

# Influence of smoking method and anatomical location on the content of polycyclic aromatic hydrocarbons in Turopolje ham

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## Abstract

In order to support a more sustainable breeding of the local Turopolje pig, the breed needs to be valorised through high added-value meat products, such as dry-cured and smoked Turopolje ham. To date, there are very few data on the quality and safety of Turopolje ham, including information on the content and distribution of potential contaminants such as polycyclic aromatic hydrocarbons (PAHs) from smoke. Hence, the aim of this study was to investigate the influence of smoking method (traditional or industrial) and anatomical site (*M. biceps femoris* - BF or *M. semimembranosus* - SM) on the content of PAHs in Turopolje ham. All hams (n=15) were produced the same way, differing only in smoking procedures; a traditional way above the firebox (group TRAD, n=5) or in the smoking chamber (group IND A - standard smoking, n=5 or B - 50 % reduced smoking, n=5). PAHs content ( $\mu\text{g}/\text{kg}$ ) was determined and quantified by high-performance liquid chromatography with fluorescence detection (HPLC-FLD). Two-way analysis of variance was performed (GLM procedure of SAS) with fixed effects of smoking (S), muscle (M) and their interaction (SxM). Of the PAHs investigated, 14 compounds were detected. The results showed that TRAD hams had higher levels of anthracene (Ant), chrysene (Chr), benzo[b]fluoranthene (BbFA), benzo[a]pyrene (BaP), and the sum of benz[a]anthracene (BaA), Chr, BbFA and BaP (PAH 4) than hams from both A and B IND groups. TRAD hams had also higher content of acenaphthene (Acp), phenanthrene (Phen), pyrene (Py), BaA, and total PAHs than IND B hams. Compared to BF, SM muscle had higher content of several PAHs i.e. naphthalene (Nap), fluorine (Flu), Acp, Phen, Ant, fluoranthene (Flt), Py, BaA, benzo[k]fluoranthene (BkFA), benzo[ghi]perylene (BghiP), and also higher total PAH content. However, a significant SxM interaction was observed for Phen and Ant content which were higher only in SM muscle of TRAD hams. In conclusion, this study shows a marked influence of smoking method and anatomical site on the PAHs content in Turopolje hams, mainly in the form of lower BaP and the sum of PAH4 content in all chamber smoked hams compared to traditionally smoked hams and higher levels of light PAHs, some heavy PAHs and total PAH content in the more exposed SM compared to the inner BF muscle. Reduced smoking in IND hams did not affect BaP and the sum of PAH4 content, but reduced the content of some individual and total PAHs content. All IND hams and TRAD hams were below the maximum permitted level for BaP. However, two out of five hams from the TRAD group exceeded the limit for the sum of PAH4 (30  $\mu\text{g}/\text{kg}$ ) set by EU legislation for traditionally smoked meat products, indicating the need for stricter control of the traditional smoking process.

**Keywords:** dry-cured ham, meat smoking, PAH compounds, muscle, Turopolje pig

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## Introduction

The Turopolje pig (TP) is an endangered local Croatian pig breed traditionally reared in the open ecosystems of the oak forests and wetlands of the Turopolje region and its surroundings (Đikić et al., 2010). To ensure a more sustainable breeding of TP, the breed needs to be valorised through high added value meat products, as recent research with consumers and stakeholders shows (Cerjak et al., 2017; Vitale et al., 2020; Rivera-Toapanta et al., 2022). So far, there is little information on the actual quality and safety of TP meat value-added products such as smoked and dry-cured Turopolje ham. In a recent study on Turopolje ham, Karolyi et al. (2024) showed that shortening the smoking time can significantly reduce smoke-derived volatile compounds and improve the texture of dry-cured ham without major changes in other quality attributes. However, the content and distribution of potentially harmful contaminants such as polycyclic aromatic hydrocarbons (PAHs) from the smoke in Turopolje ham have not yet been investigated.

PAHs are organic compounds with two or more fused benzene rings which are formed through incomplete combustion of wood and other organic substances (Purcaro et al., 2013). At high intakes many PAHs are potentially genotoxic and carcinogenic to humans (Domingo, 2017). PAHs containing more than four rings, such as benzo[a]pyrene (BaP) with five aromatic rings, are known as heavy PAHs and are generally considered more stable and toxic than light PAHs with up to four benzene rings (Lawal, 2017). Therefore, the European Food Safety Authority (EFSA) recommended using the cumulative concentration of four heavy PAHs: BaP, chrysene (Chr), benzo[a]anthracene (BaA) and benzo[b]fluoranthene (BbF), referred to as PAH4, in addition to the individual concentration of BaP, as a primary indicator of PAH contamination in food (Alexander et al., 2008). Accordingly, in the European Union (EU), the maximum level for BaP and the sum of PAH4 for smoked meat and smoked meat products was set at 2.0 µg/kg and 12.0 µg/kg respectively (EC, 2011). For traditionally smoked products, however, these maximum levels cannot be reached without significantly altering the organoleptic properties of the product. Therefore, in some Member States including Croatia, where smoking of meat has a long tradition, a deroga-

tion has been made for traditionally smoked meat and smoked meat products, which allows maximum levels of 5.0 µg/kg for BaP and 30.0 µg/kg for the sum of PAH4 (EC, 2023).

