PAPER

POLYCYCLIC AROMATIC HYDROCARBONS IN SELECTED FOOD ITEMS COMING FROM THE CROATIAN MARKET

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ABSTRACT

The study aims to evaluate the presence of 15 polycyclic aromatic hydrocarbons (PAHs) in fishery products, shellfish, meat products and spices (n = 140). Benzo[a]pyrene (BaP) was detected in the mean concentration of 0.11 μg/kg in mussels to 4.85 μg/kg in spices. However, none of the samples exceeded the maximal BaP and ∑PAH4 limit, set out under the European legislation, although high values determined in some food, especially dried herbs and spices, pointed towards a heavy contamination and the necessity for systematic controls. The study showed that processed food samples contained significantly higher ($p<0.05$) PAH levels in comparison to food coming from environmental sources.

Keywords: Croatian market, dried herbs and spices, fresh shellfish, meat products, polycyclic aromatic hydrocarbons, smoked fish

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are represented by roughly 660 different compounds and include highly hydrophobic and diverse organic compounds that have two or more fused aromatic rings (ZELINKOVA and WENZL, 2014; SINGH *et al.*, 2016). They pose as ubiquitous environmental pollutants that can be found in fresh water and marine sediments, the atmosphere and ice. The major reason for concern as regards human exposure to PAHs arises on the grounds of their carcinogenic, mutagenic and teratogenic effects (FALCÓ *et al.*, 2005; REINIK *et al.*, 2007; RENGARAJAN *et al.*, 2015). The United States Environmental Protection Agency (US-EPA) tagged 16 PAHs as priority pollutants based on the frequency of their occurrence and their carcinogenicity (EPA, 1994). The 16 PAHs include acenaphthene (ACE), acenaphthylene, anthracene (ANTHR), benz[a]anthracene (BaA), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[ghi]perylene (B[ghi]P), benzo[a]pyrene (BaP), chrysene (CHR), dibenz[a,h]anthracene (D[ah]A), fluoranthene (F), fluorene (FLR), indeno[1,2,3cd] pyrene (I[cd]P), naphthalene (NAP), phenanthrene (PHEN) and pyrene (PYR). Among them, those containing up to four fused benzene rings are known as light PAHs, while those containing more than four benzene rings are known as heavy PAHs and are more toxic and more stable as compared to their light counterparts.

Food consumption represents the main route of PAH exposure for non-smokers and non-occupationally exposed adults (ALOMIRAH *et al.*, 2011). The routes of PAH food contamination include direct contamination from natural and anthropogenic environmental sources present in air, water and soil, as well as contamination with PAHs formed throughout thermal food processing (e.g. drying, smoking, heating, baking, frying, roasting, grilling) (EFSA, 2008). PAHs most commonly found in food are BaP, BaA, CHR, D[a,h]H, PYR, ANTHR, F and BbF (YEBRA-PIMENTEL *et al.*, 2015). Available profile studies of both unprocessed (seafood, mussels) and processed food have shown the predominance of light over heavy PAHs. However, the presence of toxicologically important heavy (high molecular weight) PAHs that include CHR, BaA, BaP and BbF, has often been reported in certain whole smoked meat products (smoked pork speck, smoked chicken, smoked pork) and chopped meat products, such as various sausages (REINIK *et al.*, 2007; PURCARO *et al.*, 2009; KUBIAK *et al.*, 2015; ROZENTÄLE *et al.*, 2015; ROZENTÄLE *et al.*, 2018). Dominating PAHs of toxicological concern present in mussels are CHR, BbF and BkF (MERCOGLIANO *et al.*, 2016). It has been revealed that spices and herbs, which are important ingredients of many processed food items, are often contaminated with PAHs of the similar low molecular profile, with the prevalence of CHR similar to that in the above-mentioned foodstuffs (ROZENTÄLE *et al.*, 2018).

In order to protect public health and to keep food contaminants at toxicologically acceptable levels, food authorities of different countries have established the maximum levels (MLs) of PAHs for different food categories in which higher PAH levels are to be expected. The European Union legislation stipulates the limits for BaP and the sum of four PAHs - ∑PAH4 (BaA, CHR, BbF, BaP) in more than 10 groups of foodstuffs and is therefore the most comprehensive applicable regulation worldwide (EC REGULATION No. 835/2011, ZELINKOVA and WENZEL, 2016). Bivalve molluscs (fresh, chilled or frozen) in which environmental pollution might result in high contamination levels, are allowed MLs of 5μg/kg for BaP and 30 μg/kg for ∑PAH4. The MLs of PAHs in processed fish and meat products are set at 2 μg/kg for BaP and 12 μg/kg for ∑PAH4. However, there exists the list of EU countries that are allowed to continue using traditionally smoked fish and smoked meat products containing higher PAH levels (5 μg/kg for BaP and 30 μg/kg for ∑PAH4) (EC REGULATION No. 1327/2014). Despite the application of good smoking practices, lower PAH levels in these traditional foodstuffs have not been

achieved yet, since these products require smoking practices that significantly change their organoleptic characteristics. A three-year derogation from the obligation to observe and respect lower BaP and ∑PAH4 MLs expired in the autumn of 2017, but in half of the Member States the former MLs are still in force, pending the adoption of new, reassessment-based provisions. Recently, MLs of 10 μg/kg and 50 μg/kg were set out for BaP and ∑PAH4 in dried herbs and spices (EC REGULATION No. 1933/2015) in response to high PAH levels determined in the above in the recent years due to poor drying practices.

The main objective of this study was to evaluate the level of contamination of certain food items coming from the Croatian market with the ∑PAH15 referred to above (except for the non-fluorescent acenaphthylene), and to compare the levels of contamination from two major sources (environmental source *vs* food processing technique). In order to establish the impact of environmental sources, bivalve molluscs have been investigated, whereas the food processing impact was investigated in fishery products, meat products and spices. In order to establish the occurrence and toxicity of PAHs in food under study, low molecular PAH (ACE, ANTHR, BaA, CHR, F, FLR, NAP, PHEN, PYR), high molecular PAH (BbF, BAP, BkF, B[ghi]P, D[a,h]A, I[cd]P), ∑PAH4 (BaA, CHR, BbF, BaP) and ∑PAH8 (BaA, CHR, BbF, BaP, BkF, B[ghi]P, D[a,h]A, I[cd]P) contents were determined. Although the assessment of dietary exposure to PAHs does not fall within the scope of this study, for the sake of comparison the PAH contents and the respective sums are also expressed as benzo[a]pyrene equivalents (BaPE), so as to illustrate the toxicity of the investigated PAHs, as well as to simplify the interpretation of real-life risk for human health.

2. MATERIAL AND METHODS

2.1. Sampling and sample preparation

Food samples (n = 140) were obtained from the Croatian market during 2017 – 2018 and divided into four groups, as follows: fresh shellfish (n = 42), smoked fishery products (n = 8), meat products (n = 70) and dried herbs & spices (n = 20). Sample collection and storage were performed in accordance with the European legislation (EC REGULATION No. 333/2007, EC REGULATION No. 836/2011) so as to avoid PAH losses (ZELINKOVA and WENZEL, 2016).

Five fresh bivalve species (n = 42), including mussels (*M. galloprovincialis*, n = 29), oysters (*O. edulis*, n = 2*)*, variegated scallops (*C. varia*, n = 4), warty venus shells (*V. verruscosa*, n = 3) and smooth clam (*C. chione,* n = 4) were collected from local markets along the Croatian coastline. Samples containing approximately 4 kg of shellfish were transported to the Laboratory in cooled dim containers within 24 hours post sampling. Twenty five pieces of variegated scallops, mussels and warty venus shells and fifteen pieces of oysters and smooth clams of similar shell lengths were randomly selected from each sample and put together for the analysis; shells were than discarded, while soft tissues were homogenized (Grindomix, GM 200, Retsch, Haan, Germany) and stored at -20°C pending analysis.

Smoked fishery products (n = 8), including hot-smoked sea bass (n = 3) and sea bream (n = 1) fillets, cold smoked trout (n = 1) and tuna (n = 1) fillet, smoked sardine in sunflower oil (n = 1) and smoked salmon pate (n = 1), were obtained from the local Croatian market. In order to ensure a representative sample of fishery products, the whole package content was homogenized (Grindomix, GM 200, Retsch, Haan, Germany) and stored at -20°C pending analysis.

