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Reduction of Carcinogenic Polycyclic Aromatic Hydrocarbons in Meat by Sugar-Smoking and Dietary Exposure Assessment in Taiwan

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ABSTRACT: Polycyclic aromatic hydrocarbons (PAHs) represent an important pollutant in foods and/or the environment. This study aimed to determine the PAH contents in sugar-smoked meat by employing a quick, easy, cheap, effective, rugged, safe (QuEChERS) method combined with a GC-MS technique and assess the dietary exposure of PAHs in Taiwan. Results showed that the longer the sugar-smoking duration, the more the total PAH formation. By sugar-smoking for 6 min, the total PAH contents generated in red meat (33.9 ± 3.1−125.5 ± 9.2 ppb) were higher than in poultry meat (19.1 ± 2.0−28.2 ± 1.2 ppb) and seafood (9.1 ± 1.4−31.8 ± 1.8 ppb), with lamb steak containing the largest amount of total PAHs. Most importantly, the highly carcinogenic benzo[*a*]pyrene remained undetected in all of the sugar-smoked meat samples. In addition, the cancer risk due to dietary PAH exposure based on total intake of meat in Taiwan was $<2 \times 10^{-7}$. This outcome demonstrates that sugar-smoking can be adopted to replace the traditional smoking process with wood as smoke source.

KEYWORDS: *PAHs, sugar-smoking, QuEChERS, GC-MS, dietary exposure assessment*

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants that can enter food via contaminated soil, polluted air, and water.[1] More than 100 PAHs have been characterized in nature, of which benzo[*a*]anthracene, cyclopenta[*c,d*]pyrene, chrysene, 5-methylchrysene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*c,d*]pyrene, dibenzo[*a,h*]anthracene, benzo[*g,h,i*]perylene, dibenzo[*a,l*]pyrene, dibenzo[*a,e*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,h*]pyrene have been shown to possess carcinogenic, cytotoxic, and mutagenic activities.[2]

The formation of PAHs in food products during processing is mainly due to pyrolysis of organic components including fat, protein, and carbohydrate at temperatures >200 °C, especially at 500−900 °C.[3,4] More specifically, lipids may drip onto the flame, generating PAHs in the smoke during heating, which in turn adhere to the food surface.[5] In addition, the incomplete combustion of charcoal can induce PAH formation, finding their way onto the food surface.[6,7] Among the various PAHs, the low-molecular-weight ones with two or three aromatic rings were shown to be more labile to formation during grilling of meat.[8] However, those PAHs are classified as probably (group 2A) or possibly (group 2B) carcinogenic to humans and are less toxic as compared to the highly toxic benzo[*a*]pyrene (known as carcinogens in humans, group 1) with five aromatic rings.[9]

Meat consumption in Taiwan among men and women is estimated to be 202.53 and 129.83 g/day, respectively, with smoked meats becoming increasingly popular both at home and in restaurants because of their unique aroma and taste. However, smoked foods often pose an elevated risk to human health due to the presence of high PAH contents.[4,10−14]

Smoking is one of the oldest food preservation methods. An official survey of PAH levels in Swedish smoked meat and fish reported many products contained benzo[*a*]pyrene ranging from 6.6 to 36.9 μg/kg.[14] Among the various products, ham processed by the traditional sauna smoking contained the highest level of benzo[*a*]pyrene, apparently caused by direct exposure to smoke from a flaming log. Additionally, the combustion temperature is particularly critical in affecting PAH formation in food products.[15] For instance, hot-smoked fish was shown to contain a higher level of PAHs than cold-smoked fish.[16] In addition to temperature, the amount of PAHs formed in smoked fish can be dependent upon type of fish, smoking methods, wood variety, smoke composition, and degree of exposure to smoke. For example, a high content of benzo[*a*]pyrene (50 ppb) was observed in fish skin smoked heavily in a traditional kiln, whereas a much lower content (<0.1 ppb) was shown in fish smoked mildly in a wood-containing house.[16] Likewise, benzo[*a*]pyrene (>18 ppb) was generated in direct-smoked belly ham, but could be reduced to <0.3 ppb with an indirect smoking process.[17] Additionally, the wood nature, such as resin content, could favor PAH formation in smoked meat.[13] In a study dealing with the effect of the industrial smoking process on PAH formation, with liquid smoke the PAH contents could be reduced substantially.[18] Nevertheless, a high degree of contamination may occur because of smoldering and friction. There are also differences in PAH contents in different types of meat with similar surface/mass ratio, as evident by a higher level of PAHs in beef hams processed in a traditional smokehouse than in an industrial smokehouse. However, there was no significant difference in PAH contents in pork between those two processing methods.[12]

Sugar-smoking, a popular cooking method involving precooking of meat in a marinade and then exposure to sugar for smoking in Taiwan, has been frequently used as it imparts a characteristic flavor and tenderness to meat products. However, no information is available as to PAH generation in sugar-smoked

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meat products. The objectives of this study were to determine the PAH contents in sugar-smoked poultry meat, red meat, and seafood products by employing a quick, easy, cheap, effective, rugged, safe (QuEChERS) method combined with a GC-MS technique. Meanwhile, the dietary exposure assessment of PAHs in Taiwan was conducted.

