SURVEY OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN GERMAN SMOKED MEAT PRODUCTS

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***Abstract*—Contents of the 15+1 EU priority PAH were analysed from 113 representative commercial smoked German meat products collected in the year 2006 with a Fast-GC/HRMS method. The median of benzo[a]pyrene content was 0.03 µg/kg and therefore more than a factor of 100 below the maximum level of 5 µg/kg. The highest content of benzo[a]pyrene was detected in a Frankfurter type sausage (0.43 µg/kg). The sum content of benzo[a]pyrene, benzo[a]anthracene, chrysene and benzo[b]fluoranthene ("PAH4") as proposed by the European Food Safety Authority (EFSA) to be a good marker for PAH in food was 0.28 µg/kg in median, the sum content of the 15+1 EU priority PAH was 0.64 µg/kg in median. The analysed smoked meat products showed an increasing presence of PAH in the following order: cooked ham (n = 17) < raw sausages (n = 25) < liver sausages (n = 25) < raw ham (n = 23) < Frankfurter type sausages (n = 23). The correlation coefficient (R) between BaP and the sum of the 15+1 EU priority PAH was 0.90. To increase the safety of the consumer a lowering of the BaP maximum level to 1 µg/kg is proposed and critical aspects using "PAH4" as a marker for PAH in food surveillance are discussed.**

***Index Terms*—polycyclic aromatic hydrocarbons, EU priority PAH, smoked meat products, Germany**

I. INTRODUCTION

In the European Union a maximum level of 5 µg/kg benzo[a]pyrene (BaP) in smoked meats and smoked meat products is existing (European Commission, 2006). Also the Scientific Committee on Food (SCF) recommended the member states of the European Union to analyse the contents of 15 PAH compounds, which are classified as priority (15 SCF-PAH) and to check the suitability of BaP as a marker for the occurrence and impact of carcinogenic PAH in food (European Comission, 2005). Additionally, the European Food Safety Authority (EFSA) recommends to analyse benzo[c]fluorene (BcL) assessed to be relevant by the Joint FAO/WHO Experts Committee on Food Additives (JECFA, 2005). The 15+1 EU priority PAH that are recommended to be analysed include 15 SCF-PAH and JECFA PAH, which are: BcL, benzo[a]anthracene (BaA), cyclopenta[c,d]pyrene (CPP), chrysene (CHR), 5-methylchrysene (5MC), benzo[b]fluoranthene (BbF), benzo[j]fluoranthene (BjF), benzo[k]fluoranthene (BkF), BaP, benzo[g,h,i]perylene (BgP), dibenzo[a,h]anthracene (DhA), indeno[1,2,3-cd]pyrene (IcP), dibenzo[a,e]pyrene (DeP), dibenzo[a,h]pyrene (DhP), dibenzo[a,i]pyrene (DiP) and dibenzo[a,l]pyrene (DlP).

In a previous study 22 smoked raw meat products (mainly raw smoked hams) were analysed with respect to their contents of 15+1 EU priority PAH compounds (Jira, Ziegenhals and Speer, 2008). Within this study a median content for BaP below 0.15 µg/kg and a median sum content of the 15+1 EU priority PAH of 1.5 µg/kg was determined. The main objective of the present study was to determine the contents of the 15+1 EU priority PAH compounds in representative samples of German smoked meat products.

II. MATERIALS AND METHODS

In order to collect representative samples of smoked meat products in Germany, a total of 113 samples of smoked meat products (raw sausages (N=25), raw ham (N=23), cooked ham (N=17), Frankfurter type sausages (N=23) and liver sausages (N=25)) were analysed. These samples originated from the different states in Germany taking into consideration the various populations in the single states.

A. Accelerated solvent extraction (ASE)

About 5 g homogenised ham or 3 g homogenised other meat products were mixed with a defined amount of a specific drying material: poly(acrylic acid), partial sodium salt-*graft*-poly(ethylene oxide). The resulting material was poured into 33-mL cells, which were locked with glass microfiber filters at the outlet end of the extraction cells. Afterwards, 50 µL of the PAH standard mixture containing isotope labelled (^{13}C and ^{2}H) and fluorinated PAH

compounds were added as internal standard. The extraction was performed with an ASE 200 from Dionex (Sunnyvale, USA) and carried out with n-hexane at 100 °C and 10 MPa at a static time of 10 min. The flush volume was 60% and the purge time 120 s. Two static cycles were accomplished. The solvent of the extract was evaporated in a water bath (40 °C) using a nitrogen stream.