The meat sample pool (n = 70) consisted of 10 samples of fermented sausages (Istrian Rožica, dry homemade sausage, Kulen, tea sausage and other salami and sausages), 10 samples of semi-dry smoked sausages (Bodulska, ham, homemade sausage, Kranj sausage, Kvarner sausage, grill sausage and peasant sausage), 35 dry-cured meat samples (buđola, dry bacon, dry ham, dry sirloin and other products) and 15 semi-dry-cured meat samples (dry ham, smoked chops, smoked dry porcine shank, smoked rack, smoked ribs, smoked rolled shoulder with skin and other products). The samples were homogenized (Grindomix GM 200, Retsch, Haan, Germany) at different speeds for a different length of time depending on the type of meat product, and then stored at -20°C pending analysis.
The selected dried herb and spice samples (n = 20) included clove (*Caryophyllus aromaticus)* (n = 1), grounded garlic (*Allium sativum*) (n = 2), grounded ginger (*Zingiber officinale*) (n = 1), laurel (*Laurus nobilis*) (n = 1), grounded red paprika (*Capsicum spp*) (n = 2), grounded smoked red paprika (*Capsicum spp*) (n = 4), grounded black pepper (n = 4), mixed pepper (*Piper nigrum*) (consisting of black, green, white and red pepper, n=1), mixed dried spices (consisting of tomato, rosemary, basil and oregano) (n = 1), parsley (*Petroselinum crispum*) (n=1) and rosemary (*Rosmarinus officinalis*) (n = 2). Ungrounded samples were cut or crushed and then sieved through a 1.5-mm sieve.

2.2. Standards and reagents

All chemicals used (e.g. dichloromethane, acetonitrile) were of a HPLC grade. Ultrapure water was produced by a Millipore, Direct-Q 3 UV system (Millipore, Molsheim, France). The glassware was washed with a detergent and water, rinsed with acetone and dichloromethane, and dried at 50°C for an hour before use (European Commission, 2007).
The certified standard mix solution 16 Priority PAH, Cocktail 3, 16 comp.(10 μg of each/mL in acetonitrile) containing ACE, acenaphthylene, (ANTHR), (BaA), (BbF), (BkF), (B[ghi]P), (BaP), (CHR), (D[ah]A), (F), (FLR), I[cd]P, (NAP), (PHEN) and (PYR), was obtained from the Chiron, Trondheim, Norway. Standard mix working solutions (concentration ranges 0.15 μg/kg to 8.50 μg/kg) containing PAH16 were prepared by virtue of diluting the stock solution with acetonitrile and then stored at + 4°C in darkness. Benzo[b]chrysene (BbC) was supplied by Interchim (Montlucon, France).
The reference materials of (frozen) bivalve molluscs (ILC1060, ID025), smoked meat (ILC 424, ID109) and smoked black pepper (ILC 334, ID087), were supplied by the European Commission Joint Research Centre European Union Reference Laboratory for Polycyclic Aromatic Hydrocarbons (Institute for Reference Materials and Measurements, Geel, Belgium), while the smoked fish reference material (T0672QC) was purchased from FAPAS (Sand Hutton, York, UK).

2.3. Extraction and clean-up

PAHs were determined using the slightly modified method described by WEGRZYN and co-authors (2006) PAH isolation involves a preparative size-exclusion chromatography allowing for the efficient single-step lipid removal without saponification; within this frame, benzo[b]chrysene is used as an internal quantification standard. A homogenized sample (1 g) was spiked with BbC (50 μg/L, 100 μL) and diluted with dichloromethane to the final 4-mL volume. A sample was homogenized and vortexed for 10 min. The obtained mixture was centrifuged for 10 min at 3 500 rpm and 4°C. The supernatant was decanted and filtered through a 0.22-μm PTFE syringe on-line filter (Phenomenex, Torrance, USA) and transferred into a glass HPLC vial. Sample extracts were injected into a HPLC Agilent 1200 Series system (Agilent, Singapore, Singapore) for size-exclusion chromatography (SEC) with a fraction collector (Gilson, FC203B, Middleton, USA). The preparative SEC

was performed under isocratic conditions (100% dichloromethane) at the flow rate of 1 mL/min and room temperature using 2 size-exclusion columns connected in series (packed with PL gel based on PS/DVB, 300 × 7.8 mm i.d., 5 μm particle size and 50 Å) provided by Phenomenex (Phenomenex, Torrance, USA). The injection volume was 400 μL. Chromatograms were monitored at 254 nm and fractions were collected within 18 - 24 min timeframe. Aliquots were evaporated to dryness in a rotational vacuum concentrator (RCV2-18HCL; Christ, Osterode am Harz, Germany) at the speed of 1,300 rounds per minute; this stage took 40 min and went on at 20°C. The residue was dissolved in 100 μL of acetonitrile, so as to undergo chromatographic analysis (UPLC-FLD).

2.4. UPLC-FLD analysis

The UPLC PAH analysis was performed using an Ultra Pressure Liquid Chromatograph (Agilent 1290 Infinity UHPLC, Agilent, Singapore) equipped with a binary gradient pump (G4220A) and an auto-sampler having a thermostated sample compartment (G4226A), a thermostated column compartment (G1316C) and a fluorescence detector (G1321B). The separation of compounds was done in a Hypersil Green C18 PAH analytical column (150 mm x 3.0 mm i.d., 3.0 μm particle size) with a C18 guard Hypersil Green PAH column (10 mm x 3.0 mm i.d., 3.0 μm particle size) supplied by Thermo Scientific (Thermo-Scientific, Germany), the maintained temperature thereby being 30°C and the injection volume being 15 μL. The mobile phase consisted of a mixture of acetonitrile and acetonitrile/water (1/1) and was operated in the gradient mode at the flow rate of 0.8 mL/min (WEGRZYN *et al*., 2006). The initial composition of 100 % of acetonitrile/water (1/1) increased to 100% of acetonitrile in 30 min. The initial conditions were reached in 5 min. The total run time was 35 min. The excitation and emission wavelength pairs (excitation-Ex, emission-Em) used with fluorescence detection were as follows: Minute 2: Ex = 270 nm, Em = 340 nm for NAP; Minute 6.5: Ex = 250 nm, Em = 310 nm for FLR, ACE; Minute 8.5: Ex = 250 nm, Em = 380 nm for PHEN, ANTHR; Minute 11.0: Ex = 250 nm, Em = 460 nm for F, Minute12.1: Ex = 270 nm, Em = 385 nm for PYR; Minute: 15.5 Ex = 256 nm, Em = 395 nm for BaA, CHR; Minute 19.0: Ex = 295 nm, Em = 466 nm for BbF; Minute 21.5: Ex = 250 nm, Em = 410 nm for BkF BaP, D[ah]A, B[ghi]P; Minute 27.4: Ex = 295 nm, Em = 500 nm for I[cd]P; Minute 28.3: Ex = 460 nm, Em = 250 nm for BbC. The compounds were quantified using internal calibrations curves plotted for each of the 15 PAHs at seven concentration levels ranging from 0.15 to 8.5 μg/kg. Standard mix working solutions containing PAHs in different concentrations and a fixed amount of internal standard (5 μg/kg) were prepared in acetonitrile and injected in duplicates (15 μL per injection) so as to be able to come up with the linear regression lines.
Each sample of food products under study was analyzed in duplicate; the final PAH content was calculated as the mean of two parallel runs and expressed in μg/kg (of wet weight). The PAH content calculation also included the toxic equivalency factors (TEF) approach, so that PAH concentrations are also expressed as benzo[a]pyrene toxic equivalents; to that effect, the converting factors referred to by LAW *et al.* (2002) were used. The benzo(a)pyrene equivalent concentration (BaPE), expressed in μg/kg of food, is calculated as follows:

$$BaPE = \sum(BaPE) = \sum(C_{PAHi} \times TEF_{PAHi}),$$

where C_{PAHi} represents the concentration of the PAH congener i in food (μg/kg, while TEF_{PAHi} represents the toxic equivalency factor of the PAH congener i.

In order to illustrate the toxic potency of the investigated PAHs, the ∑PAH15 concentration, ∑PAH8 concentration and ∑PAH4 concentration are expressed as BaPE ($BaPE_{\sum PAH4}$, $BaPE_{\sum PAH8}$, $BaPE_{\sum PAH15}$).

