

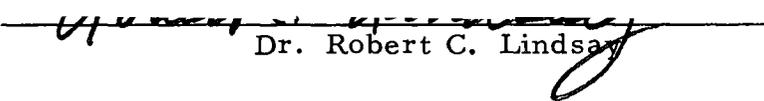
AN ABSTRACT OF THE THESIS OF

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Title: FLAVOR CHEMISTRY OF POPCORN

Abstract approved:


Dr. Robert C. Lindsay

Popcorn was popped conventionally in oil and in a microwave oven without oil. Volatile components were collected by condensation of popper headspace gases and by vacuum steam distillation of a slurry of ground, popped corn in distilled water. The organic compounds were extracted from the aqueous condensate and vacuum distillate with diethyl ether. The concentrated ether extracts were analyzed by a coupled gas chromatograph-mass spectrometer utilizing three different procedures. These were: (1) a 300 foot by 0.01 inch ID capillary column, into which samples were injected with a 50:1 inlet split, with the effluent end connected directly to the ion source of the mass spectrometer; (2) the same directly coupled capillary column, but with samples previously trapped from a packed column introduced without splitting; (3) mass spectrometric analysis of a portion (5 to 10%) of the effluent stream from a 12 foot by 1/8 inch OD packed column.

Thirty-six compounds were positively identified by comparison

of their spectra with reference spectra. Relative retention time coincidence with known compounds confirmed the mass spectral identifications. Those compounds positively identified include pyrazine, 2,5-dimethyl pyrazine, 2,3-dimethyl pyrazine, 2-ethyl-5-methyl pyrazine, 2-ethyl-3,6-dimethyl pyrazine, 2-pentyl furan, furfural, 2-acetyl furan, 5-methyl furfural, furfuryl alcohol, 5-hydroxymethyl furfural, acetaldehyde, 2-methyl propanal, 3-methyl butanal, pentanal, hexanal, benzaldehyde, phenylacetaldehyde, diacetyl, ethyl acetate, methyl palmitate, diethylphthalate, ethanol, hexane, acetic acid, hexanoic acid, palmitic acid, γ -butyrolactone, N-furfuryl pyrrole, N-furfuryl pyrrole-2-aldehyde, 2-acetyl pyrrole, pyrrole-2-aldehyde, 4-vinyl-2-methoxyphenol, 4-vinylphenol, and vanillin. Another 20 compounds were tentatively identified on the basis of their mass spectra. These were 2,3,5-trimethyl pyrazine, 2-acetyl pyrazine, (5-methyl-2-furyl)-(2-furyl)-methane, 5-methyl-2-acetyl furan, bi-(5-methyl-2-furyl)-methane, 4-hydroxy-2,5-dimethyl-3 (2H)-furanone, heptanal, ethyl formate, pyridine, thiazole, benzothiazole, ethylbenzene, a dichlorobenzene, N-ethyl-pyrrole-2-aldehyde, N-isoamyl-pyrrole-2-aldehyde, 5-methyl-pyrrole-2-aldehyde, 2-formyl-5-methylthiophene, 2-ethyl-5-butylthiophene, phenol, and 2-methoxyphenol.

It is believed that pyrazines, furans, carbonyls, and phenolic compounds make important contributions to the flavor and aroma of

popcorn. The presence of many of the compounds identified can be explained by their production during popping from precursors in the popcorn kernels by Maillard browning reactions, Strecker degradation of amino acids, thermal oxidation and hydrolysis of lipids, and direct thermal degradation of plant constituents.

Flavor Chemistry of Popcorn

by

John Pierce Walradt

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FLAVOR CHEMISTRY OF POPCORN

INTRODUCTION

Popcorn is one of many popular food items in which the characteristic flavor is produced during heat processing. Modifications of the typical popcorn flavor can be made by altering the type of popping oil used, and by the addition of certain seasonings.

Since the unique flavor and aroma of popcorn has such wide appeal, it would be useful to learn the identity of the principal chemical compounds which contribute to the flavor. Perhaps, then, this flavor could be more carefully controlled and exploited more fully as a flavoring agent for completely new, fabricated food products. Such a study should also contribute to the understanding of the mechanisms by which heat-induced flavor changes occur in other foods.

REVIEW OF LITERATURE

Composition of Corn

In a study involving natural products, it is instructive to know something about the gross composition of the raw material as a step toward defining the possible reactants in flavor production. The following proximate analyses compare mean values for popcorn with those for sweet corn and dent corn grains (52, p. 6).

| | <u>Popcorn</u> | <u>Sweet corn</u> | <u>Dent corn</u> |
|----------------|----------------|-------------------|------------------|
| Dry matter | 89.7% | 90.9% | 86.2% |
| Crude protein | 12.2 | 12.8 | 10.4 |
| Ether extract | 5.6 | 8.7 | 4.5 |
| Crude fiber | 2.0 | 2.8 | 2.4 |
| Ash | 1.6 | 2.1 | 1.5 |
| N-free extract | 78.6 | 73.6 | 81.2 |

The carbohydrate composition for corn grain, all analyses (52, p. 496), is also of interest.

| | |
|--------------|-------|
| dry matter | 86.2% |
| lignin | 0.6% |
| total sugars | 1.9% |
| pentosans | 6.2% |
| starch | 71.8% |

While no values were given specifically for popcorn, they would not be expected to differ greatly.

Flavor Investigations in Heat Processed Foods

Since no basic studies identifying the flavor components of popcorn were found in the literature, a survey was made of the available information on other food items which have received a high heat treatment, usually in a low moisture state, and which might be expected to develop similar types of flavor compounds by analogous pathways.

Cooked Corn

Steinke and Paulson (76) identified p-vinylphenol and 4-vinylguaiacol in condensed steam from cooking corn. The assumption that these phenols could arise by thermal decarboxylation of p-coumaric acid and ferulic acid, respectively, was supported by the production of phenolic compounds in an autoclaved, dilute, aqueous solution of the acids. Ferulic acid, p-coumaric acid, and vanillin were determined in corn before and after cooking. All three compounds showed five- to ten-fold increases on cooking. Concentration estimates for each compound were between 15 and 39 μg per gram of corn cooked at 250° F., and between 27 and 52 μg per gram of corn cooked at 280° F.

Dimethyl sulfide has been reported as a constituent of canned or heated sweet corn (4). The authors also showed the presence of S-methylmethionine sulfonium salt, a heat-labile compound which

decomposes to yield homoserine and dimethyl sulfide in unheated corn.

Roasted Barley

Roasted barley (Mugi-cha) flavor has been investigated by Japanese workers (70, 87) with the resulting identification of 35 compounds. The earlier study (70) identified only NaOH and Na₂CO₃ soluble components. Thus, all the 28 reported compounds were carboxylic acids, phenols, or phenolic acids. The second paper (87) reported the identification of furfural, 2-methylbutanal, 2-methylpropanal, 3-methylbutanal, 2,3-pentanedione, ethylglyoxal, and pyruvaldehyde as their mono- or bis-2,4-dinitrophenylhydrazones. Removal of the carbonyl compounds resulted in loss of the characteristic roasted barley aroma.

Bread

The literature on bread flavor was reviewed through 1965 by Coffman (11) with the observation that, in spite of the number of compounds identified up to then, a synthetic bread flavor could not yet be formulated. The development of flavor in bread is attributed to two main sources: the products of the yeast fermentation, and products of the Maillard browning reaction in crust formation. The Strecker degradation of amino acids during baking is also an important source of aldehydes derived from the parent amino acids as

shown in both model systems (64) and bread systems (66).

Two compounds with "cracker or bread-like" aromas have been isolated from freshly baked bread and proline-containing model systems (34, 33). They are 1, 4, 5, 6-tetrahydro-2-acetopyridine and N-acetonylpyrroline.

Potato Products

Self (68) reviewed the knowledge of flavor in potato products through 1965 including a discussion of amino acids, sugars, and lipids as possible precursors of flavors produced in cooking. The flavor of potato chips has been studied (10, 16, 53) with the result that 2, 4-decadienal and seven substituted pyrazine isomers are believed to make the most important contribution to desirable potato chip flavor. The pyrazines found were 2, 5-dimethyl-, 2, 6-dimethyl-, 2-ethyl-, 2, 3, 5, trimethyl-, 2-ethyl-5-methyl-, 2, 5-diethyl-, 2-ethyl-3, 6-dimethyl pyrazine. Methods for synthesis of these compounds are also given (10). The 2, 4-decadienal is believed to originate mainly from the decomposition of linoleic acid under the conditions of deep fat frying. Its flavor and odor properties are described as "deep fat fried" in character with a flavor threshold in water of 0.5 parts per billion (53). The flavor of 2, 5-dimethylpyrazine at a concentration of 10 p. p. m. in oil was described as "earthy" and "raw potato" (16).

The use of microwave finishing of potato chips has been

reported (22) to yield more uniformly colored chips than conventional deep frying even when reducing sugars were as high as 0.5%. The microwave finished chips showed lower losses of amino acids and sugars than those prepared by conventional deep fat frying.

