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POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN SELECT COMMERCIALLY PROCESSED FOOD ITEMS IN FINLAND AND THE MUTAGENIC POTENTIAL OF THESE FOOD ITEMS

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Abstract:

Commercially processed meat and fish products are common sources of human exposure to chemical food mutagens. In this study, we investigated the mutagenic potential of 21 different commercially processed meat and fish products (7 product types with 3 lots of each), along with the presence of 4 principal polycyclic aromatic hydrocarbons (PAHs) (benzo[*a*]pyrene [BaP], benzo[*b*]fluoranthene [BbF], benzo[*a*]anthracene [BaA] and chrysene [CHR]) in them. Sample extraction was carried out by an accelerated solvent extraction method, while the concentrations of the 4 PAHs were determined by gas chromatography-tandem mass spectrometry (GC-MS/MS). The mutagenic potential of food extracts was assessed by the standard plate incorporation assay (Ames test) using two strains of *Salmonella* Typhimurium (TA 100 and TA 98) both in the presence and absence of metabolic activation (S9-mix). The results show that in the majority of food items investigated, PAH levels were below the limit of quantification, except for smoked fish, one batch of which even exceeded the maximum limits for both the sum of the 4 PAHs and BaP. Furthermore, all 3 batches of smoked fish were also found to be mutagenic on both strains of *Salmonella*, both in the presence and absence of metabolic activation. Overall, the data from both assays were in a fairly good agreement with one another, suggesting that PAHs are major contributors to mutagenicity of processed food products and the set maximum levels for PAHs are usually protective against food mutagenicity, although food samples harboring PAHs at levels approaching the maximum limits may exhibit mutagenic potential. Since the number of samples investigated was relatively small, further studies are warranted to verify the conclusions.

Keywords: Benzo[a]pyrene; Processed food; Genotoxicity; Ames test; Food safety

Introduction

PAHs are condensed compounds of linked aromatic rings and are formed by incomplete combustion of organic materials (Samanta et al., 2002; Farhadian et al., 2011). Some of these chemicals are known carcinogens in humans (Samanta et al., 2002) and cause e.g. mammary tumours in laboratory animals (el-Bayoumy et al., 1995; Hecht, 2002). The occurrence of PAHs in processed food items is mainly due to processing techniques, such as grilling, barbecuing, smoking and frying (Djinovic et al., 2008; Farhadian et al., 2010; Wretling et al., 2010; Alomirah et al., 2011; Essumang et al., 2012), and their formation and concentration are dependent on the type/method of processing, processing time and the type of food being processed. For example, heating highly fatty food directly by smoking is known to produce high levels of PAHs (Djinovic et al., 2008; Chung et al., 2011; John et al., 2011). Of the known PAHs, 15 are genotoxic and carcinogenic (Scientific Committee on Food [SCF], 2002; EFSA, 2008). Of the known genotoxic and carcinogenic PAHs, benzo[*a*]pyrene (BaP) is the most commonly studied and has shown various toxicological effects in experimental animals (SCF, 2002; Schneider et al., 2002). For this reason, BaP has been used as a marker of carcinogenic PAHs in food since 2002. However, this compound alone is not a sufficient indicator for the presence of PAH in foods, and in 2008, the European Food Safety Authority (EFSA) suggested the use of the sum of four carcinogenic PAHs (PAH4): BaP, BbF, BaA and CHR, as a marker for PAH concentrations (EFSA, 2008). This has recently been implemented in EU regulations (European Commission, 2011). The maximum allowable concentration for PAH4 is 30 μg/kg and that for BaP 5 μg/kg. Epidemiological studies show an increased risk of intestinal, breast, bladder, prostate, stomach, oesophageal and pancreatic cancers after consumption of high levels of processed meat, particularly well-done fried and barbecued red meat (Navarro et al., 2004; Norat et al., 2005; Sinha et al., 2009; Ferguson, 2010; John et al., 2011; Berjia et al., 2014), and PAHs are likely contributors to this association. Although the concentrations of

PAHs in commercially processed fish and meat products in Finland are monitored, mutagenicity is not assessed concomitantly. Previously, we showed that although the mutagenic activity of Finnish processed foods appeared to be low, some food items (cold cuts of cold-smoked beef, grilled turkey, and smoked chicken) were mutagenic on the TA 100 strain with and without metabolic activation. As we did not conduct a chemical analysis of those samples, we do not know whether the mutagenicity found emanated from PAHs or some other genotoxic substances. Therefore, the main objectives of this study were to investigate the concentrations of four principal PAHs (BaP, BbF, BaA, CHR) in selected commercially processed fish and meat products in Finland, and to determine their mutagenic potential. The results were compared to evaluate the link between the PAH concentrations and the mutagenicity observed and to further our understanding of the current situation regarding consumer exposure.

