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Polycyclic aromatic hydrocarbons in four different types of Croatian dry-cured hams

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) represent an important contaminant group in a variety of food products. Since PAHs are commonly present in meat products, this study aimed to determine PAH content in four types of Croatian dry-cured hams (Dalmatian, Drniš, Krk and Istrian) produced by four different processing methods. Determination and quantification of PAHs were performed by High performance liquid chromatography with fluorescence detection (HPLC-FLD). Out of 15 investigated PAHs, 13 compounds were detected. The total average benzo(a)pyrene (BaP) and PAH4 levels obtained from dry-cured hams ranged from 0.05-0.10 μg/kg and 0.41-0.67 μg/kg, respectively. Even though Krk and Istrian dry-cured ham manufacturing processes do not include the smoking phase, no significant differences were found between investigated dry-cured hams in terms of BaP, PAH4, PAH8 and PAH 15 contents. The presence of detected PAHs in non-smoked dry-cured hams could be a result of the addition of spices in the salting phase. BaP and PAH4 contents found in dry-cured ham samples did not exceed the currently legal levels according to the European legislation.

Keywords: dry-cured ham, polycyclic aromatic hydrocarbons, benzo(a)pyrene, HPLC-FLD

INTRODUCTION

Croatian dry-cured hams are one of the most appreciated traditional food products. Dry-cured ham has specific characteristics, while the major impact on final quality have features of the raw material (such as genetic type, rearing system or feed) and manufacturing process (Toldra, 2010). Among four types of Croatian dry-cured hams, Istrian dry-cured ham is registered at EU level with Protected Denomination of Origin (PDO), while Dalmatian, Drniš and Krk dry-cured hams are protected within Protected Geographical Indication (PGI) (Cerjak et al., 2014; Petričević et al., 2018).

Smoking is one of the oldest technologies for food conservation, often used in the production of various traditional cured meat products in Croatia (Pleadin and Kovačević, 2016). In modern times,

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the application of smoking in terms of the extension of shelf-life of products is no longer essential. So, smoking is mostly applied for the enrichment of food products with specific organoleptic properties, such as desirable aroma and appearance (Jaffe et al., 2017; Fasano et al., 2016; Petričević et al., 2018). As a result of cellulose and hemicellulose pyrolysis, a great number of carbonyl compounds are formed, which provide a desirable brownish color at the meat surface. In addition, lignin pyrolysis leads to phenolic compounds formation, which are known to possess bacteriostatic and antioxidant effects (Baines et al., 2016; Malarut and Vanganai, 2007; Mičulis et al., 2011).

On the other hand, special attention should be given to the smoking process, since it may lead to the contamination of meat products with polycyclic aromatic hydrocarbons (PAHs). Polycyclic aromatic hydrocarbons comprise a large group of toxicants which represent relevant compounds from the point of food safety and toxicity. PAHs are composed of two or more fused aromatic rings made up of carbon and hydrogen atoms (Abdel-Shafya and Monsour, 2016; Alomirah et al., 2011; EFSA, 2008). They are ubiquitous environmental carcinogens whose deleterious effects are well established in laboratory animals (Yebra-Pimentel et al., 2015). In general, significant amounts of PAHs can be produced as a result of incomplete combustion of wood or other types of organic matter like fossil fuels (Škaljac et al., 2018; Zelinkova and Wenzl, 2015, Hokkanen et al., 2018). Due to their chemical inertness and lipophilic and water-insoluble character, PAHs are very persistent in the environment (Yebra-Pimentel et al., 2015; Abdel-Shafya and Mansour, 2016).

Food chain contamination with PAHs arises mostly from natural and anthropogenic sources (Šimko, 2018, Rozentale et al., 2018, Bogdanović et al., 2019). Although humans may be exposed to PAHs through different sources, dietary intake of PAHs poses a primary route of exposure for non-smokers (EFSA, 2008; Singh and Agarwal, 2018; Bogdanović et al., 2019). Origins of PAHs in food are diverse, however, thermal treatments (such as barbecuing, smoking, drying or roasting) represent the main sources of contamination (Singh et al., 2016; Ciecierska and Obiedziński, 2007; Yebra-Pimentel et al., 2015). There are several factors affecting the amount of PAHs generated during thermal food processing, while the most prominent are: temperature, duration of the treatment, distance from the source of heating, oxygen accessibility, fat content and type of combustible used (Sojinu et al., 2019; Škaljac et al, 2014; Hitzel et al., 2013; Yurchenko, 2005).

There are a number of studies that reported the PAH presence and dietary exposure from smoked meat products (Bogdanović et al, 2019; Fasano et al., 2016; Alomirah et al., 2011; Mičulis et al., 2010) and there are a few recent studies (Škaljac et al., 2018; Hokkanen et al., 2018; Ledesma et al., 2016) regarding the influence of different production methods on levels of PAH contamination in meat products. Yet, in reviewing the literature, no data was found regarding the influence of different processing methods on levels of PAH contamination in dry-cured hams. In recent years, the presence of PAH compounds in herbs and spices represents a matter of great concern (Di Bella et al., 2019; van Asselt, 2016; Schaarschmidt, 2016; EFSA, 2008). Since one of the phases of the production of Istrian and Krk dry-cured ham include salting with the addition of spices, the determination of PAH compounds in non-smoked hams is also relevant. So, the aim of this study was to determine the levels of benzo(a)pyrene (BaP) as well as the sum of PAHs; PAH4, PAH8 and PAH15 in Croatian dry-cured hams produced by four different processing methods (with differences in primary leg treatment, salting and smoking phase).

