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SMOKE

Composition of Hickory Sawdust Smoke. Low-Boiling Constituents

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Thirteen volatile components derived from hickory wood smoke have been separated by gas-liquid chromatography. Acetaldehyde, acetone, methyl formate, methyl acetate methyl alcohol, biacetyl, and acetol were each identified by both gas-liquid chromatographic retention time and infrared spectrum. Carbon monoxide, carbon dioxide, and methane were identified by infrared only, while furan and/or propionaldehyde, 2-methylfuran, isopropyl alcohol, ethanol and/or isovaleraldehyde, methyl vinyl ketone, and crotonaldehyde were identified tentatively by GLC retention time. The formation of methyl acetate and methyl formate and the decrease in concentration of methyl vinyl ketone occur as secondary reactions in the smoke condensate upon standing. The nature and extent of these chemical changes have not been reported previously.

Wood smoke, as used for the processing of foodstuffs, is a complex mixture of many classes of compounds. The authors have reported the identification of a number of phenolic and furyl compounds present in a fraction of the hickory sawdust smoke (*1*). Although others have claimed that the smoky aroma of foodstuffs exposed to smoke is due to the phenols (*5, 11*), volatile components such as acids, alcohols, and carbonyls are present also. These may modify the effect of the phenols in the development of the characteristic aroma.

The acids, from C_1 to C_{10}, have been identified (*2, 4, 5, 8*), as well as a number of ketones and aldehydes (*3, 6, 7, 8, 10*). A number of alcohols also have been reported (*3, 7, 12*). However, as observed for the phenols, the number of components reported by most of the previous investigators has been limited, and only a few of the compounds have been identified in more than one smoke preparation. The most extensive study of low-boiling smoke components has been that of Hoff and Kapsalopoulou (*3*), who identified 20 compounds by the combined use of a syringe microtechnique—in which classes of compounds were removed from the sample—and gas-liquid chromatography (GLC). Variations in the parameters of producing smoke and the differences in the techniques of isolating and identifying the components make it difficult to extrapolate the results reported in the literature to other smoke preparations. Since studies of meat processing are likely to involve a knowledge of smoke composition and reactions, this laboratory is engaged in the development of reproducible methods of smoke generation which can be used to study the effects of variables such as combustion temperature, air flow, sawdust source, and others on the chemical composition of smoke in order to determine optimum parameters for the most desirable smoke. The data reported in this paper present further information on the composition of controlled, reproducibly generated hickory sawdust smoke consisting of the volatile components present in smoke preparations, as well as some chemical changes which occur upon standing.

Experimental

The smoke used in this study was generated in a laboratory apparatus (Figure 1) in which various parameters of smoke generation could be controlled. The smoke generator was constructed of a borosilicate glass tube 12 inches long by $2^1/_4$ inches in diameter and fitted with stainless steel ends. The cylinder was wrapped with a 2-foot × 1-inch, 110-volt, 192-watt flexible heating tape and the rate of heating controlled with a Variac. The sawdust charge was placed in the vertically positioned generator over a 3-inch layer of borosilicate glass wool covering the bottom plate. Three thermocouples were fitted into the apparatus to measure the temperatures at the center and at the periphery of the sawdust bed as well as the temperature of the smoke 1 inch above the sawdust bed. Twenty-gram quantities of hickory sawdust having a moisture content of about 7% were heated to temperatures ranging between 250° and 450° C. Air was passed through the generator at a constant flow of 800 ml. per minute.

