FRACTIONATION AND IDENTIFICATION OF SOME COMPOUNDS IN WOOD SMOKE

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ABSTRACT

FRACTIONATION AND IDENTIFICATION OF SOME COMPOUNDS IN WOOD SMOKE

by Roy Wayne Porter

A smoke generator was constructed with which the generation temperature could be controlled within reasonable limits. The total steam volatile and non-steam volatile phenols, acids, and carbonyls were determined on the condensates of whole smoke and the vapor phase generated at 350, 400, 450, 500 and 550°C. The steam volatile monocarboxylic acids generated at 450°C were separated on silicic acid-glycine columns buffered at a given pH. These acids were identified by paper chromatographing their ammonium salts along with the salts of known acids. The steam volatile monocarbonyls generated at 550°C were separated as their 2, 4 dinitrophenylhydrazone derivatives on nitromethane-hexane-celite columns. The chain lengths of the parent carbonyls were determined by comparing their column and paper chromatographic behavior with known derivatives. The classes of the parent carbonyl compounds were determined by comparing the absorption maxima of neutral solutions of their 2, 4 dinitrophenylhydrazone derivatives with the derivatives of known carbonyls. Final identification was made by observing a lack of depression in the melting points of mixtures of known and unknown derivatives in the instances where the unknown was present in sufficient quantity.

The condensates from whole smoke and the vapor phase were analyzed for 3, 4 benzopyrene by the use of activated alumina columns and the tars from the inside of a commercial smoke generator were analyzed in the same manner.

Roy Wayne Porter

A limited study of the comparison of smoke flavor intensity and preference as judged by a taste panel was conducted on cheddar cheese and bacon smoked with whole smoke and the vapor phase.

The total phenol production was the highest at a generation temperature of 500°C, total acids were the highest at 450°C, and the peak production of carbonyls was at 550°C. The C_1 to C_{10} aliphatic monocarboxylic acids were identified in the steam volatile portion of the whole smoke, with acetic, formic, propionic and butyric acids in decreasing order of occurrence making up the largest portion of the acids. The C_1 to C_4 acids only were detected by these methods in the vapor phase and their concentrations ranked in the same order as in the whole smoke. The steam volatile monocarbonyls identified were 2-pentanone, valeraldehyde, 2-butanone, butanal, acetone, propanal, crotonaldehyde and ethanal, with isovaleraldehyde and methanal being tentatively identified. No 3, 4 benzopyrene was identified in the condensate from either whole smoke or the vapor phase by the procedure used. A small amount was detected in the tars from a conventional smokehouse generator with the isolated sample showing some contamination as evidenced by its spectrum.

The cheese smoked with the vapor phase had a more intense smoke flavor than that smoked with whole smoke according to taste panel evaluations, with the opposite being indicated in the case of the smoked bacon. Yet, no preference was indicated for the cheese or bacon smoked by either the whole smoke or the vapor phase. This indicated that the taste panel did not consider the intensity of smoke flavor to be the major factor in determining preference.

FRACTIONATION AND IDENTIFICATION OF SOME COMPOUNDS IN WOOD SMOKE

By

Roy Wayne Porter

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INTRODUCTION

It is well known that the smoking of food products has a preserving effect on them as well as imparting a desirable color and flavor to them. The preservation is due mainly to the bacteriostatic, antioxidant and drying effect imparted to the food during the smoking process. However, the compounds responsible for these attributes are not well known.

There is considerable evidence that such factors as generation temperature and availability of air influence the composition of the smoke produced. Also, it has been shown that smoke is an aerosol containing condensed particles suspended in a continuous vapor phase. Evidence indicates that the compounds responsible for smoke flavor are imparted by vapor absorption into the surface and interstitial water during the cold smoking of fish. Fish smoked with the vapor phase only do not appear to differ in color, flavor and keeping quality from those smoked with whole smoke. Evidence has shown that the particulate phase of smoke plays a minor role in imparting smoke flavor to foods.

Several workers have indicated that the carbonyl, phenol, and acid groups of compounds are important flavor constituents. The steam volatile portion of smoke condensate appears to contain most of the characteristic odor and flavor constituents. The phenolic compounds have received the most attention as the constituents most likely responsible for smoke flavor from both a qualitative and quantitative standpoint. However, since the acids and carbonyls appear to be present in smoke in larger quantities than the phenols, a more detailed study of them would seem appropriate.

The objectives of this study were to:

(1) Construct a smoke generating unit with which the temperature of smoke generation could be controlled and the particle and vapor phase of the smoke separated electrostatically.

(2) Determine if the total amount of carbonyls, phenols, and acids were maintained in the steam distillate of the condensed vapor phase as well as the whole smoke generated at varying temperatures.

(3) Study the composition of the steam volatile acids and monocarbonyls by the use of column and paper chromatography.

(4) Analyze for the presence of 3, 4 benzopyrene in both the vapor phase and whole smoke.

REVIEW OF LITERATURE

Composition of Wood

The main components of wood are cellulose, lignin and hemicelluloses and these are located in the cell wall. When considering wood in general, Wise (1952) stated that one can assume it to be roughly one-half cellulose, one-fourth hemicellulose and one-fourth lignin. Cellulose is made up of a linear arrangement of D-gluco-pyranose units with B (1-4) glycosidic bonds according to Fruton and Simonds (1958). Lignin is defined by Brauns and Brauns (1960) as that constituent of wood when oxidized with nitrobenzene yields vanillin and syringaldehyde. The structure of lignin is unknown and Brauns and Brauns (1960) have presented a discussion of the various proposed theories. The hemicelluloses of wood are made up of the non-cellulose polysaccharide components of the cell wall according to Browning (1952). Montgomery et al. (1956) have shown that the hemicelluloses of the corn hull contain uronic acid units and give D-glucuronic acid and D-xylose on acid hydrolysis.

The principal carbohydrate components of wood are those aforementioned and they are located in the cell wall. However, according to Wise (1952), the water extract of wood contains starch, arabogalactans (yield arabinose and galactose on hydrolysis), pectic materials, and several glycosides. Wise (1952) also stated that aliphatic and aromatic hydrocarbons, terpenes, aliphatic and aromatic acids and their salts, alcohols, phenols, aldehydes, ketones and quinones, esters and ethers may be present in the extraneous components of wood and that certain woods may contain oils, resin acids, sterols, tannins, and cyclic polyhydric alcohols.

Hardwoods are used mostly for the generation of smoke for use in smoking foods. Hamilton and Thompson (1959) reported hardwoods to contain 40-45% cellulose and 20-30% hemicelluloses. The following data are presented from an analysis of several American hardwoods by Freeman and Peterson (1941) expressed as a percentage of dry wood:

	Sapwood (%)	Heartwood (%)
Holocellulose	76.20 - 80.96	70.85 - 81.58
Cellulose	55.58 - 62.67	50.96 - 64.42
Lignin	12.27 - 20.61	14.82 - 22.26
Ash	0.11 - 0.32	0.21 - 0.84
Soluble in hot water	1.30 - 4.03	0.43 - 2.48
Soluble in alcohol-benzene	0.97 - 3.31	0.96 - 6.44

where:

Holocellulose = all polysaccharides in the wood

soluble in hot water - simpler carbohydrates and soluble dyes

soluble in alcohol-benzene = waxes and fats

Holocellulose less cellulose = hemicellulose

Thermal Decomposition of Wood

It is necessary to distinguish between the combustion and destructive distillation of wood. Hawley (1952) stated that destructive distillation precedes combustion and is the release of volatile combustible and incombustible products as well as the formation of charcoal when wood is heated in a restricted supply of air. The same author indicated that combustion is the actual burning of the combustible products of destructive

distillation (volatile combustibles and charcoal) when their ignition temperature is reached and there is sufficient air available. The smoke used for smoking foods is generally produced, according to the same author, during limited combustion due to minimum air supply, thus allowing a large portion of the volatile combustible products to escape unburned.

The terms pyrolysis, thermal decomposition, carbonization, dry distillation, and destructive distillation have all been used quite interchangeably in the literature, thus, destructive distillation will be used to cover all of these terms.

According to Hawley (1923), the actual destructive distillation of wood begins at about 250°C; soon becomes exothermic (around 280°C); and is completed at about 350°C. Fraps (1901), and later, Goos (1952) have summarized a list of over 200 compounds that are formed by the destructive distillation of wood and found in the condensable pyroligneous acid and tars. Moisture tends to decrease the speed at which destructive distillation takes place according to Hawley (1923). Later, Hawley (1952) stated that moisture also serves to retard the rate at which combustion can proceed in wood.

Cellulose

Cellulose, when heated, first breaks down to 1, 6 anhydro-glucose according to Pictet and Sarasin (1918). Irvine and Oldham (1921) have suggested that this is the result of a reaction involving two steps, the first being the hydrolysis of cellulose to glucose and the second the dehydration of glucose to 1, 6 anhydroglucose. Pictet and Sarasin (1918)

also indicated that further heating causes 1, 6 anhydroglucose to quickly break down into products such as acetic acid, phenols, water and acetone.

Hemicellulose

The hemicellulose from hardwoods is made up mainly of pentosans and the characteristic thermal breakdown products expected are furan and its derivatives according to Goos (1952). However, Merritt and White (1943) obtained a yield of only slightly above 4% furfural from the destructive distillation of oak wood. Goos (1952) indicated that the low yields of furfural are probably attributable to secondary reactions and that under conditions of destructive distillation, the pentosans break down into simpler compounds. Goos (1952) also stated that the pentosans yield larger amounts of acids than cellulose or lignin. Hawley and Wiertelak (1951) and Merritt and White (1943) have shown that the hemicelluloses are the least heat stable of the components of wood and tend to break down first.

Lignin

In the case of hardwoods, the phenolic compounds are the typical thermal breakdown products formed on the thermal decomposition of lignin according to Goos (1952). The same author indicated that a great many of the phenolic compounds consist of guaiacol and of pyrogallol 1, 3- dimethyl ether, their homologues and derivatives.

Fletcher and Harris (1952) destructively distilled lignin at 400-445°C for 7.5 hours and obtained 15-25% aqueous distillate from which a heavy tar separated. The aqueous solution contained methanol, acetone

and the following acids: formic, acetic, propionic, butyric, valeric, caproic, enanthic, caprylic and benzoic. Steam volatile phenols included phenol, O and P cresol, guaiacol, 2, 4-Xylenol and 4-methyl-, 4-ethyl- and 4-propyl-guaiacol. In addition, there was a quantity of non-steam volatile phenols. Gilbert and Lindsey (1957) found that lignin and cellulose both formed polycyclic hydrocarbons when heated at 650°C for 1 hour in a nitrogen atmosphere.

Smoke Generators

Smoldering wood

Pettit and Lane (1940) developed an experimental kiln for smoking fish wherein the air-flow during the generation of smoke could be controlled. Hicks et al. (1941) described a smoking unit which utilized smoldering wood and allowed for live steam to be incorporated into the smoke stream to increase the relative humidity of the smoke and which used high speed fans to move the smoke at high velocities.

Nicol (1960) reported the design of a smoke generator utilizing a new principle whereby sawdust was maintained in a state of violent agitation in a vertical tube by a continuous blast of hot air. The density, and to a certain degree, the composition of the smoke was regulated by the temperature of the air and the amount of sawdust fed into the stream.

For experimental purposes, Spanyar et al. (1960) built a generator consisting of a tube 18 cm. long and 3 cm. in diameter wrapped with a heating tape and insulation. Sawdust was burned in 0.5 gm. charges and the range of combustion temperatures was measured by a potentiometer.

Air was metered through the tube during the combustion process. A similar arrangement was used by Husaini and Cooper (1957) in their study. These workers used an iron pipe 36 inches long and 3 inches in diameter packed loosely with sawdust. A stream of air (1-2 p.s.i.) was introduced at one end, the sawdust ignited and allowed to burn from the other end. However, no arrangement was made to measure the actual combustion temperature.

Simpson (1961) later described a two-stage experimental smoke generator designed in Poland whereby blocks of wood were pyrolyzed in a stream of inert gas at any desired temperature up to 450°C and the resulting smoke oxidized in the second stage by mixing with controlled quantities of heated air.

Friction Generators

Anonymous (1956) described a friction-type smoke generator which utilized a 6 x 6 x 35 inch piece of hardwood standing vertically on a turning disc. The disc was 9 1/2 inches in diameter and consisted of 8 teeth of carbonized steel blades turning at 1750 r.p.m. The amount of smoke produced was controlled by the weights applied to the top end of the piece of wood. The smoke was sprayed with water and then filtered through a 5 ply metal screen to remove sparks.

Husaini and Cooper (1957), using the same principles described above, constructed a small laboratory scale friction smoke generator, which utilized 1.5 x 1.5 x 6 inch blocks of wood, to compare the smoke produced with that from smoldering sawdust. With slight modifications, this type

generator was used by Weir et al. (1961) to determine the suitability of smoke produced by friction generation for use in processing frankfurters.

Smoke Generation

The smoke used for smoking foods, according to Hawley (1952) is produced during limited combustion in a minimum air supply that allows a large portion of the volatile combustible products to escape unburned. Various workers have shown that generation temperature influences the composition of the resulting smoke and there is general agreement on the optimum range of generation temperature. Tilgner (1958) recommended a generation temperature below 300°C and Kuriyama (1960) suggested the best range to be 260°C to 310°C. Rusz (1960) indicated 280°C to 350°C to be the most applicable for smoke generation.

Tilgner et al. (1960a) used a two-stage smoke generator and suggested a destruction temperature of 400°C and an oxidation temperature of 250°C for the production of a good phenol in the resulting smoke. These workers found that the best conditions for acid production was 50°C lower for both the destruction and oxidation stages in smoke generation. Tilgner et al. (1960b), in another study, found that both the phenol and acid content of the smoke increased in the 300°C to 400°C range. They also found that in the oxidation step the phenol level increased between 125°C and 200°C and then fell below the original level and that the acid content increased through the entire range of oxidation temperatures studied (125°C - 300°C). Spanyar et al. (1960b) found that the products of incomplete combustion remained constant from wood decomposed between 180°C and 440°C.

Pettit and Lane (1940) reported that an air flow of 800 cubic feet per hour was required to produce the desired smoke constituents in maximum amounts. These workers found formaldehyde, diacetyl, acetone, furfuraldehyde, 5-methylfurfuraldehyde, methyl alcohol, ethyl alcohol, phenols, acetic acid and formic acid to be present in smoke condensed in traps. They also found that under conditions favoring the destructive distillation of wood, the production of phenols and particularly the production of aliphatic acids is greatly enhanced. Tilgner et al. (1960a) stated that an air surplus factor of 8 was required for optimal production of phenolic compounds in smoke. Spanyar et al. (1960b) noted that secondary chemical reactions took place in the smoke, notably the polymerization of oxy- and di- carbonyl compounds.

Rusz (1960) studied the composition of smokes produced by various hard and soft woods and found little difference between them. Spanyar et al. (1960b) reported no consistent variation in the smokes produced by hard and soft woods. Rusz (1960) and Spanyar et al. (1960b) also indicated that variations in water content of the sawdust did not alter the chemical composition of the smoke.

Husaini and Cooper (1957) found that smoke produced by a friction generator contained four times as many steam volatile acids, eight times as many steam volatile carbonyl compounds and more steam volatile phenols than smoke from smoldering sawdust. These workers stated that most of the smoke odor and flavor occurred in the steam volatile fraction of the smoke condensate, while the non-steam volatile fraction retained most of the color, but negligible smoke odor and flavor. They also found that

most of the acids in the smoke condensate were steam volatile and of these acids, acetic occurred in the highest concentration, followed by formic, propionic and butyric in decreasing amounts.

Rusz (1960) reported smoke produced by a friction generator to contain four times as many aldehydes, twice as many acids and 15% more phenols than smoke produced by smoldering sawdust. In a similar study, Tilgner et al. (1960c) indicated that dense smoke from a friction generator contained 1.8 times as many carboxylic acids, 1.2 times as many phenols and twice as many carbonyl compounds as smoke from smoldering sawdust. In another study, Tilgner et al. (1960d) found that friction produced smoke contained 1.3 times as many acids and 1.5 times as many carbonyls as smoke from smoldering sawdust.

Tilgner (1958b) stated that benzopyrene and dibenzanthracene are carcinogenic compounds found in wood smoke. He indicated that the formation of these compounds can be minimized by a destruction temperature below 300°C or redistilling the smoke condensate under 200°C.

Smoke Deposition

Foster (1959) reported that smoke consists of minute visible particles condensed around nuclei which are suspended in a continuous vapor phase. He indicated that the average radius of these suspended particles was between 0.1 and 0.2 microns.