MATERIALS AND METHODS

Materials. Sixteen PAH standards, including acenaphthene, acenaphthylene, anthracene, benzo[*a*]anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*g*,*h*,*i*]perylene, chrysene, dibenzo[*a*,*h*]anthracene, fluoranthene, fluorene, indeno-[1,2,3-*c*,*d*]pyrene, naphthalene, phenanthrene, and pyrene, were obtained from Supelco Co. (Bellefonte, PA, USA). The QuEChERS kits used to extract and purify PAHs from meat included a 50 mL Teflon centrifuge tube containing 6 g of magnesium sulfate ($MgSO_4$) and 1.5 g of sodium acetate (CH_3COONa) and a 15 mL Teflon centrifuge tube packed with 1200 mg of $MgSO_4$, 400 mg of primary−secondary amine (PSA), and 400 mg of end-capped C18, both of which were purchased from Agilent Technologies (Palo Alto, CA, USA). The HPLC grade solvents (acetonitrile and acetone) were from Merck (Darmstadt, Germany). Deionized water was made using a Barnstead Easypure II water purification system (Thermo Scientific Co., Waltham, MA, USA). Soy sauce was from Gin-Lan Food Co. (Taoyuan, Taiwan), whereas crystal sugar was from Taiwan Sugar Co. (Tainan, Taiwan).

Meat Samples. Meat samples were purchased from a local butcher shop in New Taipei City, Taiwan. Chicken drumstick, duck drumstick, chicken breast, chicken gizzard, and chicken heart were selected as they represented the most popular poultry commodities in Taiwan. Likewise, red meat including pork chop, beef steak, lamb steak, ham, and pork knuckle were chosen, whereas salmon, shrimp, squid, octopus, and oyster were procured for seafood. Prior to processing, all meat samples were washed with tap water and weighed, followed by dividing each meat commodity into two portions and packaging into two separate plastic bags and storing at −20 °C freezer. Among all of the meat samples, only oyster was purchased before cooking as freezing may cause a texture change. The fat contents in all of the meat samples were determined by an ethyl ether extraction method[19] using a Soxhlet apparatus (HT1043 Extraction Unit, Tecator, Sweden).

Sugar-Smoking. Smoking is commonly used to process meat products in Taiwan to extend shelf life and impart characteristic flavor and tenderness. Unlike the traditional smoking process with wood as smoke source, in this study we used a sugar-smoking process instead. In the beginning, a fixed number of each meat commodity with a total amount of 0.2, 0.8, 1.0, 1.8, 1.8, 1, 1, 0.8, 0.3, 1, 1, 0.2, 1, 0.6, and 0.1 kg for chicken heart, chicken gizzard, chicken breast, chicken drumstick, duck drumstick, pork steak, steak, lamb steak, ham, pork knuckle, salmon, shrimp, octopus, squid, and oyster, respectively, was premarinated in a 3-fold volume (based on sample weight) of juice containing 10% soy sauce and 1% crystal sugar. The premarinating time was selected on the basis of the internal temperature of various meat samples as recommended by the U.S. Department of Agriculture: 30 min and 81 °C for chicken drumstick, 25 min and 82 °C for duck drumstick, 10 min and 90 °C for chicken breast, 10 min and 94 °C for chicken gizzard, and 5 min and 90 °C for chicken heart; 10 min and 82 °C for pork chop, 15 min and 96 °C for beef steak, 10 min and 85 °C for lamb steak, 5 min and 84 °C for ham, and 20 min and 90 °C for pork knuckle; 10 min and 84 °C for salmon, 3 min and 85 °C for shrimp, 15 min and 80 °C for octopus, 10 min and 90 °C for squid, and 5 min and 91 °C for oyster. After premarinating, about 112.5 g of sugar was poured onto the bottom of a smoking pot, with one meat commodity being placed evenly on a metal screen above the pot. Then the pot was covered with a lid with the smoking time being 3 or 6 min. After smoking, all of the meat samples were deboned, vacuum-packaged, and stored at −20 °C prior to PAH analysis.

Extraction and Purification of PAHs. All of the meat samples were removed from the freezer, thawed at 4 °C, and then ground by a mechanical blender (model 890-68, Oster Co., WI, USA) prior to extraction and purification, which was performed according to a method described in a previous study.[20] A portion of 5 g of ground meat sample was taken and placed into a tube and then homogenized in 10 mL of deionized water for 1 min, followed by the addition of 10 mL of acetonitrile and vigorous shaking for 1 min. Then the QuEChERS containing 6 g of $MgSO_4$ and 1.5 g of CH_3COONa was added, after which the tube was shaken for 1 min and centrifuged at 4000 rpm for 5 min. Subsequently, 6 mL of the supernatant was collected and added to a QuEChERS cleanup tube containing 400 mg of PSA, 1200 mg of $MgSO_4$, and 400 mg of C18EC for purification, followed by centrifuging at 4000 rpm for 5 min. The supernatant was collected, and a 1 μL aliquot was taken for injection into GC-MS for PAH analysis.