The smoke samples were obtained in two ways: Whole smoke condensate was collected by passing the smoke first through a water-cooled condenser and air-cooled trap, then through two dry ice–acetone traps. The condensates were

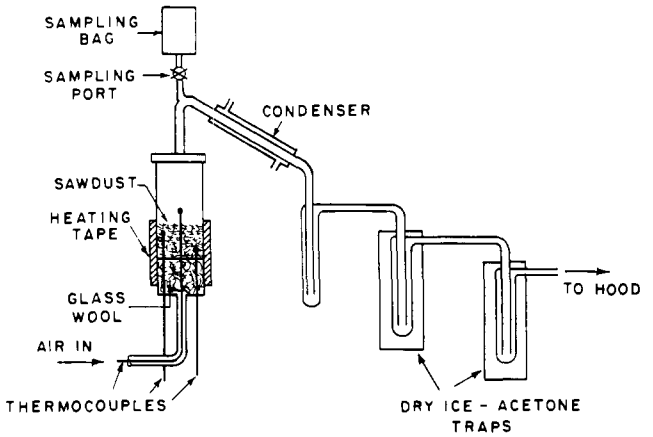

Figure 1. Smoke generator and traps for the production and collection of smoke and smoke condensates

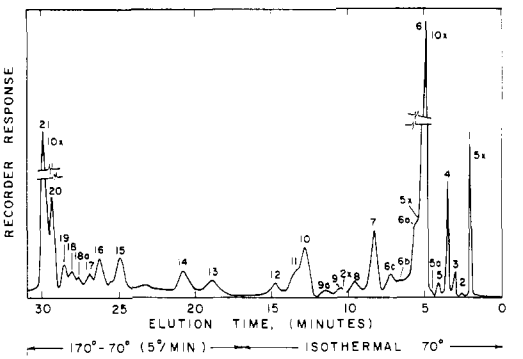

Figure 2. Gas-liquid chromatogram of the volatile components present in hickory sawdust smoke condensate

combined and had a total volume of about 5 ml. Smoke vapor was collected adjacent to the exit port of the generator in a polyethylene bag. The sampling port was approximately 12 inches from the hot reaction zone. About 3 minutes after heavy smoke evolution began, a portion of the smoke was diverted into the bag for a period of 1 minute. The total heating time was 45 minutes. Heavy smoke was visible for about 15 minutes during this period.

The whole smoke and the condensate were subjected to gas-liquid chromatography. Components of the smoke were separated using a Perkin-Elmer Model 800 gas chromatograph with a flame ionization detector and fitted with a 4 to 1 ratio stream splitter. A single column $10^1/_2$ feet long by $^1/_4$ inch in diameter, packed with 30% Carbowax 20M on 60- to 80-mesh Gas Chrom P was used. The operating conditions were: isothermal heating at 70° C. for 17 minutes, then programmed heating at a rate of 5° per minute to 170° C., helium carrier gas flow at 100 ml. per minute and the injection port and detector temperatures at 210° and 200° C., respectively. Sample size used for analysis was 10 μl. of smoke condensate and 5 ml. of whole smoke.

As only one type of column was used for the gas chromatographic studies, the chromatograms of the components were arbitrarily divided into fractions to simplify the analysis of the smoke. Thus, the term "volatile fraction" as used in this paper refers to that portion of the smoke components up to and including the peak that elutes at 30 minutes, which includes virtually all the aliphatic aldehydes, alcohols, and ketones. As this classification is based on the elution time of the components, the authors recognized that with another column and under different operating conditions the composition of their volatile fraction could vary.

Preliminary identification of the various smoke components was made by comparing the retention times with those of known compounds. Many of the individual components were trapped in dry ice–chilled capillary tubes that were attached to the exit port of the gas chromatograph. The tubes were rinsed with approximately 4 μl. of carbon tetrachloride and the solutions transferred to 0.1-mm. path length NaCl type D microcavity cells obtained from the Barnes Engineering Co., Stanford, Conn. The infrared spectra were obtained on a Perkin-Elmer Model 421 spectrophotometer fitted with a beam condenser. Several of the low-boiling components of the volatile portion were extremely difficult to trap and transfer at dry ice temperatures. For these compounds, the GLC effluent was led directly into a 7.5-cm. path length stainless steel infrared gas cell equipped with Irtran windows. The cell could be cooled by direct contact with dry ice. Valves on the cell ports permitted the trapping of the desired peak. The cell served both as a trap and an analytical chamber and eliminated sample losses due to transfer operations.

