CARCINOGENIC SUBSTANCE ASSESSMENT OF CONTENT IN SMOKED MEAT PRODUCTS

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Abstract – This paper describes the mass spectrometric methods of carcinogenic substances detection in smoke fume. The factors influencing their quantitative content were determined; the technological methods of reduction of carcinogenic PAHs residue content are presented. The indicative role of PAHs is evaluated.

Key Words - polycyclic aromatic hydrocarbons

I. INTRODUCTION

Nowadays, the majority of available methods for detection of benzo[*a*]pyrene and other PAHs in food products are based on HPLC with a fluorescence detector [1, 2, 3]. Sensitivity of HPLC with a fluorescence detector enables identification of trace elements of PAHs; however, when recovering PAHs from complex matrices such as food products, prepared samples can contain foreign organic impurities, and errors can occur due to the presence of substances that give cross signals. During the last decades, the method of gas chromatography–mass spectrometry (GC-MS) has been especially actively developed and introduced into the practice of analytical laboratories. Regarding PAHs, identification by mass spectral libraries does not exclude the possibility of errors, as mass spectra of the substances such as benzo[*b*]fluoranthene, benzo[*k*]fluoranthene and benzo[*a*]pyrene are practically identical, despite the fact that these compounds differ fundamentally in structure. Low selectivity of separation of some pairs of PAHs also makes contribution to identification errors. The capacity of selection and unambiguity of the qualitative analysis make the method of liquid chromatography–tandem mass spectrometry (LC-MS/MS) an optimal technique for PAHs analysis. The method of ionization became the main difficulty in PAHs detection by LC-MS/MS. At present, despite the well-known carcinogenic activity of PAHs, there are practically no developed criteria for evaluation of the individual carcinogenic potential of the substances of this class. The performed research on PAHs profile identification enabled calculation of the sum carcinogenic hazard of a food product, as well as determination of the PAHs presence indicators. The regularities of residue PAHs content in dependence of smoking type, packaging material and formulation of meat product were established.

II. MATERIALS AND METHODS

Analysis of PAHs was performed by LC-MS/MS on the chromatograph Agilent 1200 with the triple quadrupole detector Agilent 6400B. Chromatographic separation was conducted on the reversed phase chromatography column C18 chemically modified with phenyl groups Agilent Eclipse PAH 2.1 x 50 mm, 1.8 μm. Electrospray (ESI) was used as an ionization source, and chemical ionization was carried out at atmospheric pressure (APCI). Samples were prepared by QuEChERS [4] method with use of Octadecyl (C18(EC)) and ethylenediamine-N-propyl (PSA) sorbents. Analysis of phenols was performed using GH-MS Agilent 7890A with MSD Agilent 5975C.

III. RESULTS AND DISCUSSION

Electrospray ionization (ESI) requires the presence of an analyzing substance in a solution in an ionic form. As PAHs are the non-polar substances, ionization in a solution does not occur. In order to obtain a molecular ion, derivatization is necessary. To this end, the post-column derivatization with silver nitrate ($AgNO_3$) was used. Derivatives $[PAH+Ag]^+$ and $[2PAH+Ag]^+$, which were formed in this process, permitted an identification of PAHs; however, their relative percent content in a solution was not stable. As a result, reproducibility

of the measurement results was low. The disadvantage of the method was also the use of the additional channel of the chromatographic pump for derivatization. In connection with the unambiguity of the generating results, APCI method was chosen, which enabled ionization of non-polar substances with the corona discharge. Parameter optimization made it possible to obtain an intensive molecular ion for each PAHs; however, in the multiple reaction monitoring (MRM) mode, PAHs practically did not undergo fragmentation. Table 1 presents the PAHs molecular ions in the selected ion monitoring (SIM) mode under the conditions of APCI ionization with registration of positive ions.

Table 1 Parameters of PAHs identification in SIM mode

Analyte	Molecular ion, m/z
Cyclopenta[*c*,*d*]pyrene	227.3
Benzo[*a*]anthracene	229.3
Chrysene	229.3
5-methyl chrysene	243.3
Benzo[*j*]fluoranthene	253.3
Benzo[*b*]fluoranthene	253.3
Benzo[*k*]fluoranthene	253.3
Benzo[*a*]pyrene	253.3
Dibenzo[*a*,*l*]pyrene	303.4
Dibenzo[*a*,*h*]anthracene	279.4
Benzo[*ghi*]perylene	277.3
Indene[1,2,3-*cd*]pyrene	277.3
Dibenzo[*a*,*e*]pyrene	303.4
Dibenzo[*a*,*i*]pyrene	303.4
Dibenzo[*a*,*h*]pyrene	303.4

The fragmentor (Frag) voltage 90-135 V; evaporator temperature 380ºC; desolvation gas temperature 320ºC; desolvation gas flow rate 8 l/min.; nebulizer needle pressure 30 psi; capillary voltage 4.5 kWt.