Roasted Peanuts

Pyrazine derivatives identified from roasted peanuts (methyl-, 2,5-dimethyl-, 2 methyl-5-ethyl-, trimethyl-, and either 2,5-dimethyl-3-ethyl- or 2,6-dimethyl-3-ethyl pyrazine) have been deemed to be among the "character impact" compounds of typical roasted peanut flavor (50). N-methyl pyrrole, identified along with the pyrazines, was not considered to be an important contributor to roasted aroma. Based on quantitative studies of the amino acid and monosaccharide content of peanuts before and after roasting and the previously identified flavor compounds, a possible mechanism for the formation of pyrazine isomers from those precursors was postulated (57). From low water content model system studies where amino acids were reacted with ^{14}C -labeled glucose (and vice-versa, with labeled amino acids and unlabeled glucose), it was determined that the structure of the nitrogen source influenced the quantitative distribution of pyrazines formed but that the major source of carbon atoms in pyrazine molecules was the sugar (43). Ammonium ions were shown not to be the common intermediate through which nitrogen

entered the ring.

Benzaldehyde, phenylacetaldehyde, 2-methyl propanal, 2-methyl butanal, and 3-methyl butanal were among other compounds identified from roasted peanuts (51). Phenylacetaldehyde was believed to be of considerable importance to the sweet bouquet of roasted peanut aroma while the low molecular weight aldehydes contributed to the harsh aroma associated with warm, freshly roasted peanuts.

Pecan nut volatiles have been studied (65) and acetaldehyde, dimethyldisulfide, and methanol identified in the unroasted nuts. Nine pyrazine compounds from roasted pecans were tentatively identified from their mass spectra. Several compounds, presumed to be artifacts, including carbon disulfide, Freon 11, chloroform and other laboratory solvents, were encountered in this work. Rubber tubing used in the sweeping gas line was thought to be the source of carbon disulfide.

Cocoa and Chocolate

Several research groups have recently reported a large number of compounds identified in work on the flavor of cocoa and chocolate (84, 85, 23, 49, 60). Van Praag, Stein, and Tibbetts (85) suggested that the following compounds contributed to cocoa aroma: acetaldehyde, 2-methyl propanal, 2-methyl butanal, benzaldehyde, phenylacetaldehyde, 5-methyl-2-phenyl-2-hexenal, 2-furfural, methyl disulfide, 11

alkyl-substituted pyrazines, acetic acid, and isopentyl acetate.

The possible precursors for formation of flavor in roasting of cocoa beans have been studied and amino acids and sugars have been shown to be important precursors (63). Van Praag, Stein, and Tibbetts (85) in model system studies observed a similar series of pyrazines formed from each of six different neutral amino acids and ammonia when reacted with fructose in aqueous solutions. They assumed that ammonia was an intermediate and stated that, qualitatively, the composition of the pyrazine mixture did not depend significantly upon the amino acid involved.

Coffee

The accumulated information available on coffee flavor up to 1966 was reviewed by Gianturco (25) with a total of 103 reported compounds listed. Stoffelsma et al. (78) have concluded that at least 318 volatile constituents are known to occur in roasted coffee up to late 1968. They have also stated that at least a qualitative similarity exists between the aroma oil prepared from a steam distillate of roasted and ground coffee and the aroma complex obtained by stripping the volatile fraction of expelled coffee oil.

In spite of the 318 constituents known in coffee volatiles, it has not yet been possible to reconstitute a complete coffee aroma. Some reasons for this (which apply to most complex flavors and aromas)

are (a) no single component responsible for the aroma of coffee has been found, (b) problems of aroma component stability must be solved, and (c) probably important aroma components remain to be detected and identified (78).

Feldman, Ryder, and Kung (19) have reported on a study undertaken to determine the importance of non-volatile compounds to the flavor of coffee. Non-volatile acids, both phenolic and non-phenolic, are reported to be important in the flavor along with non-volatile products from the Maillard browning reactions.

Many of the recent publications on coffee flavor (78, 77, 24, 7, 28, 79) contain valuable data (mass and infrared spectra, GLC retention times, synthetic methods) which have been especially helpful in the current investigation of popcorn flavor.

Tea

Although tea is not a high carbohydrate product processed strictly by heating as most of the products reviewed, many of the volatile compounds identified (8, 6) are common to the group. While furan compounds are common in tea, pyrazine derivatives have not yet been identified as important flavor compounds. This absence (or very low level) must surely be one of the significant differences when the flavors and aromas of coffee, peanuts, and tea are compared, for example.

Volatiles from Cooking Oils

The oils used in cooking of foods such as potato chips or popcorn are a possible source of flavor compounds. The flavorful components could be contained in the oil, generated from precursors in the oil during heating, or they could be the result of autoxidative processes during and after cooking. Nawar (55) has recently reviewed thermal degradations of lipids with much of the reported work being done with model systems.

Crude coconut oil volatiles were investigated by Allen (1) who identified the C₇, C₉, C₁₁, C₁₃, and C₁₅ methyl ketones and the C₆, C₈, C₁₀, C₁₂, and C₁₄ delta-lactones. The flavor properties of lactones, also found in butter and heat-treated dairy products, have been reported by Siek et al. (72). The presence of lactones and methyl ketones in coconut oil and their desirable flavor contribution probably accounts for the preference of many popcorn processors for coconut oil.

The volatile decomposition products of corn oil in a simulated restaurant deep frying use were analyzed with the resultant identification of 30 acidic (39) and 45 non-acidic compounds (44). Another 20 compounds were tentatively identified. In a similar study using a hydrogenated cottonseed oil, 38 acidic compounds (88) and 61 non-acidic compounds (58) were identified. The studies indicated that the

hydrogenated cottonseed oil was more stable towards autoxidation, but more susceptible to hydrolysis, than unhydrogenated corn oil. From a flavor viewpoint, many of the compounds identified would be expected to contribute significantly to the flavor and aroma of food products cooked under commercial conditions in the oils. In cooking popcorn, specifically, the oil is actually used only once for a short period of time at the high (180° C to 260° C) temperatures employed (82). This type of use is not apt to result in the formation of oil decomposition products to the extent indicated in the studies on corn and hydrogenated cottonseed oil used in deep frying.

Flavor Studies in Model Systems

Since the production of flavor in heat-induced or non-enzymatic browning reactions has appeared so complex, various types of model systems have been devised in an attempt to simplify the study of such reactions. Hodge (32) has reviewed the literature through 1965 as it pertains to flavor production from caramelization of sugars, Maillard browning reactions of reducing sugars and amines, and Strecker degradation of amino acids. All the pathways, as defined by the above mentioned names, in most cases occur in an inseparable combination. For instance, the Strecker degradation requires a conjugated dicarbonyl to react with the α -amino acids in order to produce aldehydes of one less carbon atom. Such dicarbonyls (and

furfurals) are produced as products and intermediates in caramelization and Maillard reactions. Hodge (33) considers the Maillard reactions to be the most important flavor producing reactions in foods on drying, processing, and cooking. The reaction of proline and hydroxyproline with carbohydrates is considered very important among model systems that represent cereal flavor producing reactions (33).

An interesting side effect of non-enzymatic browning reaction products is their antioxidant effect on unsaturated fats. Evidence has been published (42) which implies that the antioxidant activity is more strongly associated with the non-dializable melanoidin fraction than with reductones as previously thought.

Sugar-amine interactions in model systems were reviewed in depth by Reynolds in 1968 (59), drawing together the present knowledge in this field. The author points out that the results of some model system studies must be interpreted somewhat carefully when extrapolating the results back to food products because of the different reaction conditions which prevail. Recent publications dealing with volatile compounds from heated glucose (86) and base-catalyzed fructose degradation (79) have appeared.

The studies of pyrazine formation from sugar-amino acid systems (43, 85) have been mentioned previously with respect to the work on peanut and chocolate flavor precursors. These studies have been some of the most interesting and fruitful from the standpoint of

partially defining formation mechanisms of roasted-type-flavors.

Since pyrrole compounds, especially 2-acetyl pyrrole and derivatives of pyrrole-2-carboxaldehyde have been identified as products from sugar-amine browning systems (37, 46), a further study was made by Kato and Fujimaki (38). Their isolated products were N-substituted pyrrole-2-aldehydes where the N-alkyl groups was from the original alkyl amine. Reactivities of amines in terms of yield of pyrrolealdehyde and absorbance at 470 m μ were in the order of n-butylamine > methylamine, β -alanine > glycine > DL-alanine. Their results indicate that furfural and pyrrolealdehydes are by-products in melanoidin formation formed possibly from a 3, 4-dideoxy-pentosulose-3-ene intermediate.

