Transilvania University of Brasov
FACULTY OF MECHANICAL ENGINEERING

ICMS 2019 & COMEC 2019
Brasov, ROMANIA, 21-22 November 2019

STUDY CONCERNING THE MONITORING OF POLYCYCLIC AROMATIC HYDROCARBONS CONTAINED IN MEAT PRODUCTS

Cristina Canja[1]
[1] Transilvania University of Brașov, Brașov, ROMANIA, canja.c@unitbv.ro

Abstract: *Polycyclic aromatic hydrocarbons are a type of complex chemical substances which are formed and are released during incomplete combustion or pyrolysis of organic matter, such as waste or food, during industrial processes. Polycyclic aromatic hydrocarbons also form in natural processes, such as carbonization.*
Contamination can occur through aerial environment, soil, water or during preparation. Processing procedures such as smoking, drying and food preparing are considered the main sources for food contamination with PAH.
The purpose of the research is to determine the value of polycyclic aromatic hydrocarbons in smoked meat products, improving the smoking process in order to minimize the harmful effects of consuming smoked products.
Keywords: *polycyclic aromatic hydrocarbons, pyrolysis, smoking, chromatography.*

1. INTRODUCTION

Food products represent the material goods resulting from human activity through the application of a technological process or a heat treatment, which is vitally important because it constitutes the daily food of the people providing the necessary energy and nutrients. The traits of food products maintain, improve or can affect the health. [8]
Meat and meat products are indispensable foods in human life, bringing to the body a source of proteins of special biological value, containing all the essential amino acids, vitamins and minerals.
Human nutrition has been based on quality since ancient times, the quality of the raw material is crucial, because it transfers to the finished product both vitamins, calories and proteins, so consumers have become more and more attentive to the quality of the food they consume. It is concerned with quality control, research, assurance and maintenance, starting from the raw material, the processing method, to the final product.
Meat products are having a composition rich in water, protein substances and fats become a proper environment for the development of microorganisms, to minimize their activity, heat treatment is applied - smoking.
Smoking is a process applied from the earliest times to meat products aimed at preserving and improving the taste, respectively their color. [8]
Smoking is the operation through which a food product is treated using the action of smoke - aerosol. The smoke being obtained by the combustion of wood materials is also a generator of PAHs, while obtaining high quality products. [1]
Following the combustion process CO_2, H_2O, ash and a quantity of heat are formed. The complete combustion process is carried out when the temperature of the wood is ignited and in contact with air. Following the process, useful substances are formed, but due to the high air intake, these substances are rusted to CO_2 and H_2O as the temperature rises very quickly. [2]
As a result of the thermal decomposition still bearing the name and pyrolysis results in coal, water vapor, gases and organic substances in the form of vapors, these are formed when the wood is heated in the absence of air. Polycyclic aromatic hydrocarbons are widespread in the environment and humans inevitably come into contact with these compounds that affect their health because they have a pronounced carcinogenic potential. Being found in air, soil, water and food, human exposure is made by inhalation of air being polluted by incomplete combustion processes, ingestion of food containing PAHS or by dermal contact. Polycyclic aromatic hydrocarbons (PAHs) constitute a large class of organic compounds containing two or more linked aromatic nucleus.[3] These have a wide spread on the food chain, where they are born during the process of incomplete combustion or pyrolysis of the organic substances involved in the natural processes. The arrival of polycyclic aromatic hydrocarbons in the body after ingestion, respectively inhalation is detectable in the internal organs rich in adipose tissue because they have a high lipophilic character. Their presence in the body causes a metabolic activation of the oxidase class enzymes, which biocatalysis the epoxidation reactions. These compounds are primarily composed of carbohydrates from foods at high temperatures and lack of oxygen. Free radicals generated during high-temperature food combustion are subjected to recombination to form light PAH, followed

