



## EVALUATING THE CONTENT OF POLYCYCLIC AROMATIC HYDROCARBONS IN MEAT PRODUCTS BY USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY

Iuliana MANEA,<sup>a,\*</sup> Valentina COATU,<sup>b</sup> Laur Constantin MANEA<sup>c</sup> and Daniela ȚIGĂNUȘ<sup>b</sup>

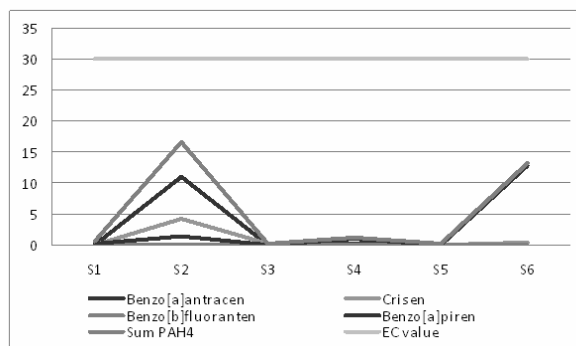
<sup>a</sup>Valahia University of Târgoviște, Faculty of Environmental Engineering and Food Science, Food Engineering Department, 13 Sinaia Alley Street, Târgoviște, Roumania

<sup>b</sup>“Grigore Antipa” National Institute for Marine Research and Development, 300 Mamaia Bd., Constanța, Roumania

<sup>c</sup>Valahia University of Târgoviște, Faculty of Environmental Engineering and Food Science, Environment Engineering Department, 18-24 Unirii Bd., Târgoviște, Roumania

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The purpose of this study is to determine the content of polycyclic aromatic hydrocarbons (PAH) in meat, precooked and finished products resulted from flow technology of meat products. PAH were dosed in pork and beef, groats and emulsion used to obtain finished products and three types of meat preparations. The method used was gas chromatography-mass spectrometry (GC-MS). Fourteen PAH were determined. A number of PAH have shown carcinogenic effects in experimental animals and it has been concluded that benzo[a]pyrene is a probable human carcinogen. Several of them are known to be potential human carcinogens including benzo[a]anthracene, chrysene, benzo[b]fluoranthene and benzo[a]pyrene. The pork had a content of benzo[a]pyrene 1.5  $\mu\text{g}/\text{kg}$  and is superior to beef which was 0.2  $\mu\text{g}/\text{kg}$ . Benzo(a)pyrene (BaP) has had in smoked product the highest levels compared to raw materials. The causes of PAHs increase are their presence in smoke and cumulative character in smoked meat products due the high solubility in lipids.



### INTRODUCTION

The improvement of health and life standards realized by ensuring high-quality products is a process that involves the removal of some toxic substances which are associated with a powerful carcinogenic and embryotoxicogen effect. From this point of view, because of the high risk of polycyclic aromatic hydrocarbons (PAH), this study proposes monitoring their level in flow technology for obtaining the meat products.

Polycyclic aromatic hydrocarbons are complex substances that may have carcinogenic, mutagenic, teratogenic effect and have a high bioaccumulation capacity.<sup>1-4</sup>

In order to facilitate the revision of Directive 2004/107/CE of The European Parliament and the Council in 2010, the member states must have as a goal the encouragement of the research on the effects of the PAH on human health and on the environment, especially because of their depositions. In the EFSA Journal<sup>5</sup> it is stipulated

\* Corresponding author: iulia.manea10@yahoo.com

that the PAH were evaluated by the International Programme on Chemical Safety (IPCS)<sup>6</sup>, the Scientific Committee on Food (SCF)<sup>7</sup> and by the Joint FAO/WHO Expert Committee on Food Additives.<sup>8</sup> Polycyclic Aromatic Hydrocarbons are a group of organic compounds, very stable, composed by at least two fused aromatic rings that have within their composition only carbon and hydrogen atoms. These can penetrate into the aliments during the technological processes. A part of the polycyclic aromatic hydrocarbons can cause cancer and DNA mutations.<sup>9-11</sup> Maternal smoking during pregnancy adversely influences the teeth forming cells, the baby tooth crown being reduced.<sup>12</sup> In nature PAH have been identified in air, soil, surface waters as a result of technological processes from the petroleum industry, natural gas, in the processing of coal, the obtaining of iron, aluminum, steel, in power station that work with wood, gas or coal. Exhaust gases from cars, forest fires and the waste burning are other important sources of environmental contamination with PAH.<sup>13-16, 21</sup>