Tilgner (1958a) stated that the gaseous components of wood smoke are more important than the colloidal smoke components. Foster and Simpson (1961) found no significant differences in appearance, flavor and

keeping quality of normally smoked and vapor smoked kippers. They concluded that in the cold smoking process used for fish that the deposition of smoke flavor appears to be a vapor absorption process. They also found that the absorption of smoke vapors was enhanced by increased water content in the fish and increased smoke velocity in the smoking chamber. A limited study with bacon by the same workers indicated that the process of smoke absorption was similar to that in fish. Foster et al. (1961), in a study to determine the role of the particle phase in smoke deposition, indicated that it can serve as a reservoir of compounds, which are released into the vapor phase when the supply of the latter is diminished in the smoke. These authors stated that this contribution amounts to only about 5% of the smoke vapors absorbed.

Tucker (1942), using phenol content as an indication of smoke penetration, found most of the phenol concentrated on the surface of smoked hams. Linton and French (1945) reported the steam distillable phenols to be a satisfactory measure of the degree of smoking, acetone not as accurate as phenols and, although considerable quantities of steam volatile acids are taken up by fish during smoking, their amount was not a good criteria for degree of smoking. These workers also indicated that the rate of deposition of smoke on fish increased with increasing smoke velocity and, although phenol content is not directly related to color development during smoking, it does increase with the appearance of the yellow smoke color.

Rusz (1959) described a procedure for distilling the phenols and carbonyls from meat tissue and found it to contain 0.5 - 5.0 mg. % phenols, and traces to 1.0 mg. % of carbonyl compounds. Proctor (1959) has

presented an evaluation of various methods of estimating the compounds deposited during the smoking process. Spanyar and Kevei (1961) conducted a study using 10% gelatin and lard as model absorbents for smoking. They found that gelatin absorbed more acids and carbonyls but less phenol compounds than the lard and that placing the gelatin in a natural casing considerably reduced the smoke constituents absorbed. Also, they indicated that most of the absorption was on the surface of the gelatin models and gelatin smoked without a casing and allowed to stand 5 days showed a considerable loss in amount of smoke constituents originally absorbed, yet during this same period of time there was a slight migration of some of the compounds toward the center. These workers found that various natural and synthetic sausage casings possess different characteristic abilities to absorb various groups of compounds in smoke.

Modification of the Smoking Process

Watts and Faulkner (1954) stated that liquid smokes usually are made from a base of a modified form of smoke condensate from hardwood heated in a closed container. Lapshin and Flekchanov (1960) have described the preparation of a liquid smoke which is used with conventional smoking in the smoke-curing of meats.

Hanley et al. (1955a) developed a process whereby smoke particles are charged by passing the smoke between opposed plates with wire grids carrying a high electrical potential (40,000 V). Bacon, maintained at ground potential was suspended between the opposing plates and the charged smoke particles were deposited on the bacon by electrical attraction.

Design and application of the equipment for a continuous smoking system utilizing this principle, with an infrared oven for heating the product, has been reported by Hanley et al. (1955b). The authors reported that a processing shrink of less than 0.5% can be obtained with this system. Foster (1959) has reported the same principle of electrostatic smoking being used in smoking fish, sausages and other meat products.

Properties acquired During Smoking

Cooper and Linton (1936) described the optimum smoking of fillets to be the point where they possess a smooth, glossy surface, a characteristic smoke flavor, a golden color, and are dried during the process. Hanley et al. (1955a) stated that the functions of a smokehouse in the meat packing industry are smoking, heating and drying. Smoking is intended to improve the color and palatability as well as the keeping quality of the product, according to Tilgner (1957).

White et al. (1942) reported that the smoking of bacon reduced the number of surface bacteria 10,000 times, permitted the fat to resist the formation of rancidity, and was superior in flavor when compared with unsmoked bacon. These workers concluded that smoked Wiltshire bacon should keep approximately twice as long as unsmoked bacon. In a later study, White (1944) found that smoked Wiltshire bacon could be stored at least 2 months without becoming rancid, whereas unsmoked bacon usually became rancid after 1 month of storage at the same temperature (-1°C to -18°C). Spoilage caused by the formation of free fatty acids was found to be of little importance.

Watts and Faulkner (1954) studied the antioxidant effects of four commercial liquid smokes, and found them to vary from no effect to a pronounced antioxidant effect. These workers also found that smoke and ascorbic acid have a synergistic effect as antioxidants. Erdman et al. (1954) used liquid smoke to determine the preservative effect of smoke on fatty fish. They reported much lower peroxide values for fish with added smoke flavor than without. Also, they found that fish, both salted and smoked, had better keeping qualities than fish which had only been salted. The same authors stated that ascorbic acid added to the fish along with salt did not protect the fish against the development of rancidity. However, when small amounts of smoke were added, the ascorbic acid acted synergistically with the smoke, resulting in a strong antioxidant effect. These workers also found that liquid smoke in concentrations of 0.4% and 0.8% proved toxic to pure cultures of Staphylococcus aureus, Bacillus subtilis and Proteus vulgaris after being exposed to the liquid smoke for various lengths of time. A liquid smoke concentration of 0.08% decreased bacterial growth but did not stop the growth of the three organisms studied.

Kemp et al. (1961) found that smoking decreased the development of rancidity and the formation of free fatty acids in dry-cured hams. These effects increased as smoking temperature increased. Also, they found that increasing temperature of smoking had a tenderizing effect on the hams.

Hess (1928) reported a pronounced bacteriostatic action of smoke on the growth of organisms in fish fillets, but noticed a decided resistance of bacterial spores to the smoke. Jensen (1943) stated that the nonsporing

psychrophiles active during the curing of bacon are destroyed along with the nonsporing mesophiles in the course of the smoking process. However, thermophiles in sausage items can spoil the food product if not inhibited by 3.5% sodium chloride, and molds will also grow in many cured and smoked meats, according to the same author.

Gibbons et al. (1954) found that the smoking of bacon resulted in considerable weight loss and destruction of bacteria. Smoke density and temperature were the most important factors affecting the bactericidal effect of the smoke.

Hedrick et al. (1960a) in a study to improve the flavor of smoked cheddar cheese found that a moderate smoke flavor in a medium cured cheese was favored over the same smoke intensity in aged cheese. This final product was best obtained when 2 lb. bars of cheese were exposed to smoke from hardwood sawdust for 4-6 hours at a smokehouse temperature below 85°F. Surface mold occurred after extended storage at temperatures favorable to mold growth. The addition of liquid smoke concentrate to whole milk prior to setting did not produce an equally acceptable product.

In a later study, Hedrick et al. (1960b) reported that smoke from hardwood sawdust had a bactericidal effect on the surface organisms of cheddar cheese. Most of the destruction of surface bacteria occurred in the first 3 hours of exposure to the smoke. One hour of exposure to smoke was effective in destroying viable-yeast and mold on the cheese surfaces.

EXPERIMENTAL PROCEDURE

Smoke Generator

It was necessary to design a smoke generating unit which would produce sufficient smoke to smoke food products for taste panel evaluation, provide a means of removing the particle phase of the smoke, and allow control of the generation temperature of the smoke to be analyzed. To accomplish this, an electric hotplate was employed to heat sawdust which was sifted onto the top of the hotplate by a vibrating pan suspended from a hopper. The amount of smoke produced was regulated by the rate at which the sawdust was sifted onto the hotplate. To separate the particle phase from the whole smoke, an electrostatic air filter (Trion 1962) was attached to the enclosed smoke chamber around the hotplate. An on-off type controller utilizing an expanding gas sensing element on the hotplate, wired into the line supplying current to the hotplate gave insufficient control of the generation temperature. Thus, a variable rheostat was wired into the power line to the hotplate and the smoke generation temperature was maintained by manually adjusting the rheostat at a constant rate of sawdust flow to the hotplate. The smoke generation temperature was measured by a Leeds and Northrup, null-current potentiometer, calibrated in degrees Fahrenheit with a thermocouple lead going to the surface of the hotplate at the point where the sawdust dropped from the vibrating pan.

A schematic diagram of the smoke generating unit including the electrostatic air filter is presented in figure 1.

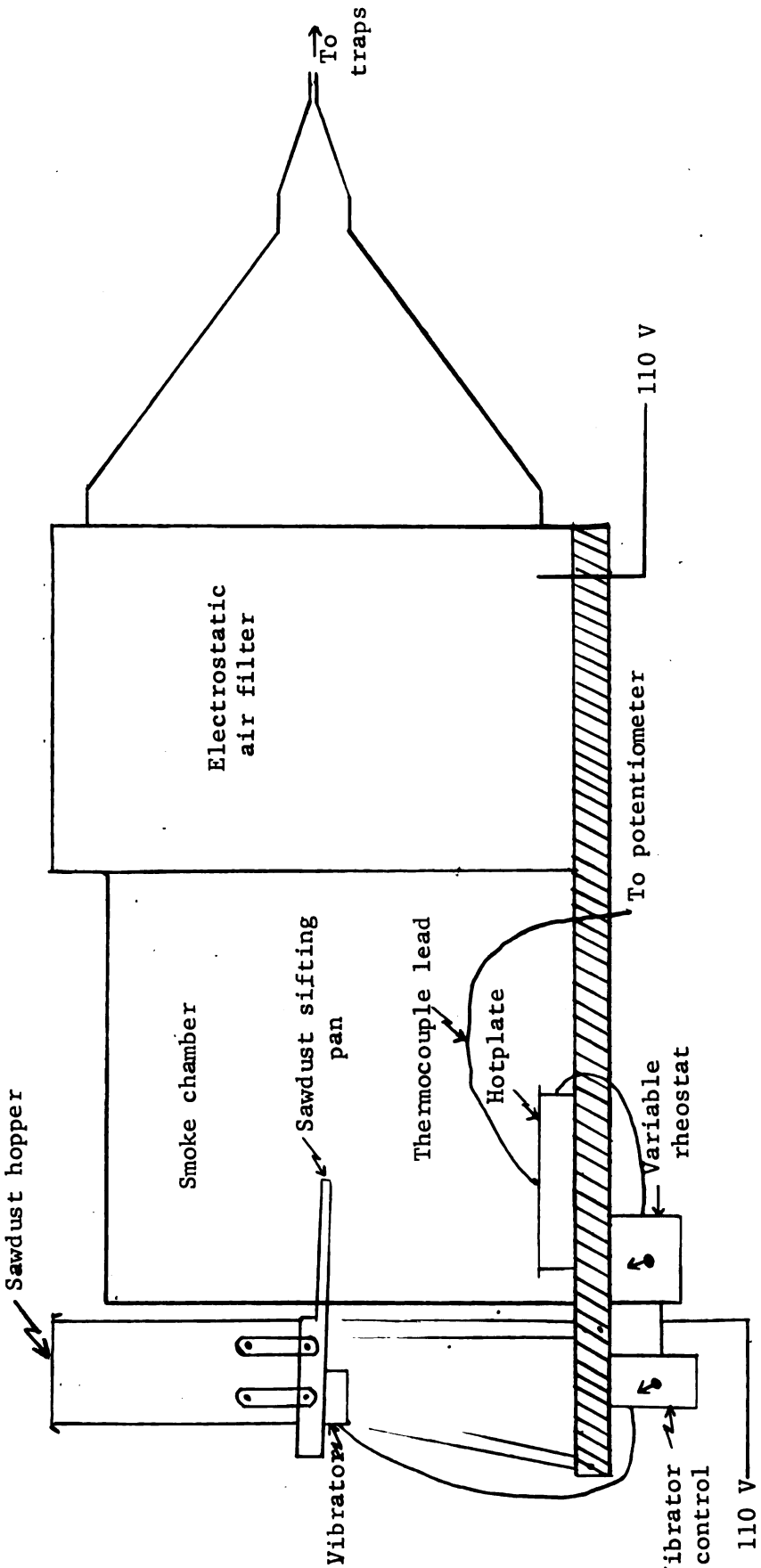

Figure 1. Schematic diagram of smoke generating unit.

Source and Treatment of Sawdust

The sawdust used in this study was from hard maple wood (acer saccharum), obtained at a local sawmill. The sawdust was screened twice through hardware cloth having one-quarter inch mesh. It was then stored in a loosely covered drum and contained 12% moisture before storage. Prior to being used for the production of smoke the sawdust was heated at 85°C for 12 hours in an open pan and allowed to cool in the atmosphere at room temperature resulting in a moisture content ranging from 3.50 to 5.18%.

Smoke Generation and Collection

Before each run the moisture content of the sawdust was determined by heating previously weighed duplicate samples for 24 hours at 100°C, after cooling in a dessicator the samples were reweighed and the weight loss was calculated as the percent moisture in the original sample.

Smoke was generated in the previously described generator at five different generation temperatures, beginning at 350°C and increasing by 50°C intervals up to 550°C. The lower limit of 350°C was established by the fact that below this temperature, the production of smoke was too inefficient to have any practical application for the smoking of food products. This conclusion was arrived at following the heating of sawdust in a 200 ml. boiling flask placed in a mineral oil bath, with the boiling flask connected to a coiled trap placed in an ethanol-dry ice bath. Heating different samples of sawdust each for a 4 hour interval at temperatures starting at 250°C and increasing to 300°C by 10°C steps did not show any condensable products until 290°C, and was more pronounced at

300°C. However, complete breakdown did not occur until the bath temperature was raised to 350°C. The upper limit of 550° was the highest temperature of generation which could be adequately controlled.

The smoke was generated at these temperatures with the sawdust flow as uniform as possible. The sawdust flow rate was determined before collection of the smoke was started by collecting the sawdust being sifted onto the hotplate over two 5 minute intervals, and sawdust flow was expressed as grams per hour. The temperature of the surface of the hotplate was observed every 15-20 minutes and only the rheostat setting was varied to maintain the desired temperature.

Collection of the smoke was accomplished by four 250 ml. suction flasks connected in series and placed in an ethanol-dry ice bath. The last flask was connected to a vacuum line and the vacuum adjusted so that there was less than 50 ft. per min. air flow at the outlet of the smoke generator. This resulted in an air flow around the hotplate which was too small to be measured by an air flow meter. The collection period was 12 hours and the extremes observed in the generation temperature over this period were recorded in each case. Duplicate samples were collected at each generation temperature of both whole smoke and of the vapor phase, the latter obtained by precipitating out the particle phase with the electrostatic air cleaner, which remained off while collecting whole smoke.

Analysis of Smoke Condensate

The condensed smoke was allowed to thaw at 4°C, and the volume of total condensed liquid was measured. Two 5 ml. aliquots of the condensed

smoke were each steam distilled at the rate of 550 ml. per hour, until 50 ml. of steam distillate was collected. Steam distillation was done immediately after thawing the smoke condensate. The 50 ml. of steam distillate contained the steam volatile portion of the smoke condensate and was treated as follows: 25 ml. was analyzed for total phenols; 10 ml. was diluted up to 50 ml. with distilled water and analyzed for total acids; 10 ml. was analyzed for total carbonyl compounds; the remaining 5 ml. was discarded. The portion remaining in the distillation flask represented the non-steam volatile portion of the smoke condensate and was diluted up to the same volume of 250 ml. in each case with distilled water. This was treated as follows: 100 ml. was analyzed for total phenols; 100 ml. was used for the analysis of total acid content; 25 ml. was employed in the analysis for total carbonyl compounds. The remaining 25 ml. was discarded in each case.

Total Phenols

The steam volatile samples to be analyzed for total phenols were first extracted by a modification of the method of Braus et al. (1952). After diluting up to 25 ml. with distilled water, the steam volatile sample was saturated with NaCl and made slightly acid with 0.1 N. HCl. It was then extracted four times with 25 ml. aliquots of diethyl ether in a separatory funnel. The ether layers were then pooled and subsequently washed with 15 ml. of a 5% HCl solution and the HCl layer was then discarded. At this point the combined ether layers were extracted with four 50 ml. portions of a 5% NaOH solution and the ether layer was then discarded. The NaOH extract was cooled and saturated with Co_2 by adding dry

ice in small pieces until the temperature reached 5°C. This NaOH extract was then extracted four times with 25 ml. aliquots of diethyl ether and the combined ether layers were then dried over anhydrous Na_2SO_4. The ether extract was then placed in a 250 ml. boiling flask which was connected to a water cooled condenser and the ether was distilled off by placing the boiling flask in an electric heating mantle. The last of the ether was taken off very slowly, and the residue comprised the phenolic compounds which was taken up by thoroughly washing the boiling flask with three 15 ml. aliquots of distilled water. Although some of the phenolic compounds are more soluble in water than others, it was quite easy to completely dissolve the residue by letting the first 2 portions of water soak in the flask for a few minutes.

The non-steam volatile samples for phenol analysis were extracted in much the same manner except that the solution was not diluted. Also, four 50 ml. aliquots of diethyl ether were used to extract the solution after it was saturated with NaCl and made acid with HCl. The rest of the procedure was the same as that used for the steam volatile samples. The solution containing the phenolic compounds was then analyzed for total phenols.