A study of thermal degradation of amino acids (62) by pyrolysis followed by analysis in a coupled GC-mass spectrometer showed the following compounds to be unique for each amino acid listed:

| | |
|-----------------|--------------------------|
| glycine | acetone |
| alanine | acetaldehyde |
| β alanine | acetic acid |
| valine | 2-methyl propanal |
| norvaline | n-butanal |
| leucine | 3-methyl butanal |
| isoleucine | 2-methyl butanal |
| serine | pyrazine |
| threonine | 2-ethylethyleneimine |
| taurine | thiophene |
| methionine | methyl propyl sulfide |
| cystine | methyl thiophene |
| phenylalanine | benzene |
| tyrosine | toluene |
| tryptophan | ammonia, CO ₂ |

proline
hydroxyproline

pyrrole
methanol, N-methyl pyrrole

Acetonitrile and carbon dioxide also appeared in the pyrolyzate of all amino acids.

Volatile compounds produced from the thermal degradation of starch at temperatures between 175 ° and 400 ° C. have been reviewed by Greenwood (29). Those compounds known include acetaldehyde, furan, propanal, acetone, acrolein, 2-methyl furan, butanal, 2-butanone, 2,5-dimethyl furan, pentanal, 2-pentanone, 3-pentanone, 2-propanone, formaldehyde, formic acid, acetic acid, and furfural.

Ferulic acid and p-coumaric acid occur widely as esters in vascular plants (56) along with a variety of similar compounds implicated as intermediates in the biosynthesis of lignins. The thermal decomposition of ferulic acid (4-hydroxy-3-methoxycinnamic acid) has been investigated (21) and the following compounds identified as thermal decomposition products: 4-methylguaiacol, 4-ethylguaiacol, 4-vinylguaiacol, vanillin, 4-hydroxy-3-methoxyacetophenone, and vanillic acid. Many of these and other phenolic compounds were identified in hickory smoke condensates (20). They are now quite well established as being produced from lignin or lignin precursors present in the plant tissue.

EXPERIMENTAL

Receipt and Storage of Popcorn

Fifty pounds of hybrid popcorn¹ was received and sealed in number 10 "C" enameled cans under nitrogen. The cans were stored at ambient temperature in the laboratory and opened just prior to use. These steps were taken to preserve the proper moisture content and to minimize chances of contamination from sources such as solvents in laboratory air.

Analysis of Popper Headspace Condensate

Popping Apparatus - - Oil Popped Corn

An electric home popcorn popper of 2 1/2 qt capacity made of aluminum was utilized. The cover was modified to accept sweeping gas inlet and outlet tubes of 1/8 inch OD stainless steel tubing. After being swept from the popper by gentle suction from an aspirator, volatiles were collected in a trap filled with glass beads which was cooled in a bath of dry ice and 2-methoxy-ethanol (methyl cellosolve). A water safety trap was used to prevent back surges from the aspirator into the cold trap. The entire apparatus was placed in a clean

¹Robust white hullless, lot #36735, White Cloud variety, supplied by Crookham Company, Caldwell, Idaho.

fume hood in a laboratory where solvents were not used.

The popper was charged with approximately 40 ml of a commercial cottonseed oil² and allowed to preheat for 2 to 3 min at which time 60-70 g of popcorn was added. Suction was applied from the time the first kernel popped until popping was judged complete.

Aqueous Condensate Analysis--Oil Popped Corn

The volatile material collected in the glass bead cold trap from three batches of popcorn totaled 20-25 ml in volume and was termed the aqueous condensate. The aqueous condensate was subjected to gas-liquid chromatography (GLC) utilizing the on-column trapping technique of Morgan and Day (54). Twelve ml samples of aqueous condensate were added to screw-capped vials (Kimble No. 60957, size 1) containing approximately 1 mg of tetradecanol to control foaming during collection of volatiles. The vials were sealed with screw caps fitted with silicone rubber liners and shaken vigorously to disperse the tetradecanol. Operating parameters for the analysis were as follows:

Entrainment apparatus

| | |
|------------------------|--------------------------------------|
| purge time and rate | 10 min at 8 ml/min of N ₂ |
| water bath temperature | 80° C ± 3° C |

²Wesson Sales Co., Fullerton, California. Purchased in gallon lots from local suppliers.

GLC apparatus

| | |
|--------------------------|---|
| Instrument | Aerograph 204-1B |
| Detector and temperature | Flame ionization, 210 ° C |
| Injector temperature | 200 ° C |
| Column | 3% 1, 2, 3- <u>Tris</u> (2-cyanoethoxy) propane on 100-120 mesh AW- DMCS Chromosorb G, 12 feet x 1/8 inch OD |
| Flow rate | 20 ml/min of N ₂ |
| Column temperature | Programmed from 40 ° C to 125 ° C at 2 ° /min, then held at 125 ° C |

Seven fractions were trapped as they eluted from the effluent splitter in stainless steel traps six inches long and 0.03 inch ID which were cooled with dry ice. These fractions were transferred, as described by Scanlan, Arnold, and Lindsay (67), to a capillary column connected to the high vacuum system of an Atlas CH-4 mass spectrometer.

Mass Spectrometry. The operating conditions for the gas chromatographic-mass spectral (GC-MS) analysis of traps designated 1, 2, 5, and 7 obtained as outlined above are given below. Traps 3, 4, and 6 were not used because water eluted along with the other components in them. Trap 5 was found to contain a large amount of water when analyzed.

GC

| | |
|--------------------|---|
| Instrument | F and M 810, inlet system modified as in (67) |
| Column | 300 feet x 0.01 inch ID coated with butanediol succinate (BDS) |
| Column temperature | 60° isothermal for traps 1, 2, and 5 87° isothermal for trap 7 |
| Flow rate | 1 ml/min of Helium |

MS

| | |
|----------------------|--|
| Filament current | 20 eV source 45 μ A 70 eV source 10 μ A |
| Electron voltage | 20 eV and 70 eV |
| Accelerating voltage | 3000 V |
| Analyzer pressure | 1.5×10^{-6} Torr |
| Multiplier voltage | 1.60 KV |
| Scanning speed | 2.5 sec from m/e 25 to m/e 250 and 5.0 sec from m/e 25 to m/e 250 for compounds with longer retention times |

The Atlas CH-4 is a Nier-type (9 inch, 60 degree sector), single-focusing mass spectrometer. It was equipped with a double

ion source into which the total effluent was directed from the capillary column. Since the 20 eV source operates at less than the ionization potential of the carrier gas (Helium), but above that of organic compounds, it is used as a GC detector. The 70 eV source provides the ionization used to obtain mass spectra.

Spectra were taken whenever the 20 eV readout gave any indication of a peak, with an effort made to obtain spectra at the peak apex and, where possible, on the leading and trailing edges to help pinpoint incompletely resolved components. Spectra were also taken at intervals during a run at points where there appeared to be no peaks; these spectra served as backgrounds enabling one to monitor and subtract column bleed and other background mass peaks from the sample component spectra.

Ether Extract Analysis--Oil Popped Corn

Steam Stripping of Oil. Since a GC analysis of the fresh commercial cottonseed oil by the on-column trapping technique had shown several peaks, the oil was steam stripped under vacuum to remove those volatile compounds which did not originate in the popping operation itself.

The apparatus utilized for vacuum steam stripping of the commercial cottonseed oil was the same as that described by Siek and Lindsay (71) for removal of volatiles from butter oil. Six liters of

oil placed in the 12 liter flask were degassed for one hour at room temperature. The temperatures were then raised on the steam generator and oil flask. With a system pressure of 1-2 m Hg (at the pump end of the system), the oil was stripped at 200° C for 1 hr; the temperature was then lowered to 180° C and stripping continued for 3 hr. The system was opened and 1.2 ml (about 0.02%) of Tenox 2 antioxidant³ in 3 ml of ethanol was added to the oil. With power to the steam generator and oil flask heating mantles off, the oil was distilled for one hour to remove the ethanol. The oil was cooled and stored in sealed glass bottles in the dark. The flavor of the stripped oil was much more bland than that of the original oil.

Collection of the Volatiles--Ether Extraction. Water eluting from the column in the gas chromatographic analysis of aqueous condensate by the on-column trapping procedure interfered with mass spectrometric analysis of the eluted fractions. For this reason, a source of volatile compounds free from water was desired. Ether extraction of the aqueous condensate provided a water-free sample, and furthermore, allowed the recovery of larger quantities of material, especially higher boiling components.

Corn was popped as previously described using the steam

³Eastman Chemical Products, Inc., Kingsport, Tenn. Tenox 2 is a food grade antioxidant containing 20% BHA, 6% propyl gallate, 4% citric acid, and 70% propyleneglycol.

stripped cottonseed oil, and the aqueous condensate was collected in the same manner. The contents of the cold trap (glass beads and aqueous condensate) were extracted three times with 65 ml portions of diethyl ether.⁴ The ether extract was dried over anhydrous sodium sulfate in the cold for at least four hours, then filtered through glass wool plug. Ether was removed by slowly refluxing through a small condenser with a calcium chloride drying tube. When concentrated to about 5 ml, the extract was transferred to a chromatographic storage tube⁵ and further concentrated with a slow stream of dry Nitrogen gas to a volume of 20-50 μ l. The sample was held at 5-6° C until used for GC analysis.

GC and Mass Spectrometric Analysis. Samples of the ether extract prepared from corn popped in stripped cottonseed oil were chromatographed twice on each of two different packed columns.