Materials and methods

Food samples:

A total of seven meat and fish samples, each comprising three lots, were purchased from a grocery shop (Prisma, Viikki), in Helsinki, Finland. The samples were freeze-dried and homogenized before conducting chemical analyses and screening for their possible mutagenicity.

Extraction of food samples:

The extraction of PAHs was performed by accelerated solvent extraction to remove fat and other interferences. An additional purification step using solid phase extraction was needed before the final detection and quantification of the analytes by GC-MS/MS.

Chemical analyses (GC-MS/MS):

Gas chromatography-tandem mass spectrometry (GC-MS/MS) analysis was performed using a gas chromatograph (Agilent, 6890N; Agilent Technologies, Santa Clara, CA, USA) coupled with a Micromass Quattro Micro GC triple-quadrupole analyser (Waters, Micromass; Waters Corp., Milford, MA, USA), using an Agilent J & W Select PAH (30 m x 0.25 mm x 0.15 µm) column, helium as the carrier gas, at a flow rate of 1.0 ml/min, with the following conditions:

Splitless injection, injection volume, 1 µl. Electron ionization, Injector temperature 300 ˚C, transfer-line temperature 300 °C, ion source temperature 275 °C. The column temperature programme was as follows: Initial temperature 110 °C (0.7 min), 85 °C/min to 180 °C, 3 °C/min to 230 °C (7 min), 28 °C/min to 280 °C (15 min), 14 °C/min to 350 °C (5 min).

Mutagenicity assay:

The mutagenicity of food extracts was determined by the standard plate incorporation assay (Ames test) as described by Maron and Ames (1983) using two strains of *Salmonella* (TA 100 and TA 98), with and without metabolic activation (S9 mix). The amount of S9 used in the S9 mix was 10%. Water and DMSO were used as negative controls for both strains while sodium azide (0.04 mg/mL) and 2-aminoanthracene (0.02 mg/mL) served as positive controls for TA 100 and TA 98, respectively, as previously described (Omoruyi et al., 2014). The results of the mutagenic activities are presented as the number of revertant colonies per gram of food sample. Only the mean and standard deviation of the highest concentration for all food extracts are shown. A total of 4 different concentrations of each food extract were used in this study.

Statistics:

The mutagenic potency of each food sample was determined from the linear slope of the dose-response curve by linear regression analysis using Prisma 4.0 (GraphPad software Inc. San Diego, CA). In addition to the statistically significant ($p < 0.05$) dose-response effect, samples were only considered mutagenic when the highest test concentration generated at least twice as many revertants as the negative control (DMSO and water).

Results and Discussion

Commercially processed meat and fish products are continuous sources of health concerns globally. They are consumed in large amounts and the presence of chemical contaminants in them is difficult to regulate since the contaminants are mainly inadvertent or formed as a result of processing. Therefore, regular monitoring studies are warranted. The current study aimed at investigating the levels of 4 PAHs in fish and meat products sold in Finland using the GC-MS/MS method and also, to determine their mutagenic potential by the standard plate incorporation assay (Ames test).

The results of seven processed meat and fish samples (with 3 lots of each) showed that the majority of Finnish food samples contained very low levels of PAHs (BaP, BaA, CHR and BbF), except for a single batch of smoked fish (Table 1). In this sample lot, PAH concentrations exceeded the maximum levels (ML) of 5 μg/kg and 30 μg/kg for BaP and PAH4 sum, respectively (EU 835/2011). Concerning the other two lots of the same product, their PAH levels were also higher than in any of meat samples tested, but yet slightly lower than the MLs for BaP and PAH4 sum. These findings were reflected in the mutagenicity assay outcome, with all 3 lots of hot smoked fish producing revertants over two-fold higher than that of the negative control (DMSO), indicating that all 3 lots of the smoked fish product contain mutagens and are thus of public health concern. Although using the standard Ames test for meat and fish samples carries the risk of generating false positive results due to the possibility of leaching histidine to the plates, this did not prove the case here as all the samples with low PAH levels were also negative in the Ames test. The number of revertants generated for the meat-based products were low (always less than 2-fold the DMSO control value), both with and without metabolic activation (S9-mix), and in both *Salmonella* TA 100 and TA 98 strains (Table 2).