The total phenol content of these samples was determined by the method of Warshowsky et al. (1948). The sulfanilic acid reagent used in this procedure was prepared daily as follows: 0.9 gms. of sulfanilic acid was mixed with 9 ml. of 12 N. HCl and diluted up to 100 ml. with distilled water in a volumetric flask. In another 100 ml. volumetric flask, 5 gms. of $NaNO_2$ was dissolved in distilled water and diluted up to the 100 ml.

mark. Three ml. of the sulfanilic acid solution was then cooled in a 100 ml. volumetric flask in an ice water bath. After cooling, 5 ml. of the $NaNO_2$ solution was added and the mixture was allowed to cool for another 5 minutes, after which time an additional 12 ml. of the $NaNO_2$ was added. This final mixture was made up to 100 ml. with distilled water and this was designated as the final sulfanilic acid reagent. This solution was kept in a cooler at 4°C and was used after standing at least 15 minutes.

The colorimetric determination on the unknown phenol solutions was carried out as follows: aliquots containing 1, 3, 5 and 7 ml. of the unknown solutions were pipetted into separate test tubes and 1 ml. of a 20% $Na_2 CO_3$ solution was added to each sample aliquot. These tubes were then diluted up to 8 ml. by adding distilled water. Two ml. of the cold, final sulfanilic acid reagent was then added to each test tube. The tubes were then shaken for 2-3 minutes and read against a blank at 425 mu. The blank contained 1 ml. of 20% $Na_2 CO_3$, 2 ml. of the cold, final sulfanilic acid reagent, and 7 ml. of distilled water. The optical densities of the various unknown solutions were compared with a standard curve to determine the concentration of phenols in the solution. This standard curve was prepared from phenol solutions varying from 1 to 20 micrograms per ml. The concentration of total phenols was then calculated as micrograms of phenol (steam volatile or non-steam volatile) per ml. of the original sample of smoke condensate subjected to steam distillation.

Total Acids

The steam volatile samples for total acid analysis were placed in

100 ml. beakers and titrated to pH 7.0 with an automatic titrator. The non-steam volatile samples were placed in 250 ml. beakers for titration. In each case 0.1 N NaOH which was previously standardized against standard dilute (0.1 N) H_2SO_4 was used to titrate the samples. The concentration of total acids was expressed as milliequivalents of acid (steam volatile and non-steam volatile) per ml. of the original sample of smoke condensate which was steam distilled.

Total Carbonyls

The total carbonyl content was determined by the gravimetric procedure of Iddles and Jackson (1934). The 10 ml. samples from the steam volatile portion of the smoke condensate were each pipetted into large test tubes, followed by the addition of 10 ml. of a saturated solution of 2, 4 dinitrophenylhydrazine in 2 N. HCl. This reaction mixture was then allowed to stand for one hour in an ice-water bath. The precipitate was then retained on Whatman #42 acid washed filter paper which had been previously folded and weighed. The hydrazones were then thoroughly washed with 2 N. HCl and then with water before drying in a dessicator over $CaCl_2$ and under a slight vacuum. When the hydrazones reached a constant weight, they were weighed on an analytical balance.

The non-steam volatile samples for total carbonyl analysis (25 ml.) were pipetted into 125 erlenmeyer flasks and had 25 ml. of the 2, 4 dinitrophenylhydrazine solution added to them. Otherwise, the treatment of these samples was identical to that of the samples from the steam volatile portion of the smoke condensate.

The relative concentration of total carbonyl compounds was expressed as milligrams of acetaldehyde (steam volatile and non-steam volatile) per ml. of the original condensed smoke sample subjected to steam distillation. This was done by multiplying the weight of the 2, 4 dinitrophenylhydrazone derivative obtained, by the following ratio:

$$\frac{\text{molecular wt. of acetaldehyde (44.05)}}{\text{molecular wt. of acetaldehyde 2, 4 dinitrophenylhydrazone (224.18)}} = .197$$

Chromatography of Volatile Acids

The steam volatile acids produced at a combustion temperature of 450° C were subjected to chromatographic analysis. This particular generation temperature was chosen for chromatographic analysis because it was at this temperature that the production of total acids appeared to be the highest.

The method of Corcoran (1956) was used for the partition chromatography of the volatile monocarboxylic acids. This method employed the use of acid-washed, chromatographic grade, 100 mesh silicic acid as a supporting medium for a stationary phase of 2 M glycine. The glycine had been previously adjusted with concentrated NaOH or HCl to a selected pH. To effectively cover the range of volatile monocarboxylic acids from formic to capric, it was necessary to use three different columns, pH 2.0, pH 8.4 and pH 10.0.

Preparation of Silicic Acid

Silicic acid, Mallinckrodt chromatography grade, 100 mesh in 200 gm. lots as needed, was suspended in 10 N. HCl for a period of 36 hours in a

600 ml. beaker covered by a large watchglass. After the suspension period, the supernatant liquid was decanted and the silicic acid washed with water to remove the HCl. Approximately 25 to 30 washings with water were necessary to make the gel acid free. At this point the silicic acid was washed with absolute methanol until the alcohol washings were neutral to litmus paper and generally required 2 to 3 such washings. The silicic acid was then washed with anhydrous ether and placed in an open dish in a dessicator over phosphorous pentoxide. A slight vacuum was maintained in the dessicator, and the silicic acid was dried for 3 days with several changes of phosphorous pentoxide. The silicic acid was then sufficiently dry and was stored in airtight glass jars prior to use.

Preparation of the Aqueous Phase

A 2 M stock solution of the glycine stationary phase was prepared and stored in a refrigerator. The pH of the stationary phase was very critical and it was necessary to standardize the pH meter before each titration of aliquots of the glycine phase. Also, it was necessary to use standard buffer solutions to standardize the pH meter which were close to the pH desired in the aqueous glycine phase to be titrated. Before titrating an aliquot of the 2 M stock solution of the glycine phase, it was taken from the refrigerator and allowed to come to room temperature.

The glycine phase at pH 2.0 was prepared by adding 1.0 N. HCl to a 25 ml. aliquot of the stock solution until pH 2.0 was reached. The glycine phase at pH 8.4 and pH 10.0 were prepared by adding 1.0 N. NaOH to

25 ml. aliquots of stock solution until the desired pH was reached. The glycine phase in each case was titrated just prior to preparation and development of the respective columns.

Preparation of the Columns

To prepare the column, 22 ml. of the appropriate pH glycine phase and 25 gms. of the acid-washed silicic acid were ground together in a 250 ml. beaker for 5 minutes, using a test tube as a pestle. Seventy-five ml. of the first eluent 1% n-butanol - 99% chloroform, was gradually introduced with constant stirring to this misture to form a smooth slurry. A sintered glass disc was placed in the bottom of a chromatographic column (2.0 cm. x 42 cm.) to retain the silicic acid. The slurry was then added in 4 to 5 small increments, with compaction by a wooden cork on the end of a glass rod between each increment, and the excess solvent is allowed to drain from the end of the column. Care was taken throughout never to allow the column packing to become dry, and this was accomplished by keeping a slight excess of eluent on the top of the column at all times. At this point, two thicknesses of a paper milk filter disc were cut to fit the inside of the column and were pressed onto the top of column to firm and level the top layer.

Preparation of known Acids and Operation of Columns

Chloroform solutions of reagent pure monocarboxylic acids were prepared and a composite solution of the acids was made, and the concentration of each acid was varied to simplify their identification when eluted from the column. Composite samples of formic, acetic, propionic and butyric

acids were prepared to run on the pH 2.0 columns. Samples containing butyric, valeric, caproic and heptylic acids were prepared for the pH 8.4 columns. Composite samples containing heptylic, caprylic, nonylic and capric acids were also prepared to run on the pH 10.0 columns.

An aliquot of the composite sample prepared for the respective pH column was pipetted onto the top of the proper column and allowed to percolate into the silicic acid. Simultaneously, the fraction collector (Rinco VE 2002-B24) was started and the collection of 5 ml. fractions begun. The sides of the column were washed three times with 5 ml. aliquots of the first eluent and these washings were each allowed to percolate into the column. The column was then filled with 1% n-butanol 99% chloroform and the rest of the eluent placed in a 300 ml. separatory funnel connected to the column by a tygon tube. A slight amount of nitrogen pressure was applied, sufficient to maintain a flow rate of approximately 5 ml. per minute. A total of 200 ml. of each of the three eluents was used, the concentrations of n-butanol being 1%, 10%, and 25% in chloroform, and was the same for every column.

One-hundred and twenty fractions were collected from each column and each fraction was titrated with approximately .03 N NaOH in absolute methanol, using 1% m-cresol purple in 95% methanol as the indicator. A stream of nitrogen was applied through a glass tube drawn out to a fine tip to each test tube for agitation during the titration to exclude the carbon dioxide from the air. The time necessary to complete the development of one column was approximately 2 hours. To prevent broadening of the peaks eluted from the columns, it was found to be necessary to apply

the sample and develop the column as soon as possible after the column was prepared.

The efficiency of the various pH columns was determined by checking the recovery of various charges of known acids from these columns. The recovery of the known acids placed on the columns is given in table 1 along with the particular column on which the acids were resolved.

Table 1. Recovery of monocarboxylic acids placed on various pH columns.

Acid	pH of column	Meq. of acid		
		Charge	Recovery	Difference
formic	2.0	.5550	.5498	-.0002
acetic	2.0	.3661	.3654	-.0007
propionic	2.0	.2655	.2666	+.0011
butyric	8.4	.2007	.2018	+.0011
valeric	8.4	.1674	.1688	+.0014
caproic	8.4	.1290	.1305	+.0015
heptylic	10.0	.0650	.0670	+.0020
caprylic	10.0	.0951	.0969	+.0018
nonylic	10.0	.0573	.0592	+.0019
capric	10.0	.0766	.0757	-.0009

Preparation of Unknown Acids

The steam volatile monocarboxylic acids were prepared for chromatography directly as their sodium salts by a modification of the method of Buyske *et al.* (1957). The condensed smoke was extracted immediately upon thawing with four 50 ml. aliquots of ether. The ether was immediately

extracted five times with 100 ml. portions of 0.5% NaOH and these basic extracts were pooled and the pH adjusted to 8.0 with H_2SO_4. To avoid the addition of too much H_2SO_4, the pH was adjusted down to approximately 8.5 with 24 N. H_2SO_4 and then 1 N. H_2SO_4 was used to further lower the pH to 8.0. This solution was then concentrated under vacuum using a rotary evaporator, to a volume of less than 100 ml. The pH of the concentrated solution was then adjusted to 2.5 with a saturated solution of tartaric acid. This mixture was then steam distilled until a volume of 500 ml. of distillate had been collected. The steam distillate was then titrated to pH 7.0 with 0.3 N NaOH and concentrated to dryness under vacuum with the use of a rotary evaporator.

Column Chromatography of Unknown Acids

The dried sodium salts of the volatile acids from a given amount of smoke condensate was taken up in a small amount of 2 M glycine (generally 4 ml.). An 0.8 ml. aliquot of the glycine phase containing the unknown acids was adjusted to pH 2.0 with 1.0 N HCl. One gram of the previously described, acid-washed silicic acid was then stirred in and the damp powder was mixed with 0.5 ml. of chloroform to produce a jelly-like consistency in the mixture. This was then transferred to the top of a previously prepared column (pH 2.0), prepared the same as described earlier except that the paper discs were placed on top of the sample and compressed with a cork on the end of a glass rod. Three 2 ml. portions of the first eluent were used to wash the sides of the column and each portion was allowed to percolate onto the column before the next was added. The fraction collector was started at the same time the first portion of eluent was placed on the

column and 5 ml. fractions were collected. The operation of the column was the same as for the known acids, except that .01 N NaOH in absolute methanol was used to titrate the fractions.

Since acetic acid was present in by far the greatest quantity in the samples of smoke condensate, it was necessary to devise a means of obtaining the longer chain acids in conveniently titratable amounts without flooding the column with acetic acid. This was accomplished by first placing the sodium salts from 15 ml. of smoke condensate on pH 2.0 columns as described above, and collecting the first 60 ml. of eluate from the column and neutralizing this with 0.1 N NaOH. By repeating this procedure and pooling the neutralized fractions and taking this to dryness under vacuum, it was possible to concentrate the sodium salts of the acids butyric and higher, since these acids come off first, leaving propionic, acetic and formic behind. Samples of the sodium salts of the acids prepared in this manner were taken up in 0.8 ml. of 2 M glycine. The pH of this mixture was adjusted to that of the stationary phase of the column on which it was to be placed (pH 8.4 or 10.0) by the addition of 1.0 N NaOH. The remainder of the preparation of these samples and development of the columns was identical with that of the samples run on the pH 2.0 columns. Elution patterns for the columns were prepared by plotting milliliters of base required for each 5 ml. fraction collected against the accumulated volume of effluent passing through the column. This was performed on samples from both whole and the vapor phase of smoke.

Identification of Unknown Acids

Identification of the acids from the smoke samples was based on a

comparison of their retention volumes with those of known acid mixtures when run on separate, but identically prepared columns. This served as tentative identification, and conclusive evidence for the identification of these acids was gained by chromatographing the unknown acids along with known acids on paper as their ammonium salts. This was accomplished by pooling each elution peak, concentrating under vacuum, making acid with 0.1 N HCl and steam distilling the acid from the indicator. The steam distillate was then made basic by adding excess concentrated NH_4OH and was then concentrated under vacuum to a small convenient volume of approximately 25 ml.

The prepared ammonium salts of the unknown acids were first chromatographed by the method described by Buyske et al. (1957) and were spotted along with the ammonium salts of known acids, on a line 4 cm. from the bottom of a 30 cm. x 43 cm. sheet of Whatman No. 1 paper. The spots were placed 3 cm. apart and the paper was coiled into a cylinder with the edges stapled for rigidity. These sheets were then allowed to stand in a cylindrical jar, 24 cm. x 46 cm. containing a solvent system of n-butanol-water-propylamine (100-15-1, V/V). These chromatograms were developed for 16 hours in an ascending direction using 1500 ml. of solvent, and the excess primary amine was removed by placing the developed chromatogram in a 100°C oven for 12 minutes. The acids were then located as their primary amine salts by dipping the paper into a solution of 0.1% ninhydrin in chloroform giving a purple color on a white background. The spots were then circled for permanent record, because they faded after a few days.

The method of Kennedy and Barker (1951) involving a solvent system of 95% ethanol and concentrated NH_4OH (100-1, V/V) was used in addition. This method allowed the use of the same samples of ammonium salts that were prepared for the butanol-water-propylamine solvent system. Whatman No. 1 paper was thoroughly washed in a shallow pan with 1% oxalic acid and rinsed several times with copious amounts of distilled water and hung by clips to dry at room temperature. Next, 25 x 45 cm. sheets of this paper were spotted 2.5 cm. from the bottom and the spots were placed 3 cm. apart. The paper was coiled into a cylinder and stapled for rigidity, then stood in a 24 x 46 cm. jar containing 300 ml. of solvent and developed in an ascending direction for 7 hours. The developed chromatograms were then placed in a 100°C oven for 5 minutes. The spots were located by spraying the chromatogram with a solution of 50 mg. of bromophenol blue in 100 ml. of water made acid with 200 mg. of citric acid. The spots showed up blue on an orange-yellow background and these spots were circled for permanent record. This solvent system worked well except that it would not resolve nonylic and capric acid mixtures, whereas the butanol-water-propylamine system would.

All of the paper chromatograms were developed at room temperature. The R_f values were calculated for both the known and unknown acids run in both solvent systems, R_f being the ratio of the distance the acid moved to the distance moved by the solvent front.

Separation and Identification of Volatile Monocarbonyls

The steam volatile monocarbonyls from condensed whole smoke generated at 550°C were converted to their 2, 4 dinitrophenylhydrazones and subjected

to the column partition chromatographic procedure of Day et al. (1960). This is a modification of the method of Kramer and Van Duin (1954) and involves the use of nitromethane as the immobile phase supported on celite, with hexane serving as the mobile phase through the chromatographic column.

The carbonyls from whole smoke generated at 550°C were selected for study because this temperature more closely resembled commercial smoke generating conditions than lower temperatures. One hundred ml. of this condensate was steam distilled at atmospheric pressure in two 50 ml. portions for 4 hours. Each time the distillate was allowed to react with 2 liters of a solution of 2 gms. of 2, 4 dinitrophenylhydrazine per liter of 2 N HCl. This reaction mixture was allowed to stand for 12 hours, after which the 2, 4 dinitrophenylhydrazones were exhaustively extracted from the aqueous mixture with chloroform. The chloroform extract (2 liters) was washed four times with 500 ml. aliquots of distilled water to remove the residual HCl. A dry mixture of the 2, 4 dinitrophenylhydrazones was obtained by evaporating the chloroform under vacuum. This dry mixture was then extracted with hexane equilibrated with 2% of its volume of nitromethane (nitromethane layer then discarded), the hexane having first been redistilled from KOH pellets (Day et al., 1960) and the fraction boiling between 68 and 70°C collected. The equilibrated hexane extract concentrated to 320 ml. under vacuum, contained the major portion of the monocarbonyl derivatives. The remaining residue was made up of excess reagent and bis -2, 4 dinitrophenylhydrazones (Day et al., 1960) and was discarded.