Materials and methods

Samples

For the purpose of this study, four types of Croatian dry-cured hams were sampled, of which three of them were registered with Protected Geographical Indications (PGI) sign (Krk, Dalmatian and Drniš dry-cured ham) and one with Protected Designation of Origin (PDO) (Istrian dry-cured ham). Hams were obtained from Duroc × (Yorkshire × Landrace) breed, while the pigs were reared and fed under the same regimes. The research was carried out on a total of 24 dry-cured hams (6 hams per 4 different processing methods). Raw hams were processed to dry-cured ham in the traditional way, characteristic for each type of ham. The processing of dry-cured hams was performed following the four protocols, as shown in Fig. 1. In the phase of primary leg treatment, in the case of Dalmatian, Drniš and Krk dry-cured hams, pelvic bones and skin with the subcutaneous adipose tissue are left on the ham. On the other hand, during the production of

Istrian dry-cured ham, skin and the subcutaneous adipose tissue are removed. Following the primary leg treatment, the surface of the hams was rubbed with sea salt or with a mixture of sea salt and spices, depending on the type, as presented in Fig. 1. This operation is carried out in cooling chambers (0–5 °C, 80–90 % RH) for a period of up to 1 month. The salting phase is followed by the pressing phase in the duration of 7–10 days, in order to achieve the shape of ham and to squeeze the excess of blood and meat juice. During the drying phase, Dalmatian and Drniš dry-cured hams are subjected to cold smoking (T < 22 °C) for about 20 days in the smoke produced by burning wood or plants from species typical for the region. After the ripening in a cellar (12–15 °C, RH 65–75%.), hams were sampled for analysis. Samples were vacuum packed, coded and stored at −18 °C. Prior to analysis, samples were thawed for 24 h at 4 °C. The determination of PAH content was carried out on muscle biceps femoris. Each sample of ham was analysed in duplicate, accordingly, the determined values of PAH concentrations presented in this study represent the mean values of two parallel analyses.

Materials and reagents

All chemicals used in sample preparation and HPLC analysis were of HPLC grade. Ultrapure water was obtained using a Millipore water purification system (Millipore Direct-Q 3 UV, Merck Millipore, Molsheim, France). Smoked meat reference material was purchased from FAPAS (T0655QC, FAPAS, Sand Hutton, England). PAH standard solutions in acetonitrile (ACN) were supplied from Sigma Aldrich which contained: naphthalene (Nap), acenaphtalene (Acp), fluorene (Flu), phenanthrene (Phen), anthracene (Ant), fluoranthene (Flt), pyrene (Py), benzo(a)anthracene (BaA), chrisene (Chr), benz(b) fluoranthene (BbF), benz(k)fluoranthene (BkF), benz(a)pyrene BaP, dibenzo(a,h)anthracene (Dba), benzo(g,h,i)perylene (BghiPer), indeno(1,2,3 cd) pyrene (IP) (Sigma Aldrich, Laramie, Wyoming, USA). Standard solutions for each PAH were prepared and their concentration ranged from 0.25-20 μg/kg.

HPLC-FLD analysis

The presence of PAHs was determined using the method published by Bogdanović et al. (2019)

