by measuring the temperature of the meats with the infrared thermometer, Fluke of Everett (WA, USA). The meats were grilled at a distance of 8 cm from the charcoal for 12 min for well-done, following flames' subsidence (Fig. IV-1). The meats were turned over four times after each side was cooked for 3 min, and no salt and oil were added during grilling them. Grilled meats were homogenized and prepared according to the method referred above.

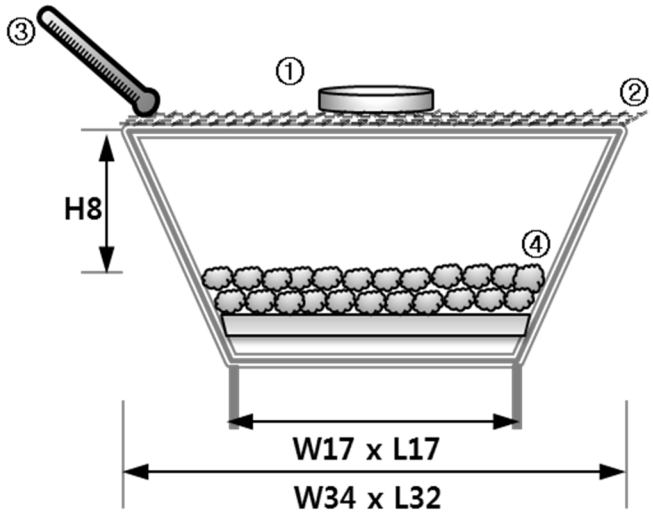


Fig. IV-1. The designs of outdoor barbecue griller (① meat, ② grill, ③ thermometer, ④ charcoal.

Conditions of grilling temperature and time. The meats were grilled for four different time periods with different grilling temperature to see the effects on the occurrence of PAHs. The first grilling period was during 12 min following ignition of charcoal for 2 min with increasing grilling temperature between 127 ℃ and 320 ℃. The second grilling period was for 12 min after the first period, and it had grilling temperature from 320 ℃ to 285 ℃. The third grilling period was also for 12 min from the end of the second period, and its temperature was decreased from 285 ℃ to 250 ℃. The last period was for 24 min following the third period with the declining temperature between 250 ℃ and 200 ℃. In this period, the flames were gradually extinguished. These grilling experiments were repeated 6 times and the meats were cooked for well-done. Figure IV-2 shows the grilling temperature according to the cooking time.

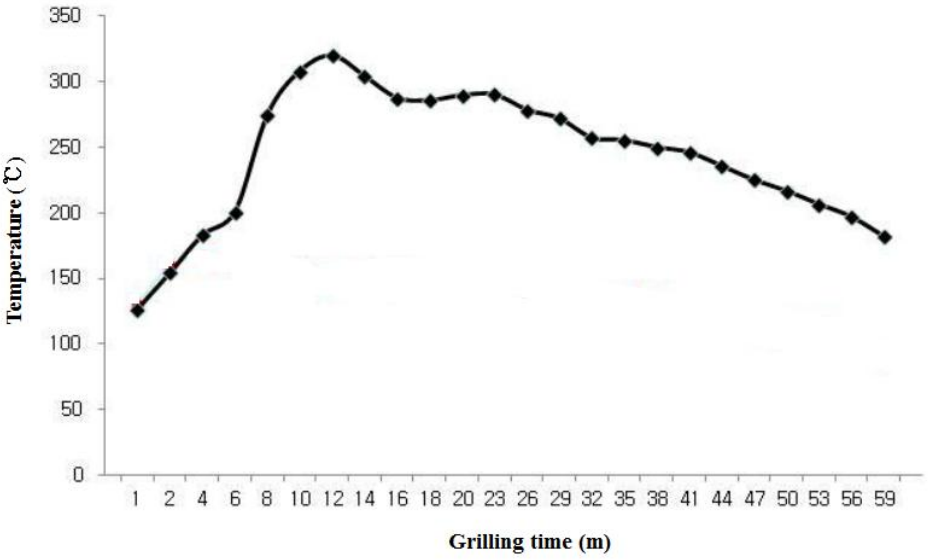


Fig. IV-2. The change of grilling temperature according to the temperature.

Conditions of removing meat drippings. The barbeque grillers were modified to remove meat drippings and charcoal smoke. For removing the meat drippings, a hole with a diameter of 9.8 cm was bored on the bottom of the barbecue griller, and an aluminum tube with a height of 12 m was attached to the hole to make a way which the drippings pass through and to make charcoal not to block the hole (Fig. IV-3). A beaker was placed under the hole to collect the drippings in an ice bucket. The meats were grilled in the middle of the griller during the second period and the experiment was repeated 6 times again.

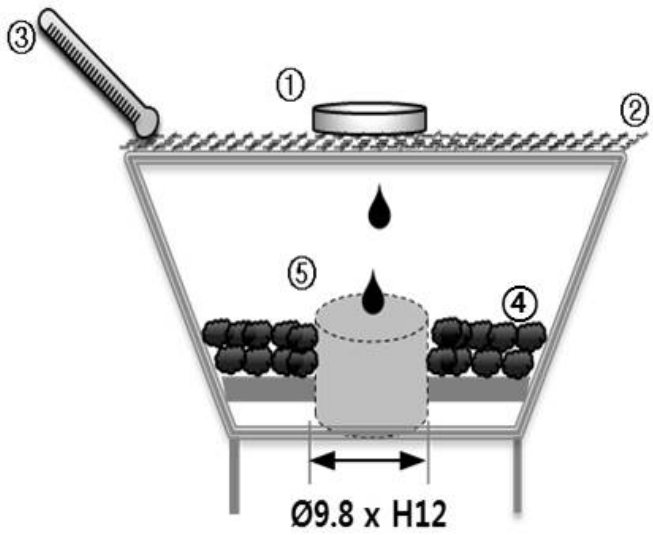


Fig. IV-3. The designs of outdoor barbecue griller for removing the meat drippings (① meat, ② grill, ③ thermometer, ④ charcoal, ⑤ aluminum tube.

Conditions of removing smoke from flame of charcoals. The barbeque griller equipped a ventilation duct to remove smoke from charcoal. The duct inlet was placed between flame and the meat, and the diameter and the length of duct were 20.8 cm and 300 cm, respectively (Fig. IV-4). The meats were grilled during the second grilling period and were repeated 6 times.

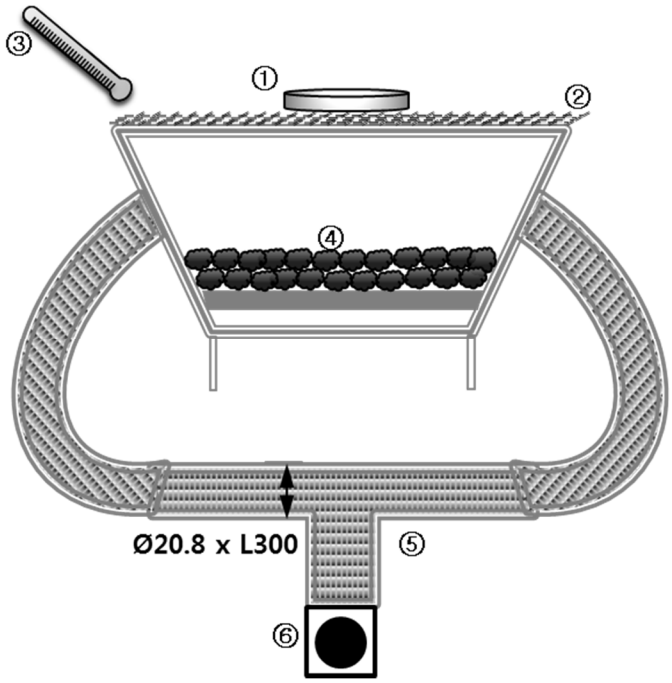


Fig. IV-4. The designs of outdoor barbecue griller for removing the meat drippings (① meat, ② grill, ③ thermometer, ④ charcoal, ⑤ aluminum duct, ⑥ vacuum pump).