Column Chromatography of Unknown Monocarbonyls

The equilibrated hexane extract (320 ml.) was first fractionated into a poorly separated forerun containing the 2, 4 dinitrophenylhydrazones of the longer chain carbonyls, and individual fractions of the C_1 to C_4 carbonyl derivatives. This was done by chromatographing 20 ml. aliquots of this extract on 20 gm. columns and required a total of 16 columns. These columns were prepared by the method of Day et al. (1960) by placing 20 gms. of celite (Johns Manville Analytical Filter Aid) in the mixing bowl of a Waring Blendor, followed by 200 ml. of equilibrated hexane. The speed of the Waring Blendor was regulated by a rheostat until the mixture showed a distinct rolling action, at which time 15 ml. of nitromethane was slowly added to the slurry and the mixing was continued for 10 minutes. After mixing the slurry was placed in a 250 ml. beaker for subsequent addition to the chromatographic column. The column (2.5 x 19.5 cm.) was prepared by first tamping a glass wool plug into the constricted end and aliquots of the slurry were placed in the column. Between aliquots a slight amount of air pressure was applied to compact the slurry. Care was taken always to maintain a solvent layer above the celite to prevent it from becoming dry and to avoid air pockets in the column during packing. When all of the slurry had been transferred to the column, it was packed with air until the solvent layer was about 1 cm. above the celite. A paper milk filter disc was cut to fit the inside diameter of the column (2.5 cm.) and the final compaction of the column was accomplished by compressing this paper disc onto the top of the celite with a cork fastened to the end of a glass rod. The paper disc served two functions by providing

a means of forming a firm, level surface of the celite and allowed the application of the sample and subsequent washing of the column without disturbing the surface of the celite. The solvent used in packing the column was discarded in each case. The finished column was packed to a height of approximately 15 cm.

A slight amount of pressure was then applied to the column to lower the solvent layer level with the surface, at which time a 20 ml. aliquot of the equilibrated hexane extract was placed on the top of the column and gently forced into the surface of the celite with air pressure. Two 5 ml. aliquots of equilibrated hexane were used to wash the sides of the column with each being forced into the celite with a slight amount of air pressure. The top of the column above the celite was then filled with equilibrated hexane, and a separatory funnel (1 liter) was then filled with equilibrated hexane and allowed to drain into the top of the column through a tygon tube and the column allowed to develop. The fractions (forerun and individual fractions) were collected visually from the column as it developed, and the corresponding fractions from all 16 columns were pooled. Each of the individual pooled fractions were concentrated under vacuum to as small a volume as possible without allowing crystallization to occur. These concentrated fractions were then rechromatographed in 15 ml. aliquots on identical (20 gm.) columns as described above and further fractionated into individual bands. The like bands from this second fractionation were then concentrated and applied again to columns (20 gm.) identical to those used in the two previous fractionations. At this point, no further separation occurred, and the volume of mobile phase

required to move each band to the lower edge of the column (when first color is observed in the eluate) was carefully measured for each band. This was used to calculate the threshold volumes for each band for the particular column used. The threshold volume was calculated as the volume of mobile phase (in this case, equilibrated hexane) required per gram of celite to move the band to the lower edge of the column. This value was highly reproducible for the fractions obtained and could be related to the structure of the derivatives (Van Duin, 1957) (Day et al., 1960). The individual bands which occurred close to each other on the column in the last two final fractionations (only 2 of which did) so as to not be adequately separated for visual collection, were collected in 25 ml. fractions of the eluate and their absorption was scanned in the 325 to 400 mu region with a Beckman DU spectrophotometer. The fractions having maximum absorption at the same wavelength were pooled and were subsequently rechromatographed and by this procedure sufficient quantities of the pure fractions were obtained for further study (Day and Lillard, 1960). After separation of the 2, 4 dinitrophenylhydrazones by column partition chromatography, the eluates were dried under vacuum and when possible, the residues were recrystallized from low boiling petroleum ether and the resulting crystals or residue was saved for later study.

The pooled forerun fraction obtained from the first column (20 gm.) fractionation was then concentrated under vacuum to a conveniently small volume, without allowing crystallization to occur. This was subjected in 15 ml. aliquots to column partition chromatography designed for C_5 and longer derivatives by another type of column described by Day et al. (1960).

These columns were prepared by placing 60 grams of celite in a Waring Blendor and adding 600 ml. of the redistilled hexane. Mixing was increased by adjusting the rheostat until the mixture exhibited a distinct rolling action, then 66 ml. of nitromethane was slowly added to the slurry and the mixing continued for 10 minutes. This slurry was then packed in a 3.0 x 60 cm. column containing a sintered glass disc at the bottom to retain the celite. The packing procedure, sample application, and development was carried out the same as for the 20 gram columns, except that redistilled hexane was used as the mobile phase. This fractionation resulted in a diffuse forerun which was barely recognizable (no attempt was made to identify this fraction), and individually separated bands. The corresponding bands were pooled, concentrated under vacuum and rechromatographed on identically prepared (60 gm.) columns. The volume of mobile phase required in each case, to move a particular band to the lower edge of the column was noted for the purpose of calculating the threshold volume of each band. The eluates of the separated bands having the same threshold volumes were pooled, dried under vacuum and, where possible, the residues were recrystallized and saved for further study.

The threshold volumes of the individual fractions were compared with literature values for various 2, 4 dinitrophenylhydrazones on the particular columns used. The 2, 4 dinitrophenylhydrazone derivatives of known carbonyl compounds having similar threshold volumes with the unknown fractions were prepared by the method of Shriner et al. (1956). To prepare the 2, 4 dinitrophenylhydrazine solution, 0.4 gm. of 2, 4 dinitrophenylhydrazine was placed in a 25 ml. Erlenmeyer flask followed by the

addition of 2 ml. of 12 N H_2SO_4. Next, 3 ml. of water was added dropwise with swirling until solution was complete, followed by the addition of 10 ml. of 95% ethanol to this solution. To prepare the carbonyl solution, 0.5 gm. of the pure carbonyl compound was dissolved in 20 ml. of 95% ethanol. The preparation of the 2, 4 dinitrophenylhydrazone derivative was accomplished by adding the freshly prepared 2, 4 dinitrophenylhydrazine to the carbonyl solution and the mixture was allowed to stand at room temperature. Crystallization of the hydrazone in each case generally occurred within 10 minutes and the mixture was allowed to stand overnight. The derivative was then retained on acid-washed filter paper and allowed to air dry. Recrystallization of the derivatives was accomplished by placing them in a 125 ml. Erlenmeyer flask with 30 ml. of 95% ethanol and heating it on a steam bath. If the derivative went into solution immediately, water was slowly added until the cloud point was reached or, until 5 ml. of water had been added. In certain instances when the hydrazone did not dissolve, ethyl acetate was added slowly to the hot mixture until the derivative was dissolved. The resulting hot solution was then filtered through fluted filter paper and allowed to stand at room temperature until crystallization occurred (about 12 hours). The melting points of these known derivatives were determined by using a modified Thiele apparatus (Robertson and Jacobs, 1962) containing mineral oil as the bath liquid and a micro Bunsen burner for the heat source. A small amount of the derivative was tapped into the fused end of a 1 mm diameter capillary which was fastened to a thermometer by a rubber band, with care being taken to have the sample next to the thermometer bulb. This was suspended in

the bath and the temperature of the bath was then raised at about 2°C per minute. The temperature at which the sample started to liquefy and at which it was completely liquid was noted. The derivatives were considered pure when a constant melting point or range was reached which compared favorably with literature values. Generally, 2 to 3 recrystallizations were sufficient to purify the known derivatives.

The known 2, 4 dinitrophenylhydrazones were then chromatographed on 20 or 60 gram celite columns as suggested by Day et al. (1960) and their threshold volumes were determined. These columns were prepared and developed exactly as previously described for the unknown derivatives.

Identification of the Unknown 2, 4 Dinitrophenylhydrazones

Evidence for the identification of the 2, 4 dinitrophenylhydrazone derivatives was obtained by first determining the chain length and the class of the parent carbonyl compound. Next, the melting points of the unknown derivatives were obtained before submitting the small amounts of the remaining derivatives to mixed melting point determinations.

The determination of the chain length of each of the parent carbonyl compounds was based on the comparison of the threshold volumes of the known and unknown derivatives on identically prepared celite columns as previously described. Further evidence for the chain lengths of the carbonyl compounds was based on the paper chromatographic behavior of the 2, 4 dinitrophenylhydrazone derivatives. Two procedures were used for the paper chromatography of the derivatives, the first was the original method of Huelin (1952) as extended by Schepartz (1961), and the second was the reverse phase procedure of Seligman and Edmonds (1955).

Methanol solutions of the unknown 2, 4 dinitrophenylhydrazone derivatives were chromatographed along with the knowns exactly as defined by Schepartz (1961), except that no attempt was made to regulate the temperature of the chromatographic chamber. In the procedure of Seligman and Edmonds (1955), the solvent system of methyl acetate-water (10:7) was used in an ascending direction, with Whatman No. 1 paper impregnated with a 5.1% solution of olive oil (U.S.P. grade) in carbon tetrachloride exactly as suggested by the authors.

The absolute R_f values were not very reproducible for both chromatographic procedures used, yet the relative movement of the various derivatives with respect to each other was observed to be quite reproducible in most instances. Considerable difficulty was experienced at first by the author in obtaining clear, well separated spots especially in the reverse phase procedure. This problem was solved by the application of as small a sample as possible which could be observed under an ultraviolet light.

The class of the parent carbonyl compound was determined by observing the absorption of the 2, 4 dinitrophenylhydrazone derivatives in the 250 mu to 450 mu range. Chloroform solutions of the unknown as well as the known derivatives were scanned on a Beckman DU spectrophotometer. As reported by Jones et al. (1956), the absorption peak of the derivative in neutral solution (chloroform), is of considerable value in determining the class of carbonyl compound involved.

The samples of unknown derivative remaining from the column fractionation, where present in sufficient quantity, was subjected to a

melting point determination by the identical procedure described earlier. When all of the data representing chain length (column and paper chromatography), class of carbonyl (maximum absorption), and melting point of the 2, 4 dinitrophenylhydrazone derivative for all of the unknowns were compared with the same data for the known derivatives, strong evidence was available for identification. However, conclusive identification was obtained when the mixed melting points of the known and unknown derivatives did not show a depression from the melting point of the known derivative. Preparation of the mixture for the mixed melting points was done by placing approximately the same amount of the known and unknown derivatives on the concave side of a watchglass and grinding the sample together by turning another watchglass inside of the first. This mixture was then tapped into the end of a 1 mm. capillary tube which had been previously fused off and the melting point determined in a modified Thiele apparatus as described earlier.

Determination of 3, 4 Benzopyrene in Smoke Condensate

The method of Latarjet et al. (1956) was used to determine the presence of 3, 4 benzopyrene in the smoke condensate. This method involves the fixation of the hydrocarbon on activated alumina and the eluate checked for its fluorescence in the same region as known 3, 4 benzopyrene.

Extraction

Whole smoke generated at 550°C in the smoke generator described earlier was condensed in ethanol-dry ice traps as described for the routine analysis of the smoke condensate, except, that the first collection flask contained glass wool to retain most of the tars. The vapor phase of smoke

generated at the same temperature was condensed in the same manner. The smoking period was 12 hours in each case and the traps were weighed before and after the smoke was condensed to determine the amount of condensate.

The condensing flasks were thoroughly rinsed with a mixture of 400 ml. of acetone and 50 ml. of water and the condensate was completely dissolved. This solution was then extracted with four 150 ml. portions of cyclohexane in a separatory funnel. The cyclohexane extract was in turn washed with two 250 ml. aliquots of water to remove the residual acetone from the cyclohexane extract. The final cyclohexane extract was then dried over $CaCl_2$ to remove the last traces of water.

Chromatography

Alumina (Alcoa, grade F-20) was heated at 180°C for 13 hours in an oven to facilitate activation. After activation, the alumina was stored in a dessicator over phosphorous pentoxide prior to use. A chromatographic column 2.0 x 60 cm was packed to a height of 40 cm with the dry, activated alumina with the column being tapped with a table knife between aliquots to insure compaction. A close fitting paper disc was pressed onto the top of the compacted column with a cork on the end of a glass rod. This served to level and firm the top surface of the column and did not allow the alumina to be distubred when the sample was applied.

The cyclohexane extract was then poured onto the column and allowed to percolate through the alumina. Since 3, 4 benzopyrene has its maximum absorption at 385 mu (Latarjet et al., 1956), the proportion of the compounds absorbing at this wavelength which were fixed on the activated alumina was determined by comparing the absorbance of the cyclohexane

extract at this wavelength to that of the cyclohexane recovered from the end of the column. In each instance 100% of the compounds which absorb at this wavelength were fixed on the column of activated alumina. Two 10 ml. portions of cyclohexane were used to wash the inside of the top of the column and the first was allowed to enter the column packing before the second was applied. After washing, the column was filled with benzene and a separatory funnel filled with benzene was attached to the top of the column with tygon tubing. A slight vacuum was applied at the bottom of the column by attaching a water aspirator to a 125 ml. suction flask, which was in turn attached to the column by means of a cork with the column outlet protruding through it. Two suction flasks were used and each was marked at the 25 ml. level and by alternating the two flasks at the base of the column, 25 ml. fractions were collected.

The fluorescence of the fractions was checked with a Fisher Nefluoro-Photometer using a Mercury arc light source. The center filter used was 365 mu, the right filter 450 + mu, and the left filter 425 mu (operating manual, Fisher Nefluoro-Photometer). The generating solution contained 2.56×10^{-5} gms/ml. of 3, 4 benzopyrene (Nutritional Biochemicals) in benzene and fractions containing 2.30×10^{-8} gms. of 3, 4 benzopyrene per ml. were set to show 100% fluorescence, thus, fractions containing 2.30×10^{-10} gms. of 3, 4 benzopyrene would show 1% fluorescence.

The extracts of both whole smoke and the vapor phase did not show any fluorescence in the same region as 3, 4 benzopyrene at these concentrations. However, a yellow substance which came off in the first few fractions did not fluoresce. The fractions containing this were pooled

(these fractions were chosen visually) and concentrated under vacuum and their absorption scanned in the ultraviolet region on a Beckman DU spectrophotometer in 10% benzene and 90% cyclohexane.

Analysis of Tar from Conventional Smoke Generator

A sample of tar from the sides of the outlet of a smoldering sawdust smoke generator was dissolved in the acetone-water mixture and extracted exactly as the smoke condensates.

The extract was placed on the 2.0 x 40 cm. activated alumina column described above. It was eluted with benzene and the 25 ml. fractions collected exactly as before. The fractions were checked for fluorescence with the same instrument described earlier. The filter arrangement and light source was the same as described before. The generating solution contained 2.72×10^{-5} gms/ml. of 3, 4 benzopyrene and the sample set at 100% fluorescence contained 2.72×10^{-9} gms/ml. of 3, 4 benzopyrene. The fractions which showed fluorescence were pooled and concentrated under vacuum using a rotary evaporator with the evaporation flask immersed in 50°C water. The residue was taken up by washing the flask with three 10 ml. portions of cyclohexane. This solution was then placed on a 1.2 cm. diameter column packed to a height of 20 cm. with the dry, activated alumina. This was then eluted with successive mixtures of 5 ml. benzene and 95 ml. cyclohexane, 10 ml. benzene and 90 ml. cyclohexane, 15 ml. benzene and 85 ml. cyclohexane, ----- etc., until no more fluorescence was observed in the 25 ml. fractions collected under vacuum and observed in the Nefluoro-Photometer as before. The fractions showing fluorescence were pooled and concentrated the same as for the second chromatography,

the residue was again taken up in 30 ml. of cyclohexane and poured on a column 0.5 cm. in diameter and packed to a height of 10 cm. with the activated alumina. This was eluted with successive mixtures of benzene and cyclohexane exactly as described above. The fractions showing fluorescence were pooled and concentrated as before, taken up in 20 ml. of cyclohexane and subjected to two successive chromatographies on columns 2 cm. in diameter packed to a height of 7 cm. with dry, silicic acid (Mallinckrodt chromatography grade, 100 mesh). Elution, collection of fractions and observation of fluorescence was performed exactly as in the operation of the last two activated alumina columns except that vacuum was not used.

After the fluorescent fractions from the last silicic acid column were pooled and concentrated, the residue was taken up in 10% benzene and 90% cyclohexane. The ultraviolet absorption spectrum of this solution was obtained using a Beckman DU spectrophotometer. Comparison of the ultraviolet absorption spectrum of the isolated compound with that of pure 3, 4 benzopyrene in 10% benzene and 90% cyclohexane gave a good indication of the purity of the isolated compound. The concentration of 3, 4 benzopyrene in the solution containing the isolated compound was determined by comparing the absorbance of this solution against that of solutions containing known concentrations of 3, 4 benzopyrene.