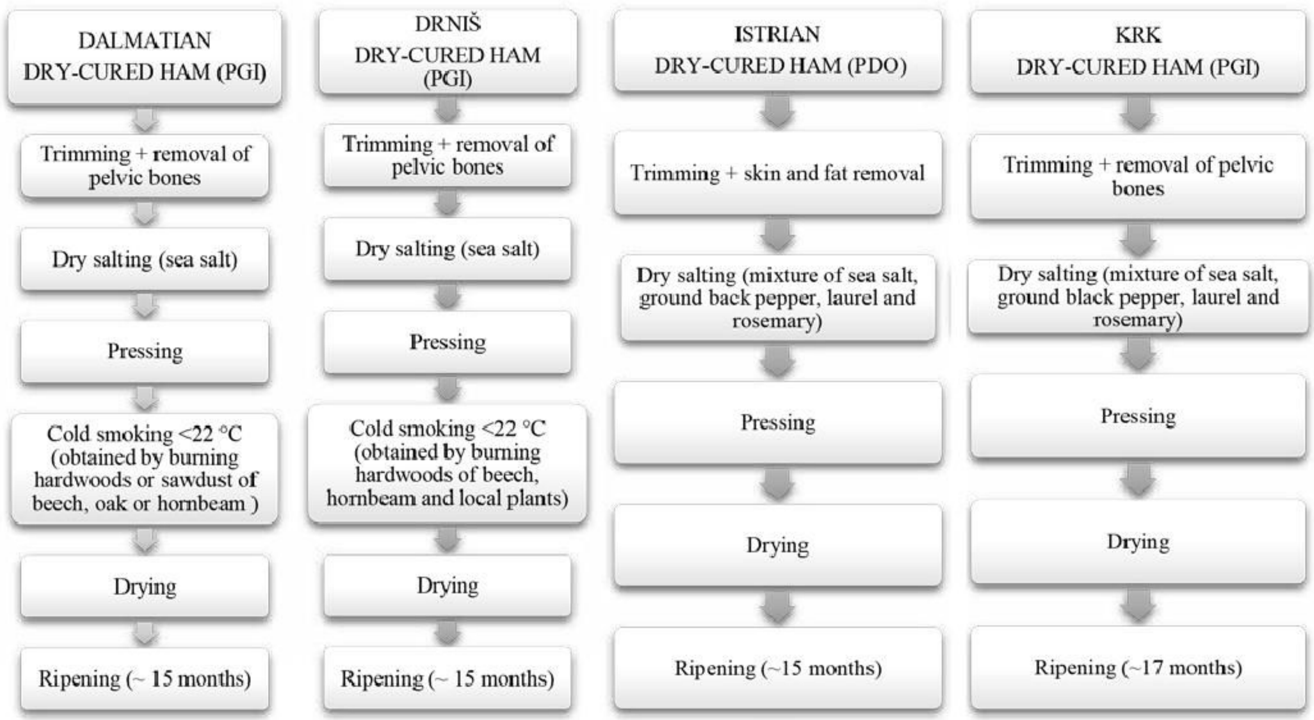

Fig. 1 Schematic diagrams of the technological processes of Dalmatian, Drniš, Istrian and Krk dry-cured ham production (Petričević et al., 2018).

Slika 1. Shematski prikaz tehnološkog procesa proizvodnje dalmatinskog, drniškog, istarskog i krčkog pršuta (Petričević i sur., 2018).

with slight modifications. The samples of dry-cured hams were homogenized using a Grindomix GM 200 (Grindomix GM 200, Retsch, Haan, Germany). 1 g of homogenized biceps femoris muscle sample was saponified with ethanolic 2 N potassium hydroxide solution in a water bath (SW22 Julabo, JULABO GmbH, Seelbach, Germany) at 80 °C for 2 h. After cooling, liquid-liquid extraction with cyclohexane was performed to isolate PAHs containing an unsaponifiable fraction. Using a rotary evaporator (Heidolph rotary evaporator, Laborota 4002, Schwabach, Germany), the eluate was reduced to about 0.2 mL and under reduced pressure at 40 °C. The residue was dissolved in 3 mL of ACN and the mixture was injected on the Sep-Pak (500 mg/3 mL) Vac Silica cartridges (Waters, Milford, Massachusetts, USA) preconditioned with 3 mL ACN and eluted with 3 mL ACN. The SPE clean-up step was carried out in a 20-port solid-phase extraction Vacuum-manifold (VacElut 20, Agilent, Santa Clara, California, USA). The eluate was then evaporated on a rotational vacuum concentrator (RCV2-18HCL, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) at 1300 rpm for 90 min at 50 °C. Following the evaporation step, the residue was dissolved in 1 mL of ACN. The solution was then filtered using a 0.2 μm-nylon syringe on-line filter (Phenex™ Filter Membranes, Nylon, 0.2 μm, Phenomenex, Torrance, California, USA) and analyzed on Ultra High Performance Liquid chromatography system (Agilent 1290 Infinity UHPLC, Santa Clara, CA, California, USA) equipped with a fluorescence detector (Agilent 1260 Infinity Fluorescence Detector, Santa Clara, California, USA). The separation of the compounds was performed in a Hypersil Green PAH C18 analytical column, 150 mm × 3 mm, particle size 3 μm (Hypersil™ Green PAH LC Column, Thermo Fisher Scientific, Waltham, MA, Massachusetts, USA). The mobile phase was a mixture of ACN and water in a gradient mode. The initial composition of the mobile phase was 100% ACN:water=1:1, changing after to 40 % ACN: water= 1:1 in 20 min, with the final 10 min-hold. The injection volume was 15 μL. The flow rate was kept at 0.8 mL/min (Wegrzyn et al., 2006) during the run and the temperature of the column was maintained at 30 °C. The fluorescence detection conditions (excitation-Ex/ emission- Em) were as 270/340 Nap, Acp; 250/310 Flu; 250/380 Phen, Ant; 250/465 Flt; 270/385 Py, BaA, Chr; 256/466 BbF; 295/400 BkF, BaP, Dba, BghiPer; 300/500 IP.

The identification of PAHs was performed by comparison of retention times of analyzed PAHs with those obtained by injecting standards in the same conditions with ±2,5 % and comparing fluorescence spectra with positive control sample and/or recorded spectra. Quantification was performed using external calibrations curves plotted for each PAH and covering for seven concentration levels ranging from 0.25-20 μg/kg. Duplicate 15 μL-injections were used to construct linear regression lines (reporting peak area vs PAH concentration).

Statistical analysis

The obtained results were statistically analyzed using the SPSS Version 9.0 program (SPSS Statistics 9.0, StatSoft Inc, Tulsa, Oklahoma, USA). Data from the PAH analysis were submitted for a one-way Variance Analysis (ANOVA) considering dry-cured hams (4) as factor and PAHs as dependent variables. Statistically significant differences were estimated at the level of probability of 0.05. Tukey's honest significant difference test was carried out when the ANOVA showed a significant effect ($p < 0.05$).