2.5. Validation of the method and analytical quality assurance

In-house validated method for the respective matrices, i.e. dried herbs and spices, fishery products, shellfish and meat products, was applied. The performance assessment criteria included applicability, the limit of detection (LOD), the limit of quantification (LOQ), precision ($HORRAT_r$, $HORRAT_R$), specificity, linearity and recovery. The limit of detection (LOD) was calculated from the average of ten PAH15-negative samples (consisting of shellfish, smoked fish, smoked meat or spices), earlier analysed for PAH presence and used for validation as the blank material; to the above average, the tripled standard deviation was added (LOD = mean ± 3SD) (EC REGULATION No 582/2016). In order to determine the limit of quantification (LOQ), the mean concentration determined in ten PAH-negative samples of each matrix was summed up with the six-fold standard deviation (LOQ = mean ± 6SD). The precision of the method was assessed using the Horwitz equation as the Horrat value descriptive of each PAH at three concentration levels under repeatable (HORRATr) and reproducible ($HORRAT_R$) conditions. Food samples were spiked at the concentrations of 0.5, 1 and 1.5 of the MLs defined for 4 PAHs in triplicate. For each PAH, the mean recovery of a 36-sample set was calculated and used for the accuracy assessment, evaluated based on the intra-laboratory coefficient of variation. The specificity was checked by analyzing 15 PAHs in each of the ten blank samples per tested matrix and verifying the presence of interferences in the region of interest where single PAHs were expected to elute. The linearity was checked through the regression coefficients of determination (r^2) of the analytical curves using the standard mix working solution containing 15 PAHs at seven concentration levels ranging from 0.15 to 8.5 μg/kg. In this study, the presence or absence of matrix effects was identified using calibration curves and a fixed amount of internal standard (5 μg/kg) obtained with matrix-matched calibration standards (all matrices of food groups investigated in the study) and calibration solutions in the solvent. In the first step a three-point calibration curve (1, 2 and 4 μg/kg for meat and fish products; 2.5, 5 and 10 μg/kg for dried herbs and spices and shellfish products) was plotted using linear regression with the calibration standards in the solvent solution. In the next step, another three-point calibration curve of the same concentrations per food group and a fixed amount of internal standard (5 μg/kg) was plotted based on the measurement data of the matrix-matched calibration standards. The slopes of the regression curves representative of the two sets of calibration solutions were evaluated statistically. Internal quality control was pursued with each analytical batch using the available reference material (frozen mussels, ILC1060, ID025; T0672QC smoked fish, smoked meat, ILC 424, ID109 and ILC 334 and ID087 smoked black pepper), and was carried out by virtue of spiking the food samples so as to obtain the concentration of 2 μg/kg. Within each analytical series, the reference materials and the spiked food samples were analysed in duplicate and checked for recovery. The interpretation of validation and quality assurance results was performed as proposed by the EC REGULATION No 836/2011.

2.6. Statistical analysis

Data analysis was carried out using the XLSTAT 2018.3.51141 Software package (Addinsoft, New York, USA). The Kruskal-Wallis one-way analysis of variance was

performed for each data set so as to detect differences among food groups, considered to be statistically significant if estimated at the level of probability of $p = 0.05$.

3. RESULTS AND DISCUSSION

3.1. Validation of the method and quality assurance results

The results concerning linearity, LOQ, recovery and $HORRAT_R$ are presented in Table 1 and Fig. 1 shows a chromatogram descriptive of the target PAHs in a standard (c = 1.06 μg/kg) and a smoked ham sample spiked at the level of 1.96 μg/kg. The LOD established for the studied food groups ranged from 0.02 to 1.00 μg/kg, while the LOQ varied from 0.15 to 1.84 μg/kg.
The recoveries obtained within the frame of internal quality control spanned from 50 to 120 %, which is in accordance with the criteria established by the EC REGULATION No. 836/2011. The mean slopes of the regression curves for the standard and matrix sets of calibration solutions were not significantly different. Therefore, matrix-matched standard calibrations were not used. The applied analytical method fulfils all methodological requirements set out by the EC REGULATION No. 836/2011 and can therefore be considered as suitable for the determination of 15 PAHs in food categories under this study.

3.2. Shellfish products

Bivalve molluscs, posing in this study as unprocessed food representatives, are exposed to PAHs ubiquitously present in the marine environment due to polluted sediments, spill residues, shipping activities (de-ballasting waters), industrial and urban runoff, and atmospheric pollution (SORIANO *et al*., 2006). Furthermore, they are widely used in coastal monitoring programmes and pollution assessment studies as filter-feeders having a slow rate of detoxification and the ability to accumulate many toxic contaminants.
In this research, shellfish products were divided into two groups: mussels and other shellfish. The mussels were produced on farms, while other shellfish came from natural habitats in the Adriatic Sea. In cultured mussels, the predominance of PHEN, FLR, F and PYR was established (Table 2).
Other shellfish showed the abundance of PHEN, ACE and F, with the highest PHEN and ACE levels found in warty venus and the F levels in oysters. Statistically significant percent-shares of low molecular (78.9 % in mussels and 69.6 % in other shellfish) (Fig. 2) as compared to those of high molecular PAHs, obtained in this study (Fig. 3), were also reported in the studies by BIHARI *et al.* (2007), PERUGGINI *et al.* (2007), SERPE *et al.* (2011), and MERCOGLIANO *et al.* (2016) for shellfish coming from other Adriatic and Mediterranean areas. The majority of bivalve species collected from the Croatian market contained the investigated ∑PAH15 in concentrations ranging from 0.71 μg/kg to 14.49 μg/kg in mussels, and from 6.07 μg/kg to 27.45 μg/kg in other shellfish products (Table 3).
As for toxicologically important PAH markers, significantly higher ∑PAH4 and ∑PAH8 were found in other bivalve species, in particular in oysters, with a predominance of I[cd]P (6.99 μg/kg), BbF (4.02 μg/kg) and CHR (3.16 μg/kg). High- and medium-molecular PAHs in marine ecosystems are mostly of pyrolytic origins (MERCOGLIANO *et al*., 2016), so that their occurrence in other bivalve species under this study may also come from pyrolytic sources (antropogenic pollution coming from the mainland). A statistically significant difference in BaP content was found amongst the investigated bivalve

molluscs, above all when it comes to the smooth clam (Table 3). This study confirmed that farmed shellfish species (mussels) are characterized with lower levels of toxicologically important PAHs as compared to native shellfish species, which is in line with the results obtained in the studies by MERCOGLIANO *et al.* (2016) and ZELINKOVA *et al.* (2015). The majority of BaP and ∑PAH4 levels found in shellfish harvested along the Croatian coast were far below the MLs of 5 μg/kg and 30 μg/kg, respectively EC REGULATION No. 835/2011, as can be seen in Table 3, and are in accordance with the findings in mussels harvested along the Adriatic, the Campanian and the Ionian coasts of Italy (STORELLI and MARCOTRIGIANO, 2011; SERPE *et al.*, 2010). The highest ∑PAH15 (27.5 μg/kg and 26.5 μg/kg) and ∑PAH8 (14.3 μg/kg) were determined in oysters and warty venus, whereas mussels showed the highest BaP content. Significant differences in $BaPE_{\Sigma PAH4}$, $BaPE_{\Sigma PAH8}$ and $BaPE_{\Sigma PAH15}$ values were observed, with the highest values established in oysters. Literature sources have reported $BaPE_{\Sigma PAH15}$ values of 1.56 μg/kg ww determined in Mediterranean mussels coming from the Adriatic Sea (PERUGINI *et al.*, 2007) and ranges of 0.1 to 4.5 μg/kg dry wt in shellfish coming from the Red Sea (EL NEMR *et al.*, 2016). PAH uptake depends on the physiology of the up-taking organisms and cyclic annual variations. In accordance with the EU legislation, monitoring of aqua-cultured shellfish is carried out along the Croatian coast, so as to ensure that PAH levels are within the consumer safety limits (BOGDANOVIĆ *et al.*, 2014). The study results revealed that sampling techniques developed for PAH monitoring in cultivated and wild shellfish found along the Croatian coast, minimize any risk for human health coming from the seafood consumption which has generally been tagged as one of the main sources of human exposure to severe pollutants.

3.3. Smoked fish

The majority of PAHs present in smoked food originate from wood smoke. While the amounts of PAHs in raw fish are very low due to the fish ability to oxidize and further metabolise PAHs absorbed from the environment, cold and hot-smoked fish are generally characterised with higher PAH contents that depend on fish properties, methods and parameters of fish smoking, composition of the smoke and the level of exposure of edible fish parts to the smoke released (DUEDAHL-OLESEN *et al.*, 2010; STOLYHWO *et al.*, 2005; ZELINKOVA *et al.*, 2015). Furthermore, if a fishery product is canned in oil, the contamination may arise due to vegetable oil. Similar to meat products, the skin acts as a barrier against smoke particles, hence preventing any significant PAH penetration into fish muscles.