Combustion of fossil fuels and inhalation of combustion Emissions and consumption in the diet are potential sources for the formation of PAH intermediates, which then turn into different PAH.<sup>17</sup> The acute oral toxicity of PAH appears to be low to moderate, but adverse hematological effects, dermal effects, immunosuppressive as well as reproductive and fetal effects are observed after long-term administration in experimental animals.<sup>4</sup> The aliments can be contaminated from the environment, through the air, water, soil or during their culinary processing and preparation. The major risk of contamination of the aliments with the PAH is possible during the technological stages (of drying, of smoking) and the preparation of these at high temperatures.<sup>18-23</sup> Charcoal-grilled and smoked meats, especially pork, beef and sausage are produced with high content of BaP.<sup>24</sup> Absorption of PAH from the gastro-intestinal tract appears to vary per animal species, time of ingestion, and the nature of lipid compounds present in food.<sup>25-27</sup> Grova showed that activity from radio-labeled BaP was not traced in blood and milk from orally exposed lactating goats.<sup>26, 28, 29</sup>

Hoogenboom<sup>28</sup> said that the PAH with big molecular mass are apparently not absorbed from the gastro-intestinal tract (and transferred to milk). They tend to be stored mostly in the kidneys, liver, and fat. Smaller amounts are stored in the spleen, adrenal glands, and ovaries.<sup>11</sup> A number of PAH have shown carcinogenic effects in experimental

animals and it has been concluded that BaP is a probable human carcinogen. BaP has been chosen as an indicator substance for this group of compounds by numerous national and international bodies, and the mixture of many PAH increases the risk of cancer.<sup>7, 31, 32</sup> For this reason the content of PAH in milk powder for children, in commercial roasted coffee beans, Frankfurter-type sausages is closely monitored.<sup>33-35</sup>

Recent conclusions of The World Health Organization/Food and Agriculture Organization's Joint Expert Committee on Food Additives and Contaminants (JECFA) and the EFSA Panel on Contaminants in the Food Chain have indicated however that BaP may not be the most appropriate marker for PAH in food.<sup>16,36-38</sup> PAH are characterized by high hydrophobicity, resistance to natural degradations and carcinogenic properties. Detoxification of PAH is complicated, and is performed by various enzymatic and nonenzymatic reactions. PAH are converted to arene oxide intermediates followed by formation of derivatives of *trans*dihydrodiols, phenols, and quinones. These intermediate products are known to be toxic, carcinogenic, and/or mutagenic. Results from animal studies show that PAH do not tend to be stored in the body for a long time. Most PAH that enter the body leave within a few days, primarily via feces and urine.<sup>11</sup> A number of polyphenols in fruit and vegetables, such as the protocatechuic acid, tannic acid, resveratrol, have inhibitory action on BaP and 7.12-dimethylbenz [a] anthracene (DMBA).<sup>39, 40</sup>

They also modulate the activity of enzymes involved in the activation BaP and DMBA, which further justifies the consumption of grapes containing resveratrol, green tea and tannins from different sources. The aim of this study was to identify the sources of meat products contamination with PAH.

## EXPERIMENTAL

### 1. Materials

Samples starting with the refrigerated raw material were taken from a meat factory: beef pulp (S1) and pork pulp (S2) in the refrigerated state. From the technological flow, there were taken samples of beef (S3) and pork emulsion (S4), obtained from the analyzed raw material. From the precooked products mentioned above (beef groats and emulsion) the following finished were obtained: home-made sausages (S5), semi-smoked sausage (S6). Home-made sausages are fresh sausages made from groats beef and fat without smoked. Semi-smoked beef sausage is made from groats and emulsion and are hot smoked.

## 2. Method

A method developed by NIMRD (“Grigore Antipa” National Institute for Marine Research and Development Constanța) based on the methods described in the IAEA-MEL/Marine Environmental Studies Laboratory, “Training manual on the measurement of organochlorine and petroleum hydrocarbons in environmental samples”, 1995, was used to determine the PAH.<sup>41</sup>

The solvents used were gas – chromatography grade manufactured by Merck Company. A standard mixture manufactured by LGS Standards GmbH, containing 16 priority PAH dissolved in toluene in individual concentrations of 100 µg/mL was used to calibrate the GS-MS.