GC-MS Analysis. Identification and quantification of PAHs were accomplished using a GC-MS method as described previously.[20] An Agilent 30 m HP-5MS column (0.25 mm i.d., 0.25 μm film thickness) connected with a 5 m guard column was installed to extend column life. Samples were injected in splitless mode with helium as carrier gas at a flow rate of 1.0 mL/min. The GC operation conditions were as follows: injector temperature at 290 °C, oven temperature at 70 °C initially, raised to 195 °C at 15 °C/min and maintained for 2.5 min, raised to 240 °C at 15 °C/min and maintained for 17 min, raised to 270 °C at 5 °C/min and to 310 °C at 15 °C/min with a holding time for 10 min; with these conditions a total of 16 PAHs were separated within 40 min. For MS conditions, the interface temperature was 270 °C with an electron multiplier voltage of 70 eV, and detection was performed by selected ion monitoring (SIM) mode according to elution time and m/z of various PAHs. Additionally, each PAH in samples was identified by comparing retention time and mass spectra of unknown peaks with those of authentic standards.

For quantification, 14 concentrations (0.1−120 ng/mL) of each PAH (including naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, and benzo[*b*]-fluoranthene) were prepared, whereas 13 concentrations (0.2−120 ng/mL) of benzo[*a*]anthracene and chrysene, 10 concentrations (1.0−120 ng/mL) of benzo[*k*]fluoranthene and benzo[*a*]pyrene, 11 concentrations (0.5−120 ng/mL) of indeno[1,2,3-*c*,*d*]pyrene and dibenzo[*a*,*h*]anthracene, and 12 concentrations (0.3−120 ng/mL) of benzo[*g*,*h*,*i*]perylene were also prepared. The 16 PAH standard curves were thus obtained by plotting concentration against integrated peak area, and the amount of each PAH was calculated on the basis of its respective calibration curve. The method validation was not carried out as high accuracy and precision have been demonstrated in a previous study.[20]

Risk Assessment of Dietary Exposure of PAHs. The approach used for carcinogenic risk assessment of PAHs in this study is based on the toxic equivalency factors (TEFs), and comparative potency of various PAHs is estimated using benzo[*a*]pyrene as a surrogate.[21] The potency toxicity of benzo[*a*]pyrene is characterized with TEF as 1, which is then applied as a basis for calculation of toxicity equivalent (TEQ) of the other PAHs, including highly carcinogenic dibenzo-[*a*,*h*]anthracene (TEF = 1), benzo[*a*]anthracene, benzo[*b*]-fluoranthene, benzo[*k*]fluoranthene, and indeno[1,2,3-*c*,*d*]pyrene (TEF = 0.1 each); midcarcinogenic (TEF = 0.01) acenaphthylene, anthracene, fluoranthene, chrysene, and benzo[*g*,*h*,*j*]perylene; low-carcinogenic (TEF = 0.001) acenaphthylene, acenaphthene, fluorene, phenanthrene; and noncarcinogenic naphthalene.

The TEQ value for determination of dietary exposure assessment of PAHs was calculated as

$$\mathrm{TEQ}_{\mathrm{B[}a\mathrm{]P}} = \sum_{i=1}^{n} (C_i \times \mathrm{TEF}_i)$$

where $\mathrm{TEQ}_{\mathrm{B[}a\mathrm{]P}}$ is the total TEQ level, converting as BaP using the TEFs of PAHs in food, C_i is the concentration of PAH congener i in food, and TEF_i is the TEF of PAH congener i in food.

To assess cancer risk, the dietary exposure was calculated by prorating the total cumulative intake level over a lifetime to give lifelong average daily intake (LADI, ng/kg BW/day)[22,23] using the formula

$$\mathrm{LADI} = \sum_{j=1}^{n} [(\mathrm{TEQ}_k \times \mathrm{IR}_{kj} \times \mathrm{ED}_j)/(\mathrm{BW}_j \times \mathrm{AT})]$$

where TEQ_k is the total TEQ levels of PAHs in food k (μg TEQ/kg); ED_j is the exposure duration j (year); IR_{kj} is the average intake of food k in exposure duration j (g/day) based on daily food consumption data of a nationwide dietary survey conducted in Taiwan, 2008, provided by the Academia Sinica, Taiwan;[23] BW_j is the average body weight during exposure duration (kg); and AT is the average lifespan for carcinogen (79 years in Taiwan).

Statistical Analysis. Duplicate analyses were conducted for all meat samples, and the mean values were subjected to ANOVA and Duncan's multiple-range test for significant difference ($p < 0.05$) among samples using SAS software (ver. 9.2).[24]

RESULTS AND DISCUSSION

PAHs in Sugar-Smoked Meat Products. Table 1 shows the PAH contents in sugar-smoked poultry products. Among the various PAHs, only naphthalene was detected in chicken drumstick (0.9 ± 0.2 ng/g) and chicken breast (1.5 ± 0.9 ng/g) after 30 and 10 min of premarinating, respectively. However, following sugar-smoking for 3 min, four new PAHs (acenaphthene, fluorene, phenanthrene, and anthracene) were generated in chicken heart, three PAHs (acenaphthylene, fluorene, and phenanthrene) each in chicken drumstick and chicken breast, six PAHs (naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, and fluoranthene) in chicken gizzard, and five PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, and phenanthrene) in duck drumstick. After extensive sugar-smoking for 6 min, several more PAHs were produced including acenaphthylene and pyrene in chicken gizzard and acenaphthene in chicken breast and chicken drumstick, accompanied by a loss of anthracene in chicken heart and phenanthrene in duck drumstick (Table 1), probably caused by degradation or conversion to some other PAH derivatives. Most importantly, benzo[*a*]pyrene remained undetected in sugar-smoked poultry meat, demonstrating a much safer processing method of sugar-smoking when compared to traditional smoking method with wood as smoke source. For total PAHs, a pronounced rise was observed for all five poultry meat commodities after sugar-smoking for 3 or 6 min (Table 1).