The mean PAH contents determined in smoked fish samples in this study are reported in Table 2. PAH profiling of fishery products coming from the Croatian market revealed the predominance of four light PAHs (ACE, PHEN, FLR and ANTHR) (Table 2, Fig. 2), which is in accordance with the results reported for commercial smoked fish (VARLET *et al.*, 2007; ZELINKOVA *et al.*, 2015; DUEDAHL-OLESEN *et al.*, 2018). PHEN, ANTHR, PYR and F were detected in all samples, the highest concentration of PHEN thereby being detected in smoked salmon pate (5.76 μg/kg). I[1cd]P (6.63 μg/kg) was determined in only one smoked sea bass sample. The analysis of the selected fishery products revealed the highest ∑PAH15 values in smoked salmon pate (118.05 μg/kg), followed by smoked sea bream (35.29 μg/kg) and smoked trout (32.13 μg/kg), while the lowest value was established in smoked tuna (1.17 μg/kg). The PAH amounts found in fish samples positively correlate with the lipid content of the same (SINGH *et al.*, 2016), which may explain the highest ∑PAH15 values obtained in smoked salmon pate within our study frame. As stated above, the amount of PAHs transferred by smoke particles into the final fishery product depends on several processing parameters including smoking technology,

combustion temperature, smoke composition, type of wood used, and the level of exposure of edible fish parts to the smoke released (STOLYHWO *et al*., 2005, DUEDAHL-OLESEN *et al*., 2010). Among the mutagenic/carcinogenic PAHs analysed, the highest BaP (0.76 μg/kg), ∑PAH4 (1.70 μg/kg) and ∑PAH8 (11.56 μg/kg) (Table 3) were detected in smoked sea bass fillets, while the lowest BaP (< LOD μg/kg), ∑PAH4 (0.18 μg/kg) and ∑PAH8 (0.22 μg/kg) were determined in smoked tuna. The aggregated average ∑PAH8 content decreased in the following order: I[cd]P, B[bghi]P, CHR, D[ah]A, B[b]F, B[a]P, B[a]A and B[k]F (Tables 2 and 3). The investigated fishery products complied to the PAH MLs of 2 μg/kg for B[a]P and 12 μg/kg for ∑PAH4, laid down under the EU legislation. BaP-based toxic equivalency factors calculated for ∑PAH4, ∑PAH8 and PAH15 showed a similar decreasing pattern starting with the highest values found in smoked sea bass that declined over smoked sardine in sunflower oil and smoked salmon pate down to the lowest values in smoked trout (Table 3). Smoked salmon pate, most heavily contaminated with PAH15, was characterized with low BaPE levels, while smoked sea bass fillets, moderately contaminated with PAH15, showed the highest BaPE levels, which can be attributed to the presence of heavy PAHs that have higher TEF values. A similar $BaPE_{PAHtotal}$ trend has been observed in different food items, for instance in the study by PERUGINI *et al.* (2007) in fish and shellfish, in the study by SANTOS *et al.* (2011) in meat products, and in the study by GOMES *et al.* (2013) in traditional meat sausages.

3.4. Meat products

Smoked meat products represent the principal source of PAHs that generate during an incomplete wood combustion (ALVES *et al.* 2018). As already well known, the amounts and types of PAHs present in contaminated smoke meat products depend on a number of factors, such as the fuel used, the smoking technique, the temperature at which the pyrolysis takes place, the air flow through the smoke generator, the distance between the meat sample and the heat source, the smoking chamber design, the smoked meat fat content, the duration of smoking, and the cleanness and maintenance of the equipment used (CODEX ALIMENTARIUS COMMISSION, 2009). Data on PAHs in smoked meat products are highly variable, this discrepancy being explained by differences in food smoking procedures and meat product characteristics (whole meat *versus* chopped meat products). Spices used in smoked meat production can also be contaminated with PAHs, therefore further increasing the levels of those hazardous contaminants.
Smoked meat products investigated in this study were divided into four groups, consisting of either whole meat samples in terms of dry-cured and semi-dry-cured meat products, or chopped meat products in terms of dry-fermented and semi-dry sausages. ∑PAH4 and ∑PAH8 composition pattern was dominated by light, harmless PAHs (93.0 % in semi-dry meat products to 98.3 % in dry-fermented sausages). Amongst them, the four PAHs most abundantly present in dry-cured meat products were (in descending order) PHEN, FLR, ACE and ANTHR. Similarly, dry-fermented sausages were also characterized by light PAHs supremacy, their representation thereby following virtually the same PHEN-ACE-FLR-ANTHR decreasing pattern described above, while both semi-dry-cured meat products and semi-dry sausages showed a little bit different PAH presence pattern dominated by PHEN, F, ACE and FLR in decreasing order for the former meat products and by PHEN, FLR, F and PYR in decreasing order of the latter meat products (Table 2, Fig. 2).

Table 1. Selected performance indicators of the method in use: linearity, limit of quantification (LOQ), recovery and precision (HORRAT$_R$).

PAHs [a]	Linearity (r^2)[b]	Dried herbs and spices			Shellfish			Smoked fish			Smoked meat		
		LOQ[c] (μg/kg)	Recovery[d]	HORRAT$_R$[e]	LOQ (μg/kg)	Recovery[d]	HORRAT$_R$[e]	LOQ (μg/kg)	Recovery[d]	HORRAT$_R$[e]	LOQ (μg/kg)	Recovery[d]	HORRAT$_R$[e]
NAP	0.998	3.14	71.22	1.88	1.85	76.18	1.66	2.90	77.72	0.99	2.97	119.2	1.08
FLR	0.999	3.30	103.5	0.99	3.23	113.9	0.76	2.94	76.88	0.76	3.30	120.0	1.33
ACE	0.999	3.27	91.03	1.15	0.63	102.2	1.02	2.61	71.08	0.98	3.00	88.20	1.25
PHEN	1.000	0.30	69.77	0.79	0.79	117.0	1.32	0.40	82.95	1.44	0.79	98.25	1.06
ANTHR	1.000	0.43	86.61	1.36	0.50	112.9	0.85	0.69	64.21	0.85	0.50	100.0	0.85
F	0.997	0.50	89.61	2.00	0.40	116.5	1.65	0.59	92.05	1.77	0.30	100.0	0.86
PYR	0.999	0.59	92.06	0.86	0.86	93.87	1.14	0.89	80.34	1.14	0.86	100.0	1.14
B(a)A	0.999	0.26	75.56	1.28	0.07	116.0	1.89	0.23	68.56	1.89	0.07	90.40	1.89
CHR	1.000	0.17	94.54	1.66	0.07	117.2	1.18	0.13	76.82	1.18	0.07	88.80	1.18
B(b)F	1.096	0.23	99.99	0.89	0.26	106.4	0.87	0.17	71.86	0.87	0.26	94.71	0.08
B(k)F	1.000	0.23	83.66	1.33	0.20	93.55	1.11	0.17	78.73	1.11	0.20	100.0	1.11
B(a)P	1.000	0.26	76.99	1.65	0.10	94.15	0.71	0.40	75.84	0.71	0.10	82.00	0.71
D[ah]A	0.998	0.63	88.77	1.18	0.66	88.48	0.84	0.69	81.05	0.84	0.76	77.00	0.84
B[ghi]P	0.972	0.56	70.66	1.44	0.56	86.49	1.13	0.53	81.99	1.13	0.50	100.0	1.13
I[cd]P	0.973	0.26	69.05	0.88	0.30	78.32	0.14	0.36	85.24	1.14	0.30	68.75	0.14

[a]Polycyclic aromatic hydrocarbons (PAHs): acenaphthene (ACE), acenaphthylene, anthracene (ANTHR), benz[a]anthracene (BaA), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[ghi]perylene (B[ghi]P), benzo[a]pyrene (BaP), chrysene (CHR), dibenz[a,h]anthracene (D[ah]A), fluoranthene (F), fluorene (FLR), indeno[1,2,3cd] pyrene I[cd]P, naphthalene (NAP), phenanthrene (PHEN) and pyrene (PYR); [b]Determination coefficients (r^2) of the analytical seven-point curves constructed for standard solutions (0.25-8.50 μg/kg);[c]Limit of quantification; [d]Mean recovery at three concentrations used in the precision assessment (selected food categories were spiked at the concentrations of 0.5, 1 and 1.5 of MLs); [e]Horrat coefficient (EC REGULATION No. 836/2011) for each PAH at three concentrations (selected food categories were spiked at the concentrations of 0.5, 1 and 1.5 of MLs value) in reproducibility (R) conditions used for the method precision assessment.