The presence of PAHs in smoked foods is highly variable and is influenced by many factors such as the product size (i.e. surface/mass ratio), the type and composition of the wood used for smoking, the type of smoke generator (internal or external), the accessibility of oxygen, the temperature of smoke generation and the smoking time (SCF, 2002). In industrial meat plants, the smoking of meat is strictly controlled on a regular basis, and the separation of the smoke generators from the smoking chambers facilitates the removal of potentially undesirable compounds from the smoke. However, traditional smoking over an open fire can lead to higher PAH contamination of meat products as the smoking process is difficult to control and the smoked item is more directly exposed to combustion products (Šimko, 2009). Previous studies on smoked ham have shown that the traditional smoking method has been associated with increased PAHs levels (Ciecierska and Obiedziński, 2007; Kartalović et al., 2015), including a higher incidence of hams that exceed the legal limits set for maximum BaP and/or PAH4 levels (Mastanjević et al., 2020a; Mastanjević et al., 2020b). On the other hand, when smoking ham under more controlled conditions, such as in the production of dry-cured hams with a protected geographical indication (PGI) (Poljanec et al., 2019) or in the industrial production of smoked ham (Tăbăran et al., 2018), the permitted legal limits were not exceeded.

The usual trimming and boning of the pelvic bones before curing gives the fresh ham a characteristic appearance. On the lateral side, the surface of the thigh is covered with skin and subcutaneous fat, while on the medial side, it is open and consists of exposed muscle tissue. The differences between the inner, skin-covered muscles, such as the *biceps femoris* (BF), and the outer, non-skin-covered muscles, such as the semimembranosus (SM), therefore exist in the rate and degree of dehydration, salt uptake and the dynamics of the biochemical changes that take place during the processing of the dry-cured ham (Petrova et al., 2015; Marušić-Radovčić et al., 2021; Poljanec et al., 2021), but also in relation to the absorption

of smoke and the deposition of PAHs, as recently shown by Marušić-Radovčić et al. (2024).

Given the above considerations, this study aimed to investigate the influence of the smoking method (traditional or industrial) and the anatomical location (BF or SM muscle) on the PAH content in the production of smoked Turopolje ham.

## Materials and methods

### Animals and Ham Processing

The production of pigs for ham processing was described by Karolyi et al. (2024). All hams used in the present study were processed the same way and differed only in the smoking method: in the traditional way with an internal firebox (TRAD group, n=5) or in the controlled smoking chamber with an external smoke generator (IND group: A - standard smoking, n=5 or B - 50 % reduced smoking, n=5). In brief, raw TP hams (mean weight  $6,5 \pm 0,85$  kg) were shaped and manually salted with a mixture of commercial curing salt (NaCl with 0.6 %  $\text{NaNO}_2$ ) and spices (black pepper, garlic, and paprika), piled into large PVC tubs and placed in the cold for salting ( $T=4$  °C,  $\text{RH}=85$  %). After salting, the IND hams were smoked in the chamber ( $T=20$  °C,  $\text{RH}=80$  %) a total of eight times (IND A) or four times (IND B) for 60 min with smoke produced by burning commercial hardwood pellets for meat smoking in an external smoke generator, while the TRAD hams were traditionally craft smoked (daily for 2-3 h) for two weeks in cold smoke produced by burning beech logs in an internal firebox. After smoking, all hams were placed to the drying and ripening chamber under controlled conditions ( $T=14$  °C,  $\text{RH}=72$  %). The sampling of BF and SM was carried out when the dry-cured hams were 15 months old (Photo 1). After excision, the samples are coded, vacuum-packed and frozen at  $-20$  °C until analysed.

### Determination of PAHs content

PAH content ( $\mu\text{g}/\text{kg}$ ) was determined and quantified by high-performance liquid chromatography with fluorescence detection (HPLC-FLD), according to Bogdanović et al. (2019). PAH isolation involves a preparative size-exclusion chromatography. Briefly, a homogenized sample (1 g) was spiked with internal standard BbChr ( $50 \mu\text{g}/\text{L}$ ,  $100 \mu\text{L}$ ) and diluted with dichloromethane. The obtai-



**Photo 1** Turopolje hams from different smoking methods (TRAD – traditional, IND A – industrial, IND B – industrial 50% reduced)

**Fotografija 1.** Turopoljske šunke iz različitih metoda dimljenja (TRAD – tradicionalno, IND A – industrijsko, IND B – industrijsko 50% reducirano)

ned mixture was centrifuged and filtered. Sample extracts were injected into a HPLC Agilent 1200 Series system (Agilent, Singapore, Singapore) for size-exclusion chromatography (SEC). The preparative SEC was performed under isocratic conditions (100% dichloromethane) at the flow rate of 1 mL/min and room temperature. The PAH analysis was performed using an Ultra Pressure Liquid Chromatograph (Agilent 1290 Infinity UHPLC, Agilent, Singapore) equipped with 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  $\mu\text{m}$  particle size) supplied by Thermo Scientific (Thermo-Scientific, Germany), the maintained temperature thereby being 30 °C and the injection volume being 15  $\mu\text{L}$ . 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 and the compounds quantification were as described by Bogdanović et al. (2019).

### Statistical analysis

Microsoft Excel was used for descriptive statistics (mean values, standard deviations) and graphical representations of BaP, the sum of PAH4 and the total PAHs content. For the comparison of the smoking methods and the muscles, the data on

PAH profiles were analysed by a two-way analysis of variance using the general linear model procedure (PROC GLM) of the SAS 9.4 statistical software (SAS Institute Inc., Cary, NC, USA) with fixed effects of smoking (S), muscle (M) and their interaction (S x M). Differences were considered statistically significant if  $P < 0.05$ .