Application of Whole Smoke and Vapor Phase to Food Products

Smoking of Cheese

A limited study was undertaken to determine the difference, if any, which existed between the organoleptic quality of aged cheddar cheese smoked with whole smoke when compared with that smoked with the vapor

phase. Two 1 lb. blocks of cheese were suspended on a hardware cloth rack in a laboratory dessicator. A rubber stopper having a glass intake tube, glass outlet tube and a thermometer protruding through it, was placed in the opening of the dessicator cover. The glass intake tube was connected to the outlet of the laboratory smoke generator by a tygon tube, and the other end of the intake tube continued below the level of the hardware cloth and terminated about 1 1/2 inches above the bottom of the dessicator. The outlet tube, which was connected to a vacuum line, just protruded through the rubber stopper. Thus, by applying a slight vacuum on the outlet tube, the smoke entered the bottom of the dessicator and filled the dessicator. This procedure was followed for the smoking of aged cheddar cheese from the Michigan State University Dairy. Two 1 lb. blocks of cheese were smoked for 4 hours with whole smoke and two 1 lb. blocks were smoked for 4 hours with the vapor phase. The sawdust flow rate remained constant throughout the smoking period and the smoke was generated at 550°C, and the electrostatic air cleaner was turned off when smoking with whole smoke. The temperature inside the dessicator was 25°C at the start of the smoking period and reached a maximum of 31.5°C in each instance. After smoking, the cheese was wrapped in paper and stored in a 4°C refrigerator.

Organoleptic Evaluation of the Smoked Cheese

The smoked cheese was taken from the cooler and the outside 1/16" layer was removed with a knife. Cross sections of the 2" x 2 1/2" bars were then cut approximately 1/8" thick to be subjected to taste panel evaluation. One slice of cheese smoked by each of the two methods was

given each of twelve taste panel judges. Each panel member was given two score sheets, on one sheet the panel member was instructed to rank the two samples on the intensity of smoke flavor present from 1 to 10 (1 least, 10 most); on the other sheet the panel member was instructed to score the samples as to preference on a hedonic scale (9-like extremely, 5 neither like nor dislike, 1-dislike extremely).

Smoking of Bacon

A similar approach was undertaken to study the relationship between the organoleptic quality of normally cured bacon, smoked with whole smoke and the vapor phase. The bellies from the right and left sides of three hogs were obtained from the Michigan State University Meat Laboratory. These bellies were dry cured in a commercial cure at random with several other bellies. After remaining in cure for 15 days the bellies were removed and soaked for 1 hour in warm (35°C) water. The bellies were then hung by the flank end in a 4°C. cooler until chilled and dried for 6 hours, before the first one was taken out to be smoked. The 6 bellies were put into cure, taken out of cure and soaked all at the same time, however, they were smoked separately.

One side of each pair of bellies (right or left) was smoked with whole smoke and the other pair member was smoked with the vapor phase. The smoking chamber used was the Meats Laboratory air-conditioned smokehouse and the smoke was provided by connecting the previously described smoke generator to the smokehouse. In order to provide sufficient smoke density, the sawdust flow rate onto the hotplate was too great to facilitate the regulation of the generation temperature. Therefore, the

generation temperature was not controlled and only a comparison between the whole smoke and the vapor phase could be made. The collection plates of the electrostatic air cleaner were removed and this unit remained off when smoking with whole smoke. The collection plates were replaced in the electrostatic air cleaner and the unit turned on when smoking with the vapor phase. Once the sawdust flow rate was set, it was never changed until all the bellies were smoked.

The individual belly to be smoked was placed in the smokehouse with a thermometer inserted into the center. The smokehouse was controlled at 136-150°F until the bacon reached an internal temperature of 125°F, at which time the smokehouse temperature was reduced to 130-140°F until a total of 7 hours had lapsed, and the bacon was then removed from the smokehouse and stored at 4°C. Smoke was applied throughout the 7 hour period and smoking of the six bellies was completed in a 48 hour period. The treatment of the various bellies is summarized in table 2.

Table 2. Smoke treatment of the various bellies.

Identification	Smoke treatment
15 E left	vapor
15 E right	whole
05 E left	whole
05 E right	vapor
16 E left	vapor
16 E right	whole

Organoleptic Evaluation of the Smoked Bacon

The bacon slabs were sliced anterior to the last rib and baked on a rack at 275°F for 50-55 minutes before serving to the taste panel judges. The bacon was presented to the taste panel so that the pairs were compared. The panel members were instructed to evaluate the bacon in the same manner as in the case of the smoked cheese. Again, twelve taste panel judges were used for evaluation of each pair of smoked bellies and the same panel members were not necessarily used in all three comparisons.

RESULTS AND DISCUSSION

Effect of Generation Temperature on Total Phenols, Acids and Carbonyls

The extremes in generation temperatures observed, sawdust flow rate and moisture content and volume of condensate collected for analysis for total phenols, acids, and carbonyls are presented in table 3.

Table 3. Summary of collection data for samples subjected to analysis for total phenols, total acids and total carbonyls (average of duplicate 12 hr. runs).

Gen. temp. (°C)	Observed temp. extremes (°C)	Sawdust flow-rate gms/hr.	Sawdust moisture content (%)	Liquid condensate collected (ml.)
350[a]*	341 - 354	59.5	4.31	22.2
350[b]**	343.5 - 353.3	63.6	3.96	23.3
400[a]	394.5 - 403.5	60.8	4.64	21.7
400[b]	394.5 - 406.5	60.4	3.86	21.5
450[a]	433 - 451.5	61.1	4.08	23.7
450[b]	432.4 - 452.6	61.3	4.06	22.5
500[a]	486.2 - 500	62.6	4.19	21.4
500[b]	485 - 501.4	59.5	4.07	22.8
550[a]	537.6 - 551	58.8	4.39	23.6
550[b]	540.5 - 553.5	62.1	4.43	24.2

a* = vapor phase
b** = whole smoke

Considerable difference was observed in the appearance of the liquid condensates from the vapor phase and whole smoke. The condensate from whole smoke was extremely dark brown with a somewhat oily appearance,

while that from the vapor phase was much lighter in color. Only minute tar deposits were observed in the cold traps in a few instances after collecting the vapor phase. But considerable deposition of tars was observed in the cold traps after every collection of whole smoke. This indicated that most of the particles were removed by the electrostatic air cleaner during collection of the vapor phase. Also, it must be emphasized that only the portion of the condensate which was liquid after the sample thawed was analyzed. Thus, the insoluble tars (present mainly in whole smoke) was not analyzed. However, the liquid portion condensed around the tarry nuclei of the particles (Foster 1959) was included in the portion of whole smoke analyzed. This indicates that only the most volatile portion of the smoke was analyzed in the case of the vapor phase.

The relative amounts of steam volatile and non-steam volatile phenols in both the vapor phase and whole smoke generated at different temperatures are summarized in table 4.

Table 4. Total phenol concentration in smoke generated at various temperatures. (u gms. phenol/ml.)

Gen. temp. (°C)	Type of smoke			
	Whole		Vapor	
	S. V.*	N. S. V.**	S. V.*	N. S. V.**
350	88.0	1.7	56.4	1.2
400	90.8	1.6	69.2	1.3
450	153.0	2.9	138.0	1.9
500	196.5	4.7	160.9	1.7
550	165.4	3.5	151.7	1.9

*steam volatile
**non-steam volatile

The values of total phenols were found to be approximately the same for both types of smoke produced at 350 and 400°C. A significant increase was observed at 450°C and the highest phenol level was obtained at 500°C, with a slight decrease at 550°C. The generation temperatures at which the highest phenol concentrations were observed are higher than those suggested by Tilgner et al. (1960a) and Tilgner et al. (1960b).

Essentially no differences were found in the proportion of non-steam volatile phenols from the vapor phase of smoke over the range of generation temperatures studied. Whole smoke generated at 450°C and above showed a slight increase in non-steam volatile phenols. From the data in table 4 it is obvious that the largest proportion of phenols in both the vapor phase and whole smoke are steam volatile, corresponding with the observations of Husaini and Cooper (1957).

The relative amounts of steam volatile and non-steam volatile acids found in whole smoke and the vapor phase generated at different temperatures are presented in table 5.

Table 5. Total acid concentration in smoke generated at various temperatures (Meq. acids/ml.)

Gen. temp. (°C)	Type of smoke			
	Whole		Vapor	
	S. V.*	N. S. V.**	S. V.*	N. S. V.**
350	.082	.015	.066	.011
400	.077	.019	.071	.014
450	.121	.023	.115	.019
500	.104	.024	.110	.022
550	.112	.027	.106	.023

*steam volatile
**non-steam volatile

The production of total acids showed the largest value at the 450°C generation temperatures and tended to fall off slightly at temperatures above this in both the vapor phase and whole smoke. The non-steam volatile acids, although a small proportion of the total acids, increased in amount as the generation temperature increased in both cases. The total amount of acids in the whole smoke was slightly higher than in the vapor phase, except for the steam volatile acids generated at 500°C. Thus, it appears that the production of total titratable groups reached a peak at 450°C and above this temperature they possibly started to become oxidized to more stable compounds. Also, the increase in non-steam volatile acids as generation temperature increased would seem to indicate the formation of larger acid compounds at increased generation temperatures. Again, as in the case of the phenolic compounds, most of the acids were steam volatile.

The total carbonyl compounds found in the steam volatile and non-steam volatile portions of whole smoke and the vapor phase are presented in table 6.

Table 6. Total carbonyl concentration in smoke generated at various temperatures. (mg. acetaldehyde/ml.)

Gen. temp. (°C)	Type of smoke			
	Whole		Vapor	
	S. V.*	N. S. V.**	S. V.*	N. S. V.**
350	19.95	3.10	12.38	1.46
400	20.18	4.75	19.01	3.43
450	27.29	7.71	25.93	5.07
500	31.17	9.64	27.90	7.77
550	37.18	13.34	34.26	10.11

*steam volatile
**non-steam volatile

The results in table 6 indicated that there was a distinct tendency for the concentration of total carbonyl compounds to increase as the generation temperature increased for both the whole smoke and vapor phase. This was true for both the steam volatile and non-steam volatile carbonyls. The carbonyl content of both fractions was slightly higher in the whole smoke than in the vapor phase. This seemed to indicate that at least a part of the carbonyl compounds are retained in the particle phase. It would be interesting to know if this increase in total carbonyl compounds reached a threshold or peak with increasing generation temperatures above 550°C.

Separation and Identification of Steam Volatile Acids generated at 450°C.

The steam volatile monocarboxylic acids C_1 to C_{10} were successfully separated on silicic acid-glycine columns buffered at pH 2.0, 8.4 and 10.0. Typical elution patterns for mixtures of known acids are presented in figures 2, 3 and 4. As indicated in these diagrams the longer chained acids are eluted first and the shorter ones last in each case. On the pH 8.4 column, isovaleric and valeric acids were not separated and butyric and isobutyric acids also were not separated. Therefore, the C_4 peaks were labelled butyric-isobutyric and the C_5 peaks were labelled valeric-isovaleric. The elution patterns for the steam volatile monocarboxylic acids from various amounts of whole smoke are presented in figures 5, 6 and 7, with the vapor phase being represented in figures 8 and 9. Elution peaks corresponding with all of the C_1 to C_{10} known acids were obtained from the whole smoke (figures 5-7), whereas, elution peaks corresponding to the C_1 to C_4 known acids only were obtained for the vapor phase (figures

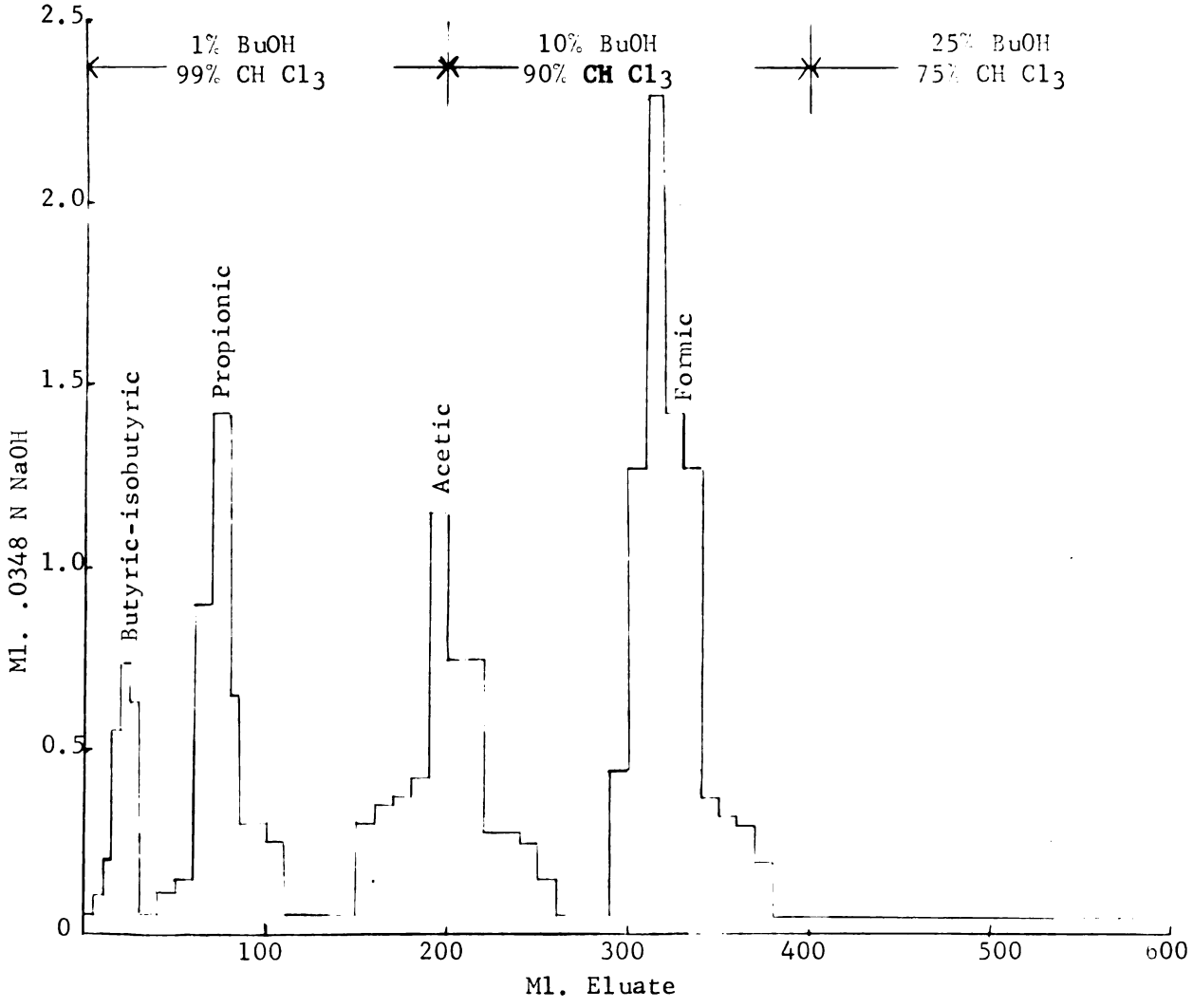

Figure 2. Silicic acid-glycine column pH 2.0 (known acids)

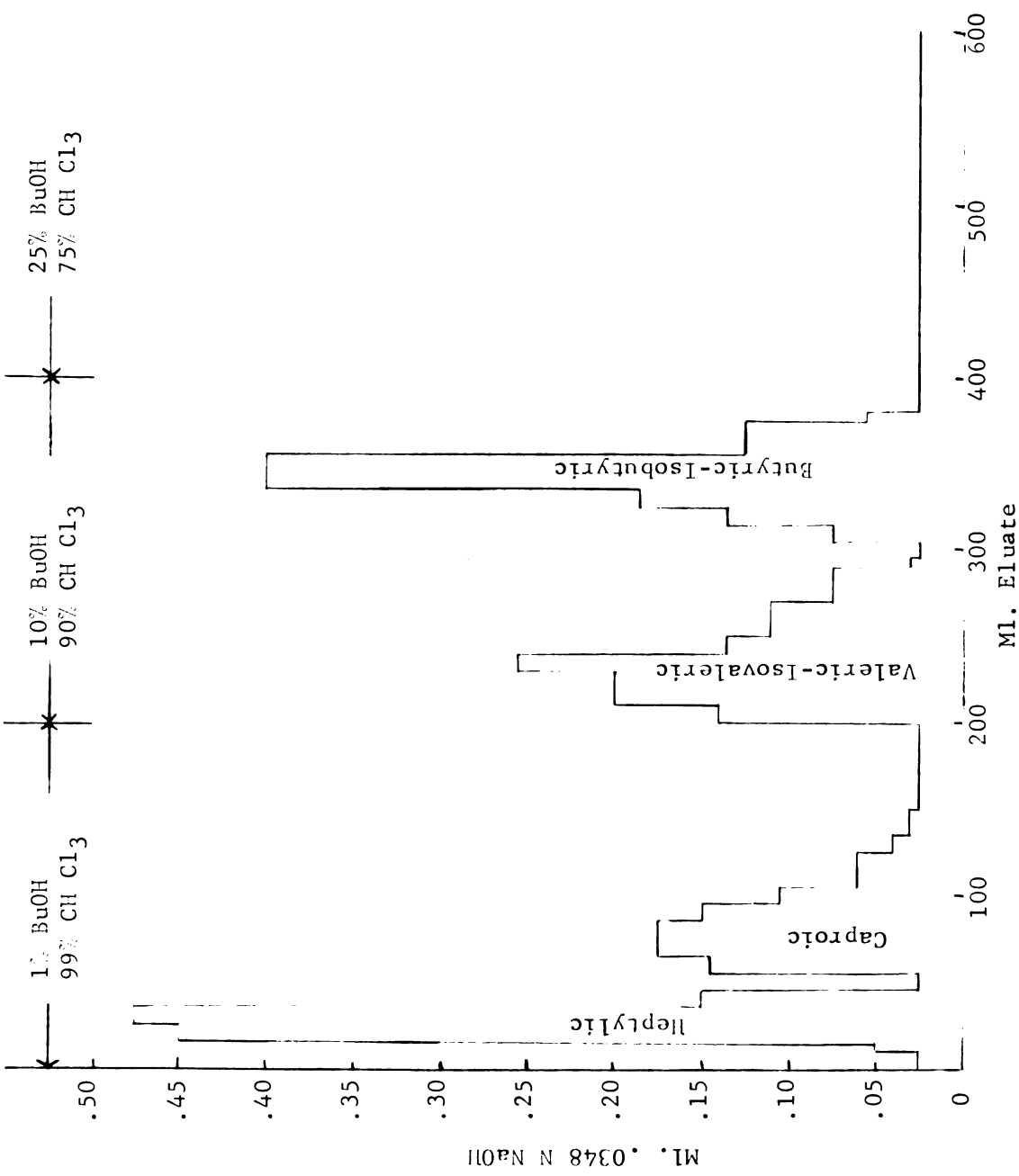

Figure 3. Silicic acid-glycine column pH 8.4 (known acids)

