HARDWOOD	ALDER
LIGNIN	PYROLYSIS
METHOXYPHENOLS	GC – MS
SEPARATION	IDENTIFICATION
PEROXIDATION	PROTECTION

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A related basic study of the most prominent dimethoxyphenols:
Phenolic antioxidants in wood smoke

Front page 2010 - Göran Petersson

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Abstract

Alder wood smoke in a large sausage-curing chamber was analysed with regard to its

content of methoxyphenol antioxidants. The use of Tenax adsorbent cartridges permitted

simultaneous quantitative sampling of phenols condensed on smoke particles as well as gaseous

compounds. The analytical determinations were performed by thermal desorption combined with

gas chromatography and mass spectrometry. In addition to methoxyphenols, several other key

components were assessed, including 1,6-anhydroglucose, 2-furaldehyde, furans and

hydrocarbons. Benzene was the most prominent hydrocarbon. The concentrations of polycyclic

aromatic compounds were low, due to a low smoke generation temperature.

Predominant smoke components were the 2,6-dimethoxyphenols, which are characteristic

thermal degradation products from hardwood. The 2,6-dimethoxyphenols are stronger

antioxidants than the 2-methoxyphenols present in lower amounts. The particularly active

antioxidants with a 4-alkenyl side-chain constituted 20–30% of total methoxyphenols, which is

much more than normally reported for liquid smoke. The phenolic antioxidants may be important not only for the preservation of foods, but also for health as dietary components.

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1. Introduction

Smoke curing of food, including ham, sausages and fish, is an old technique for flavouring and preservation, which is presently extensively used in industrial modified versions. The chemical aspects of meat smoking have been reviewed (Toth & Potthast, 1984), and the chemical composition of smoke condensates and liquid smoke has been assessed in many studies, including some recent investigations (Guillén & Ibargoitia, 1998, 1999). Methoxyphenols are major components of great importance for the smoke flavour and for the preserving antioxidant effect of the smoke. High antioxidant activities on lipid peroxidation have recently been demonstrated in model assays for several specific methoxyphenols related to those occurring in wood smoke (Barclay, Xi & Norris, 1997; Ogata, Hoshi, Shimotohno, Urano & Endo, 1997).

The chemical composition of freshly generated smoke in curing chambers is more difficult to assess than that of liquid smoke. Phenols and other smoke components occur either gaseous or condensed on particles, or both (Hawthorne, Krieger, Miller & Mathiason, 1989). The aim of this study was to apply an analytical technique permitting determination of all prominent methoxyphenols and a range of other compounds. Similar assessments have previously been

made for smoke from forest biomass burning (Kjällstrand, Ramnäs & Petersson, 2000) and for chimney emissions of wood smoke (Kjällstrand & Petersson, 2001a). An additional aim was to elucidate relative proportions and effects of curing smoke antioxidants. The antioxidant properties of specific methoxyphenols in birchwood smoke were recently characterised (Kjällstrand & Petersson, 2001b).

2. Materials and methods

2.1 Smoke generation

Curing smoke was generated continuously by smouldering combustion in a 5-10 cm thick bed of commercial pea-size wood particles, including bark, from alder (*Alnus glutinosa*). An airflow through the enclosed smoke generator transported smoke into the curing room (120 m^3, 24°C) filled with sausages hanging on mobile equipment. In our laboratory, smoke was generated by choked burning of match-size wood sticks, as previously described for other biomass materials (Kjällstrand et al., 2000).

2.2 Analytical methods

Samples of 4–5 ml were taken during approximately 1 min with a conventional air pump. The adsorbent cartridges were injector glass liners (0.8 mm i.d.) filled with the porous polymer Tenax TA (60-80 mesh). In the laboratory, thermal desorption was performed at 220°C in the injector of the gas chromatograph (Varian 3800) with the helium carrier gas. The separations

were made on a fused silica open tubular column (30 m × 0.25 mm i.d.) with a cyanopropylphenylsilicone (Rtx 1701, 0.25 μm layer) as the stationary phase. The oven temperature was increased 10°C min^{-1} from –50°C to 50°C and then 5°C min^{-1} from 50°C to 250°C. The column outlet was connected to a Varian Saturn 2000 ion trap mass spectrometer. Electron impact mass spectra (70 eV, m/z 35-400) were recorded. Concentrations were calculated using the response of methoxyphenols (Kjällstrand et al., 2000) for all compounds. The reported methoxyphenols were previously identified in smoke from forest biomass (Kjällstrand et al., 2000), and their identities were confirmed by retention times and mass spectra. Remaining assessed compounds were unequivocally identified by their mass spectra and are known prominent wood smoke components from previous studies of hydrocarbons (Barrefors & Petersson, 1995), furans (Barrefors, Björkqvist, Ramnäs & Petersson, 1996) and chimney emissions (Kjällstrand & Petersson, 2001a)