The hickory sawdust used in this work was obtained as such from Koch Supplies, Inc. and is the same as used in commercial smoking operations. Authentic samples of chemicals used for the identification of smoke components were purchased from commercial sources.

Results and Discussion

Component Identification. Figure 2 is a representative chromatogram of the volatile components of whole smoke condensate analyzed 80 minutes after collection. The identification of the components, both positive and tentative, is listed in Table I. Identification of a component is considered positive only when both the GLC elution time and the infrared spectrum agree with those of an authentic sample. The volatile composition of the smoke from successive runs was qualitatively reproducible. The major components of the condensate, based on peak area, are acetaldehyde, acetone, methanol, biacetyl, and acetol. The following were tentatively identified, based on GLC retention time: peak 3, furan and/or propionaldehyde; peak 5a, 2-methylfuran; peak 6a, isopropyl alcohol; and peak 6b, ethanol and/or isovaleraldehyde. Peak 11 was tentatively identified as crotonaldehyde based on retention time and the fact that it formed a hydrazone when bubbled through 2,4-

Table I. Low-Boiling Components of Hickory Smoke

Peak No.	Identity	GLC	IR	Other
A	Carbon monoxide, carbon dioxide, methane		+	
1	Acetaldehyde	+	+	
2	Methyl formate[a]			
3	Furan and/or propionaldehyde	+		
4	Acetone, methyl acetate[a]	+	+	
5				
5a	2-Methylfuran	+		
6	Methanol	+	+	
6a	Isopropyl alcohol	+		
6b	Ethanol and/or isovaleraldehyde	+		
6c	Methyl vinyl ketone	+		
7	Biacetyl	+	+	Odor
8–10				
11	Crotonaldehyde	+		Positive DNP reaction
12–20				
21	Acetol	+	+	

[a] These two substances form upon standing.

dinitrophenylhydrazine reagent, thus indicating a carbonyl compound.

Attempts at trapping the remaining minor components were unsuccessful since these compounds were present in too low a concentration to yield recognizable infrared spectra. To complicate the identification of the minor components further, water—which does not produce a signal with the flame ionization detector—eluted during a large part of the 70° C. isothermal program and was trapped in the capillary collecting tube together with the desired substance. When the tube was rinsed with carbon tetrachloride, distribution of the compound between the water and the solvent could occur, reducing the amount of material available for infrared analysis. This would decrease the infrared absorption intensities to a point where spectral interpretation would become virtually impossible.

While the collection of smoke condensate is a convenient procedure for the study of smoke components, food products are generally exposed to whole smoke during processing. To determine, therefore, whether there were any differences between the composition of whole smoke and the condensate, the whole smoke was collected in a polyethylene bag and a 5.0-ml. volume introduced into the gas chromatograph by means of a gas-tight syringe. Figure 3 is a representative chromatogram of this material. Comparison of the chromatograms of the whole smoke and condensate (Figure 2) reveals several apparent differences. Peak A is a major constituent of the whole smoke but is not present in the condensate. This peak consists of several gases noncondensable at dry ice–acetone temperature at the partial pressures found in wood smoke. The gases comprising peak A were trapped in a 7.5-cm. path length infrared gas cell and the spectrum recorded. Since small molecules in the gas phase yield relatively simple yet extremely characteristic spectra, positive identification of the components of peak A was possible even though several compounds were present together. Substances identified were methane, carbon monoxide, and carbon dioxide. Peak 3—furan and/or propionaldehyde—is a major component of the whole smoke but is only a minor constituent of the condensate; peak 5a—2-methylfuran—is present also in greater relative concentration in the smoke. On the other hand, acetone (peak 4), methanol (peak 6), and biacetyl (peak 7) are major components of the condensate but not of the whole smoke. The minor components between peaks 10 and 21, which appear in the condensate, were not detected in the whole smoke.

Although quantitative calculations from the data are not possible, the differences in relative composition between the whole smoke fraction and the smoke condensate appear to be real.