Table 2. Determination of PAHs levels in selected food from Croatian market.

	Fresh shellfish						Meat products									
	Mussels (n^a = 29)		Other shellfish (n^a = 13)		Smoked fish (n^a = 8)		Dry-cured meat products (n^a = 35)		Semi-dry cured meat products (n^a = 15)		Dry-fermented sausages (n^a = 10)		Semi-dry sausasges (n^a = 10)		Dried herbs and spices (n^a = 20)	
PAHs	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
NAP	$<0.56^{C}$	<0.56-6.62	$<0.56^{C}$	<0.56	<0.95C	<0.88-0.79	1.58^{B}	<0.55-27.60	$<0.95^{C}$	<0.55-4.09	2.55^{A}	<0.55-11.12	$<0.55^{C}$	<0.55-0.71	1.93^{B}	<0.95-27.68
ACE	0.24^{E}	<0.19-6.85	2.00^{D}	<0.19-8.11	22.30^{B}	<0.79-99.67	2.75^{D}	<0.91-25.97	1.56^{D}	<0.91-16.81	11.25^{C}	<0.91-45.69	$<0.91^{E}$	<0.91-1.01	76.34^{A}	<0.99-807.68
FLR	1.21	<0.98-4.01	<1.00	<0.98-1.27	2.79^{BC}	<0.89-9.86	4.15^{B}	<1.00-51.13	1.04	<1.00-2.33	11.14^{A}	<1.00-42.95	1.26^{C}	<1.00-4.48	6.56^{AB}	<1.00-31.74
PHEN	1.56^{E}	<0.24-4.17	2.16^{DE}	<0.24-6.29	3.14^{CDE}	<0.12-5.76	8.88^{BC}	<0.91-54.86	3.95^{CD}	<0.91-12.93	14.31^{B}	<0.91-63.54	3.19^{D}	<0.91-9.10	55.44^{A}	<0.09-473.05
ANTHR	0.16^{F}	<0.15-3.20	0.28^{EF}	<0.15-1.88	0.87^{DE}	<0.21-2.00	2.10^{CD}	<0.24-13.88	0.78^{E}	<0.24-3.38	5.38^{B}	<0.24-21.95	0.71^{E}	<0.24-2.78	13.24^{A}	<0.13-74.55
F	0.58^{CD}	<0.12-2.21	1.89^{B}	<0.12-6.96	0.56^{D}	<0.18-1.08	1.54^{BC}	<0.15-6.39	2.05^{B}	<0.15-10.22	1.80^{B}	<0.15-5.39	1.12^{BC}	<0.15-3.45	22.87^{A}	<0.15-124.63
PYR	0.49^{D}	<0.26-1.21	0.84^{CD}	0.30-1.94	0.78^{D}	<0.27-1.40	1.13^{C}	<0.26-10.88	1.02^{CD}	<0.26-4.27	1.94^{B}	<0.26-7.07	0.89^{C}	<0.26-2.71	26.31^{A}	<0.18-163.86
B[a]A	0.10^{C}	<0.02-0.62	0.22^{B}	<0.02-0.75	0.10^{C}	<0.07-0.19	0.15^{C}	<0.02-1.41	0.14^{C}	<0.02-0.66	0.09^{C}	<0.02-0.28	0.07^{C}	<0.02-0.21	5.76^{A}	<0.08-25.39
CHR	0.45^{B}	<0.02-0.98	0.53^{B}	<0.02-3.16	0.27^{CD}	<0.04-0.61	0.30^{CD}	<0.02-1.9	0.20^{CD}	<0.02-0.65	0.27^{CD}	<0.02-0.57	0.18^{D}	<0.02-0.38	10.15^{A}	<0.05-45.68
B[b]F	0.40^{BC}	<0.08-1.27	0.76^{B}	<0.08-4.02	0.19^{DE}	<0.05-0.52	0.26^{DE}	<0.08-1.78	0.13^{DE}	<0.08-0.49	0.14^{DE}	<0.08-0.30	0.08^{E}	<0.08-0.25	4.83^{A}	<0.07-20.86
B[k]F	0.23^{B}	<0.05-0.71	0.40^{B}	<0.05-2.14	$<0.05^{C}$	<0.05-0.09	0.15^{BC}	<0.06-1.55	0.10^{C}	<0.06-0.58	0.09^{C}	<0.06-0.17	$<0.06^{C}$	<0.06-0.19	1.55^{A}	<0.07-11.02
B[a]P	0.11^{C}	<0.12-0.69	0.21^{B}	<0.12-0.46	0.16^{BC}	<0.12-0.76	0.19^{B}	<0.03-1.47	0.11^{C}	<0.03-0.40	0.12^{C}	<0.03-0.30	0.15^{BC}	<0.03-0.37	4.85^{A}	0.15-21.88
D[ah]A	0.11^{D}	<0.20-1.39	0.48^{B}	<0.20-1.67	0.26^{C}	<0.21-1.59	$<0.23^{D}$	<0.23-1.72	$<0.23^{D}$	<0.23-0.52	$<0.23^{D}$	<0.23-0.78	$<0.23^{D}$	<0.23-0.25	8.68^{A}	<0.19-39.74
B[ghi]P	0.29^{C}	<0.17-1.32	0.69^{B}	<0.17-1.81	0.40^{C}	<0.16-1.63	0.31^{C}	<0.15-1.54	0.37^{C}	<0.15-0.70	0.30^{C}	<0.15-0.66	0.44^{C}	<0.15-1.45	3.70^{A}	<0.17-14.29
I[cd]P	0.24^{C}	<0.09-2.66	1.14^{B}	<0.09-6.99	0.83^{B}	<0.11-6.63	0.11^{C}	<0.09-1.29	$<0.09^{C}$	<0.09-0.59	$<0.09^{C}$	<0.09-0.53	0.29^{C}	<0.09-1.67	3.80^{A}	<0.08-22.10

[a]n – number of samples; [b]PAHs - polycyclic aromatic hydrocarbons, acenaphthene (ACE), acenaphthylene, anthracene (ANTHR), benz[a]anthracene (BaA), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[ghi]perylene (B[ghi]P), benzo[a]pyrene (BaP), chrysene (CHR), dibenz[a,h]anthracene (D[ah]A), fluoranthene (F), fluorene (FLR), indeno[1,2,3cd] pyrene I[cd]P, naphthalene (NAP), phenanthrene (PHEN) and pyrene (PYR); Values expressed as < (less than) denote values lower than the detection limit. Superscript uppercase letters A, B, C, D denote statistically significant difference ($p < 0.05$) in the investigated polycyclic aromatic hydrocarbons.

Table 3. Minimum, maximum, median and mean concentrations (μg/kg) of BaP[a], ∑PAH4[b], ∑PAH8[c] and PAH15[d] with benzo(a)pyrene toxic equivalents (BaPE[e], μg/kg) detected in different food from Croatian market

	Concentration μg/kg						
	BaP[a]	**∑PAH4[b]**	**∑PAH8[c]**	**PAH15[d]**	**[e]BaPE$_{PAH4}$**	**[e]BaPE$_{PAH8}$**	**[e]BaPE$_{PAH15}$**
			Mussels (n = 29)				
Minimum	<0.03	<0.02	0.18	0.71	<0.03	<0.03	0.03
Maximum	0.69	3.56	7.70	14.49	0.63	7.96	8.12
Median	0.06	0.95	1.38	5.28	0.12	0.15	0.21
Mean	0.11^{D}	1.06^{BC}	1.93^{CD}	6.55^{C}	0.17^{BC}	0.75^{DE}	0.82^{D}
			Other shellfish (n=13)				
Minimum	0.09	0.58	1.60	6.07	0.12	0.13	0.20
Maximum	0.46	7.94	14.33	27.45	0.83	9.31	9.44
Median	0.17	1.34	2.75	9.98	0.32	1.67	1.72
Mean	0.21^{AB}	1.72^{AB}	4.42^{AB}	12.08^{C}	0.31^{A}	2.84^{AB}	2.96^{AB}
			Smoked fish (n=8)				
Minimum	<0.12	0.18	0.22	1.17	0.05	0.05	0.14
Maximum	0.76	1.70	11.56	118.05	0.80	9.44	9.64
Median	<0.12	0.59	0.86	18.91	0.11	0.14	0.28
Mean	0.16^{BC}	0.72^{CD}	2.23^{BC}	29.64^{B}	0.19^{B}	1.58^{BC}	1.72^{B}
			Dry-cured meat products (n = 35)				
Minimum	<0.03	0.07	0.09	4.50	<0.03	<0.03	<0.03
Maximum	1.47	6.28	12.13	125.17	1.80	10.54	10.81
Median	0.08	0.55	0.92	13.21	0.12	0.19	0.32
Mean	0.19^{CD}	0.90^{DE}	1.59^{E}	22.04^{C}	0.24^{BC}	0.85^{E}	1.05^{D}
			Semi-dry cured meat products (n=15)				
Minimum	<0.03	<0.02	0.31	3.09	<0.03	<0.03	0.18
Maximum	0.40	2.15	3.68	30.15	0.48	3.15	3.28
Median	0.04	0.36	0.86	9.21	<0.08	<0.08	0.14
Mean	0.11^{D}	0.58^{E}	1.18^{E}	12.10^{C}	0.14^{C}	0.44^{DE}	0.59^{CD}