Information on the concentrations of PAHs in commercially processed Finnish meat and fish products is generally lacking in scientific literature. However, the Finnish Food Safety Authority (EVIRA) reported in 2006, low levels of BaP in 62 smoked fish samples examined. The low levels recorded were attributed to the removal of the fish skin prior to extraction and analysis (EVIRA, 2006). In support of this, Omoruyi and Pohjanvirta (2014) reported smoked, unskinned fish samples to be mutagenic in the *Salmonella* TA98 strain, and mostly in the absence of metabolic activation (S9 mix). Regarding PAHs in meat, Reinik et al. (2007) reported 3.4% (n = 32) of commercially cured meat products investigated in Estonia to contain BaP greater than the ML of 5 µg/kg, with the highest concentration occurring in home-grilled pork samples. Similarly, Elhassaneen (2004) determined 11 PAHs in charcoal-broiled beef burgers, and reported a PAH range from 0.31 to 14.95 µg/kg, with pyrene and BaP being the most frequently detected PAH compounds. The concentrations of BaP in that study were 0.99–4.8 µg/kg, with the highest concentration of BaP being slightly lower than the ML. The contents of 16 PAHs in seven different barbequed meat sausages in Swiss have also been determined (Mottier et al., 2000). The highest concentrations were found for phenanthrene and naphthalene, while BaP concentrations varied between "not detected" and 2.81 µg/kg (Mottier et al., 2000).

In meat, the highest reported concentrations of PAHs have been found in food cooked over open flames (Reinik et al., 2007). For example, in barbequed meat, total PAHs were found to be present at levels of up to 164 µg/kg, with BaP concentration amounting to 30 µg/kg (Panalaks, 1976). Results for the total sum of PAHs are difficult to compare between studies due to variations in analyzed compounds and to several non-carcinogenic PAHs which have been included in earlier works.

More recently, Miculis et al. (2011) reported 4 PAHs in smoked fish and meat from Latvia. The 4 PAHs (BaP, BaA, BaF and CHR) were exactly those examined in the current study. All

meat samples investigated in that study had total PAHs between 1.18–8.22 µg/kg. Meanwhile, herring had PAH4 of 20.20 µg/kg. In a similar study in Poland, Kubiak et al. (2015) reported BaP concentration in bacon, jalowcowa sausage and chicken sausage to be 8.49, 8.41 and 7.68 µg/kg, respectively. All values were greater than those reported in our study, except for a single batch of smoked fish, where we reported the BaP concentration of 8.20 g/kg. In addition, Kubiak et al. (2015) concluded that in the inner parts of all meat products, PAH levels were higher in products smoked by traditional methods compared with products processed industrially.

Elsewhere, Mihalca et al. (2011) reported six fish samples (out of 15 samples investigated) to contain BaP levels in excess of 5.0 µg/kg. Interestingly, these samples were processed by traditional smoking, where the food was directly exposed to hot smoke from a burning log fire. In contrast, all fish samples smoked by indirect technique, using smoke from an external smoke generator, had BaP levels below the limit of quantification (0.3 µg/kg) in that study. In further support of these findings, Ciecierska and Obiedzirinski (2007) reported that the traditional processing method of smoking significantly elevated the concentrations of PAHs in meat products.

Although the concentrations of PAHs in processed meat and fish products are well documented, there are, however, only a few reports on the mutagenic potential of such food items globally. We have previously reported 40 % and 27 % of commercially processed Finnish meat/fish samples to be mutagenic on the *Salmonella* TA 100 and TA 98 strains, respectively, with or without metabolic activation (Omoruyi and Pohjanvirta, 2014). Some of the samples that contributed to the outcome in that study included cold cuts of cold-smoked beef, grilled turkey and smoked chicken. Using the same methodology, two-third of all Nigerian food varieties investigated (hamburger, chin-chin, doughnut, suya, bean cake, French fries, potato chips and fried chicken) were found to be mutagenic (Omoruyi et al., 2014). In the present

study, the mutagenicity data were generally in agreement with the chemical analysis results. This implies that 1) PAHs appear to be a major source of food mutagenicity in the case of commercially processed meat and fish products, and 2) although in most cases the MLs for PAH4 and BaP seem to be appropriately set to protect the consumer from food mutagenicity, food samples harboring PAHs at levels approaching these limits may exhibit mutagenic potential. However, because our sample size was fairly small, further studies are warranted to verify these conclusions.