B. Gel permeation chromatography (GPC)

The evaporated ASE extract was dissolved in 4.5 mL cyclohexane/ethylacetate (1:1, v/v) and filtered through a polytetrafluoroethylene (PTFE) filter with a pore size of 1 µm. The GPC column (25 mm i.d.) was filled with 60 g Bio-Beads S-X3. Samples were eluted at a flow rate of 5 mL/min applying cyclohexane/ethylacetate (1:1, v/v). The waste time was 0–36 min and the collect time 36–65 min. The GPC solvent was removed with a rotary evaporator, and the eluate was dried in a nitrogen stream. The dried GPC eluate was dissolved in 1 ml cyclohexane.

C. Solid phase extraction (SPE)

The SPE was performed automatically with a modified ASPEC Xli (automatic sample preparation with extraction columns) from Gilson (Bad Camberg, Germany). A total of 1 g silica dried for 12 h at 550 °C and subsequent deactivating with 15% bidestilled water was filled into commercial 8-mL SPE columns (12 mm i.d.). After conditioning of the columns with 3 mL cyclohexane the samples were applied and eluted with 10 mL cyclohexane.

D. Preparation for GC/MS analysis

The dried eluate of SPE was dissolved in 1 mL isooctane and 50 µL of the PAH-recovery standard mixture and transferred to a 1 mL tapered vial. The remaining sample was carefully concentrated in a nitrogen stream to a volume of about 50 µL.

E. Fast-GC/HRMS analysis

Fast GC/HRMS was performed using a Trace-GC chromatograph (ThermoFisher Scientific, Milan, Italy) equipped with a split/splitless injection port. Separation was performed on a TR-50MS column (10 m x 0.1 mm x 0.1 µm) (ThermoFisher Scientific, Bremen, Germany) (Ziegenhals, Hübschmann, Speer and Jira, 2008). Injection temperature was 320 °C; injection volume was 1.5 µL (splitless). Helium with a constant flow of 0.6 mL/min was used as carrier gas. The following temperature program was used: isothermal at 140 °C for 1 min, at 10 °C/min to 240 °C, at 5 °C/min to 270 °C, at 30 °C/min to 280 °C, at 4 °C/min to 290 °C, at 30 °C/min to 315 °C and at 3 °C/min to 330 °C.

Identification of PAH by GC/HRMS was performed using a sector mass spectrometer DFS (ThermoFisher Scientific, Bremen, Germany) working in the electron impact (EI) positive ion mode and applying an electron energy of 45 eV. The temperatures of the source and the transfer line were heated up to 280 °C and 300 °C. The resolution of the MS was tuned to 8.000 (10% valley definition).

III. RESULTS AND DISCUSSION

In this investigation 113 samples of smoked meat products were analysed and the contents of the 15+1 EU priority PAH were determined. The median of BaP contents was 0.03 µg/kg and consequently more than a factor of 100 below the maximum level of 5 µg/kg. The 95-percentile (P 95) was 0.14 µg/kg and maximum value was 0.43 µg/kg, which was still more than a factor of 10 below the maximum level. The highest PAH contents were observed for BcL and CHR+TP which were the only PAH compounds with median contents above 0.1 µg/kg. Dibenzpyrenes (DeP, DhP, DiP and DlP) were observed in only a few samples. In most samples contents of dibenzpyrenes were below the LOD of 0.01 µg/kg.

EFSA concluded, that BaP is not a suitable indicator for the occurrence of PAH in food and assessed, that the sum content of the four PAH compounds BaP, CHR, BaA and BbF ("PAH4") is the most suitable indicator of PAH in food (EFSA, 2008). The median contents of "PAH4" were 0.28 µg/kg, P 95 was 1.19 µg/kg and maximum value was 2.46 µg/kg. Because of the above mentioned coelution of CHR and TP also "PAH4" includes contents of TP. In a previous study the TP/CHR-ratio in smoked meat products was varying in the range of 0.1 to 2.7 (Jira, Ziegenhals and Speer, 2008). The median of the sum content of 15+1 EU priority PAH was 0.64 µg/kg, P 95 was 2.58 µg/kg and the maximum value was 5.47 µg/kg.

The highest BaP levels were detected in raw ham and Frankfurter type sausages with median concentrations of about 0.05 µg/kg. The highest content of BaP was detected in a Frankfurter type sausage (about 0.4 µg/kg). The lowest BaP contents were detected in cooked ham (median: 0.01 µg/kg). Extreme values for BaP contents in cooked ham were in the range of 0.02 to 0.05 µg/kg. The median content of BaP was 0.02 µg/kg for raw sausages and 0.03 µg/kg for liver sausages.

The highest “PAH4” levels were observed in Frankfurter type sausages. Within this group of hot smoked meat products median “PAH4” contents of 0.6 µg/kg were observed. The median “PAH4” contents of raw ham and liver sausages were both in the range of 0.3 µg/kg. Raw sausages had a median of 0.2 µg/kg. The lowest “PAH4” levels were observed in cooked ham (median: 0.1 µg/kg). In only one Frankfurter type sausage the “PAH4” level was above 2 µg/kg.