by heavy PAH that moves into hydrophobic compartments with food chain, most often contained in high-fat foods. [4]
Marker contaminant is considered: benzo (a) pyrene and the sum of 4 PAH: benzo (a) pyrene ($C_{20}H_{12}$), benzo (a) anthracene ($C_{18}H_{12}$), benzo (b) fluoranthene ($C_{20}H_{12}$) and chrysene ($C_{18}H_{12}$).
Smoking is a process of food preservation that generates PAHs. The accumulation of PAHs in smoked meat products depends on how they are smoked, the nature of the fuel, the temperature and the duration of smoking.
Wood quality plays an important role, pyrolysis of hardwood generates poorer smoke in PAHs than softwood.
Their indirect presence, sometimes appreciable, in food (related to polluting sources in the food industry, but also to our normal nutrition): in vegetables, meat, milk derivatives, etc. and especially in the smoked products, which, through the trophic cycle mentioned, are then found in our body. [5]
Smoke is an aerosol composed of an air mixture with the products of incomplete combustion of wood or sawdust.
The content of the different groups of chemical compounds of smoke, g/ 100 g of wood is given in table 1.

Table 1: The content of different chemical compounds in smoke, g/ 100g wood

Groups of chemical compounds	%
Acids (in acetic acid)	3,65-5,24
Phenols (in phenol)	0,12-0,30
Carbonyl compounds (in percent of acetic aldehyde)	1,14-9,03
Carbonyl compounds (in percent of acetic aldehyde)	10,0-18,0
Esters	6,7
Benzpiren (in cellulose smoke)	19,10-6
Benzpiren (in lignin smoke)	28,10-6

Factors that influence the smoke composition:

- smoke production technology - classic or friction generators are used for smoke production; its advantage is that the smoke is richer in volatile acids, carbonyls, phenols and poorer in PAH;
- wood (sawdust) type - two categories are distinguished: soft wood and hard wood; hardwood (beech, oak, maple, ash) generates smoke richer in aromatic compounds and acidic substances as compared to soft (resinous) essences which give a mediocre, sometimes unpleasant aroma containing a large amount of polycyclic aromatic hydrocarbons;
- wood (sawdust) moisture - it must have a humidity of 30%, the lower it is, the higher the amount of phenols and tar, but the amount of condensable substances decreases;
- the disintegration temperature of the wood (sawdust) - must be lower than 450 ºC; at temperatures above 600 °C the oxidation of the useful substances in the smoke takes place, so the quality decreases;
- air intake - must be maximum 20%, its insufficiency leads to a dense smoke, which worsens the sensory qualities of the smoked product (dark color);
- smoke purification - is used to remove suspended solids (sawdust, ash, soot, tar).

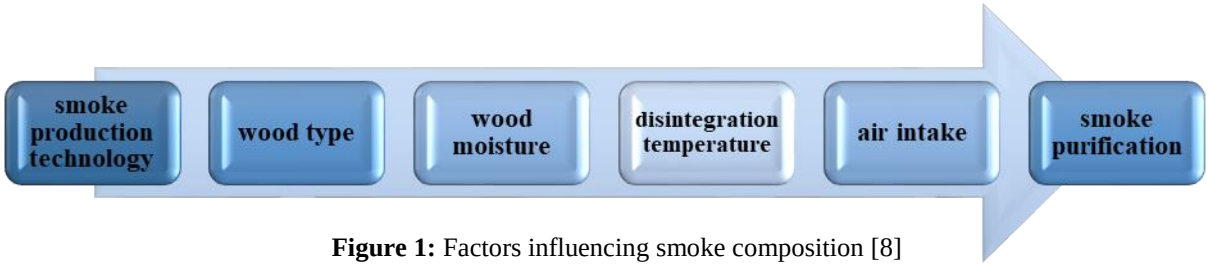

Figure 1: Factors influencing smoke composition [8]

The smoke has antiseptic action being determined by the components of the smoke and exposure to moderate temperatures. Although smoke has a low phenolic content, it has a strong bactericidal action. Some molds have a high heat resistance, whereas the other microorganisms are destroyed in 1-2 hours.
Due to the hydrocarbons in the smoke, it is considered to be carcinogenic affecting human health and the safety of smoked meat products. [6]
As a result of smoking, the product undergoes both negative and positive changes. The negative ones are represented by the deterioration of the hygienic qualities by the accumulation of PAHs and formic aldehyde, respectively the degradation of the amino acids. In order to avoid them, the following precautions can be taken: lowering the pyrolysis temperature of the wood fuel, purifying the smoke before entering the smoking room.