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Table 1: Levels of PAHs in commercially processed Finnish foods

		PAHs (µg/kg)				
Food item	Batch	Bap	BaA	CHR	BbF	Sum
Smoked ham	1	ND	< LOQ	< LOQ	ND	0
	2	ND	ND	< LOQ	ND	0
	3	ND	ND	< LOQ	ND	0
Honey-roasted chicken	1	ND	< LOQ	< LOQ	ND	0
	2	ND	ND	< LOQ	ND	0
	3	< LOQ	< LOQ	< LOQ	ND	0
Grilled turkey	1	ND	0.81	0.84	ND	1.60
	2	ND	ND	ND	ND	0
	3	ND	ND	< LOQ	ND	0
Pepper salami	1	ND	< LOQ	0.88	ND	0.88
	2	ND	< LOQ	< LOQ	ND	0
	3	ND	< LOQ	< LOQ	ND	0
Cold-smoked beef	1	ND	< LOQ	< LOQ	ND	0
	2	< LOQ	< LOQ	< LOQ	ND	0
	3	ND	ND	< LOQ	ND	0
Sauna-smoked ham	1	NA	NA	NA	NA	NA
	2	ND	ND	< LOQ	ND	0
	3	ND	ND	< LOQ	ND	0
Hot Smoked fish	1	4.7	4.5	4.7	4.5	18.40
	2	8.2	15	15	5.8	44
	3	1.0	3.9	3.0	0.8	8.7

Key: ND: Not detected; NA: Not analyzed; LOQ: Limit of quantification. LOQ (BaP, BaA, CHR, BbF) = 0.8 µg/kg

Table 2: Results of the mutagenicity assay on *Salmonella* TA 100 and TA 98 strains, both in the presence and absence of metabolic activation (S9 mix)

		Mutagenicity (revt/kg)			
		TA 100		TA 98	
Sample	Batch	+S9	-S9	+S9	-S9
Water		151± 2.3	74 ± 10.4	24 ± 0.2	15 ± 1.4
Dimethylsulphoxide		160 ± 10.7	81 ± 5.6	25 ± 2.2	13 ± 0.0
Smoked ham	1	201 ± 9.1	128 ± 10.5	34 ± 1.5	21 ± 0.8
	2	174 ± 12.5	106 ± 9.8	32 ± 0.8	19 ± 2.2
	3	189 ± 7.9	115 ± 8.4	37 ± 4.1	15 ± 0.8
Honey-roasted chicken	1	247 ± 11.0	98 ± 8.6	25 ± 2.1	20 ± 1.0
	2	198 ± 15.0	124 ± 15.2	41 ± 6.4	19 ± 4.8
	3	258 ± 9.3	104 ± 9.4	34 ± 4.0	19 ± 2.1
Grilled turkey	1	297 ± 19.8	132 ± 8.1	45 ± 0.0	14 ± 1.8
	2	225 ± 0.0	132 ± 3.2	39 ± 3.4	17 ± 2.8
	3	188 ± 10.1	130 ± 10.8	27 ± 1.5	16 ± 2.4
Pepper salami	1	241 ± 14.6	120 ± 12.0	32 ± 4.9	21 ± 3.0
	2	168 ± 8.7	123 ± 8.2	30 ± 0.6	18 ± 2.1
	3	200 ± 4.9	104 ± 4.1	34 ± 3.2	16 ± 2.1
Cold-smoked beef	1	209 ± 5.1	132 ± 0.0	39 ± 5.0	14 ± 1.5
	2	188 ± 8.7	124 ± 12.4	28 ± 0.0	20 ± 4.2
	3	188 ± 0.0	108 ± 8.4	29 ± 2.1	15 ± 3.1
Sauna-smoked ham	1	NA	NA	NA	NA
	2	268 ± 11.2	140 ± 12.4	42 ± 1.2	13 ± 0.0
	3	158 ± 3.8	132 ± 14.5	28 ± 1.2	15 ± 2.4
Smoked fish	1	392 ± 12.0*	201 ± 16.2*	51 ± 4.7*	40 ± 5.4*
	2	478 ± 41.23*	224 ± 21.4*	64 ± 4.9*	46 ± 4.2*
	3	401 ± 22.8*	214 ± 18.0*	55 ± 2.0*	40 ± 4.8*

Key: *: mutagenicity; NA: Not analyzed

The data are given as the mean ± SD (n = 3).

The asterisk indicates a mutagenic response (>2-fold higher number of revertants vs. DMSO control, combined with statistical significance [$p<0.05$]).