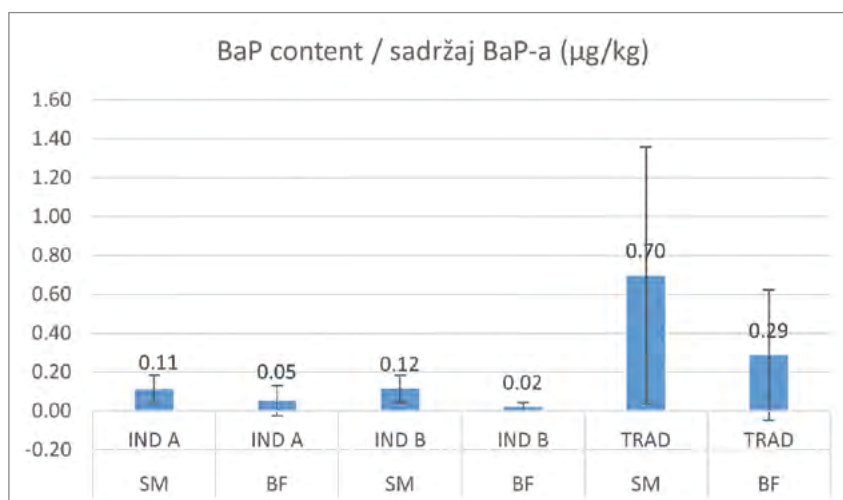
## Results and discussion

Of the PAHs analysed, 14 compounds were detected: naphthalene (Nap), fluorene (Flu), acenaphthene (Acp), phenanthrene (Phen), anthracene (Ant), fluoranthene (Flt) and pyrene (Py) in the group of light PAHs, as well as benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbFA), benzo[k]fluoranthene (BkFA), benzo[a]pyrene (BaP), dibenz[a,h]anthracene (DbahA) and benzo[ghi]perylene (BghiP) in the group of heavy PAHs, with the limits of quantification (LOQ) ranging from 0.10 (BaP) to 3.30  $\mu\text{g}/\text{kg}$  (Flu).

The mean values ( $\pm$  standard deviation) for BaP, the sum of BaA, Chr, BbFA and BaP (PAH4) and the total PAHs content ( $\mu\text{g}/\text{kg}$ ) in SM and BF muscles of TRAD and IND A and IND B Turopolje hams are presented in Graphs 1 to 3.

The mean BaP content ranged from 0.02  $\mu\text{g}/\text{kg}$  in the BF muscle of IND B ham to 0.70  $\mu\text{g}/\text{kg}$  in the SM muscle of TRAD ham. The mean values for the sum of PAH4 and the total PAHs content followed the same pattern and ranged from 1.16

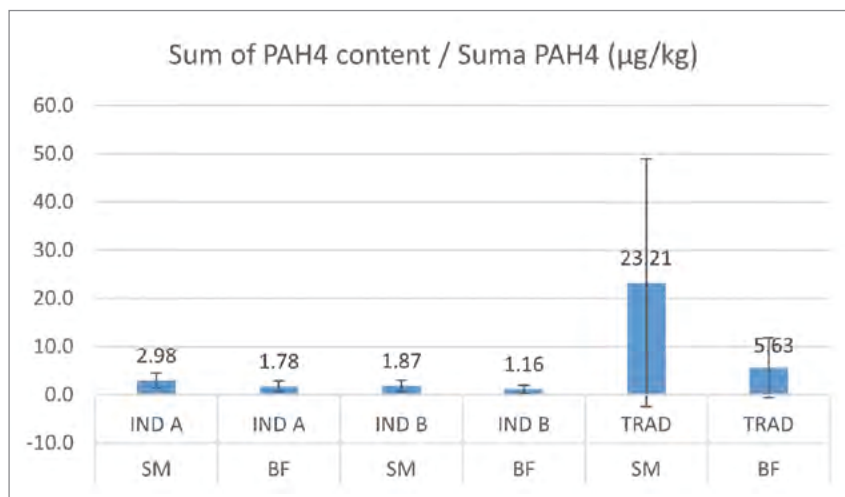
and 22.75  $\mu\text{g}/\text{kg}$  in the BF of IND B ham to 23.21 and 247.17  $\mu\text{g}/\text{kg}$  in the SM of TRAD hams. From an EU regulatory perspective, the reported group mean values for BaP and the sum of PAH4 were below the permitted limits for all smoking methods examined ( $< 2.0 \mu\text{g}/\text{kg}$  and  $< 12.0 \mu\text{g}/\text{kg}$  for industrial smoking and  $< 5.0 \mu\text{g}/\text{kg}$  and  $< 30.0 \mu\text{g}/\text{kg}$  for traditional smoking, respectively). Individually (data not shown), all samples were below the maximum permitted level for BaP. However, the samples of SM from two out of five TRAD hams exceeded the allowed limit of 30.0  $\mu\text{g}/\text{kg}$  for PAH4 content by 1.4 and 1.9 times and can therefore be considered potentially unsafe for consumption. This result corroborates previous report by Mastanjević et al. (2020b) for traditionally smoked *Slavonska šunka*, where almost  $\frac{1}{4}$  of the analysed samples exceeded the prescribed PAH4 content, some of them up to 3.3 times, confirming that the potential health risks associated with traditional smoking still exist in practise. Compared to other smoked hams, the mean BaP and PAH4 levels in IND A and IND B hams were similar for BaP or somewhat higher for PAH4 than those reported by Poljanec et al. (2019) for smoked Croatian PGI hams (i.e., 0.05 – 0.07  $\mu\text{g}/\text{kg}$  for BaP and 0.41 – 0.44  $\mu\text{g}/\text{kg}$  for PAH4, BF muscle), while the TRAD hams were generally well above these ranges and more comparable to the average BaP and PAH4 levels (i.e. 0.65  $\mu\text{g}/\text{kg}$  and 5.24  $\mu\text{g}/\text{kg}$ , respectively) found in some specimens of household smoked dry-cured ham *Hercegovački*