3. Results and discussion

Concentrations of total methoxyphenols and key species of other compound classes are given in Table 1 for three smoking chamber samples, and one laboratory sample of smoke from choked burning of alder wood. Percent compositions of the methoxyphenols are given in Table 2 for the same samples. The total ion chromatogram in Fig. 1, for the second smoke sample, illustrates the separation of prominent assessed smoke components.
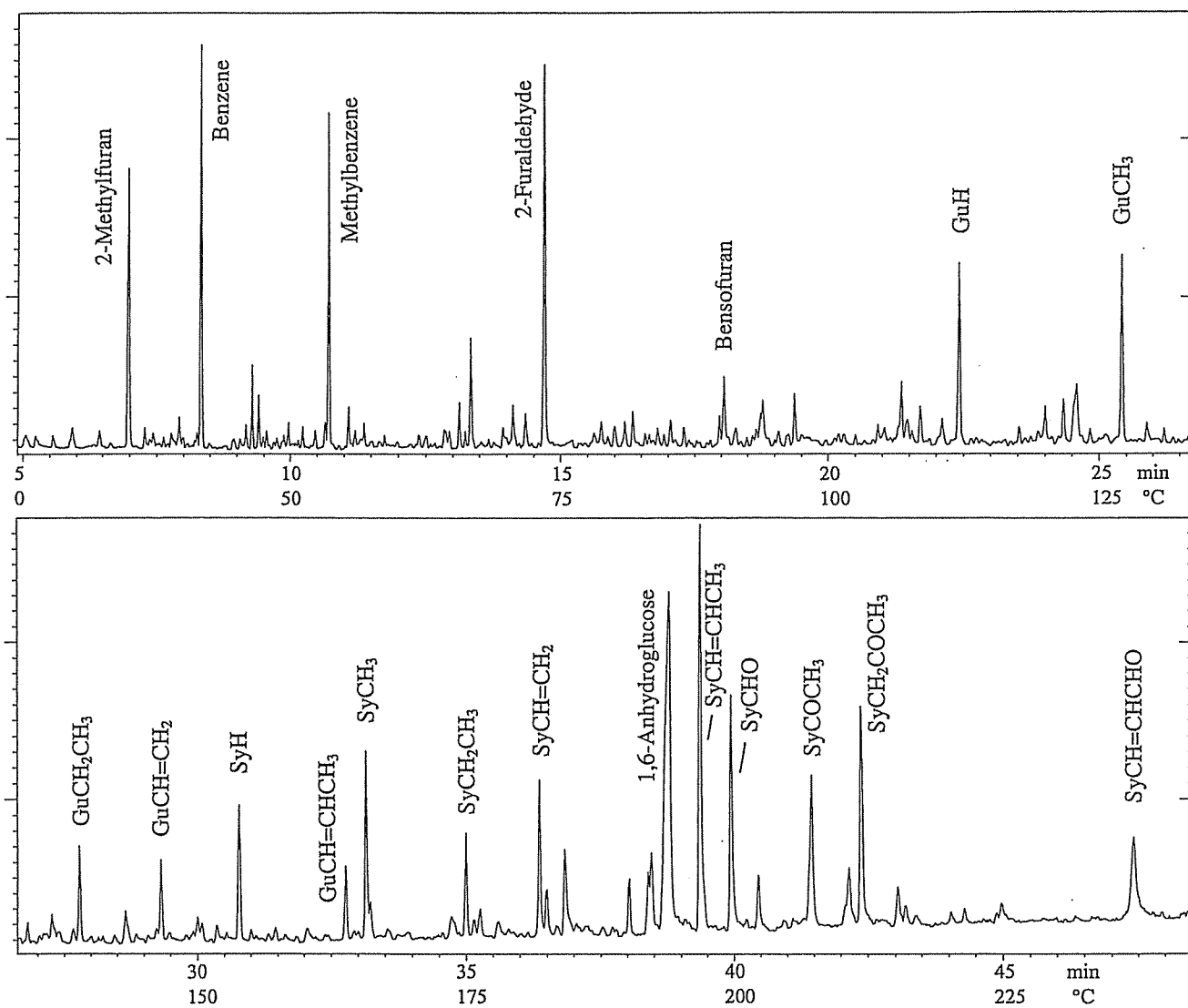
Fig. 1. Total ion chromatogram illustrating the separation of components of alder smoke from a curing chamber (second sample in Tables 1 and 2; the same attenuation for the two halves of the chromatogram).

