

IDENTIFICATION OF PHENOLIC COMPOUNDS IN A POLISH SMOKE FLAVOUR EXTRACT

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A Polish smoke flavour extract was analyzed with the aim to identify its phenolic compounds. All phenols liberated from an alkaline millieu at pH = 11 were extracted with diethyl ether and analyzed by means of a combined gas chromatography - mass spectrometry technique.

Best separation conditions were obtained when using PTGC with Cromosorb W-DMSS glass columns, coated with XE-60 or OV-225 stationary phases. The analyses were run on a Du Pont model no. 21-492 mass spectrometer equipped with Varian model no. 2700 gas chromatograph and a data system.

Basing on the obtained analytical results altogether 39 phenolic and 4 non-phenolic compounds were identified. The identification was based on the respective mass spectra and retention volumes, which were compared with data gathered for standard substances and/or with data published elsewhere.

IDENTIFICATION DES COMPOSÉS PHÉNOLIQUES DANS LE PRODUIT POLONAIS DE LA FUMÉE "LIQUIDE"

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Le produit polonais de la fumée "liquide" a été étudié pour identifier des composés phénoliques. Phénols ont été libérés en milieu alcalin / pH = 11/ par extraction d'oxyde d'éthyle et puis analyses par la méthode chromatographie en phase gazeuse et spectrométrie de masse combinée.

Les analyses ont été effectuées avec spectromètre de masse Du Pont, modèle No 21-492 équipé en chromatographie en phase gazeuse Varian, modèle No 2700 et en système automatique transformation les résultats.

Les meilleures séparations de constituants ont été obtenues dans les conditions expérimentales suivantes: colonne chromatographique avec XE-60 sur OV-225 sur chromosorb W-DMCS, température programmée.

En résultat des recherches effectuées on a identifié 39 composés phénoliques et 4 composants non phénoliques. Identification a été fait par comparaison des spectres de masse et de volumes de rétention de composés étudiés avec les substances purs et références bibliographiques.

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DIE IDENTIFIKATION VON PHENOLISCHEN VERBINDUNGEN IN EINEM POLNISCHEN RÄUCHERRAUCHEXTRAKT

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Die phenolischen Verbindungen, anwesend in einem polnischen Räucherrauchextrakt, wurden einer Identifikationsanalyse untergeben. Für diesen Zweck waren alle Phenole, die sich aus einem alkalischen Millieu bei pH=11 befreien, mit Diethylether extrahiert und in einem kombinierten Gaschromatographen-Massenspektrometer analysiert.

Die besten Separationsbedingungen erhielt man mit PTGC auf Chromosorb W-DMCS, bedeckt mit XE-60 oder OV-225. Die Analysen wurden auf einem Du Pont Model No 21-492 Massenspektrometer, versehen mit einem Varian Model No 2700 Gaschromatographen und einem Datasystem durchgeführt.

Auf Grund der Resultate dieser Analysen wurden insgesamt 39 phenolische und 4 nichtphenolische Verbindungen identifiziert. Die Identifikation beruhte auf dem Vergleich der erhaltenen Massenspektren und Retentionsvolumen mit denen, die für Standardsubstanzen bekommen worden waren, wie auch mit Daten, die in Fachliteratur publiziert worden sind.

## ИДЕНТИФИКАЦИЯ ФЕНОЛОВЫХ СОЕДИНЕНИЙ В ПОЛЬСКОМ ПРЕПАРАТЕ КОПТИЛЬНОГО ДЫМА

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Польский препарат коптильного дыма анализировано с целью идентификации его феноловых соединений. Фенолы освобождённые от щелочной среды для pH = II экстрагировано этиловым эфиром и анализировано с помощью газового хроматографа соединенного с масс-спектрометром.

Найлучшие разделения получено применения программированную температуру на хромосорбе W - DMCS с нанесенной неподвижной фазой XE-60 или OV- 225.

Анализы исполнено на масс-спектрометре фирмы Du Pont модель № 21-492 снабженным газовым хроматографом фирмы Varian модель № 2700, а также системой обработки данных.

Опираясь на полученных результатах идентифицировано 39 феноловых соединений и 4 нефеноловые соединения.

Идентификацию проведено на массовых спектрах и удерживаемых объёмах, которые подвергались сравнению со стандартными веществами а также с литературными данными.

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The contribution of phenolic compounds to the overall sensory and preserving features of flavourings derived from wood smoke is widely known. However, the role of individual compounds in this overall effect is poorly recognized, among others also due to gaps existing in our knowledge concerning the chemical identity of these compounds.

