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Kurze Mitteilung — Communication brève

Polycyclic Aromatic Hydrocarbons in Dried Mushrooms

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Introduction

As the polycyclic aromatic hydrocarbons (PAH) represent an important group of potential carcinogens, their occurrence in foodstuffs has been extensively studied (1). Various amounts of PAH's have been found in vegetables (2), smoke flavours (3), herbs and spices (3), coffee (3, 4), tea (3, 5), vegetable oil (3, 6), grilled meat (7), smoked fish (8, 9), smoked meat (10) and smoked cheese (11). Numerous analytical methods have been published for the analysis of PAH's (12).

In this laboratory, routine survey of benzo[a]pyrene (BaP) and benzo[e]pyrene (BeP) in meat is done according to the method of *Grimmer and Böhnke* (9), with some modifications. The lack of informations about dried mushrooms led us to test the applicability of the method to that matrix. The result of this study is reported below.

Experimental

Chemicals

Cyclohexane RPE-ACS (Carlo Erba).

Methanol p. a. (Merck).

Potassium hydroxide p. a. (Merck).

Silica gel (Merck 7734), dried 4 hours at 360 °C and deactivated with 8% distilled water.

Benzo[a]pyrene (BCR).

Benzo[e]pyrene (BCR).

PAH standard mix (Supelco 4-8905).

Naphtalene, Phenanthrene, Chrysene, Picene (Fluka).

Water, distilled.

A blank was run with each series of analyses to check the purity of the reagents.

Gas Chromatography/Mass Spectrometry

A Hewlett-Packard 5790 gas chromatograph coupled to a Hewlett-Packard 5970 mass detector was used throughout this study. The mass detector is driven by a Vector-1 interface and data system (Teknivent, St. Louis, MO., USA). Column: DB-1, 30 m, 0.32 mm ID, $df = 0.25 \mu\text{m}$ (J + W Scientific, Folsom, CA, USA).

Carrier gas: Helium at 140 kPa (constant pressure).

On-column injector: OCI-3 (SGE, Ringwood, Australia).

Temperature program: 75 °C to 320 °C at 10°/min rate then hold 10 minutes.

Sampling

The samples have been purchased on the local market, most of them were imported from the Middle or Far East.

Saponification and extraction

The procedure is largely adapted from the one published by *Grimmer and Böhnke* (9).

5 g of finely ground sample are introduced into a 250 ml roundbottomed flask and mixed with 70 ml of methanolic 2 m KOH. The resulting suspension is then refluxed for 3 hours. 70 ml of cyclohexane are added through the condenser and reflux is continued for 15 minutes. The mixture is then transferred, while hot, to a 250 ml separatory funnel. The flask and the condenser are rinsed with 30 ml of methanol:water 9:1 and the rinsing solution is added to the funnel. After shaking the phases are allowed to separate. The methanolic phase is transferred to another funnel and extracted with 50 ml cyclohexane. The methanolic phase is discarded. The combined cyclohexane extracts are washed with 50 ml of distilled water. The cyclohexane extract is then carefully concentrated to about 1 ml on a rotary evaporator. The DMF/cyclohexane partition step described in the original method, used for meat, is not necessary for dried mushrooms.

Silica gel clean-up

For an extensive description of this clean-up see *Larsson* (8). The final volume is adjusted to 1 ml.

Capillary GC-MS

2 μl of the extract or the standard solutions are injected in the on-column mode. The standard solutions are prepared in cyclohexane according to classical

methods. The PAH's are identified by their mass spectra and their retention indices. The retention indices have been calculated using the method proposed by Lee (13, 14, 15). For the quantitation of BeP and BaP, the detector was used in single-ion mode, the ions at m/e 125, 126, 250 and 252 being used for this purpose. For the identification of the various PAH's the detector was used in total ion mode, i. e. from m/e 40 to m/e 350.

Detection limit and recovery

The detection limit for the benzopyrenes is 20 pg in the single ion mode. The recovery for BaP and BeP has been measured using spiked samples containing no initial detectable amounts of BaP and BeP. The mean recovery for the benzopyrenes was $82 \pm 7\%$.

Results and discussion

23 samples of dried mushrooms have been analysed for their contents in BaP and BeP. The results are summarized in table 1.