The next step in the investigation was the detection of the factors that influence the PAHs quantitative content. First of all, it was necessary to evaluate the impact of different wood species on PAHs content. When wood species that are most widely used for food products smoking were heated for 20 min. at 300 ºC, the following results of PAHs content (μg/kg) were obtained in the generated pyrolysis liquid: hazel-wood – 30.47; beech wood – 21.07; apple tree – 28.75; cherry – 17.21. The high PAHs content generated from hazel-wood is possibly associated with the higher content of substances with quinoid structure, which is typical for hazel-wood. The process of wood heating in time also resulted in increase in PAHs content. Temperature rise from 450°C to 700°C leads to two- to threefold increase in PAHs content in a product.

Generated pyrolytic woodsmoke was analyzed on dispersity by correlation spectroscopy using a laser instrument with the system of photon counting from Malvern Instruments with image receiving analyzing block – Malvern Correlator K7023 (UK).

Generation of smoke fume particles occurs during heating; therewith, the higher the temperature, the less the smoke fume particle size [5]. With that, the concentration of PAHs in the fume phase decreased, and reduction of the fume particle size led to increase in the penetrative capacity. Table 2 presents the data of the dependency of the PAHs sum content on the nominal fume phase particle size.

Table 2 Impact of particle size of fume from smouldering beech wood sawdust on PAHs generation

Name	Average size of fume particles, nm			
	1000	700	250	100
Sum concentration of PAHs in fume phase, ng/m3	4820	3980	3770	3640
Mass fraction of benzo[*a*]pyrene, % of total PAHs	1.2	0.5	0.62	0.52

A significant effect on the level of PAHs absorption from vapor-gas phase upon thermolysis of wood has fat content in processing produce, which increases a degree of PAHs absorption up to ten times, all other things being equal. It is necessary to note the increased content precisely of the most carcinogenic PAHs with high molecular weight in fat tissue. The differences in PAHs residue content between pork and beef are not so significant. With that, phenolic substances in the back fat samples after smoking were found in trace quantities, which can indicate the chemical neutrality of fat with respect to phenolic components, which are

responsible for taste and aroma of smoked products. Reduction of the proportion of back fat content in uncooked smoked sausages will not lead to deterioration of product organoleptic characteristics; however, it will significantly improve its safety.

A casing is a barrier on the way of smoking substances penetration. The most permeable for PAHs is a natural casing, which is traditionally produced from intestines of farm animals. Protein and fibrouse casings are denser in structure, and penetration of PAHs through these barriers is largely hampered. A fibrouse casing is capable to ensure PAHs decrease in a product up to 40% compared to natural.

A complex assessment of PAHs content involved detecting the indicators of the presence of the substances of this class in a product. Selection of the individual PAHs was based on the frequency of the repetition of their results that were higher than the detection limit. Eight PAHs, namely benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*g,h,i*]perylene, chrysene, dibenzo[*a,h*]anthracene, and indene[1,2,3-*cd*]pyrene, were detected most frequently. Analysis of the frequency of the positive detection of four PAHs, which are controlled under the EC norms (EC 835/2011 of August 19, 2011) [6], in smoked meat products, namely, benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene and chrysene, showed that the sum content of the abovementioned four PAHs was within the limits 60±5% of the total content of 15 PAHs. Benzo[*a*]pyrene content was 6±0.5% relative to analyzing 15 PAHs.

The question of the detection of the sum carcinogenic hazard of PAHs is rather debatable, and several researchers developed so called Toxicity Equivalence Factors ("TEFs") of PAHs. The data obtained during the investigation of PAHs by California EPA's Office of Environmental Health Hazard Assessment (OEHHA) was used as the Toxicity Equivalence Factors ("TEFs").

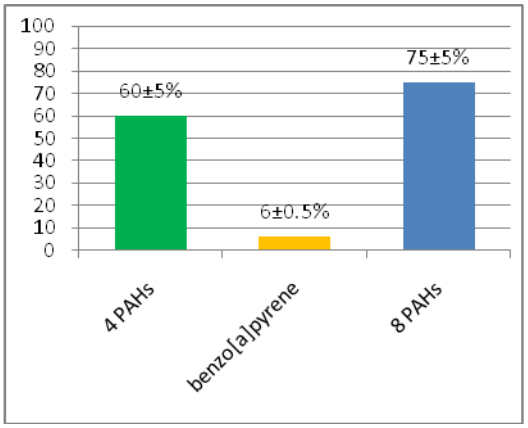

Figure 1. Qualitative content of 4 and 8 PAHs relative to the sum of 15 PAHs

For an assessment of the total carcinogenicity (K, arb. unit) of a smoked meat product, the following equation was used:

$$K = [\Sigma(PAHi) \times Qi)], \quad [1]$$

Where, PAHi is the mean concentration of an individual PAH, μg/kg;

Qi - Toxicity Equivalence Factor of an individual PAH.

