# Phenolic Components of Smoked Meat Products

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Phenolic concentrates were isolated from uncured pork belly strips smoked in the laboratory and from a commercially prepared summer sausage product. Sufficient quantities of phenolic fractions were isolated from 100-g meat samples to permit identification by combined gas chromatography-mass spectrometry and estimation of quantities of individual components. Odors of phenolic concentrates closely resembled those of the smoked products. The phenolic fraction from both samples contained 4-methylguaiacol, phenol, 4-ethylguaiacol, *m*- and *p*-cresol, *cis*- and *trans*-isoeugenol, syringol, 2,6-dimethoxy-4-allylphenol, vanillin, acetovanillone, and cyclotene. Eugenol, 4-vinylguaiacol, syringaldehyde, acetosyringone, and maltol were recovered from the smoked pork belly strips but not from the summer sausage. Two major phenolic smoke components, coniferaldehyde and sinapaldehyde, were not recovered from smoked pork belly, indicating reaction of these compounds with meat components. Approximately 50% of the recovered phenols were para-substituted and would, therefore, not be determined by conventional colorimetric methods.

The nature of specific compounds responsible for the changes induced in foods by wood smoke is not well established. Ockerman *et al.* (1964) and Howard *et al.* (1966) identified individual carbonyl compounds and polycyclic aromatic hydrocarbons, respectively, in smoked foods. Most workers, however, have analyzed classes of smoke components rather than individual smoke compounds deposited in foods (Bratzler *et al.*, 1969; Proctor *et al.*, 1959; Shewan, 1953; Tucker, 1942). Group analyses provide little information on the nature of specific smoke components and reaction products responsible for the characteristic properties of smoked foods. Chemical, physical, and physiological properties of individual compounds within a given group cannot be validly represented by the behavior of one compound used as a standard in the analysis. Quantitative estimates of total phenols in smoked foods are usually derived from colorimetric reactions (the 4-aminoantipyrene and the Gibbs colorimetric reactions) which are insensitive to phenolic compounds substituted in the para-position (Emerson, 1943; Mohler and Jacob, 1957). Para-substituted phenols have been identified in wood smoke (Fiddler *et al.*, 1966; Lustre and Issenberg, 1969) and may be the major components. It is thus doubtful that the estimates of total phenols reported are true measures of the concentrations of these compounds in food products. The objective of this investigation was to determine which of the previously identified phenolic components of hardwood sawdust smoke (Lustre and Issenberg, 1969) could be recovered from a model food smoked in the laboratory and from a commercial smoked product.

## METHODS AND MATERIALS

Uncured rindless pork belly was purchased locally (Armour and Co., Boston, Mass.). Analysis showed that it had a moisture content of 38.7% and a fat content of 41.6%. The commercial product, a smoked summer sausage ("Beef Stick," Hickory Farms of Ohio, Toledo, Ohio), was purchased from a local Hickory Farms store. Samples were stored at 0° C prior to use.

**Smoking of Samples.** Uncured pork belly was smoked in the apparatus shown schematically in Figure 1. Smoke was generated in an all glass generator previously described (Lustre and Issenberg, 1969). The smokehouse was an oven (H) (Model No. JD1202 WH; General Electric Co., Louisville, Ky.), 52 cm × 47 cm × 45 cm in interior dimensions. Samples to be smoked were suspended from a stainless steel rack located at the top. The rack and the inside walls of the oven were completely lined with Teflon (41/1612; Chemical Rubber Co., Cleveland, Ohio) to minimize absorption of smoke components on these surfaces. Glass socket connections (E and F) were fitted in the center of both sides of the oven wall to serve as the inlet and outlet for the flowing smoke. An open glass Petri dish (K), 8 cm in diameter, was located on the bottom of the oven and served as a nonspecific absorber during the smoking period. Smoke flowed from the oven into a solid carbon dioxide–ethanol cooled trap (L) located at the outlet. All connections were made of glass.