3.1 Smoke composition

As seen from Table 1, the methoxyphenols are major smoke components. The 2-methoxyphenols constitute 20–30% and the 2,6-dimethoxyphenols 70-80% of total methoxyphenols. A high proportion of 2,6-dimethoxyphenols is characteristic of hardwood smoke, whereas smoke from softwood biomass contains 2-methoxyphenols only (Kjällstrand et al., 2000). The smoke methoxyphenols reflect the structure of the corresponding units in the plant lignin. Liquid smoke from hardwood often contains lowered proportions of 2,6-dimethoxyphenols (Tóth & Potthast, 1984; Guillén & Ibargoitia, 1999). This is probably explained by losses with removed particles during production.

The concentration of phenol as well as of methylphenols, dimethylphenols and ethylphenols was very low. With increasing combustion temperature, the proportion of these phenols increases relative to the methoxyphenols (Alén, Kuoppala & Oesch, 1996). The much higher proportions of phenol and alkylphenols reported for many liquid smoke preparations (Tóth & Potthast, 1984) are therefore explained by higher smoke generation temperatures than in the studied alder smoke generator.

The major carbohydrate-derived smoke component was 1,6-anhydroglucose, as expected from its well-known formation from cellulose and its presence in smoke from a wide variety of plant materials (Simoneit et al., 1999). The very polar structure makes 1,6-anhydroglucose difficult to analyse by gas chromatography, and it is often not reported. The 2-furaldehydes are less polar and more volatile carbohydrate degradation products. Furfural, 2-furaldehyde, is the major compound, which may be even more prominent in liquid smoke (Guillén & Ibargoitia, 1999).

Table 1. Concentrations (mg m^{-3}) of selected components of alder smoke during industrial curing (24°C) of sausages.

| | Smoke curing chamber Consecutive samples | | | Choked lab. burning |
	4.1 ml	4.3 ml	4.5 ml	4.2 ml
Phenols				
2,6-Dimethoxyphenols	16.8	16.1	12.1	143
2-Methoxyphenols	5.3	5.3	4.6	39
Phenol	0.2	0.2	0.2	1.7
Anhydrosugars				
1,6-Anhydroglucose	5.8	6.0	4.8	-
Furaldehydes				
2-Furaldehyde	2.0	2.5	2.1	12
5-Methyl-2-furaldehyde	0.3	0.3	0.2	0.9
Furans				
2-Methylfuran	1.9	1.6	1.5	1.3
2,5-Dimethylfuran	0.3	0.3	0.3	0.5
Bensofuran	0.4	0.4	0.4	0.7
Hydrocarbons				
Benzene	2.2	2.2	1.9	6.8
Methylbenzene	1.5	1.4	1.3	4.4
Styrene	0.2	0.2	0.2	1.3
Naphthalene	0.2	0.2	0.2	2.0

The most prominent volatile alkylfuran was 2-metylfuran. Furan is probably formed in similar amounts but was partially lost by break-through on sampling. Characteristic proportions between furan and several alkylfurans were given in a previous study of smoke from smouldering combustion (Barrefors, Björkqvist, Ramnäs & Petersson, 1996). The proportions of the furans are lower in smoke from flaming combustion.

Benzene was the major aromatic hydrocarbon in the smoke, followed by methylbenzene (toluene), dimethylbenzene (xylene), ethylbenzene and ethenylbenzene (styrene), in accordance with previous studies of wood smoke (Barrefors & Petersson, 1995). As seen from Table 1, the proportions of the volatile hydrocarbons relative to the methoxyphenols were lower for choked burning. Complementary samples also demonstrated lower proportions of the volatile furans and hydrocarbons in the smoke generator than in the smoking chamber. It is concluded that the gaseous compounds are absorbed less efficiently than the particle-associated compounds during smoke curing. Liquid smoke prepared by aqueous extraction is not expected to contain significant amounts of volatile furans and hydrocarbons.