Determination of PAHs using GC-MS. PAHs was determined and measured by using a GC chromatography, CP-3800 of Varian (CA, USA) with MS spectrometry, 1200L of Varian (CA, USA). An auto-sampler, Combi-PAL of CTC Analytics (Zwingen, Switzerland) was used based on the Korean Food Code method. A DB-5ms GC capillary column from Agilent technologies (CA, USA) with length of 30 m, inner diameter of 25 μm and film thickness of 0.25 μm was equipped. The oven was heated at a rate of 4 ℃/min to 245 ℃ following being at 80 ℃ for 1 min, and it was finally increased to 270 ℃ at 30 ℃/min followed by being held for 10 min. Helium carrier gas was flowing at 1.5 mL/min. A injector was at the temperature of 320 ℃ and 1 μL of samples were injected with splitless mode. Mass spectrometry was operated with source temperature of 250 ℃ and electron ionization (EI) of 70 eV. Signals are acquired by selective ion monitoring (SIM) mode with dwell time of 0.1 s. The 4 PAHs were quantified by comparison of retention times and ion masses of selected ions to those of the 4 PAHs in standards and qualified by calculating their levels with calibration curve (Table IV-1).

Table IV-1. **Retention time and m/z value of quantitative and qualitative ions of 4 PAHs and 2 deuterated PAHs**

PAHs	Retention time(min)	Quantitative Ions(m/z)	Qualitative Ions(m/z)
B[a]A	40.63	228	229, 226
Chr	40.81		
B[b]F	45.22	252	253,250
B[a]P	46.51		
Chr-d12	40.67	240	241, 236
B[a]P -d12	46.41	264	265, 260

Quality control. The analytical methods were developed by validating parameters: specificity, detection limits, linearity of standard curve and accuracy including recovery and precision according to the guidelines for validation of analytical methods recommended by the IUPAC and AOAC (Thompson, Ellison, & Wood, 2002; Taverniers, Loose, & Bockstaele, 2004).

The specificity for ions of 4 PAHs and 2 deuterated PAHs was confirmed by comparing the ion information of blank samples fortified with standards to beef loin and pork neck samples fortified with standards. For detection limits, limit of detection (LOD), the lowest level to detect the analytes, was estimated by multiplying standards deviations derived from analyzing 7 samples containing 4 PAHs of 1 μg/kg by 3. And limit of qualification (LOQ), the lowest level to determine the concentration of analytes, was estimated by multiplying LOD by 3. The linearity of calibration curve was evaluated by calculating regression coefficient (R^2). Calibration curves were obtained from a regression equation calculated with 5 different levels of 4 PAHs ranged from 10 to 500 μg/L (10, 50, 100, 200 and 500 μg/L) by the regression analysis. The accuracy was validated with recovery and precision, and Precision was composed of repeatability and reproducibility.

Recovery is obtained by comparing the levels of PAHs of blank samples and fortified samples with mixed standards of 2 μg/kg and deuterated

internal standards of 4 μg/kg. The recovery test was performed 5 times and the average of relative recovery was calculated. Relative standard deviations (RSD^r) of each PAH in recovery tests were used for the repeatability. The reproducibility was validated by calculating relative standard deviations (RSD^R) of results of analyzing the samples for recovery tests by 5 different laboratories.

Analysis of meat droppings. Meat drippings collected during grilling on the condition of removing meat dripping (Fig. IV-1) were analyzed 3 times to determine the amount of moisture and fat. The level of moisture and fat were determined by air oven drying method (AOAC Method 950.46) and the Roese-Gottlieb method (AOAC Method 905.02), respectively.

Statistical analysis. The student's t-test was carried out to assess the significant differences of the occurrence of PAHs in different grilling conditions by using Microsoft Office Excel 2007 (WA, USA). Standards deviations are obtained for averaging analysis data.

IV-3. Results and Discussion

Quality control. All parameters of method validation were shown in table 2 (Table IV-2) and chromatograms of 4 PAHs and 2 deuterated PAHs were present in Figure 5 (Fig. IV-5). The method was properly validated by the criteria recommended by the AOAC and IUPAC (Thompson et. al., 2002; Taverniers et. al., 2004). The peaks of 4 PAHs and 2 deuterated PAHs were properly separated each other, and there were no significant noises interrupting isolation of the peaks. The linearity of the standards curves were suitable to calculated the levels of PAHs with high correlation coefficient value of more than 0.99. LODs and LOQs of 4 PAHs were ranged from 0.08 to 0.21 μg/kg and from 0.24 to 0.63 μg/kg, respectively. The relative recoveries of 4 PAHs were from 90.2 to 111.0% in beef samples fortified with 4 PAHs of 2.0 μg/kg and from 83.4 to 110.7% in PAHs-fortified pork samples. The repeatability was satisfied with RSD^rs of 4 PAHs ranged from 1.23 % to 9.32 % and the reproducibility was also good enough to obtain reliable data with RSD^Rs ranged from 6.60 % to 14.58 %.

Table IV-2. **The values of validation parameters (linearity, detection limits, and accuracy)**

PAHs	Linear equation	Regression coefficient	Matrix	LOD (µg/kg)	LOQ (µg/kg)	Relative recovery (%)	RSD ^{r} (%)	RSD ^{R} (%)
B[a]A	$y = 0.0068x - 0.0593$	0.9999	Beef	0.09	0.27	111.0	1.23	6.60
			Pork	0.15	0.45	110.7	1.91	8.08
Chr	$y = 0.0119x + 0.3210$	0.9974	Beef	0.09	0.27	101.7	1.46	9.92
			Pork	0.08	0.24	92.2	2.05	8.91
B[b]F	$y = 0.0083x - 0.0165$	0.9997	Beef	0.20	0.60	99.4	7.88	14.58
			Pork	0.18	0.54	99.9	5.45	9.21
B[a]P	$y = 0.0119x - 0.0739$	0.9999	Beef	0.18	0.54	90.2	9.32	8.11
			Pork	0.21	0.63	83.4	4.55	7.34

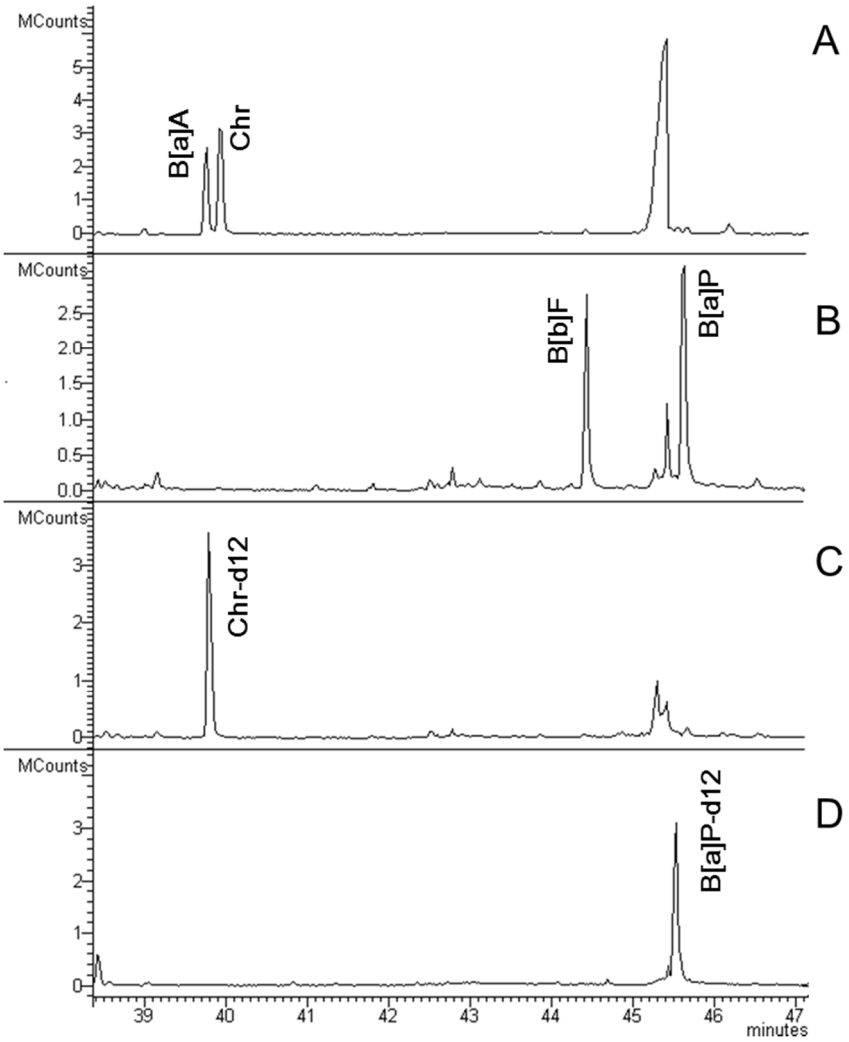


Fig. IV-5. The Chromatograms of GC-MS of 4 PAHs and deuterated 2 PAHs with selected ion monitoring (SIM) mode at m/z of 228 (A), m/z of 252 (B), m/z 240 (C) and m/z 264 (D).