The PAH content changes in premarinated and sugar-smoked red meat are shown in Table 2. Likewise, after premarinating, only two PAHs (naphthalene and fluoranthene) were detected in beef steak, two PAHs (naphthalene and fluorene) in pork knuckle and pork chop, and one PAH (naphthalene) in both ham and lamb steak. However, after sugar-smoking for 3 min, some other PAHs were produced: acenaphthylene, acenaphthene, fluorene, and anthracene in beef steak; fluoranthene in pork knuckle; acenaphthene and phenanthrene in ham; fluorene in lamb steak; and acenaphthylene and fluoranthene in pork chop. Likewise, after prolonged sugar-smoking for 6 min, several new PAHs were shown: phenanthrene and pyrene in beef steak; acenaphthene, phenanthrene, anthracene, and pyrene in pork knuckle; fluorene in ham; acenaphthene, phenanthrene, anthracene, fluoranthrene, and pyrene in lamb steak; acenaphthene, phenanthrene, anthracene, and pyrene in pork chop (Table 2). Like poultry meat, no benzo[*a*]pyrene was detected in all five red meat commodities. For total PAHs, a marked increment was found following sugar-smoking over a 6 min period.

A similar tendency was observed in PAH varieties and contents during premarinating and sugar-smoking of seafood products (Table 3). After premarinating, only naphthalene was detected in oyster, octopus, salmon, and shrimp, whereas no PAHs were detected in squid. However, after sugar-smoking for 3 min, seven new PAHs including acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene were generated in oyster only, whereas no PAHs were detected in squid. After extensive sugar-smoking for 6 min, three more PAHs (acenaphthene, fluorene, and phenanthrene) were produced in octopus, as were three PAHs (acenaphthylene, acenaphthene, and fluorene) in salmon, four PAHs (acenaphthylene, acenaphthene, fluorene, and phenanthrene) in shrimp, and three PAHs (acenaphthene, fluorene, and anthracene) in squid (Table 3). Also, no benzo[*a*]pyrene was detected in all five seafood commodities. With the exception of oyster, a significant rise was shown for total PAHs in the other four seafood products over a sugar-smoking period for 6 min.

Comparatively, after sugar-smoking for 6 min, the total PAHs were produced in largest amount (125.5 ± 9.2 ppb) in lamb steak, followed by pork chop (75.4 ± 0.8 ppb), beef steak (42.4 ± 3.0 ppb), pork knuckle (33.9 ± 3.1 ppb), oyster (31.8 ± 1.8 ppb), chicken gizzard (28.2 ± 1.2 ppb), ham (27.1 ± 0.4 ppb), chicken heart (25.7 ± 1.7 ppb), chicken drumstick (24.5 ± 2.3 ppb), chicken breast (24.3 ± 3.4 ppb), salmon (20.9 ± 2.2 ppb), duck drumstick (19.1 ± 2.0 ppb), squid (10.8 ± 1.2 ppb), octopus (9.2 ± 0.6 ppb), and shrimp (9.1 ± 1.4 ppb). More specifically, of the eight PAHs detected in chicken gizzard, beef steak, pork chop, and oyster, only fluoranthene was classified as carcinogenic by the U.S. Environmental Protection Agency (EPA),[25] implying the PAHs present in most meat commodities in this study did not show carcinogenicity. As fluoranthene was present in minor amounts ranging from 0.2 to 1.7 ppb, this should not pose any health risk to human. With the exception of benzo[*a*]pyrene, the other PAH levels were lower than those in meats treated with traditional wood smoking.[12]

Among the various PAHs, the noncarcinogenic naphthalene was the most susceptible to formation during premarinating and subsequent sugar-smoking. The formation mechanism of naphthalene has been well elucidated previously,[10] demonstrating that lipid oxidation and degradation products such as cyclohexane or hydroperoxide may undergo further oxidation or cyclization to form naphthalene or naphthalene-like compounds during heating of model lipids or food lipids. More specifically, the formation of cyclic compounds can also be due to the interaction between oleic acid and linoleic acid through Diels–Alder reaction during heating, which in turn undergoes further polymerization to form PAHs or PAH derivatives.[10] Thus, the fat content in meat products used in our study should play a vital role in PAH formation. In addition to fat content, the fatty acid composition is also important for PAH formation. However, with the exception of lamb steak (12% fat), the other 14 meat samples contained only a low amount of fat (0.04–7.06%), which should explain why lamb steak contained a larger amount of total PAHs than the other meat commodities. Theoretically, the highly toxic benzo[*a*]-pyrene containing five aromatic rings should be more difficult to generate than those PAHs with two to four rings. Therefore, only under drastic heating conditions more lipid oxidation or degradation products were produced, and then the formation of benzo[*a*]pyrene could be possible.[10] As both the premarinating and sugar-smoking conditions are quite mild in this study, the formation of carcinogenic benzo[*a*]pyrene should be difficult.

