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The Relationship Of Nano-Microcomponent Composition Of Smoke And The Content Of Polyaromatic Hydrocarbons In Smoked Food Products.

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ABSTRACT

The problem of use of products of thermal decomposition of wood for reception of food is considered. It is shown, that contents of polycyclic aromatic hydrocarbons (PAH) in food depends from the nano and micro sizes of smoke particles. The paper examines the dependence on the nature of the wood used for smoking on the formation of fifteen PAHs. Eight PAHs (benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[ghi]perylene, chrysene, dibenzo[*a,h*]anthracene, indene[1,2,3-*cd*]pyrene) are the indicators of presence of dangerous substances in food, selected as markers of PAHs.
Keywords: polycyclic aromatic hydrocarbons, PAH, smoking, nano, micro particles, wood, meat products

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INTRODUCTION

Deep processing of wood are used in the production of food technologies, pharmaceutical and medical devices. Traditional is the use of wood for the manufacture of products of thermolysis of smoking. Degradation of hardwood when heated under conditions of lack of oxygen leads to education of the smoke, which contains substances decaying wood, part of which has unsafe properties [1, 2]. Once in the smoked food, smoky fires of wood components in-give it a nice flavor, but also substances are formed, causing pyrolysis products in the greater content of harmful impurities, especially polycyclic aromatic hydrocarbons (PAHs) [3, 4, 5].

Risk of PAHs has been evaluated by the international programme on chemical safety (International Programme on Chemical Safety, IPCS) of the who and the EU Scientific Committee on food safety (Scientific Committee on Food, SCF) [6]. Their results showed that 15 PAHs are formed in smoke fluids and fall in food products, namely, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, benzo[*a*]pyrene, chrysene, dibenzo[*a,h*]pyrene, dibenzo[*a,h*]anthracene, dibenzo[*a,e*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene, 5-methyl chrysene, indene[1,2,3-*cd*]pyrene and cyclopenta[*c,d*]pyrene have a pronounced carcinogenic, mutagenic and teratogenic effect in humans [7]. These substances are genotoxic carcinogens for which you cannot install pre-release component of secure content, below which appears carcinogenic potential of [8, 9].

Stringent controls on PAHs is very important because the oncology, along with cardiovascular disease and diabetes is today the highest risk to humans [10, 11]. In the Russian Federation is normalized only one representative of this group – benzo[*a*]pyrene, which maximum concentration limit makes of 0,001 mg/kg for food production, for drinking water 0.6 µg/kg.

In the European Union today must control 4 PAHs. The permitted levels of benzo[*a*]pyrene in smoked meat to 5.0 µg/kg. The 1.9.2014 is reduced to 2.0 µg/kg. The permitted total maintenance of 4 PAH (benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene and chrysene) in food products the EU reduced to 30 µg/kg to 12 µg/kg [12].

For definition of PAH the chromatography methods are used. Features of chemical structure of PAH from products of disintegration of wood cause their high sensitivity the analysis with the fluorescent detector in comparison with diode-matrix or spectrometric detectors [13, 14, 15]. It was of interest to estimate the influence of conditions of formation of components of smoky compositions and their dispersivenesses on the contents of dangerous PAH in smoked products.

The problem of the given research consist in an estimation of influence of smoky components of disintegration of wood on a safe level of the contents of aromatic substances in smoked food production depending on the nominal size of particles.

MATERIALS AND METHODS

Thermal decomposition of wood spent, heating up wood sawdust of different breeds at 200–700°C with the subsequent processing by the received smoky fraction of foodstuff during 1–24 ч. Muscle samples were subjected to smoke for food industry pork *Longisimus Dorsy* landras age 1 year.

The analysis of PAH carried out by means of chromatograph Ultimate 3000 (Dionex) with fluorescent detector RF2000 (Dionex) and Supelco LC-PAH column, 150x4.6mm, 5mkm. PAH extracted twice on 50 ml of cyclohexane from 1 g sample, washing of an extract consistently 50 ml HPLC water and 50 ml 50 % water methanol.