GC Conditions

| | |
|--------------------------|--------------------------|
| Instrument | Aerograph 204-1B |
| Detector and temperature | Flame ionization, 230° C |
| Injector temperature | 230° C |
| Effluent splitter | 9:1 ratio, 225° C |

⁴B and A Anhydrous Reagent, Allied Chemical Co., Morristown, N. J. Purchased from local suppliers in 1 lb containers.

⁵Kontes Glass Company, Vineland, N. J. Catalog number K-42256.

| | |
|---------------------|---|
| BDS column (B) | 2.5% Butanediol succinate on 100-120 mesh AW-DMCS Chromosorb G, 12 feet long x 1/8 inch OD, Flow rate--20 ml/min at 100 ° C. |
| Apiezon column (A) | 2.5% Apiezon L on 100-120 mesh AW-DMCS Chromosorb G, 12 feet long x 1/8 inch OD, Flow rate--20 ml/min at 100 ° C. |
| Temperature program | 55 ° C to 200 ° C at 2 ° /min, hold at 200 ° C. Same for both columns. |

The first run on each column (2.5-3 μ l sample size) was made with two persons evaluating the aroma of the gas stream from the effluent splitter. Subsequently, a 6 μ l sample was injected on each column and a fraction trapped from each column during the time that the most popcorn-like aromas had previously been noted as eluting. These fractions were stored in a freezer until subjected to GC-MS analysis. Operating conditions for the GC-MS were the same as previously described except for the capillary column operating temperatures. These isothermal temperatures were 83 ° C for the fraction trapped from the BDS column and 100 ° C for the fraction trapped from the Apiezon L column.

Popping Apparatus--Microwave Oven

Because several compounds had been encountered in the previous work which were thought to arise from the oil used in popping (40, 45), a method was needed to pop the corn without the need for oil as a medium for heat transfer. The microwave oven unit available, a Model 1161 Ratheon Radarange, fulfilled this need. The oven was thoroughly cleaned and an all glass apparatus assembled for containing the popping corn and collecting the headspace volatiles.

A round Pyrex glass 2 qt pan seven inches in diameter and four inches deep with a small pouring lip served as the popping container with the lid being a piece of plate glass eight inches square. Two pieces of 3 mm glass tubing were inserted through the stainless steel mesh grill in the oven door and into the pouring lip opening in the pyrex popper. One of these tubes carried Nitrogen sweep gas into the popper, and the other was connected to the cold traps and aspirator to allow collection of the volatiles produced. Two cold traps were connected in series: the first contained no packing material and was cooled by an ice-water and salt mixture, while the second was filled with glass beads and cooled by a dry ice-ethanol bath. The trapping system was all glass, fitted by means of standard taper or ball and socket joints which were not greased. A one inch long piece of Tygon tubing was used to connect the first cold trap to the

glass tube reaching through the oven door into the popper. Suction was supplied by a water aspirator connected to the exit of the second cold trap.

Before each use all the glassware, including the glass beads, was washed thoroughly in hot detergent solution, rinsed in tap and distilled water, and dried in a 100° C hot air oven. After removal from the oven, each piece was rinsed with diethyl ether to insure removal of any traces of grease.

Approximately 600 g of corn could be popped before the second trap accumulated enough water to freeze solid. The corn was popped in batches of 60 to 70 g which required about 80 sec at the "high" heat setting on the oven. Nitrogen was bled slowly into the popper to reduce the risk of drawing contaminated room air into the cold traps. Aspirator suction was applied from the time the first kernel popped until the oven timer turned off.

Corn popped in the microwave oven did not expand quite as fully as that popped in oil, and approximately 10% of the kernels did not pop. If the oven were left on until all the kernels popped, considerable scorching of the earlier popped kernels occurred. This was accompanied by a strong burned odor.

Ether Extract Analysis--Microwave Popped Corn

Trapped Fractions by GC-MS Analysis. The aqueous

condensate obtained from the headspace as the corn was popped in the microwave oven was extracted with diethyl ether as described for the oil popped corn. The extract was dried, concentrated, and chromatographed on the BDS and Apiezon L packed columns in the Aerograph 204 under the same conditions outlined on page 19. After an initial run to evaluate the aroma of the eluting fractions, another run was made in which successive fractions were trapped in the stainless steel traps for GC-MS analysis in the manner previously described. Instrumental conditions were the same except for the BDS capillary column temperature which was controlled isothermally as shown below.

| | |
|------------------------------|---------|
| Traps A-1, B-1, and B-2 | 90 ° C |
| Traps B-3, B-4, and B-5 | 147 ° C |
| Traps B-6, B-7, B-8, and B-9 | 180 ° C |
| Traps B-10 and B-11 | 185 ° C |
| Trap B-12 | 175 ° C |

The fraction designated A was trapped from the Apiezon L packed column, and those designated B were obtained from the BDS column.

Whole Extract by Coupled Capillary Column GC-MS. The aqueous condensate from microwave popped corn was extracted for 24 hours in a 250 ml continuous liquid-liquid extractor with freshly

distilled anhydrous reagent diethyl ether.⁶ The extract was dried over Na_2SO_4 for 12 hr at 5-6° C. The ether was removed by distillation through a 1 cm x 60 cm glass helix-filled column with a fractionating head. The drop ratio at the fractionating head was controlled electronically at a ratio of about 3:1 with the larger part being directed back down the column. Ten to 12 hr was required to remove 200 ml of ether. The extract was evaporated to 5-10 ml, then transferred to a chromatographic sample tube and further concentrated in a slow Nitrogen stream to about 20 μl .

Preliminary investigation allowed conditions to be established utilizing a Perkin-Elmer model 226 gas chromatograph with a 300 foot x 0.01 inch ID capillary column coated with butanediol succinate.

BDS Capillary--Perkin Elmer 226

| | |
|-----------------------------|--|
| Injection block temperature | 230° C |
| Detector temperature | 240° C |
| Injection Split | 100:1 |
| Carrier gas | Nitrogen, 40 psi (approx. 2 ml/min) |
| Temperature Program | 60° C to 200° C at 1° / min, hold at 200° C |

⁶A control sample of the B and A Anhydrous Reagent ether, when evaporated to 20-50 μl , was found to contain BHT. This was removed by a simple distillation.

Retention time data were obtained on the BDS capillary column using the above conditions. These retention times were used to substantiate those obtained on the capillary column GC-MS system which was operated under the conditions outlined below for three separate runs.

GC Conditions

| | |
|-----------------------------------|---|
| Instrument | F and M 810 |
| Column and dimensions | Butanediol succinate 300 foot x 0.01 inch ID |
| Temperature program | First and second runs: 60° C to 200° C at 1° /min--hold Third run: 150° C hold for 10 min, then 4° /min to 200° C and hold |
| Flow rate | 2 ml/min, initially, of Helium |
| Injector split and temperature | 50:1, 230° C |

MS Conditions

| | |
|------------------|---|
| Filament current | 20 eV source 50 μ A 70 eV source 25 μ A, 1st run 40 μ A, 2nd run 34 μ A, 3rd run |
|------------------|---|

All other operating conditions for the Atlas CH-4 mass spectrometer were the same as previously listed on page 18.

Ether extracts were evaporated under a Nitrogen stream until no further volume reduction could be achieved. Sample sizes injected for the three GC-MS analyses were 1 μ l, 4.5 μ l, and 3 μ l.

Acidic and Basic Fractions by Packed Column GC-MS. The aqueous condensate from microwave popped corn (pH approx. 7.0) was acidified to pH2 (pH paper) with 10% HCl, then extracted three times in a separatory funnel with 25 ml portions of freshly distilled diethyl ether to yield a fraction enriched in acidic and neutral compounds. The aqueous solution was then adjusted to pH 9-10 with sodium carbonate powder and extracted four times with 25 ml portions of freshly distilled diethyl ether to yield a fraction enriched in basic compounds.

Ether was removed from both fractions on the column and fractionating head as previously described; they were then transferred to chromatographic sample tubes and concentrated under a Nitrogen stream as much as possible prior to GC-MS analysis as described below.

The Atlas CH-4 mass spectrometer was fitted with an EC-1 inlet valve which could be adjusted to allow from 5% to 10% of the GC column effluent to enter the dual ion source. The remaining 90-95% of the effluent was directed through a heated tube so that the odor of the components being analyzed could be evaluated simultaneously. The strip chart output from the 20 eV ion source

provided a gas chromatogram. In actual operation, the 20 eV source signal/noise ratio was extremely small, rendering all but the largest peaks indistinguishable. As a result, reliance was placed on odor assessment to detect the presence of components in the effluent.