Dry-fermented sausages (n=10)							
Minimum	<0.03	0.09	0.31	9.26	<0.03	<0.03	0.18
Maximum	0.30	0.97	2.01	171.48	0.33	4.24	4.51
Median	0.09	0.67	1.20	28.04	0.13	0.25	0.63
Mean	0.12^{D}	0.61^{CDE}	1.21^{DE}	44.59^{AB}	0.14^{BC}	0.90^{CD}	1.26^{BC}
Semi-dry sausasges (n=20)							
Minimum	<0.03	0.16	0.17	0.28	<0.03	<0.03	<0.03
Maximum	0.37	1.10	4.32	23.02	0.39	1.84	2.24
Median	0.14	0.35	1.03	6.48	0.15	0.29	0.38
Mean	0.15^{BCD}	0.49^{E}	1.29^{CDE}	8.76^{C}	0.17^{BC}	0.36^{DE}	0.51^{D}
Dried herbs and spices (n=20)							
Minimum	0.15	1.24	3.63	7.22	0.21	0.31	0.48
Maximum	21.88	113.12	207.46	1009.53	27.27	278.36	300.76
Median	0.81	4.54	8.38	49.00	1.16	8.02	10.75
Mean	4.85^{A}	25.60^{A}	43.34^{A}	246.03^{A}	6.09^{A}	49.93^{A}	53.64^{A}

[a]BaP: Benzo(a)pyrene; [b]∑PAH4: The sum of benzo(a)anthracene - BaA, chrysene - CHR, benzo(a)pyrene - BaP and benzo(b)fluoranthene - BbF; [c]∑PAH8: The sum of benzo(a)anthracene - BaA, chrysene - CHR, benzo(a)pyrene - BaP and benzo(b)fluoranthene – BbF, benzo[k]fluoranthene (BkF), benzo[ghi]perylene (B[ghi]P) dibenz[a,h]anthracene (D[ah]A) and indeno[1,2,3cd] pyrene I[cd]P; d∑PAH15: The sum of acenaphthene (ACE), anthracene (ANTHR), benz[a]anthracene (BaA), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[ghi]perylene (B[ghi]P), benzo[a]pyrene (BaP), chrysene (CHR), dibenz[a,h]anthracene (D[ah]A), fluoranthene (F), fluorene (FLR), indeno[1,2,3cd] pyrene I[cd]P, naphthalene (NAP), phenanthrene (PHEN) and pyrene (PYR) and [e]BaPE: The benzo(a)pyrene based toxic equivalency factors expressed to ∑PAH4, ∑PAH8 and ∑PAH15. Values expressed as < (less than) denote values lower than the detection limit. Different superscript uppercase letters A, B, C, D, E denote statistically significant difference ($p < 0.05$) in the investigated polycyclic aromatic hydrocarbons and benzo(a)pyrene based toxic equivalency factors of selected food groups (marked in columns).

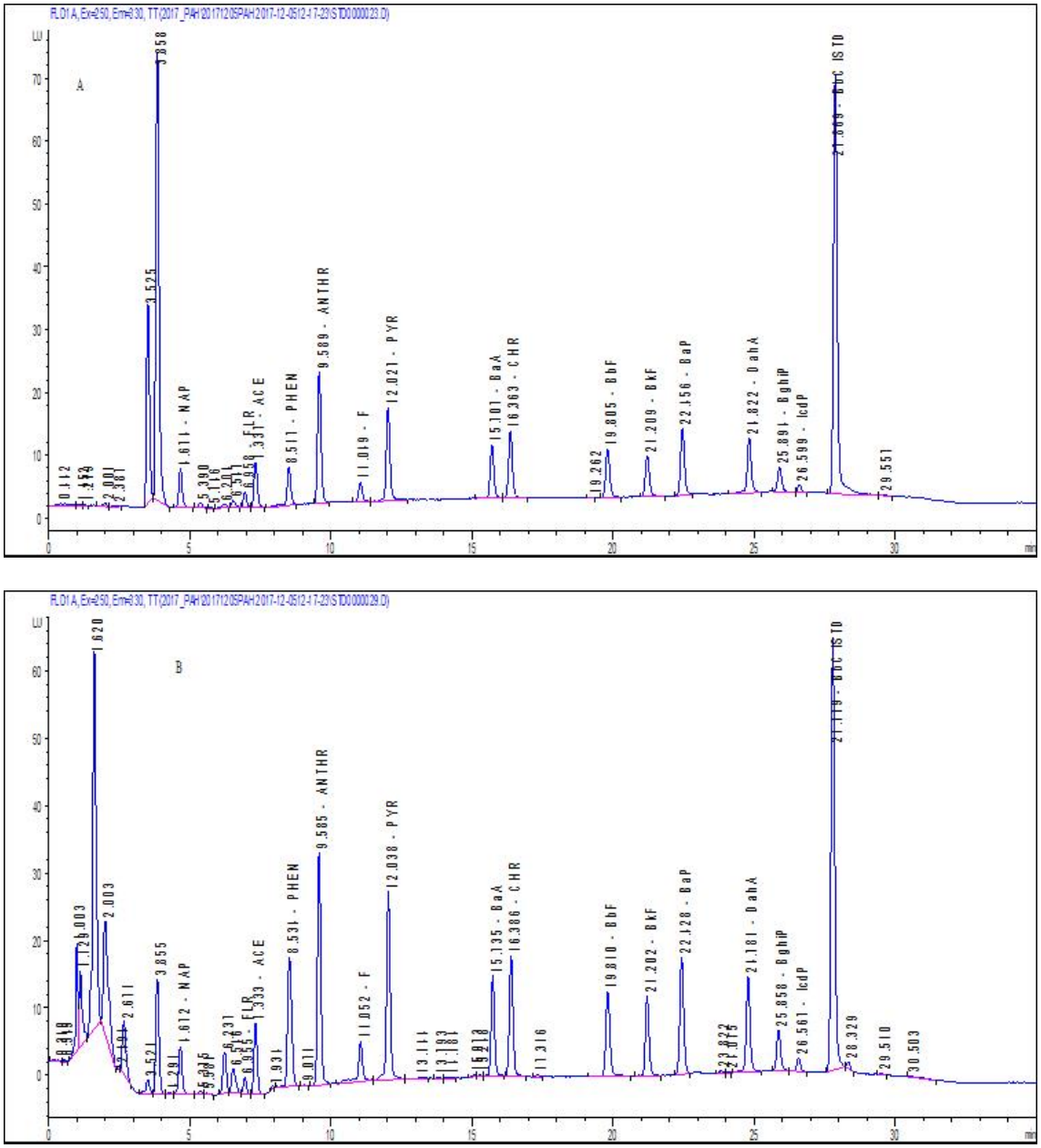

Figure 1. Chromatogram of the target PAHs (acenaphthene (ACE), anthracene (ANTHR), benz[a]anthracene (BaA), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[ghi]perylene (B[ghi]P), benzo[a]pyrene (BaP), chrysene (CHR), dibenz[a,h]anthracene (D[ah]A), fluoranthene (F), fluorene (FLR), indeno[1,2,3cd] pyrene I[cd]P, naphthalene (NAP), phenanthrene (PHEN) and pyrene (PYR) in standard sample at the level of 1.06 μg/kg (16 Priority PAH, Cocktail 3, Chiron) (A) and smoked ham sample spiked at the level 1.96 μg/kg (B)

A similar PAH profile was reported in the studies by SANTOS *et al.* (2011) and ROSEIRO *et al.* (2012), in which the prevalence of light PAHs was attributed to the unique sensorial properties of Portuguese traditional meat sausages. ALVES *et al.* (2017) also confirmed the similar pattern of low molecular PAHs' domination in fermented sausages of distinctive Portuguese and Serbian origin. Regarding the carcinogenic/mutagenic PAHs, BaP, ∑PAH4 and ∑PAH8 levels determined in this study were very low, with the highest values in sirloin (1.47 μg/kg for BaP, 6.28 μg/kg for ∑PAH4 and 12.13 μg/kg for ∑PAH8).