50 ml of a mix 9:1 N,N-dimethyl formamide – water was added to the washed out extract, separating a bottom layer to which added 50 ml of a 1% sodium chloride solution and 75 ml cyclohexane. The top layer filtered through waterless sodium sulfate and evaporated dry on the rotational evaporator. The dry rest dissolved in 3 ml of cyclohexane and passed through silica. The cartridge for extraction was filled with 200 mg of a sorbent (Silica-SPE Bulk Sorbent, Agilent), then conditioned playing through 5 ml of cyclohexane, put 3 ml of test and eluted 15 ml of cyclohexane. Solvent evaporated at 40°C dry, the dry rest for analysis HPLC dissolved in 500 mkl acetonitrile (Figure 1, 2).

Conditions of the HPLC analysis: a gradient eluent A – water, for 20 min from 40% up to 0%, B – acetonitrile from 60% up to 100%, test 20 mkl, a stream – 1.2 ml/min, pressure 600–1300 psi, absorption length λ_1 240–293 nm, radiations λ_2 376–485 nm. A standard mix of 15 PAH-Mix 170 (Dr. Ehrenstrofer) was used.

The resulting charcoal smoke were subjected to analysis of pyrolytic on dispersion method of correlation spectroscopy in laser installation with the system for counting photons Malvern Instruments with the foto analyses block – Malvern K7023 (United Kingdom).

RESULTS AND DISCUSSION

Results of determination of PAH contents in pyrolized liquid are presented (see Table I). Liquid formed at warming up of food production most used for smoking of wood during 20 min at 500°C. You can see that the heat treatment of various species results in the formation of significant amounts of PAHs, which are processed through the smoke gas phase in a lot of the food. A large number of PAH formed from walnut, possibly associated with increased content of substances hinoid structure, typical of nut trees. The process of heating wood in time also led to increased content of PAHs. Plenty PAH which has formed wood of a nut, probably, it is connected with the increased contents of quinoid substances, characteristic for nut breeds. Process of heating of wood in time also led to growth of contents of PAH.

Table 1: Formation of PAH at thermal processing wood

Name	PAH in pyrolized wood, mkg/kg			
	The nut wood	The beech	The apple-tree	The cherry
Cyclopenta[*c,d*]pyrene	18.66	9.75	1.6	1.05
Benzo[*a*]anthracene	9.03	0.24	0.54	0,77
Chrysene	53.24	2.47	7.25	2.37
5-methyl chrysene	102.36	2.77	2.12	7.34
Benzo[*j*]fluoranthene	47.67	2.2	3.81	1.87
Benzo[*b*]fluoranthene	1.09	1.24	1.21	0.06
Benzo[*k*]fluoranthene	0.98	0.35	0.77	0.1
Benzo[*a*]pyrene	7.2	0.21	0.17	0.38
Dibenzo[*a,l*]pyrene	1.62	0.35	0.08	0.02
Dibenzo[*a,h*]anthracene	2.45	0.54	0.20	0.24
Benzo[*ghi*]perylene	6.57	0.03	0.37	1.32
Indene[1,2,3-*cd*]pyrene	1.2	0.2	0.1	0.12
Dibenzo[*a,e*]pyrene	9.56	0.72	1.67	0.45
Dibenzo[*a,i*]pyrene	3.24	0	0.57	0
Dibenzo[*a,h*]pyrene	35.6	0	8.29	1.12
Σ	300.47	21.07	28.75	17.21

On Figure 1 it is shown the chromatogram of 15 PAH standard mix which are necessary for analysis of safety of the food production subjected to smoky processing by decaying wood. The typical picture of PAH contents in the extract of smoked meat production (pork) is given on Figure 1. The analysis shows, that in smoked food production practically at the majority of modes of smoking full spectrum PAH in which the most significant weight is benzo[*a*]pyrene.