RESULTS AND DISCUSSION

Due to the wide variety of meat products on the European market and, most of all, their popularity in certain EU countries, the control of PAHs presence in this type of products is essential in order to improve their safety and quality (Pleadin and Bogdanović, 2016; Jira, 2010; Lorenzo et al., 2011; Škaljac et al., 2014; Hokkanen et al., 2018). During the Dalmatian and Drniš dry-cured ham production, the method of cold smoking is performed and it is obtained by burning hardwoods or wood chips of beech (*Fagus* sp.), oak (*Quercus* sp.) or hornbeam (*Carpinus* sp.) and local plants in case of Drniš dry-cured ham. If the traditional method of smoking is performed, special attention must be given to the temperature in the smoking chamber not exceeding 22 °C. As previously mentioned, a non-desired effect of smoking is the formation of carcinogenic PAHs (Abdel-Shafy and Mansour, 2016). Unlike the Dalmatian and Drniš dry-cured ham, Krk and Istrian are not subjected to cold smoking during the production process. However, during their production, both undergo salting phase with the addition of spices which are often contaminated with PAHs (EFSA, 2008; Di Bella et al., 2019; van Asselt, 2016; Schaarschmidt, 2016). Results of the determination of individual PAH compounds (mean ± standard deviation) in Croatian dry-cured hams (µg/kg) are presented in Table 1. As shown in Table 1., out of 15 investigated PAHs, 13 compounds were detected and quantified. The two following compounds were not detected; BaA and IP. This result is in accordance with those observed in earlier studies (Jira, 2004; Bogdanović et al., 2019), in which BaA was also not detected.

Table 1 Content of individual PAH compounds (mean ± standard deviation) in Croatian dry-cured hams (µg/kg).
Tablica 1. Udio pojedinačnih PAH spojeva (srednja vrijednost ± standardna devijacija) u uzorcima hrvatskih pršuta (µg/kg).

PAH compound	Abbrev	[1]LOD	Istrian dry- cured ham	Krk dry -cured ham	Dalmatian dry-cured ham	Drniš dry-cured ham	p-value
Naphthalene	Nap	0.55	2N.D.a	0.26 ±0.09^{b}	N.D.^{a}	0.14±0.06^{b}	*0.000*
Fluorene	Flu	0,25	0.32±0.03^{a}	0.16±0.02^{b}	0.14±0.04^{b}	0.14±0.01^{b}	*0.002*
Acenaphthene	Acp	0.91	3.95±0.76^{a}	1.44±0.19^{b}	1.41 ± 0.29^{b}	1.56±0.15^{b}	*0.007*
Phenanthrene	Phen	0.24	1.37±0.10^{a}	1.85±0.53b^{c}	2.81 ± 0.42^{b}	1.18±0.05^{c}	*0.000*
Anthracene	Ant	0.15	0.16±0.04^{a}	N.D.b	0.42 ± 0.20^{c}	1.25±0.14^{d}	*0.000*
Fluoranthene	Flt	0.09	0.51±0.18	0.40±0.16	0.45 ± 0.13	0.80±0.21	*0.329*
Pyrene	Py	0.26	0.31±0.04^{a}	0.30 ±0.12^{b}	0.44 ± 0.12^{b}	0.61±0.11^{b}	*0.039*
Benz[a]anthracene	BaA	0.02	N.D.	N.D.	N.D.	N.D.	-
Chrysene	Chr	0.02	0.20±0.04	0.26 ±0.11	0.21 ± 0.05	0.19 ±0.01	*0.074*
Benzo[b]fluoranthene	BbF	0.08	0.17±0.04	0.31±0.10	0.16 ± 0.05	0.17±0.04	*0.148*
Benzo[k]fluoranthene	BkF	0.06	0.09±0.02^{a}	0.35±0.09^{b}	0.08 ± 0.03^{a}	0.22±0.08b	*0.000*
Benzo[a]pyrene	BaP	0.03	0.08±0.01	0.10±0.06	0.07 ± 0.02	0.05±0.01	*0.133*
Dibenz[a,h]anthracene	Dba	0.23	0.54±0.08^{a}	N.D.^{b}	0.09 ± 0.04^{c}	0.14±0.06^{c}	*0.012*
Benzo[ghi]perylene	BghiPer	0.15	0.29±0.08	0.47±0.12	0.27 ± 0.08	0.32±0.02	*0.164*
Indeno[1,2,3—cd] pyrene	IP	0.09	N.D.	N.D.	N.D.	N.D.	-

Determined values represent the mean values of the 6 samples analyzed by the ham type. Results are expressed as the means of values detected in two parallel analyses for each sample. / Prikazane vrijednosti predstavljaju srednje vrijednosti od 6 analiziranih svakog tipa pršuta. Rezultati su izraženi kao srednje vrijednosti dva paralelna mjerenja provedena za svaki uzorak.

