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HYGIENIC ASPECTS OF LIQUID SMOKE

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SUMMARY

We have tried to prepare the liquid smoking agent from wood tar oil. From several separated fractions we have selected the most suitable one, judging both the sensory qualities as well as the content of basic phenolic components. The content of PAH, an important value from hygienic point of view, has been determined by GLC equipped with a capillary column coated with methyl-phenyl silicone stacionary phase SE-52.

We identified 17 PAH by means of standard samples. Quantitative analysis was done by "in situ" method based on TLC separation with UV fluorescence detection combined with GLC having 50 ng sensitivity.

The results showed PAH content in the smoke flavour to be lower than 50 ng sensitivity treshold. Special attention was given to benzo/a/pyrene, the content of which was found to be aproximately 2 ng.100^{-1}ml /0,2 ul.l^{-1}/ or 0,2 ppb, and was lower than official 1 ppb limit.

The prepared smoke flavour thus conforms both sensoric and hygienic standards.

INTRODUCTION

The question of smoke as an additive substance in foodstaf especially in meat and meat products is permanently topical. The fact that the smoke produced from any raw material contains a mixture of chemical compounds which may be harmful to human body makes the application of the smoke in the production process very questionable. It is known, that polycyclic aromatic hydrocarbons /PAH/, a fraction of substances of the smoke, include some components with obvious or possible carcinogenic effect. The way of removing these substances from the smoke process is very important. The application of the liquid smoke preparations of a known and controlled composition, from which harmful constituents were removed, is one of the ways of this problem solution.

Since a liquid smoke product hasn't been produced in Czechoslovakia till now, we are working on its preparation at our department at this time. We used beech wood tar as a raw material. Our aim was to gain a suitable fraction organoleptically effective and hygienically faultless. We verified the hygienic faultlessness by identification of some PAH, especially of benzo/a/pyrene. The presence of benzo/a/pyrene is a measure of hygienic value of a liquid smoke product.

We have chosen capillary-gas-chromatography /GLC/ and thin-layer-chromatography /TLC/ as the most suitable and most effective analytical methods from many other methods quoted in expert literature.

MATERIAL

We utilized beech wood tar industrialy gained at the temperature from 400 to 450 °C as a raw material for the liquid smoke preparation processing. We verified the contents of PAH in a fraction which was optimal in organoleptic features and with its proportional contents of the important compounds. It means that this fraction would be convenient as a smoke liquid.

We applied a standard mixture of PAH for identification: Naphtalene, Acenaphtalene, Acenaphtylene, Fluorene, Phenantrene, Anthracene, Fluoranthrene, Pyrene, Benzo/a/anthracene, Chrysene, Benzo/a/fluoranthrene, Benzo/b/fluoranthrene, Benzo/j/fluoranthrene, Benzo/a/pyrene, Benzo/e/pyrene, Dibenzo/a,h/anthracene, Indeno/1,2,3,-c,d/pyrene, Benzo/g,h,i/perylene. /Fs: Fluka /Buchs, Switzerland/, Aldrich Chem. Co. /Milwauke, Wisc. USA/, Analab. Inc. /North Haven, Conn., USA/, Sigma /St. Louis, MO., USA/ Carlo Erba /Milano, Italy/.

METHODS:

Isolation of PAH

We employed a method by TOTH and BLAAS /1/ for isolation. The principle of this method is in repeted extraction of PAH from the raw material with cyclohexan. Fenol substances are removed by the solution of NaOH /8%/. The extract is dried and concentrated to the bulk of 0,1 to 0,2 ml.

Separation PAH by TLC

We made separation of PAH by the KADAR et al. /2/ method using TLC. For the preparation of the plates there was used silica gel Kieselgel 60 GF_{254}. The thickness of the layer was from 0,1 to 0,25 mm. The plates were developed in a mixture benzen - hexan /1:4/ for 30 min at the temperature 20 °C. The detection was made by UV light by sight. Dichlormethane was used for the PAH extraction.

Proof and determination of PAH by GLC

We employed the GLC with capillary column for the determination of the isolated extracts of the PAH on gas-chromatograph Fractovap mod 2350 /Carlo Erba, Milan/. The capillary column /lenght 40 m/ had been impregnated in a static way by SE-52 as a liquid phase and had been conditioned at 260 °C taken twice in the course of 30 minutes. A detector FID /the temperature 275 °C/ was used. The temperature of the injector was 250 °C. The sensibility was 16 x 1. Recorder Speedomax XL 681 was used and the motion of paper was 1 $m.min^{-1}$.

Evaluation of the GLC records

The GLC records were evaluated by the method of standard additions. The chromatographic record of a synthetic standard mixture of SUPELCO-FLUK was a base of this evaluation.

