POLYCYCLYC AROMATIC HYDROCARBONS AND PHENOLS IN SMOKED ZLATIBOR BACON

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The gas chromatographic analysis of the components of smoke isolated from Zlatibor bacon was performed. A complex mixture of aliphatic and aromtic hydrocarbons was obtained. The compounds were identified by a GC-MS-C combination. More than 60 compounds including alkanes, cycloparaffins, aromatic hydrocarbons and hydrocarbons with two-, three- or four-condensed rings were identified. The amount of components with condensed rings is 33,4%, of cycloparaffins 20,6% and aromatic hydrocarbons 12,6%. The phenol extraction was performed with acetone. After analysing the results of thin layer chromatography, gas chromatographic analysis was also performed. The quantitative content of following phenols in the 1 cm surface layer and sample residue was determined: phenol, guayacol, m-, and p-cresol, p-ethylphenol, 2,5-xylenol and 2,6 xylenol.

INTRODUCTION

The production of Zlatibor bacon has a long and rich tradition in the region of Mount Zlatibor. It is manufactured according to traditional technology which has been preserved and passed on from generation to generation. The product is characterised by outstanding organoleptic properties (appearance, colour, taste, aroma, etc.).

Smoking is the factor that influences the characteristics aroma and taste to the greatest degree. The comoposition of the wood and the combustion conditions are the most important factors which determine the chemical nature of the formed smoke. Although the approximate chemical composition of the individual types of trees used to produce smoke by pyrolysis is the same or very similar, there are considerable differences between soft and hard wood, between wood from various areas, etc [1]. Only hard types of wood are used as a source of heat and smoke in the production of Zlatibor bacon (beech, oak). However, besides contributing to the quality and maintaining an antioxidative and bactericidal effect, the smoke also produces undesired substances which are cancerogenic. These substances are polycyclic aromatic hydrocarbons (PAHs). Among them, 3,4-benzopyrene has been studied the most, because it is the first compound in this group to have been proved to be cancerogenic [2].

Knowing the composition of the smoke components, which represent potential consumer health hazard, is very important. As there are no such data in the literature regarding Zlatibor bacon, the goal of this study was to isolate and identifiy the polycyclic aromatic hydrocarbons formed during the process of smoking Zlatibor bacon.

EXPERIMENTAL

Zlatibor bacon was manufactured in Cajetina at the Cajetina Meat Industry Plant. The back bacon of dometstic white meaty hogs, about 6 months old, was used. The production proceeded according to the following stages-sample preparation, dry salting (21 days) and

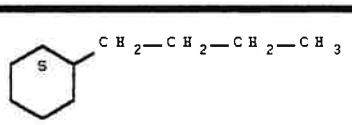
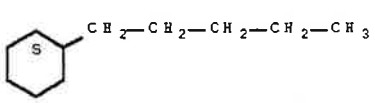
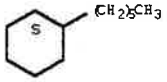
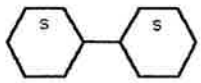
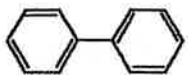
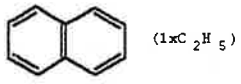
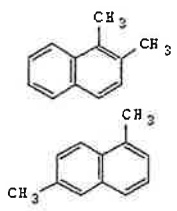
cold smoking-drying at 10-15°C (30 days). The smoked samples of bacon were ground homogenised and packed into jars. The Grimmer and Bohke method was used to isolate the polycyclic aromatic hydrocarbons[3]. GC- analysis was performed on a Varian 3400 gas chromatograph under the following conditions: column DB-5, film thickness 10 μm, lenght 30 m, ID 0,25 mm, carrier gas, N_2, detector FID, temperature program 50-310°C, 4 °/min, injector temperature 250°C, temperature of detector 300°C, integrator Spectra-Physics System I.
The compounds were identified by a GC-MS-C combination Varian 3700-MS311-A instrument, by comparing them with reference supstances and mass spectra obtained by comparison with library mass spectra from Gottintgen University [4,5].