There are, of course, data available in literature<sup>1-9</sup>, which pertain to some of these compounds, and in particular data published by Knowles et al.<sup>8,9</sup> deal with a smoke flavouring obtained by a method identical with that employed in this paper<sup>11</sup>. In that particular product altogether 18 phenolic compounds were identified. As this number is smaller than the number of peaks found during routine GLC analyses of said product, it was thought that further investigations can give more information on the constitution of this group of compounds.

#### EXPERIMENTAL

Isolation of phenols :

The tarry fraction from a smoke condensate obtained according to a patented method<sup>12</sup> was dissolved in a 5% NaOH solution in water, using the tar to the NaOH solution in a ratio as 1 : 3 w/w. This solution was extracted 5 times with diethyl ether using the ether volume to the volume of the water fraction in a ratio of 1:1. The ether extracts were discarded and the aqueous fraction acidified with a 5% solution of sulphuric acid until a pH = 11 was reached. Thereafter this solution was again extracted 5 times with diethyl ether in aliquots equal in volume to the volume of the aqueous fraction. The ether extracts were collected and combined and thereafter washed twice with distilled water. The ratio of water to ether was in this case as 1:3 v/v. After discarding the water layer the ether extract was desiccated over anhydrous sodium sulphate overnight. Thereafter it was filtered through a Whatman no.3 filter and the solvent was distilled off. The bulk amount of ether was distilled off under normal pressure, however, in the final stage vacuum was applied / down to 10 mm Hg / and the bath temperature was raised up to 80°C. The remainder after distillation was then weighed and dissolved in acetone so as to obtain a 10% w/v solution.

Gas chromatography :

For choosing the best conditions of chromatographic separation of the above mentioned solution of phenols in acetone, a preliminary test was run on a variety of columns. The results are gathered in table no.1.

Basing on these results the following two sets of conditions were taken as optimal to run the GLC analyses :

1/ Glass column 1.8 m in length, 0.2 mm ID, packed with chromosorb W-DMCS of 100/120 mesh grade, coated with 6% w/w of XE-60. Programmed temperature in the column oven within the range 40-230°C raised at a rate of 2°C/min. Carrier gas: helium at a rate of 40 ml/min, stream splitter with a ratio of 1:1, flame ionization detector thermostated at 250°C.

2/ Glass column 1.8 m in length, 0.2 mm ID, packed with chromosorb W-DMCS of 100/120 mesh grade, coated with 6% w/w OV-225. Programmed temperature in the column oven within the range 100-240°C raised at a rate of 1°C/min. Carrier gas: helium at a rate of 40 ml/min, stream splitter with a ratio of 1:1, flame ionization detector thermostated at 250°C.

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The analyses were run on a Varian model no. 2700 gas chromatograph linked in line to the mass spectrometer.

### Mass spectrometry:

The effluents from the GCL columns were introduced through a stream splitter and an interconnecting glass tube onto a DuPont model no. 21-492 B mass spectrometer equipped with a data system model no. 21-094 B. The stream splitter, the interconnecting tube and the jest separator were all glass made.

The following operation conditions were used :

- temperature of connecting line and separator: 250°C
- temperature of the ion source : 250°C
- ionization energy : 70 eV
- number of scans per minute: 6
- scanning speed : 2 seconds/decade
- scanning range : 2 - 1000 amu
- resolving power: 1:1000 / 10% valley /
- acquisition range: 27 - 600 amu
- acquisition rate : 4
- threshold : 1

### Discussion of results

The separation achieved on the GLC columns is shown on fig. no.1 for XE-60, and on fig. no.2 for OV-225, respectively. Identification of the individual compounds was done by comparing the mass spectra either with spectra of known substances and/or with data kept in the library of the data system, or by comparing them with data published in literature. In all cases also the retention volumes were taken in account as an additional criterion for identity. The results of the performed identification are given in table no. 2.

All the compounds listed in table no.2 can be in general split up into the following four groups: alkylphenols, alkylmethoxyphenols, alkyldimethoxyphenols, and others.

In the first group /alkylphenols/ we were able to confirm the presence of the following substances already reported by Knowles<sup>8,9</sup> phenol, 3-; 4-; 2-methylphenol. Besides that we found the following additional substances, namely: 2,5-; 2,3-; 3,5-; 3,4-dimethylphenol, 2,4,6-trimethylphenol, 4-ethylphenol, 4-vinylphenol, 4-isopropylphenol. In this number the presence of 2,5-dimethylphenol and 4-isopropylphenol is reported here for the first time. On the contrary, we could not confirm the presence of 2,6-; 2,4-dimethylphenol, 2-; 3-ethylphenol, 3-ethyl 5-methylphenol, tert-butylphenol, which substances were reported elsewhere<sup>3,4,5,7</sup>.