According to previous literature reports, a high amount of benzo[*a*]pyrene could be generated during the traditional smoking process with wood as the major smoke source because

Table 1. Concentrations of PAHs (Nanograms per Gram)[a] in Premarinated and Sugar-Smoked Poultry Meat

	chicken heart			chicken drumstick			chicken gizzard			chicken breast			duck drumstick		
PAH	premarin 5 min[b]	3 min[c]	6 min[c]	premarin 30 min	3 min	6 min	premarin 10 min	3 min	6 min	premarin 10 min	3 min	6 min	premarin 25 min	3 min	6 min
naphthalene	nd[d]	18.7 ± 0.8Aa	19.6 ± 1.3Aa	0.9 ± 0.2	8.8 ± 0.8Ba	17.8 ± 2.0Aa	nd	9.0 ± 2.0Ba	17.0 ± 0.4Aa	1.5 ± 0.9	12.3 ± 1.7Aa	16.7 ± 2.3Aa	nd	3.0 ± 0.3Ba	11.3 ± 1.2Aa
acenaphthylene	nd	nd	nd	nd	1.8 ± 0.0Bb	2.8 ± 0.0Ab	nd	nd	2.5 ± 0.2c	nd	1.8 ± 0.0Ab	2.4 ± 0.6Abc	nd	1.5 ± 0.1Bb	3.6 ± 0.1Ab
acenaphthene	nd	1.0 ± 0.0Bc	3.8 ± 0.1Ab	nd	nd	2.4 ± 0.1bc	nd	1.8 ± 0.2Bbc	3.7 ± 0.2Ab	nd	nd	3.2 ± 0.2b	nd	0.9 ± 0.1Bc	2.7 ± 0.4Ac
fluorene	nd	2.1 ± 0.1Ab	2.1 ± 0.3Ac	nd	0.6 ± 0.0Bb	1.3 ± 0.1Abc	nd	2.6 ± 0.4Ab	2.9 ± 0.4Ac	nd	1.3 ± 0.0Ab	1.5 ± 0.2Acd	nd	0.6 ± 0.1Bcd	1.5 ± 0.3Ac
phenanthrene	nd	0.5 ± 0.0Ac	0.3 ± 0.1Ac	nd	0.3 ± 0.0Ac	0.3 ± 0.1Ac	nd	1.3 ± 0.1Abc	1.4 ± 0.1Ad	nd	0.7 ± 0.4Ab	0.5 ± 0.2Ad	nd	0.2 ± 0.0d	nd
anthracene	nd	0.4 ± 0.0c	nd	nd	nd	nd	nd	0.5 ± 0.0Ac	0.4 ± 0.0Ae	nd	nd	nd	nd	nd	nd
fluoranthene	nd	nd	nd	nd	nd	nd	nd	0.3 ± 0.0Ac	0.4 ± 0.0Ae	nd	nd	nd	nd	nd	nd
pyrene	nd	nd	nd	nd	nd	nd	nd	nd	0.1 ± 0.0e	nd	nd	nd	nd	nd	nd
benzo[*a*]anthracene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
chrysene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
benzo[*b*]fluoranthene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
benzo[*k*]fluoranthene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
benzo[*a*]pyrene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
indeno[1,2,3-*c,d*]pyrene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
dibenzo[*a,h*]anthracene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
benzo[*g,h,i*]perylene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
total	nd	22.7 ± 0.9B	25.7 ± 1.7A	0.9 ± 0.2	11.5 ± 0.8B	24.5 ± 2.3A	nd	15.4 ± 3.0B	28.2 ± 1.2A	1.5 ± 0.9	16.1 ± 2.1B	24.3 ± 3.4A	nd	6.1 ± 0.6B	19.1 ± 2.0A

[a]Mean of duplicate analyses ± standard deviation. Means of the same kind of poultry in the same row with different letters (A, B) are significantly different ($p < 0.05$). Means bearing different letters (a–e) in the same column are significantly different ($p < 0.05$). [b]Pre-marinating time. [c]Sugar-smoking time. [d]nd, not detected, below limit of quantification (QL)

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Table 2. Concentrations of PAHs (Nanograms per Gram)[a] in Premarinated and Sugar-Smoked Red Meat

