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EVALUATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN PORK MEAT PRODUCTS OBTAINED IN TRADITIONAL SYSTEMS IN ROMANIA

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ABSTRACT. The aim of this study was to determine the polycyclic aromatic hydrocarbons (PAH) levels in the smoked meat products obtained in the traditional system in Romania. The importance of this study resides in the fact that these compounds are known to be carcinogenic and their levels should be strictly monitored so as to ensure the safety of the consumers. Until now, no study was performed in Romania that addresses this issue in the traditional pork meat products. The study was performed on 60 meat products samples (sausages, bacon, ham) taken from a certified traditional meat processing unit but also from retail units that delivered industrially processed meat products. The method used in the detection of the 15 PAHs analysed was HPLC. Our results showed that the highest percent of total PAHs is found in the traditionally obtained smoked bacon and the lowest in industrially produced pork meat sausage. None of the samples examined exceeded the limit imposed by the European legislation for benzo[a]pyrene (BaP), but the smoked bacon revealed higher values for PAH4 than the allowed limit. Following this study we suggest that a more careful attention should be paid to all factors and apply such smoking conditions that result in the lowest possible contamination with PAH in pork meat products obtained in the traditional system in Romania.

Keywords: *polycyclic aromatic hydrocarbons, traditional, industrial, smoked, product.*

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INTRODUCTION

Traditional products represent an important part of Romanian culture, having a major role in the preservation of various technologies of processing, distinctive in many regions and with many identity trade markers. At the level of the agricultural economy in Romania, traditional products account for only 2.5% of the total sales [1], while in the European countries there is a growing trend of production and consumption of these types of products [2]. Various researches have been performed on a number of traditional meat products obtained in different regions, underlining their unique flavours and nutritional benefits [3,4]. Still, it is important also to focus the studies on their biochemical particularities resulting after the traditional processing, that sometimes may affect their quality and protection of consumers.

Cured meat products are usually processed through a series of technological steps, like: salting, drying and smoking. In Romania, traditional cured meat products have the characteristic of being processed in a particular way, depending on the region, leaving them to dry and smoke for a larger amount of time and using natural wood, which defines their specific organoleptic quality and stability requirements. Previous studies have been carried out to evaluate the impact of these technological steps on the microbiological and compositional modifications [5, 6] but very few focused on the aspect of potentially toxic residues that may exceed their limits imposed by the current legislation, like the polycyclic aromatic hydrocarbons (PAHs) levels.

Polycyclic aromatic hydrocarbons (PAHs) comprise the largest class of chemical compounds known to be cancer causing agents [7]. Due to these effects they are considered to be top of the list of the most hazardous substances [8]. Smoking influences the levels of PAHs in products, and that is why the period and conditions in which this step is made holds great importance. Unfortunately, traditional products are not strictly monitored and this step evaluated only from the aspect of sensorial characteristics not also the biochemical changes that affect these functional products.

During the smoking process, hundreds of individual PAHs are formed and released during the incomplete combustion or thermal decomposition (pyrolysis) of the organic material. Among the PAHs released in the product, benzo[*a*]pyrene can be used as a specific biochemical marker for the occurrence and impact of these carcinogenic factors [9]. Thus, the European Food Safety Authority (EFSA) recommends analysis of benzo[*c*]fluorene (B*c*L) assessed to be relevant by the Joint FAO/WHO Experts Committee on Food Additives (JECFA) [10]. Recently another PAH has been the major interest for toxicological evaluations, dibenzo[*a*,*l*]pyrene, being considered to have a much stronger carcinogenic potential than benzo[*a*]pyrene [11]. In 2005, the European Commission issued a Recommendation [12] that Member States should perform random monitoring for the presence of PAHs in foodstuffs. The Panel found that PAH4 (the sum of benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene and chrysene) and PAH8 (the sum of benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[ghi] perylene, chrysene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene) were the most suitable indicators for PAHs in food, with PAH8 not providing much added value compared to PAH4.

The estimated contribution of meat products to the overall intake of PAHs also differs between countries: from very low for the UK to 21% in USA and 27% in France, resulting in the second contributing food group after bread and cereals [13, 14].The study of PAH levels in Romania is interesting and very important in order to be able to improve the quality and safety of the traditional products that use smoking as main step in processing. Given that some traditional products are in the course of obtaining the protected designated origin trade marks (PDO), it is imperious to check the compliance with the EU regulations. These types of products are also highly consumed in Romania so any potential harmful effect is mandatory to be exposed and assessed. The most consumed smoked meat product in Romania is the pork meat sausage, which is distinguishable by a particular taste, high nutritional value and large variety of processing technologies.

The aim of this study was to investigate the contents of PAH compounds in three different meat products (pork sausage, pork ham, pork bacon) and to evaluate the effect of traditional smoking process with the industrial one.