## 3. Sample preparation for the analysis

Sub-samples of the meat and meat products were weighed and anhydrous sodium sulphate was added. Internal standard 9, 10 dihydroanthracene was added to the samples for quantifying the overall recovery of the analytical procedures. Samples were Soxhlet extracted for 8 h with 250 mL of methanol. The extracts were then saponified by adding 20 mL of 0.7 M KOH and 30 mL of water (both pre extracted) and refluxing for 2 h. The resulting mixture was transferred into a separatory funnel and extracted 3 times with hexane (once with 90 ml, twice with 50 mL). Then the extracts were combined, filtered through glass wool and dried with anhydrous sodium sulfate. The extracts were concentrated by rotary evaporation down to 15 mL, and then further concentrated to about 5 ml under a gentle flow of clean nitrogen. Finally, the extract was cleaned up and fractionated

by passing it through a silica/alumina column in which the silica and alumina were activated first at 200°C for 4 h and partially deactivated with 5% water. The chromatography column was prepared by slurry packing 10 ml of silica, then 10 mL of alumina and finally 1 g sodium sulphate was added to the surface to avoid disturbance of the top layer when pouring the solvent. Elution was performed using 20 mL of hexane to yield the first fraction (containing the aliphatic hydrocarbons), then 30 ml of hexane: methylene chloride (90:10) and followed by 20 mL of hexane: methylene chloride (50:50). These two eluents containing the aromatic hydrocarbons (PAH) were combined for analysis.

Appropriate blanks were analyzed with each set of samples. The fraction containing PAHs was evaporated under a weak (flow) of nitrogen to 1 mL and it was subjected to qualitative and quantitative analysis on GC/MS Perkin Elmer Clarus 500. The following analytical conditions were used: capillary column Elite 35 MS, stationary phase: Dimethylpolysiloxane (35% Diphenil), length 30 m, internal diameter 0.32 mm, film thickness 0.25 µm; carrier gas – helium, rate – 1 cm<sup>3</sup>/min, injector split/splitless in split mode, split flow 15 cm<sup>3</sup>/min, sample volume – 2µL, injector temperature – 300°C, temperature program – initial temperature 100°C, 5 min, heating rate – 6°C\*min<sup>-1</sup>, first isotherm – 250°C for 0 min, heating rate – 10°C\*min<sup>-1</sup>, second isotherm – 330°C for 10 min, ionization – E +70 eV, interface temperature – 330°C, temperature of source – 270°C,– data collection method – SIR. The PAH were identified by retention times and characteristic ions of identified compounds (Table 1).

Table 1

Characteristic PAH ions

Compound	Quantitation ion (m/z)	Confirmation ions (m/z)
Naphtalene	128	127;129
Acenaphthylene	152	153;151
Acenaphthene	154	153;152
Fluorene	166	165;167
Phenanthrene	178	176;179
Anthracene	178	89;179
Fluoranthene	202	203;101
Pyrene	202	203;101
Benzo[a]anthracene	228	229;114
Crysene	228	229;114
Benzo[b]fluoranthene	252	253;126
Benzo[k]fluoranthene	252	253;125
Benzo[a]pyrene	252	253;126
Benzo (g,h,i)perylene	276	138;227
Dibenzo(a,h)anthracene	278	139;279
Indeno(1,2,3-c,d)pyrene	276	138;277

## RESULTS AND DISCUSSION

The results are shown in Fig. 1. The sum of the quantities of PAH in all products analyzed varies between 0.7  $\mu\text{g}/\text{kg}$  beef groats and 133.3  $\mu\text{g}/\text{kg}$  to pork pulp. The pork has had a content of PAH (133.3  $\mu\text{g}/\text{kg}$ ), which means that there was a source of contamination before processing meat. PAH are liposoluble and their content in pork was higher than in beef (3.0  $\mu\text{g}/\text{kg}$ ). The beef had a content of fat 14.8 g/100g, compared with 48.3 g/100 g in pork.

The beef groats were obtained from beef pulp by pre-mincing and mixing with the salt. In this case the content of PAH decreased by 76.66%, but

contained 3% sodium chloride and 75.6% water, compared to 66.8% in pulp beef.

Since four of the PAH are considered the most carcinogenic, in Fig. 2 the sum of four substances can be seen (PAH4): BaP, benz(a)anthracene, benzo(b)fluoranthene and chrysene. In all samples the analyzed PAH4 is less than EC value 30.0  $\mu\text{g}/\text{kg}$ , as is stipulated in Commission Regulation (EU) No 835/2011.<sup>42</sup> After 9/1/2014 maximum level for PAH4 is 12.0  $\mu\text{g}/\text{kg}$ . The semi-smoked sausage (13.3  $\mu\text{g}/\text{kg}$ ) and pork (16.7  $\mu\text{g}/\text{kg}$ ) have had the highest content of PAH4. Semi-smoked sausage allows the absorption of large quantities of PAH from smoke, because the section has a small diameter and have natural casings from sheep gut, therefore are permeable.