**In the same row, different superscript letters (a,b,c,d) means that values are significantly different*

*(Tukey honest Test, p ≤ 0.05); [1]LOD: limit of detection, ~~nd~~ [2]N.D.-not detected. / *Različite oznake u istom redu (a,b,c,d) označavaju statistički značajnu razliku (Tukey test, p ≤ 0.05); [1]LOD: limit detekcije, [2]N.D.-nije identificiran*

PAHs containing up to four fused benzene rings are known as the light PAHs, whereas those containing more than four benzene rings are described as the heavy PAHs. Heavy PAHs are recognized as more stable and toxic than light ones (Wenzl et al. 2006; Singh et al., 2016). Results of this study show the prevalence of light PAHs over the heavy ones, which is in agreement with earlier studies (Alomirah et al., 2011; Lorenzo et al., 2011; Gomes et al., 2013; Škrbić et al., 2014) on PAH profiles in smoked meat products.

Concerning the light PAH group, average Nap levels obtained from Krk and Drniš dry-cured ham ranged from 0.14-0.26 µg/kg. Statistically significant differences ($p < 0.05$) between these two types of dry-cured ham were found. On the other hand, the presence of Nap was not detected in Istrian and Dalmatian dry-cured ham samples. Statistically significant differences ($p < 0.05$) between the Istrian and the other types of investigated dry-cured hams were found by means of Flu (0.14-0.33 µg/kg), Acp (1.41-3.95 µg/kg) and Py (0.30-0.61 µg/kg) content.

Average Phen levels ranged from 1.18-2.80 µg/kg, while statistically significant differences ($p<0.05$) between Istrian (1.37 ± 0.10 µg/kg) and Krk (1.85 ± 0.53 µg/kg), as well as between Dalmatian (2.80 ± 0.42 µg/kg) and Drniš dry-cured hams (1.18 ± 0.05 µg/kg) were found. The presence of Ant was not detected in Krk dry-cured ham. In contrary, Ant was detected in Istrian (0.16 ± 0.04 µg/kg), Dalmatian (0.42 ± 0.20 µg/kg) and Drniš (1.25 ± 0.14 µg/kg) dry-cured ham, while statistically significant differences ($p < 0.05$) were found among these type of samples by means of Ant content. Regarding Flt and Chr levels, no significant differences ($p>0.05$) were found between four groups of samples. The total average Flt and Chr levels obtained from investigated dry-cured hams ranged from 0.40-0.80 µg/kg and 0.19-0.26 µg/kg, respectively.

Regarding the heavy PAH group, the mean BghiPer and BbF levels ranged from 0.27-0.47 µg/kg and 0.16-0.31 µg/kg, respectively, while no significant differences ($p>0.05$) were found among four groups of samples by means of BghiPer and BbF content. Statistical analysis revealed a significant difference ($p<0.05$) in BkF levels among dry-cured ham samples. The highest BkF level was observed in Krk dry-cured ham (0.35 ± 0.09 µg/kg), followed by Drniš dry-cured ham (0.22 ± 0.08 µg/kg), whose BkF levels statistically differ from those observed in Istrian (0.09 ± 0.02 µg/kg) and Dalmatian (0.08 ± 0.03 µg/kg) dry-cured ham. Presence of Dba was not detected in Krk dry-cured ham, while statistically significant differences ($p<0.05$) were observed between Istrian (0.54 ± 0.08 µg/kg) and Dalmatian (0.09 ± 0.04 µg/kg), as well as between Istrian and Drniš (0.14 ± 0.06 µg/kg) dry-cured ham. BaP is still widely used as a marker in scientific investigations owing to its carcinogenic and mutagenic properties. Yet, Andree et al. (2010) suggests dibenzo(a,l)pyrene as a compound of scientific interest, due to the results of toxicological investigations that pointed its carcinogenic potential as possibly much stronger than that of BaP. The highest BaP levels were detected in Krk dry-cured ham (0.10 ± 0.06 µg/kg), followed by Istrian (0.08 ± 0.01 µg/kg) and Dalmatian dry-cured ham (0.07 ± 0.02 µg/kg), while Drniš had the lowest (0.05 ± 0.01 µg/kg) BaP content. No significant differences ($p> 0.05$) were observed among four types of dry-cured ham samples by means of BaP content. Average mean levels of BaP (0.05-0.10 µg/kg) are below those reported by other authors (Jira, 2004; Rozentale et al., 2015; Šimko, 2002) and in agreement with those reported by Bogdanović et al. (2019).

The PAH group consists of around 660 different compounds, while some representatives show carcinogenic properties. As mentioned above, the best known carcinogenic PAH compound is BaP (EFSA, 2008; Andree et al., 2010; Yebra-Pimentel et al., 2015). However, as stated by EFSA (EFSA, 2008), BaP alone is not a suitable marker for the occurrence of PAH in food. Therefore, EFSA offers the sum content of the four PAH compounds (PAH4) (BaP, BaA, BbF and Chr) or PAH8 (sum of PAH4, BkF, BghiPer, Dba and IP) as more suitable indicators of PAHs in food (Bogdanović et al., 2019; Jira, 2010, Yebra-Pimentel et al, 2015; EFSA, 2008). In accordance with Regulation (EU) No. 835/2011 of 19 August 2011 (EC, 2011), a maximum acceptable concentration of 2.0 µg/kg BaP (a marker of the presence of PAH) in smoked meat and smoked meat products has been set from 1/9/2014 on. Concerning the permissible sum of PAH4 in these food products, the acceptable limit of 12.0 µg/kg in wet weight has been defined (EC, 2011; Ledesma et al, 2016; Hitzel et al., 2013). Content of PAH4, PAH8 and PAH15 (mean ± standard deviation) in Croatian dry-cured hams (µg/kg) are presented in Table 2.

