

## INFLUENCE OF TEMPERATURE OF WOOD PYROLYSIS ON THE CONTENT OF POLYCYCLIC AROMATIC HYDROCARBONS IN SMOKED MEATS

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### ABSTRACT

The aim of the study was to evaluate the effect of wood pyrolysis temperature on the content of polycyclic aromatic hydrocarbons in selected sausage products. The study material consisted of rural sausage smoked in traditional way in two chambers. The tested PAH content of the samples was determined by liquid chromatography (HPLC). Studies have shown that running a smoking process at an average temperature of 551.2°C resulted in a significant reduction in the concentration of polycyclic aromatic hydrocarbons compared to their content in the article smoked at an average temperature of 675.2°C. The concentration of benzo(a)pyrene in smoked sausage at lower temperatures was in accordance with the applicable standards. And the sum of the four PAHs, both at higher and lower temperatures, exceeded the current recommendations.

**Key words:** smoking, smoking temperature, deli meats, polycyclic aromatic hydrocarbons

### INTRODUCTION

One of the commonly used methods of preserving meat products is smoking, which, in addition to prolonging the shelf life, allows obtaining certain sensory characteristics of meat products [Bilska et al. 2017]. From consumer research [Kowalski and Pycz 2006, Migdał et al. 2014] it appears that the original sensory value of meat products can be obtained by the traditional method of smoking. At the time of traditional smoking, meat products are subjected to the simultaneous action of heat and chemical compounds contained in smoke, usually obtained by the pyrolysis of wood and the oxidation of gaseous decomposition products occurring in the diffusion zone over the furnace [Bagnowska et al. 2011]. The smoke generated by this process is a complex aerosol containing more than 1000 different chemical compounds that are derived from aliphatic and aromatic hydrocarbons, phenolic, carbonyl and organic acids. Apart from the constituents of smoke, which have a preservative and sensory effect, particular attention is paid to the presence of smoke compounds in the smoke which may adversely affect the health of consumers [Yang et al. 1991, Yang et

al. 2010, Kuna 2011]. The group includes these compounds (PAHs – polycyclic aromatic hydrocarbons) which exhibit mutagenic, carcinogenic and teratogenic effects on the human body [SCoF, 2002]. Due to these threats, the European Commission has issued a recommendation to monitor concentrations of benzo(a)pyrene and other PAHs selected by the EU Scientific Committee on Food (SCF), in selected groups of foodstuffs, including cold meats as defined in Commission Regulation (EU) No. 835/2011 of 19 August 2011 [EC, 2005]. According to Commission Regulation (EU) No. 835/2011 in addition to the level of B(a)P [ML 2 µg · kg<sup>-1</sup>], the total content of 4 PAHs [ML 12 µg · kg<sup>-1</sup>; Benzo(a)anthracene, chrysene, benzo(a)pyrene and benzo(b)fluorantene] [(UE) NR 835/2011] should also be monitored.

One of the factors that determine the PAH content of smoked meat products is the temperature of the pyrolysis process of the wood when it is smoked. This is due to the fact that these compounds arise during incomplete combustion of organic matter, primarily during the thermal decomposition of lignin [Djinovic et al. 2008, Rey-Salgueiro et al. 2009 a, 2009 b, Arias et al. 2010, Ghasemzadeh-Mohammadi 2012, Yebra-Pimentel

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et al. 2012, Yerba-Pimentel et al. 2014, Rozentāle 2015, Shrestha et al. 2015, Pongpiachan 2015]. According to numerous studies [Ciecierska and Obiedziński 2007, Kuhn et al. 2009, Bagnowska et al. 2011], of Ledesma et al. [2014] it is shown that at a temperature above 500°C in smoke there are visible amounts of PAH in the chromatographic spectra. At about 1000°C, PAHs are produced in large quantities, and aliphatic hydrocarbons are also present in products smoked at such temperatures [Alén et al. 1996, Varlet et al. 2007, Guillén et al. 2000 a, 2009 b, Stolyhwo and Sikorski 2005, Niewiadomska et al. 2016].

Due to the significant influence of the increase in wood burning temperature during smoking on the amount of PAHs deposited in the product, it is recommended that the temperature of this process does not exceed 425 to 450°C [McGrath et al. 2003, Kowalski and Pyczc 2006]. However, as experience shows, keeping the smoking temperature in this range is difficult, and traditional smoking typically takes place in temperatures far above the recommended level, which may result in PAH accumulation in the products to be treated.