RESULTS AND DISCUSSION

The mean PAH levels obtained at the assessment of the traditional products compared to the industrial ones are shown in table 1, 2 and 3. For each product tested the evaluation of PAH level was made from the external layer as well as from the internal one. In the case of traditional pork sausage, among the 15 PAHs investigated, 14 compounds were detected within the external layers and 7 also from the inner layers. The sum of PAH evaluated from the external layers was 80.52 $\mu g/kg^{-1}$, and from the content 3.48 $\mu g/kg^{-1}$. The quantity of benzo[*a*]pyrene (BaP) was found to be in an average 1.86 $\mu g/kg^{-1}$, a value that was relatively high compared to the industrially obtained products. When compared to the industrially obtained pork sausage, we found that the 12 PAHs values at the external surface of the products were lower (65.9 μg / kg^{-1}). From the inner part of the product, the levels of PAHs were also in a lower amount (2.25 $\mu g/kg^{1}$), as well as the BaP levels (0.45 $\mu g/kg^{-1}$).

Table 1. PAH level in the traditional sausage produced in a traditional and industrial system

No.	PAH	Traditional smoked pork sausage ($\mu g \cdot kg^{-1}$)	Industrial smoked pork sausage ($\mu g \cdot kg^{-1}$)	ANOVA
1	Naphthalene	11.8	11.54	*
2	Acenaphthene	3.23	4.14	**
3	Fluorene	<LQ	<LQ	NP
4	Phenanthrene	14.91	17.54	**
5	Anthracene	2.18	2.45	*
6	Fluoranthene	0.59	0.47	**
7	Pyrene	0.37	0.84	***
8	Benz[a]anthracene	0.04	<LQ	NP
9	Chrysene	0.18	0.26	*
10	Benzo(b)fluoranthene	8.19	9.45	**
11	Benzo(k)fluoranthene	1.16	0.84	***
12	**Benzo[*a*]pyrene**	**1.86**	**0.45**	***
13	Dibenz(a,h)anthracene	3.29	2.44	**
14	Benzo(ghi)perylene	32.66	15.48	***
15	Indeno(1,2,3cd)pyrene	LQ	<LQ	-
Total value PAHs		**80.52**	**65.98**	*

Significance, NP, not performed; * $P< 0.05$; ** $P<0.01$; *** $P<0.001$; Data is presented as least square mean.

The total values of PAHs are different according to the smoking technology, the statistical analysis showing significant variations between the levels of PAHs in traditional pork sausage and the industrial one. It was obvious that the traditional smoking process affects in a high amount the concentration of PAHs in the product, therefore making it from this point of view more dangerous.

The PAHs values obtained in the Romanian traditional pork meat sausages are also higher than the ones revealed in the Spanish traditional pork meat sausages [15], where the BaP levels were only 0.02 $\mu g / kg^{-1}$, much lower than the one revealed by our study (1.86 $\mu g / kg^{-1}$). Also, when analysing the PAH4 levels (the sum of benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene and chrysene) we revealed that the number obtained (10.27 $\mu g / kg^{-1}$) is high and very close to the limit (12 $\mu g \cdot kg^{-1}$) imposed by the current EU legislation [9].

Table 2. PAH concentrations in traditionally and industrially produced smoked bacon

No.	PAH	Traditional smoked pork bacon (μg·kg^{-1})	Industrial smoked pork bacon (μg·kg^{-1})	ANOVA
1	Naphthalene	6.96	10.32	**
2	Acenaphthene	17,55	<LQ	NP
3	Fluorene	<LQ	<LQ	NP
4	Phenanthrene	45.93	37.79	**
5	Anthracene	1.20	0.47	*
6	Fluoranthene	3.03	1.16	**
7	Pyrene	3.15	3.65	***
8	Benz[a]anthracene	0.75	3.33	**
9	Chrysene	0.47	4.09	*
10	Benzo(b)fluoranthene	9.47	9.51	**
11	Benzo(k)fluoranthene	2.05	17.90	***
12	**Benzo[*a*]pyrene**	**1.87**	**0.32**	***
13	Dibenz(a,h)anthracene	7.63	3.84	**
14	Benzo(ghi)perylene	7.40	7.02	***
15	Indeno(1,2,3cd)pyrene	0.12	0.60	*
Total value PAHs		**107.57**	**100**	*

Significance, NP, not performed; * P< 0.05; ** P<0.01; *** P<0.001; Data is presented as least square mean.