**Graph 1** BaP (mean  $\pm$  st.dev.) in SM and BF muscles of Turopolje ham from different smoking methods (TRAD – traditional, IND A – industrial, IND B – industrial 50% reduced)

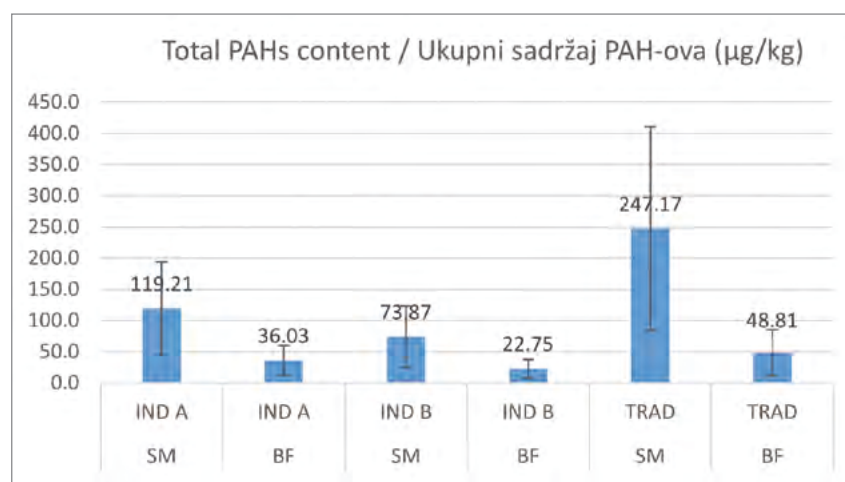
**Grafikon 1** BaP (srednja vrijednost  $\pm$  SD) u SM i BF mišićima turopoljske šunke iz različitih metoda dimljenja (TRAD – tradicionalno, IND A – industrijsko, IND B – industrijsko 50% reducirano)





**Graph 2** PAH4 (mean  $\pm$  st.dev.) in SM and BF muscles of Turopolje ham from different smoking methods (TRAD – traditional, IND A – industrial, IND B – industrial 50% reduced)

**Grafikon 2.** PAH4 (srednja vrijednost  $\pm$  SD) u SM i BF mišićima turopoljske šunke iz različitih metoda dimljenja (TRAD – tradicionalno, IND A – industrijsko, IND B – industrijsko 50% reducirano)



**Graph 3** Total PAHs (mean  $\pm$  st.dev.) in SM and BF muscles of Turopolje ham from different smoking methods (TRAD – traditional, IND A – industrial, IND B – industrial 50% reduced)

**Grafikon 3.** Ukupni PAH-ovi (srednja vrijednost  $\pm$  SD) u SM i BF mišićima turopoljske šunke iz različitih metoda dimljenja (TRAD – tradicionalno, IND A – industrijsko, IND B – industrijsko 50% reducirano)

*pršut* (Mastanjević et al., 2020a).

The analysis of variance (Table 1) showed that TRAD hams had significantly higher levels of Ant, Chr, BbFA, BaP, and the sum of BaA, Chr, BbFA and BaP (PAH4) than hams from both A and B IND groups. TRAD hams had also higher content of Acp, Phen, Py, BaA, and total PAHs than IND B hams. Compared to BF, SM muscle showed significant

ly higher content of several PAHs, i.e. Nap, Flu, Acp, Phen, Ant, Flt, Py, BaA, BkFa, BghiP, and also higher total PAH content, which is consistent with the recent observations of Marušić Radovčić et al. (2024) on smoked dry-cured ham *Dalmatinski pršut*. However, a significant S x M interaction was observed for Phen and Ant content which were higher only in SM muscle of TRAD hams (Table 2).

**Table 1** Least-squares means for PAHs content (µg/kg) in Turopolje ham according to smoking method and anatomical site

**Tablica 1.** Srednje vrijednosti procijenjene metodom najmanjih kvadrata za sadržaj PAH-ova (µg/kg) u turopoljskoj šunki prema metodi dimljenja i anatomskej lokaciji