Samples to be smoked were sliced as strips from the frozen pork belly slab. Each strip was 1–2 mm thick, 10 mm wide, and 25 to 30 cm long. Twelve strips weighing 635 g were smoked at one time. Individual strips were held at one end with straightened paper clips and suspended from the rack (I) located at the top of the smokehouse. All samples were thawed and equilibrated at room temperature for at least 30 min after cutting.

Smoke was produced in the generator under conditions previously described (Lustre and Issenberg, 1969). Mixed hardwood sawdust was used and the apparent combustion temperature was 350° to 450° C. Air flow rate, from a compressed air cylinder, was 650 cc per min.

Samples were smoked at a temperature of 65° C for 10 hr. During this period, there was smoke and moisture condensation on the oven walls and drippings of fat from the samples. The time and temperature conditions of smoking were similar to those used in the commercial preparation of bacon (Rowe, 1969). Sawdust in the generator had to be replenished three times during the smoking period. The inlet of the smokehouse and the outlet of the carbon dioxide–ethanol trap were closed for 30 min while the sawdust was changed. This period was considered part of the 10-hr smoking time.

After smoking, the strips of pork belly were considerably shrunken, having lost 36% of their weight through moisture evaporation and loss of liquid fat. The lean tissue was brownish red in color and the fat was yellowish. The samples had a pleasant, heavily smoked aroma. They were stiff and oily, indicating some cooking had occurred. The smoked samples were wrapped in aluminum foil and kept overnight at 4° C prior to analysis.

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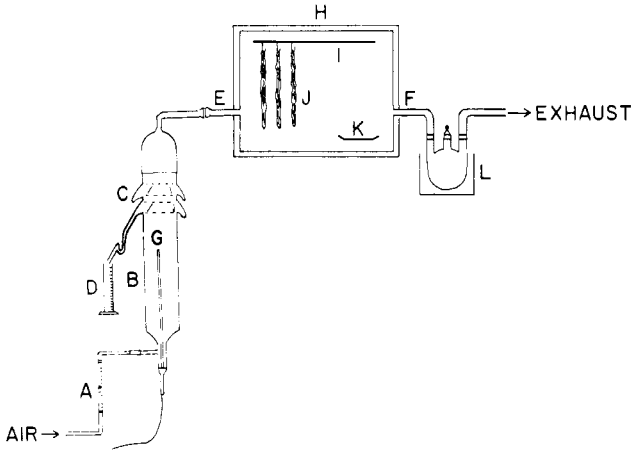


**Figure 1. Apparatus for the smoking of meat samples. A. flowmeter B. combustion chamber C. condensers D. overflow drain E. smoke inlet F. smoke outlet G. thermocouple H. smokehouse I. sample rack J. pork belly strips K. Petri dish (nonspecific absorber) L. solid carbon dioxide–ethanol trap**

**Collection of Smoke Condensates from the Smoking System.** Smoke condensates for analysis were collected from various parts of the smoking system shown in Figure 1. Condensate was collected from the condensing chambers of the smoke generator (C), from the nonspecific absorber located inside the smokehouse (K), and from the carbon dioxide–ethanol cooled trap (L) located at the smokehouse outlet. Individual condensates were transferred with acetone or distilled water to flasks sealed with Parafilm (American Can Co., Neenah, Wis.) and stored under an atmosphere of nitrogen at 4° C until analyzed.

**Preparation of Unsmoked Meat Blank.** Strips of pork belly (160 g ) were hung in the smokehouse and exposed to experimental conditions identical to those used in the smoking process, except that no smoke was used. Samples were exposed to an air flow of 650 cc per min at a temperature of 65° C for 10 hr. The air passed through a bed of sawdust in the smoke generator prior to entering the smokehouse.

At the end of the 10-hr period, the samples were partially cooked and had lost 34% of their weight by the evaporation of water and the loss of liquid fat. The lean tissue was brownish red and the fat was white. Samples had an aroma similar to that of roast pork.

**Separation of an Aqueous Extract of Smoke Components from Smoked Pork Belly.** Smoked pork belly (100 g) was cut into 5- to 10-mm pieces and mixed with 200 ml of 5% sodium hydroxide in a Waring Blendor. The mixture was blended for 5 min until it was homogeneous.