Compared to smoked sausage, smoked bacon contains higher levels of PAHs. We revealed that the total value of the 15 PAHs in the traditional smoked bacon is 107.57 μg / kg^{-1}, much higher compared to the traditional smoked sausage (80.52 μg / kg^{-1}). The difference in PAHs levels between the two types of processing (industrial and traditional) is not so high in case of bacon (107.57 μg / kg^{-1} vs. 100 μg / kg^{-1}). Literature data are very diverse for this type of products; this fact could be explained by the different smoking technologies used. An essential role in the total content of PAHs is held by the type of wood used in smoking, quantity of oxygen, type of procedure (direct or indirect) and period [13].

All the values obtained in the analysis of PAHs concentration in the traditionally obtained smoked bacon and the industrially obtained one has shown statistically significant differences (table 2). The value of BaP in the industrially obtained smoked bacon showed a markedly lower level (0.32 μg / kg^{-1}), than in the case of traditional smoked bacon where the value is almost six time higher (1.87 μg / kg^{-1}). The maximum acceptable level for BaP in food products is 2 μg / kg^{-1}, and even though none of the samples exceeded this limit imposed, the values are very high. Even though the value of BaP in the industrial smoked bacon was relatively low compared to the traditional one, in case of PAH4 the values were much higher (17.25 μg / kg^{-1} vs. 12.56 μg / kg^{-1}). Both products exceed the limit (12 μg·kg^{-1}) imposed by the current EU legislation.

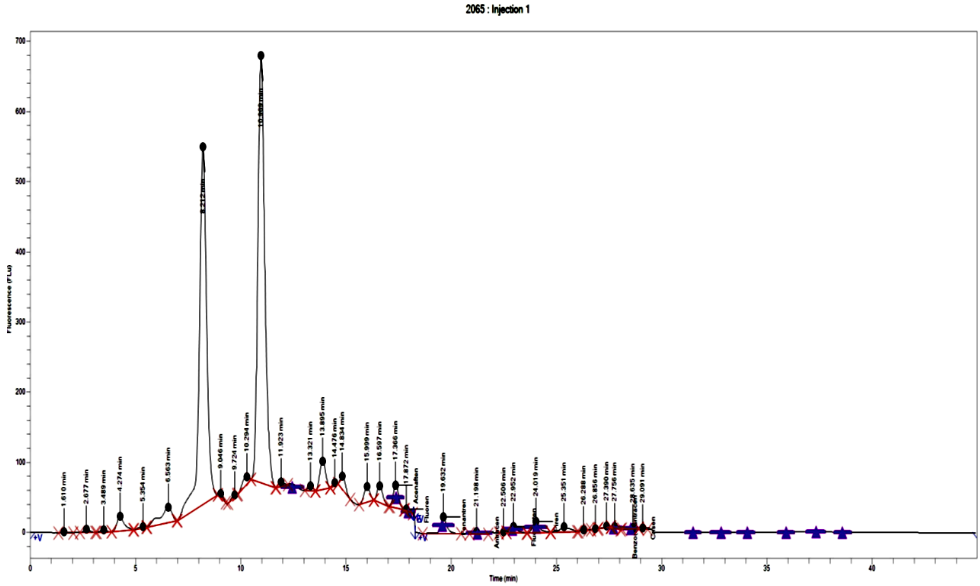

Fig. 1. Representative chromatogram obtained in a sample of traditional smoked bacon

Compared to smoked sausage and smoked bacon, pork ham contains the lowest levels of BaP (Table 3), not detectable by our method because of the low quantity (LQ). The highest level among the 15 PAHs analysed was the phenanthrene, which showed in both types of products (traditional and industrial ones) a level that exceeded 60 $\mu g / kg^{-1}$. We revealed that the total value of the 15 PAHs in the traditional smoked ham was 92.69 $\mu g / kg^{-1}$, not statistically different from the industrial one (97.15 $\mu g / kg^{-1}$).

The PAHs values determined in Romanian traditional pork meat sausage were lower than the ones reported in Portuguese traditional smoked sausage [16] but when compared to other studies made in Italy and Spain, our values were much higher [15, 17]. The content of BaP in the Romanian traditional sausage was much higher than the one revealed in "Pitina" (traditional Italian smoked sausage) (0.8 $\mu g / kg^{-1}$) [15], Serbian smoked sausage (0.24 to 0.33 $\mu g / kg^{-1}$) [8], Swedish sausage (below the limit of detection) [18]. Although the level of BaP were much higher than the other traditional products from other countries, none of the products investigated exceeded the maximum limit (2 $\mu g / kg^{-1}$) imposed by the current legislation in what concerns the BaP levels [9].