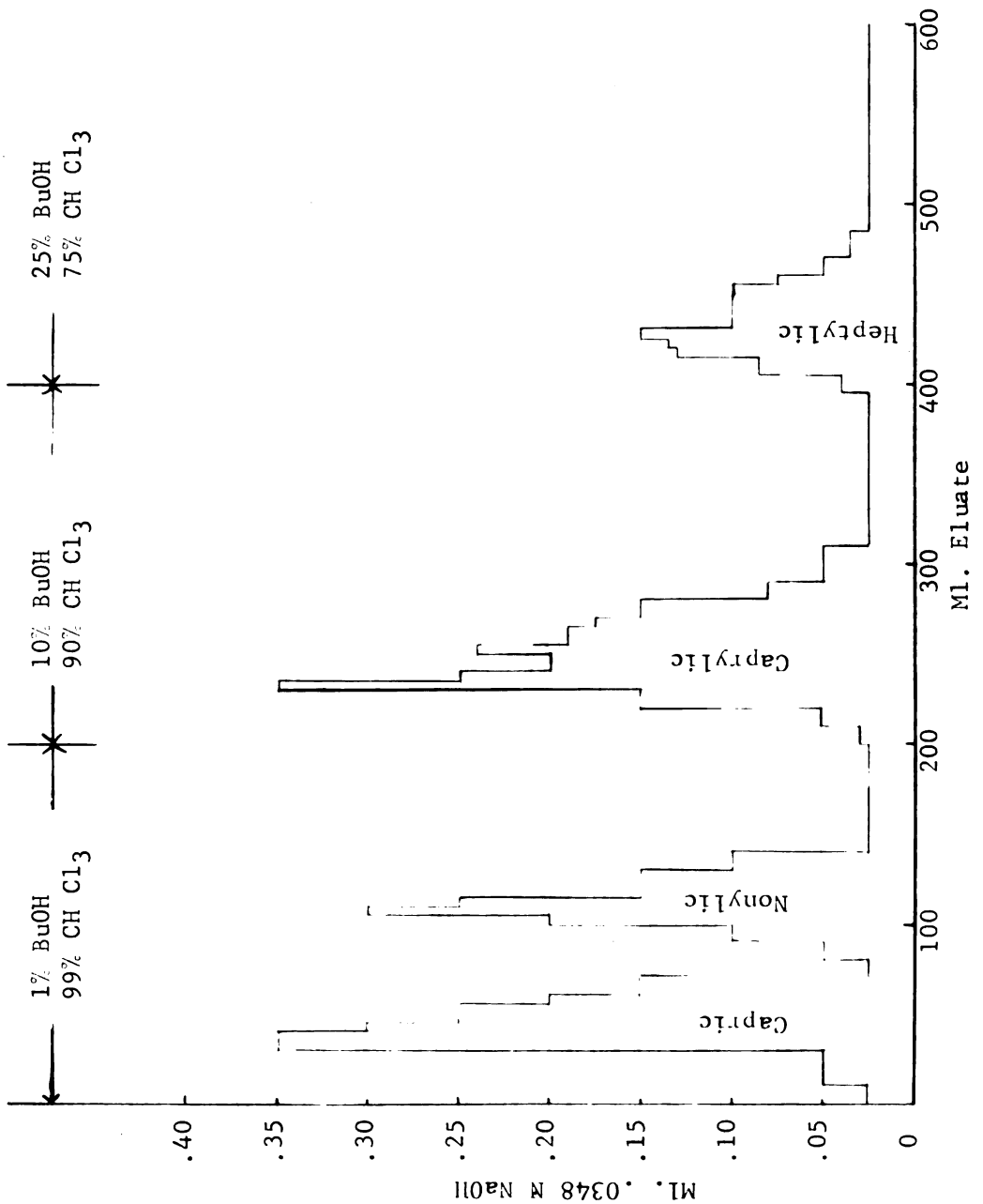

Figure 4. Silicic acid-glycine column pH 10.0 (known acids)

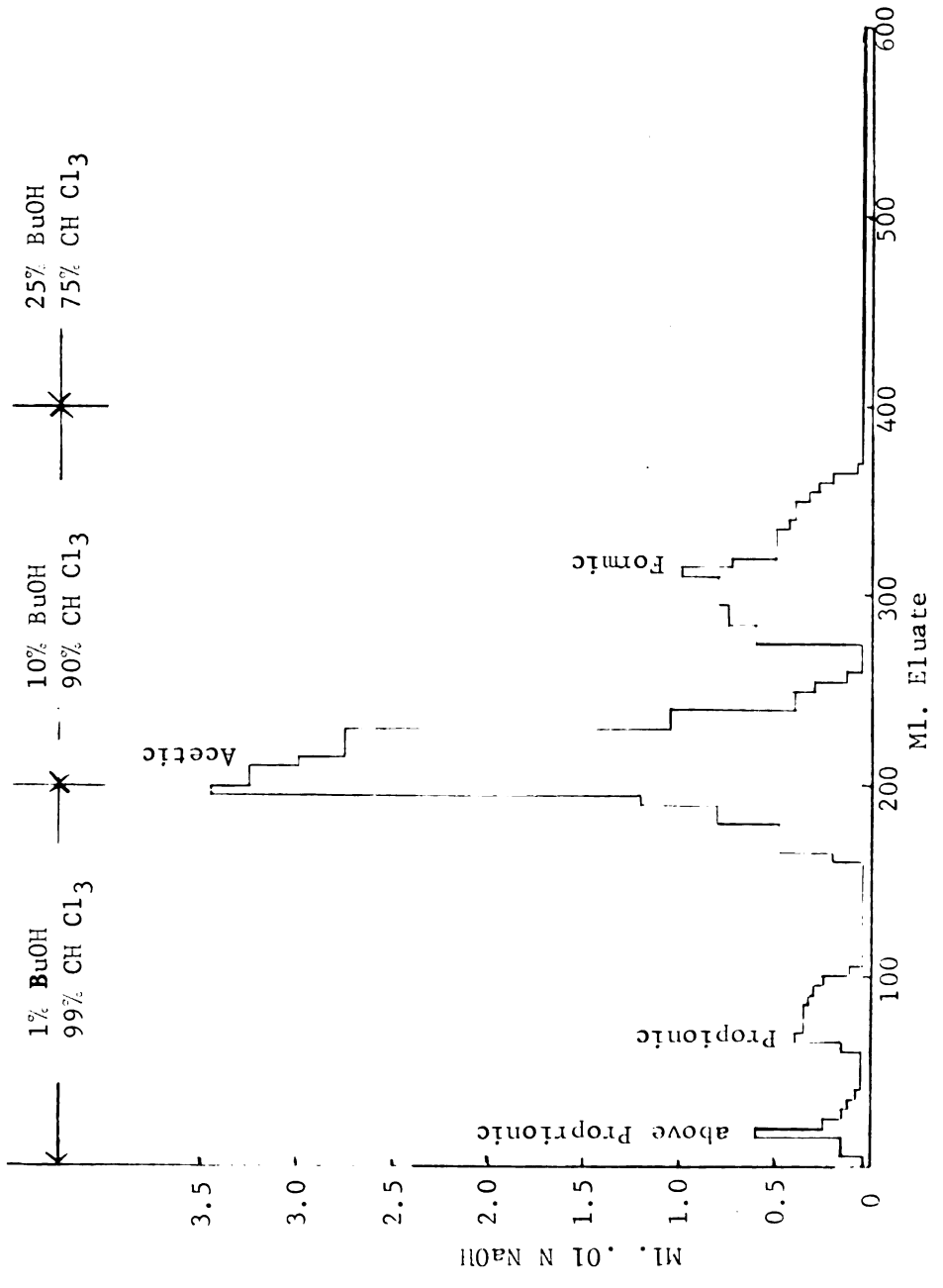

Figure 5. Silicic acid-glycine column pH 2.0 (4.0 ml. whole smoke)

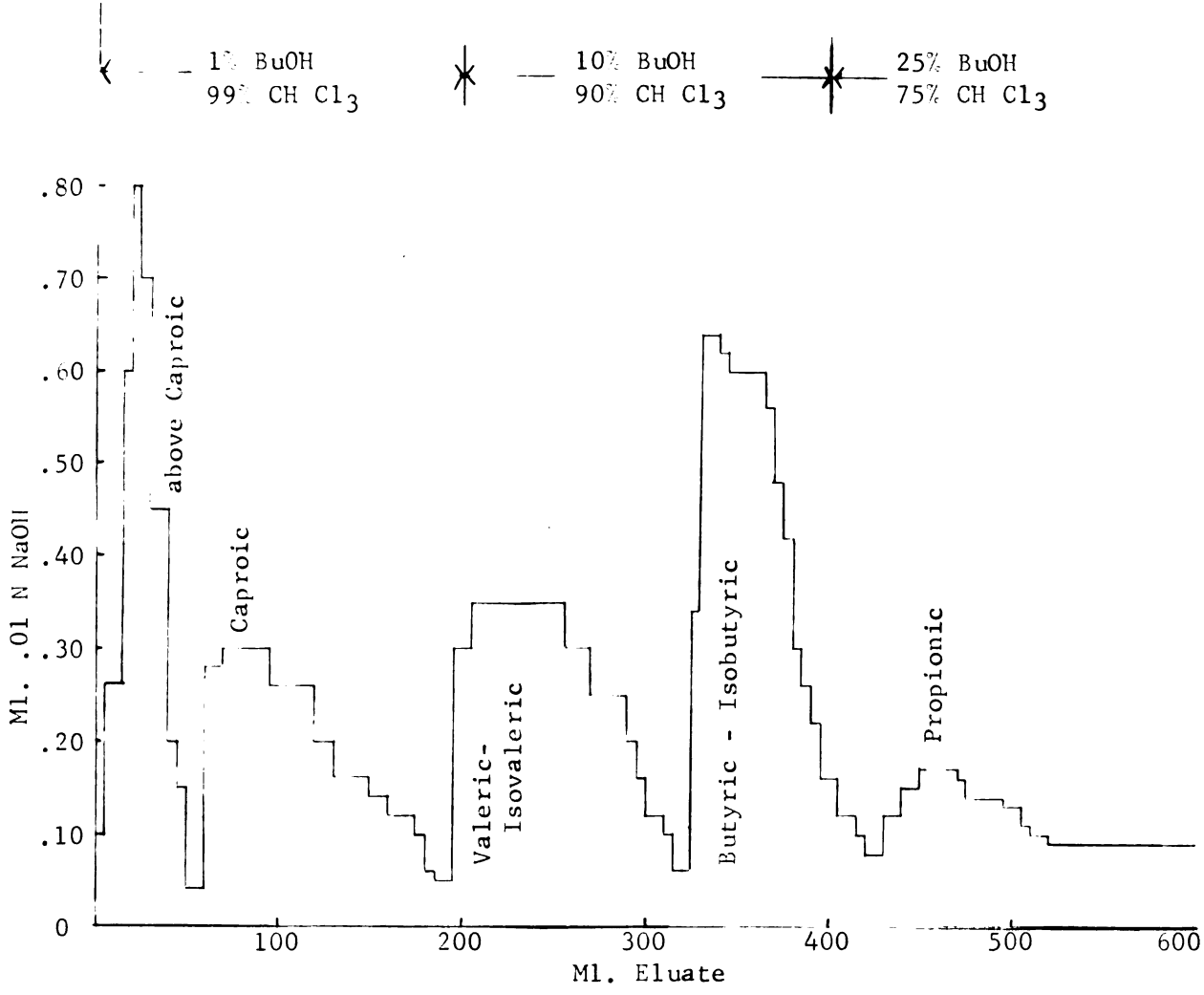

Figure 6. Silicic acid column pH 8.4 (45 ml. whole smoke)

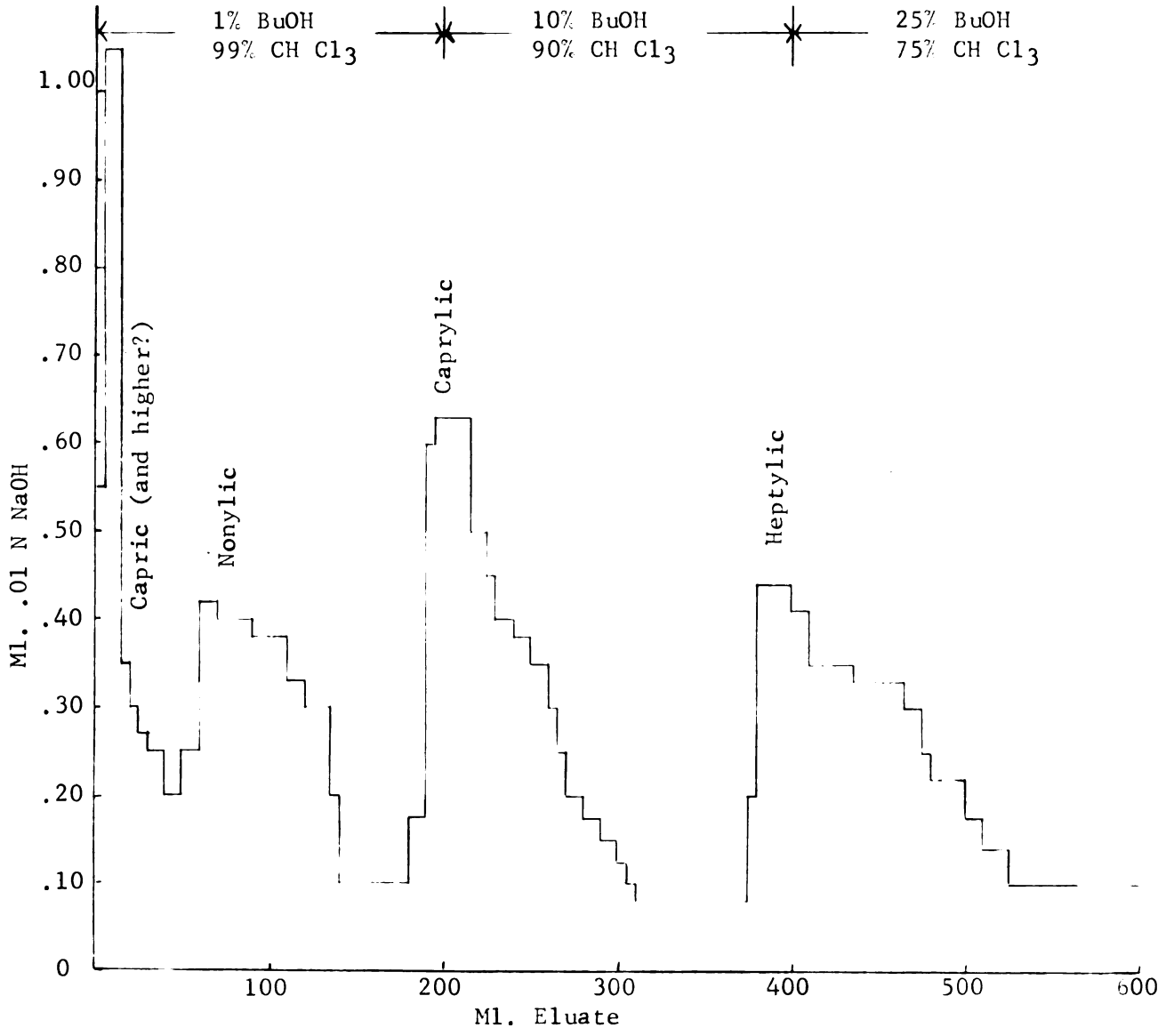

Figure 7. Silicic acid - glycine column - pH 10.0 (105 ml. whole smoke)

