THE EVALUATION OF THE AROMATIC POLYCYCLIC HYDROCARBONS CONTENT OF MEAT PRODUCTS OBTAINED BY DIFFERENT METHODS

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Abstract
The aim of the present study was to assesse the content of Polycyclic Aromatic Hydrocarbons (PAHs) in different types of meat products obtained by traditional method, in order to identify sources of contaminated PAH from meat products. The analysed meat products were purchased from specialized stores. The analysed products have had different composition and structure: sausages, salami, neck, muscle, ribs. The thermal treatment applied was different depending on the assortment, either by hot smoking or cold smoking or pasteurization. These were obtained either by cold smoking or hot smoking or pasteurisation. Romanian meat products obtained through traditional methods do not contain chemical additives and are processed by cold smoking. Minimal heat treatment, such as cold smoking, presents the lowest risks with regard to the accumulation of Aromatic Polycyclic Hydrocarbons. The method used for PAHs determination was gas chromatography - mass spectrometry (GC-MS). Fourteen PAHs were determined. The benzo[a]piren (BaP) values were lower than the maximum 2µg/kg, in home made sausages (0,1 µg/kg), spices sausages (1.2 µg/kg), summer salami (1,8 µg/kg), and smoked neck, home made smoked pork sausages and pork pastrami showed the highest level 3.9 µg/kg, 3.4 µg/kg respectively 3.0 µg/kg.

Keywords: polycyclic aromatic hydrocarbons, meat products, gas chromatography - mass spectrometry

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1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are chemicals composed of two or more combined aromatic rings and have a carcinogenic effect.
HAPs are formed during incomplete combustion or pyrolysis at high temperature of fuels, in the aluminium, iron, steel industry, during the operation of thermal power stations and through combustion from motor vehicles. Also, PAHs may be present in the environment after incineration of waste and cigarette smoke, there are over 100 complex compounds (Mottier et al. 2000).
Food can be contaminated by PAHs that are present in air, soil, or water, or during food processing and cooking. PAHs contaminates food through various thermal treatments performed incorrectly, such as smoking, drying and cooking. The amount of PAHs depends on the method used, the temperature, the exposure time, the fat drainage and the distance from heat source (Kazerouni et al., 2001; Husam et al., 2011; O. Viegas et al., 2012; Tsai et al., 2012). The studies showed that barbecued beef burgers contained the highest levels of PAHs, with benzo[a]pyrene concentrations of up to 29 µg/kg. Beef burgers cooked at a larger distance from charcoal (above 7 cm) contained higher PAHs concentrations than those cooked at a smaller distance (above 4 cm) (Zelinkova and Wenzl, 2015).
Also the content of PAHs depends on the type of meat and processing method, thus Fei et al., 2017 obtained different values for PAHs by processing pork, chicken, salmon. A recent study in China showed that the concentration of PAHs in smoked meat products ranged from 14.4 to 56.3 µg kg-1 (Jiafu et al., 2016). The studies conducted by Jahurul (2013) showed that grilled meat contained high levels of PAHs.
To comply with the level of PAHs in food, Regulation (EC) No 1881/2006 of 19 December 2006 on the maximum level for certain contaminants in food has been replaced by

Regulation 835/2011 of 19 August 2011. The most significant change introduces new markers of this PAHs and establishes the maximum level of four PAHs (4PAHs): benzo (a)pyrene, benz(a)anthracene, benzo(b) fluoranthene, crisen.
In order to reduce the PAHs content, it is recommended to marinate the meat, with 1.2% lemon juice, before the heat treatment (Farhadian et al., 2012). Another method of reducing PAHs in smoked products is proposed by Shaun et al, 2013, and consists in the replacement of wood-fired smoking with sugar-smoking.
The aim of this study was to identify the processing method that accumulates the lowest PAHs content in meat products.

MATERIAL AND METHODS

2.1. Meat products sampling

The products under this study were purchased from specialist stores. The quotation used for the samples was the following:
S1 – home made smoked pork sausages;
S2 – spices sausages;
S3 – dry salami;
S4 – smoked neck;
S5 – pork pastrami;
S6 – home made sausages;
S7 – summer salami;
S8 – semi-smoked sausages.
The characteristics of the meat products were the following: S3, S7- pasteurized, hot and cold smoked products; S1, S2, S4, S5, S6 - cold smoked products; S8 – hot smoked product.

2.2. Method

This method was developed by NIMRD (National Institute for Marine Research and Development "Grigore Antipa" Constanta) (UNEP/IOC/IAEA, 1995).
The solvents used were gas –chromatography grade manufactured by Merck Company. For calibrate the GS-MS was used a standard mixture with containing 16 PAHs (Table 1).