Table 3. PAH concentrations in traditionally and industrially produced smoked ham

No.	PAH	Traditional smoked ham (µg·kg^{-1})	Industrial smoked ham (µg·kg^{-1})	ANOVA
1	Naphthalene	6.66	4.41	**
2	Acenaphthene	6.30	5.07	**
3	Fluorene	16.63	18.51	NS
4	Phenanthrene	60.50	67.62	**
5	Anthracene	0.23	0.64	*
6	Fluoranthene	1.21	0.60	NS
7	Pyrene	0.94	0	NP
8	Benz[a]anthracene	0.18	<LQ	NP
9	Chrysene	<LQ	<LQ	NP
10	Benzo(b)fluoranthene	<LQ	<LQ	NP
11	Benzo(k)fluoranthene	<LQ	<LQ	NP
12	**Benzo[*a*]pyrene**	<LQ	<LQ	NP
13	Dibenz(a,h)anthracene	<LQ	<LQ	NP
14	Benzo(ghi)perylene	<LQ	<LQ	NP
15	Indeno(1,2,3cd)pyrene	<LQ	<LQ	NP
Total value PAHs		**92.69**	**97.15**	**NS**

Significance, NP, not performed; NS – no statistical significance (P>0.05); * P< 0,05; ** P<0.01; *** P<0.001; Data is presented as least square mean.

PAH4 is a more suitable indicator for the occurrence of PAHs, with a maximum allowed content in smoked meat products of 12 µg / kg^{-1} [9]. In the studied products we revealed that traditional and industrial pork meat sausage and ham do not exceeded this limit and there is no hazard concerning this aspect. In the traditionally and industrially smoked bacon the levels of PAH4 were higher than the limit imposed by the current EU legislation which constitutes a great concern regarding the safety of these products.

CONCLUSIONS

According to the results obtained in this study, Romanian sausage and ham, smoked in traditional and industrial conditions, are safe for its consumers regarding European regulation on PAHs content. In case of pork bacon produced in Romania, PAH4 levels exceed the imposed European limit and further investigation for identification of optimal smoking conditions in order to minimize levels are needed. We suggest that a more careful attention should be paid to all factors and apply such smoking conditions that result in the lowest possible contamination with PAH in pork meat products obtained in the traditional system in Romania, given the fact that in all types of products the levels of BaP were higher than in the industrially obtained ones.

EXPERIMENTAL SECTION

Sampling

The study was performed on a number of 60 meat product samples, which were taken from a certified traditional pork meat processing unit and retail units found in Salaj County. The following meat products were examined: traditional pork meat sausage (n=10), industrial pork meat sausage (n=10), traditional pork meat bacon (n=10), industrial pork meat bacon (n=10), traditional pork meat ham (n=10), industrial pork meat ham (n=10). All the samples taken were kept in their original package and at a temperature of 0-4 ˚C until their analysis in the laboratory.

The analysis of PAHs through HPLC method

The analysis of the 15 PAHs in the meat products was carried out at the Research Institute for Analyses Instrumentation Cluj-Napoca, Romania. The Perkin Elmer 200 Series High Performance Liquid Chromatograph (HPLC) with FLD detector was used to determine the following PAHs: Naphthalene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benz[a]anthracene, Chrysene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[a]pyrene, Dibenz[a,h]anthracene, Benzo[ghi]perylene, Indeno[1,2,3-cd]pyrene. For sample preparation, the method described by Ojaveer and Tanner (1996) [19] was used. The extraction method used was the following one: 10 g of sample was homogenized in a blender and 50 ml of KOH solution 0.4M in ethanol and water (9:1) for saponification was added. The reaction was performed in an ultrasound bath for 30 minutes at 60˚C, after which filtration was made using a filtering paper. The obtained product was extracted twice using 15 ml cyclohexane and the supernatant was purified on a Florisil column. After this step the sample was evaporated under nitrogen flow and finally recaptured with 1 ml of acetonitrile. Before injection, the samples were filtrated again on cartridges of 0.45 µm.

For the recuperation study, a sample of meat product was taken and contaminated with a standard solution that contained all the 15 PAHs in the same proportion, dissolved in acetonitrile. Afterwards, at the 10 g of sample, 1 ml of standard solution with a concentration of 30µg/ml for each PAH was added. In parallel, a blank solution was analysed in order to calculate with accuracy the recuperation levels. In table 4, the levels of recuperation are presented.

Table 4. The recuperation level for PAH through liquid/liquid extraction from food samples

No.	PAH	Recuperation (%)
1	Naphthalene	81.2
2	Acenaphthene	75.4
3	Fluorene	73.2
4	Phenanthrene	69.8
5	Anthracene	77.9
6	Fluoranthene	73.8
7	Pyrene	71.3
8	Benz[a]anthracene	84.3
9	Chrysene	78.4
10	Benzo(b)fluoranthene	75
11	Benzo(k)fluoranthene	79.5
12	Benzo[*a*]pyrene	77.1
13	Dibenz(a,h)anthracene	75.8
14	Benzo(ghi)perylene	69.9
15	Indeno(1,2,3cd)pyrene	84.5

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