Significantly higher amounts were reported for BaP in smoked meat products produced in Latvia (from <0.05 μg/kg to 6.03 μg/kg) (ROZENTĀLE *et al.*, 2015) and traditional smoked meat products from the Baltic States (from 0.05 μg/kg to 166 μg/kg) (ROZENTĀLE *et al.*, 2018), with ∑PAH4 in ranges from 0.15-34.65 μg/kg and 0.42-628 μg/kg, respectively. None of the samples investigated in our study exceeded the MLs for BaP and ∑PAH4 stipulated by the pertaining legislation (2 μg/kg and 12 μg/kg, respectively) (EC REGULATION No. 835/2011). When comparing the investigated meat products based on their PAH contents expressed as benzo(a)pyrene toxic equivalencies (BaPE) (Table 3), a slightly higher $BaPE_{\sum PAH15}$ and $BaPE_{\sum PAH8}$ were established in dry-fermented sausages, while a slightly higher $BaPE_{\sum PAH4}$ was seen in dry-cured meat products. It should also be mentioned that literature sources have revealed significantly higher BaPE concentrations than those measured in whole meat and chopped meat products analysed in our study; for instance, SANTOS *et al.* (2011) and GOMES *et al.* (2013) reported that ∑PAH16 BaPE in Portuguese traditional meat/blood products range from 2.74 μg/kg to 52.38 μg/kg, while ∑PAH8 spans from 0.59 to 55.33 μg/kg and ∑PAH4 from 1.43 μg/kg to 20.18 μg/kg. As for the mean total 15 PAHs content in the studied meat products, statistically significant differences were observed. The highest average 15 PAHs sum of 171.48 μg/kg established in dry-fermented sausages comes as the consequence of the highest low molecular PAH content. When it comes to the total PAH levels, dry-cured, semi-dry-cured meat products and semi-dry sausages differed significantly from dry-fermented sausages ($p<0.05$). Statistically significant differences in toxicologically important BaP, ∑PAH4 and ∑PAH8 ($p>0.05$) failed to be found within the whole meat products' groups. Therefore, data on Croatian meat products examined within the frame of this study argue against the need for exceptions stated under EC REGULATION No. 1327/2014 under which the MLs for BaP and ∑PAH4 in traditional smoked meat and fish products is set at 5 μg/kg and 30 μg/kg, respectively. However, determination of PAHs in processed meat is a permanent process. A true assessment of risk resulting from the constant presence of PAHs in food chain requires a versatile and precise analytical method capable of measuring the level of a number of toxic PAH compounds, with the possibility of extension to additional compounds in accordance with the recommendation of the Scientific Committee on Food. Furthermore, according to the recommendation of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), benzo(c)fluorene as a compound usually, however inappropriately omitted from PAH food analyses, should be included due to its carcinogenic effects and scarce data on its occurrence in food.

3.5. Dried herbs and spices

Dried herbs and spices are defined as vegetable products, or mixtures thereof, which are free from any extraneous matter whatsoever, and are used for flavouring, seasoning and imparting the food aroma; therefore, they are classified as "all natural" (IS0, 1995; TORRE TORRES *et al.*, 2015). Since all spices come from plants, they have generally been recognised as safe (GRAS). However, even though used in small amounts, spices have also been recognized as a potential source of chemical hazards (ROZENTĀLE *et al.*, 2017). Very high levels of PAHs detected in herbs and spices present in foodstuffs (DG SANCO, 2004; EFSA, 2008) have recently resulted in new legislation requirements for BaP content, which should not exceed 10 μg/kg, and the sum of BaP, BaA, BbF and BaP, which should not exceed 50 μg/kg EC REGULATION No. 1933/2015).

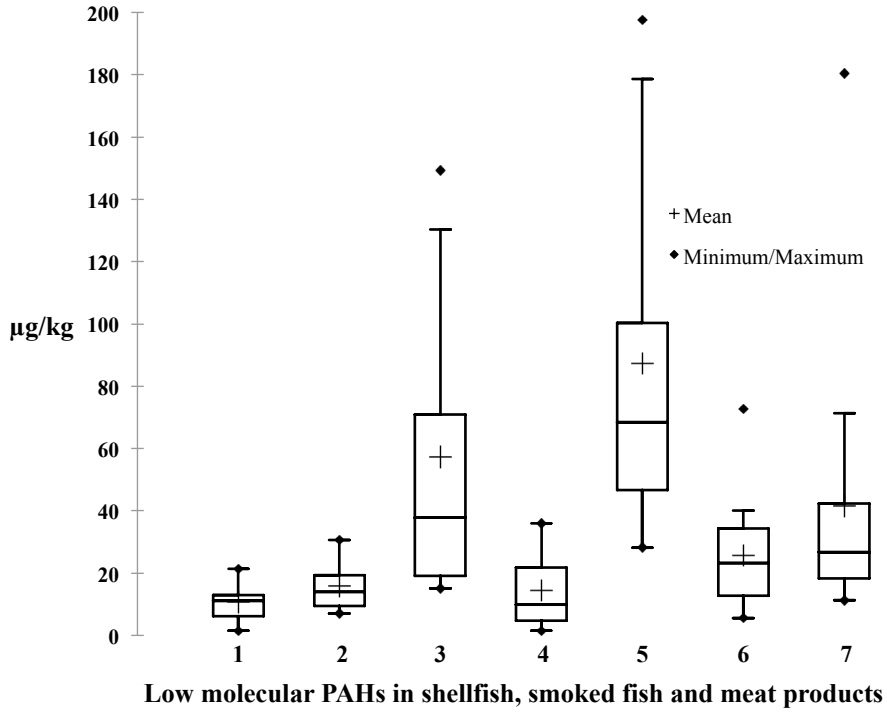

Figure 2. Contents (µg/kg fresh weight) of low molecular PAHs (acenaphthene (ACE), anthracene (ANTHR), benz[a]anthracene (BaA), fluoranthene (F), fluorene (FLR), naphthalene (NAP), phenanthrene (PHEN) and pyrene (PYR) in selected food from Croatian market (Food – 1: mussels; 2: other shellfish; 3:smoked fish; 4: semi dry sausages; 5: semi-dry cured meat products; 6: dry-fermented sausages; 7: dry-cured meat products).

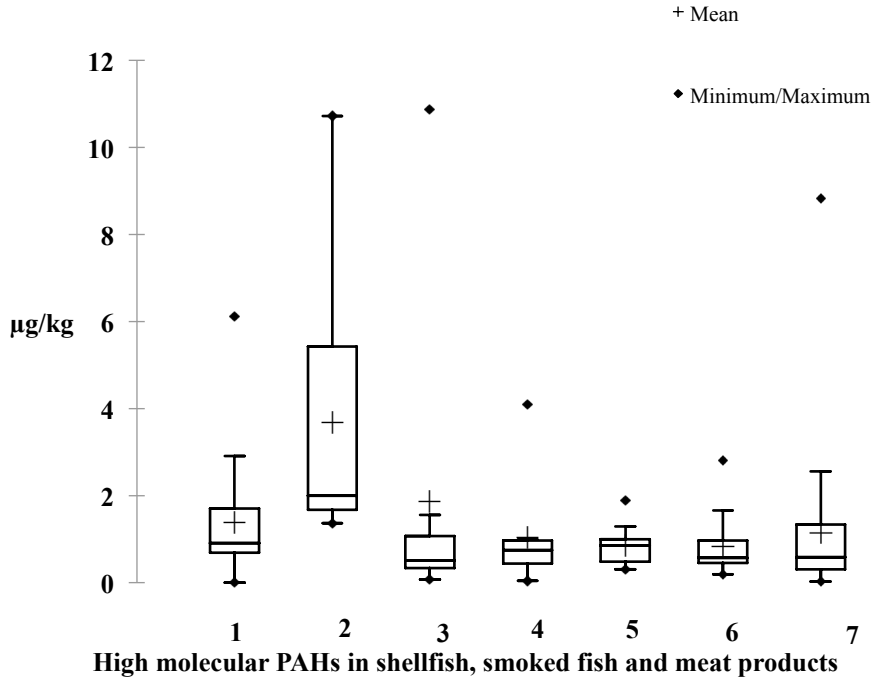

Figure 3. Contents (µg/kg fresh weight) of high molecular PAHs (benzo[b]fluoranthene (BbF), benzo[a]pyrene (BaP), benzo[k]fluoranthene (BkF), benzo[ghi]perylene (B[ghi]P), dibenz[a,h]anthracene (D[ah]A), indeno[1,2,3cd] pyrene I[cd]P) in selected food from Croatian market (Food – 1: mussels; 2: other shellfish; 3:smoked fish; 4: semi dry sausages; 5: semi-dry cured meat products; 6: dry-fermented sausages; 7: dry-cured meat products).

