An Analytical Method for Polycyclic Aromatic Hydrocarbons and their Derivatives in Fish Oil Derived from Grilled Fish.

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Introduction

Polycyclic aromatic hydrocarbons (PAHs), some of which are carcinogens and/or mutagens [1], can be unintentionally produced during cooking such as grilling and charcoal-broiling [2]. On the other hand, some halogenated PAHs (XPAHs), which are compounds whose one to several hydrogen atoms are substituted to chlorine or bromine atom of PAHs, are more hazardous than their parent PAHs [3,4]. Given that XPAHs could be generated by the reaction of PAHs with halogens (e.g., chlorine atom) [5], they are likely to be also unintentionally produced during cooking (e.g., grilling and charcoal-broiling with salt as a source of chlorine atom). However, information on the occurrence of XPAHs in foods is limited due to the lack of established analytical methods. Especially, oil in food is suspected to interfere with the analysis of PAHs and XPAHs, which makes it more difficult to develop the analytical method.

In this study, we have developed an analytical method for PAHs and XPAHs in fish oil derived from fish grilled with salt. To achieve this, we have firstly developed the appropriate pretreatment method to remove oil. As candidates to effectively remove oil, we have tested a sulfoxide column, which is used for the analysis of polychlorinated biphenyl (PCB) in transformer oil, and a potassium hydroxide (KOH) silica gel column, which is used for the dioxin analysis.

Materials and methods

Chemicals

Target compounds were 12 PAHs and 38 chlorinated PAHs (ClPAHs), whose analytical standards were purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan) or Sigma-Aldrich (St. Louis, MO, USA), or prepared by organic synthesis. They are listed in Table 1. The solvents for extraction and clean-up (acetone, hexane, and dichloromethane) were obtained from Wako Pure Chemical Industries, Ltd. (residual pesticide analysis grade).

Standard mixture for recovery test

The standard mixture (100 μL), which included each target compound (100 ng/mL), was prepared and added to sardine purified oil (0.5 g). The mixture was diluted by 1 mL of 10% dichloromethane/hexane, then it was pre-treated by the methods described below.

Pretreatment with sulfoxide column

A Sulfoxide column was washed with 20 mL of acetone, 40 mL of hexane, and 10 mL of 10% dichloromethane/hexane, successively. One milliliter of the standard mixture for recovery test was loaded in the column. Target compounds were eluted with 30 mL of 10% dichloromethane/hexane. Its eluted solution was fractionated by 1 mL. Each fractionated solution was concentrated and 2 ng of fluoranthene-d_{10} were spiked.

Table 1: List of target PAHs and XPAHs.

Compound	Abbreviation	Compound	Abbreviation
ClPAHs		BrPAHs	
9-chlorofluorene	9-ClFle	9-bromophenantherene	9-BrPhe
9-cholorophenantherene	9-ClPhe	1-bromoanthracene	1-BrAnt
2-choloroanthracene	2-ClAnt	2-bromoanthracene	2-BrAnt
9-choloroanthracene	9-ClAnt	9-bromoanthracene	9-BrAnt
3,9-dichlorophenantherene	3,9-Cl_2Phe	1,5-dibromoanthracene	1,5-Br_2Ant
1,9-dichlorophenantherene	1,9-Cl_2Phe	9,10-dibromoanthracene	9,10-Br_2Ant
9.10-dichloroanthracene	9,10-Cl_2Ant	2,6-dibromoanthracene	2,6-Br_2Ant
9,10-dichlorophenantherene	9,10-Cl_2Phe	1-bromopyrene	1-BrPyr
3-chlorofluoranthene	3-ClFlu	7-bromobenz[a]anthracene	7-BrBaA
8-chlorofluoranthene	8-ClFlu	7,11-dibromobenz[a]anthracene	7,11-Br_2BaA
1-chloropyrene	1-ClPyr	5,7-dibromobenz[a]anthracene	5,7-Br_2BaA
3,9,10-trichlorophenantherene	3,9,10-Cl_3Phe	4,7-dibromobenz[a]anthracene	4,7-Br_2BaA
1,5,9-trichloroanthracene	1,5,9-Cl_3Ant	PAHs	
3,8-dichlorofluoranthene	3,8-Cl_2Flu	fluorene	Fle
1,3-dichloropyrene	1,3-Cl_2Pyr	phenantherene	Phe
1,6-dichloropyrene	1,6-Cl_2Pyr	anthracene	Ant
1,8-dichloropyrene	1,8-Cl_2Pyr	fluoranthene	Flu
6-chlorochrysene	6-ClChr	pyrene	Pyr
7-chlorobenz[a]anthracene	7-ClBaA	benz[a]anthracene	BaA
1,5,9,10-tetrachloroanthracene	1,5,9,10-Cl_4Ant	chrysene	Chr
trichloropyrene	Cl_3-Pyr	benzo[b]fluoranthene	BbF
6,12-dichlorochrysene	6,12-Cl_2Chr	benz[a]pyrene	BaP
7,12-dichlorobenz[a]anthracene	7,12-Cl_2BaA	indeno[1,2,3-cd]pyrene	IDP
tetrachloropyrene	Cl_4Pyr	benzo[g,h,i]perylene	BghiP
6-chlorobenz[a]pyrene	6-ClBaP	dibenz[a,h]anthracene	DBahA