PAH Compounds / PAH spojevi	Smoking (S) / Dimljenje (S)			Muscle (M) / Mišić (M)			Significance / Značajnost		
	TRAD (n=5)	IND A (n=5)	IND B (n=5)	SM (n=15)	BF (n=15)	RMSE	S	M	S x M
Naphthalene / Naftalen	1.41	0.93	0.76	1.39	0.67	0.63	t	**	ns
Fluorene / Fluoren	89.02	55.75	35.30	98.58	21.46	51.18	t	**	ns
Acenaphthene / Acenaften	8.06 <sup>a</sup>	3.51 <sup>ab</sup>	1.86 <sup>b</sup>	7.13	1.82	4.78	*	**	t
Phenanthrene / Fenantren	23.43 <sup>a</sup>	9.69 <sup>ab</sup>	6.25 <sup>b</sup>	21.02	5.24	13.15	*	**	*
Anthracene / Antracen	2.20 <sup>a</sup>	0.75 <sup>b</sup>	0.68 <sup>b</sup>	2.05	0.37	1.05	**	**	*
Fluoranthene / Fluoranten	3.47	1.95	0.33	3.29	0.54	2.93	t	*	ns
Pyrene / Piren	2.78 <sup>a</sup>	1.45 <sup>ab</sup>	0.72 <sup>b</sup>	2.52	0.78	1.81	*	*	t
Benz[a]anthracene / Benz[a]antracen	1.77 <sup>a</sup>	0.39 <sup>ab</sup>	0.16 <sup>b</sup>	1.37	0.18	1.43	*	*	t
Chrysene / Krizen	5.67 <sup>a</sup>	0.57 <sup>b</sup>	0.70 <sup>b</sup>	3.12	1.50	4.56	*	ns	ns
Benzo(b)fluoranthene / Benzo(b)fluoranten	8.37 <sup>a</sup>	1.34 <sup>b</sup>	1.15 <sup>b</sup>	4.56	2.68	5.48	**	ns	ns
Benzo(k)fluoranthene / Benzo(k)fluoranten	0.24	0.05	0.10	0.20	0.06	0.19	t	*	ns
Benzo[a]pyrene / Benzo[a]piren	0.55 <sup>a</sup>	0.08 <sup>b</sup>	0.07 <sup>b</sup>	0.31	0.16	0.32	**	ns	ns
Dibenz(a,h)anthracene / Dibenz[a,h]antracen	0.17	0.63	0.00	0.48	0.05	1.14	ns	ns	ns
Benzo(ghi)perylene / Benzo[ghi]perylene	0.69	0.54	0.24	0.73	0.24	0.63	ns	*	ns
Indeno(1,2,3-cd)pyrene / Indeno[1,2,3-cd]piren	nd	nd	nd	nd	nd	-	-	-	-
PAH41	16.36 <sup>a</sup>	2.38 <sup>b</sup>	2.08 <sup>b</sup>	9.36	4.53	11.37	*	ns	ns
Total PAHs / Ukupni PAH-ovi	147.82 <sup>a</sup>	77.62 <sup>ab</sup>	48.31 <sup>b</sup>	146.75	35.75	78.06	*	**	ns

Naphthalene (Nap), Fluorene (Flu), Acenaphthene (Acp), Phenanthrene (Phen), Anthracene (Ant), Fluoranthene (Flt), Pyrene (Py), Benz[a]anthracene (BaA), Chrysene (Chr), Benzo[b]fluoranthene (BbFA), Benzo[k]fluoranthene (BkFA), Benzo[a]pyrene (BaP), Dibenz[a,h]anthracene (DBaHA), Benzo[ghi]perylene (BghiP), Indeno[1,2,3-cd]pyrene (IP) TRAD – traditional, IND A – industrial, IND B – industrial reduced (50%); SM – semimembranous, BF – biceps femoris; 1PAH4: the sum of BaA, Chr, BbFA and BaP; RMSE – root mean square error; \*\*P≤0.01; \*P≤0.05; t - P<0.10; ns - P>0.05; nd - not detected; a,b within a row for smoking (S) least-squares means lacking a common superscript letters differ (P≤0.05) Naftalen (Nap), Fluoren (Flu), Acenaften (Acp), Fenantren (Phen), Antracen (Ant), Fluoranten (Flt), Piren (Py), Benz[a]antracen (BaA), Krizen (Chr), Benzo[b]fluoranten (BbFA), Benzo[k]fluoranten (BkFA), Benzo[a]piren (BaP), Dibenz[a,h]antracen (DBaHA), Benzo[ghi]perilen (BghiP), Indeno[1,2,3-cd]piren (IP) TRAD – tradicionalno, IND A – industrijsko, IND B – industrijsko reducirano (50%); SM – semimembranous, BF – biceps femoris; 1PAH4: zbroj BaA, Chr, BbFA i BaP; RMSE – srednja kvadratna pogreška; \*\*P≤0.01; \*P≤0.05; t - P<0.10; ns - P>0.05; nd – nije utvrđeno; a,b unutar redova za dimljenje (S) srednje vrijednosti najmanjih kvadrata bez zajedničkog indeksa značajno se razlikuju (P≤0.05)

**Table 2** Interaction of smoking method and anatomical site (SxM) for PAHs content (µg/kg) in Turopolje ham  
**Tablica 2.** Interakcija metode dimljenja i anatomske lokacija (SxM) za sadržaj PAH-ova (µg/kg) u turopoljskoj šunki

PAH Compounds / PAH spojevi	Muscle (M) / Mišić (M)						SE
	SM			BF			
	Smoking (S) / Dimljenje (S)						
	TRAD (n=5)	IND A (n=5)	IND B (n=5)	TRAD (n=5)	IND A (n=5)	IND B (n=5)	
Phenanthrene / Fenantren	40.03 <sup>a</sup>	15.04 <sup>ab</sup>	7.97 <sup>b</sup>	6.83 <sup>b</sup>	4.34 <sup>b</sup>	4.54 <sup>b</sup>	5.88
Anthracene / Antracen	3.80 <sup>a</sup>	1.17 <sup>b</sup>	1.17 <sup>b</sup>	0.60 <sup>b</sup>	0.33 <sup>b</sup>	0.19 <sup>b</sup>	0.47

TRAD – traditional, IND A – industrial, IND B – industrial reduced (50%); SM – semimembranous, BF – biceps femoris; SE – standard error; a,b within a row least-squares means lacking a common superscript letters differ (P≤0.05)

TRAD – tradicionalno, IND A – industrijsko, IND B – industrijsko reducirano (50%); SM – semimembranous, BF – biceps femoris; SE – standardna greška; a,b unutar redova srednje vrijednosti najmanjih kvadrata bez zajedničkog indeksa značajno se razlikuju (P≤0.05)

## Conclusion

This study shows a marked influence of smoking method and anatomical location on PAH content in Turopolje ham, mainly in the form of lower BaP and PAH4 content in all chamber-smoked hams compared to traditionally smoked counterparts and higher levels of light PAHs, some heavy PAHs and total PAH content in the more exposed SM compared to the inner BF muscle. Reduced smoking by half in IND hams did not affect BaP and PAH4 content, but reduced the content of some individual and total PAHs contents. Finally, it should be emphasised that all IND hams and TRAD

hams were below the maximum permitted level for BaP. However, two out of five hams from the TRAD group exceeded the limit for the sum of PAH4 (30 µg/kg) set by EU legislation for traditionally smoked meat products, indicating the need for stricter control of the traditional smoking process.