	beef steak			pork knuckle			ham			lamb steak			pork chop		
PAH	premarin 15 min[b]	3 min[c]	6 min[c]	premarin 20 min	3 min	6 min	premarin 5 min	3 min	6 min	premarin 10 min	3 min	6 min	premarin 10 min	3 min	6 min
naphthalene	8.4 ± 0.1a	29.1 ± 2.2Aa	28.9 ± 1.3Aa	1.6 ± 0.5a	18.7 ± 0.2Ba	27.8 ± 2.4Aa	4.8 ± 1.6	10.9 ± 0.3Ba	20.0 ± 0.3Aa	1.3 ± 0.1	32.4 ± 2.0Ba	101.2 ± 7.1Aa	11.5 ± 0.9a	40.5 ± 0.8Ba	62.7 ± 0.1Aa
acenaphthylene	nd[d]	2.0 ± 0.2Ab	2.0 ± 0.1Acd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.2 ± 0.0Ab	2.1 ± 0.2Acd
acenaphthene	nd	1.2 ± 0.4Bb	3.1 ± 0.6Acd	nd	nd	1.2 ± 0.2c	nd	1.5 ± 0.1Bb	3.7 ± 0.1Ab	nd	nd	3.6 ± 0.4c	nd	nd	2.4 ± 0.2c
fluorene	nd	0.9 ± 0.1Bb	4.5 ± 0.4Ab	0.4 ± 0.0b	1.1 ± 0.1Bb	3.5 ± 0.3Ab	nd	nd	1.9 ± 0.1c	nd	0.6 ± 0.1Bb	13.2 ± 0.9Ab	0.5 ± 0.0b	0.7 ± 0.0Bc	5.0 ± 0.2Ab
phenanthrene	nd	nd	2.3 ± 0.4c	nd	nd	0.6 ± 0.2c	nd	0.2 ± 0.0Bc	1.6 ± 0.0Ac	nd	nd	5.4 ± 0.7c	nd	nd	1.9 ± 0.1d
anthracene	nd	0.2 ± 0.0Bb	1.0 ± 0.1Bde	nd	nd	0.4 ± 0.0c	nd	nd	nd	nd	nd	1.3 ± 0.0c	nd	nd	0.8 ± 0.0e
fluoranthene	0.2 ± 0.0b	0.2 ± 0Bb	0.5 ± 0.1Ae	nd	0.2 ± 0.0Ac	0.3 ± 0.0Ac	nd	nd	nd	nd	nd	0.5 ± 0.0c	nd	0.2 ± 0.0Bc	0.4 ± 0.0Af
pyrene	nd	nd	0.3 ± 0.1e	nd	nd	0.1 ± 0.0c	nd	nd	nd	nd	nd	0.4 ± 0.1c	nd	nd	0.2 ± 0.0f
benzo[*a*] anthracene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
chrysene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
benzo[*b*] fluoranthene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
benzo[*k*] fluoranthene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
benzo[*a*]pyrene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
indeno[1,2,3-*c,d*]pyrene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
dibenzo[*a,h*] anthracene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
benzo[*g,h,i*] perylene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
total	8.6 ± 0.1	33.5 ± 2.9B	42.4 ± 3.0A	2.0 ± 0.5	19.9 ± 0.3B	33.9 ± 3.1A	4.8 ± 1.6	12.6 ± 0.4B	27.1 ± 0.4A	1.3 ± 0.1	33.0 ± 2.1B	125.5 ± 9.2A	12.0 ± 0.9	43.6 ± 0.8B	75.4 ± 0.8A

[a]Mean of duplicate analyses ± standard deviation. Means of the same kind of meat in the same row with different letters (A, B) are significantly different ($p < 0.05$). Means bearing different letters (a–f) in the same column are significantly different ($p < 0.05$). [b]Premarinating time. [c]Sugar-smoking time. [d]nd, not detected, below limit of quantification (QL).

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Table 3. Concentrations of PAHs (Nanograms per Gram)[a] in Premarinated and Sugar-Smoked Seafood

	oyster			octopus			salmon			shrimp			squid		
PAH	premarin 5 min[b]	3 min[c]	6 min[c]	premarin 15 min	3 min	6 min	premarin 10 min	3 min	6 min	premarin 3 min	3 min	6 min	premarin 10 min	3 min	6 min
naphthalene	6.9 ± 1.1	19.1 ± 0.1Aa	18.8 ± 0.6Aa	2.2 ± 0.3	3.0 ± 0.2B	6.2 ± 0.4Aa	5.3 ± 0.7	7.9 ± 0.8B	15.3 ± 1.8Aa	2.2 ± 0.2	2.6 ± 0.4B	5.7 ± 1.0Aa	nd[d]	nd	8.3 ± 1.1a
acenaphthylene	nd	2.2 ± 0.1Ab	2.3 ± 0.2Ac	nd	nd	nd	nd	nd	1.7 ± 0.2b	nd	nd	1.0 ± 0.2b	nd	nd	nd
acenaphthene	nd	3.2 ± 0.4Ab	3.1 ± 0.7Ab	nd	nd	1.0 ± 0.0c	nd	nd	2.5 ± 0.2b	nd	nd	1.0 ± 0.0b	nd	nd	1.2 ± 0.0b
fluorene	nd	3.6 ± 0.6Ab	2.5 ± 0.0Bbc	nd	nd	0.2 ± 0.1d	nd	nd	1.5 ± 0.0b	nd	nd	1.3 ± 0.2b	nd	nd	1.0 ± 0.0b
phenanthrene	nd	2.4 ± 0.1Ab	2.1 ± 0.2Ac	nd	nd	1.9 ± 0.1b	nd	nd	nd	nd	nd	0.2 ± 0.0b	nd	nd	nd
anthracene	nd	0.6 ± 0.1Ab	0.5 ± 0.0Ad	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.3 ± 0.1b
fluoranthene	nd	1.2 ± 0.0Bb	1.7 ± 0.1Ad	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
pyrene	nd	0.7 ± 0.0Bb	1.0 ± 0.0Ad	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
benzo[*a*]anthracene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
chrysene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
benzo[*b*]fluoranthene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
benzo[*k*]fluoranthene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
benzo[*a*]pyrene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
indeno[1,2,3-*c,d*]pyrene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
dibenzo[*a,h*]anthracene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
benzo[*g,h,i*]perylene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
total	6.9 ± 1.1	32.9 ± 1.3A	31.8 ± 1.8A	2.2 ± 0.3	3.0 ± 0.2B	9.2 ± 0.6A	5.3 ± 0.7	7.9 ± 0.8B	20.9 ± 2.2A	2.2 ± 0.2	2.6 ± 0.4B	9.1 ± 1.4A	nd	nd	10.8 ± 1.2

[a]Mean of duplicate analyses ± standard deviation. Means of the same kind of meat in the same row with different letters (A, B) are significantly different ($p < 0.05$). Means bearing different letters (a–d) in the same column are significantly different ($p < 0.05$). [b]Premarinating time. [c]Sugar-smoking time. [d]nd, not detected, below limit of quantification (QL).