From the presented data it is visible, that the used method of the analysis allows to identify reliably the full list harmful PAH. The contents of some of them which are usually not controllable, appears enough big (Table 2).

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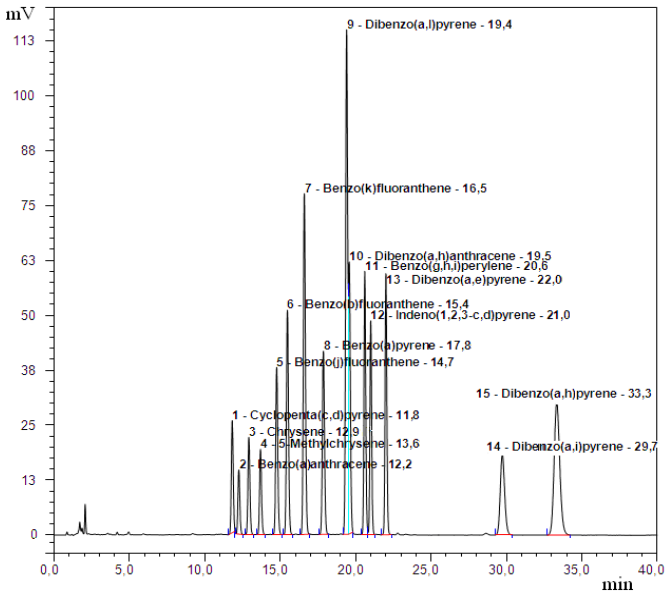

Figure1: The chromatographic separation of 15 PAH calibrating mixes on column Supelco LC-PAH (150x4.6mmx5mkm). A solution of 1 ng/ml in acetonitrile (times of an exit are specified)

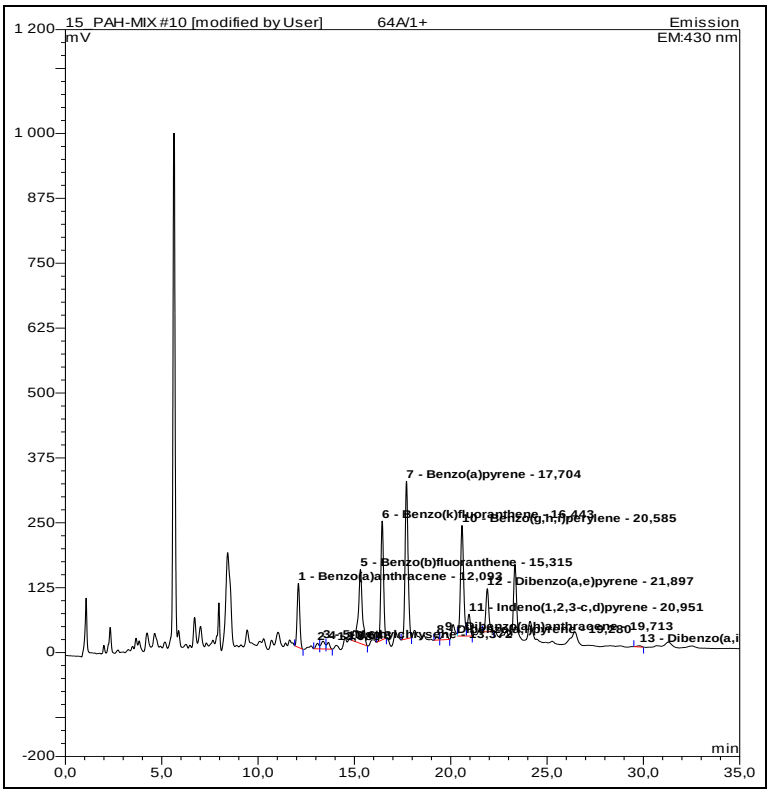

Figure 2: The chromatographic tests of a smoked pig after solid-phase extraction

Table 2: The contents of fifteen of the most dangerous PAH in smoked pork depending on the temperature of Smoking