As shown in Table 2., no statistically significant differences were found among dry-cured ham samples by means of PAH4, PAH8 and PAH15 levels. PAH15 ranged between 7.0-8.80 µg/kg, with the highest mean PAH15 level observed in Istrian dry-cured ham (8.80 ± 0.93 µg/kg), followed by Drniš (7.53 ± 0.05 µg/kg), Dalmatian (7.07 ± 1.85 µg/kg) and Krk (7.00 ± 1.43 µg/kg) dry-cured ham. Ciecierska and Obiedziński (2007) performed a similar experiment. Their results showed that the sum of 15 PAHs was equal to 2.78 µg/kg in the case of industrially smoked ham. In the case of hams, for which the traditional method of smoking was applied, 3.26 µg/kg PAH15 was found. The levels of PAH15 detected in this investigation are above those observed by Ciecierska and Obiedziński (2007). Mean PAH8 levels ranged from 0.73-1.28 µg/kg, with the highest mean level observed in Istrian dry-cured ham (1.28 ± 0.42 µg/kg), followed by Krk (1.10 ± 0.15 µg/kg), Drniš (0.88 ± 0.04 µg/kg) and Dalmatian dry-cured ham (0.73 ± 0.12 µg/kg), where the lowest PAH8 level was observed. Detected levels of PAH8 are far below those reported by Alomirah et al. (2011). Similar results were observed in dry-cured hams by means of PAH4 content, where the non-smoked hams had

Table 2 Content of PAH4, PAH8 and PAH15 (mean ± standard deviation) in Croatian dry-cured hams (μg/kg).
Tablica 2. Udio PAH4, PAH8 i PAH15 (srednja vrijednost ± standardna devijacija) u uzorcima hrvatskih pršuta (μg/kg).

PAH compound	Abbrev	Istrian dry-cured ham	Krk dry-cured ham	Dalmatian dry-cured ham	Drniš dry-cured ham	p-value
∑ BaA, Chr,BbF,BaP	PAH4[1]	0.66±0.15	0.67±0.13	0.44± 0.08	0.41±0.02	*0.108*
∑ BaA, Chr, BbF,BkF,Bap,	PAH8[2]	1.28±0.42	1.10±0.15	0.73±0.12	0.88±0.04	*0.068*
∑ 15 PAH	PAH 15[3]	8.80±0.93	7.00±1.43	7.07±1.85	7.53±0.05	*0.212*

Determined values represent the mean values of the 6 samples analyzed by the ham type. Results are expressed as the means of values detected in two parallel analyses for each sample. [1]PAH4: the sum of benzo(a)anthracene-BaA, chrysene-Chr, benzo[b]fluoranthene-BbF, benzo[a]pyrene-BaP, 2PAH8: the sum of BaA, Chr, BbF, BaP, benzo[k]fluoranthene-BkF, dibenz[a,h]anthracene- Dba, benzo[ghi]perylene-BghiPer, indeno[1,2,3—cd]pyrene-IP, [3]PAH15: the sum of BaA, Chr, BbF, BaP, BkF, Dba, BghiPer, IP, naphthalene-Nap, fluorene-Flu, acenaphthene-Acp, phenanthrene-Phen, anthracene-Ant, fluoranthene-Flt, pyrene-Py. / Prikazane vrijednosti predstavljaju srednje vrijednosti od 6 analiziranih uzoraka svakog tipa pršuta. Rezultati su izraženi kao srednje vrijednosti dva paralelna mjerenja provedena za svaki uzorak. [1]PAH4: suma benzo(a)antracena-BaA, krizena-Chr, benzo[b]fluorantena-BbF, benzo[a]pirena-BaP, [2]PAH8: BaA, Chr, BbF, BaP , benzo[k]fluorantena-BkF, dibenz[a,h]antracena- Dba, benzo[ghi]perilena-BghiPer, indeno[1,2,3—cd]pirena-IP, [3]PAH15: suma BaA, Chr, BbF, BaP , BkF, Dba, BghiPer, IP , naftalena-Nap, fluorena-Flu, acenaftena-Acp, fenantrena-Phen, antracena-Ant, flourantena-Flt, pirena-Py.

the highest mean PAH4 levels.