Trichloroacetic acid (40%, 200 ml), was slowly added, with stirring, to the mixture in the Blendor to precipitate the proteins. The mixture was transferred to two 250-ml centrifuge bottles using three 20-ml portions of 40% trichloroacetic acid to wash the Blendor. The washings were added to the solutions in the centrifuge bottles. After centrifugation (Model PR-6, International Equipment Co., Needham Heights, Mass.) at 0° C and 1800 rpm (700 × g) for 10 min, a thick upper layer of fat separated and the precipitated proteins formed a thick cake at the bottom. The upper layer of solid fat was broken and the middle aqueous layer was decanted. It was then filtered under suction, using S&S No. 595 filter paper, into a 1-l. flask. The resulting filtrate was clear and yellow in color.

**Isolation of Phenols from the Meat Extract.** A phenolic fraction was isolated from the meat extract by fractionation based on acidity (Braus and Middleton, 1952). The meat extract (300 ml) was brought to pH 12 with 15 ml of 40% sodium hydroxide and extracted consecutively with two 300-ml and one 150-ml volumes of ether. The ether layer, which contained most of the fat and neutral compounds, was discarded. Phenols were regenerated from their sodium salts in the alkaline aqueous layer by saturating with carbon dioxide at 0° C to pH 6.8. They were then extracted with two 300-ml and one 150-ml volume of ether. Free acids remained in the aqueous phase.

The ether solution of phenols was dried with anhydrous sodium sulfate and transferred in 400-ml portions to a 1-l. round bottomed flask. After concentration to a volume of 5 to 10 ml in a rotary evaporator at 15° C, the solution was transferred to a 15-ml centrifuge tube using three 10-ml volumes of acetone to rinse the round bottomed flask. Volume was reduced to approximately 3 ml with a stream of prepurified nitrogen between additions of washings. The solution, in the centrifuge tube, was concentrated to 2 ml by careful purging with prepurified nitrogen. Fat-like material which crystallized at this degree of concentration was removed by centrifugation of the mixture at 0° C and 2500 rpm (950 × g) for 5 min. The clear yellow supernatant was decanted into another 15-ml centrifuge tube. The precipitate was washed three times by resuspension in 2 ml of acetone and recentrifugation at 0° C and 2500 rpm for 5 min. The washings were added to the original supernatant. Final concentration of the solution to 0.05 ml was accomplished without further precipitation of material. The concentrate was yellowish brown in color and had a smoked meat aroma. It was stored as a dilute solution in acetone under an atmosphere of nitrogen in a glass stoppered centrifuge tube sealed with Parafilm.

**Separation of Phenols from the Aqueous Smoke Condensates.** Phenols were isolated from the aqueous smoke condensates collected from various parts of the smoking system. The procedure followed was similar to that used for the separation of phenols from the meat extract. The sample was diluted to 100 ml with distilled water and brought to pH 12 with 15 ml of 40% sodium hydroxide. Neutral compounds were extracted with two 200 ml and one 100 ml volume of ether. Phenols were removed from the alkaline aqueous layer by saturation with carbon dioxide at 0° C to pH 6.8 and extraction with two 200 ml and one 100 ml volumes of ether. The combined ether extracts were dried with anhydrous sodium sulfate and concentrated in a rotary evaporator at 15° C. This concentrate was transferred to a 15-ml centrifuge tube with acetone. Final concentration to the desired volume was accomplished by careful purging with prepurified nitrogen.

**Analysis of Meat Blank.** These samples were treated in a manner identical to the procedure used for the extraction and isolation of phenols from smoked meat samples.

**Analysis of Commercially Smoked Sausage.** The casing was removed from the sausage and 100 g of surface tissue, 3 mm thick (representing 30% of the total weight of the meat), was sliced from the edges.

Extraction and isolation of a phenolic concentrate was identical to the methods used for smoked pork belly.