The analysed samples of meat products showed the increasing presence of sum contents of 15+1 EU PAH (median values; maximum without outliers and extreme values) in the following order: cooked ham (0.3 µg/kg; 0.4 µg/kg) < raw sausages (0.5 µg/kg; 0.7 µg/kg) < liver sausages (0.7 µg/kg; 1.8 µg/kg) < raw ham (0.8 µg/kg; 1.8 µg/kg) < Frankfurter type sausages (1.2 µg/kg; 3.3 µg/kg).

The contribution of the four PAH compounds proposed by EFSA (BaP, BaA, CHR and BbF) to the sum content of the 15+1 EU priority PAH in median was 4% for BaP, 10% for BaA, 19% for CHR and 7% for BbF. The contribution of “PAH4” to the sum content of the 15+1 EU priority PAH in median was 42%. The sum content “PAH4” was dominated by CHR (median: 47%), followed by BaA (24%), BbF (17%) and BaP (10%).

The sum content of the 15+1 EU priority PAH was correlated with the contents of the single PAH contents. Correlation coefficients higher than 0.85 were found for BaP (0.90), the three benzofluoranthenes (BbF: 0.88; BjF: 0.89; BkF: 0.86), BcL (0.88), BaA (0.97) and CHR (0.95). Especially for the dibenzpyrenes the correlation coefficients were very low, because for nearly all samples of smoked meat products their contents were below the LOD. For the sum content (“PAH4”) a correlation coefficient of R=0.99 was calculated.

The investigation of representative samples of smoked meat products showed median BaP contents of 0.03 µg/kg, which are more than a factor of 100 below the maximum level of 5 µg/kg. The highest observed BaP content in a Frankfurter type sausage was 0.43 µg/kg, which is still more than a factor of 10 below the maximum level. The observed correlation coefficient between the sum content of the 15+1 EU priority PAH and BaP of R=0.90 is an indicator for a suitability of BaP as a marker substance for PAH in smoked meat products. A better correlation coefficient of R=0.99 for the sum content (“PAH4”) is due to a relatively high contribution of “PAH4” to the sum content of the 15+1 EU priority PAH of 42% (median). A substantial disadvantage of using “PAH4” instead of BaP as a marker substance for PAH in food surveillance is the insufficient chromatographic separation of CHR and TP, which is only feasible with a time-consuming GC temperature programme running more than one hour, which is not suitable for routine measurements. In contrast to a sufficient gaschromatographic separation of BaP also the separation of CHR and CPP respectively BbF, BjF and BkF appears problematic. Furthermore an important disadvantage of using “PAH4” as an indicator of PAH in food is, that PAH compounds with very different carcinogenic potential are summed up to a total content without weighting. A very different carcinogenic potential of these four PAH compounds was not only established by IARC (2009), but also by other researchers (Nisbet and LaGoy, 1992; Bostrom, Gerde et al., 2002), who assessed a toxicological potential for BbF, BaA and CHR, which was more than a factor of 10 lower as observed for BaP. Because within the presented study a median contribution of only 10% for BaP to “PAH4” was determined, this sum content is dominated by PAH compounds with lower toxicological relevance. In order to evaluate the suitability of BaP as a marker in addition to representative samples also suspicious samples of smoked meat products should be analysed for their contents of the 15+1 EU priority PAH compounds.

IV. CONCLUSION

The observed median BaP contents of 0.03 µg/kg demonstrate clearly, that the production of smoked meat products with BaP levels below 1 µg/kg is possible without any problems. Considering the genotoxic and carcinogenic properties of several PAH compounds the Scientific Committee on Food (SCF) recommended, that the PAH contents in smoked meat products should be as low as reasonably achievable (ALARA) (SCF, 2002). Actually the Codex Alimentarius Commission (2008) works on a proposed draft for a „Code of Practice for the Reduction of Contamination of Food with Polycyclic Aromatic Hydrocarbons (PAH) from Smoking and Direct Drying Processes” with the objective of lowering PAH contents in foods (e.g. smoked meat products). The unreasonably high BaP maximum level of 5 µg/kg is in conflict with efforts to reduce the PAH contents in smoked meat products. Therefore a lowering of the maximum level for BaP from 5 µg/kg to 1 µg/kg seems to be advisable.

BaP seems to be a good marker for PAH in smoked meat products and is quite easy to be analysed. In contrast a regulation of “PAH4” suffers from analytical problems and a dominating sum content by lower toxic PAH compounds.

ACKNOWLEDGEMENT

The authors thank E. Klötzer for the excellent technical assistance.

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