Operating conditions for the mass spectrometer were the same as described on page 18 except that the 70 eV filament current was 30

μ A. Operating conditions for the gas chromatograph were as follows:

| | |
|----------------------------|--|
| Instrument | F and M 810 |
| Injection port temperature | 232 ° C |
| Transfer line temperature | 210 ° -250 ° C |
| Column | 5% Diethyleneglycol succinate (DEGS) on 100-120 mesh AW- DMCS Chromosorb G |
| Column dimensions | 12 feet x 1/8 inch OD stainless steel |
| Flow rate | 25 ml/min Helium measured at 100 ° C |
| Temperature program | 60 ° C to 200 ° C at 2 ° /min, hold at 200 ° C |

Infrared Spectra. Infrared spectra were obtained for some of the large peaks late in the DEGS column chromatogram from the ether extract of microwave popped corn aqueous condensate. The compounds were trapped from the effluent splitter on the Aerograph

204 in 1/16 inch by 12 inch glass capillary tubes cooled with crushed dry ice. The compounds were washed carefully from the tubes onto a 5 mm etched square in the center of a sodium chloride plate with a small amount of ether or acetone. Careful evaporation of the solvent left a thin film of the compound suitable for obtaining the infrared spectrum using a Beckman Model IR5 spectrophotometer equipped with a 5x KBr lens type beam condenser.

Preparation and Analysis of Vacuum Steam Distillate

Distillation

A diagram of the reduced pressure steam distillation apparatus used for recovery of volatiles from popped corn is shown in Figure 1. The foam traps were included as a precautionary measure, but no foaming occurred. They did serve to prevent any particulate material from the bumping action from being carried over into the condensate traps.

Popped corn was prepared by grinding a total of approximately 900 g (weight before popping) in an auger-type food grinder (Enterprise Model 2112, 1/3 HP) so that the largest particles were 2-4 mm in size. The ground popcorn was then slurried in nine liters of glass-distilled water and poured into the 22 liter distillation flask. To the steam generator was added 1500 ml of glass distilled water and a

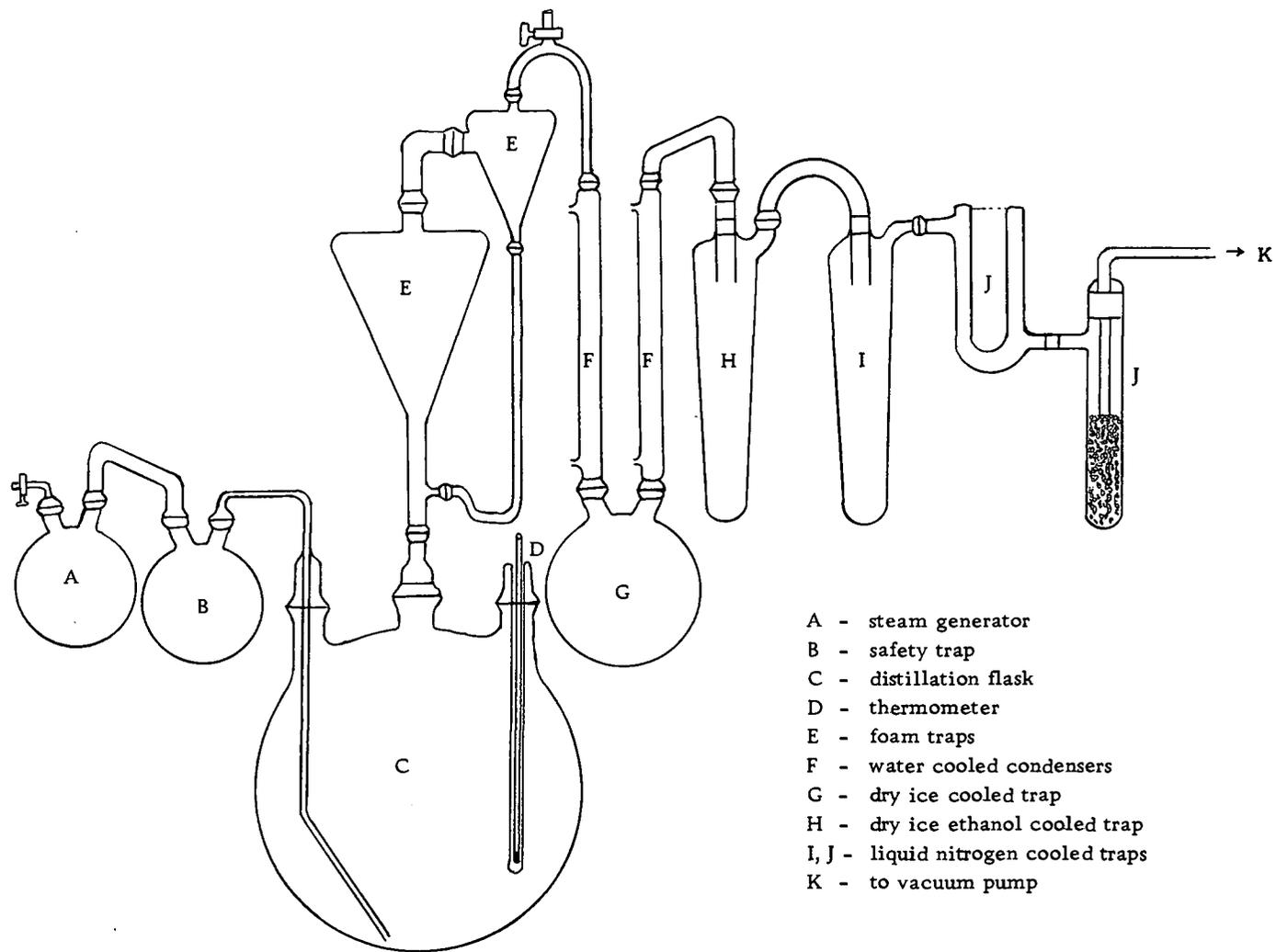


Figure 1. Vacuum steam distillation apparatus.

few boiling chips. The cold traps were cooled and degassing begun slowly to prevent violent bumping of the popcorn slurry. Power was applied to the heating mantle on the distillation flask at a rate sufficient to maintain the slurry at a temperature of 20-24° C. The steam generator was heated sufficiently to keep the water boiling at a steady rate. With a system pressure of near 1 mm Hg at the pump end of the system, distillation was continued for 4 hr.

Extraction

The aqueous condensate from traps G, H, and I in Figure 1 was combined, saturated with sodium chloride, placed in a 3 liter capacity liquid-liquid extractor, and extracted with a total of 500 ml of freshly distilled anhydrous reagent diethyl ether for 24 hr. The ether extract was dried over sodium sulfate and concentrated to approximately 15 ml using the column and fractionating head as described on page 22. The extract was then divided into two equal portions in separate chromatographic samples tubes, one of which was stored without concentrating at 5-6° C for future packed column GC-MS analysis. The other portion was concentrated and subjected to GC analysis on several different columns.

Packed Column GC-MS Analysis

The ether extract of the aqueous distillate obtained from

microwave popped corn was analyzed under conditions identical to those described for the acidic and basic fractions on page 28 and 29.

The ether extract of the distillate from oil-popped corn was analyzed by gas chromatography only. The chromatograms were compared to those obtained from microwave popped corn under similar conditions.

Synthesis of Reference Compounds

Furan-2-glyoxal

The method of Kipnis and Ornfelt (41) involving selenium dioxide oxidation of the methyl group of 2-acetyl furan to an aldehyde was used to prepare furan-2-glyoxal. In a 250 ml 3-neck flask fitted with a reflux condenser, sealed stirrer, and thermometer was placed 8.75 g (0.087 mole) of SeO_2 , 55 ml dioxane and 1.6 ml of water. The mixture was stirred at 50 °C until solution was complete. Then 9.5 g (0.086 mole) of 2-acetyl furan was added and the mixture refluxed with stirring for four hours. The reaction mixture was filtered through a bed of Celite filter aid to remove the SeO_2 , and the solvent was removed on a rotary evaporator. Vacuum distillation of the residue through a 10 cm Vigreux column yielded 5 g of a yellow oil (46% yield) which boiled at approximately 59 °C at a pressure of 4 mm. A mass spectrum of the product was obtained by GC-MS

utilizing the 300 foot x 0.01 inch ID BDS capillary column operated isothermally at 149° C. Packed column GC was used to determine product purity.

N-furfuryl pyrrole

The method reported by Gianturco et al. (27) was utilized. One-half mole each of furfurylamine (48.5 g) and 2,5-dimethoxy-tetrahydrofuran (66.0 g) was mixed with 15 ml of glacial acetic acid and refluxed for 30 min. The mixture was cooled and adjusted to pH 9 with 30% NaOH. The mixture was steam distilled, the distillate was extracted with redistilled diethyl ether, and the ether was removed on the rotary evaporator. The yield was 52 g (70%) of a clear liquid. The product was repeatedly chromatographed on a 12 foot x 1/8 inch OD, 5% DEGS column, and the pure N-furfuryl pyrrole was trapped in glass melting point capillaries. The above pure samples were used to obtain mass and infrared spectra as well as a refractive index.