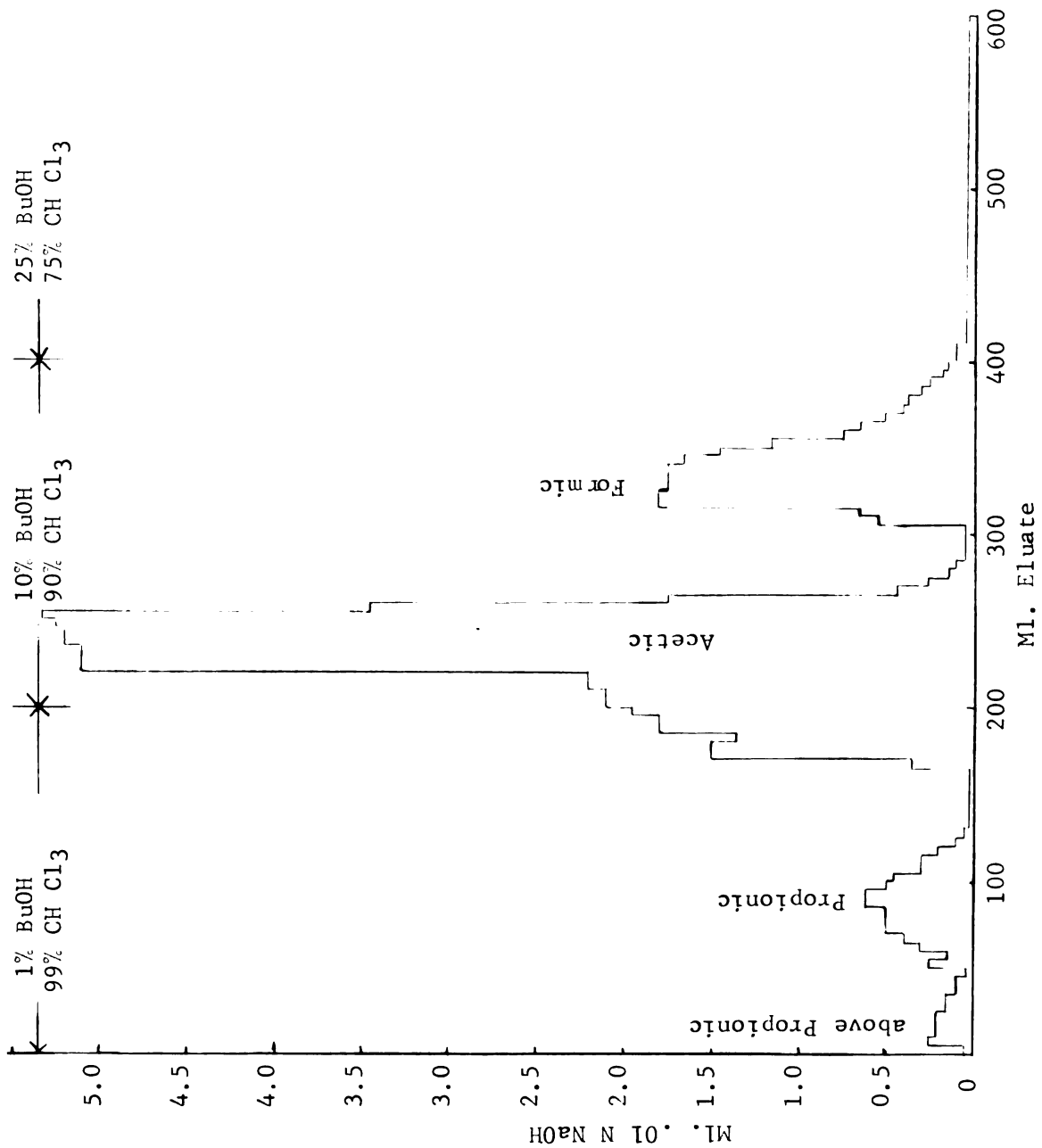

Figure 8. Silicic acid - glycine column pH 2.0 (8.0 ml. vapor phase)

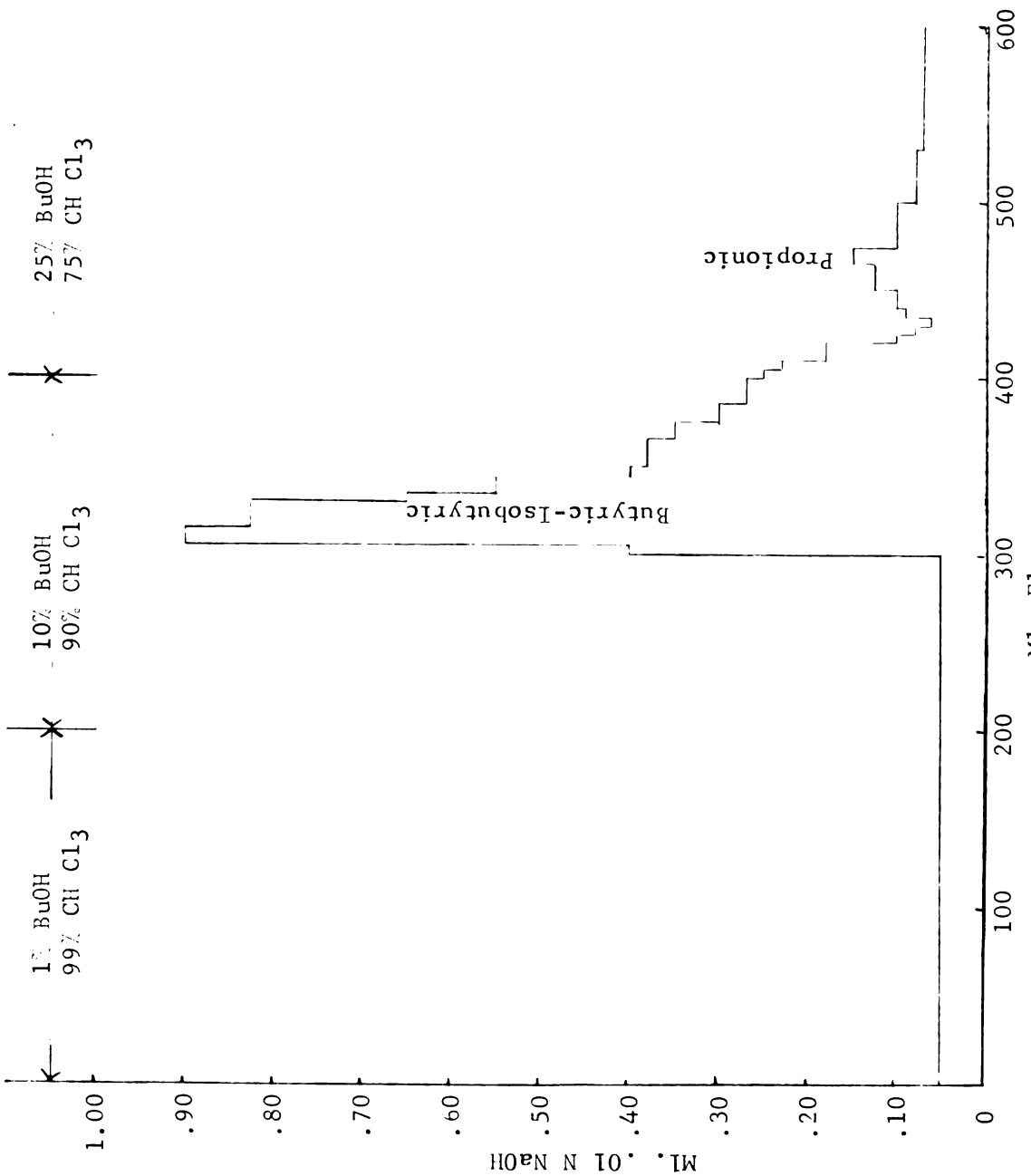

Figure 9. Silicic acid - glycine column pH 8.4 (90 ml. vapor phase)

8 and 9). This indicated that the longer chained acids (C_5 and above) probably were condensed on the particles removed during the electrostatic filtering process.

The acids were tentatively identified by comparing the eluate volumes of the peaks obtained from the smoke samples with those of the known acids chromatographed on identically prepared columns. Final identification was accomplished by paper chromatographing the ammonium salts of the acids from a given elution peak along with the ammonium salts of the corresponding known acids. A comparison of the eluate volumes of the known and unknown acids from the various silicic acid-glycine columns is presented in table 7, and a summary of the paper chromatographic data is shown in table 8.

Table 7. Comparison of column chromatographic data for known acids and acids from smoke samples.

Acid	Column pH	Eluate volume (ml.)		
		Known	Whole smoke	Vapor phase
formic	2.0	290 - 380	275 - 370	305 - 410
acetic	2.0	150 - 260	160 - 260	165 - 285
propionic	2.0	40 - 110	60 - 105	50 - 130
butyric isobutyric	8.4	305 - 380	325 - 420	300 - 430
valeric isovaleric	8.4	200 - 295	195 - 315	-
caproic	8.4	55 - 150	60 - 185	-
heptylic	10.0	395 - 485	375 - 525	-
caprylic	10.0	200 - 310	180 - 310	-
nonylic	10.0	80 - 140	50 - 140	-
capric	10.0	10 - 70	0 - 40	-

Table 8. Comparison of R_f values for known acids and acids from smoke samples

Acid	Solvent A*		Solvent B**	
	Unknown	Known	Unknown	Known
formic	.31	.32	.31	.31
acetic	.36	.34	.35	.36
propionic	.39	.39	.49	.48
butyric isobutyric	.50	.48	.54	.54
valeric isovaleric	.57	.58	.64	.62
caproic	.67	.66	.68	.69
heptylic	.75	.76	.72	.71
caprylic	.79	.80	.76	.76
nonylic	.82	.82	.82	.81
capric	.84	.85	.82	.82

*butanol : H_2O : propylamine
**95% ethanol : NH_4OH (would not resolve nonylic and capric)

Again as in the column separation mixtures of butyric and isobutyric acids and of valeric and isovaleric acids could not be separated by the paper chromatographic procedures employed. The ethanol-NH_4OH solvent system also was incapable of resolving nonylic and capric acids, whereas the butanol-H_2O-propylamine solvent was capable of separating the 2 acids. Table 8 shows that the mobility of the acids increased directly with their increase in chain length.

Considerably larger quantities of samples were required to obtain easily titratable amounts of the longer chained acids separated on the pH 8.4 and 10.0 columns than the shorter acids separated on the pH 2.0 columns.

The pH 8.4 columns for the unknown samples (figures 6 and 9) both indicated some propionic acid to be carried over from the initial screening procedure.

Husaini and Cooper (1957) reported the presence of formic, acetic, propionic, butyric and 2 unidentified acids to be present in both smoldering sawdust and friction generated smoke. These authors reported that all of the total titratable acidity was attributed to these acids. They found acetic acid to be present in the greatest quantity followed by formic, propionic and butyric in decreasing amounts.

The quantitative recovery of the various acids identified in the present study is summarized in table 9 for the steam volatile acids generated at 450°C. The results shown in table 9 show acetic acid to be present in by far the largest concentration, followed by formic, propionic, butyric (and isobutyric) acids in decreasing amounts. The acids higher than butyric were detected in extremely small quantities in the whole smoke and were not detected in the vapor phase by the methods used in this study. Formic acid occurred in larger amounts in the vapor phase than in the whole smoke, otherwise, there was little difference observed in the relative proportions of the C_1 to C_4 acids in both the vapor phase and whole smoke. In both instances of the whole smoke and vapor phase, all of the titratable acidity in the steam volatile portion of the smoke condensate was not accounted for by the monocarboxylic acids identified (table 9). This would suggest the possibility of the presence of aromatic and even dicarboxylic acids which were not detected by the methods employed. Also, the phenolic compounds present would tend to be weakly acidic in nature, accounting for some of the titratable acidity in the smoke condensate.

Table 9. Quantitative recovery of steam volatile monocarboxylic acids from smoke samples

Acid	Meq. acid/ml. smoke condensate	
	Whole smoke	Vapor phase
formic	.0193	.0235
acetic	.0699	.0700
propionic	.0065	.0067
butyric isobutyric	.0013	.0011
above butyric	.0039	---
Total	.1009	.1013
Titrated directly	.1210	.1150

Separation and Identification of Steam Volatile Monocarbonyls from Whole Smoke Generated at 550°C.

The steam volatile monocarbonyl compounds were first separated on nitromethane-hexane-celite columns as their 2, 4 dinitrophenylhydrazone derivatives. The chain lengths of the parent carbonyl compounds were determined by comparing threshold volumes of the unknown derivatives with those of known derivatives on identically prepared columns and by comparing paper chromatographic data on the unknown and known derivatives. A summary of the threshold volumes of the various unknown and known derivatives together with the type of column used is presented in table 10.

The unknown bands were easily collected visually except for the acetone and propanal bands. This is evidenced by the similarity in the threshold volumes recorded for these compounds (table 10). As described previously, these 2 bands were collected in 25 ml. fractions and their absorbance scanned in the 325 to 400 mu region. The fractions having the

Table 10. Column chromatographic data for the 2, 4 dinitrophenylhydrazone derivatives

Column band No.	2, 4 dinitrophenylhydrazone derivative	Type column	Threshold volume (ml.) Unknown	Known
Forerun	unidentified	60 gm.	-	-
1a	2-pentanone	60 gm.	15.5	15.6
b	isovaleraldehyde	60 gm.	21.8	21.4
c	valeraldehyde	60 gm.	22.5	22.5
2a	2-butanone	20 gm.	23.0	23.4
b	butanal	20 gm.	28.7	28.9
3a	acetone	20 gm.	38.3	38.1
b	propanal	20 gm.	38.5	38.3
c	crotonaldehyde	20 gm.	43.0	43.4
4a	ethanal	20 gm.	51.6	51.2
b	methanal	20 gm.	90.3	91.5

most absorbance at 363 mu (acetone) were pooled and those absorbing most at 357 mu (propanal) were also pooled. By subsequent rechromatographing these fractions and repeating this procedure each time, it was possible to separate the 2 bands in quite pure form. The bands were considered pure when only one absorption maxima was present at either 357 or 363 mu.

At this point, there appeared to be at least four carbonyl compounds of C_5 or longer separated on the 60 gm. columns. This included the forerun fraction which was barely recognizable and no attempt was made to identify this fraction. The 20 gm. columns resolved the mixture into seven additional carbonyl compounds. The threshold volumes of the unknown

derivatives compared well with the known derivatives as indicated in table 10. As chain length of the parent carbonyl compound decreased, the threshold volume of the 2, 4 dinitrophenylhydrazone derivative increased (table 10).

Further evidence for the chain length of the monocarbonyl derivatives was obtained by observing their movement on paper chromatograms along with known derivatives. Since the R_f values were not reproducible between runs for either method used, the results of duplicate runs are presented in each case. The results of the paper chromatography of the derivatives utilizing the heptane-methanol solvent system are summarized in table 11. The results in table 11 show that it was possible to judge only the relative chain length of the unknown derivatives when their R_f values were compared with the known derivatives. As the chain length of the parent carbonyl increased, the R_f values tended to be higher. The opposite was true when the derivatives were paper chromatographed using the methyl acetate-water solvent system and the paper impregnated with olive oil (table 12). Again, the R_f values were not sufficient for complete identification and were used to aid in confirming the chain length of the parent carbonyls.

Jones et al. (1956) reported that the wavelength of maximum absorbance of the 2, 4 dinitrophenylhydrazone derivatives in neutral solutions is a good criterion for establishing the class of the parent carbonyl compound. Aliphatic aldehyde derivatives have their maximum absorbance at 344-358 mu, aliphatic ketones at 364-367 mu, and mono-unsaturated aliphatic aldehydes have maximum absorbance at 373 mu. Similar values were reported earlier by Roberts and Green (1946).