The levels of the 4 PAHs according to different conditions of grilling temperature and time. The levels of the 4 PAHs of the meats grilled by different temperature and time conditions were shown in table 3. Beef loin samples showed the highest levels of 4 PAHs at 28.70 μg/kg during period 1. And beef rib samples produced the highest levels of 4 PAHs at 23.81 μg/kg during period 1. Meanwhile, 4 PAHs ranged from 10.93 μg/kg to 16.91 μg/kg and from 9.20 μg/kg to 12.70 μg/kg were occurred in beef loin and beef rib during other periods, respectively. Similarly, in pork samples, 4 PAHs was highly contaminated at the level of 16.96 μg/kg in neck lean during period 1, and the levels from 4.38 to 7.63 μg/kg were occurred in other periods. 4 PAHs of pork belly was 33.17 μg/kg during 1st period, and from 11.44 μg/kg to 21.77 μg/kg in other periods. The levels of PAHs between the period 1 and the other periods had significant differences with p-value less than 0.05 in beef ribs and pork neck leans while there were no significant differences among the period 2, 3 and 4 with p-value higher than 0.05. The reason why high level of PAHs was occurred in the first period may be because of the incomplete combustion of charcoal. PAHs occurred from charcoal might be carried to meat during grilling as the smoke forms. The amount of PAHs occurred in the charcoal and carried to meat was

Table IV-3. **The concentrations of the total and each of 4 PAHs in different conditions of grilling temperature and time (n=6)**

Meats		Period	PAHs (μg/kg)				
			B[a]A	Chr	B[b]F	B[a]P	Total
Beef	Loin	1	6.96 (±3.84)	7.14 (±4.17)	9.54 (±7.26)	5.07 (±6.27)	28.70 (±20.09)
		2	3.62 (±1.94)	3.80 (±1.22)	6.25 (±2.09)	3.23 (±1.85)	16.91 (±5.62)
		3	2.15 (±1.01)	2.67 (±1.20)	3.91 (±1.46)	2.20 (±1.58)	10.93 (±3.89)
		4	2.68 (±1.88)	2.95 (±1.84)	4.59 (±2.86)	2.48 (±2.62)	12.70 (±8.07)
	Rib	1	7.45 (±1.63)	9.24 (±2.53)	2.31 (±0.68)	4.81 (±1.32)	23.81 (±4.97)
		2	3.21 (±0.96)	2.27 (±0.88)	2.19 (±1.08)	3.30 (±1.13)	10.98 (±3.69)
		3	2.76 (±0.82)	2.14 (±0.76)	1.95 (±0.48)	2.35 (±0.86)	9.20 (±2.43)
		4	2.89 (±2.27)	2.65 (±2.58)	2.81 (±2.33)	4.35 (±4.13)	12.70 (±9.62)
Pork	Neck lean	1	5.45 (±1.64)	2.80 (±1.12)	5.66 (±3.66)	3.04 (±1.43)	16.96 (±7.61)
		2	1.75 (±0.62)	0.94 (±0.64)	1.71 (±0.82)	1.21 (±0.49)	5.60 (±2.51)
		3	2.24 (±0.68)	0.97 (±0.40)	2.70 (±0.84)	1.73 (±0.62)	7.63 (±2.06)
		4	1.14 (±0.30)	1.30 (±0.80)	1.26 (±0.54)	0.68 (±0.21)	4.38 (±1.04)
	Belly	1	10.27 (±5.40)	7.97 (±4.00)	8.93 (±4.18)	5.99 (±3.32)	33.17 (±15.43)
		2	3.09 (±2.15)	4.15 (±1.93)	8.77 (±4.86)	5.76 (±3.53)	21.77 (±10.56)
		3	2.45 (±1.35)	2.16 (±1.11)	3.81 (±1.69)	3.03 (±0.98)	11.44 (±4.42)
		4	3.13 (±3.05)	2.33 (±2.31)	3.27 (±1.66)	2.81 (±1.73)	11.54 (±8.47)

decreased as it started being combusted completely (Costa, Viegas, Melo, Petisca, Pinho, & Ferreira, 2009; Hassan, Magda, & Awad, 2010). To reduce the occurrence of PAHs in grilling meats, one of the most important things is to start grilling meats after the charcoal combusted completely. The consumers would be able to figure out when the charcoal is completely combusted by the color of the flame and the amount of generated smoke.

However, in the beef loin and pork belly, there were no significant differences of occurrence of PAHs between periods 1 and 2, although the levels of 4 PAHs were decreased from period 1 to period 2. This is because the high amount of fat in beef loin and pork belly might cause high variations in statistical analysis. According to the Korean food composition table, beef loin and pork belly contain fat of 31.7% and 26.4 %, respectively. Therefore, it is necessary to consider the fat contents in meats to reduce occurrence of PAHs in grilling them.

Occurrence of PAHs in different fat contents. To determine the change of occurrence of PAHs according to the fat contents, the level of PAHs in beef and pork samples grilled during period 2 for 12 min were compared. Grilled pork belly was contaminated by the highest levels of PAHs at 21.77 μg/kg with significant differences from the other meats.

B[a]A, Chr, B[b]F and B[a]P in pork belly samples were 3.09, 4.15, 8.77, 5.76 µg/kg, respectively. Grilled beef loin samples were following the pork belly samples with 4 PAHs of 16.91 µg/kg, B[a]A of 3.62 µg/kg, Chr of 3.80 µg/kg, B[b]F of 6.25 µg/kg and B[a]P of 3.23 µg/kg. Pork neck lean was the least PAHs contaminated meat with PAHs of 5.60 µg/kg, B[a]A of 1.76 µg/kg, Chr of 0.94 µg/kg, B[b]F of 1.71 µg/kg and B[a]P of 1.21 µg/kg. Levels of B[a]P in grilled beef and pork were similar to those in other research showing the levels of 2.3 to 6.1 µg/kg (Larsson, Sahlberg, Eriksson, & Busk, 1983). Olatunji et. al. (2014) also determined B[a]P in grilled beef and pork. The levels of B[a]P in beef and pork were 2.74 µg/kg and 1.75, respectively. Furthermore, the content of 4 PAHs in barbecued beef was 10 µg/kg. The reason why level of PAHs in grilled Pork belly is the highest in grilled meats would be that pork belly contains the largest amount of fat of 26.4%. The occurrence of PAHs in charcoal grilling meat is affected on by the content of fat in the meat, not by protein and carbohydrate (Saito, Tanaka, Miyazaki, & Tsuzaki. 2014). Fat dripping down from the meat during grilling is incompletely combusted and pyrolyzed on the flame of charcoal to produce PAHs. The PAHs were carried into the meat by smoke (Chen & Chen, 2001; Chung et. al., 2011). Another study showed that the PAHs in grilled meat were lower when they cooked the meat contained less fat

content (Lijinsky & Ross, 1967).

Analysis of meat dripping. The meat drippings were analyzed to determine their components followed investigating the influence of removing meat dripping on the reduction of occurrence of PAHs in grilled meat during grilling meat. Dripping from Beef loin for first period of 4 min from staring grilling was composed of fat of 55.8 %, moisture of 28.2 %, and others such as protein etc. Dripping collected for second period of 4 min after first collection was composed of fat of 64.5 %, moisture of 27.2 % and others. Dripping occurred for third period of from 9 to 12 min during grilling beef loin contained fat of 76.2 %, moisture of 9.3 % and others. As the time had gone by, the amount of fat had been increased while moisture had been decreased. With pork belly, dripping of first period was composed of fat of 76.8 %, moisture of 18.7 % and others. Those of second and third periods contained fat of 95.3 % and 99.0 %, moisture of 2.2 % and 0.5. The levels of fat had been increased as time had gone by same as the trends in beef loin. Therefore, drippings were mainly composed of fat and a little moisture. And the more meat contained fat, the more dripping contained fat (Fig. IV-6).