N-furfuryl pyrrole-2-carboxaldehyde

This method, utilizing a Vilsmeier reaction to formylate N-furfuryl pyrrole prepared previously, was adapted from that of Silverstein, Ryskiewicz, and Willard (74). In a 250 ml 3-neck flask fitted with a condenser and drying tube, a sealed stirrer, and a

dropping funnel was placed 11.7 g (0.16 mole) of dimethylformamide. The solution was stirred and cooled on an ice bath to 5 ° C while 24.6 g (0.16 mole) of phosphorous oxychloride was added slowly. The ice was removed and the mixture stirred 10 min. The temperature was increased slowly while 22.1 g (0.15 mole) of N-furfuryl pyrrole was added dropwise over a period of about 30 min. The mixture was stirred for an additional hour with the temperature held at 60 ° C. A cold solution of 85 g of sodium acetate in 100 ml of water was added through the condenser. The mixture was heated to boiling, held for 10 min, then cooled to room temperature. The mixture was extracted with redistilled diethyl ether, dried over sodium sulfate, and the ether removed to yield about 5 g of viscous, dark colored product. Vacuum distillation of the crude product was attempted, but it did not completely separate the reaction products. The 2 g of recovered material formed mixed crystals on standing which were analyzed by preparative GC. The two predominant components were trapped for infrared and mass spectrometric analysis.

2, 3-Dihydrobenzofuran

The synthesis of 2, 3-dihydrobenzofuran was accomplished by catalytic hydrogenation of benzofuran in ethyl acetate solution. Ten milligrams of the catalyst, 5% Palladium on BaSO₄, was added to

15 ml of ethyl acetate containing 1 ml of benzofuran in a 50 ml flask. A teflon coated stirring bar was added, and the flask was connected to a hydrogenation apparatus described by Farquhar et al. (18). The hydrogen pressure over the surface of the stirred solution at room temperature corresponded to a 30 cm head of water. No further hydrogen was taken up after 18 hr at 25° C, so the flask was removed, the solution filtered to remove the catalyst, and the ethyl acetate taken off on the rotary evaporator. The remaining liquid was analyzed by preparative gas chromatography with the 2, 3-dihydrobenzofuran peak being trapped for mass spectral analysis.

4-Vinyl-2-methoxyphenol

The preparation of 4-vinyl-2-methoxyphenol (p-vinyl guaiacol) was performed by the thermal decarboxylation of 4-hydroxy-3-methoxycinnamic acid (ferulic acid) (21). The reaction was performed in a nine inch disposable glass pipette (Scientific Products no. P-5210-2) into which a loose glass wool plug was inserted as far as possible from the large end. Approximately 300 mg of ferulic acid was placed in the large end against the plug and a length of heater tape was wrapped tightly around the area containing the sample and the glass wool plug. A thermocouple was placed against the glass beneath the heater tape and connected to a pyrometer for a temperature monitor. A powerstat was used to control the current

through the heater tape to maintain the temperature between 200 ° C and 250 ° C. Dry Nitrogen gas at a flow rate of 20 ml/min was passed through the reaction chamber to sweep the liberated p-vinyl guaiacol out through the drawn-out portion of the pipette into a 1/16 inch x 12 inch glass capillary tube connected by means of a short sleeve of 1/16 inch ID Teflon tubing. The droplets were collected in the capillary tube. When the reaction was completed, one end of the tube was sealed in a flame and the tube was centrifuged to concentrate the product at the sealed end. The product purity was greater than 99% as judged by gas chromatography. Infrared and mass spectra were obtained on the product without further purification.

4-Vinylphenol

The thermal decarboxylation of p-hydroxycinnamic acid (p-coumaric acid) to yield p-vinylphenol was performed in the same apparatus under the same conditions as described for preparation 4-vinyl-2-methoxyphenol. The product, which formed a white solid at room temperature, was obtained in about 95% purity as shown by GC. Mass spectra and infrared spectra were obtained on the product.

2, 3 Dimethyl-5, 6-dihydropyrazine

This compound, an intermediate in the synthesis of 2, 3-dimethyl pyrazine, was prepared as described by Ishiguro and

Matsumura (35). Briefly, the method consisted of slowly adding 20 g of diacetyl in 160 ml of ether to a stirred solution of 18.5 g of ethylenediamine and 200 ml of ether. A white precipitate formed which was recovered by filtration of the ether solution. Several attempts to recrystallize the pure dihydropyrazine from a mixture of ether and isopropanol were made. Mass spectra of the material obtained indicated that any heating of the reaction mixture (which eventually resulted in a clear solution) decreased the percentage of the 2,3-dimethyl-5,6-dihydropyrazine, and concurrently produced 2,3-dimethyl pyrazine.

RESULTS AND DISCUSSION

Identification of Compounds

Aqueous Condensate--Oil Popped Corn

Of the fractions trapped from the direct analysis of the aqueous condensate, only the first two did not contain a considerable quantity of water which tailed extremely on the packed Tris column. The main constituents of traps 1 and 2 were pentanal and hexanal, respectively, as determined by comparison of their mass spectra with reference spectra (2). Acetaldehyde, acetone, diacetyl, 3-methyl butanal, and Freon 12, all present in small amounts, were also identified on the basis of their mass spectra.

The presence of water eluting from the packed Tris column interfered with the mass spectral analysis of components eluting after about six minutes from the starting point. Thus, those components which were judged to have more important odor properties could not be subjected to mass spectrometry with this technique.

The presence of Freon 12 was observed frequently when samples were introduced into the mass spectrometer from the 1/16 inch OD by six inch stainless steel traps. The origin of the Freon 12 could not be determined, although the dry ice used to cool the traps was suspected as a possible source. A silicone contaminant (3) was also

frequently observed early in the capillary column chromatogram when using the trapping technique.

The presence of the large amounts of pentanal and hexanal relative to other components in the packed column chromatogram was taken as an indication of oxidative breakdown of the oil used (40). It appeared that these compounds would have to be minimized to facilitate analysis for the compounds produced from the popcorn itself. For this reason a quantity of the cottonseed oil was vacuum steam stripped to remove at least those volatile compounds which were initially present. The steam stripped cottonseed oil had a very bland taste compared to a sample of untreated oil. Later, the microwave oven proved to be effective in popping the corn without the use of oil.

Ether Extract--Oil:Popped Corn

The ether extraction of organic compounds from the aqueous popper headspace condensate allowed the isolation of volatile organic compounds free from water and in higher yield than the previous method. The ether extract aroma was intensely "nutty and popcorny." Sniffing the effluent gas stream from the ether extract on the BDS and Apiezon L packed columns indicated that there was at least one area of each chromatogram which had odors reminiscent of popcorn. These areas were trapped in the stainless steel traps and subjected to GC--MS analysis. Furfural and 2-pentyl furan were identified in

both fractions on the basis of their mass spectra with retention times in agreement with those of known compounds run on both the packed columns and a BDS capillary column. In addition, acetyl pyrazine- (MW 122), 2-pentyl thiophene-(MW 154), and n-dodecane-MW 170) were tentatively identified from their mass spectra. The largest component in each fraction was 2-pentyl furan which has been reported as being responsible for the "grassy or beany" character of reverted soybean oil (75). The 2-pentyl furan was also isolated from slightly oxidized soybean and cottonseed oils as well as thermally oxidized corn oil and hydrogenated cottonseed oil (45). The flavor threshold of 2-pentyl furan in oil was reported to be 1 ppm. In the chromatograms of popcorn flavor isolates, popcorn-like aromas were noted slightly before and after the 2-pentyl furan peak. However, it is believed that 2-pentyl furan does not, itself, contribute significantly, to the rich, desirable, fresh popcorn aroma.

Ether Extract--Microwave Popped Corn

Trapped Fractions by GC--MS. Figure 2 shows the chromatogram of the ether extract obtained on the BDS packed column and shows the areas which were trapped for subsequent GC--MS analysis. Table 1 lists the compounds identified in each trap and the information on which the identification was based. The very large quantities of 3,5-di-tert-butyl-4-hydroxytoluene (BHT) were traced to the anhydrous diethyl ether (47). In all subsequent work the ether