2.3. Sample preparation for the analysis

The samples of the meat products were weighed, anhydrous sodium sulphate was added, Soxhlet extracted with methanol, saponified by adding KOH and water and refluxing for 2 h. The resulting mixture was transferred into a separatory funnel and extracted 3 times with hexane.
Then the extracts were combined, filtered, dried with anhydrous sodium sulfate, concentrated by rotary evaporation first , and then under a gentle flow of clean nitrogen. Finally, the extract was cleaned up and fractionated.
The chromatography column was prepared and the elution in three steps: with hexane, with hexane:methylene chloride (9:1) and with hexane:methylene chloride (5:5) was performed. 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.

Table 1. Characteristic PAH ions

PAHs	**A**	B
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

A. **Quantitation ion (m/z)**
B. **Confirmation ions (m/z)**

The following analytical conditions were used: stationary phase: Dimethylpolysiloxane (35% Diphenil), capillary column Elite 35 MS, length 30 m, film thickness 0,25 μm, internal diameter 0,32 mm; carrier gas - helium,

injector split/splitless in split mode, sample volume – 2µl, injector temperature – 300°C, 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)

3. RESULTS AND DISCUSSION

In Fig.1 is presented the chromatogram obtained through the analysis of the home-made sausages samples. After analyzing the results, the S6 sample presented the lowest concentration of PAHs. Values above the detection limit were found for benzo[a]pyrene (0,1 µg/kg), benzo[a]antracene (0,1µg/kg), antracene (6,2µg/kg), naphtalene (9,6µg/kg), fluorantene (0,5µg/kg), dibenzo(a,h)antracene (0,1µg/kg). From all of them only benzo[a]pyrene and benzo[a]antracene are shown to be carcinogenic, but the limit allowed isn't exceeded. Other PAHs monitored were below the limit of detection.

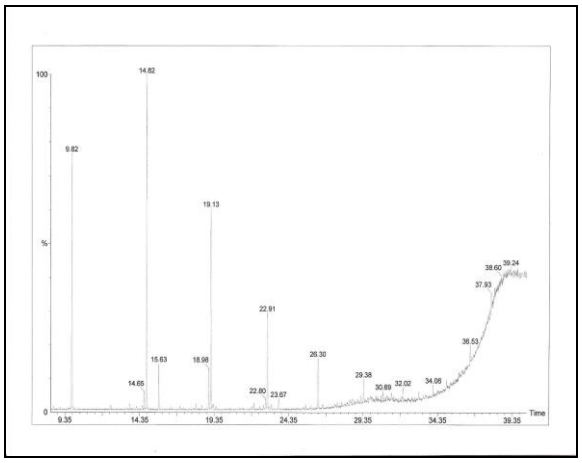

Fig. 1 The GC/MS chromatogram obtained for the Home-made sausages

The resulting chromatogram for the sample Summer salami is shown in Fig.2. Except acenaphthylene, benzo[a]anthracene, benzo[k]fluoranthene were not detected, all monitored PAHs were present, but their amounts did not exceed regulated limits. Benzo[a]pyrene was 1,8 µg/kg and did not exceed the maximum value stated by the European regulations. Benzo[b]fluorantene was 3,6 µg/kg the highest in all samples.

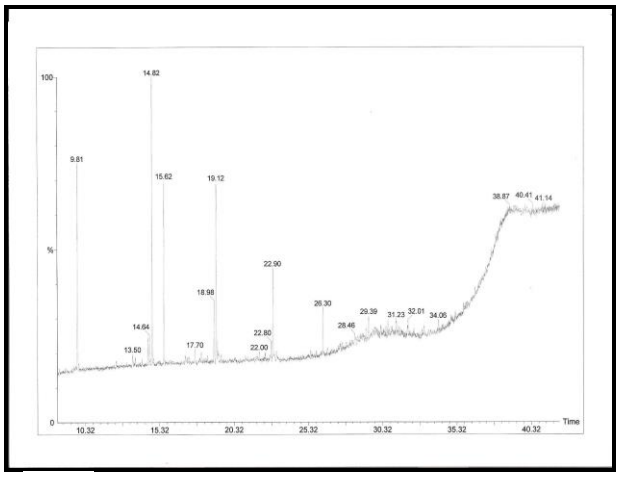

Fig. 2 The GC/MS chromatogram obtained for the summer salami

The semi-smoked sausages had the highest amount of Benzo[a]pyrene 12,9 µg/kg. This can be explained by low water content 56.74%, high temperature in smoking (70^{o}C) and high permeability of natural membranes. The largest amount of PAHs is located on the membrane surface, their degree of diffusion depends on the smoke humidity and fat content of sausages. All PAHs are potentially carcinogenic, so starting with the 2014, the maximum limit in food is less than at present. Commission regulation (EU) No 835/2011 of 19 August 2011 establishes maximum levels of benzo[a]pyrene in smoked meats and smoked meat products 2 µg/kg wet weight (figure 3).