The aim of the study was to evaluate the effect of wood pyrolysis temperature on the content of polycyclic aromatic hydrocarbons in selected sausage products.

## MATERIAL AND METHODS

Experiment was carried out in one of the meat plants specialized in the production of conventional meat products.

The study material consisted of 42 samples of rural sausage smoked in traditional way [Migdał et al. 2014]. Smoked sausage samples were collected systematically every two weeks in the amount of about 1.5 kg.

To verify the assumed research goal, which is the influence of temperature during the smoking process on the level of contamination of the smoked product with polycyclic aromatic hydrocarbons a rural sausage was selected, as the whole heat-setting process was carried out in the smoke chamber. At the same time, our own research [Choroszy and Tereszkiwicz 2017] has shown that the product has been found to exceed the maximum limit of four PAHs and B(a)P [EU Commission Regulation 2011].

The smoking of the tested product was carried out using a traditional method of producing smoke in a furnace located at distance of 50 cm from the smoke chamber and an inlet duct connected thereto. The smoke in the furnace was produced by pyrolysis of air-dry beech and alder blocks. Produced in such way smoke aerosol was directed to the chamber. Smoking was carried out in parallel in two smoke chambers where, by regulating the amount of wood burned and the air supply, a varied temperature of the wood pyrolysis was achieved. The combustion process in the furnace of the first chamber was carried out by using the incandescent method, while in

the furnace of the second chamber by using the flame method. The applied measures allowed to maintain the temperature in the first chamber (Chamber I) at an average level of 551.2°C. In this chamber, measures were taken to regulate the intensity of the wood burning process by limiting air access and the amount of combustible material. In contrast, in the second chamber (Chamber II) the combustion proceeded in an unregulated manner and the average temperature of the furnace was 675.2°C.

The wood burning temperature was controlled during the entire smoking process using the LB-707 thermometer-hygrometer-barometer reading at 15-minute intervals. The smoking process lasted 270 minutes. The wood pyrolysis temperature control record is presented in Table 1.

After the smoked and cooled products from both chambers samples were taken to determine PAH content. The samples were taken in accordance with Polish Standard PN-EN ISO 15753. Subsequently, the samples were homogenized in full, then 20 g of sample was taken from the homogenizer into a sterile container and frozen at –25°C. Each trial was performed in two identical replicates.

The tested PAH content of the samples was determined by liquid chromatography (HPLC). The content of the following hydrocarbons was determined: benzo(a)pyrene – B(a)P, chrysene – CHR, benzo(a)anthracene – B(a)A, benzo(b)fluoranthene – B(b)F, benzo(c)fluorene – B(c)F, benzo(g,h,i)perylene – B(g,h,i)P, benzo(j)fluorantene – B(j)F, benzo(k)fluoranthene – B(k)F, dibenzo(a,e)pyrene – DB(a,e)P, dibenzo(a,h)anthracene – DB(a,h)A, dibenzo(a,h)pyrene – DB(a,h)P, dibenzo(a,i)pyrene – DB(a,i)P, dibenzo(a,l)pyrene – DB(a,l)P, indene(c,d)pyrene – I(c,d)P. The hydrocarbons designated for their construction and chemical properties were divided into light and heavy according to the criteria given by Kubiak [2013].

The direct PAH designation included the extraction of the fat fraction by the Soxhlet method. The extraction was done in a 100 cm<sup>3</sup> camera using hexane + DCM in a ratio of 3 + 1 (v/v) for 8 hours. The concentrated sample was purified by SEC/GPC exclusion chromatography. A Bio-Beads SX-3 bed was placed in a 50 cm × 1.5 cm column. The solvent was a 1:1 mixture of cyclohexane and ethyl acetate (volume), flow was 2 cm<sup>3</sup> · min<sup>-1</sup>. The 60 cm<sup>3</sup> pre-fraction was discarded. Another 60 cm<sup>3</sup> PAH containing fraction was collected. The concentrate was then purified by solid phase extraction (SPE) using a 6 cm<sup>3</sup> SPE column containing 1000 mg silica gel. The column was conditioned with a mixture of hexane and dichloromethane 1 + 1 (v/v). A 3 × 1 cm<sup>3</sup> sample was then applied, and washed with 2 × 5 cm<sup>3</sup>. The final 8 cm<sup>3</sup> fraction was concentrated.