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## Utjecaj metode dimljenja i anatomske lokacije na sadržaj policikličkih aromatskih ugljikovodika u turopoljskoj šunki

### Sažetak

Kako bi se podržao održiviji uzgoj lokalne turopoljske svinje, pasminu je potrebno vrednovati kroz mesne proizvode visoke dodane vrijednosti, kao što je dimljena i sušena turopoljska šunka. Do danas postoji vrlo malo spoznaja o kvaliteti i sigurnosti dimljene turopoljske šunke, uključujući podatke o sadržaju i distribuciji potencijalnih kontaminanata poput policikličkih aromatskih ugljikovodika (PAH) iz dima. Stoga je cilj ovog rada bio istražiti utjecaj načina dimljenja (tradicionalnog ili industrijskog) i anatomske lokacije (M. biceps femoris - BF ili M. semimembranosus - SM) na sadržaj PAH-ova u turopoljskoj šunki. Sve šunke (n=15) proizvedene su na isti način, a razlikovale su se samo u postupku dimljenja; tradicionalni način iznad ložišta (skupina TRAD, n=5) ili u komori za dimljenje (skupina IND: A - standardno dimljenje, n=5 ili B - 50% smanjeno dimljenje, n=5). Sadržaj PAH (μg/kg) određen je i kvantificiran metodom tekućinske kromatografije visoke učinkovitosti s fluorescencijskom detekcijom (HPLC-FLD). U analizi podataka korištena je dvostruka analiza varijance (GLM procedura statističkog paketa SAS) s fiksnim efektima dimljenja (S), mišića (M) i



njihove interakcije (SxM). Od istraživanih PAH-ova detektirano je 14 spojeva. Rezultati su pokazali da TRAD šunke imaju više razine antracena (Ant), krizena (Chr), benzo[b]fluorantena (BbFA), benzo[a]pirena (BaP) i zbroja benz[a]antracena (BaA), Chr i BaP (PAH 4) u odnosu na šunke iz A i B IND skupine. TRAD šunke također su imale veći sadržaj acenaftena (Acp), fenantrena (Phen), pirena (Py), BaA i ukupnih PAH-ova nego IND B šunke. U usporedbi s BF, SM mišić je imao veći sadržaj naftalena (Nap), fluorena (Flu), Acp, Phen, Ant, fluorantena (Flt), Py, BaA, benzo[k]fluorantena (BkFA), benzo[ghi]perilena (BghiP), kao i viši sadržaj ukupnih PAH-ova. Međutim, primijećena je značajna interakcija SxM za sadržaj Phen i Ant, koji je bio veći samo u SM mišiću TRAD šunki. Zaključno, provedeno istraživanje pokazuje značajan utjecaj načina dimljenja i anatomske lokacije na sadržaj PAH-ova u analiziranim turopoljskim šunkama, poglavito u obliku nižeg sadržaja BaP-a i PAH4 u svim industrijski dimljenim šunkama u usporedbi s tradicionalno dimljenim šunkama, te višim razinama lakih PAH-ova, pojedinih teških PAH-ova i ukupnih PAH-ova u vanjskom SM mišiću u usporedbi s unutarnjim BF mišićem. Smanjeno dimljenje u IND šunkama nije utjecalo na sadržaj BaP-a i PAH4, ali je smanjilo sadržaj nekih pojedinačnih i ukupnih PAH-ova. Sve TRAD šunke i IND šunki imale su razine BaP-a ispod dopuštenih granica. Međutim, dvije od pet šunki iz TRAD skupine premašile su limit za ukupni sadržaj PAH4 (30 µg/kg) koji dozvoljava zakonodavstvo EU za tradicionalno dimljene mesne proizvode, što ukazuje na potrebu strože kontrole tradicionalnog procesa dimljenja.

**Ključne riječi:** šunka, dimljenje mesa, PAH spojevi, mišić, turopoljska svinja

## Einfluss der Räucher- und der anatomischen Stelle auf den Gehalt an polyzyklischen aromatischen Kohlenwasserstoffen in Turopolje-Schinken