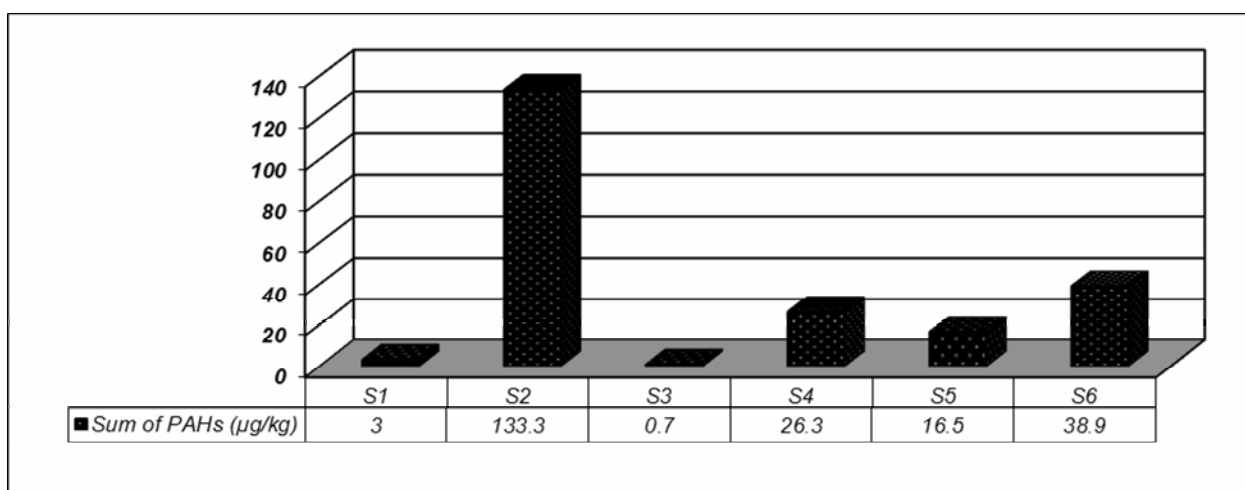


Fig. 1 – The total content of PAH in the analyzed sample.

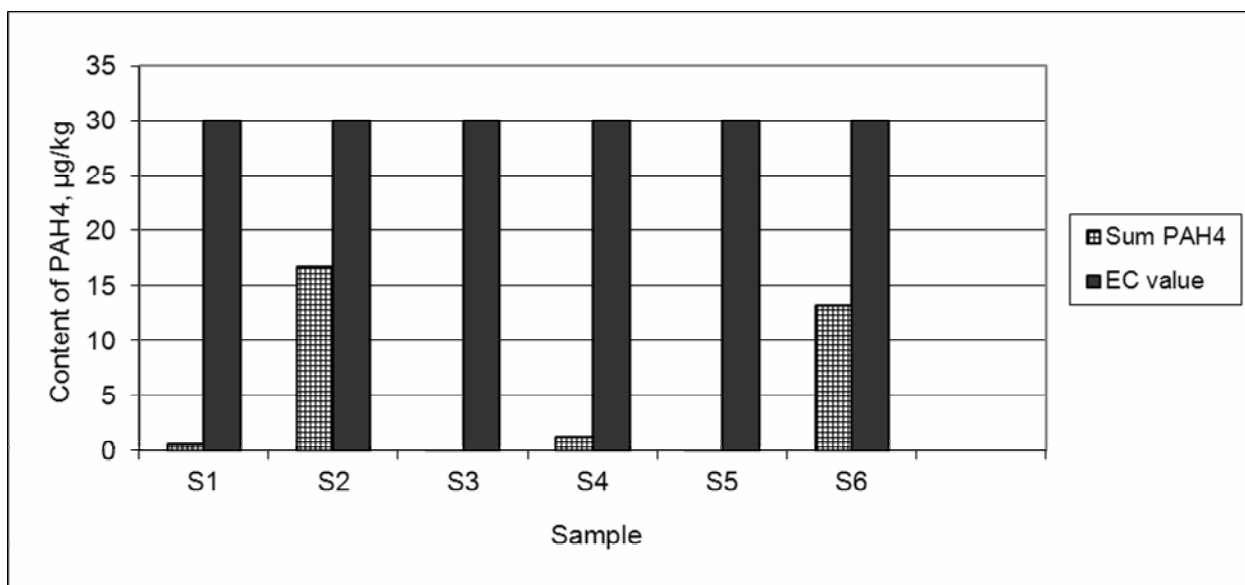


Fig. 2 – The sum of BaP, benz(a)anthracene, benzo(b)fluoranthene and chrysene.