**Gas Chromatography of Phenolic Fractions.** The concentrated phenolic fractions were separated by gas chromatography on a 2.3 mm i.d. × 1.83 m 5% Carbowax 20M TPA on 100/120 mesh Chromosorb W column. Helium flow rate was 40 cc per min. Sample volumes of 0.1 μl were injected. Column temperature was isothermal at 100° C for 10 min and then programmed at 4° C per min to 220° C. Injector temperature was 250° C. Detector temperature was 270° C.

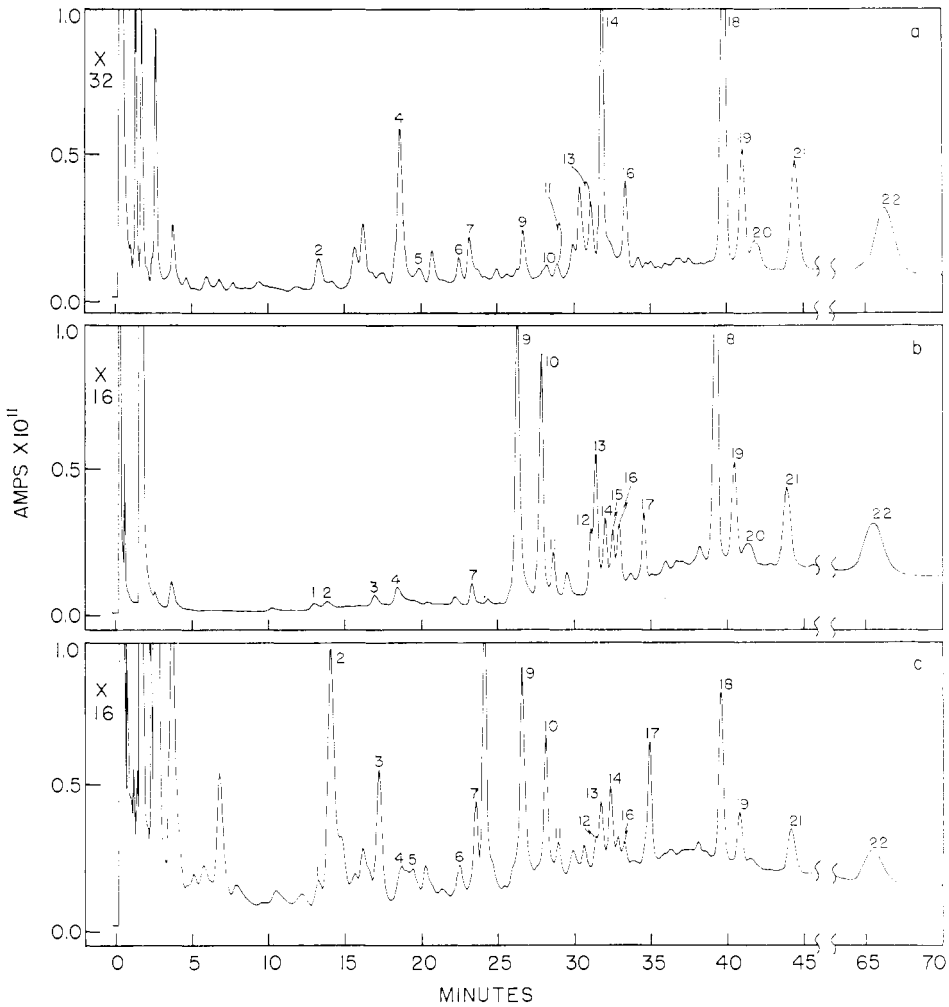


**Figure 2. Gas chromatograms of phenolic fractions prepared from: A. generator condensers B. nonspecific absorber inside smokehouse C. solid carbon dioxide–ethanol trap at smokehouse outlet. Component identities are given in Table I**

An Aerograph Model 1200 gas chromograph (Varian Aerograph, Walnut Creek, Calif.) equipped with a flame ionization detector was used.

Chromograms of phenolic fractions of smoke condensates collected from various parts of the smoking system are shown in Figure 2. A chromatogram of the phenolic fraction of smoked pork belly is shown in Figure 3. Figure 4 is a chromatogram of the phenolic fraction isolated from the commercial summer sausage.