### Zusammenfassung

Um eine nachhaltigere Zucht des lokalen Turopolje-Schweins zu unterstützen, muss die Rasse durch Fleischprodukte mit hohem Mehrwert, wie trocken gepökelten und geräucherten Turopolje-Schinken, aufgewertet werden. Bislang gibt es nur sehr wenige Daten über die Qualität und Sicherheit von Turopolje-Schinken, einschließlich Informationen über den Gehalt und die Verteilung potenzieller Schadstoffe wie polyzyklische aromatische Kohlenwasserstoffe (PAK) aus dem Rauch. Ziel dieser Studie war es daher, den Einfluss der Räucher- und der anatomischen Stelle (M. biceps femoris - BF oder M. semimembranosus - SM) auf den PAK-Gehalt im Turopolje-Schinken zu untersuchen. Alle Schinken (n=15) wurden auf die gleiche Weise hergestellt und unterschieden sich nur in den Räucher- und der anatomischen Stelle: auf traditionelle Weise über dem Feuer (Gruppe TRAD, n=5) oder in der Räucher- und der anatomischen Stelle (Gruppe IND A - Standardräucherung, n=5 oder B - 50 % reduzierte Räucherung, n=5). Der PAK-Gehalt (µg/kg) wurde durch Hochleistungsflüssigkeitschromatographie mit Fluoreszenzdetektion (HPLC-FLD) bestimmt und quantifiziert. Es wurde eine zweifache Varianzanalyse durchgeführt (GLM-Prozedur von SAS) mit festen Effekten des Rauchens (S), des Muskels (M) und ihrer Wechselwirkung (SxM). Von den untersuchten PAKs wurden 14 Verbindungen nachgewiesen. Die Ergebnisse zeigten, dass TRAD-Schinken höhere Gehalte an Anthracen (Ant), Chrysen (Chr), Benzo[b]fluoranthren (BbFA), Benzo[a]pyren (BaP) und die Summe von Benz[a]anthracen (BaA), Chr, BbFA und BaP (PAH 4) aufwies als Schinken aus den IND-Gruppen A und B. TRAD-Schinken wiesen auch einen höheren Gehalt an Acenaphthen (Acp), Phenanthren (Phen), Pyren (Py), BaA und PAK insgesamt auf als IND B-Schinken. Im Vergleich zu BF wies SM-Muskel einen höheren Gehalt an mehreren PAK auf, d. h. Naphthalin (Nap), Fluor (Flu), Acp, Phen, Ant, Fluoranthren (Flt), Py, BaA, Benzo[k]fluoranthren (BkFA), Benzo[ghi]perylen (BghiP), und auch einen höheren Gesamtgehalt an PAK. Eine signifikante SxM-Interaktion wurde jedoch für den Phen- und Ant-Gehalt beobachtet, der nur im SM-Muskel von TRAD-Schinken höher war. Zusammenfassend zeigt diese Studie einen deutlichen Einfluss der Räucher- und der anatomischen Lage auf den PAK-Gehalt in Turopolje-Schinken, vor allem in Form eines niedrigeren BaP- und PAK4-Gehalts in allen in der Kammer geräucherten Schinken im Vergleich zu traditionell geräucherten Schinken und eines höheren Gehalts an leichten PAKs, ei-

nigen schweren PAKs und dem Gesamtgehalt an PAKs in den stärker exponierten SM-Muskeln im Vergleich zu den inneren BF-Muskeln. Das reduzierte Räuchern von IND-Schinken hatte keinen Einfluss auf den BaP- und den PAH4-Gehalt, verringerte jedoch den Gehalt einiger einzelner PAKs und den Gesamtgehalt an PAKs. Alle IND-Schinken und TRAD-Schinken lagen unter dem zulässigen Höchstwert für BaP. Allerdings überschritten zwei von fünf Schinken aus der TRAD-Gruppe den in den EU-Rechtsvorschriften für traditionell geräucherte Fleischerzeugnisse festgelegten Grenzwert für die Summe der PAK4 (30 µg/kg), was auf die Notwendigkeit einer strengeren Kontrolle des traditionellen Räucherverfahrens hinweist.

**Schlüsselwörter:** trockener Schinken, Fleischräucherung, PAK-Verbindungen, Muskel, Turopolje-Schwein

## Influencia del método de ahumado y la ubicación anatómica en el contenido de hidrocarburos aromáticos policíclicos en el jamón de Turopolje

### Resumen

Con el fin de promover una cría más sostenible del cerdo autóctono de Turopolje, es necesario valorizar la raza mediante productos cárnicos de alto valor añadido, como el jamón de Turopolje curado en seco y ahumado. Hasta la fecha, existen pocos datos sobre la calidad y seguridad del jamón de Turopolje, incluyendo la información sobre el contenido y la distribución de posibles contaminantes como los hidrocarburos aromáticos policíclicos (PAHs, por sus siglas en inglés) provenientes del humo. Por lo tanto, el objetivo de este estudio fue investigar la influencia del método de ahumado (tradicional o industrial) y el sitio anatómico (M. biceps femoris - BF o M. semimembranosus - SM) en el contenido de PAHs en el jamón de Turopolje. Todos los jamones (n=15) se produjeron de la misma manera, diferenciándose únicamente en los procedimientos de ahumado: un método tradicional sobre la caja de fuego (grupo TRAD, n=5) o en una cámara de ahumado (grupo IND: A - ahumado estándar, n=5 o B - ahumado reducido en un 50 %, n=5). El contenido de PAHs (µg/kg) se determinó y cuantificó mediante cromatografía líquida de alta resolución con detección por fluorescencia (HPLC-FLD). Se realizó un análisis de varianza de dos vías (procedimiento GLM de SAS) con efectos fijos de ahumado (S), músculo (M) y su interacción (SxM). De los PAHs analizados, se detectaron 14 compuestos. Los resultados mostraron que los jamones TRAD tenían niveles más altos de antraceno (Ant), criseno (Chr), benzo[b]fluoranteno (BbFA), benzo[a]pireno (BaP) y la suma de benz[a]antraceno (BaA), Chr, BbFA y BaP (PAH4) en comparación con los jamones de ambos grupos IND (A y B). Además, los jamones TRAD presentaron mayores contenidos de acenafteno (Acp), fenantreno (Phen), pireno (Py), BaA y PAHs totales en comparación con los jamones IND B. En comparación con el músculo BF, el músculo SM mostró un mayor contenido de varios PAHs, como naftaleno (Nap), fluoreno (Flu), Acp, Phen, Ant, fluorantheno (Flt), Py, BaA, benzo[k]fluoranteno (BkFA), benzo[ghi]perileno (BghiP) y mayor contenido total de PAHs. Sin embargo, se observó una interacción significativa SxM en los contenidos de Phen y Ant, que fueron más altos únicamente en el músculo SM de los jamones TRAD. En conclusión, este estudio muestra una marcada influencia del método de ahumado y del sitio anatómico en el contenido de PAHs en los jamones de Turopolje, destacando niveles más bajos de BaP y PAH4 en todos los jamones ahumados en cámara en comparación con los ahumados tradicionalmente, así como mayores niveles de PAHs ligeros, algunos PAHs pesados y contenido total de PAHs en el músculo SM más expuesto en comparación con el músculo interno BF. El ahumado reducido en los jamones IND no afectó el contenido de BaP ni de PAH4, pero sí redujo el contenido de algunos PAHs individuales y totales. Todos los jamones IND y TRAD estuvieron por debajo del nivel máximo permitido para BaP. Sin embargo, dos de los cinco jamones del grupo TRAD excedieron el límite para la suma de PAH4 (30 µg/kg) establecido por la legislación de la UE para productos cárnicos ahumados tradicionalmente, lo que indica la necesidad de un control más estricto del proceso de ahumado tradicional.