RESULTS AND DISCUSSION

We chose a procedure of separation and identification using the chromatography

method by TOTH and BLAAS /1/ and KADAR et al. /2/ for determination PAH in the liquid smoke preparations. The PAH were isolated from samples by a combination of extract methods: liquid-liquid and TL chromatography. We employed silica gel as an absorbent. The best results were obtained with silica gel Merck Kieselgel 60 GF 254.

A critical point of this part of our work was the extraction of the separated PAH from the material of the thin layer. The use of dichlormethane in the amount of 4 ml proved to be good.

For identification and quantitative evaluation of the individual PAH capillary GLC was used. The choice of a sufficiently selective and effective capillar chromatography column was of great importance. As it was nessesery to work at higher temperatures, we employed a capillary column from borosilicate glass /HRIVŇÁK/ /3/

Identification of investigated substances was made by capillary GLC with a standard mixture of PAH. On the fig. 1 there is a chromatographic record of the standard mixture and elution waves of individual PAH. As could be seen, the high effective capillary GLC employed under mentioned circumstances enabled a separation of all studied PAH. On the fig. 2 there is a chromatographic record of the investigated fraction of wood tar after TLC separation. Next there is the record of the same sample enriched with the standard mixture of PAH /fig. 3/. As it can be seen from the records we identified according to retentive times the folloving PAH: 5,7,8,9,10,11,12,13,14 that is Phenantrene, Fluoranthrene, Pyrene, Benzo/a/anthracene, Chrysene, Benzo/a/fluoranthrene, Benzo/b/fluoranthrene, Benzo/e/pyrene, Benzo/a/ pyrene. We paid special attention to benzo/a/pyrene /elution wave 14/, the presence of which is from the hygienic point of view dominant.

As it can be seen from the appertaining chromatographic records /fig. 2., 3./ the contents of this PAH was in the investigated fraction very small. Therefore we applied UV detection on the thin layer /TLC/ /fig. 4/ in combination with capillary GLC /fig. 5/ for the quantitative evaluation.

The detection of PAH on the thin layers was made at 360 nm. The amount /expressed in ul/ of the PAH standard mixture was calculated on the basis of the inner standard method. The results from the calculation has been that the contents of PAH in 100 ml investigated sample was smaller than 50 ng. For the elution wave 14 /the wave for benzo/a/pyrene/ was a quantity of 2 $ng.100\ ml^{-1}$.

Because this quantity is according to KADAR et al. /2/ below the threshold of the method sensitivity, we can say that the contents of benzo/a/pyrene was in the investigated fraction of wood tar, therefore in the processed liquid smoke preparation, substantially lower than the tolerated value of 1 ppb.

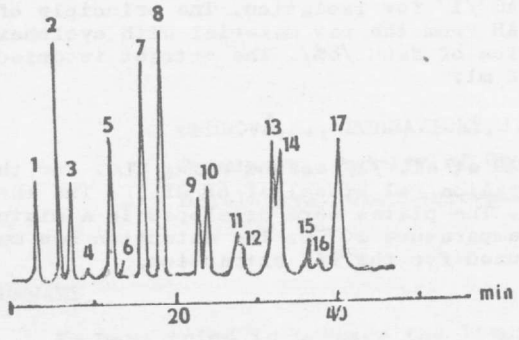


Fig. 1. Standard mixture PAH

Elution wave:

1 - Naphtalene
2 - Acenaphtalene
3 - Acenaphtylene
4 - Fluorene
5 - Phenantrene
6 - Anthracene
7 - Fluoranthrene
8 - Pyrene
9 - Benzo/a/anthracene
10 - Chrysene
11 - Benzo/a/fluoranthrene
12 - Benzo/b/fluoranthrene
13 - Benzo/e/pyrene
14 - Benzo/a/pyrene
15 - Dibenzo/a/anthracene
16 - Indeno/1,2,3,-c,d/pyrene
17 - Benzo/g,h,i/perylene