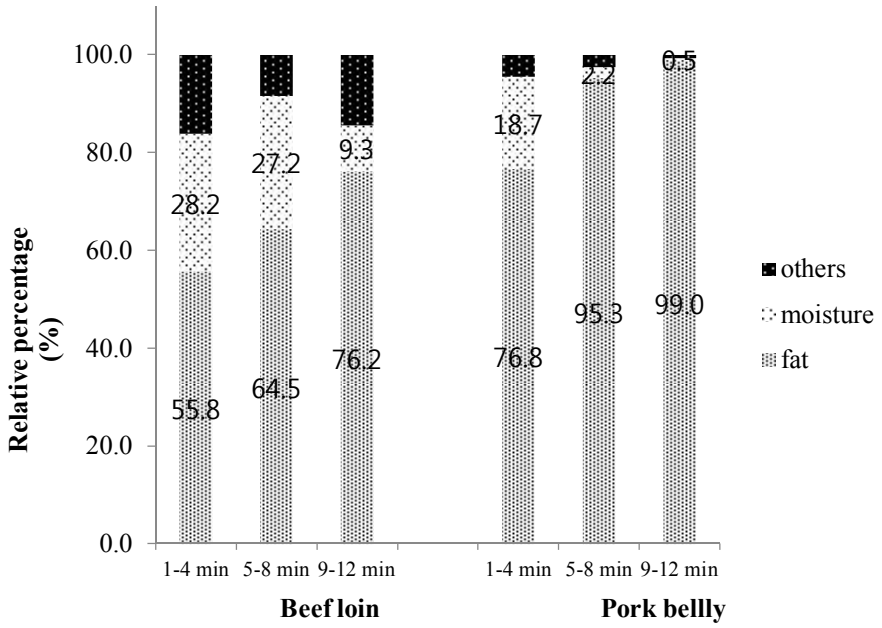


Fig. IV-6. The amount of fat, moisture and others in dripping of meat during grilling (n=3).

Reduction of occurrence of PAHs by removing meat dripping. To reduce the occurrence of 4 PAHs during grilling the meat, meat dripping was removed. The levels of 4 PAHs in grilled meat were reduced by removing meat dripping (Fig. IV-7). By removing meat dripping, the mean contents of 4 PAHs, B[a]A, Chr, B[b]F and B[a]P in beef loin were decreased from 16.91 μg/kg , 3.62 μg/kg, 3.80 μg/kg, 6.25 μg/kg and 3.23 μg/kg to 2.47 μg/kg , 0.60 μg/kg, 0.27 μg/kg, 0.81 μg/kg, and 0.78 μg/kg, respectively. PAHs were approximately reduced by from 76 % to 93 %. Meanwhile, the formation of 4 PAHs, B[a]A, Chr, B[b]F and B[a]P in beef rib were reduced from 10.98 μg/kg, 3.21 μg/kg, 2.27 μg/kg, 2.19 μg/kg and 3.30 μg/kg to 2.61 μg/kg , 0.63 μg/kg, 0.14 μg/kg, 0.70 μg/kg, and 1.13 μg/kg, respectively. The reduction rates of PAHs by removing fat dripping in beef rib were from 66% to 94%. Regarding to pork meat, 4 PAHs, B[a]A, Chr, B[b]F and B[a]P in neck lean were reduced from 5.60 μg/kg, 1.75 μg/kg, 0.94 μg/kg, 1.71 μg/kg and 1.21 μg/kg to 2.93 μg/kg , 1.05 μg/kg, 0.44 μg/kg, 0.89 μg/kg, and 0.56 μg/kg by removing fat dripping during grilling meat. The reduction rates were ranged from 40% to 54%. And in pork belly, removing fat dripping reduced the formation of PAHs during grilling it by rates of 87% to 91%. 4 PAHs of 21.77 μg/k, B[a]A of 3.09 μg/kg, Chr of 4.15 μg/kg, B[b]F

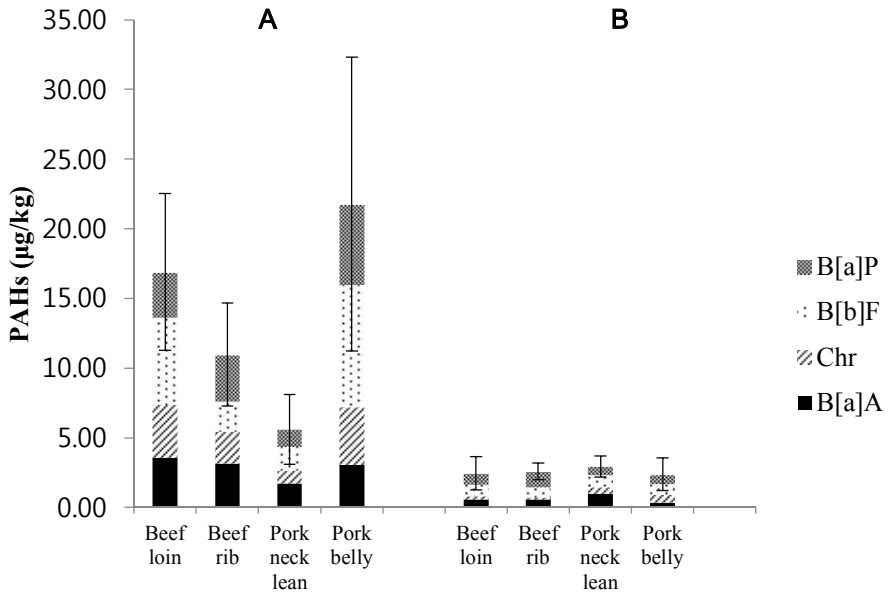


Fig. IV-7. The reduction of occurrence of PAHs in grilled meat with different grilling conditions: (A) control, (B) removing fat drippings. The bars are the standard deviations (n=6).

of 8.77 μg/kg and B[a]P of 5.76 μg/kg were changed to 2.38 μg/kg , 0.40 μg/kg, 0.54 μg/kg, 0.78 μg/kg, and 0.66 μg/kg, respectively. The more fat meat contained, the more reduction rate the meat had by removing the fat dripping.

In a recent research, it was found that wrapping meat samples with aluminum foil and banana leaves reduced the occurrence of the PAHs by 45.7% and 39.9 % during grilling them. This reduction of the occurrence of the PAHs is because wrapping meat also prevents fat from falling on the fire and combusting incompletely (Farhadian et. al., 2011). Furthermore, a grill called as 'Safe Grill' was invented in the USA (US Patent 5,331,886). It is equipped with a filter between meat and fire and it prevents fat from dripping on the fire. However, the ways to remove fat dropping such as using the filter or wrapping meat also prevent grilled meat from having grilled flavor with block the meat from the flavor from the fire. Therefore, removing the fat dripping through the aluminum tube in this study would be one of the best ways to reduce the PAHs during grilling with good test.

Reduction of occurrence of PAHs by removing smoke from flame of charcoals. To reduce the occurrence of PAHs in grilled meat, the smoke from flame of charcoals was removed by the ventilation duct placed between

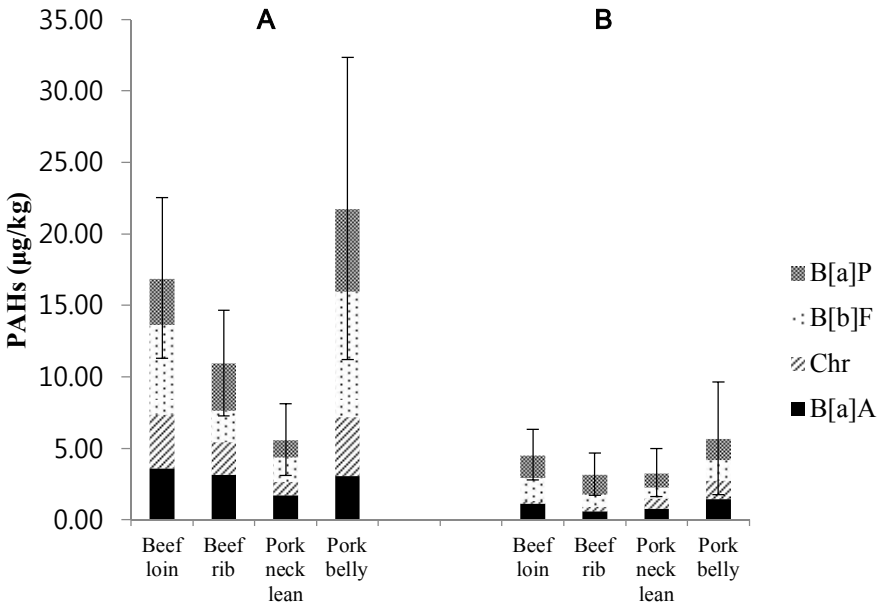


Fig. IV-8. The reduction of occurrence of PAHs in grilled meat with different grilling conditions: (A) control, (B) removing smoke. The bars are the standard deviations (n=6).