Qualitative and quantitative PAH designations were performed using a Waters Alliance 2695 High Performance Liquid Chromatograph equipped with an Agilent PAH 5 Pursuit C18 column measuring 250×4.6 mm and 5 m grains. 50 l of the sample was dosed and the column temperature was 30°C. The mobile phase flow was 1 cm<sup>3</sup> · min<sup>-1</sup>. The gradient program was as follows: the analysis was conducted using internal standards AS-BbC – 05.05.15. PAH identification was carried out using a fluorescent detector with excitation and emission wave programming. The signal of (a) anthracene corresponds to wave length: 286/408, – benzo(a)pyrene signal corresponds to wave length 380/406, – benzo(b)fluoranthene signal corresponds to wave length 304/433 and chrysene signal corresponds to wave length 266/408.

The PAH content in µg/kg of sample was determined using the 15 WWA v 2 program (Tables 2, 3).

The results were analyzed statistically using the STATISTICA 12 program, calculating the mean, standard deviation, minimum-maximum range. The evaluation of the effect of wood pyrolysis on the level of PAH concentration in the examined product was made by Student-test for two independent trials with a significance level of 0.05.

## RESULTS AND DISCUSSION

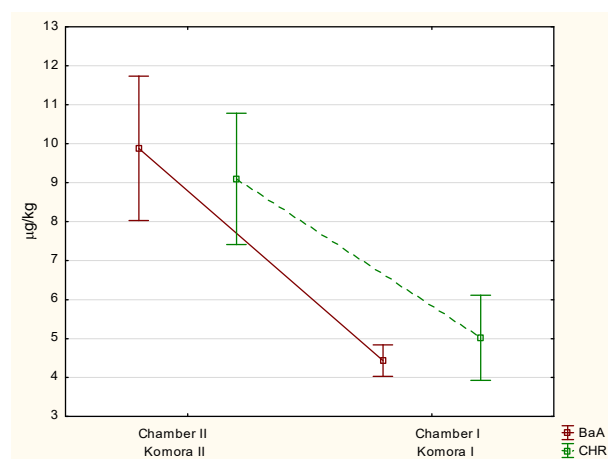
It has been observed from the conducted own studies that the combustion process used to control the temperature of wood pyrolysis varied between the chambers. The average temperature in chamber I was 551.2°C and ranged from 500–590°C. The temperature in this chamber was lower than the average temperature of 675.2°C in chamber II by about 124°C. The highest temperature in chamber II was recorded at 150 minutes of smoking and was 760°C (Table 1).

The results of the experiment are presented in Tables 4, 5 and in Figures 1, 2 and 3.

In our own study, nine PAHs were identified in the entire PAH profile, while the remaining six in all tested samples were below the detection limit. It should therefore be assumed that the rural sausage contained no dibenzo(a,e)pyrene, dibenzo(a,h)anthracene, dibenzo(a,h)pyrene, dibenzo(a,i)pyrene, dibenzo(a,l)pyrene. In the group of nine hydrocarbons found in the tested product, two (B(a)A and CHR) belonged to the light PAHs and the remaining seven to the heavy PAHs.

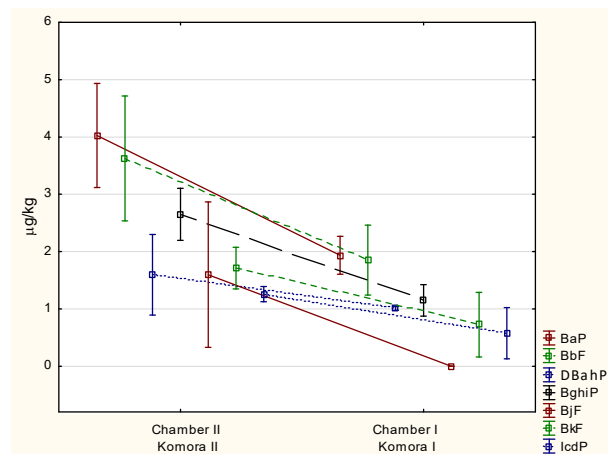
In analyzing the content of PAHs present in the product, the influence of differentiated smoking temperatures was significant. The average content of each of the nine PAHs, both light and heavy in smoked sausage in a chamber where the temperature significantly exceeded the maximum temperature for PAH formation, was statistically significantly higher, mostly twice the PAH con-