Name	Relative danger	PAH, mkg/kg		
		250°C	350°C	600°C
Benzo[*a*]anthracene	0.01–0.15	2.75	2.90	3.01
Benzo[*b*]fluoranthene	0.08–0.12	0.48	0.60	0.66

Benzo[j]fluoranthene	0.08–0.12	0.04	0.05	0.07
Benzo[*k*]fluoranthene	0.004–0.05	0.12	0.14	0.16
Benzo[*ghi*]perylene	0.01–0.02	0.54	0.72	0.79
Benzo[*a*]pyrene	1.0	0.47	0.59	0.66
Chrysene	0.001–0.12	2.33	2.50	2.71
Dibenzo[*a,h*]pyrene	–	0.08	0.10	0.12
Dibenzo[*a,h*]anthracene	0.7–1.11	0.17	0.20	0.22
Dibenzo[*a,e*]pyrene	–	0.46	0.60	0.64
Dibenzo[*a,i*]pyrene	–	0.04	0.05	0.06
Dibenzo[*a,l*]pyrene	–	0.02	0.03	0.04
5-methyl chrysene	–	0.13	0.15	0.16
Indene[1,2,3-*cd*]pyrene	0.01–0.28	0.48	0.50	0.55
Cyclopenta[*c,d*]pyrene	–	1.39	1.6	1.68

The increase of contents of PAH with growth of temperature of heat treatment of wood and corresponding hit PAH in processable production is typical. The growth of concentration of 15 PAH and separately benzo[*a*]pyrene depending on increase in temperature of formation of a smoke is shown on Figure 3.

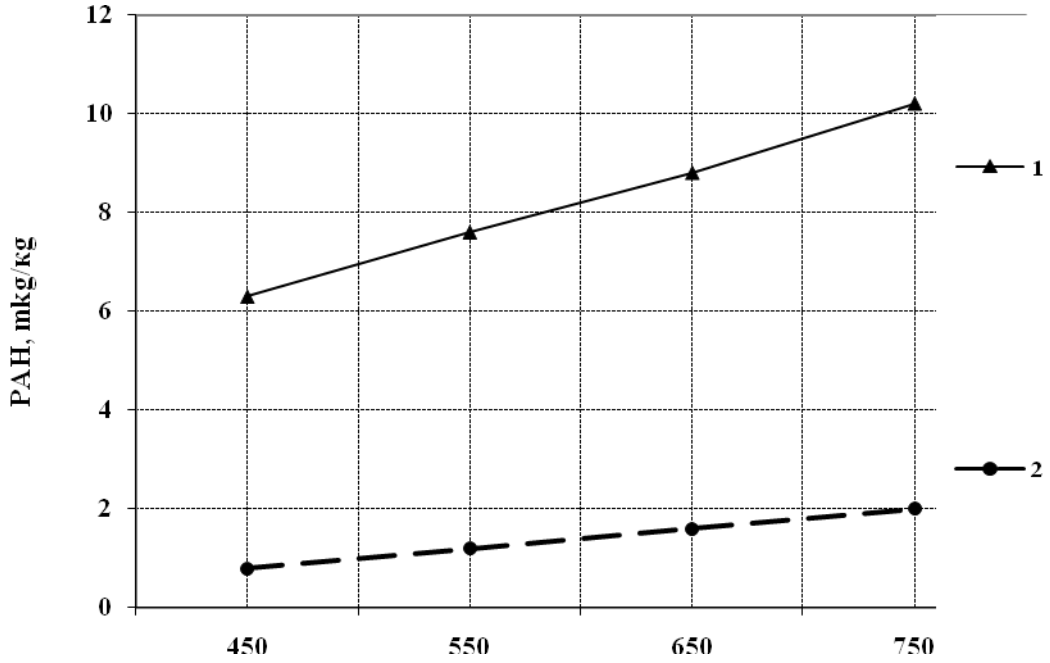

Figure 3: The dependence of the contents of the sum of 15 PAH (mkg/kg of weight of wood) – 1 and benzo[*a*]pyrene – 2 on the temperature of formation of a smoke beechen wood