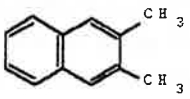
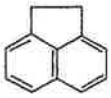
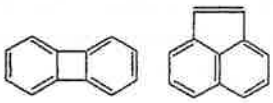
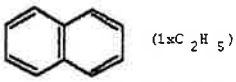
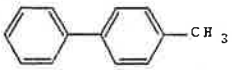
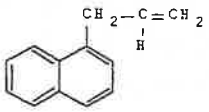
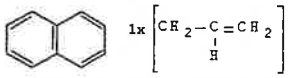
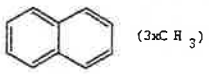
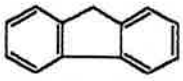
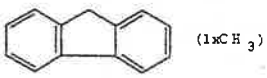
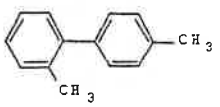
Phenols-isolation and determination
Ten ml of 0,1 mol/l NaOH are added to a well homogenized sample (about 5g) and mixed on a shaker for about 15 min at 25°C. After filtering through folded filter paper, the aqueous layer is tranferred to a separating funnel and brought to pH=1-2 by HCl solution (5mol/l). $(NH4)_2SO_4$ is added to the solution until a saturated solution is formed (about 75g of salt per 100 ml of solution), as well as 1 ml of acetone. The sample is put on a shaker for 10 min in order to extract the phenol by acetone. The acetone extract is separated from the water phase and evaporated to a volume of 0,5 ml [6].
In order to identify the phenol 2 μm of the concentrated acetone extract are transferrd to a thin layer chromatography plate (Silufol, Czechoslovakia). Toluene was used as the eluent. The spots were stained by a 2% solution of 4-aminoantipyrine and an 8% solution of ammonium persulfate in the presence of ammonia vapour. Phenol standards were also analysed. On the basis of the R_f values the phenol type was determined. In order to quantitatively determine phenol, 1 μl of the concentrated acetone extract was injected into an LXM-80 gas chromatograph under the following conditions: glass column, lenght 2m, inner diameter 3mm, Reoplex-400 phase on Polysorb-I, gas flow (helium, hydrogen and air) 30 cm^3/min, detector temperature 190°C, injector temperature 190°C, column temperature from 150°C to 200°C (at 6-9 °/min). The internal standard method was used in the GC method of quantitavely determining phenol.

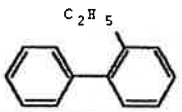
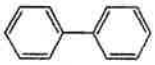
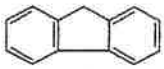
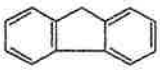
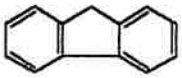
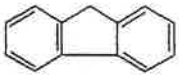
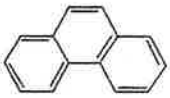
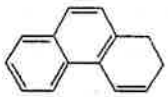
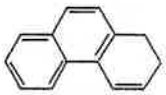
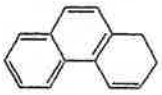
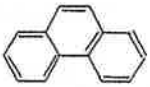
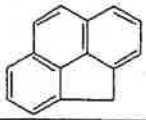
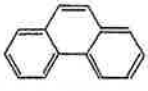
RESULTS AND DISSCUSION
More than 60 compounds consisting of alkanes, cycloparaffins, aromatic hydrocarbons and hydrocarbons with two-, three- or four-condensed rings were identified (Table 1). The major fraction (33,4%) is made up of hydrocarbons with condensed rings, 20,2%, with two-, 12,9%, with three- and 0,2% with four-condensed rings. The fraction of cycloparaffins is 20,6% and of aromatic hydrocarbons 12,6%.
Certain polycyclic aromatic compounds, i.e. those with four- to seven-condensed polycyclic aromatic hydrocarbons is 3,4-benzopyrene, for which it has been shown that it belongs to this group of compounds. However, it should be emphasized that our investigations did not show the presence of this compound in smoked Zlatibor bacon. The reason why 3,4-benzopyrene, as well as other polycyclic aromatic hydrocarbons are not formed, which are more or less hazardous to human health, is probably related to the type of smoking.
According to the investigations of some researchers, the formation of benzopyrene and other polycyclic aromatic hydrocarbons with cancerogenic properties strongly depends on the smoking temperature and type of wood. Softwood (fir, pine) forms 1.5 to 4.5 times more 3,4-benzopyrene in regard to hardwood (beech, oak)[7]. According to the findings of other researchers, these compounds are produced by the pyrolysis of wood at temperatures above 400°C, by so-called hot smoking. Below this temperature, i.e. when cold smoking is

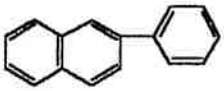
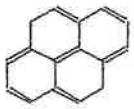
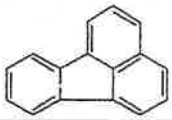
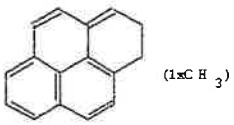
applied, the danger of the formation of these compounds is minimal [8-14]. In the production of Zlatibor bacon the procedure of cold smoking was applied and hardwood tree types were used which would explain why minimal amounts of polycyclic aromatic hydrocarbons were formed and that the potentially most dengerous ones, i.e. 3,4-benzopyrene and its derivatives were not among them.