tent in the same sausage after chilling in the chambers with controlled thermal conditions. The average content of benzo(a)anthracene in a rural sausage smoked at pyrolysis temperatures above 600°C was 9.88 µg · kg<sup>-1</sup> on average and was 5.45 µg · kg<sup>-1</sup> higher than the average content of this hydrocarbon in smoked sausage at an average temperature of 551°C. The mean chrysene content in chamber II was 4.08 µg · kg<sup>-1</sup> higher than its content in the smoked samples in the chamber at which the temperature was lower.



**Fig. 1.** Influence of temperature of wood pyrolysis on the content of light PAHs in rural sausage

**Rys. 1.** Wpływ temperatury pirolizy drewna na zawartość lekkich WWA w kiełbasie wiejskiej



**Fig. 2.** Influence of temperature of wood pyrolysis on the content of heavy PAHs in rural sausage

**Rys. 2.** Wpływ temperatury pirolizy drewna na zawartość ciężkich WWA w kiełbasie wiejskiej

Studies have shown that rural sausage smoked with use of traditional method cumulates PAHs at levels close to those of PAHs in sausage tested by other authors

**Table 1.** The course of the smoking process with temperature control of wood pyrolysis, °C

**Tabela 1.** Przebieg procesu wędzenia z kontrolą temperatury pirolizy drewna, °C

Chamber I – Komora I			Chamber II – Komora II		
$\bar{x}$	min	max	$\bar{x}$	min	max
551.2	500	590	675.2	563	760

**Table 2.** Gradient elution programme, % by volume

**Tabela 2.** Program gradientowy eluentu, % objętościowy

Time, min Czas, min.	Acetonitrile Acetonitryl	Water Woda	Ethyl acetate Octan etylu
0	75	25	0
15	75	25	0
55	93	7	0
56	100	0	0
60	100	0	0
75	50	0	50
80	100	0	0
85	75	25	0
90	75	25	0

**Table 3.** The Waters 2475 Fluorescence Detector Programme

**Tabela 3.** Program detektora fluorescencyjnego – Waters 2475

Time, min Czas, min.	Channel A – Kanał A	Channel B – Kanał B	Channel C – Kanał C	Channel D – Kanał D
	Wave length: excitation/emission, nm – Długość fal: wzbudzenie/emisja, nm			
0.0	266/408	286/408	310/357	380/406
16.0	–	–	315/510	–
22.0	–	304/433	–	–
40.0	286/420	300/500	315/422	362/408
60.0	281/434	295/408	–	–

**Table 4.** Influence of temperature of wood pyrolysis on the content of PAHs in rural sausage

**Tabela 4.** Wpływ temperatury pirolizy drewna na zawartość WWA w kielbasie wiejskiej

Light PAHs, $\mu\text{g} \cdot \text{kg}^{-1}$ Lekkie WWA, $\mu\text{g} \cdot \text{kg}^{-1}$	Chamber I – Komora I				Chamber II – Komora II				Test results Wyniki testowania		
	Statistical Measures Miary statystyczne				Statistical Measures Miary statystyczne				t	p	
	$\bar{x}$	min	max	SD	$\bar{x}$	min	max	SD			
BaA	4.43	3.91	4.70	0.41	9.88	7.64	11.72	1.85	7.04	0.000	
CHR	5.02	4.09	6.40	1.09	9.10	7.09	10.83	1.69	4.97	0.001	
Heavy PAHs, $\mu\text{g} \cdot \text{kg}^{-1}$ Ciężkie WWA, $\mu\text{g} \cdot \text{kg}^{-1}$	Chamber I – Komora I				Chamber II – Komora II				Test results Wyniki testowania		
	Statistical Measures Miary statystyczne				Statistical Measures Miary statystyczne				t	p	
	$\bar{x}$	min	max	SD	$\bar{x}$	min	max	SD			
	BaP	1.94	2.32	1.58	0.33	4.03	5.01	2.98	0.91	5.29	0.000
	BbF	1.85	1.14	2.50	0.61	3.63	2.31	4.71	1.09	3.48	0.006
	DBahP	1.01	0.97	1.04	0.03	1.60	1.05	2.50	0.70	2.04	0.069
	BghiP	1.15	0.80	1.37	0.27	2.65	2.22	3.21	0.45	6.93	0.000
	BjF	0.00	–	–	0.00	1.60	0.0	2.71	1.27	–	–
BkF	0.73	1.26	2.05	0.56	1.71	0.0	1.10	0.36	3.60	0.005	
IcdP	0.58	1.17	1.43	0.45	1.26	0.0	0.91	0.13	3.58	0.05	