Mean PAH concentrations obtained in this study in certain food categories are shown in Table 2. The majority of PAHs found in randomly sampled spices and herbs circulating on the Croatian market were low molecular ACE, PHEN and PYR, present in the total PAH15 content in the percent-share of 31.02 %, 22.53%, and 10.69%, respectively. As for the heavy ∑PAH4 and ∑PAH8, they were detected in all investigated herbs and spices (Fig. 4).

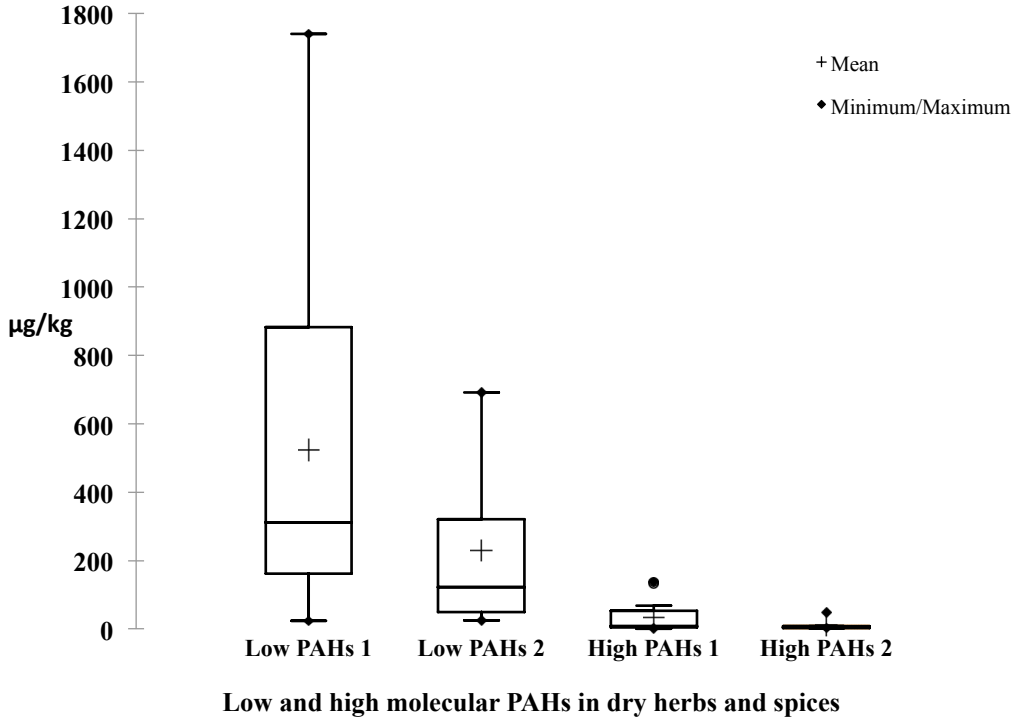

Figure 4. Contents (µg/kg fresh weight) of low (acenaphthene (ACE), anthracene (ANTHR), benz[a]anthracene (BaA), fluoranthene (F), fluorene (FLR), naphthalene (NAP), phenanthrene (PHEN) and pyrene (PYR and high molecular PAHs (benzo[b]fluoranthene (BbF), benzo[a]pyrene (BaP), benzo[k]fluoranthene (BkF), benzo[ghi]perylene (B[ghi]P), dibenz[a,h]anthracene (D[ah]A), indeno[1,2,3cd] pyrene I[cd]P) in selected dry herbs and spices from Croatian market.

The highest levels of the above were established in smoked paprika (∑PAH4 113.12 µg/kg, ∑PAH8 207.46 µg/kg) and the lowest in rosemary (∑PAH4 1.24 µg/kg, ∑PAH8 3.63 µg/kg) and garlic (∑PAH4 2.27 µg/kg, ∑PAH8 5.64 µg/kg). BaP concentration ranged from 0.15 µg/kg in garlic and 0.21 µg/kg in rosemary to 21.88 µg/kg in smoked paprika (Table 3). ∑PAH8 most abundantly present in the examined herbs and spices were CHR, D[ah]A, B[a]A, B[b]F and B[a]P (in decreasing order) (Table 2). The highest CHR, D[ah]A and B[a]A values (Table 2) were witnessed in smoked paprika, followed by mixed pepper (5.02 µg/kg, 6.09 µg/kg and 2.67 µg/kg, respectively) and rosemary (4.81 µg/kg, 37.92 µg/kg and 0.67 µg/kg, respectively).

In the study by ROZENTĀLE *et al.* (2017), the occurrence of four EU-regulated PAHs (B[a]P, B[a]A, CHR and B[b]F) was checked in 3 groups of herbs and 3 groups of spices. According to the authors, PAH concentration found to be the highest in almost all analysed seasonings, with the mean values ranging from 1.73 µg/kg (nutmeg) to 8.68 µg/kg (thyme), was that of CHR, its mean values established in red paprika and black pepper thereby being 3.18 µg/kg and 4.63 µg/kg, respectively. Similar to our study, the investigated seasonings showed variations in PAH levels. Contrary to the results of our

study, in the investigation by ROZENTÄLE *et al.* (2017) the highest BaP contamination was detected in black pepper at the level of 6.60 μg/kg, while in thyme the ∑PAH4 contamination of 37.39 μg/kg was determined. When it comes to $BaPE_{\Sigma PAH4}$, $BaPE_{\Sigma PAH8}$ and $BaPE_{\Sigma PAH15}$ calculated within this study frame, the obtained results exhibited a similar descending pattern with the highest results of 27.27 μg/kg, 278.36 μg/kg and 300.76 μg/kg, respectively, for smoked paprika, to the lowest BaPE values of 0.21 μg/kg, 0.31 μg/kg and 0.48 μg/kg, respectively, detected in garlic (Table 3). Traditional smoking and processing methods applied in the production of smoked paprika and smoked cardamom resulted in high PAH levels. However, given that the consumption of these spices is low, and to enable these smoked products to remain on the market, they were exempted from the maximal levels set out by the EC (2015). In summary, our results showed statistically significant ($p < 0.05$) shares of low molecular as compared to high molecular PAHs (Figure 2). However, the investigated herbs and spices were contaminated with PAHs at levels lower than the maximum levels established by the EU.

4. CONCLUSIONS

The results of this study confirmed that certain food items circulating on the Croatian market are contaminated with PAHs at levels below the established maximal limits set out under the pertaining legislation. A statistically significant difference ($p<0.05$) in toxicologically important PAH markers were found across the investigated food categories. With regard to BaP and ∑PAH4 contents, only certain spices showed significantly higher levels of the latter. Other shellfish, smoked fishery products and spices were characterized with higher ∑PAH8 marker values as compared to mussels and meat products. The highest total PAH amounts were found in dried herbs and spices, followed by meat sausages and smoked fish. The present study showed that processed foodstuffs are more severely contaminated with PAHs in comparison with food contaminated from environmental sources. Independent of food category, PAH composition pattern was dominated by low molecular PAHs. Being aware of the fact that the scope of the food-governing legislation is limited mostly due to the difficulty to define safe levels for complex PAH mixtures, future analyses should be extended to additional PAH compounds. A special attention should be paid to benzo[c]fluorene (BcF) as recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), since data on its occurrence in food are still scarce, but the levels of benzo[c]fluorene-derived adducts are much higher than those of benzo[a]pyrene-derived adducts. Also, the margin of exposure (MOE) approach would be of interest for our further studies intended to evaluate certain food consumption patterns pursued in Croatia.

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