Prior to gas chromatographic analysis, all samples were concentrated until the odor of solvent could no longer be detected while purging with nitrogen. Final volumes were approximately 0.05 ml for the condensate collected from the nonspecific absorber inside the smokehouse, 0.25 ml for the condensate collected from the carbon dioxide–ethanol trap at the smokehouse outlet, 0.4 ml for the condensate at the generator condensers, and 0.05 ml for phenolic fractions isolated from the smoked meat products. Absolute yields of concentrates from each sample thus differed, depending on the quantity of phenolic fractions present. However, the relative concentrations of individual compounds in each concentrate may be compared. Concentrations of individual compounds identified were measured by comparing the peak heights of the separated components with those of known concentrations of standard compounds chromatographed under identical conditions.

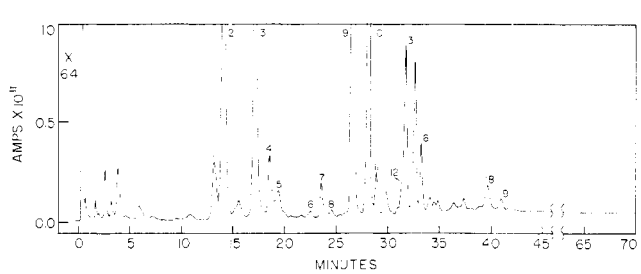


**Figure 3. Gas chromatogram of phenolic fraction prepared from pork belly strips exposed to hardwood sawdust smoke. Component identities are given in Table I**

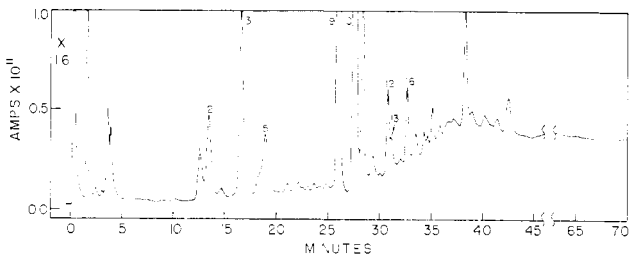


**Figure 4. Gas chromatogram of phenolic fraction isolated from commercially prepared hickory smoked summer sausage. Component identities are given in Table I. Identities of compounds eluted after peak 16 are unknown**

**Table I. Components of Phenolic Fraction**

| Peak Number[a] | Identity[b] |
|---|---|
| 1 | 2-Hydroxy-3-methylcyclopent-2-en-1-one (Cyclotene) |
| 2 | *o*-Methoxyphenol (Guaiacol) |
| 3a | 2-Methoxy-4-methylphenol (4-Methylguaiacol) |
| 4 | 3-Hydroxy-2-methylpyrone (Maltol) |
| 5a | *m*-Methylphenol (*m*-Cresol) |
| 5b | *p*-Methylphenol (*p*-Cresol) |
| 5c | 2-Methoxy-4-ethylphenol (4-Ethylguaiacol) |
| 6 | 2-Methoxy-4-allylphenol (Eugenol) |
| 7 | 2-Methoxy-4-vinylphenol (4-Vinylguaiacol) |
| 8 | 2-Methoxy-4-*cis*-propenylphenol (*cis*-Isoeugenol) |
| 9a | 2,6-Dimethoxyphenol (Syringol) |
| 9b | 2-Methoxy-4-*trans*-propenylphenol (*trans*-Isoeugenol) |
| 10 | 2,6-Dimethoxy-4-methylphenol |
| 11 | 2,6-Dimethoxy-4-ethylphenol |
| 12 | 2,6-Dimethoxy-4-allylphenol |
| 13 | 4-Hydroxy-3-methoxybenzaldehyde (Vanillin) |
| 14 | 2,6-Dimethoxy-4-vinylphenol |
| 15 | 2,6-Dimethoxy-4-*cis*-propenylphenol |
| 16 | 4′-Hydroxy-3′-methoxyacetophenone (Acetovanillone) |
| 17 | 2,6-Dimethoxy-4-*trans*-propenylphenol |
| 18 | 4-Hydroxy-3,5-dimethoxybenzaldehyde (Syringaldehyde) |
| 19 | 4′-Hydroxy-3′,5′-dimethoxyacetophenone (Acetosyringone) |
| 20 | 4′-Hydroxy-3′,5′-dimethoxypropiophenone (Propiosyringone) |
| 21 | 3-(4′-Hydroxy-3′-methoxyphenyl)-2-propenal (Coniferaldehyde) |
| 22 | 3-(4′-Hydroxy-3′.5′-dimethoxyphenyl)-2-propenal (Sinapaldehyde) |