**Table 5.** Influence of temperature of wood pyrolysis on the content the PAHs in rural sausage identified by the European Commission

**Tabela 5.** Wpływ temperatury pirolizy drewna na zawartość WWA określonych w wymaganiach Komisji UE w kiełbasie wiejskiej

Content of PAHs, $\mu\text{g} \cdot \text{kg}^{-1}$ Zawartość WWA, $\mu\text{g} \cdot \text{kg}^{-1}$	Komora I – Chamber I		Komora II – Chamber II		Maximum limits as required by the European Commission Maksymalne limity według wymagań Komisji Europejskiej
	$\bar{x}$	min	$\bar{x}$	min	
BaP	1.94	1.58	4.03	2.98	2.00
BaA	4.43	3.91	9.88	7.64	–
BbF	1.85	1.14	3.63	2.31	–
CHR	5.02	4.09	9.10	7.09	–
Sum 4 PAHs – Suma 4 WWA	13.22	10.90	26.65	18.65	12.00

Lorenzo et al. [2010] showed that the total content of fifteen PAHs in the chorizo sausage was between 30 and 35  $\text{g} \cdot \text{kg}^{-1}$ . In turn, Kubiak [2012] reports a very different level of PAH accumulation – from 18.5  $\mu\text{g} \cdot \text{kg}^{-1}$  in silesian sausage to 77.1  $\mu\text{g} \cdot \text{kg}^{-1}$  in dried hunter's sausage.

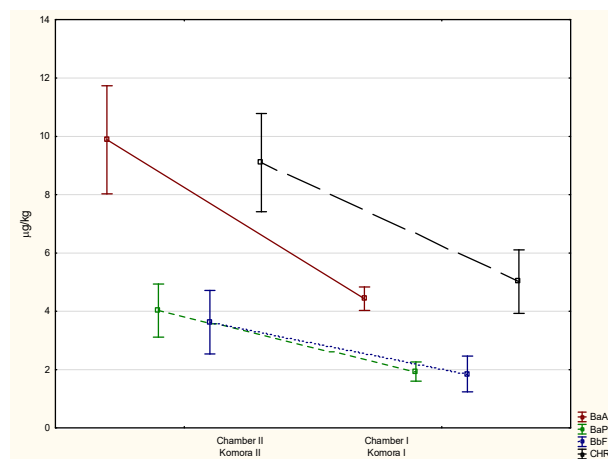
As shown by Šimko et al. [1991], smoking by the warm method, which is based on a higher temperature compared to the cold smoking method, generates significantly higher levels of polycyclic aromatic hydrocarbons in smoked meat products in the long smoking period. This is confirmed by studies by other authors [Afolabi et al. 1983, Alonge 1987, Roda et al. 1999].

centration in the wood pyrolysis chamber was about 2.5 times higher than that of sausage smoked in chamber 1 (aver 1.15  $\mu\text{g} \cdot \text{kg}^{-1}$  for B(g,h,i)P and 1.85  $\mu\text{g} \cdot \text{kg}^{-1}$  for B(b)F) (Table 4). The results of the study also indicate the changes caused by the tested factor in the benzo(j)fluorantene content. It was found that smoking at a temperature of over 600°C generated an average content of 1.6  $\mu\text{g} \cdot \text{kg}^{-1}$ , but the presence of this PAH in the smoked sausage at lower temperatures of wood pyrolysis was not identified. The pyrolysis temperature also significantly influenced the concentration of benzo(k)fluoranthene and indene(c,d)pyrene. In chamber II, the concentration of these PAHs was 1.71  $\mu\text{g} \cdot \text{kg}^{-1}$  and 1.26  $\mu\text{g} \cdot \text{kg}^{-1}$ , respectively. Half of indene(c,d)pyrene, and a reduction to 0.73  $\mu\text{g} \cdot \text{kg}^{-1}$  of benzo(k)fluorantene concentration were recorded in case of smoked sausage in chamber I. The differences found were statistically significant (Table 4). There was no statistically significant effect of the wood pyrolysis temperature on DB(ah)P content, for which the probability level was 0.069. However, it can be said that the trend of its changes was similar to the other PAHs evaluated.