In the second group / alkylmethoxyphenols / we confirmed already found by Knowles<sup>8,9</sup>, 2-methoxyphenol, 2-methoxy 4-methylphenol, 2-methoxy 4-vinylphenol, 2-methoxy 4-ethylphenol, 2-methoxy 4-allylphenol, 2-methoxy 4-/cis and trans/ propenylphenol, 2-methoxy 4-propylphenol. Besides that we found, not yet reported, 4-methoxyphenol. On the contrary, we could not confirm the presence of 3-methoxyphenol, 2-methoxy 4-isopropylphenol, which were reported by Hruza et. al.<sup>7</sup>

In the third group / alkyldimethoxyphenols / we confirmed already found by Knowles<sup>8,9</sup>, 2,6-dimethoxyphenol, 2,6-dimethoxy 4-methylphenol, 2,6-dimethoxy 4-ethylphenol, 2,6-dimethoxy 4-allylphenol, 2,6-dimethoxy 4-cispropenylphenol.

On the contrary, we could not confirm the presence of 2,6-dimethoxy 4-vinylphenol, 2,6-dimethoxy 4-transpropenylphenol, which substances were reported elsewhere<sup>5,6,9</sup>. The presence of 2,6-dimethoxy 4-isobutylphenol is reported here for the first time.

In the last group / others / we were able to confirm the presence of 3,5-dimethoxy 4-hydroxybenzaldehyde already reported by Knowles<sup>8,9</sup>. In this group the presence of: 3,5-dimethoxybenzaldehyde, pyrocatechol, 3-methoxy 4-hydroxybenzoic acids, 3-methyl-pyrocatechol, 4-vinylbenzaldehyde, 5-methylpyrocatechol, 3-methoxy 4-/propan 2-one/ phenol, 1-naphol, 2-methoxy 4-acetic acid phenol, 1-allylnaphthalen, is reported here the first time.

Besides that, there are several substances in minor quantities, which could not be identified for certain. These substances are listed in table no.3 as "unknown".

Concluding one can say that in the analysed phenolic fraction altogether 43 individual compounds could be identified and for 12 additional substances the molecular weight could be given although their chemical composition remains obscure. This is almost twice as much as was found by Knowles in a similar product. Among the identified compounds there are 13 of them, which are reported in this paper for the first time. It is hoped that the results given in this paper can be useful in further research concerning the sensory, antioxidative, bactericidal and biological activity of the flavouring in question.

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Table no.1

Results of the preliminary test for choosing best separation conditions on GC columns

No.	Stationary phases	Coated	Support /100-120 mesh /	Temperature /°C/	Remarks
1	Silicone OV-3	5	Gas Chrom Q	PTGC 100°-250°C, 2°C/min.	poor separation, short Rt
2	Silicone OV-17	5	Diatomite CQ	PTGC 100°-250°C, 2°C/min.	" " short Rt
3	Silicone OV-225	6	Chromosorb W-DMCS	PTGC 100°-240°C, 1°C/min.	very good separation
4	Silicone SE-52	5	Diatomite CQ	PTGC 100°-220°C, 2°C/min.	poor separation, short Rt
5	OS-138	3	Chromosorb G-DMCS	205°C	poor sep.; long Rt
6	Silicone XF-1152	7	Chromosorb W-DMCS	PTGC 100°-175°C, 2°C/min.	good sep.; column bleeding
7	Silicone XE-60	6	Chromosorb W-DMCS	PTGC 40°-230°C, 2°C/min.	very good sep.; short Rt
8	Silicone AN-600	7	Gas Chrom Q	PTGC, 80°-250°C, 2°C/min.	good sep.; short Rt
9	Apolar 10C	7	Anakrom SD	180°C	poor sep.
8	N G A	7	Chromosorb W-DMCS	180°C	good sep.; long Rt
9	N G A + Bentonite	3+1	Chromosorb W-DMCS	180°C	poor sep.; long Rt
10	P E G A	10	Diatomite C-AW	180°C	good sep.; long Rt
11	P E G A + H <sub>3</sub> PO <sub>4</sub>	10+1	Chromosorb W-AW	PTGC 120-200°C, 2°C/min.	good sep.; column bleeding
12	B D S	10	Diatomite C-AW	180°C	good sep.; long Rt
13	D E G A	10	Diatomite C-AW	180°C	good sep.; long Rt
14	Carbowax 20M+TPA	7	Chromosorb W-AW	PTGC 145-240°C, 2°C/min.	good sep.; column bleeding
15	FFAP	5	Chromosorb W-HP	PTGC 150-240°C, 2°C/min.	good sep.
16	SP - 1000	5	Chromosorb G-HP	183°C	poor sep.