PAH4 ranged between 0.41-0.67 μg/kg with the highest mean level observed in Krk dry-cured ham (0.67±0.13 μg/kg), followed by Istrian (0.66 ± 0.15 μg/kg), Dalmatian (0.44 ± 0.08 μg/kg) and Drniš (0.41±0.02 μg/kg) dry-cured ham. This is a rather surprising result since Krk and Istrian dry-cured ham manufacturing process does not include the smoking phase. It can be concluded that the presence of PAH compounds in non-smoked dry-cured hams is could be a result of the addition of spices in the salting phase, such as ground black pepper, laurel and rosemary in case of Krk and Istrian dry cured ham. Recently, Rozentale et al. (2018) investigated the occurrence of PAH4 in 150 samples of different dried herbs and spices (oregano, basil, thyme, black pepper, pepper and nutmeg). PAHs were detected in 86% of samples, while BaP content ranged from non-detectable levels to 6.60 μg/kg. High levels of PAHs found in dried herbs and spices are related to the application of inappropriate drying process (Schaarschmidt, 2016; Tripathy et al., 2015; Zelinkova and Wenzl, 2015a, 2015b). To this date, several studies have been carried out (Di Bella et al., 2019; van Asselt et al., 2016; EFSA, 2008) in order to evaluate the incidence and the levels of PAHs and revealed that spices and herbs are frequently highly contaminated with PAH compounds. Therefore, the new permissible levels of 10 μg/kg BaP and 50 μg/kg PAH4 in herbs and spices have been set (EC, 2015; van Asselt et al., 2016). The production process of black pepper, used in the salting phase during the production of Krk and Istrian dry-cured ham, includes an additional step of dipping greenish yellow pepper spikes in boiling water (Abdulazeez et al., 2015). This production step may be the cause of extra contamination of black pepper with PAHs, and therefore, may also represent a potential risk of additional contamination of Krk and Istrian dry-cured ham with PAHs.

Previous studies reported that casings in case of smoked sausages and skin and subcutaneous fat tissue in case of dry-cured ham or bacon act as barriers by preventing PAH diffusion into the product's inner layers (Bogdanović et al., 2019; Škaljac et al, 2014; García-Falcón and Simal-Gándara 2005; Djinović et al. 2008). Therefore, it can be concluded that the highest concentration of PAH15 found in Istrian dry-cured ham (8.79 ± 0.93 μg/kg) is a result of processing this type of ham without skin and subcutaneous fat tissue. BaP and PAH4 contents found in dry-cured ham samples did not exceed the currently legal levels according to the European legislation. The results in this study are in arrangement with other studies (Hokkanen et al., 2018; Šimko, 2002; Šimko, 2018) and indicate that cold smoking under controlled technological conditions leads to lower concentrations of PAHs. In contrary, direct smoking obtained under uncontrolled technological conditions, like ones that are often applied in households, give rise to significant amount of PAH content in smoked foods (Zelinkova and Wenzl, 2015a; Yebra-Pimentel et al., 2015; Wretling et al., 2010; Fasano et al., 2016; Škaljac et al, 2018).

CONCLUSION

The results of this investigation show that BaP and PAH4 contents found in dry-cured ham samples did not exceed the currently legal levels according to the European legislation. No significant differences were found among studied dry-cured hams by means of BaP, PAH4, PAH8 and PAH15 levels. The majority of PAHs detected in non-smoked dry-cured hams originates are assumed to originate from spices added in the salting phase of production. The research results, therefore, highlight the importance of systematic control of herbal and spice contamination with PAHs in order to protect consumer health. In conclusion, findings in this study demonstrate that lower PAH levels can be obtained if the smoking is carried out under controlled conditions and if good manufacturing practice is applied. Further studies need to be carried out in order to more accurately validate the potential risk of dietary exposure to PAHs through different spices used in the production of other meat products.

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Policiklički aromatski ugljikovodici u četiri vrste hrvatskih pršuta

Sažetak

Policiklički aromatski ugljikovodici (PAH-ovi) česti su kontaminanti u prehrambenim proizvodima. S obzirom da mesni proizvodi u značajnoj mjeri mogu biti kontaminirani PAH-ovima, cilj ovog rada bio je odrediti njihov udio u četiri vrste hrvatskih pršuta. Istraživanje je provedeno na Dalmatinskom, Drniškom, Krčkom i Istarskom pršutu, čiji se tehnološki procesi proizvodnje razlikuju. Analiza PAH spojeva provedena je primjenom visokoučinkovite tekućinske kromatografije sa flourescentnom detekcijom (HPLC-FLD). Od 15 istraživanih PAH spojeva, identificirano je i kvantificirano 13 spojeva. Prosječna koncentracija benz(a)pirena (BaP) iznosila je 0,05-0,10 µg/kg, dok su prosječne količine PAH4 spojeva iznosile 0,41-0,67 µg/kg. Unatoč tome što proces proizvodnje Krčkog i Istarskog pršuta ne uključuje fazu dimljenja, nisu određene statistički značajne razlike u udjelima BaP, PAH4, PAH8 i PAH15 spojeva između svih istraživanih pršuta. Kontaminacija PAH-ovima kod nedimljenih pršuta je mogla bi biti posljedica primjene začina u fazi soljenja. Koncentracije PAH spojeva određene u uzrocima hrvatskih vrsta pršuta nisu prekoračile dopuštene razine određene trenutno važećim zakonodavstvom Europske Unije.