The used HPLC method with the fluorescent detector was sensitive enough and allowed to determine all group of 15 PAH with concentration in analyzed test considerably below established maximum concentration limits. The limit of detection of 15 PAH in conditions of the analysis made for cyclopenta[*c,d*]pyrene and benzo[*j*]fluoranthene 1 ng/ml, for benzo[*a*]pyrene 0.01 ng/ml, for the others PAH 0.02 ng/ml. A degree of extraction separate PAH from a matrix of an analyzed product varied from 48.2 up to 90.5%, average value of a degree of extraction has made 79.16 %. The benzo[*b*]chrysene as the internal standard was used.

Results of researches have confirmed applicability of the used technique of allocation, clearing and analysis of PAH for an estimation of their contents in the smoked food production received in various conditions.

From the data of Table 2 it is visible, that traditionally used modes of smoking allow to receive production which under the contents of benzo[*a*]pyrene corresponds to normative requirements. At more heats degradation of wood at smoking, the amount of benzo[*a*]pyrene in meat production can exceed an admissible level. From the data Table 2 it is visible also the real pictures of "safety" of smoked production. The control only behind one parameter, the maintenance of benzo[*a*]pyrene which toxicity is conditionally accepted for 1, is insufficient as presence of the others PAH in view of the factor of their relative danger are undesirable. The total contents of data PAH in smoked production many times over exceeded a level of benzo[*a*]pyrene. It once again confirms the thesis about necessity of the constant control over contents of PAH for food production.

The dependences of total contents of sum PAH from the nominal sizes of particles of a smoky phase are presented in Table 3.

Table 3: Influence of particle size of smoke from sawdust of beech trees on PAH formation

Name	The average diameter of smoky particles, nm				
	1000	700	250	80	50
Total concentration of PAH in a smoky phase, ng/m^3	4820	3980	3770	3640	3500
Mass fraction of benzo[*a*]pyrene, % from sum PAH	1.2	0.5	0.62	0.52	0.5

Particle smoke formation occurs when heated, and the higher the temperature, the smaller the smoke particles. The concentrations of PAHs in the phase of decline and smoky smaller particles of smoke led to increased penetration. This leads to increased content of PAHs in food-smoked with increasing temperature (Table 2).

The analysis of quantitative contents of PAH in smoked meat production within the limits of certification has shown, that most often found out were 8 PAH, namely benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, chrysene, dibenzo[*a,h*]anthracene and indene[1,2,3-*cd*]pyrene. Total percentage set forth above 8 PAH averaged more than 75% from total PAH. Thus the mass fraction of cyclopenta[*c,d*]pyrene which is considered the least cancerogenic and does not possess mutagen properties, by results of researches, was in a range of 10–15%. One more factor in favour of a choice set forth above 8 PAH as the indicator of presence of group PAH, is the high degree of their extraction which varies within the limits of from 79.2 up to 91.4%. While for dibenzo[*a,l*]pyrene, dibenzo[*a,e*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,h*]pyrene, the degree of extraction made from 48.2 up to 64.2%.

CONCLUSION

Thus, the dependence of contents of PAH in smoked food production from technology of processing was established and indicators of presence of PAH in smoked production on the basis of meat raw material which eight are set forth above PAH are revealed. The actual data on the content of PAHs confirm the importance of monitoring the content of polycyclic compounds harmful wood that can fall into food and to cause harm to human health.