the fire and the meat during grilling meat. The levels of PAHs in grilled meats were significantly reduced by removing the smoke (Fig. IV-8). By removing smoke, the mean contents of 4 PAHs, B[a]A, Chr, B[b]F and B[a]P in beef loin were decreased from 16.91 μg/kg , 3.62 μg/kg, 3.80 μg/kg, 6.25 μg/kg and 3.23 μg/kg to 4.56 μg/kg, 1.17 μg/kg, 0.20 μg/kg, 1.60 μg/kg, and 1.58 μg/kg, respectively. PAHs were approximately reduced by from 51 % to 95 %. Meanwhile, the formation of 4 PAHs, B[a]A, Chr, B[b]F and B[a]P in beef rib were reduced from 10.98 μg/kg, 3.21 μg/kg, 2.27 μg/kg, 2.19 μg/kg and 3.30 μg/kg to 3.20 μg/kg, 0.64 μg/kg, 0.32 μg/kg, 0.84 μg/kg, and 1.40 μg/kg, respectively. The reduction rates of PAHs by removing smoke in beef rib were from 58% to 86%. Regarding to pork meat, 4 PAHs, B[a]A, Chr, B[b]F and B[a]P in neck lean were reduced from 5.60 μg/kg, 1.75 μg/kg, 0.94 μg/kg, 1.71 μg/kg and 1.21 μg/kg to 3.31 μg/kg, 0.84 μg/kg, 0.68 μg/kg, 0.78 μg/kg, and 1.03 μg/kg by removing smoke during grilling meats. The reduction rates were ranged from 15% to 54%. And in pork belly, removing smoke reduced the formation of PAHs during grilling it by rates of 52% to 84%. 4 PAHs of 21.77 μg/k, B[a]A of 3.09 μg/kg, Chr of 4.15 μg/kg, B[b]F of 8.77 μg/kg and B[a]P of 5.76 μg/kg were changed to 5.69 μg/kg, 1.48 μg/kg, 1.31 μg/kg, 1.42 μg/kg, and 1.48 μg/kg, respectively.

Viegas Novo and his colleagues recently determined the contents of

PAHs in grilled salmon. They used the coconut charcoal to grille the salmon samples, and they figured out that the levels of PAHs in grilled salmon were lower because the coconut charcoal absorbed the fat from the salmon without flame and smoke. This study also shows that removing smoke is one of the best ways to reduce the occurrence of PAHs in grilling meat.

IV-4. Conclusion

PAHs are one of unintentionally occurring carcinogenic and genotoxic hazards in food. And people have been concerning about the risk of them in food. A number of countries have been monitoring PAHs in food and setting maximum limits in some food groups. And they are also trying to reduce the exposure to PAHs as 'ALARA principle'. Grilled and barbequed meat is one of the most consuming foods and contributing foods regarding to exposure to PAHs via food intake. Some practices of reduction have been introduced in recent studies to reduce the formation of PAHs in food (Bansal & Kim, 2015). Cooking in a low temperature and avoiding the direct contact of high temperature flame decreased the PAHs level in food (Lijinsky et. al., 1964; Chen et. al., 1997; Farhadian et. al., 2010; Farhadian et. al., 2011; Chen et. al., 1997). However, there were still questions about the reason why PAHs were occurred in grilling meat and what were the main sources of this PAHs production. We determined the changes of levels of PAHs during grilling meats by removing meat dripping and smoke from the charcoal. One of the biggest factors contaminating PAHs in grilled meat was the carry-over from the charcoal to meat. In the beginning of grilling meat, charcoal combusted

incompletely and high level of PAHs were occurred. But the levels of PAHs decreased after 12 minutes from starting flame when charcoal started to combust completely. Therefore, it is one way to reduce the occurrence of PAHs to start grilling the meat after charcoal combusts completely.

Another factor forming PAHs in meat during grilling is fat content of the meat. Pork and beef containing different amount of fat were grilled after flame combusted completely and analyzed to compare the levels of PAHs. Pork belly and beef loin with higher fat contents had higher levels of PAHs rather than pork neck lean and beef ribs. It had better select meats with less fat contents to reduce exposure to PAHs by consuming grilled meats.

Smoke from charcoal and meat dripping are also important factors producing PAHs during grilling meats. Smoke carries the PAHs formed from charcoal to the meat. And meat drippings are incompletely combusted on the charcoal when they directly fall on the charcoal. PAHs occurred from the incomplete combustion are deposited on the meat through the smoke. 4 PAHs were decreased by 48 % to 89 % with preventing meat dripping from directly falling on the charcoal and by 41 % to 74 % with ventilating smoke from the charcoal. By the way, if only smoke from charcoal effects on the occurrence of PAHs after charcoal is combusted completely, PAHs should be higher than this when just removing meat dripping because the smoke still

occurred. Therefore, smoke from the incomplete combustion of the meat dripping is more important factor rather than just smoke from charcoal when the charcoal is completely combusted.

In conclusion, the PAHs are formed during grilling meats by several factors such as smoke from charcoal, the fat of meats and meat dripping. Especially, he most important factor is the incomplete combustion of meat dripping. Therefore, to reduce the PAHs contamination in grilled meats and exposure to PAHs by consuming them, it need to prevent meat dripping from falling on the charcoal directly.

References

Abd El-Aty AM, Choi JH, Rahman MM, Kim SW, Tosun A, Shim JH., 2014, Residues and contaminants in tea and tea infusions: a review. Food Additives & Contaminants: Part A. 31(11), 1794-1804.

Adisa A, Jimenez A, Woodham C, Anthony K, Nguyen T, Saleh MA., 2015, Determination of polycyclic aromatic hydrocarbons in dry tea. Journal of Environmental Science and Health, Part B. 50, 552-559.

Alomirah H, Al-Zenki S, Husain A, Sawaya W, Ahmed N, Gevao B, Kannan K., 2010, Benzo(a)pyrene and total polycyclic aromatic hydrocarbons (PAHs) levels in vegetable oils and fats do not reflect the occurrence of the eight genotoxic PAHs. Food Additives and Contaminants. 27(6), 869-878.

AOAC (Association of official analytical chemists). 2000. Official methods of analytical of AOAC international. (17th ed.). Maryland: AOAC

international, (Chapter 33).

AOAC (Association of official analytical chemists). 2000). Official methods of analytical of AOAC international. (17th ed.). Maryland: AOAC international, (Chapter 39).

Balenovic J, Petrovic I, Perkovac M., 1995, Determination of Polycyclic Aromatic Hydrocarbons in Vegetable Oils. pp. 275-281. In: The European Conference on Food Chemistry. Vienna, Austria. Euro Food Chem. VIII

Banerjee H, Ganguly P, Roy S, Banerjee D, Paramasivam M, Banerjee T, Sharma KK., 2010, Persistence and safety risk assessment of propineb in Indian tea. Environ Monit Assess. 170, 311-314.

Bartle, K. D. 1991. Analysis and occurrence of PAH in food. In C. S. Creaser, & R. Purchase (Eds.), *Food contaminants: Sources and surveillance*, Cambridge: Royal Society of Chemistry. 41-60.

Basel, R. M. 1994. Grill apparatus for reducing carcinogens in grilled goods.

US Patent 5,331,886.

Benford, D., Bolger, P.M., Carthew, P., Coulet, M., DiNovi, M., Leblanc, J.C., Renwick, A.G., Setzer, W., Schlatter, J., Smith, B., Slob, W., Williams, G., & Wildemann, T., 2010a, Application of the Margin of Exposure(MOE) approach to substances in food that genotoxic and carcinogenic. Food and chemical Toxicology, 48, S2-S24.

Benford D, DiNovi M, Setzer RW., 2010b, Application of the margin-of-exposure (MoE) approach to substances in food that are genotoxic and carcinogenic e.g.: Benzo(a)pyrene and polycyclic aromatic hydrocarbons. Food and Chemical Toxicology. 48, S42-S48.

Boffetta P, Jourenkova N, Gustavsson P., 1997, Cancer risk from occupational and environmental exposure to polycyclic aromatic hydrocarbons. Cancer Causes and Control. 8, 444-472.

Butt MS, Sultan MT., 2009, Green tea: nature's defense aginst malignancies. Critical reviews in Food Science and Nutrition. 49, 463-473.