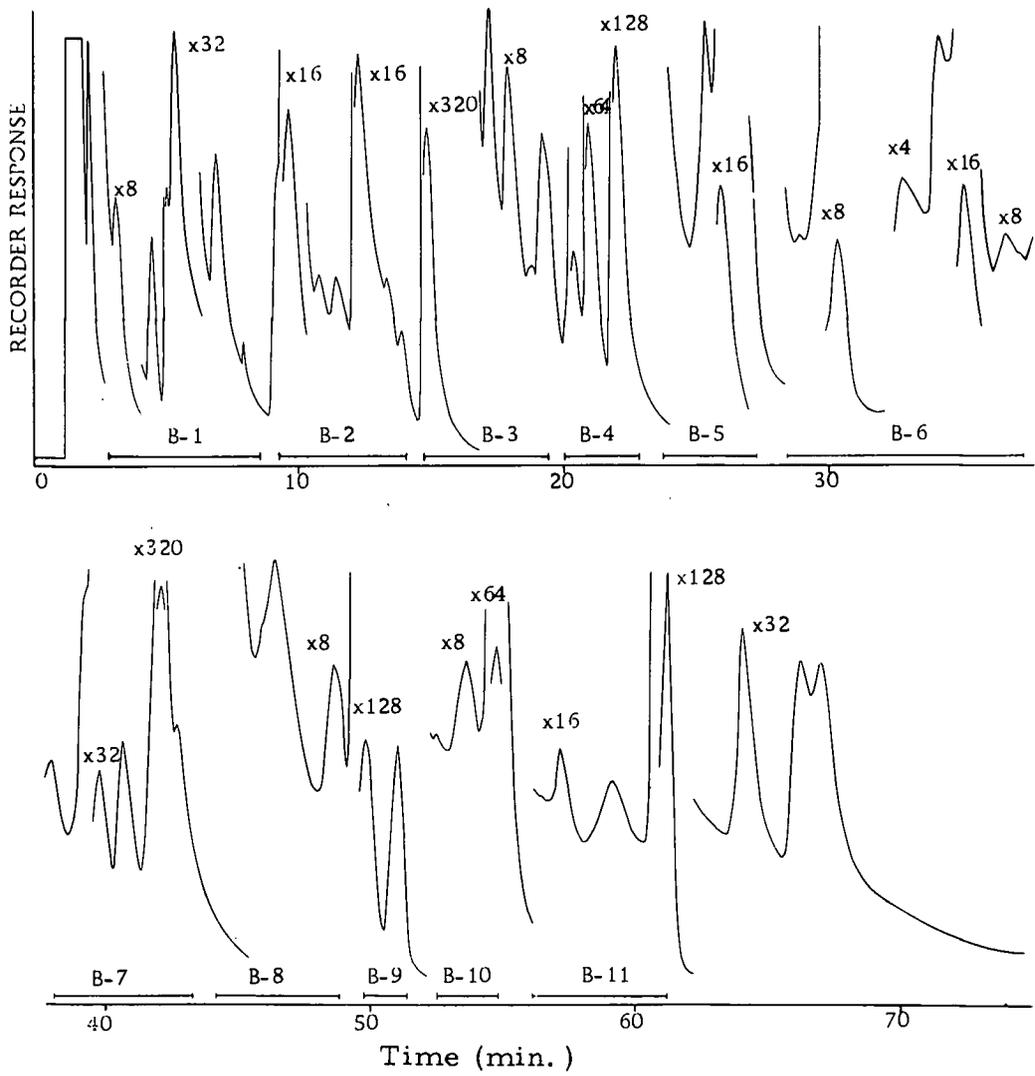


Figure 2. Chromatogram of microwave popped corn ether extract on the BDS packed column.

Table 1. Compounds identified by GC-MS of trapped fractions.

| Fraction | Compound | MS identification | MS reference | Fraction | Compound | MS identification | MS reference |
|----------|---------------------------|-------------------|-------------------|-------------------------|-------------------------------------|-------------------|--------------|
| A-1 | 2-Methyl propanal | + | 13 | B-4 | 2-Pentyl furan | + | 6 |
| | Diacetyl | + | 13 | | Furfural | + | 13 |
| | Ethanol | + | 13 | | 2-Ethyl-3, 6-dimethyl pyrazine | tentative | 7 |
| | Ethyl benzene | tentative | 13 | | 2-Acetyl furan | tentative | 13 |
| | Heptanal | tentative | 13 | | Benzaldehyde | + | 13 |
| | 2-Acetyl furan | + | 13 | | Furfuryl alcohol | + | 13 |
| | 2-Acetyl pyrazine | tentative | | | 5-Methyl furfural | tentative | 13 |
| B-1 | Ethanol | + | 13 | B-5 | Furfural | + | 13 |
| | 2-Methoxy ethanol | + | 13 | | 5-Methyl furfural | + | 13 |
| B-2 | 2-Pentyl furan | + | 6 | | Furfuryl alcohol | + | 13 |
| | 2, 3 Dimethyl pyrazine | tentative | 7 | | 2-Methoxy phenol | tentative | 13 |
| | 2, 5 Dimethyl pyrazine | + | 7 | | γ-butyrolactone | + | 14 |
| B-3 | | | | | Bi-(5-methyl-2-furyl)-methane | + | 79 |
| | Diacetyl | + | 13 | | B-6 | γ-butyrolactone | + |
| | Hexane | + | 13 | 5-Methyl-2-acetyl furan | | tentative | 79 |
| | a-dichloro benzene | tentative | 13 | Hexanoic acid | | + | 13 |
| | 2-Ethyl-5-methyl pyrazine | tentative | 7 | B-7 | 3, 5-di-tert-butyl-4-hydroxytoluene | + | 13 |
| | Furfural | + | 13 | | 2-Acetyl pyrrole | + | 9 |
| | 2-Acetyl furan | + | 13 | | Pyrrole-2-carboxaldehyde | + | 9 |
| | Benzaldehyde | + | 13 | | B-11 | Methyl palmitate | + |
| | | | Diethyl phthalate | + | | 13 | |

was freshly distilled before each use. Even though the ether had been redistilled, small quantities of BHT were still observed occasionally. The 2-methoxy ethanol is believed to be an artifact since considerable quantities of it were in use in the laboratories during this phase of the work.

Whole Extract by Coupled Capillary Column GC--MS. By directly injecting samples of the ether extract into the capillary column (50:1 inlet split ratio) and utilizing a temperature program, it was hoped to obtain a more coherent picture of its composition. Three separate analyses were made using this technique. Table 2 lists the compounds identified by comparison of their mass spectra with reference spectra and supporting retention times where compounds were available. GC confirmation (+) indicates that relative retention times for authentic compounds were within 5% of the values for the compounds in the extract.

The number of peaks obtained on the capillary column was considerably fewer than observed on a packed column with the same ether extract. Apparently, the limited capacity and dynamic range of the 0.01 inch ID capillary columns allows only the larger components to be seen when much of the volume of sample injected is solvent. Every attempt was made to reduce the ether volume, but the extract became brown or black, very viscous, and difficult to handle with a syringe. At that point, ether and furfural made up a large part of the mixture.

Table 2. Compounds identified by coupled capillary column GC-MS.

| Compound | MS identification | MS reference | GC confirmation |
|--------------------------------|----------------------|-----------------|--------------------|
| Acetaldehyde | + | 13 | + |
| Ethyl formate | tentative | 13 | |
| Ethyl acetate | + | 13 | + |
| 2- or 3-Methyl butanal | tentative | 13 | + |
| Diacetyl | + | 13 | + |
| Ethanol | + | 13 | + |
| 2, 5-Dimethyl pyrazine | + | 7 | + |
| 2, 3-Dimethyl pyrazine | tentative | 7 | |
| 2-Ethyl-5-methyl pyrazine | tentative | 7 | |
| 2-Ethyl-3, 6-dimethyl pyrazine | tentative | 7 | |
| Furfural | + | 13 | + |
| 2, 3, 5-Trimethylpyrazine | tentative | 7 | |
| Benzaldehyde | tentative | 13 | |
| Acetyl furan | tentative | 13 | + |
| 5-Methyl furfural | + | 13 | + |
| Furfuryl alcohol | + | 13 | + |
| Phenol | tentative | 13 | |
| γ -Butyrolactone | + | 14 | + |
| BHT | + | 13 | + |
| 2-Acetyl pyrrole | + | 9 | + |
| Pyrrole-2-carboxaldehyde | tentative | 9 | + |
| Methyl palmitate | + | 14 | + |
| Diethyl phthalate | + | 13 | + |

A 600 foot x 0.02 inch ID capillary column was tried without an injection split and found to have considerably better dynamic range than the 0.01 inch ID columns. Operating difficulties involved when this column was connected to the mass spectrometer were not completely resolved. A small bore packed column (3% Carbowax 20-M on 100-120 mesh Aeropak 30, 20 feet x 0.047 inch ID) was tried and found to be quite effective in separating the components. However, it was thought that the greater sample capacity of a 1/8 inch OD packed column would be desirable when operating in conjunction with the mass spectrometer. The 12 foot x 1/8 inch OD 5% DEGS column was found to resolve the mixture nearly as well as the smaller bore packed column, so it was utilized in subsequent GC--MS applications.