**Palabras claves:** : jamón curado, ahumado de carne, compuestos PAHs, músculo, cerdo de Turopolje

## Impatto del metodo di affumicatura e della posizione anatomica sul contenuto di idrocarburi policiclici aromatici nel prosciutto del Turopolje

### Riassunto

Per supportare una suinicoltura locale più sostenibile, è necessario valorizzare la razza del Turopolje attraverso prodotti a base di carne ad alto valore aggiunto, come il prosciutto affumicato ed essiccato del Turopolje. Ad oggi, le conoscenze sulla qualità e la sicurezza del prosciutto affumicato del Turopolje sono molto scarse, compresi i dati sul contenuto e sulla distribuzione di potenziali contaminanti come gli idrocarburi policiclici aromatici (IPA) presenti nel fumo. Pertanto, lo scopo di questo studio consisteva nello studiare l'impatto del metodo di affumicatura (tradizionale o industriale) e della posizione anatomica (M. biceps femoris/muscolo bicipite femorale - BF o M. semimembranosus/muscolo semimembranoso - SM) sul contenuto di IPA nel prosciutto del Turopolje. Tutti i prosciutti (n=15) sono stati prodotti allo stesso modo, differendo solo per il processo di affumicatura, avvenuto in modo tradizionale sul focolare (gruppo TRAD, n=5) oppure nella camera di affumicatura (gruppo IND: A - affumicatura standard, n=5 oppure B - affumicatura ridotta al 50%, n=5). Il contenuto di IPA ( $\mu\text{g}/\text{kg}$ ) è stato determinato e quantificato utilizzando il metodo della cromatografia liquida ad alte prestazioni con rilevamento della fluorescenza (HPLC-FLD). Nell'analisi dei dati è stata utilizzata un'analisi della varianza a due vie (procedura GLM del pacchetto statistico SAS) con effetti fissi del fumo (S), dei muscoli (M) e della loro interazione (SxM). Tra gli IPA studiati, sono stati rilevati 14 composti. I risultati hanno mostrato che i livelli di antracene (Ant), crisene (Chr), benzo[b]fluorantene (BbFA), benzo[a]pirene (BaP) e della somma di benz[a]antracene (BaA), Chr e BaP (PAH 4) erano più elevati nei prosciutti TRAD rispetto ai prosciutti dei gruppi A e B IND. I prosciutti TRAD avevano anche un contenuto più elevato di acenafte (Acp), fenantrene (Phen), pirene (Py), BaA e IPA totali rispetto ai prosciutti IND B. Rispetto al BF, il muscolo SM aveva un contenuto più elevato di naftalene (Nap), fluorene (Flu), Acp, Phen, Ant, fluorantene (Flt), Py, BaA, benzo[k]fluorantene (BkFA), benzo[ghi]perilene (BghiP), nonché un contenuto più elevato di IPA totali. Tuttavia, è stata osservata un'interazione SxM significativa per il contenuto di Phen e Ant, che era maggiore solo nel muscolo SM dei prosciutti TRAD. In conclusione, la ricerca condotta mostra un impatto significativo del metodo di affumicatura e della posizione anatomica sul contenuto di IPA nei prosciutti del Turopolje analizzati, principalmente sotto forma di un contenuto inferiore di BaP e IPA4 in tutti i prosciutti affumicati industrialmente rispetto ai prosciutti affumicati tradizionalmente, e livelli più elevati di IPA leggeri, di singoli IPA pesanti e di IPA totali nel muscolo SM esterno rispetto al muscolo BF interno. La riduzione dell'affumicatura nei prosciutti IND non ha influenzato il contenuto di BaP e IPA4, ma ha ridotto il contenuto di alcuni IPA singoli e totali. Tutti i prosciutti TRAD e IND avevano livelli di BaP inferiori ai limiti consentiti. Tuttavia, due prosciutti su cinque del gruppo TRAD hanno superato il limite del contenuto totale di IPA4 ( $30 \mu\text{g}/\text{kg}$ ) consentito dalla legislazione UE per i prodotti a base di carne affumicati in modo tradizionale, il che indica la necessità di un controllo più rigoroso del processo di affumicatura tradizionale.

**Parole chiave:** prosciutto, affumicatura della carne, composti IPA, muscolo, maiale del Turopolje