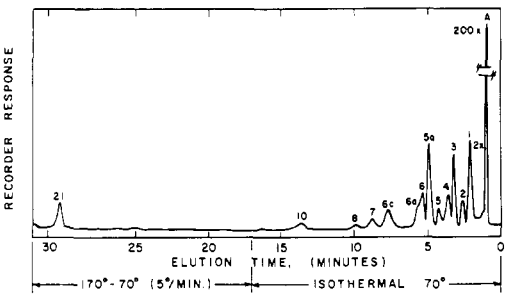

Figure 3. Gas-liquid chromatogram of volatile components present in hickory sawdust smoke

Esterification Studies. During the study of the composition of the low-boiling components of smoke, the condensates were stored at room temperature (25° C.) in tightly stoppered bottles. When they were used after several weeks of storage, some interesting changes had occurred. These changes are shown in Figure 4, *A*, *B*, and *C*.

Figure 4*A* is a chromatogram of the whole smoke condensate 2 hours after generation. Figure 4*B* shows the same condensate after 20 hours storage. There was a considerable increase in the concentration of peak 2. This was trapped subsequently in an infrared gas cell and identified as methyl formate.

After 20 hours of storage, a shoulder appeared on the left side of the acetone peak (arrow on peak 4, Figure 4*B*). After 72 hours (Figure 4*C*), this component had increased in concentration to such an extent that the acetone now appeared only as a shoulder (arrow in Figure 4*C*). Identification of this major component as methyl acetate was accomplished by trapping the eluting peaks in a gas cell and obtaining an infrared spectrum of the mixture. The infrared spectral band characteristics of acetone were subtracted from the spectrum of the mixture. The resultant spectrum was identical to the authentic sample of methyl acetate.

Figure 4 also shows the disappearance of methyl vinyl ketone (peak 6c). After 72 hours its disappearance is virtually complete.

A quantitative study was made of the formation of methyl formate and methyl acetate in the whole smoke condensate at 25° C. as a function of time. The values were obtained with GLC, using 10-μl. samples of smoke condensate at intervals over a period of 700 hours. A known amount of *p*-dioxane was added at the start as an internal standard to eliminate variations in sampling technique and gas-liquid chromatographic operating parameters. Results are shown in Figure 5. Molar concentrations of methyl formate and methyl acetate were obtained by injecting known quantities of authentic compounds into the gas chromatograph and calculating the corresponding peak areas. During the first 20-hour period the concentration of peak

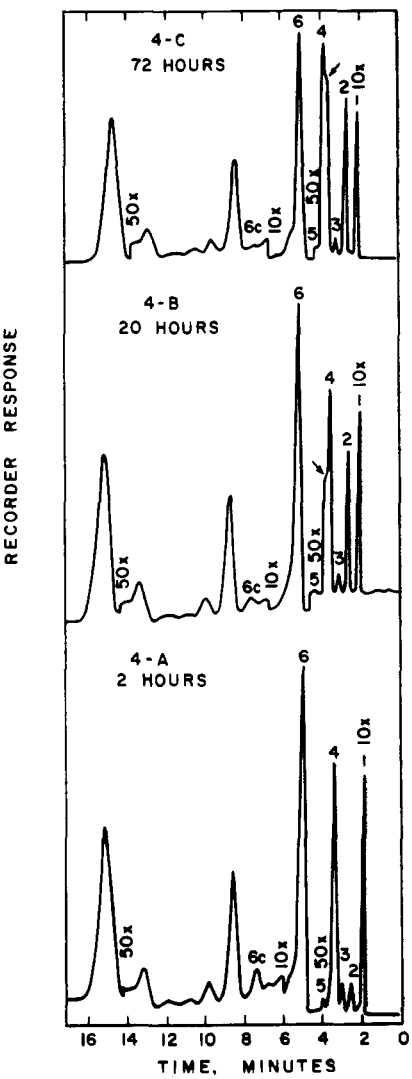

Figure 4. Gas chromatograms of the low-boiling components present in hickory sawdust smoke condensate over a period of time