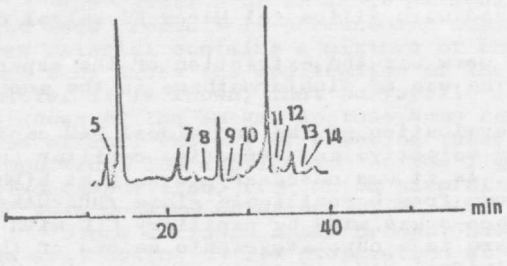


Fig. 2. Fraction of wood tar

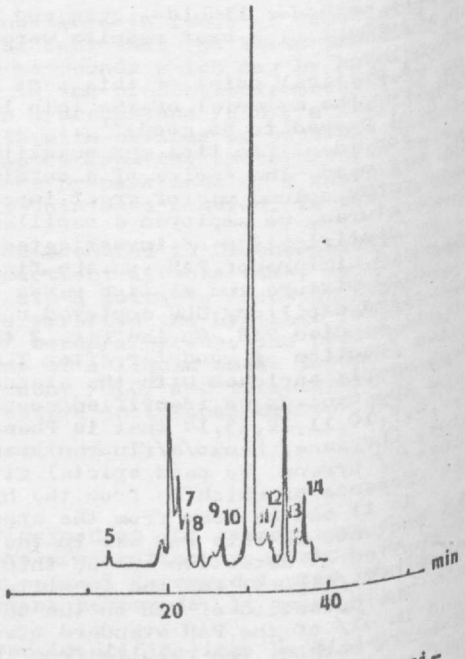
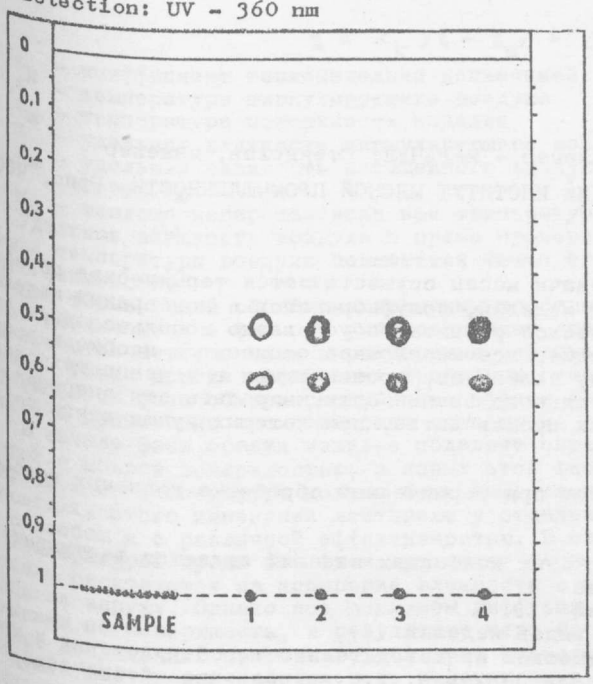


Fig. 3. Fraction of wood tar enriched with standard mixture of PAH

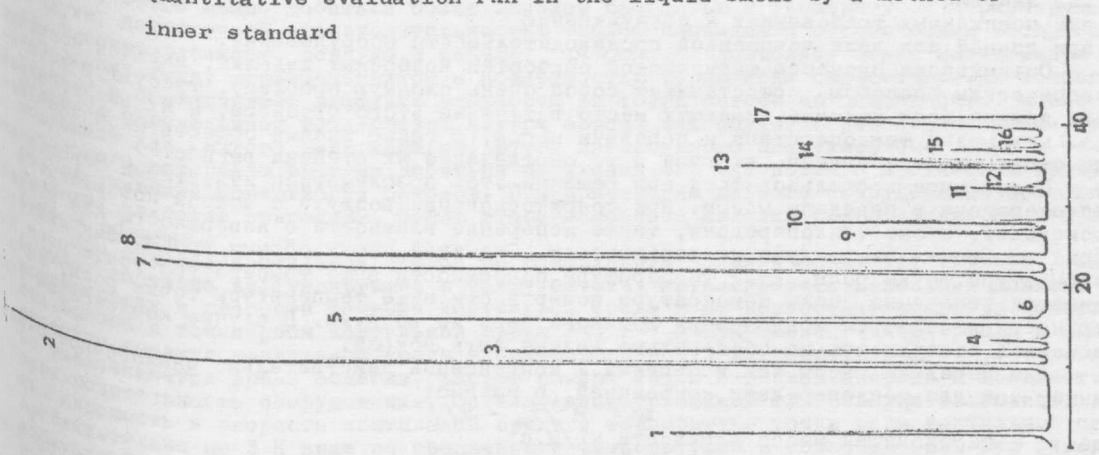
Developing mixture: benzen- n-hexan /1 : 4/
Sorbent: silica gel
Detection: UV - 360 nm



1-48,3 ng
2-97 ng
3-242 ng
4-483 ng

Fig. 4. Identification of PAH by means of "in situ" method on a layer by UV light

Fig. 5. Quantitative evaluation PAH in the liquid smoke by a method of inner standard



REFERENCES

1. Tóth, L., Blaas, W.: Fleischwirtschaft 52 /1972/ 1414-1422
2. Kadar, R., Nagy, K., Fremstad, D.: Talanta 27 /1980/ 227-230
3. Hrivňák, J.: Hydrochémia '81 Bratislava ČSVTS 1981 257-266
4. Lee, M.L. et al.: Separation and Identification of Sulphur Heterocycles in Coal - derived Produots. Fourth International Symposium on Analysis, Chemistry and Biology, Columbus, 1980