REFERENCES

[1] Farhadian, A. Effects of marinating on the formation of polycyclic aromatic hydrocarbons (benzo[a]pyrene, benzo[b]fluoranthene and fluoranthene) in grilled beef meat / A. Farhadian, S. Jinap, A. Faridah, I.S.M. Zaidul // Food Control. 2012. № 28 (2). P. 420–425.

[2] Rose, M. Investigation into the formation of PAHs in foods prepared in the home to determine the effects of frying, grilling, barbecuing, toasting and roasting / M. Rose, J. Holland, A. Dowding, S. (R.G.) Petch, D. Mortimer // Food and Chemical Toxicology. 2015. № 78. P. 1–9.

[3] José, L. Domingo Carcinogenicity of consumption of red meat and processed meat: A review of scientific news since the IARC decision / José L. Domingo, M. Nadal // Food and Chemical Toxicology. 2017. № 105. P. 256–261.

[4] Duedahl-Olesen, L. Polycyclic aromatic hydrocarbons (PAH) in Danish barbecued meat / L. Duedahl-Olesen, M. Aaslyng, L. Meinert, T. Christensen, M.L. Binderup // Food Control. 2015. № 57. P. 169–176.

[5] Silva B.O. Effects of the methods of smoking on the levels of polycyclic aromatic hydrocarbons (PAHs) in some locally consumed fishes in Nigeria / Silva B.O., Adetunde O.T., Oluseyi T.O. Olayinka K.O. Alo B.I. // African J. of Food Sci., 2011, V. 5(7), P. 384 – 391.
[6] Polycyclic aromatic hydrocarbons in food. Scientific opinion of the panel on contaminants in the food chain (Question № EFSA-Q-2007-136). The EFSA Journal. 2008. p. 724.
[7] Monographs on the evaluation of carcinogenic risks to humans. V. 92. Lyon, France: International Agency for Research on Cancer. 2010.
[8] Vasudha, B. Review of PAH contamination in food products and their health hazards / B. Vasudha, K. Ki-Hyun // Environment International. 2015. № 84. P. 26–38.
[9] Vostrikova N.L. Formation of the scientific basis of meta-data associated with estimates of «Onco-» risks linked to meat products / Vostrikova N.L., Kuznetsova O.A., Kulikovskii A.V., Minaev M.Y. // Theory and practice of meat processing. 2017. № 2(4). P. 96
[10] Silva B.O. Effects of the methods of smoking on the levels of polycyclic aromatic hydrocarbons (PAHs) in some locally consumed fishes in Nigeria / Silva B.O., Adetunde O.T., Oluseyi T.O. Olayinka K.O. Alo B.I. // African J. of Food Sci., 2011, V. 5(7). P. 384 – 391.
[11] Essumang, D.K. Polycyclic aromatic hydrocarbon (PAH) contamination in smoke-cured fish products / D.K. Essumang, D.K. Dodoo, J.K. Adjei // Journal of Food Composition and Analysis. 2012. № 27. P. 128–138.
[12] Commission Regulation (EU) № 835/2011 of August 2011 amending regulation (EC) № 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs // Official Journal of the European Union 8/20/2011. L. 215. P. 4–8.
[13] Mottier P. Quantitative determination of polycyclic aromatic hydrocarbons in barbecued meat sausages by gas chromatography coupled to mass spectrometry / Mottier P., Parisod V., Turesky R.J. // J. Agric. Food Chem. 2000. vol. 48. P. 1160 – 1166.
[14] Kulikovskii A.V. Methodology of the Determination of Polycyclic Aromatic Hydrocarbons in Foods/ Kulikovskii A.V., Vostrikova N.L., Chernukha I.M., Savtchuk S.A. // Journal of Analytical Chemistry.- 2014.- V.69.- №2.- P. 205–209.
[15] Jira W.A. GC/MS method for the determination of carcinogenic polycyclic aromatic hydrocarbons (PAH) in smoked meat products and liquid smokes / Jira W.A. // Eur. Food Res. Technol. 2004. V. 218. P. 208 – 212.