Camargo, M.C.R., Antoniolli, P.R., Vicente, E., Tfouni, S.A.V. 2011. Polycyclic aromatic hydrocarbons in Brazilian commercial soybean oils and dietary exposure. Food Additives and Contaminants: Part B. 4(2), 152-159.

CDC. Korea Centers for Disease Control and Prevention. 2008. guideline for the evaluation of the fourth Korea national health & nutrition examination survey. Osong (Korea): Korea Centers for Disease Control and Prevention.

CDC. Korea Centers for Disease Control and Prevention. 2009. guideline for the evaluation of the fourth Korea national health & nutrition examination survey. Osong (Korea): Korea Centers for Disease Control and Prevention.

CDC. Korea Centers for Disease Control and Prevention. 2010. guideline for the evaluation of the fourth Korea national health & nutrition examination survey. Osong (Korea): Korea Centers for Disease Control and Prevention

Chen, B. H., & Lin, Y. S. 1997. Formation of PAHs during processing of duck meat. *Journal of Agricultural and Food Chemistry*, 45, 1394–1403.

Chen, B. H., & Chen, Y. C. 2001. Formation of polycyclic aromatic hydrocarbons in the smoke from heated model lipids and food lipids. *Journal of Agricultural and Food Chemistry*, 49, 5238–5243.

Chung, S. Y., Yettella, R. R., Kim, J. S., Kwon, K., Kim, M. C., & Min, D. B. 2011. Effects of grilling and roasting on the levels of polycyclic aromatic hydrocarbons in beef and pork. *Food Chemistry*, 129, 1420–1426.

Codex Alimentarius Commission (CX/FAC05/37/34). 2004. Joint FAO/ WHO food standards programme Codex Committee on food additives and contaminants. Discussion paper on polycyclic aromatic hydrocarbons (PAH) contamination.

Costa, M., Viegas, O., Melo, A., Petisca, C., Pinho, O., & Ferreira, I.M.P.L.V.O. 2009. Heterocyclic aromatic amine formation in barbecued sardines (*Sardina pilchardus*) and Atlantic salmon (*Salmo*

salar). *Journal of Agricultural and Food Chemistry*, 57, 3173–3179.

Duedahl-Olesen, L., Aaslyng, M., Meinert. L., Christensen, T., Jensen, A.H., Binderup, M.-L., 2015 Polycyclic aromatic hydrocarbons (PAH) in Danish barbecued meat, *Food Control*, 57, 169-176.

Eurachem Working Group. 1998. The Fitness for purpose of analytical methods, a laboratory guide to method validation and related topics. Eurachem guide.

European Commission (EC). 2006. Regulation No 1881/2006 of 19th December, 2006, setting maximum levels for certain contaminants in foodstuffs. *Off. J. Eur. Union,* L364, 5–24.

European Union (EU). 2011. Commission Regulation No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs. *Off. J. Eur. Union,* L215, 4–8.

EFSA - European Food Safety Agency. 2008. Scientific opinion of the panel

on contaminants in the food chain on a request from the European Commission on polycyclic aromatic hydrocarbons in Food. *EFSA J.* 724, 1–114.

European Commission. 2005, Commission regulation (EC) No. 208/2005 of 4 february 2005 amending regulation (EC) No. 466/2001 as regards polycyclic aromatic hydrocarbons. Official Journal of the European Union. Brussels: European Commission

European Commission., 2011, Commission regulation (EC) No. 835/2011 of 19 august 2011 amending regulation (EC) No. 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs. Official Journal of the European Union. Brussels: European Commission

European Food Safety Authority (EFSA)., 2008, Polycyclic aromatic hydrocarbons in food. Scientific opinion of the panel on contaminants in the food chain. The EFSA Journal. 724, 1-114.

Farhadian, A., Jinap, S., Faridah, A., & Zaidul, I. S. 2010. Determination of

polycyclic aromatic hydrocarbons in grilled meat. *Food Control*, 21, 606–610.

Farhadian, A., Jinap, S., Hanifah, H.N., & Zaidul, I. S. 2011. Effects of meat preheating and wrapping on the levels of polycyclic aromatic hydrocarbons in charcoal-grilled meat. *Food Chemistry,* 124, 141–146.

Food Standards Australia New Zealand (FSANZ). 2004. Survey of polycyclic aromatic hydrocarbons (PAH) in Australian Foods – Dietary exposure assessment and risk characterization http://www.foodstandards.gov.au/science/surveillance/documents/PAH%20Survey%20for%20website.pdf

Gertz C, Kogelheide H., 1994, Investigation and legal evaluation of polycyclic aromatic hydrocarbon in vegetable fats and oils. Fat Sci Technol. 96, 175–180.

Global Evnironment Monitoring System-Food contamination Monitoring and Assessment Program (GEMS/Food)-EURO, 1995, GEMS/Food-EURO Second Workshop on Reliable evaluation of low level

contamination of food. May 26-27, Kulmbach, Germnay

Hassan, G. M., Magda, R. A., & Awad, A. A. 2010. Nutritional, biochemical and cytogenotoxicity studies on wasted fat released from chicken during grilling process. *Food Chemical Toxicology*, 48, 2675-2681.

International Agency for Research on Cancer (IARC), 2010, Monographs on the evaluation of carcinogenic risk to humans, overall evaluations of carcinogenicity. IARC Monogr. Eval. Carcinogen. Risks Hum Suppl. 92, 33-814.

Jägerstad, M. J., & Skog, K., 2005. Review genotoxicity of heat-processed foods. *Mutation Research*, 574, 156-172.

Jiang D, Xin C, Li W, Chen J, Li F, Chu Z, Xiao P, Shao L., 2015, Quantitative analysis and heatlh risk assessment of polycyclic aromatic hydrocarbons in edible oils marketed in Shandong of China. Food and Chemical Toxicology. 83, 61-67.

Jira W, Ziegenhals K, Speer K., 2008, Gas chromatography-mass

spectrometry (GC-MS) method for the determination of 16 European priority polycyclic aromatic hydrocarbons in smoked meat products and edible oils. Food Additives and Contaminants. 25(6), 704-713.

Join FAO/WHO Expert Committee on Food Additives (JECFA), 2005, Summary and conclusions of the sixty-fourth meeting of the Joint FAO/WHO expert Committee on Food Additives, WHO Food Additives Series No. 30. Geneva: World Health Organization (WHO)

Kamangar F, Schantz MM, Abent CC, Fagundes RB, Dawsey SM., 2008, High levels of carcinogenic polycyclic aromatic hydrocarbons in mate drinks. Cancer Epidemiology, Biomarkers and Prevention. 17(5), 1262-1268.

Kao, T.H., Chen, S., Huang, C.W., Chen, C.J., & Chen, B.H., 2014. Occurrence and exposure to polycyclic aromatic hydrocarbons in kindling-free-charcoal grilled meat products in Taiwan. *Food and Chemical Toxicology*, 71, 149-158.

Knize, M. G., Salmon, C. P., Pais, P., & Felton, J. S. 1999. Food heating and the formation of heterocyclic aromatic amine and PAH mutagens/carcinogens. In L. S. Jackson, M. G. Knize, & J. N. Morgan (Eds.), *Impact of processing on food safety*, New York: Kluwer Academic, 179-193.

Korean Ministry of Food and Drug Safety (MFDS). 2013. Korean Food Code. Osong (Korea): Ministry Food and Drug Safety. https://www.mfds.go.kr/eng/brd/m_15/view.do?seq=69982&srchFr=&srchTo=&srchWord=&srchTp=&itm_seq_1=0&itm_seq_2=0&multi_itm_seq=0&company_cd=&company_nm=&page=3

Krajian H, Odeh A., 2013, Polycyclic aromatic hydrocarbons in medicinal plants from Syria. Toxicology and Environmental Chemistry. 95(6), 942-953.

Kweon, S., Kim, Y., Jang, M., Kim, Y., Kim, K., Choi, S., Chun, C., Khang, Y., Oh, K. 2014. Data resource profile: the Korea National Health and

Nutrition Examination Survey (KNHANES). International Journal of Epidemiology. 43, 69-77.

Lagerqvist A, Håkansson D, Frank H, Seidel A, Jenssen D., 2011, Structural requirements for mutation formation from polycyclic aromatic hydrocarbon dihydroldiol epoxides in their interaction with food chemopreventive compounds. Food and Chemical Toxicology. 49, 879-886.

