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Note

A new method for the rapid isolation of polycyclic aromatic hydrocarbons from smoked meat products

K. POTTHAST and G. EIGNER
Institut für Chemie und Physik der Bundesanstalt für Fleischforschung, Kulmbach (G.F.R.)
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Since the occurrence of carcinogenic polycyclic hydrocarbons in smoked meat products was discovered, many methods have been developed for the determination of these compounds. In the analysis of meat products, such methods have mainly involved liquid–solid and liquid–liquid extraction of the untreated, previously ground material[1–4]. In addition, extraction procedures have been used in which the smoked or grilled meat products were subjected to prior alkali disintegration[5,6]. These procedures have, for the most part, the disadvantages of being expensive in terms of time and of the amount of materials needed; especially costly is the expense for the ultrapure extraction reagents. Another disadvantage of these procedures is the fact that the residue after the extraction must be further purified by column chromatography.

Our procedure is based on the observations that the polycyclic aromatic hydrocarbons (PAHs) are more soluble in propylene carbonate than in any other organic solvent and that extraction of the PAHs from meat products with this solvent yields a fat-free extract; this is particularly advantageous for the rapid purification and preparation of the extract.

MATERIALS AND REAGENTS

Unless otherwise specified, the materials used were obtained from Merck (Darmstadt, G.F.R.).

Celite 545 (Macherey, Nagel & Co., Düren, G.F.R.), heated at 500° for 3 h.

Sodium sulphate p.a., heated at 500° for 3 h.

Florisil, 60–100 mesh (ASTM) for column chromatography, heated at 500° for 3 h and, after cooling in a dessiccator, activated with 15% of water.

Calcium chloride p.a., powder form, water-free.

Sea sand p.a., heated at 500° for 3 h.

Propylene carbonate for synthesis (Schuchardt, Munich, G.F.R.) purified from PAH by percolation with cyclohexane for about 14 h.

Light petroleum (b.p. 40°) purified by distillation at 20° with a rotating vacuum evaporator.

Cyclohexane, Uvasol.

Chloroform p.a.

Sodium hydroxide, 10% (w/v) solution.

Chromatography columns (60 cm × 6 cm) with glass frit (pore diam. DO) and PTFE stopcocks (Normag, Hofheim, G.F.R.).

Separatory funnels (2 litre) with PTFE stopcocks and long stems (Schott, Mainz, G.F.R.).

METHOD

A 100 or 200-g sample of the pre-ground, smoked or grilled meat product was weighed into an aluminium container (capacity 1.5 litres) and stirred with an equivalent volume (100–200 ml) of chloroform by using kneading beaters and a domestic food-mixer. Sodium sulphate (half the weight of the meat) was then added, and uniform distribution of the sodium sulfate, was achieved by homogenization for 3 min. By this means, most of the fat fraction of the sample dissolved in the chloroform phase. After thorough mixing with an amount of Celite equivalent to the weight of the sample, the fat portion became uniformly distributed over the surface of the adsorbent, thus facilitating extraction of the PAHs; efficient mixing was ensured by using the food-mixer throughout.

In order that extraction of the PAHs with propylene carbonate should not be affected by the chloroform, evaporation of the latter solvent was required before further treatment (such evaporation should be conducted at low temperature, as loss of PAHs increases with increasing temperature). Removal of chloroform within 2 h was achieved by using a vacuum drying-cabinet at 40°.

The dried material was put into a chromatography column containing a 5-cm layer of Celite above the glass frit, and the column-packing material was compacted by shaking. The contents of the column were then covered with a 2-cm layer of sea sand in order to prevent the surface of the packing from being disturbed by the addition of propylene carbonate. About 500 ml of propylene carbonate was required for elution of the PAHs from the column; however, only the first 200 ml was collected. This eluate was transfered to a separatory funnel containing 600 ml of 10% (w/v) sodium hydroxide solution and about 400 ml of distilled water, and the mixture was vigorously shaken to hydrolyse the propylene carbonate. The pressure formed in the funnel was released by carefully opening the PTFE stopcock, then the funnel was cooled under running water to about 20°.

The PAHs were extracted from this alkaline aqueous solution of propylene glycol by thorough shaking with four 200–250-ml portions of light petroleum, with care to avoid formation of an emulsion. After the first shaking, the light petroleum phase contained a flocculent precipitate, which, during phase separation, should remain in the light petroleum phase. After separation of the final aqueous residue, a mixture of 70 g of calcium chloride and 30 g of Celite was added to the combined light petroleum phases in another separatory funnel, and this mixture was thoroughly shaken; the Celite–calcium chloride mixture was then allowed to settle for a few minutes. The use of calcium chloride for drying the light petroleum phase also has the advantage that residual propylene glycol, in the form of a crystalline alcohol, was bound to the calcium chloride. The addition of Celite prevented too dense a packing of the calcium chloride and also served to assist sedimentation of the flocculated component. In order to extract the remaining small amount of impurities, the light petroleum phase was drained from the separatory funnel and filtered through a layer

of Florisil (activated with 15% of water formed in a wide glass funnel loosely stoppered with glass wool) and collected in a 1-litre round-bottomed flask; the Florisil layer was moistened with distilled light petroleum before the filtration. Since the light petroleum extract, under normal pressure, usually flowed through the calcium chloride–Celite layer only very slowly, it was necessary to produce a slight pressure in the separatory funnel; this was done with the aid of a bellows, which was firmly attached to the separatory funnel by means of a glass tube passing through a rubber stopper. During opening of the separatory funnel stopcock, little or none of the calcium chloride–celite mixture flowed out. After the light petroleum phase had drained out of the separatory funnel, the remaining calcium chloride–Celite mixture was rinsed with two about 100-ml portions of light petroleum; thorough shaking was no longer necessary.

The extract collected in the flask was clear and was concentrated to a few millilitres in a rotating vacuum evaporator with a bath temperature of 20°. It was then transferred (with rinsing) into a graduated reagent tube, with a ground-glass fitting, by means of a Fortuna safety pipette and 15 ml of cyclohexane. The extract was then carefully concentrated to about 1 ml in the evaporator at 40°; this was the best concentration for thin-layer chromatography (TLC). For analysis by gas or liquid chromatography it might also be necessary to concentrate the solution still further and to add an internal standard.

RECOVERY EXPERIMENTS

To test the method, 10 μg of benzo[*a*]pyrene were added to different meat products. After extraction and concentration to 2 ml, the extract was separated by TLC. As Tóth[2] has described, a repeated development is necessary. Using these procedures, and the quantitative fluorimetric analysis method described by Tóth, we achieved a recovery of 95 to 100% of the benzo[*a*]pyrene. The time required for the extraction and further preparation is estimated to be about 4 h; thus, in comparison with earlier methods, the analysis time is greatly reduced and many samples can be treated concurrently. In addition, 2 h are required for quantitative analysis of the PAHs, so that each determination takes a total of 6 h; this time is proportionately shortened by processing several samples at the same time.

APPLICATION TO SMOKED MEAT PRODUCTS

Products examined ranged from lightly-smoked to black-smoked. The method appeared to be satisfactory, regardless of the extent of smoking. The extracts obtained were completely clear, although they occasionally contained a little yellow colour, which did not interfere with the results.

The method described here provides, in addition to a saving of time in comparison with other methods, the advantages of a relatively problem-free technique and a small expenditure for reagents.

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