Determination of Phenol, Guaiacol and 4-Methylguaiacol in Wood Smoke and Smoked Fish-Products by Gas-Liquid Chromatography

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Summary. The determination of phenol, guaiacol and 4-methylguaiacol in smoke and smoked fish products is described. An alkaline fish homogenate is extracted with ethyl ether in order to remove the lipids. After protein precipitation, the phenols are isolated by extraction with chloroform. The chloroform extract is analysed by gas chromatography using a packed column and a flame ionisation detector. Some losses of the phenols during the isolation procedure have been observed. The analytical method developed has been applied to smoke and smoked fish samples from a cold-smoking experiment with a modern smoking kiln.

Since ancient times smoking has been used as a food preservation technique, based on dehydration of the food and deposition of smoke compounds with some anti-microbial and anti-oxidant activity.

During smoke production the lignins of wood, mainly consisting of guaiacylpropane and syringyl-propane [1], are pyrolysed, giving a complex mixture of phenolic compounds, polycyclic aromatic hydrocarbons and carbonyl compounds.

It is assumed that reactions between the carbonyl compounds and proteins are mainly responsible for the colour formation on smoked surfaces [2], whilst the absorbed phenolic compounds are closely related to the flavor and aroma of the smoked product.

Colorimetric determinations of phenols with 2,6-dichloroquinone-chloroimide [3, 4] and 4-aminoantipyrine [5, 6] are usually applied for the measurement of the total phenol concentration in smoke and smoked products. The colorimetric reagents used are inactive to phenolic compounds substituted in the para-position.

However, identification of the individual phenolic components in wood smoke and smoked foods by Fiddler et al. [7] and Lustre and Issenberg [8, 9] using gas-liquid chromatography and infra-red spectroscopy, have shown that para-substituted phenols are also present.

It is obvious that quantitative analytical methods for the determination of individual phenols provide more detailed and accurate information about the behaviour of phenols during smoking.

The determination of phenols in smoked products is rather difficult, due to the necessary isolation of the phenols from the food. Some phenolic compounds are unstable or reactive to the proteins of the food.

So far, Issenberg et al. [10] investigated the recovery of some individual phenols during the isolation and concentration from smoked meat and model systems.

Phenols, like phenol itself, guaiacol and 4-methylguaiacol and syringol, were isolated with a series of ethyl ether extractions to separate the components according to acidity.

Recoveries of the above-mentioned phenols from water and triolein solutions were between 80 and 90%. The small losses were probably due to adsorption on
the filter during a filtration step and to vapourisation during transfer operations.

A large amount of \[^{14}C\]-phenol added to uncured pork belly (1 mg phenol/g) was extracted with an 50\% aqueous ethanol solution. The over all recovery, i.e., recovery of the extraction from the meat and isolation of phenol from the meat extract, was ~59\% of the added phenol. The authors suggest that the incomplete recovery may be due to an interaction of the added phenol with the proteins.

Recently steam distillation, followed by an extraction with ethyl ether, has been used by Potthast [11] for the isolation of several phenolic compounds from aqueous solutions and meat.

In the case of aqueous solutions, quantitative recovery was obtained. However by analysing mixtures of phenols added to uncured meat homogenates, much lower yields of phenolic compounds, especially those phenols with reactive carbonyl groups, were obtained.

At our Institute the formation of colour and flavour of herring during cold smoking has been investigated extensively. High concentrations of phenolic compounds, like guaiacol, phenol and 4-methylguaiacol, are to be expected in cold smoke [11], so we decided to develop a simple isolation procedure and a gas-liquid chromatographic determination of phenol, guaiacol and 4-methylguaiacol in cold smoke and cold smoked products.

Methods and Materials

1. Chemicals

The following chemicals, all of analytical grade, were used: hydrochloric acid, sodium hydroxide, chloroform, sodium chloride, phenol, guaiacol, 4-methylguaiacol, 3,5-dimethylphenol, trichloroacetic acid.