Larsson, B. K., Sahlberg, G. P., Eriksson, A. T., & Busk, L. A., 1983. Polycyclic Aromatic Hydrocarbons in Grilled Food. *Journal of Agricultural and Food Chemistry,* 31, 867-873.

Lee JG, Kim SY, Moon JS, Kim SH, Kang DH, Yoon HJ., 2016a, Effects of grilling procedures on levels of polycyclic aromatic hydrocarbons in grilled meats. Food chemistry. 199, 632-638.

Lee, J., Lim, T., Kim, S., Kang, D., Yoon, H. 2018. Determination and risk characterization of polycyclic aromatic hydrocarbons of tea by using the

Marin of Exposre (MOE) approach. Food Science and Biotechnology. 27(6), 1843-1856.

Lee JG, Park SK, Yoon HJ, Kang DH, Kim MH., 2016b, Exposure assessment and risk characterisation of ethyl carbamate from Korean traditional fermented rice wine, Takju and Yakju. Food Additives and Contaminants: Part A. 33(2), 207-214.

Lijinsky, W., & Shubik, P. 1964. Benzo[a]pyrene and other polynuclear hydrocarbons in charcoal-broiled meat. *Science*, 145, 53-55.

Lijinsky, W., & Ross, A. E. 1967. Production of Carcinogenic Polynuclear Hydrocarbons in the Cooking of Food. *Food and Cosmetics Toxicology,* 5, 343-347.

Lijinsky W., 1991, The formation and occurrence of polynuclear aromatic hydrocarbons associated with food. Mutation Research. 259, 251-261.

Lin D, Tu Y, Zhu L., 2005, Concentration and health risk of polycyclic aromatic hydrocarbons in tea. Food and Chemical Toxicology. 43, 41-48.

Lin D, Zhu L., 2006, Factors affecting transfer of polycyclic aromatic hydrocarbons from Mate tea to tea infusion. Journal of Agricultural and Food Chemistry. 54, 4350-4354.

Londoño VAG, Reynoso M, Resnik S., 2014, Polycyclic aromatic hydrocarbons (PAHs) in yerba mate (Ilex paraguariensis) from the Argentinean market. Food and Additives Contaminants: Part B. 7(4), 247-253.

Longwell JP., 1982, The formation of polycyclic aromatic hydrocarbons by combustion. Sumposium (international) on combustion. 19(1), 1339-1350.

Magnusson B, Qrnemark U., 2014, Eurachem guide: The Fitness for purpose of analytical methods, a laboratory guide to method validation and related topics. 2nd ed. Eurachem Working Group

Martí-Cid R, Llobet JM, Castell V, Domingo JL., 2008, Evolution of the dietary exposure to polycyclic aromatic hydrocarbons in Catalonia, Spain. Food and Chemical Toxicology. 46, 3163-3171.

MFDS – the Ministry of Food and Drug Safety (Republic of Korea), Food Code. 2014.

Ministry of Food and Drug Safety (MFDS), 2016, Food Code. MFDS, Seoul, South Korea

Moret, S., Dudine, A., Conte, L. S. 2000. Processing effects on the polycyclic aromatic hydrocarbon content of grapeseed oil. J Am Oil Chem Soc. 77, 1289–1292.

Moret, S., Purcaro, G., Conte, L. S. 2005. Polycyclic aromatic hydrocarbons in vegetable oils from canned foods. Eur J Lipid Sci Technol. 107(7–8), 488–496.

Nisbet ICT, Lagoy PK., 1992, Toxic equivalency factors (TEFs) for polycyclic aromatic hydrocarbons (PAHs). Regulatory Toxicology and

Pharmacology. 16, 290-300.

O'brien, J., Renwick, A. G., Constable, A., Dybing, E., Műller, D. J. G., Schlatter, J., Slob, W., Tueting, W., van Benthem, V., Williams, G. M., Wolfreys, A. 2006. Approaches to the risk assessment of genotoxic carcinogens in food: A critical appraisal. Food and Chemical Toxicology. 44(10), 1613-1635.

Olatunji, O.S., Fatoki, O. S., Opeolu, B. O., & Ximba, B. J. 2014. Determination of polycyclic aromatic hydrocarbons [PAHs] in processed meat products using gas chromatography - flame ionization detector. *Food Chemistry,*156, 296-300.

Özcan M, Arslan D, Ünver A., 2005, Effect of drying methods on the mineral content of basil (Ocimum basilicum L.). Journal of food engineering. 69, 375-379.

Pincemaille J, Schummer C, Heinen E, Moris G., 2014, Determination of polycyclic aromatic hydrocarbons in smoked and non-smoked black teas

and tea infusions. Food Chemistry. 145, 807-813.

Plaza-Bolaños P, Frenich AG, Vidal JLM., 2010, Polycyclic aromatic hydrocarbons in food and beverages. Anlytical methods and trends. Journal of Chromatography A. 1217, 6303-6326.

Ramesh A, Walker SA, Hood DB, Guillén MD, Schneider K, Weyand EH., 2004, Bioavalilability and risk assessment of orally ingested polycyclic aromatic hydrocarbons. International Journal of Toxicology. 23, 301-333.

Rey-Salgueiro, L., Martinez-Carballo, E., Garcia-Falcon, M. S., & Simal-Gandara, J. 2008. Effects of a chemical company fire on the occurrence of polycyclicaromatic hydrocarbons in plant foods. *Food Chemistry*, 108, 347–353.

Rey-Salgueiro, L., Garcia-Falcon, M. S., Martinez-Carballo, E., & Simal-Gandara, J. 2008. Effects of toasting procedures on the levels of polycyclic aromatic hydrocarbons in toasted bread. *Food Chemistry*, 108, 607–615.

Richter H, Howard JB., 2000, Formation of polycyclic aromatic hydrocarbons and their growth to soot-a review of chemical reaction pathways. Progress in Energy and Combustion Science. 26, 565-608.

Rose, M., Holland, J., Dowding, A., Petch, S., White, S, Fernandes, A., & Mortimer, D. 2015. Investigation into the formation of PAHs in foods prepared in the home to determine the effects of frying, grilling, barbecuing, toasting and roasting. *Food and Chemical Toxicology,* 78, 1–9.

RDA - Rural Development Administration. 2011. *8th Revision food composition table* (pp. 246-258), Suwon: Rural Development Administration.

Saito E., Tanaka, N., Miyazaki, A., & Tsuzaki, M. 2014. Concentration and particle size distribution of polycyclic aromatic hydrocarbons formed by thermal cooking. *Food Chemistry*, 153, 285-291.

Schulz C, Fritz H, Ruthenschrőr A., 2015, Occurrence of 15+1 EU priority

polycyclic aromatic hydrocarbons (PAH) in various types of tea (Camellia sinensis) and herbal infusions. Food Additives and Contaminants: Part A. 31(10), 1723-1735.

Simko, P., Khunova, V., Simon, P., Hruba, M. 1995. Kinetics of sunflower oil contamination with polycyclic aromatic hydrocarbons from contaminated recycled low density polyethylene film. International journal food science and technology. 30, 807-812.

Singh L, Varshney JG, Agarwal T., 2016, Polycyclic aromatic hydrocarbons' formation and occurrence in processed food. Food Chemistry. 199, 768-781.

Speer, K., Steeg, E., Horstmann, P., Ku¨hn, Th., Montag, A. 1990. Determination and distribution of polycyclic aromatic hydrocarbons in native vegetable oils, smoked fish products, mussels and oysters, and bream from the river Elbe. Journal of High Resolution Chromatography. 13, 104–111.

Starvic, B., Klassen, R. 1994. Dietary effects on the uptake of

benzo[a]pyrene. Food and Chemical Toxicology. 32, 727–734.

Sundararajan, N., Ndife, M., Basel, R., & Green, S. 1999. Comparison of sensory properties of hamburgers cooked by conventional and carcinogen reducing safe grill' equipment. *Meat Science*, 51, 289–295.

Szeliga J, Dipple A., 1998, DNA adduct formation by polycyclic aromatic hydrocarbon dihydrodiol epoxides. Chemical Research in Toxicology. 11(1), 1-11.

Taverniers, I., Loose, M. D., & Bockstaele, E. V. 2004. Trends in Quality in the Analytical Laboratory. II. Anlaytical Method Validation and Quality Assureance. *Trends in Analytical Chemistry,* 23(8), 535-552

Teixeira, V. H., Casal, S., Oliveira, M. B. P. P. 2007. PAHs content in sunflower, soybean and virgin olive oils: Evaluation in commercial samples and during refining process. Food Chemistry. 104, 106–112.