The concentration of naphthalene was only 0.2 mg m^{-3} in the smoke curing chamber, and the concentration of phenanthrene was determined by selective ion monitoring to be as low as 0.01 mg m^{-3}. This contrast sharply with the high concentrations of these three hydrocarbons in smoke from wood burning for heating purposes (Kjällstrand & Petersson, 2001a), and is explained by the rapid increase of emissions of polycyclic hydrocarbons with the combustion temperature (Alén et al., 1996). Phenanthrene is indicative of the presence of polycyclic aromatic compounds with higher molecular weight. Genotoxic and carcinogenic compounds with these structures in smoked food products (Tóth & Potthast, 1984) may constitute important health hazards, and concentrations lower than specified limits are required (García-Falcón, González-

Amigo, Lage-Yusty, López de Alda-Villaizán & Simal-Lozano, 1996). Cytotoxic effects of liquid

smoke have recently been demonstrated (Putnam, Bombick, Avalos & Doolittle, 1999).

Most of the assessed compounds are polar in nature, but are favourably separated on the

polar stationary phase used. As illustrated in Fig. 1, the 2-methoxyphenols appear before the

corresponding 2,6-dimethoxyphenols in the chromatogram, and the structural analogues within

the two compound groups elute in the same sequence. Small sampling volumes permitted

determination of the volatile compounds 2-methylfuran and benzene without significant break-

through losses. The Tenax sampling technique is favourable for compounds appearing both as

vapours and condensed on particles. Such semi-volatile compounds are easily lost when

condensates are analysed and when particulate sampling filters are used (Hawthorne et al., 1989).

3.2 Methoxyphenol proportions

The specific composition of the methoxyphenols is outlined in Table 2 as percentages of

the 21 assessed compounds. The thirteen 2,6-dimethoxyphenols differ only in the structure of the

group in *para* position relative to the phenolic hydroxyl group. All analogous 2-methoxyphenols

are formed, but those present in very small amounts are not reported. The two groups of

methoxyphenols are often viewed as 4-hydroxy-3,5-dimethoxyphenyl (syringyl, Sy) and 4-

hydroxy-3-methoxyphenyl (guaiacyl, Gu) compounds. The percentage composition is very

uniform for the three smoking chamber samples, indicating a good reproducibility for the

analytical approach.

The steady-state smouldering combustion in the smoke generator differs from the choked

laboratory burning, which includes smoke from flaming combustion at higher temperature. The

proportions of the carbonyl-containing methoxyphenols are higher in the curing smoke from

Table 2. Composition (%) of alder smoke methoxyphenols, arranged in order of estimated decreasing antioxidant activity (Sy = syringyl = 4-hydroxy-3,5-dimethoxyphenyl; Gu = guaiacyl = 4-hydroxy-3-methoxyphenyl).

Phenols	Smoke curing chamber Consecutive samples			Choked lab. burning
$SyCH{=}CHCH_3$ *(E)*	14.3	15.9	15.5	16.8
$SyCH{=}CHCH_3$ *(Z)*	1.8	1.8	1.8	2.8
$SyCH{=}CH_2$	4.8	5.8	4.9	9.6
$SyCH_2CH{=}CH_2$	1.5	1.8	1.8	3.9
$SyCH_2CH_2CH_3$	1.5	1.8	1.8	1.7
$SyCH_2CH_3$	2.9	3.3	3.1	4.3
$SyCH_3$	5.5	6.5	5.8	8.7
$SyCH_2COCH_3$	8.5	8.7	8.8	4.5
$GuCH{=}CHCH_3$ *(E)*	2.2	2.5	2.7	4.2
$GuCH{=}CH_2$	2.2	2.5	2.7	4.0
$SyCH{=}CHCHO$ *(E)*	6.6	7.6	6.6	6.6
SyH	4.0	4.3	4.4	10.0
$SyCOCH_2CH_3$	2.2	2.2	1.8	1.0
$SyCOCH_3$	6.6	5.8	6.6	2.6
$SyCHO$	15.8	9.8	9.7	6.1
$GuCH_2CH_3$	2.9	2.9	3.5	1.2
$GuCH_3$	5.1	5.4	6.6	2.6
$GuCH_2COCH_3$	1.8	1.8	1.8	1.4
$GuCH{=}CHCHO$ *(E)*	2.2	2.5	2.7	2.9
GuH	5.9	5.4	6.2	2.8
$GuCHO$	1.5	1.4	1.3	2.2

smouldering combustion only. The proportions of $SyCH=CH_2$ and SyH are higher for smoke from choked burning, which may be explained by decarboxylation of $SyCH=CHCOOH$ and $SyCOOH$ (Tóth & Potthast, 1984). Only the most volatile 2-methoxyphenols GuH, $GuCH_3$ and $GuCH_2CH_3$ are expected to be gaseous at the low temperature in the curing chamber (Hawthorne et al., 1989). The elevated proportions of these species compared with smoke from choked burning are consistent with a more rapid uptake by the sausages of the particulate methoxyphenols.