Key words: pršut, policiklički aromatski ugljikovodici, benzopiren, HPLC-FLD

Polyzyklische aromatische Kohlenwasserstoffe in vier Sorten des kroatischen Pršut

Zusammenfassung

Polyzyklische aromatische Kohlenwasserstoffe (PAH) sind Kontaminate, die in Lebensmitteln häufig erscheinen. Da Fleischprodukte häufig mit polyzyklischen aromatischen Kohlenwasserstoffen kontaminiert sein können, ist das Ziel dieser Arbeit, ihren Anteil in vier Sorten des kroatischen Pršut zu untersuchen. Die Untersuchung wurde am Dalmatiniski, Drniški, Krčki und Istarski Pršut durchgeführt, deren Technologieverfahren sich unterscheiden. Die Analyse der polyzyklischen aromatischen Kohlenwasserstoffe wurde anhand der Hochleistungsflüssigkeitschromatograhpie mit Fluoreszentdetektor (HPLC-FLD) vorgenommen. Von den 15 untersuchten PAH-Verbindungen wurden 13 Verbindungen identifiziert und quantifiziert. Die durchschnittliche Konzentration von Benz(a)pyren betrug 0,05-0,10 µg/kg, während eine durchschnittliche Menge von PAH4 –Verbindungen 0,41-0,67 µg/kg ermittelt wurde. Obwohl das Herstellungsverfahren des Krčki und Istarski Pršut keine Räucherungsphase umfasst, wurden keine statistisch relevanten Unterschiede bei den Anteilen der BaP-, PAH4-, PAH- und PAH15-Verbindungen bei den untersuchten Pršut festgestellt. Die Kontaminierung mit polyzyklischen aromatischen Kohlenwasserstoffen bei nicht geräucherten Pršut könnte die Folge der Verwendung von Gewürzen in der Salzungsphase sein. Die Konzentration der PAH-Verbindungen, die bei den Proben der kroatischen Pršut -Sorten ermittelt wurde, überschreitet nicht die zulässigen Grenzwerte, die durch die aktuell gültige Gesetzgebung der Europäischen Union vorgeschrieben sind.

Schlüsselwörter: Pršut, polzyklische aromatische Kohlenwasserstoffe, Benzopyren, HPLC-FLD

Los hidrocarburos aromáticos policíclicos en cuatro tipos de los jamones croatas

Resumen

Hidrocarburos aromáticos policíclicos (HAP) son contaminantes frecuentes en los productos alimenticios. Tomando en cuenta que los productos cárnicos pueden estar contaminados en gran medida por los HAP, el fin de este trabajo fue determinar su contenido en cuatro tipos de los jamones croatas. La investigación fue hecha en los jamones de Dalmacia, Drniš, Krk e Istria, con los procesos tecnológicos de producción diferentes. El análisis de los compuestos HAP fue hecho por la cromatografía de líquidos de alta resolución con detección por fluorescencia (HPLC-FLD). Entre los 15 compuestos HAP analizados, 13 fueron identificados. La concentración media del benz(a)pireno (BaP) fue 0,05-0,10 μg/kg, mientras la cantidad media de los compuestos PAH4 fue 0,41-0,67 μg/kg. A pesar de que los procesos de producción de los jamones de Krk y de Istria no incluyen la fase del ahumado, no fueron determinadas las diferencias estadísticamente significativas en el contenido de los compuestos BaP, HAP4, HAP8 y HAP15 en todos los jamones analizados. La contaminación por los PAH de los jamones no ahumados pudo ser la consecuencia del uso de las especies en la fase de salazón. Las concentraciones de los compuestos HAP determinadas en las muestras de los jamones de Croacia no excedieron los niveles determinados por la legislación actual de la Unión Europea.

Palabras claves: jamón, hidrocarburos aromáticos policíclicos, benzopireno, HPLC-FLD

Idrocarburi policiclici aromatici in quattro differenti tipi di prosciutto crudo croato

Riassunto

Gli idrocarburi policiclici aromatici (PAH) sono frequenti agenti contaminanti nei prodotti alimentari. Poiché i prodotti a base di carne possono essere significativamente contaminati dagli PAH, questo studio aveva come scopo quello di accertare la loro presenza e la loro percentuale in quattro differenti tipi di prosciutto crudo croato. La ricerca è stata condotta su prosciutti crudi croati differenti (dalmata, di Drniš, dell'isola di Veglia e istriano) i cui processi tecnologici di produzione si differenziano l'uno dall'altro. L'analisi tendente ad accertare la presenza dei composti PAH è stata condotta con la tecnica della cromatografia liquida ad alta prestazione con rivelatori a fluorescenza (HPLC-FLD). Dei 15 PAH oggetto della ricerca, ne sono stati identificati e quantificati ben 13. È stata rilevata una concentrazione media di benzo[a]pirene (BaP) pari a 0,05-0,10 μg/kg, contro una concentrazione media di composti PAH4 pari a 0,41-0,67 μg/kg. Nonostante il fatto che il processo di produzione del prosciutto crudo dell'isola di Veglia e del prosciutto crudo istriano non preveda la fase dell'affumicamento, non sono state accertate differenze statisticamente significative nelle percentuali dei composti BaP, PAH4, PAH8 e PAH15 tra tutti i prosciutti esaminati. La presenza contaminante d'idrocarburi policiclici aromatici nei prosciutti crudi non affumicati potrebbe essere dovuta all'aggiunta di spezie nella fase della salatura. Le concentrazioni di PAH accertate nei campioni dei prosciutti crudi croati esaminati non hanno superato i livelli consentiti previsti dalla legislazione attualmente in vigore nell'Unione Europea.

Parole chiave: prosciutto crudo, idrocarburi policiclici aromatici, benzopirene, HPLC-FLD