Pretreatment with KOH silica gel column

A 50 mL of glass column was filled with 12 g of KOH silica gel. The column was washed with 50 mL of hexane. One milliliter of the mixture for recovery test was loaded in the column. The column was eluted with 30 mL of hexane, 240 mL of 10% dichloromethane/hexane. The eluted solution of hexane (30 mL) and 10% dichloromethane/hexane (60 mL) was classified as fraction 1. And the subsequent eluted solutions were split with each 60 mL, which were called as fractions 2, 3 and 4, respectively. Each fraction was concentrated and 2 ng of fluoranthene-d_{10} were spiked.

Sample

To prepare a sample of a grilled fish, a fish was salted 2 g per each, and it was grilled for 11 min in a fish oven. Burnt fish skin was selectively collected, and brayed with anhydrous sodium sulfate. Then, the sample was extracted using a Soxhlet extraction method with 250 mL dichloromethane after spiking 2 ng each of clean-up spikes (isotope labeled phenanthrene [Phe]-$^{13}C_6$, fluoranthene [Flu]-$^{13}C_6$, chrysene [Chr]-$^{13}C_6$, benz[a]pyrene [BaP]-$^{13}C_4$, chloropyrene [ClPyr]-$^{13}C_6$). The extract was concentrated, and pre-treated by the silica gel column, which was preliminary washed with 60 mL of toluene, and adding 100 mL of toluene. And then, each sample was concentrated and spiked with 2 ng of Flu-d_{10}.

Analytical procedure

The concentrations of PAHs and XPAHs in the samples were analyzed by a gas chromatography with high resolution mass spectrometry (HRGC-HRMS) (JMS-700, JEOL, Tokyo, Japan). Gas chromatographic separation was accompanied by a 60-m BPX-DXN fused silica capillary column (0.25 mm i.d., Kanto Chemical Co., Inc, Tokyo, Japan). Two microliter of the aliquot was injected in splitless mode at 280°C. The temperature of the column oven was kept at 130°C for 1 min, and raised with a rate of 5°C/min to 250°C, and then raised with 10°C/min to 320°C. This was held for 18 min. The MS was operated in an electron-impact selected ion monitoring (SIM) at resolution R > 10,000 (10% valley). Peaks were identified by comparison of the retention time of samples to standards if the signal-to-noise (S/N) radio was > 3, and were quantified if target/qualifier ion radio were within 15% of the theoretical values.

Results and discussion

The recovery rates of PAHs and XPAHs in fish oil using the sulfoxide column were determined (data not shown). Eighty five percent of fish oil was eluted with 6 mL of 10% dichloromethane/hexane. These results indicated that PAHs were able to be analyzed from the following fractions. Although PAHs with larger rings were successfully separated from the oil, fluorene (Fle) and Phe were not separated (the recovery rates of Fle and Phe were < 10%, < 20%, respectively). It was suggested that a larger part of these compounds were eluted with the oil. Compared with PAHs, XPAHs were hardly separated from the oil. This could be attributed to higher affinities of XPAHs to the solvent. Consequently, the sulfoxide column was insufficient for the pretreatment for the analysis of PAHs and XPAHs in fish oil.

The recovery rates of PAHs and XPAHs using the KOH silica gel column are shown in Figure 1. The fish oil was not detected in all the fractions. It was considered that the fish oil was degraded and/or absorbed with the KOH silica gel column. XPAHs were recovered in fraction 1, whose recovery rates ranged 60–105%. Parent 3 or 4 rings-PAHs were also recovered in fraction 1, whose recovery rates ranged 60–70%. The other PAHs were recovered in fractions 1 and 2, whose recovery rates ranged 100–140%. In addition, PAHs and XPAHs were not detected in