B(a)P is one of the four PAHs most commonly found in food products. According to the norms [EU Regulation (UE) nr 835/2011] its maximum concentration in these products may not exceed 2  $\mu\text{g} \cdot \text{kg}^{-1}$ . The results show that B(a)P concentration in smoked sausage in chamber II was more than double that of the standard. It is worth emphasizing that lowering the smoking temperature to the parameters characteristic of chamber I resulted in a decrease in B(a)P to 1.94  $\mu\text{g} \cdot \text{kg}^{-1}$  (Fig. 3), below ML as defined in the standard.

Contamination of B(a)P in case of sausages with a similar technological process was investigated by Ledesma et al. [2015] who found that the average B(a)P content in this product was 1.60  $\mu\text{g} \cdot \text{kg}^{-1}$ .

The sum of four PAHs selected for food monitoring by the EC Commission in samples of rural sausage smoked in chamber II exceeded the Maximum Limit (12



**Fig. 3.** Influence of temperature of wood pyrolysis on the content the PAHs in rural sausage identified by the European Commission

**Rys. 3.** Wpływ temperatury pirolizy drewna na zawartość WWA określonych w wymaganiach Komisji UE w kiełbasie wiejskiej

Studies have shown that in the group of heavy PAHs that the greatest differences between the examined chambers were found for the content of benzo(g,h,i)perylene and benzo(b)fluoranthene. It was shown that their con-

$\mu\text{g} \cdot \text{kg}^{-1}$ ) and was on average  $26.65 \mu\text{g} \cdot \text{kg}^{-1}$ , with a minimum value of  $18.65 \mu\text{g} \cdot \text{kg}^{-1}$  (Table 5). Therefore, the content of these hydrocarbons in all tested samples exceeded the limit of the new standard. On the other hand, the average level of four PAHs in most smoked samples under controlled temperature also exceeded the applicable standard and amounted to  $13.22 \mu\text{g} \cdot \text{kg}^{-1}$ . The maximum limits in chamber were determined in the first place by the very high concentration of chrysene, while in chamber II by chrysene and benzo(a)anthracene (Fig. 3).

## SUMMARY

Studies have shown that running a smoking process at an average temperature of  $551.2^\circ\text{C}$  resulted in a significant reduction in the concentration of polycyclic aromatic hydrocarbons compared to their content in the article smoked at an average temperature of  $675.2^\circ\text{C}$ . The concentration of benzo(a)pyrene in smoked sausage at lower temperatures was in accordance with the applicable standards. And the sum of the four PAHs, both at higher and lower temperatures, exceeded the current recommendations.

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## **WPŁYW TEMPERATURY PIROLIZY DREWNA NA ZAWARTOŚĆ WIELOPIERŚCIENIOWYCH WĘGLOWODORÓW AROMATYCZNYCH W WYROBACH MIĘSNYCH WĘDZONYCH**

### **STRESZCZENIE**

Celem pracy była ocena zawartości wielopierścieniowych węglowodorów aromatycznych w wybranym wyrobie wędliniarskim. Materiał badawczy stanowiły próby kiełbasy wiejskiej wędzonej metodą tradycyjną w dwóch komorach, w których zróżnicowano temperaturę pirolizy drewna. Zawartość WWA w wędzonym produkcie oznaczono metodą chromatografii cieczowej HPLC. Badania wykazały, że prowadzenie procesu wędzenia w średniej temperaturze wynoszącej 551,20°C spowodowało istotne obniżenie stężenia wielopierścieniowych węglowodorów aromatycznych w porównaniu do ich zawartości w wyrobie wędzonym w temperaturze wynoszącej średnio 675,2°C. Stężenie benzo(a)pirenu w kiełbasie wędzonej w niższej temperaturze spełniało obowiązujące normy, zaś suma czterech WWA zarówno w temperaturze wyższej jak i niższej przekraczała obowiązujące zalecenia.

**Słowa kluczowe:** wędzenie, temperatura wędzenia, wędliny, WWA