Curve A, 2 hours; curve B, 20 hours; curve C, 72 hours

4 remained constant, indicating that peak 4 was due entirely to acetone, and no appreciable amount of methyl acetate was present. After 20 hours, peak 4 contained a mixture of methyl acetate and acetone. To obtain the concentration of methyl acetate alone, the concentration of the mixture was corrected for the quantity of acetone present in the

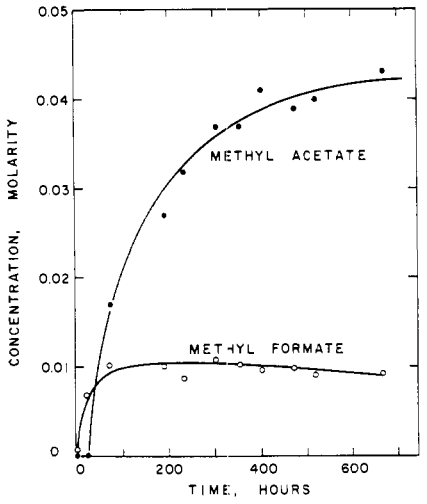

Figure 5. The formation of methyl acetate and methyl formate in whole smoke condensate as a function of time

initial period. Figure 5 also indicates that the rate of formation of methyl formate was extremely rapid and seemed to reach equilibrium after 70 hours. Methyl acetate, on the other hand, continued to form at a significant rate and had a concentration 4½ times that of methyl formate, even after a 700-hour period. Even though trace amounts of methyl acetate and methyl formate may be present in fresh smoke, the major portion results from secondary reactions which occur upon standing.

The formation of methyl acetate and methyl formate was followed in model experiments, using a mixture of methanol, formic acid, and acetic acid. The concentrations of the compounds used in the mixture were the same as those found in the smoke condensate. The quantities of methyl acetate and methyl formate formed and the rates of formation were comparable with those observed in the condensate.

The identification of methyl formate indicated the presence of formic acid in whole smoke condensate. The authors had been unable to detect this acid directly by the use of GLC. Positive identification of formic acid by GLC has not been accomplished because it decomposes on the hot metal inlet system of the apparatus (*9*). However, in other investigations on the composition of wood smoke, the presence of formic acid in substantial quantities has been established by the use of other methods (*4*, *5*, *7*, *8*).

Correlation studies between secondary reaction products and the organoleptic quality of food products treated with the condensate have not been made. The known odor qualities of the esters reported here, however, suggest the possibility that they may contribute to the development of a more mellow aroma of the smoke condensate upon standing.

Acknowledgment

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COMMUNICATIONS

ENZYME IDENTIFICATION

Nature of the Myrosinase Enzyme

Black, brown, and oriental mustard seeds were surveyed for myrosinase activity using methyl *n*-pentyl ketoxime-O-sulfonate (potassium salt) and sinigrin as the enzyme substrates. No component specific for the hydrolysis of oxime sulfonates was detected. Thus, the reported existence of a distinct enzyme, myrosulfatase, could not be confirmed.

THE ENZYME MYROSINASE is responsible for the development of the flavor and pungency of many food products, such as mustard and horseradish, by its hydrolysis of the thioglucosides. This enzyme has been considered to be a mixture of a thioglucosidase and myrosulfatase (*3*, *4*, *6*, *7*, *9*) in contrast to the concept of a single enzyme system (*2*, *5*, *8*) a thioglucosidase. The single enzyme theory describes the action of thioglucosidase on the mustard oil compounds as the hydrolysis of glucose from the thioglucoside followed by a Lossen rearrangement of the aglycone to give isothiocyanate and sulfate.

The work of Gaines and Goering (*3*, *4*) revived the controversy. These workers reported separating on diethylaminoethylcellulose (DEAE-C) the myrosulfatase and thioglucosidase com-