[a] Peak numbers are assigned only to identified components. They do not correspond to peak numbers previously reported using a different GC column (Lustre and Issenberg, 1969). [b] Identities were established by GC–MS.

**Identification of Components of the Phenolic Fraction.** Identities of components in phenolic fractions were tentatively established from retention data on the 5% Carbowax 20M TPA column and confirmed by gas chromatography–mass spectrometry (GC–MS) using an Hitachi-Perkin Elmer RMU-7 mass spectrometer. Identification was considered positive when gas chromatographic retention data and mass spectra of the unknown peak coincided with those of reference compounds or data previously collected during identification of components of the phenolic fraction of wood smoke condensates (Lustre and Issenberg, 1969).

Gas chromatographic conditions used during recording retention data and mass spectra were identical to those described above. The procedure used for recording mass spectral data has been described previously (Lustre and Issenberg, 1969).

During GC–MS analysis every peak was scanned repeatedly to check for the presence of mixtures. Table I is a list of the identities of the numbered peaks in chromatograms of phenolic fractions (Figures 2–4).

## RESULTS AND DISCUSSION

A phenolic fraction can be isolated from 100-g quantities of smoked meat by extraction with 5% sodium hydroxide. Gas chromatographic separation and mass spectral analysis were sufficient for the identification of individual components in this fraction. Interference from phenolic compounds in the meat itself was shown by blank analysis to be absent. This was expected, as phenols are not natural constituents of meat tissue.

**Table II. Concentrations of Phenolic Compounds from Smoked Pork Belly and Smoked Sausage**

| Peak No. | Compound | Concentration: Pork Belly mg/100 g of meat | Pork Belly %[a] | Summer Sausage mg/100 g of meat | Summer Sausage %[a] |
|---|---|---|---|---|---|
| 1 | Cyclotene | 1.01 | 12 | 0.08 | 3 |
| 2 | Guaiacol | 3.56 | 40 | 0.10 | 4 |
| 3a | 4-Methylguaiacol | 2.86 | 34 | 0.70 | 28 |
| 3b | Phenol | | | | |
| 4 | Maltol | 1.80 | 21 | ... | ... |
| 5a | *meta*-Cresol | | | | |
| 5b | *para*-Cresol | 0.18 | 2 | 0.07 | 2 |
| 5c | 4-Ethylguaiacol | | | | |
| 6 | Eugenol | 0.10 | 1 | ... | ... |
| 7 | 4-Vinylguaiacol | 0.60 | 7 | ... | ... |
| 8 | *cis*-Isoeugenol | 0.10 | 1 | ... | ... |
| 9a | Syringol | 8.40 | 100 | 2.45 | 100 |
| 9b | *trans*-Isoeugenol | | | | |
| 10 | 2,6-Dimethoxy-4-methylphenol | 3.56 | 43 | 1.52 | 60 |
| 11 | 2,6-Dimethoxy-4-ethylphenol | 0.87 | 10 | 0.58 | 23 |
| 12 | 2,6-Dimethoxy-4-allylphenol | 0.38 | 4 | 0.15 | 6 |
| 13 | Vanillin | 2.34 | 28 | 0.20 | 8 |
| 16 | Acetovanillone | 0.93 | 11 | 0.10 | 4 |
| 18 | Syringaldehyde | 0.83 | 9 | ... | ... |
| 19 | Acetosyringone | 0.44 | 5 | ... | ... |

[a] Relative to syringol.