Table 1. BaP and BeP contents of dried mushrooms (in mg/kg)

Sample Nb ¹	BaP	BeP
1	0.2	0.3
2	n. d. ²	n. d.
3	n. d.	n. d.
4	n. d.	n. d.
5	n. d.	n. d.
6	n. d.	n. d.
7	0.32	0.19
8	n. d.	n. d.
9	0.10	0.06
10	0.04	0.03
11	n. d.	n. d.
12	0.46	0.27
13	0.35	0.21
14	0.27	0.16
15	0.02	0.06
16	0.03	0.02
17	0.04	0.02
18	0.07	0.05
19	0.07	0.05
20	0.05	0.03
21	0.04	0.02
22	0.02	0.01
23	0.05	0.03

¹ Samples 1 to 14: *Auricularia* sp., samples 15 to 23: *Morchella* sp.

² Not detected ($< 1 \mu\text{g}/\text{kg}$).

For some samples a more complete determination of the PAH's has been done. Up to 26 PAH's have been identified, some of them have been quantified. A typical chromatogram is shown in Figure 1 and the results are summarized in table 2.

Table 2. Polycyclic aromatic hydrocarbons in dried mushrooms (in mg/kg)

Peak Nb	Hydrocarbon (PAH)	RI ¹	A	B	Sample C	D	E
1	Biphenyl	233.94	a ²	- ³	-	-	-
2	Acenaphthylene	245.48	0.4 ⁴	-	-	-	-
3	Trimethylenaphthalene	267.18	a	-	-	-	-
4	Fluorene	268.85	0.7	-	-	-	-
5	Phenanthrene	300.00	5.1	0.2	0.3	0.3	a
6	Anthracene	301.69	1.0	-	-	-	a
7	Methylphenanthrene	320.18	-	-	-	a	a
8	Methylanthracene	324.18	-	-	-	a	a
9	2-Phenyl-naphthalene	331.59	a	-	-	a	a
10	Dimethylphenanthrene	338.85	-	-	-	a	-
11	Fluoranthene	345.15	1.9	0.07	0.1	0.04	a
12	Acephenanthrylene	348.38	-	-	-	-	a
13	Pyrene	352.39	2.0	0.06	0.1	0.04	a
14	MW 218	354.55	-	-	-	-	a
	MW 218	356.55	-	-	-	-	a
	MW 218	359.17	-	-	-	-	a
	MW 218	362.40	-	-	-	-	a
15	Benzo[a]fluorene	367.03	-	-	-	a	a
16	Benzo[b]fluorene	369.65	-	-	-	a	a
17	Methylpyrene	373.65	-	-	-	a	a
18	Methylfluoranthene	374.73	-	-	-	a	a
19	Benzo[ghi]fluoranthene	391.22	-	-	-	a	a
20	Cyclopenta[cd]pyrene	397.84	-	-	-	-	a
21	Benzo[a]anthracene	398.92	0.7	-	-	a	a
22	Chrysene	400.00	0.4	-	-	-	a
23	Benzo[fluoranthene]	444.14	0.3	-	-	-	a
24	Benzo[e]pyrene	453.51	0.09	-	-	-	0.19
25	Benzo[a]pyrene	455.14	0.13	-	-	-	0.32
26	Indeno[1,2,3-cd]pyrene	495.14	-	-	-	-	a
27	Benzo[ghi]perylene	>500.0	-	-	-	-	a

¹ Retention indices calculated according to Lee (13)

² Identified, not quantified

³ Not detected (< 0.04 mg/kg)