Table 11. Paper chromatography of 2, 4 dinitrophenylhydrazones (heptane-methanol)

Column band No.	2, 4 dinitrophenylhydrazone derivative	Run 1		Run 2	
		R_f unknown	R_f known	R_f unknown	R_f known
forerun	unidentified	-	-	-	-
1a	2-pentanone	.64	.69	.71	.65
b	isovaleraldehyde	.69	.72	.65	.66
c	valeraldehyde	.66	.70	.70	.67
2a	2-butanone	.56	.55	.55	.60
b	butanal	.52	.49	.51	.48
3a	acetone	.43	.40	.46	.44
b	propanal	.38	.40	.42	.47
c	crotonaldehyde	.42	.39	.43	.40
4a	ethanal	.30	.25	.32	.28
b	methanal	.15	.19	.13	.16

Table 12. Paper chromatography of 2, 4 dinitrophenylhydrazones (reverse phase, methyl acetate-water)

Column band No.	2, 4 dinitrophenylhydrazone derivative	Run 1		Run 2	
		R_f unknown	R_f known	R_f unknown	R_f known
forerun	unidentified	-	-	-	-
1a	2-pentanone	.36	.34	.32	.37
b	isovaleraldehyde	.28	.31	.32	.30
c	valeraldehyde	.21	.24	.25	.27
2a	2-butanone	.42	.45	.46	.41
b	butanal	.38	.34	.36	.37
3a	acetone	.61	.59	.57	.58
b	propanal	.51	.48	.50	.50
c	crotonaldehyde	.40	.42	.45	.43
4a	ethanal	.58	.54	.53	.55
b	methanal	.65	.63	.61	.66

The maximum absorbance of the various 2, 4 dinitrophenylhydrazone derivatives of the monocarbonyls from the smoke samples are presented in table 13 along with those of known derivatives. These results indicated the presence of one mono-unsaturated carbonyl (band 3c), three aliphatic ketones (bands 1a, 2a and 3a), and six aldehydes (bands 1a, 1b, 2b, 3b, 4a and 4b).

Final identification of the carbonyl compounds was accomplished by observing a lack of depression of the metling points of mixtures containing the unknown and known 2, 4 dinitrophenylhydrazone derivatives when the unknowns were available in sufficient quantity. The results of the

Table 13. Absorption maxima of 2, 4 dinitrophenylhydrazones

Column band No.	2, 4 dinitrophenylhydrazone derivative	Absorption maxima (mu) Unknown	Known
forerun	unidentified	---	---
1a	2-pentanone	362	363
b	isovaleraldehyde	358	357
c	valeraldehyde	358	358
2a	2-butanone	363	363
b	butanal	358	358
3a	acetone	363	363
b	propanal	357	358
c	crotonaldehyde	373	373
4a	ethanal	354	356
b	methanal	348	348

melting point data are summarized in table 14 and the melting points are given as the uncorrected melting points. Thus, eight of the eleven observed bands from the nitromethane-hexane-celite columns were identified as indicated by the melting point data. Bands 1b and 4b were tentatively identified as isovaleraldehyde and methanal, respectively, but were not present in sufficient quantity to allow melting point determinations. The forerun fraction was quite diffuse and was present in too small a quantity to allow further study. However, one would suspect it to consist of a relatively long chained carbonyl compound(s) since it was eluted first from the column.

Table 14. Melting point data for 2, 4 dinitrophenylhydrazones

Column band No.	2, 4 dinitrophenylhydrazone derivative	Melting point (°C) Unknown	Known	Mixture
forerun	unidentified	-	-	-
1a	2-pentanone	141 - 143	142 - 143.5	140.5 -143
b	isovaleraldehyde	-	-	-
c	valeraldehyde	104 - 107	105 - 106.5	103 - 106
2a	2-butanone	112 - 113	113	110 - 113.5
b	butanal	112 - 113	114 - 114.5	112 - 114
3a	acetone	124 - 125.5	125.5	123 - 125
b	propanal	145 - 147	146.5	144 - 146
c	crotonaldehyde	186 - 188	188	185 - 188
4a	ethanal	164 - 165.5	166.5	163 - 166
b	methanal	-	-	-

3, 4 Benzopyrene Analysis

An attempt was made to isolate and determine the quantity, if present, of 3, 4 benzopyrene in the condensates of both the whole smoke and vapor phase generated at 550°C. With the procedure used, no 3, 4 benzopyrene was found in the condensate of either whole smoke or the vapor phase. The possibility exists that it could have occurred in concentrations too small to be detected by this procedure. Falk and Steiner (1952) stated that polynuclear aromatic hydrocarbons are formed during pyrolysis of many substances in a restricted supply of air in the temperature range of 750°C to 1600°C. Thus, a combustion temperature of 550°C was possibly not sufficiently high to bring about the formation of 3, 4 benzopyrene.

A yellow substance was observed in the first four to five 25 ml. fractions eluted with benzene from the 40 cm. activated alumina. These fractions were chosen visually and a spectrum was obtained in the ultraviolet region which appears in figure 10. The absorption peak at 275 mu suggests the presence of an aromatic substance, as several aromatic compounds tend to absorb strongly in this region, particularly some aromatic hydrocarbons and quinone type compounds (Friedel and Orchin 1951). The author observed that the amount of this substance found in the whole smoke was considerably greater than that which occurred in the vapor phase. This could reflect one of the largest differences between the whole smoke and the vapor phase.

Analysis of Tar from Conventional Smoke Generator

The spectra of pure 3, 4 benzopyrene (2.75 ug/ml.) along with that of the hydrocarbons isolated from the tars of a conventional smoke generator are presented in figure 11. The tars were found to contain 23.1 u gms per gm of tar of the isolated hydrocrabon(s), using pure 3, 4 benzopyrene as a standard. As indicated by the spectra in figure 11, the isolated fraction appears to consist mainly of 3, 4 benzopyrene, but appears to be contaminated possibly with anthracene, which has peaks at 372, 375.8, and 380 mu (Commins 1958). Distinct shoulders appear on the curve in the region of these wavelengths and this as well as several other hydrocarbons are known to be eluted with mixtures containing 3, 4 benzopyrene from activated alumina columns (Commins 1958). Thus, it was not possible to isolate 3, 4 benzopyrene from smoke tars in pure form by this method. If one could check the fluorescent spectrum of each fraction eluted from the

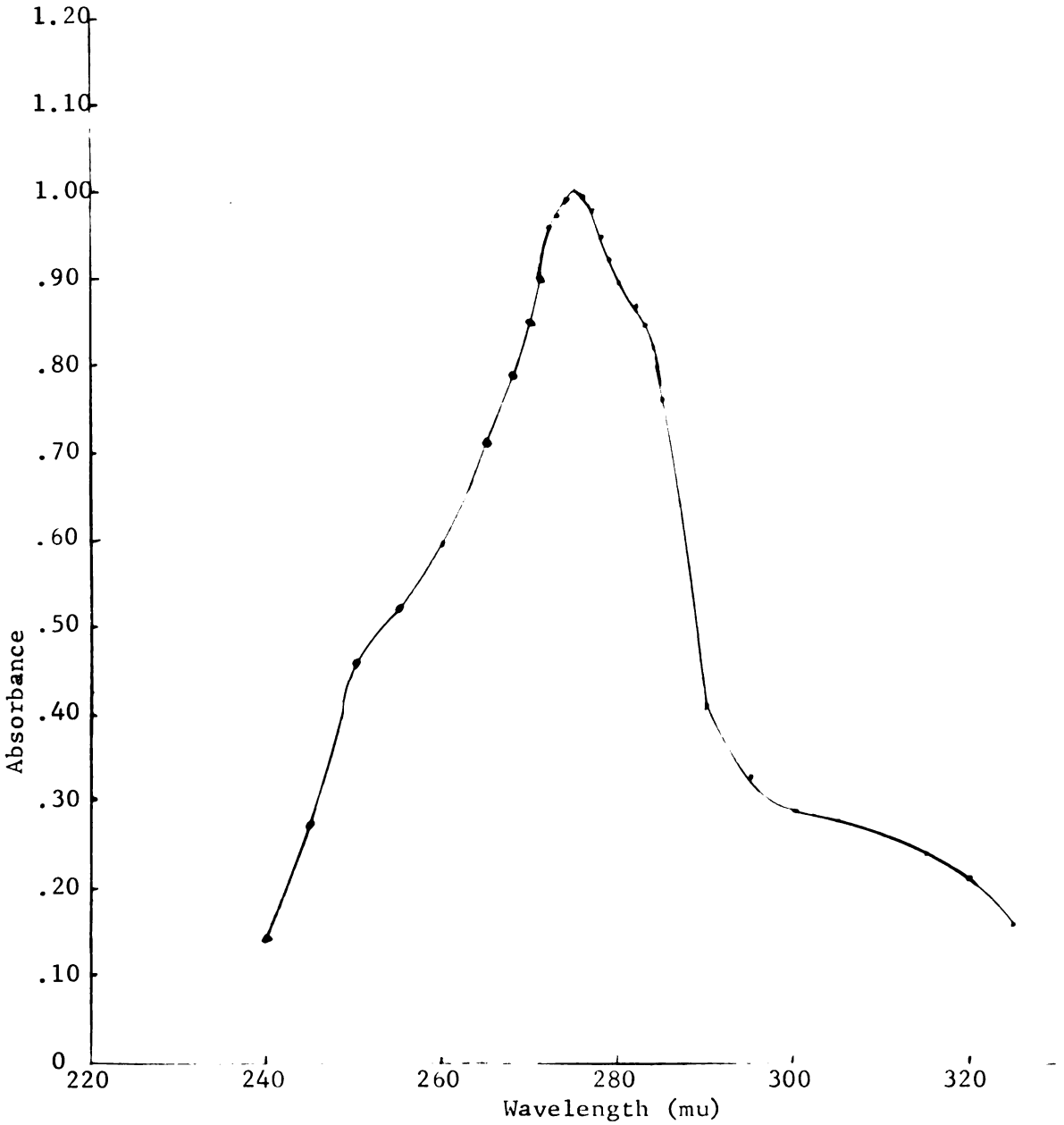

Figure 10. Absorption spectrum of forerun fraction from activated alumina column

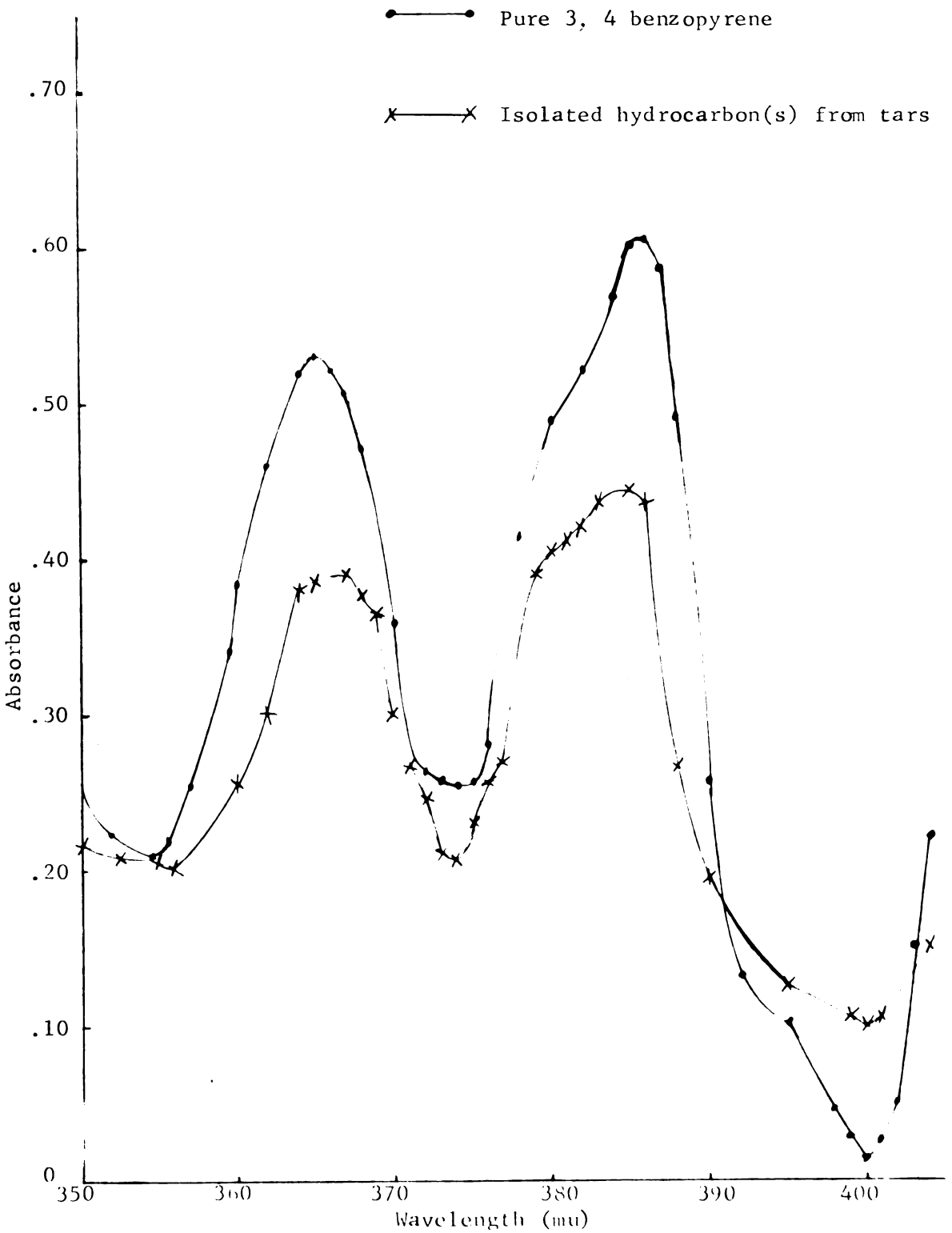

Figure 11. Absorption spectrum of isolated hydrocarbon(s) and pure 3, 4 benzopyrene.

column and collect only those whose fluorescent spectra compared with 3, 4 benzopyrene, the fractions containing compounds which have fluorescent characteristics similar, but not identical, to those of 3, 4 benzopyrene could possibly be eliminated.

Evaluation of Smoked Cheese and Bacon

The results of the taste panel evaluation of cheese smoked by both whole smoke and the vapor phase are summarized in table 15.

Table 15. Taste panel evaluation of smoked cheese

Smoking method	Smoke intensity	Preference
whole smoke	3.8	7.3
vapor phase	8.9	7.1

These results indicated that the vapor phase tended to penetrate the cheese more than whole smoke since a much greater smoke intensity was observed in the cheese smoked with the vapors only. One must keep in mind that the smoking of cheese is a "cold" smoking process (i.e. 32°C) and the more volatile constituents would tend to penetrate more readily under these conditions. A somewhat sticky coating was observed to be more prevalent on the cheese smoked with whole smoke than that smoked with the vapor phase. This might have impaired the penetration of vapors into the cheese during smoking with whole smoke. The results in table 15 indicated that level of smoke intensity did not have a great deal of influence on the preference of the cheese, since essentially no difference was found in the preference of the cheese smoked by the two methods.

The results of the taste panel study involving the smoked bacon are presented in table 16.

Table 16. Taste panel evaluation of smoked bacon

	Whole		Vapor	
	Intensity	Preference	Intensity	Preference
	5.4	7.8	3.6	7.1
	6.1	7.6	3.8	6.7
	5.8	6.3	4.8	7.6
Average	5.8	7.2	4.1	7.1

There was a reversal of the situation observed in the smoked cheese in that the bacon smoked by whole smoke was judged to have a consistently higher smoke intensity as indicated in table 16. This could be possibly due to the higher temperatures used in the smoking chambers and longer smoking time for the bacon than for the cheese. In two of the three cases observed, the bacon smoked with whole smoke was preferred to that smoked by the vapor phase whereas, in the third instance the opposite was true. However, the averages for the preferences indicated for the smoked bacon were essentially the same for both methods (table 16). Definite conclusions cannot be drawn as to the advantages of the two methods on the basis of this rather limited study of acceptance.

SUMMARY AND CONCLUSIONS

Most of the phenols, acids and carbonyls present in the smoke condensates were steam volatile. The highest total phenol level was attained at a generation temperature of 500°C, highest acid level at 450°C and highest level of total carbonyls at 550°C. Column and paper chromatography revealed the C_1 to C_{10} aliphatic monocarboxylic acids to be present in whole smoke with acetic, formic, propionic and butyric in decreasing order of concentration, making up most of the acids. The acids longer than C_4 tended to be present in decreasing amounts as chain length increased and made up a relatively small proportion of the total acids. Using the same procedures, acetic, formic, propionic and butyric in decreasing amounts were found to be present in the vapor phase. These were the only acids detected in the vapor phase by the methods employed. In either the case of whole smoke or the vapor phase, all of the titratable acidity in the steam volatile portion of the smoke condensate was not accounted for by the acids identified.

The following monocarbonyls were identified in the steam volatile portion of whole smoke: 2-pentanone, valeraldehyde, 2-butanone, butanal, acetone, propanal, crotonaldehyde and ethanal. Isovaleraldehyde and methanal were tentatively identified.

No 3, 4 benzopyrene was detected in the condensates from either the whole smoke or the vapor phase with the procedure used. A small amount (23.1 u gms/gm.) of 3, 4 benzopyrene was detected in the tars from a conventional smoke generator, although the spectrum of the sample isolated showed considerable contamination.

Cheese smoked with the vapor phase was judged to have a more intense smoke flavor than that smoked with whole smoke by a taste panel, whereas, the opposite was found for smoked bacon. However, there was no preference in either case for that smoked by either the whole smoke or vapor phase indicating that intensity of smoke flavor is not the major factor in preference.

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