The data is presented in Table 3.

Table 3. The mean content of PAH and the total carcinogenicity of a smoked meat product

PAH	PAHi, μg/kg	K, arb. unit.
Cyclopenta[*c,d*]pyrene	2.14	-
Benzo[*a*]anthracene	1.98	0.20
Chrysene	2.46	0.02
5-methyl chrysene	0.60	0.60
Benzo[*j*]fluoranthene	0.10	0.01
Benzo[*b*]fluoranthene	0.50	0.05
Benzo[*k*]fluoranthene	0.14	0.01
Benzo[*a*]pyrene	0.60	0.60
Dibenzo[*a,l*]pyrene	0.03	0.33
Dibenzo[*a,h*]anthracene	0.23	0.23
Benzo[*ghi*]perylene	0.32	-
Indene[1,2,3-*cd*]pyrene	0.36	0.04
Dibenzo[*a,e*]pyrene	0.87	-
Dibenzo[*a,i*]pyrene	0.06	0.60
Dibenzo[*a,h*]pyrene	0.13	1.30
Σ	10.52	3.99

Thus, the total carcinogenicity (K, arb. unit) of a smoked meat product is on the average 3.99 arb. units; with that, the carcinogenicity of benzo[*a*]pyrene is 0.60 arb. units; that is, the carcinogenic hazard of a meat product cannot be

assessed by the benzo[*a*]pyrene content because it is conditioned to a significant extent by the presence of other PAHs.

For regulation purposes, it is possible to apply the direct extrapolation of the carcinogenic effect obtained from dosing of experimental animals and expressed in mg/kg of body weight without incorporation of additional allowances and safety factors. The results of the calculation of human exposition to meat product contaminants are presented in table 4.

Table 4 PAHs exposure upon consumption of different meat products

	Cooked sausages	Semi-smoked	Uncooked smoked
Production, thousands of tons	1547.0	439.5	143.6
PAHs content, µg/kg	0.4	8.9	10.5
PAHs exposure µg/kg of body weight per year	0.06	0.39	0.15

On the basis of the calculation results, the main source of PAHs consumption is semi-smoked sausages. PAHs quantity in uncooked smoked sausages is higher, but their consumption is three times lower compared to semi-smoked sausages.

The undeniable advantage of this approach to the calculation of the human body exposure to PAHs is a possibility to assess the risk, exposure to chemical contaminants, which enter a human body by different ways, and evaluation of certain groups of food products that increase the risk of chemical carcinogenesis.

IV. CONCLUSION

The result of the study was the development of the method of PAHs identification by the LC–MS/MS method. The extraction selectivity was enhanced due to the use of the contemporary sorbents. The parameters of the chromato-mass-spectrometric detection of PAHs were optimized.

The factors influencing the PAHs quantitative content were determined. The dynamics of PAHs accumulation in dependence of smoking conditions, formulation, production technology and packaging material type was investgated. The technological means of carcinogenic PAHs reduction in smoked meat products, which included the complex approach both to the conditions of the fume composition formation and to the technological aspects of smoked produce production, are presented.

The data obtained enabled identification of PAHs presence indicators, determination of safety criteria for smoked meat products with consideration for the potential carcinogenic hazard of the individual PAH.

REFERENCES

1. ISO 15753:2006 Animal and vegetable fats and oils - Determination of polycyclic aromatic hydrocarbons. – Geneva: ISO 2006. - 22p.
2. ISO 15302:2007 Animal and vegetable fats and oils - Determination of benzo[a]pyrene - Reverse-phase high performance liquid chromatography method. – Geneva: ISO 2007. - 9p.
3. ISO 22959:2009 Animal and vegetable fats and oils - Determination of polycyclic aromatic hydrocarbons by on-line donor-acceptor complex chromatography and HPLC with fluorescence detection. – Geneva: ISO 2009. - 22p.
4. Amith D., Lynam K. (2011) Polycyclic Aromatic Hydrocarbon Analysis in Fish by GC/MS Using QuEChERS., Agilent Technologies publication, 5990-6668EN http://www.crawfordscientific.com/downloads/Application-Notes/Techniques/GC/5990-6668EN.pdf
5. Ivankin A.N., Kulikovsky A.V., Vostrikova N.L., Chernucha I.M., Figovsky O.L., Belaykov V.A.,. Lihanova L.M. (2013). Nano, Micro Transformations of termo degraded products of wood and their influence on the safety of food. Scientific Israel – Technological Advantages 15:2: 56–62.
6. COMMISSION REGULATION (EU) No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs. Official Journal of the European Union 20.8.2011. L 215/4 EN.