Thompson, M., Ellison, S. L. R., & Wood, R. 2002. Harmonized guidelines for single laboratory validation of methods of analysis (IUPAC

Technical Report). *Pure and Applied Chemistry*, 74, 835–855.

US Environmental Protection Agency (EPA), 1983, Guidelines establishing test procedures for the analysis of pollutants. 49,209. Fed Reg, Washington, DC, USA

Vasudha Bansal & Ki-Hyun Kim, 2015. Review of PAH contamination in food products and their health hazards. *Environment International*, 84, 26–38.

Venskutonis PR., 1997, Effect of drying on the volatile constituents of thyme (Thymus vulgaris L.) and sage (Salvia officinalis L.). Food Chemistry. 59(2), 219-227.

Veyrand B, Brosseaud A, Sarcher L, Varlet V, Monteau F, Philippe M, Andre F, Bizec BL., 2007, Innovative method for determination of 19 polycyclic aromatic hydrocarbons in food and oil samples using gas chromatography coupled to tandem mass spectrometry based on an isotope dilution approach. Journal of Chromatography A. 1149, 333-344.

Veyrand, B., Sirot, V., Durand, S., Pollono, C., Marchand, P., Dervilly-Pinel, G., Tard, A., Leblanc, J.C., Bizec, B.L., 2013. Human dietary exposure to polycyclic aromatic hydrocarbons: results of the second French total diet study. Environment International. 54, 11-17.

Viegas, O., Novo, P., Pinto, E., Pinho, O., & Ferreira, I.M.P.L.V.O. 2012. Effect of charcoal types and grilling conditions on formation of heterocyclic aromatic amines (HAs) and polycyclic aromatic hydrocarbons (PAHs) in grilled muscle foods. *Food and Chemical Toxicology*, 50, 2128–2134.

Vieira MA, Maraschin MM, Rovaris ÂA, Amboni RDDMC, Pagliosa CM, Xavier JJM, Amante ER., 2010, Occurrence of polycyclic aromatic hydrocarbons throughout the processing stages of erva-mate (Ilex paraguariensis). Food Additives and Contmainants. 27(6), 776-782.

Wang ZM, Zhou B, Wang YS, Gong QY, Wang QM, Yan JJ, Gao W, Wang LS., 2011, Black and Green tea consumption and the risk of coronary artery desease: a meta-analysis. Am J Clin Nutr. 93, 506-515.

Wenzl, T., Simon, R., Anklam, E., & Kleiner, J. 2006. Analytical methods for polycyclic aromatic hydrocarbons (PAHs) in food and the environment needed for new food legislation in the European Union. *Trends in Analytical Chemistry,* 25, 716–725.

WHO - World Health Organization. 1998. Selected non-heterocyclicpolycyclic aromatic hydrocarbons (pp. 202). Environmental Health Criteria, p. 202.

WHO - World Health Organization. 2005. Summary and conclusions of the sixty fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives (pp. 47). Rome.

Yoon, E., Park, K., Lee, H., Yang, J., Lee, C. 2007. Estimation of excess cancer risk on time-weighted lifetime average daily intake of PAHs from food ingestion. Human and Ecological Risk Assessment. 13, 669-680.

Yu L, Cao Y, Zhang J, Cui Z, Sun H., 2012, Isotope dilution-GC-MS/MS analysis of 16 polycyclic aromatic hydrocarbons in selected medicinal

herbs used as health food additives. Food Additives and Contaminants: Part A. 29(11), 1800-1809.

Zelinkova Z, Wenzi T., 2015, The occurrence of 16 EPA PAHs in food – a review. Polycyclic Aromatic Compounds. 35, 248-284.

Zhao Z, Zhang L, Cai Y, Chem Y., 2014, Distribution of polycyclic aromatic hydrocarbon (PAH) residues in several tissues of edible fishes from the largest freshwater lake in China, Poyang Lake, and associated human health risk assessment. Ecotoxicology and Environmental Safety. 104, 323-331.

Ziegenhals K, Jira W, Speer K., 2008, Polycyclic aromatic hydrocarbons (PAH) in various types of tea. Eur Food Res Technol. 228, 83-91.

국문 초록

식용유지 및 다류 중 다환방향족탄화수소의 위해성 평가 및 숯불구이 중 저감화

이 준 구
농생명공학부
식품생명공학전공
서울대학교 대학원

다환방향족탄화수소는 발암물질이면서 유전독성을 가지고 있으며, 식품가열공정 중 비의도적으로 발생한다. 식품 중 다환방향족탄화수소가 빈번히 발생하는 발생하는 식품으로 식용유지류가 있다. 식용유지 중 다환방향족탄화수소의 오염도를 조사하고 그 위해성을 평가한 연구들이 수행되어져 왔다. 그러나 대부분은 독성등가치를 이용하여 다환방향족탄화수소의 위해성을 평가한 것으로서 노출안전역(MOE)을 이용하여 위해성을 평가한 연구는 많지 않다. 또한 다류는 맛과 건강으로 국내에서 많이 소비되는 음료로서, 향을 풍부하게 하고 차 속의 효소를 파괴하기 위해 가열공정을 거치고 된다. 따라서 이런 가열공정 중

다환방향족탄화수소가 생성될 수 있다. 식용유지와 다류에서의 다환방향족탄화수소의 오염도와 위해성을 평가하기 위해 303 개의 식용유지와 468 개의 다류를 수거하여 Benz(a)anthracene (BaA), Chrysene (CHR), Benzo(b)fluoranthene (BbF) and Benzo(a)pyrene (BaP)를 분석하였다. 4 종의 다환방향족탄화수소를 분석하기 위해 가스크로마토그래피-질량분석기를 이용하였다. 식용유지에서는 불검출~12.91 ng g^{-1} 의 오염도를 보였으며, 다류에서는 불검출~4.63 ng g^{-1} 의 오염도를 보였다. 식용유지는 들기름과 참기름에서 다환방향족탄화수소가 상대적으로 높게 검출되었으며, 다류에서는 마테차와 둥굴레차가 상대적으로 높은 오염도를 보였다. 오염도와 식용유지 및 다류의 섭취량을 고려하여 노출량을 평가하고 노출안전역을 구한 결과, 10,000 이상으로 평가되어 국내 식용유지와 다류의 섭취에 다른 다환방향족탄화수소의 위해도는 우려한 수준이 아닌 것으로 나타났다. 특히 전통적인 위해평가 방법인 독성등가치를 이용한 결과에 비해 다환방향족탄화수소의 복합체를 이용한 독성평가 결과를 활용한 결과에 비해 과대평가되는 것이 확인되었다. 따라서 과거 수행되었던

다환방향족탄화수소의 위해평가 연구들은 최근 제안된 복합체의 독성평가결과를 활용하여 재평가될 필요가 있다. 또한 다환방향족탄화수소는 바비큐 등 숯불구이를 통해 많이 생성되는 것으로 알려져 있다. 따라서 이런 숯불구이 방법에 따른 다환방향족탄화수소의 생성량에 대한 영향을 조사하였다. 숯불구이 중 다환방향족탄화수소를 분석하기 위해 기체크로마토그래피-질량분석기를 이용하여 동시분석 하였다. 숯불이 안정적으로 연소될 경우에 다환방향족탄화수소의 생성량이 가장 적었으며, 특히 숯불구이 중 고기에서 육즙이 숯불에서 직접 떨어지지 않도록 하고, 숯에서 발생하는 연기를 제거하여 고기에 닿지 않도록 할 경우에 다환방향족탄화수소의 생성량이 줄어들었다. 소고기와 돼지고기 숯불구이 시 육즙이 숯불에 직접 떨어지지 않도록 할 때 다환방향족탄화수소 4 종의 합은 48~89% 저감화 되었으며, 연기를 제거할 경우에는 41~74% 저감화되었다. 따라서 숯불구이 중 다환방향족탄화수소 생성의 주요 원인은 숯불구이 중 고기에서 떨어지는 지방들이 숯불에 직접 닿아 불완전 연소하고

이때 발생하는 다환방향족탄화수소가 고기에 이행되어서 생성되는 것으로 확인되었다.

주제어: 식품안전, 식품가공, 식품 제조가공 중 발생 오염물질, 다환방향족탄화수소, 위해평가, 시험법 개발, 식용유지, 다류, 숯불구이, 다환방향족탄화수소 저감화

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