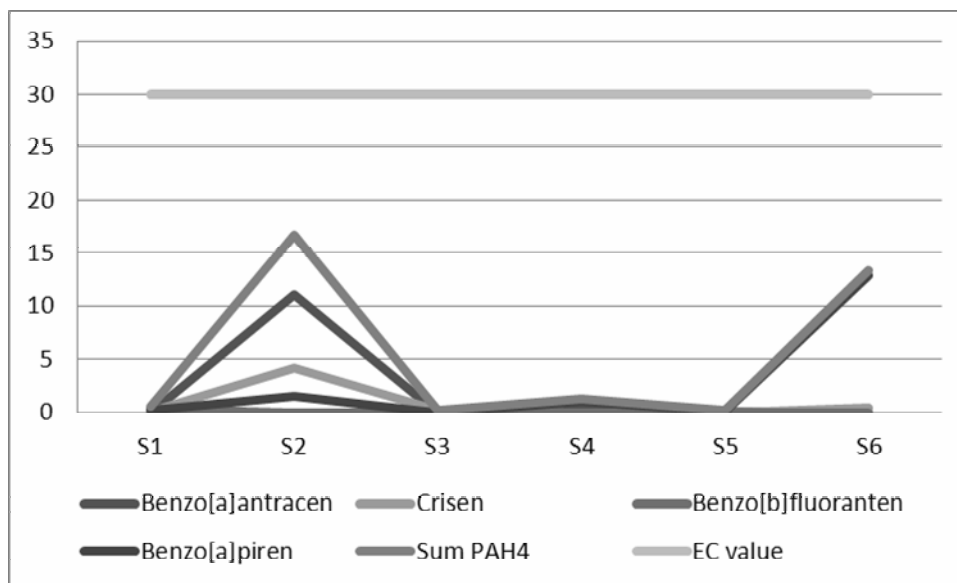


Fig. 3 – The content of PAH4 in analyzed samples.

Table 2

PAH not detected in samples

PAH not detected	Samples
Benz(a)anthracene	pulp beef, beef groats, semi-smoked sausage
Chrysene	Emulsion, home-made sausages
Benzo(b)fluoranthene	Pulp beef, beef groats, emulsion, semi-smoked sausage, home-made sausages
Benzo(a)pyrene	beef groats

In Fig. 3 it can be observed that the quantities of each of the four hydrocarbons increased carcinogenic risk. BaP has registered the highest value in pork meat (11.0 µg/kg), in other samples being smaller amounts of 0.3 µg/kg. BaP has had in smoked product the highest levels compared to raw materials. However, in semi-smoked sausage BaP was the highest (12.9 µg/kg) exceeded the 5.0 µg/kg provided in Commission Regulation (EU) No 835/2011 but the sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene is less than 30 µg/kg the maximum provided in same Order. From 1/9/2014 the maximum level for BaP is 2.0 µg/kg. Maximum levels in Regulation No. 208/2005 for BaP in smoked meats and smoked meat product is 5 µg/kg. In a series of samples were not detected potentially high carcinogenic PAH, even in smoked products (Table 2). The method detection limit for the 16 compounds investigated varies between 0.1 and 1 µg/kg.

## CONCLUSIONS

The monitoring of PAH during the technological flow of obtaining meat products is

important for finding the sources these contaminants. Among the raw materials, pork had a higher content of PAH than beef because they are fat soluble and pork has a higher fat content than beef with 33.5%.

Semi-finished products have had a PAH content lower than the raw materials from which they were obtained, which means that there was no an additional source of contaminants. Smoking was the operation which brought a quantitative increase of PAH but it was below the limit mentioned in European regulations, namely Commission Regulation (EU) No 835/2011 of amending the (EC) No 1881/2006, for the PAH4. In order to considerable reduce those contaminants, the use of modern methods of smoking, especially the use of liquid smoke which does not contain PAH, is more and more recommended.

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