The advantages of smoking are:
• enhancement of the aroma due to the following compounds: phenolics (guaiacol, 4-methyl-guaiacol, eugenol, anisol, syringol), carbonyls, lactones, esters, alcohols, furfunol, acids, etc.;
• changing the color from yellow - golden to brown due to the reaction of combining phenols with the aldehydes resulting in resins thus depositing on the membrane surface;
• the antioxidant action of the smoke compounds is due to the high boiling phenol fractions;
• antiseptic action is due to the presence of phenols, aldehydes, acids, phenols being the most active

Quality evaluation of traditional smoked sausages
Sensory properties play a major role in the decision to buy meat products. The organoleptic examination is a method of analysis that involves examining the external appearance, section appearance, consistency, color, smell and taste. In case of observing the organoleptic changes, the microbiological and physico-chemical examination is passed.

Table 2: Organoleptic examination of meat products in film – traditional smoked sausages

Depreciation factors	Relatively fresh products	Tained products
Exterior aspect of products	The membrane must be adherent, undamaged, break resistant, reddish-brick, clean	Mold appears on the membrane
Aspect in section	Brick uniform color, composition bound	Greenish-gray areas are observed
Consistency	Farm, elastic	Due to smoking, they strengthen
Taste and flavour	Pleasant smell due to the smoke	Smells of rancidity

Table 3: Physical-chemical examination of meat preparations in the membrane – traditional smoked sausages

Characteristics	Admissibility issues	Analysis methods
Water, % max.	30	STAS 9065-3/73
Fats, % max.	55	STAS 9065-2/73
Sodium chloride, % max.	6	STAS 9065-5/73
Hydrolizable nitrogen (mg NH_3-100 g) max.	200	SR 9065-7/200 OMS 611-03.04.1995
Total proteic substances, % min.	16	STAS 9065-4/81
Nitrite (NO_2 mg-100 g) max	7	STAS 9065-9/74 OMS 611-03.04.1995

Table 4: Bacteriological examination of filmed meat products - traditional smoked sausages

Microbiological conditions	Maximum limit allowed	Analysis method
Salmonella-25g	Abs.	STAS 2356/82
Staphylococcus coagulase positive max.-g	100	ISO 6888/92

2. MATERIALS AND METHODS

The researches were carried out between April 2018 and March 2019 and consisted of determining the polycyclic aromatic hydrocarbons from a series of 44 samples of traditional smoked sausages manufactured in industrial regime in one of the famous meat processing factories in Brasov.
The sampling was carried out in accordance with the provisions of EC Regulation 333/2007 regarding the sampling methods and the analysis methods for the official control of the content of Pb, Cd, Hg, and PAHs in foodstuffs, as subsequently amended and supplemented. The samples are taken under hygienic conditions in appropriate sterile, clean, food-grade, tear-resistant and mechanical shocks.
All samples taken were labeled and sealed. The samples were packaged separately so as not to contaminate or degrade between them. The transport to the analysis laboratory was performed in a short time so as not to change its integrity parameters, the temperature during the transport reaching about 2 ... 4ºC.
The samples were taken randomly, from different places of the lot, their number being given by the sampling plan applied.

References (converging from the national regulations)

EC Regulation 333/ 2007: Establishing the sampling methods and the analysis methods for the official control of the contents of Pb, Cd, Hg, 3-MCPD, and PAHs from food products, with subsequent modifications and completions;

EC Regulation 1881/2006: Establishing the maximum level for certain contaminants in food products, with subsequent modifications and completions;

ANSVSA Order no. 51/2005: The sanitary veterinary norms for the implementation of the measures of supervision and control of some substances and of their residues in live animals and the products through the performance of the analytical methods and the interpretation of the results;

SR EN ISO 15753/2007: Fats and oils of animal and vegetable origin. Determination of PAHs;

SR EN ISO 15753 / A1: 2012: Fats and oils of animal and vegetable origin. Determination of PAHs; Exclusion of olive oil obtained from pomace;

SR EN ISO 661: 2007: Fats and oils of animal and vegetable origin. Sample preparation for analysis;

SR EN ISO 661 / AC: 2011: Fats and oils of animal and vegetable origin. Sample preparation for analysis;

PS-13-DTSAONASRA: Specific procedure for sampling non-animal products;

SR EN ISO / IEC 17025: 2005: General requirements for the competence of testing and calibration laboratories.