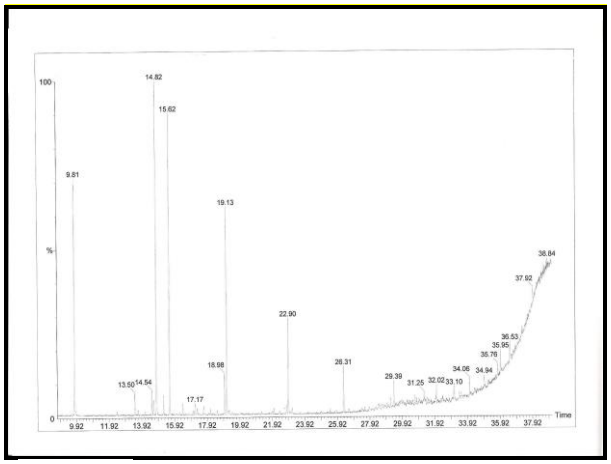

Fig. 3 The GC/MS chromatogram obtained for the Semi-smoked sausages

According with Regulation (EU) No 835/2011 sum of benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene and chrysene (4 PAHs) for smoked meat products is 12 µg/kg.
The smoked neck had the BaP value (3.9 µg/kg), more than 2 µg/kg, a potent factor is the high lipid content, because PAHs diffuses easily into lipids. Pöhlmann et al., 2012, showed that sausages with lower fat contents had the PAH contents lower. The same, the home made smoked pork sausages and pork pastrami had 3.4 µg/kg respectively 3.0 µg/kg BaP.
According to Reinik et al. (2001), BaP contents in meat products were in the range of 0.3 - 31.2 µg/kg, with a mean concentration of 0.8 µg/kg.
The maximum acceptable concentration for BaP was exceeded in mainly home-made ham, smoked meat and smoked chicken.
Sum of 4 PAHs was 0.2 µg/kg in S7, 7.2 µg/kg in S8, 7.84µg/kg in S1, and the highest in the S3 sample, 23.75 µg/kg.
Olatunde et al., (2014), reported the concentration of BkP, BaP, IP and BghiP in smoked, grilled and boiled meat samples ranged 0.64–31.54 µg/kg, 0.07–7.04 µg/kg, 0.09–15.03 µg/kg, 0.51–46.67 µg/kg and 0.01–5.11 µg/kg, respectively.

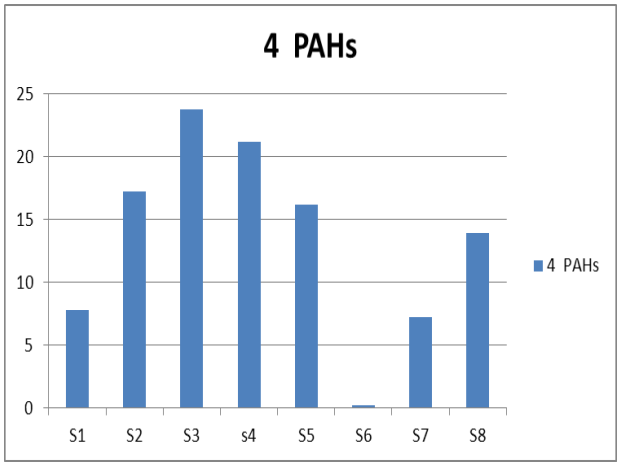

Fig. 4 Content of four PAHs in analized samples

In all sample acenaphthene, acenaphthylene, fluoranthene, pyrene, benzo[a]anthracene, benzo[b]fluoranthene were undetectable.

4. CONCLUSIONS

From 01.09.2014 the maximum level for BaP dropped from 5 to 2.0 µg/kg and sum of 4 PAHs dropped from 30 to 12.0 µg/kg.
The highest content for the four carcinogenetic PAHs was observed at pasteurized – hot smoked product. In case of the samples analyzed, sum of the four PAHs was minimal, for home made sausages, and maximum for dry salami, which is a hot smoked, pasteurized and cold smoked salami.
Heavily processed and smoked meat products had the highest PAHs content

5. ACKNOWLEDGEMENT

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