Table 1. The compounds isolated form smoked Zlatibor bacon identified by GC-MS-C.

PEAK	NAME	STRUCTURAL FORMULA	Molec. weight	% Area
1.	CYCLOHEXYL BUTANE-1		140	0.14
2.	UNDECANE	$C_{11}H_{24}$	156	0.16
3.	CYCLOHEXYL PENTANE-1		154	0.29
4.	DODECANE	$C_{12}H_{26}$	170	0.85
5.	CYCLOHEXYL HEXANE-1		168	0.6
6.	BICYCLOHEXYL		166	19.0
7.	METHYLBICYCLOHEXYL		180	0.08
8.	METHYLBICYCLOHEXYL		180	0.15
9.	METHYLBICYCLOHEXYL		180	0.4
10.	1,1'-BIPHENYL OR PHENYLBENZENE		154	1.69
11.	1-ETHYLNAPHTALENE OR 2-ETHYLNAPHTALENE (DIMETHYLNAPHTALENE)		156	0.96
12.	1,2-DIMETHYLNAPHTALENE OR 1,6-DIMETHYLNAPHTALENE		156	0.28

13.	2,3-DIMETHYLNAPHTALENE		156	1.03
14.	ACENAPHTHENE (1,2,-dihydroacenaphthene)		154	0.97
15.	BIPHENYLENE OR ACENAPHTHYLENE		152	3.72
16.	ETHYLNAPHTHALENE		156	0.5
17.	4-METHYL BIPHENYL		168	0.74
18.	METHYL BIPHENYL		168	0.6
19.	?	?	168	0.2
20.	METHYL BIPHENYL		168	4.5
21.	1-(2-PROPENYL)-NAPHTALENE		168	0.8
22.	NAPHTHYL PROPENE		168	1.4
23.	TRIMETHYL NAPHTHALENE (1,4,5-trimethyl naphthalene)		170	1.6
24.	FLUORENE		166	3.5
25.	2-METHYL FLUORENE OR 4-METHYL FLUORENE		182	1.7
26.	2.4'-DIMETHYL BIPHENYL		182	1.6

27.	2-ETHYL BIPHENYL		182	2.0
28.	ETHYL BIPHENYL		182	0.5
29.	2-METHYL FLUORENE OR 9-METHYL FLUORENE		180	1.2
30.	DIMETHYL FLUORENE		194	1.1
31.	DIMETHYL FLUORENE		194	2.3
32.	DIMETHYL FLUORENE		194	0.6
33.	PHENANTHRENE		178	8.0
34.	DIHYDRO METHYL PHENANTHRENE		194	2.3
35.	DIHYDRO METHYL PHENANTHRENE		194	0.7
36.	DIHYDRO METHYL PHENANTHRENE		194	0.3
37.	METHYL PHENANTHRENE		192	0.6
38.	4H-CYCLOPENTA(def) PHENANTHRENE		190	0.5
39.	METHYL PHENANTHRENE OR METHYL ANTRACENE		192	0.5
40.	METHYL PALMITATE	$CH_3OOC(CH_2)_{14}CH_3$	270	0.4

41.	2-PHENYL NAPHTALENE		204	0.4
42.	4,5,9,10-TETRAHYDROPYRENE		206	0.1
43.	PYRENE		202	0.2
44.	FLUORANTHENE		202	0.08
45.	?	?	202	0.06
46.	METHYL DIHYDROPYRENE		218	0.7
47.	METHYL STEARATE	$CH_3OOC(CH_2)_{16}CH_3$	298	0.5
48.	DOCOSANE	$C_{22}H_{46}$	310	0.03
49.	TRICOSANE	$C_{23}H_{48}$	324	0.02
50.	TETRACOSANE	$C_{24}H_{50}$	338	0.04
51.	PENTACOSANE	$C_{25}H_{52}$	352	0.06
52.	HEXACOSANE	$C_{26}H_{54}$	366	0.08
53.	HEPTACOSANE	$C_{27}H_{56}$	380	0.1
54.	OCTACOSANE	$C_{28}H_{58}$	394	0.1
55.	NONACOSANE	$C_{29}H_{60}$	408	0.08
56.	TRIACONTANE	$C_{30}H_{62}$	422	0.07
57.	UNTRIACONTANE	$C_{31}H_{64}$	436	0.06
58.	DOTRIACONTANE	$C_{32}H_{66}$	450	0.04
59.	CHOLESTEROL	$C_{27}H_{46}O$	386	0.02
60.	TRITRIACONTANE	$C_{33}H_{68}$	464	0.02
61.	TETRATRIACONTANE	$C_{34}H_{70}$	478	0.01
62.	PENTATRIACONTANE	$C_{35}H_{72}$	492	tr.
63.	HEXATRIACONTANE	$C_{36}H_{74}$	516	tr.