The sample from choked burning permits comparisons with the methoxyphenol composition of smoke from other biomass materials generated by the same method (Kjällstrand et al., 2000). Birchwood gives an even lower and grass and heather a much higher ratio between 2-methoxyphenols and 2,6-dimethoxyphenols. Different softwood materials give rise to 2-methoxyphenols only, with $GuCH=CHCH_3$, $GuCH=CH_2$ and $GuCHO$ as major components.

Reported methoxyphenol proportions of many liquid smoke preparations differ widely from the proportions in Table 2, although the liquids are produced from hardwood smoke (Guillén & Ibargoitia, 1998, 1999; Tóth & Potthast, 1984). Major assessed components are often GuH, $GuCH_3$, SyH and $SyCH_3$. This is probably explained by higher smoke generation temperatures and losses during the subsequent processing steps, rather than by differences in smoke composition between different hardwood species.

3.3 Antioxidant aspects

The methoxyphenols in Table 2 have been ranked according to estimated antioxidant activity (Kjällstrand & Petersson, 2001b). The additional methoxyl group in the 2,6-dimethoxyphenols makes them stronger antioxidants than the corresponding 2-methoxyphenols.

The antioxidant strength and the redox potential are governed by the stability of the phenoxy radical (Simic, 1992; Lind, Shen, Eriksen & Merényi, 1990). Conjugation between a side-chain double bond and the aromatic ring therefore increases the antioxidant activity. An electron-withdrawing α-carbonyl group decreases the antioxidant strength. The ranking is based mainly on recent studies of antioxidant activity of selected methoxyphenols and related compounds (Barclay et al., 1997; Ogata et al., 1997).

The prevention of lipid peroxidation by breaking radical reaction chains is considered to be the most important antioxidant effect of curing smoke. This effect is linked to the phenolic fraction of the smoke (Tóth & Potthast, 1984). A lipophilic character of the phenols should favour their penetration into lipids and consequently their antioxidative effect. The methoxyphenols with an alkenyl or an alkyl side-chain appear to possess both a higher antioxidant activity and a higher lipophilicity compared to the carbonyl-containing methoxyphenols.

The high content of about 15% of the *trans* isomer of $SyCH=CHCH_3$ and the total high proportion of 20-25% of the first three 1-alkenyl phenols in Table 2 are likely to contribute strongly to the antioxidant properties of the smoke. The proportion of these methoxyphenols is reported to be much lower in liquid and solid smoke (Tóth & Potthast, 1984; Guillén & Manzanos, 1997; Guillén & Ibargoitia, 1998). The proportions of GuH and SyH with low antioxidant activity are much lower in the alder smoke than in the liquid and solid smoke preparations. It is also interesting that vanillin, GuCHO, as the most well-known methoxyphenol, appears to be the weakest antioxidant.

The methoxyphenols in smoked meat products may also be important with respect to intake of antioxidants with food. Interest in dietary phenolic antioxidants for improved health and

prevention of diseases has increased sharply in recent years (Newmark, 1996). In the living cell, methoxyphenols may be important not only as chain breakers in lipid peroxidation, but also as scavengers of peroxidation-initiating aqueous superoxide ions (Ogata et al., 1997). A potential effect of the methoxyphenols depends on the uptake and distribution of the compounds in the body. For workers at the smoking plant, respiratory uptake of the methoxyphenol antioxidants should be considered as well as exposure to toxic smoke components.

3.4 Smoke curing procedure

Curing in a smoke chamber with freshly generated alder smoke, according to the investigated procedure, offers several advantages. The hardwood produces smoke with a high proportion of 2,6-dimethoxyphenols, which have stronger protective antioxidant activity than 2-methoxyphenols from softwood. The smouldering bed of alder wood particles in the smoke generator permits keeping a constantly low combustion temperature. This results in a high proportion of the particularly antioxidative methoxyphenols with an alkenyl side-chain. It also keeps the concentrations of hazardous polycyclic aromatic compounds at a very low level. A drawback is the high concentration of benzene in the curing atmosphere, but benzene appears to be much less absorbed than the major methoxyphenols, which are condensed on smoke particles.

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