2. Gas-liquid Chromatographic Analysis

Samples of phenol extracts were analysed by gas chromatography (Hewlett Packard 5700 gas chromatograph) on a glass column of 2.0 m x 2.8 mm o.d. packed with 10\% of ethylene glycol adipate on Chromosorb W-AW (80–100 mesh).

The gas-chromatographic conditions were: column temperature: isothermal at 145 °C, injection port temperature: 200 °C, flame ionisation detector temperature: 200 °C, carrier-gas flow rate: 20 ml He/min, sample volume: 1.4 μl.

3. Cold Smoking of Herring

Several experiments were carried out in a smoking kiln of our Institute. The smoke was produced by pyrolysis wood sawdust (moisture content 60\%) in a Fessman smoke generator.

About 60 kg of brined herring was smoked during 7.5 h at a relative humidity of 82\% and a temperature of 29 °C. The velocity of the smoke gas was 3.5 m/s.

At regular time intervals smoked herring samples were taken. The gaseous phase, sampled by suction of the smoke at a rate of 8.2 l/min water. Sampling time was 30 min.

Samples of the gaseous phase were taken in the smoking house of the kiln and in the supply pipe from the smoke generator to the smoking house.

4. Isolation of Phenols from an Aqueous Solution

One hundred ml of an aqueous solution of the phenols containing 6.1 mg phenol/l, 6.2 mg guaiacol/l, 5.2 mg 4-methylguaiacol/l was extracted during 5 minutes with 10 ml of chloroform. (15 mg 3,5-dimethylphenol/l CHCl₃, was used as an internal standard)

The pH of the aqueous solution was adjusted by adding hydrochloric acid or sodium hydroxide. If necessary, sodium chloride was added (0.36 g of NaCl/ml). Two ml of the chloroform layer was transferred to a gas chromatographic sample phial and prepared for the gas-chromatographic analysis.

Solutions of phenol, guaiacol and 4-methylguaiacol (1–30 μg/l) in chloroform were used as standards.

5. Determination of Phenol, Guaiacol and 4-Methylguaiacol in Wood Smoke

Fifty ml of the wood smoke sample solution was pipetted into an Erlemeyer, which contained 18 g of NaCl. Five ml of chloroform containing 15 mg 3,5-dimethylphenol/l was added to the solution and the mixture was shaken during 5 min.

Two ml of the chloroform layer was pipetted into a 3-ml sample phial, followed by the gas-chromatographic analysis.

Standard addition of the phenolic compounds concerned to the smoke water was applied in order to determine the concentration of phenol, guaiacol and 4-methylguaiacol. Solutions of the phenols in chloroform were used as standards (1–30 μg/l).

6. Determination of Phenol, Guaiacol and 4-Methylguaiacol in Smoked Fish Products

Ten grams of a homogenised smoked-herring sample was mixed with 40 ml 5% NaOH solution. Fat was removed by extracting the homogenate with two portions of 100 ml of ethyl ether.

Next, 60 ml 40% trichloroacetic acid was added, and the solution was centrifuged at 2000 r.p.m. for 20 minutes. Seventy-five ml of the supernatant liquid was pipetted in an Erlenmeyer flask, which contained 25 ml of water and 36 g of NaCl.

The pH was adjusted to ~ 6. Ten ml of chloroform was added, and the mixture was shaken for 5 minutes. Two ml of the chloroform layer was used for the gas chromatographic analysis.

The standard addition method was usually applied for the determination of the concentration of the phenolic compounds.

The above-mentioned procedure, without fish, was followed in order to determine the over all recovery of the isolation procedure.

Results and Discussion

Analytical Procedure

The extraction yields of phenol, guaiacol and 4-methylguaiacol from aqueous solutions with chloroform are given in Table 1 as a function of the pH.

Guaiacol and 4-methylguaiacol are extracted quantitatively, whilst phenol is extracted up to 25\%.
If the solution is saturated with sodium chloride the extraction yield of phenol is increased to 56\%.