* The number of CH_3- and C_2H_5-groups is established for certain but their exact position is unknown.

Phenolic components are responsible for the aroma of smoke and their antimicrobial action contributes to product longevity. Phenols also show antioxidative action. They stabilize fats due to their role of free radical acceptors by wich they stop oxidation aiready in the initiation

stage[15]. Guayacol, 4-methyl guayacol and siringol have the greatest influence on the formation of the aroma of smoked products. The smell of smoked products is attributed to siringol, while the taste originates from guayacol and its derivatives[16]. According to the findings of Issenberg et al[17], the phenol concentration in smoked meat ranges from 0,12 mg/100 g (in the centre) to 3,70 mg/100 g (on the surface).

Investigations of electrostatically smoked bacon have shown that there is relatively weak phenol diffusion into the bacon interior. Of the total amount of phenol 27% was found in the skin, 5.7% in the fatty part, in the outer meaty part exposed to smoking 48% and in the rest of the meaty part of bacon 18.6% [18].

Phenols, carbonyls and carbonic acids are mostly obtained by the pyrolysis of wood at 600°C. Variations in the temperature also change the composition of the phenol compounds. The main components obtained at lower temperatures are guayacol, acetovanillone and acetosiringone, while siringol is predominantly obtained at 760°C, the temperature of wood combustion[19].

Although 58 phenolic components have been identified in smoke, very little is still known about their formation. There are also no precise answers which active compounds form the aroma of smoked products [20].

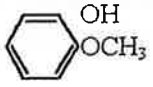
The phenol content was determined in the final product, i.e. after the 30th day of smoking. Two deskinned slabs of bacon were used for the investigations. The phenol content was determined in the outer, meaty part of the bacon of 1 cm thickness and in the rest of the sample. Parallel samples were taken from both pieces of bacon, so the final result presents the average of four determinations.

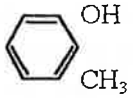
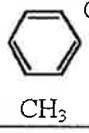
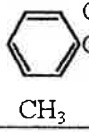
After extraction by the Korenman method[6], the phenols were analysed by gas chromatograpchy. The internal standard method was applied for quantitative determinations. The phenol content in the surface meaty layer of bacon at 1 cm thickness and in the rest of the bacon is presented in Table 2.

It is noticeable that there is a higher phenol content in the surface part of the bacon than in the rest. The phenols are initially concentrated on the bacon surface. In the course of further smoking, the water content in bacon decreases, consequently making the surface layer more compact, which slows the diffusion into the interior. Phenol and guayacol show the greatest differences in content at the surface and in the interior (about ten times). Of the first four major compounds, phenol penetrated into the bacon surface the least (about 8% in regard to the content in the surface layer, which is in agreement with the findings of Knowles et al[18]. As opposed to phenol, m-and p-cresols penetrated into the most during smoking (about 59% and 66%, respectively, in regard to the content determined in the bacon surface layer).

The determined amounts of phenol were somewhat lower compared to the findings of other researchers, according to which the average phenol content in smoked products ranges form 0,12 mg/100g in the centre to 3,70 mg/100g on the product surface[17, 18].

Table 2. Phenol content in the surface layer and rest of the sample of smoked Zlatibor bacon, mg/100g bacon).

Compound	structural formula	Surface layer of 1 cm thickness	Rest of the sample
Phenol (Hidroxybenzene)		1.50	0.12
guayacol (2-metoxyphenol)		1.56	0.22

m-kresole (3-metylphenol)		1.58	0.88
p-kresole (4-metylphenol)		1.33	0.88
p-ethylphenol		0.28	traces
2,5-xylenol (2,5-dimethylphenol)		0.31	0.07
2,6-xylenol (2,6-dimethylphenol)		0.12	traces

The technology of cold smoke is applied in the production of Zlatibor bacon, which is probably one of the reasons why such small amounts of phenol are formed. Cold smoking also influences the formation of the major components found in Zlatibor bacon (phenol, guyacol, m- and p-cresol). This was confirmed by literature data according to which eugenol, siringaledhyde and acetosiringol are the major phenols found in meat products smoked in hot smoke, while phenol, guayacol and m-cresol are the major phenols of cold-smoked meat products [1, 16].

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