Methods of analysis

High performance liquid chromatography

High performance liquid chromatography was developed after the chromatographic separation theory showed that to obtain a high separation efficiency, comparable to that achieved in gas chromatography, the use of fine-grained fillers of the order of 5-10 μm is required. However, in order to have the proper elution rates, in these situations it is necessary to use high pressures at the entrance of the eluent in the column.

The principle of the method consists in the extraction of PAHs by means of the acetonitrile/ acetone mixture, followed by two purifications on the reverse phase C18 cartridge and on the Florisil cartridge.

After separation, the liquid chromatography is determined and the fluorescence is measured at different excitation and emission wavelengths, depending on the standardized and optimized working method.

In order to obtain repeatable results, the ambient temperature in the laboratory was ≤ 20 °C, the higher temperatures, increase the solubility of short-chain fatty acids.

After accurately weighing 2g of the sample, 5 steps were taken, followed by the actual determination (optimization of the apparatus, tracing the standard curve, reading, calculating and expressing the results).

Tabelul 2: Working procedure characteristics (soft device - fluorescence detector)

Time, min.	**Excitation, nm**	**Emission, nm**	**A/ Z**
0,00	270	385	Da
5,00	270	385	Da
<14,50	256	446	Da
<19,00	292	410	Da
>25,00	292	410	Nu

Step 1 - for fat extraction, Step 2 - the fatty residue is purified by successive extractions also with mixture 1, in three stages, step 3 - purification of the fatty residue, step 4 - hexane extract was subjected to a successive extraction with Mix 3 three steps, the supernatant (3x 2 ml) was collected in a clean and weighed vial, followed by a further purification by passing the eluate on the Florisil cartridge, Step 5 - all the eluate obtained was concentrated to 1 ml.

Table 3: Maximum volume of fat residue depending on the amount of sample taken

Weight	**1g**	**1,5g**	**2g**	**2,5 g**
Fat residue Step 1 (mg), max.	320	480	640	800
Fat residue Step 2 (mg) max.	20	30	40	50

All fatty residues are weighed, evaporation to dryness is carried out with nitrogen, in water bath at 35^0C, sample preparation can take 2-3 days, the sample extracts are kept overnight under freezing conditions at -18^0C.

3. RESULTS AND DISCUSSIONS

The reading was completed by the individual chromatogram for each sample read and by an analysis report, which identifies at the retention times specific to the analyzed PAHs the concentration corresponding to each substance.
The set of determinations also read a fortified sample to the maximum admitted limit corresponding to the legislation in force (EC Regulation 1881/2006, as subsequently amended and supplemented).

Table 4: Maximum allowable limits for PAHs in smoked meat and smoked meat products

Maximum level (µg/kg)	
Benzo(a)piren	Sum of 4 PAH (benzo (a) pyrene, benzo (a) anthracene, benzo (b) fluoranthenes, crisen)
5.0 µg/ kg until 31 august 2014 **2,0 µg/ kg from 1 september 2014**	30.0 µg/kg until 31 august 2014 12,0 µg/kg from 1 september 2014

The result from the sample analysis report represents the value of the PAH concentration in the sample;
Issuing the analysis report and processing the data by correcting the value with the recovery ratio only for Benzo (a) pyrene and Sum 4PAH respectively.
For results less than LOD, in the analysis bulletin they will be passed as "undetectable", for results with values between LOD and LOQ, the results will be passed as "non-quantifiable".
The expression of the result is made with the same units and with the same number of significant figures as the maximum levels established in Regulation (EC) no. 1881/2006.
The results of laboratory determinations on smoked sausages are presented in table 5.

Table 5: The results obtained by benzo (a) pyrene and the sum of 4PAHs between April 2018 and March 2019 for traditional smoked sausages

Samples	**2018 - 2019**													**Average**
	µg/kg	Apr.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	
Traditional smoked sausages	Benzo(a) piren	1,90	1,86	1,92	1,97	1,99	2,03	2,34	2,25	1,97	1,96	1,90	1,93	2
	Sum of 4 PAH	9,46	9,16	9,45	9,12	9,65	9,86	12,3	12,2	9,30	9,76	9,61	9,68	9,96

Graphical representation of the results obtained by benzo(a)pyrene and the sum of 4 PAHs during the period April 2018 and March 2019 for traditional smoked sausages:

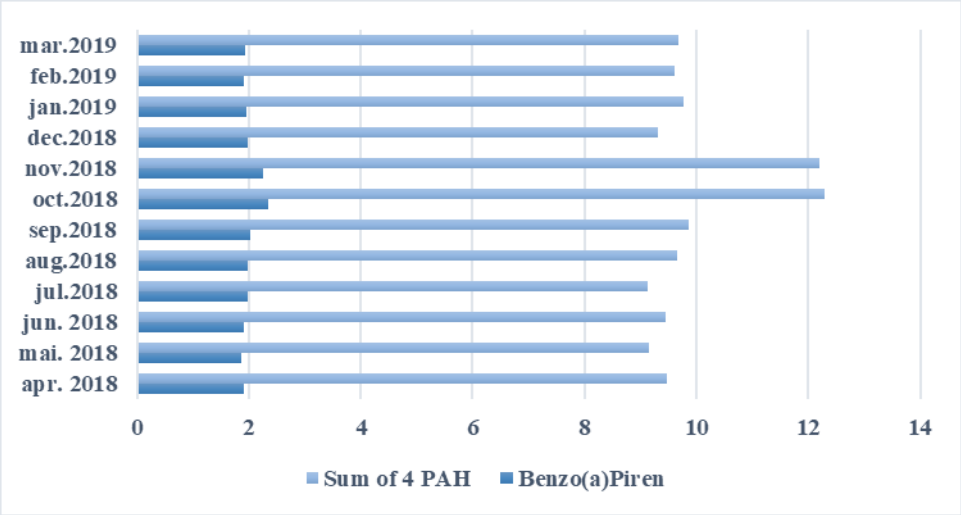

Figure 2: Values for Benzo(a)Pyrene and sum of 4 PAH from april 2018 to march

After centralizing the results of the analysis, it was concluded that during 2018 the values of Benzo(a)Pyrene increased over the admitted limit for traditional smoked sausages in September, October and November.
The values for the sum of 4 PAH were exceeded reported to the admitted limit for traditional smoked sausages in October and November.
The data was analyzed and several actions were taken – removal of the products from the trade field and imposing a more effective monitoring of the obtained values.

4. CONCLUSIONS

Polycyclic aromatic hydrocarbons are the compounds of food products which, judging by their nature, can affect the health of the consumers. Meat preserving by smoking is the main source of creating PAHs in meat products. The maximum admitted content is regulated through a series of documents and regulations that are applicable both at European level and national level.
The study was focused on monitoring the content of PAH in smoked meat products – traditional smoked sausages during a year, establishing exceptions from rules in force for samples from October and November of 2018. As a result of this exception, the producer has removed from commercialization the batches of products that were affected, evicting the negative effects both on the consumers and on his side as a producer.

REFERENCES

[1] Haritash A., K., C., P., Kaushik, 2009, Biodegradation aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A review, Journal of Hazardous Materials 169.
[2] Chen B., H., 1997, Analysis, Formation and Inhibition of Polycyclic Aromatic Hidrocarbons in Foods: An Overview, Journal of Food and Drug Ananlysis.
[3] Cerniglia Carl E., 1993, Biodegradation of polycyclic aromatic hydrocarbons, Current Opinion Biotechnology.
[4] José M., Lorenzo, L., Purriños, R., Bermudez, N., Cobas, M., Figueiredo, M., C., G., Fontán, 2011, Polycyclic aromatic hydrocarbons (PAHs) in two Spanish traditional smoked sausage varieties: ”Chorizo gallego” and ”Chorizo de cebolla”, Meat Science 89.
[5] Abdulazeez T., Lawal, 2017, Polycyclic aromatic hydrocarbons. A review, Environmental Chemistry, Pollution & Waste Management.
[6] Lochan Singh, J., G., Varshney, T., Agarwal, 2016, Polycyclic aromatic hydrocarbons` formation and occurrence in processed food, Food Chemistry 199.
[7] Regulation (UE) no. 835/2011 of the Commsion from 19 august 2011 for modifying the Regulation (CE) no. 1881/2006 concerning the maximum levels for Polycyclic aromatic hydrocarbons.
[8] Savu C., Petcu C., Igiena și controlul produselor de origine animală, Semne Publishing, Romania, 2008.