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Table 4. Total Toxicity Equivalent (TEQ)[a] in Smoked Meat Products

meat product	premarinated	TEQ (μg/kg) 3 min smoking time	TEQ (μg/kg) 6 min smoking time
poultry			
chicken heart	0.000	0.008 ± 0.000	0.006 ± 0.000
chicken drumstick	0.000	0.003 ± 0.000	0.007 ± 0.000
chicken gizzard	0.000	0.013 ± 0.002	0.018 ± 0.001
chicken breast	0.000	0.005 ± 0.001	0.006 ± 0.001
duck drumstick	0.000	0.003 ± 0.000	0.009 ± 0.001
red meat			
beef steak	0.002 ± 0.000	0.008 ± 0.001	0.026 ± 0.003
pig knuckle	0.000	0.003 ± 0.000	0.012 ± 0.001
ham	0.000	0.002 ± 0.000	0.007 ± 0.000
lamb steak	0.000	0.001 ± 0.000	0.041 ± 0.002
pork chop	0.001 ± 0.000	0.005 ± 0.000	0.024 ± 0.001
seafood			
oyster	0.000	0.030 ± 0.002	0.032 ± 0.002
octopus	0.000	0.000	0.003 ± 0.000
salmon	0.000	0.000	0.006 ± 0.000
shrimp	0.000	0.000	0.003 ± 0.001
squid	0.000	0.000	0.005 ± 0.001

[a]TEQ is calculated by summing each PAH's respective BaP toxicity equivalent factor (TEF) expressed in μg/kg.

of incomplete combustion of wood. For instance, a high level of benzo[*a*]pyrene (>18 ppb) was reported to be present in high-fat smoked belly ham by a direct smoking process.[17] However, with an indirect smoking process the benzo[*a*]pyrene content in belly ham could be reduced to 0.3 ppb. Similarly, low PAH formation was observed in meat products by employing an indirect process with smoke produced from an external smoke generator.[14] Alternatively, liquid smoke was also used to minimize PAH formation during smoking.[15] As mentioned above, the amount and kind of PAHs formed during smoking can also be greatly affected by wood variety, as shown by a larger level of total PAHs and benzo[*a*]pyrene with spruce wood when compared to apple or alder wood.[13] However, with beech wood as smoke source, only a low level of benzo[*a*]-pyrene (0.268 ppb) was formed in meat.[12] Compared to published reports, no benzo[*a*]pyrene was detected in sugar-smoked meat commodities in our study, demonstrating that sugar-smoking is superior to wood-smoking in preventing carcinogenic PAH formation, apparently due to a much milder smoking condition of the former. Although the traditional wood-smoking process was not carried out in this study, the absence of benzo[*a*]pyrene in sugar-smoked meat can still be used as a basis for comparison with that in wood-smoked meat in the literature as the GC-MS methods employed in this study can separate and quantify benzo[*a*]pyrene accurately. Thus, the sugar-smoking process can be adopted instead of the traditional smoking process for future meat processing.

Dietary Exposure Assessment of PAHs from Smoked Meat. With the exception of beef steak (0.002 ± 0.000 μg TEQ_{BaP}/kg) and pork chop (0.001 ± 0.000 μg TEQ_{BaP}/kg), the TEQ_{BaP} values in most premarinated samples remained nil, mainly due to the presence of noncarcinogenic naphthalene (TEF = 0) (Tables 2 and 4). The total PAH TEQ_{BaP} levels in meat after sugar-smoking for 3 min ranged from 0.003 ± 0.000 to 0.013 ± 0.002, from 0.001 ± 0.000 to 0.008 ± 0.001, and from 0.000 to 0.030 ± 0.002 for poultry meat, red meat, and seafood, respectively. Among the various meat commodities, oyster was shown to possess the highest TEQ_{BaP}, which should be due to the presence of midcarcinogenic (TEF = 0.01) PAHs including acenaphthylene, anthracene, and fluoranthrene (Tables 2 and 3). Additionally, an increase in sugar-smoking time (6 min) led to a higher TEQ_{BaP} level ranging from 0.006 ± 0.000 to 0.018 ± 0.001, from 0.007 ± 0.000 to 0.041 ± 0.002, and from 0.003 ± 0.000 to 0.032 ± 0.002 for poultry meat, red meat, and seafood, respectively (Table 4), which can also be