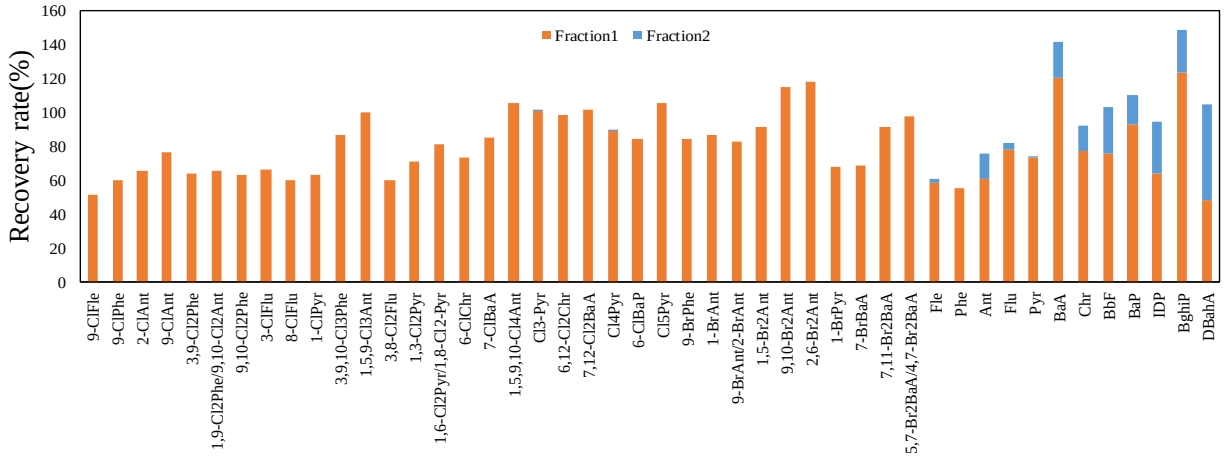

Figure 1: Recovery rates of PAHs and XPAHs using KOH silica gel column.

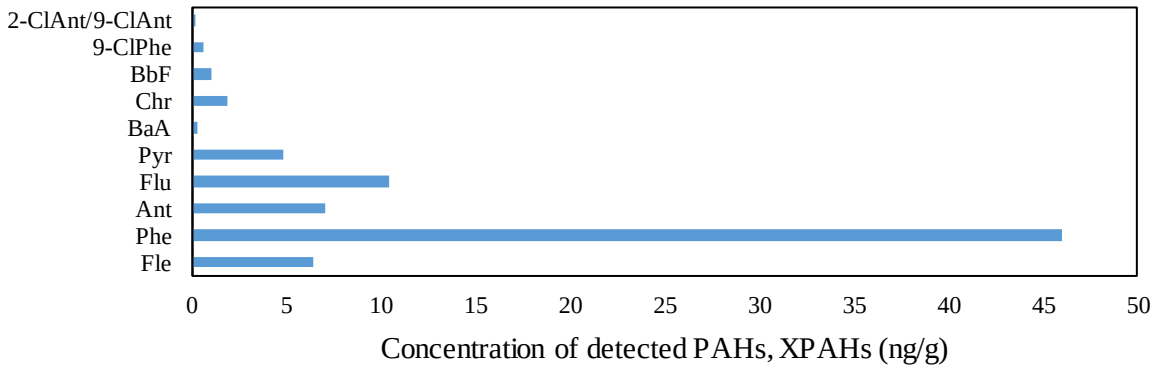

Figure 2: Concentrations of PAHs and XPAHs in fish skin grilled with salt.

fractions 3 and 4. As a result, PAHs and XPAHs were able to be recovered with 30 mL of hexane and 120 mL of 10% dichloromethane/hexane.

Using the developed pretreatment method, the concentrations of PAHs and XPAHs in the fish skin grilled with salt were determined. The results are shown in Figure 2. The recovery rates for the clean-up spikes were 150–170% (Phe-$^{13}C_6$), 130–135% (Flu-$^{13}C_6$), 130–145% (Chr-$^{13}C_6$), 130–145% (ClPyr-$^{13}C_6$) and 35–45% (BaP-$^{13}C_4$). The recovery rates of 5 ring-PAHs including BaP-$^{13}C_4$ were low, whose peaks may be interfered by degradation products derived from fish oil. Phe, Flu, anthracene (Ant), Fle, Pry, Chr, benzo[b]fluoranthene (BbF), and BaA were detected from the skin of burnt fish. Phe was the dominant PAHs. As for XPAHs, 9-ClPhe and 2-ClAnt/9-ClAnt were detected. Compared with the concentrations of XPAHs on the skin of burnt fish, those of PAHs were likely to be high. However, the sample number of this study was limited, more information is required to sure that the addition of salt can be a halogen source of XPAHs during grilling a fish.

Acknowledgements

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