The component recovered in greatest quantity from the smoked pork belly was syringol. Other compounds present in moderate amounts were guaiacol, 4-methylguaiacol, 2,6-dimethoxy-4-methylphenol, vanillin, acetovanillone, cyclotene, and maltol. Phenol was a minor component. A comparison of the relative amounts of phenol and of 4-methylguaiacol, which is eluted in the same GC peak, was made by mass spectral analysis. The molecular ion was the base peak for both compounds and the entire GC peak was scanned repeatedly during the analysis. The GC peak contained only 5% phenol.

Specific smoke components present in the phenolic fraction isolated from smoked pork belly and commercial summer sausage are reported for the first time. A list of these compounds and their approximate concentrations are shown in Table II.

Most of the compounds recovered from the smoked pork belly were also recovered from the commercial summer sausage. Syringol was also the major component. 4-Methylguaiacol, 2,6-dimethoxy-4-methylphenol, and 2,6-dimethoxy-4-ethylphenol were present in relatively large amounts. Guaiacol and vanillin were found in lower concentrations, and syringaldehyde, 4-vinylguaiacol, and maltol, which were present in the smoked pork belly product, were absent. Since the processing and storage history of this sample is not known, the significance of the absence of these compounds in the commercially smoked product cannot be determined.

All phenolic compounds, and maltol and cyclotene, which were recovered from the smoked meats, are compounds present in smoke condensates collected in this laboratory (Lustre and Issenberg, 1969). A few of the phenols present in smoke condensates were not recovered from the smoked meats. 2,6-Dimethoxy-4-vinylphenol, 2,6-dimethoxy-4-*cis*- and -*trans*-propenylphenol, propiosyringone, coniferaldehyde, and sinapaldehyde were not detected. Some of these compounds are minor components of smoke condensates and it is possible

that they were not deposited in the meat in quantities sufficient for recovery. Failure to recover coniferaldehyde and sinapaldehyde cannot be satisfactorily explained on this basis, for these compounds are major components of smoke condensates. Figure 2c shows that coniferaldehyde (peak 21) and sinapaldehyde (peak 22) were recovered from the nonspecific absorber and the condensate collected at the smokehouse outlet. This indicates that they were present in the oven and passed through the pork belly samples. Failure to recover them from the smoked product could be due to their participation in reactions with meat proteins. This is likely, as both compounds contain carbonyl groups and an activated double bond that is sensitive to addition reactions. When synthesized (Lustre and Issenberg, 1969), they were found to produce, on contact with proteinaceous tissue, an orange coloration that closely resembled the color of smoked pork belly. Their possible significance in color formation in smoked foods should be investigated.

The phenolic concentrate from smoked pork belly and from commercial summer sausage had an aroma that closely resembled the smoked product. Cyclotene, maltol, vanillin, and acetovanillone are compounds with potent characteristic aromas. Maltol and cyclotene are flavor enhancers and may have an important influence on flavor of foods even at very low concentrations.

The chromatogram in Figure 2b shows that guaiacol and 4-methylguaiacol are too volatile to condense inside the smokehouse at 65° C. They were recovered from the pork belly (Figure 3) exposed to smoke at the same temperature and for the same length of time. This suggests that these compounds were deposited or retained in meat by mechanisms other than simple condensation. For the higher boiling phenols, it is more difficult to differentiate between deposition by simple condensation or by other processes, as these compounds were condensed in the oven at the smoking temperatures used.

Total phenols recovered from 100 g of pork belly, as calculated from Table II, was 28 mg. Recovery from the summer sausage was 6 mg. Fourteen of the phenols recovered from the pork belly, representing 47% of the total weight of phenols, and seven of the phenols recovered from the summer sausage, representing 55% of the total phenols, are para-substituted. These compounds would not be detected by the 4-aminoantipyrene or the Gibbs colorimetric methods which have been used for the analysis of total phenols in food products (Tucker, 1942; Proctor *et al.*, 1959; Bratzler *et al.*, 1969). Most investigators have also used phenol as their standard in colorimetric assays. Results show that phenol is a minor component of the phenolic fraction. Its use as a standard in colorimetric estimations of total phenols could result in an underestimation of the total phenols present.


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