Table 5. Lifelong Average Daily Dietary Intake (LADI)[a] of PAHs from Sugar-Smoked Meat Products in Taiwan

meat product	smoking time (min)	LADI (ng/kg BW/day) adults (19−64 years old) males	adults (19−64 years old) females	aged (>65 years old) males	aged (>65 years old) females
poultry meat					
chicken heart	3	trace[b]	trace	trace	trace
	6	trace	trace	trace	trace
chicken drumstick	3	0.002	0.001	trace	trace
	6	0.003	0.002	0.001	0.001
chicken gizzard	3	trace	trace	trace	trace
	6	trace	trace	trace	trace
chicken breast	3	0.002	0.001	0.001	trace
	6	0.002	0.001	0.001	0.001
duck drumstick	3	trace	trace	trace	trace
	6	trace	trace	trace	trace
red meat					
beef steak	3	0.001	0.001	trace	trace
	6	0.007	0.003	trace	trace
pig knuckle	3	trace	trace	trace	trace
	6	0.002	trace	trace	0.001
ham	3	trace	trace	trace	trace
	6	trace	0.001	trace	0.001
lamb steak	3	trace	trace	trace	trace
	6	0.004	0.004	0.001	0.002
pork chop	3	0.001	trace	trace	trace
	6	0.005	0.003	0.001	trace
seafood					
oyster	3	0.001	0.001	0.001	trace
	6	0.001	0.001	trace	trace
octopus	3	trace	trace	trace	trace
	6	trace	trace	trace	trace
salmon	3	trace	trace	trace	trace
	6	trace	trace	trace	trace
shrimp	3	trace	trace	trace	trace
	6	trace	trace	trace	trace
squid	3	trace	trace	trace	trace
	6	trace	trace	trace	trace

[a]LADI is calculated by summing (the TEQ_{BaP} levels of PAHs in selected food products, μg/kg) × (exposure duration, year) × (average ingestion amount of selected food product during exposure duration, g/day)/(body weight (kg) × average life span in Taiwan, ca. 79 years). [b]Trace, LADI values <0.001 ng/kg BW/day.

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attributed to the presence of high amounts of midcarcinogenic PAHs.

Epidemiological studies have demonstrated that frequent consumption of grilled or smoked meat products prepared by traditional process involving a direct contact between food and smoke generated by combustion is responsible for the high incidence of stomach cancer.[26−28] Levels of carcinogenic benzo[*a*]pyrene in smoked meat products have been recently reported to be present in meat (0.97−1.20 μg/kg wet wt), fish (nd−0.99 μg/kg wet wt),[4] ham by direct sauna method (14.6−36.9 μg/kg), pork (nd−<0.3 μg/kg), lamb leg (0.8−0.9 μg/kg), and salmon (8.4 μg/kg by direct sauna and nd−<0.3 μg/kg by indirect smoking).[7] As indicated before, wood type, processing temperature, and smoking methods can strongly influence the amount of benzo[*a*]pyrene formed. Unlike the traditional wood-smoking process, meats that are to be sugar-smoked are subjected to premarinating and then a shortened time of subsequent smoking for prevention of benzo[*a*]pyrene formation. However, the detailed mechanism needs further investigation.

LADI regarding lifespan and dietary exposure to PAHs was evaluated to assess health risk in Taiwan, and the results indicated most LADI of PAH values were insignificant (Table 5), especially in both aged male and female subjects. The highest LADI values of 0.007 and 0.004 ng/kg BW/day were observed for male adults and female adults over a 79 year lifespan in Taiwan, respectively. Accordingly, the LADI values for adults were higher than for the elderly (Table 5). Likewise, the LADI values for male adults were larger than for female adults (Table 5). Among the various meat products, red meat (beef steak, lamb steak, and pork chop) consumed by male subjects showed a higher PAH dietary exposure, whereas seafood was the lowest in PAH dietary exposure. Subsequently, the cancer risk induced by PAHs was assessed on the basis of the carcinogenic potency factor (oral slope factor) of benzo[*a*]pyrene, 7.3 (mg/kg BW/day)$^{-1}$, as suggested by IRIS of the U.S. EPA based on the incidence of forestomach cancer in the rat.[25] Then, each PAH convener in foods was converted into TEQ_{BaP} value based on benzo[*a*]pyrene cancer potency and LADI, and the cancer risks of all sugar-smoked meat samples were estimated to be $<2 \times 10^{-7}$. According to the U.S. EPA, excess human cancer risk of one in a million over a 70 year lifespan (10^{-6}) is considered to be an acceptable or inconsequential level, whereas a one in ten thousand chance of excess cancer risk (10^{-4}) is considered to be a serious level.[25,29] The cancer risk of sugar-smoked meat is lower than the acceptable level; thus, our study demonstrates a much lower cancer risk of sugar-smoked meat. Nevertheless, we have to point out that the health risk was assessed on the basis of the total intake amount of meat as no information is available as to the consumption data of sugar-smoked meat in Taiwan. On the basis of the fact that the intake amount of sugar-smoked meat is lower than the total amount of meat, the dietary exposure of PAHs for the former should be even lower.

Conclusions. The present study demonstrates, for the first time, the PAH formation in sugar-smoked poultry meat, red meat, and seafood products. As no carcinogenic benzo[*a*]-pyrene was detected and low cancer risk was calculated, the sugar-smoking process can be adopted to replace the frequently used wood-smoking process to prevent carcinogenic PAH formation.

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Notes

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