Results of identification

Table no.2

Peak No	Identity	Mol. wt.	Relative retention volume		Reported in literature
			XE - 60	OV-225	
1	3-Methyl 2-cyclopenten-1-one	112	0,598	0,387	2 + 9
2	2-Methoxyphenol	124	0,610	0,416	2 + 9
3	1,2-Dimethoxybenzene	138	0,475	0,475	3,7
4	Phenol	94	0,682	0,450	2 + 9
5	2-Methylphenol	108	0,711	0,493	5 + 8
6	2-Methoxy 4-methylphenol	138	0,727	0,557	2 + 9
7	3-Methylphenol	108	0,777	0,611	3 + 9
	4-Methylphenol	108			3 + 9
8	2,5-Diisopropylphenol	122	0,797		7
9	2-Methoxy 4-Ethylphenol	152	0,816	0,679	2 + 9
10	2,3-Dimethylphenol	122	0,846	0,712	7
	Unknown	154			
11	3,5-Dimethylphenol	122	0,867	0,772	Not reported
	4-Ethylphenol	122			7
11	2,4,6-Trimethylphenol	152		0,750	7
12	2-Methoxy 4-propylphenol	166	0,905	0,812	2 + 9
	3,4-Dimethoxyphenol	122			3
13	2-Methoxy 4-allylphenol	164	0,915	0,837	2 + 9
	2-Methoxy 4-vinylphenol	150			2 + 8
	Unknown	168			
13	4-Vinylphenol	120		0,904	7
14	4-Isopropylphenol	136	0,950		Not reported
15	Unknown	168	0,958	0,938	

Peak No	Identity	Mol. wt.	Relative retention volume x/		Reported in literature
			XE - 60	OV - 225	
16	2-Methoxy 4-cis-propenylphenol	164	0,981	0,956	3 + 9
17	2,6-Dimethoxyphenol	154	1,000	1,000	1 + 9
18	4-Methoxyphenol	124	1,036		Not reported
19	2-Methoxy 4-transpropenylphenol	164	1,051	1,075	4 + 9
20	3,5-Dimethoxybenzaldehyde	134	1,067		Not reported
	Unknown	162			
21	2,6-Dimethoxy 4-methylphenol	168	1,086	1,152	2 + 9
	Pyrocatechol	110		1,122	Not reported
22	3-Methoxy 4-hydroxybenzoic acids	168	1,106		Not reported
23	Unknown	138	1,115		
24	Unknown	138	1,124		
	Unknown	134			
	Unknown	168			
25	3-Methylpyrocatechol	124	1,139	1,217	Not reported
	4-Vinylbenzaldehyde	132			Not reported
26	2,6-Dimethoxy 4-Ethylphenol	182	1,152	1,267	2 + 9
27	Vanillin	152	1,165		2,3,5,6
28	5-Methylpyrocatechol	124	1,191		Not reported
29	2,6-Dimethoxy 4-propylphenol	196	1,217	1,387	3,7,8
30	2,6-Dimethoxy 4-allylophenol	194	1,227	1,415	5 + 8
31	3,4-Dimethoxyacetophenone	180	1,243	1,520	5
32	Unknown	256	1,255		
	Unknown	242			
33	Unknown	194	1,287		
34	3-Methoxy 4-propan 2-onephenol	180	1,302		Not reported
35	2,6-Dimethoxy 4-cis-propenylphenol	194	1,354	1,642	5 + 9
36	1-Naph <sup>t</sup> ol	144	1,372		Not reported
37	2-Methoxy 4-acetic acid phenol	182	1,442	1,771	Not reported
38	3,5-Dimethoxy 4-hydroxybenzaldehyde	182	1,471	1,841	5,6,9
39	Unknown	218	1,495		
	Unknown	246			
40	Unknown	204	1,518		
41	2,6-Dimethoxy 4-isobutylphenol	210	1,570	2,026	Not reported
42	1-Allylnaphthalen	168	1,696		Not reported

x/ data for the relative retention volume are based  
on the retention volume of 2,6-Dimethoxyphenol.