⁴ mg/kg

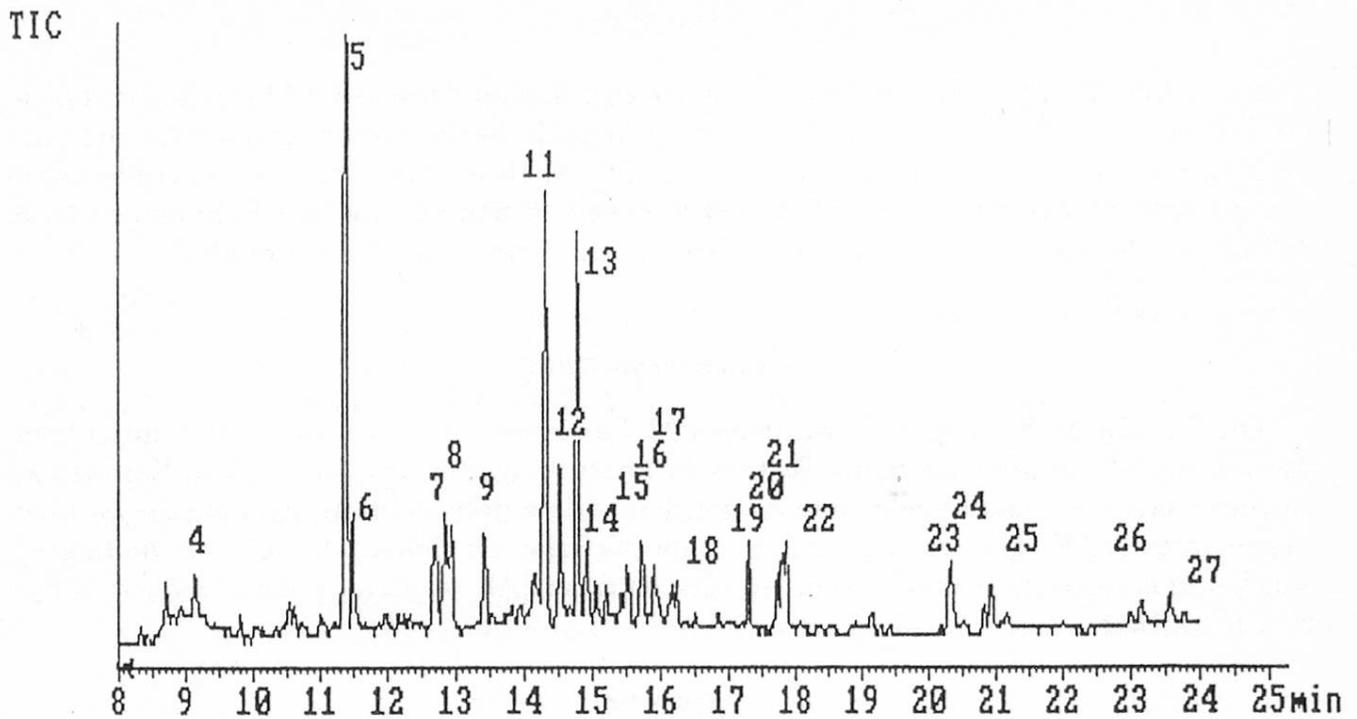


Fig. 1. Total ion chromatogram (40 to 350 amu) of an extract of *Auricularia* sp. GC conditions: see experimental part. Peak identification: see table 2

In order to investigate the intake of BaP by the consumer, the following test was applied to a sample of *Auricularia* with high BaP content.

A 5 grams portion of dry sample was soaked overnight in 125 ml of distilled water, a second 5 grams portion was soaked in 125 ml of milk (a common practice for rehydrating dry mushrooms in Switzerland). The two samples were then filtered and the mushrooms analyzed for their BeP and BaP contents. The results are summarized in Table 3.

Table 3. BaP and BeP contents (mg/kg on dry weight basis) in *Auricularia* sp. before and after overnight soaking in water and in milk

Sample	BaP	BeP
Dry sample	0.27	0.16
Soaked in distilled water	0.14	0.10
Soaked in milk	0.07	0.05

It appears that if the mushrooms are soaked in milk, 75% of the BaP can be removed.

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Summary

23 samples of dried mushrooms (*Auricularia auricula-judae* Lin. and *Morchella esculenta* Pers. ex Fr.) have been analysed for their contents in benzopyrenes and for the presence of other polycyclic aromatic hydrocarbons (PAH). Analyses have been performed by capillary gas chromatography/mass spectrometry. Levels of Benzo[a]pyrene (BaP) ranged up to 460 $\mu\text{g}/\text{kg}$. 26 PAH's have been identified in the extract of some samples.

Zusammenfassung

Die Gehalte an Benzopyrene wurden in 23 Trockenpilzproben (*Auricularia auricula-judae* Lin. und *Morchella esculenta* Pers. ex Fr.) bestimmt. Bei Anwendung von Kapillargaschromatographie/Massenspektrometrie wurden andere polycyclische aromatische Kohlenwasserstoffe (PAK) gleichzeitig erfasst. Hohe Gehalte an Benzo[a]pyren, bis 460 $\mu\text{g}/\text{kg}$, wurden in einigen Proben gefunden. 26 verschiedene PAK wurden in den analysierten Proben identifiziert.

Résumé

23 échantillons de champignons séchés (*Auricularia auricula-judae* Lin. et *Morchella esculenta* Pers. ex Fr.) ont été analysés quant à leurs teneurs en benzopyrènes et quant à la présence d'autres hydrocarbures aromatiques polycycliques condensés (PAH). Les analyses ont été réalisées par chromatographie en phase gazeuse capillaire couplée à la spectrométrie de masse. Des teneurs en benzo[a]pyrène (BaP) allant jusqu'à 460 $\mu\text{g}/\text{kg}$ ont été trouvées. 26 PAH divers ont été identifiés dans les extraits de certains échantillons.

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