Packed Column GC--MS. Table 3 includes the compounds identified using the packed DEGS column connected through the EC-1 inlet valve to the mass spectrometer. The vacuum distillate as well as the acidic and basic fractions from microwave popped corn were analyzed utilizing this system. Table 3 indicates in which of the samples each compound was found to be present. Figure 3 shows a chromatogram of the aqueous distillate ether extract from the vacuum steam distillation of microwave popped corn. The numbers of the peaks refer to the numbered compounds listed in Table 3. The table includes the relative retention time (t_R/t_R) values on the DEGS column which were calculated with furfural = 1.000. Since

Table 3. Compounds identified by packed column GG-MS.

| Compound | Peak (Fig. 3) | Relative extract | Retention authentic | MS identification | Aroma agreement | Found in | | | MS reference |
|--------------------------------|------------------|---------------------|------------------------|----------------------|--------------------|------------|-----------------|----------------|-----------------|
| | | | | | | distillate | acidic fraction | basic fraction | |
| Ether | | | | + | + | + | + | + | 13 |
| Ethyl acetate | | 0.113 | 0.097 | + | + | | | + | 13 |
| Chloroform | | 0.128 | 0.116 | + | + | + | + | + | 13 |
| Unknown, MW 98 | | 0.407 | | | | + | | + | |
| Pyridine | | 0.424 | 0.483 | tentative | | | | + | 13 |
| Unknown, MW 95 | | 0.487 | | | | + | | | |
| Pyrazine | 1 | 0.512 | 0.506 | + | + | + | + | + | 7 |
| Unknown, MW 111 | | 0.548 | | | | | | + | |
| Thiazole | | 0.562 | 0.550 | tentative | | + | | + | 14 |
| Unknown, MW 99 | | 0.562 | | | | + | | | |
| Unknown, MW 124 | | 0.562 | | | | + | | | |
| Unknown, MW 96 | | 0.575 | | | | | | + | |
| 2-Methyl pyrazine | 2 | 0.611 | 0.606 | + | + | + | | + | 7 |
| Unknown, MW 99 | | 0.622 | | | | + | | + | |
| Unknown, MW 110 | | 0.626 | | | | | | + | |
| Unknown, MW 86 | | 0.698 | | | | | | + | |
| 2, 5-Dimethyl pyrazine | 3 | 0.718 | 0.703 | + | + | + | + | + | 7 |
| 2, 3-Dimethyl pyrazine | 4 | 0.783 | 0.756 | + | + | + | | + | 7 |
| 2-Ethyl-5-methyl pyrazine | 5 | 0.807 | 0.803 | + | | + | + | + | 7 |
| 2, 3, 5-Trimethyl pyrazine | | 0.827 | 0.846 | tentative | + | + | | + | 28 |
| Acetic acid | | 0.842 | 0.835 | + | + | | + | | 13 |
| 2-Ethyl-3, 6-dimethyl pyrazine | 6 | 0.885 | 0.881 | | | + | + | + | 7 |
| Unknown, MW 122 | | 0.885 | | | | + | | | |
| Unknown, MW 140 | | 0.911 | | | | | | + | |
| Unknown, MW 150 | | 0.956 | | | | + | | | |
| Unknown, MW 169 | | 1.000 | | | | | + | + | |
| Furfural | 7 | 1.000 | 1.000 | + | + | + | + | + | 13 |
| Unknown, MW 120 | | 1.032 | | | | + | | + | |
| 2-Acetyl furan | 8 | 1.105 | 1.109 | + | | + | + | + | 13 |

Table 3. Continued

| Compound | Peak (Fig. 3) | Relative extract | Retention authentic | MS identification | Aroma agreement | Found in | | | MS reference |
|--------------------------------------|------------------|---------------------|------------------------|----------------------|--------------------|------------|-----------------|----------------|-----------------|
| | | | | | | distillate | acidic fraction | basic fraction | |
| Benzaldehyde | 8 | 1.105 | 1.090 | + | | + | + | | 13 |
| Unknown, MW 155 | | 1.108 | | | | | + | | |
| Unknown, MW 120 | | 1.144 | | | | + | | | |
| Unknown, MW 131 | | 1.160 | | | | | | + | |
| Unknown, MW 114 | | 1.178 | | | | + | | | |
| Unknown, MW 148 | | 1.204 | | | | + | | | |
| 5-Methyl furfural | 9 | 1.240 | 1.242 | + | + | + | + | + | 13 |
| Unknown, MW 133 | | 1.240 | | | | + | | | |
| Unknown, 120 | | 1.240 | | | | + | | | |
| Unknown, MW 96 | | 1.267 | | | | | + | | |
| Unknown, MW 134 | | 1.276 | | | | + | | + | |
| N-ethyl pyrrole-2-aldehyde | | 1.294 | | tentative | | + | | | 81 |
| 2-Acetyl pyrazine | | 1.305 | 1.352 | tentative | + | + | | | |
| Unknown, MW 109 | | 1.325 | | | | + | + | | |
| Unknown, MW 133 | | 1.345 | | | | + | | | |
| Unknown, MW 127 | | 1.345 | | | | + | | | |
| Phenylacetaldehyde | | 1.355 | 1.361 | + | + | + | + | + | 6 |
| (5-methyl-2-furyl)-(2-furyl)-methane | | 1.364 | | + | | + | | | 79 |
| Furfuryl alcohol | 10 | 1.374 | 1.369 | + | + | + | + | + | 13 |
| Unknown, MW 96 | | 1.364 | | | | + | | | |
| Unknown, MW 112 | | 1.421 | | | | + | + | | |
| Unknown, MW 136 | | 1.442 | | | | + | + | | |
| Unknown, MW 134 | | 1.496 | | | | + | | + | |
| γ -Butyrolactone | 11 | 1.496 | 1.520 | + | + | + | + | + | 14 |
| 2-Formyl-5-methyl thiophene | | 1.457 | | tentative | | + | | + | 79 |
| Hexanoic acid | 12 | 1.541 | 1.538 | + | + | + | + | | 13 |
| Unknown, MW 123 | | 1.598 | | | | + | + | | |
| N-isoamyl pyrrole-2-aldehyde | | 1.598 | | tentative | | + | | | 79 |
| Unknown, MW 103 | | 1.552 | | | | | | + | |

Table 3. Continued

| Compound | Peak (Fig. 3) | Relative extract | Retention authentic | MS identification | Aroma agreement | Found in | | | MS reference |
|---------------------------------------|------------------|---------------------|------------------------|----------------------|--------------------|------------|-----------------|----------------|-----------------|
| | | | | | | distillate | acidic fraction | basic fraction | |
| Unknown, MW 95 | | 1.630 | | | | | + | | |
| BHT | | 1.647 | 1.653 | + | | + | + | + | 13 |
| Unknown, MW 84 | | 1.649 | | | | | + | + | |
| N-furfuryl pyrrole | 13 | 1.682 | 1.684 | + | | + | + | | 79 |
| 2-Ethyl-5-butyl thiophene | | 1.713 | | tentative | | + | | + | 13 |
| Unknown, MW 130 | | 1.809 | | | | + | | | |
| Unknown, MW 109 | | 1.809 | | | | + | + | | |
| An unknown acid | | 1.854 | | | | | + | | |
| Benzothizole | | 1.874 | 1.802 | tentative | | + | | | 13 |
| Unknown, MW 120 | | 1.915 | | | | + | + | + | |
| 2-Acetyl pyrrole | 14 | 1.915 | 1.921 | + | + | + | + | + | 9 |
| Unknown, MW 146 | | 1.969 | | | | + | + | | |
| Pyrrole-2-carboxaldehyde | 15 | 1.995 | 2.027 | + | + | + | + | | 9 |
| Unknown, MW 160 | | 2.002 | | | | + | + | | |
| Unknown, MW 98 | | 2.027 | | | | + | | | |
| 4-Hydroxy-2,5-dimethyl-3(2H)-furanone | | 2.002 | 2.013 | tentative | | | + | | 73 |
| Unknown, MW 85 | | 2.057 | | | | + | | | |
| Unknown, MW 124 | | 2.027 | | | | | | + | |
| Unknown, MW 149 | | 2.117 | | | | + | + | | |
| 5-Methyl-2-pyrrolealdehyde | | 2.157 | | tentative | | + | + | | 81 |
| 4-Vinyl-2-methoxy phenol | 16 | 2.217 | 2.207 | + | + | + | + | | 79 |
| Unknown, MW 99 | | 2.399 | | | | + | | | |
| Unknown, MW 126 | | 2.392 | | | | | | | |
| N-furfuryl pyrrole-2-carboxaldehyde | 17 | 2.415 | 2.407 | + | | + | + | | 79 |
| Unknown, MW 164 | | 2.603 | | | | + | | | |
| 4-Vinylphenol | 18 | 2.601 | 2.434 | + | + | + | + | | |
| Unknown, MW 149 | | 2.650 | | | | + | | | |
| Unknown, MW 177 | | 2.650 | | | | + | | | |
| Unknown, MW 68 | | 2.774 | | | | | + | | |
| Unknown, MW 222 | | 2.683 | | | | + | | | |

Table 3. Continued

| Compound | Peak (Fig. 3) | <u>Relative Retention</u> | | MS identification | Aroma agreement | <u>Found in</u> | | | MS reference |
|-----------------|------------------|---------------------------|-----------|----------------------|--------------------|-----------------|-----------------|----------------|-----------------|
| | | extract | authentic | | | distillate | acidic fraction | basic fraction | |
| Unknown, MW 131 | | 2.939 | | | | + | | | |
| Unknown, MW 149 | | 3.013 | | | | + | | | |
| Unknown, MW 97 | | 3.482 | | | | | + | | |
| Vanillin | 19 | 3.578 | 3.358 | + | + | + | + | | 13 |
| A phthalate | | 3.645 | | | | + | | | 3 |
| Palmitic acid | | 4.158 | 3.634 | + | | | + | | 13 |

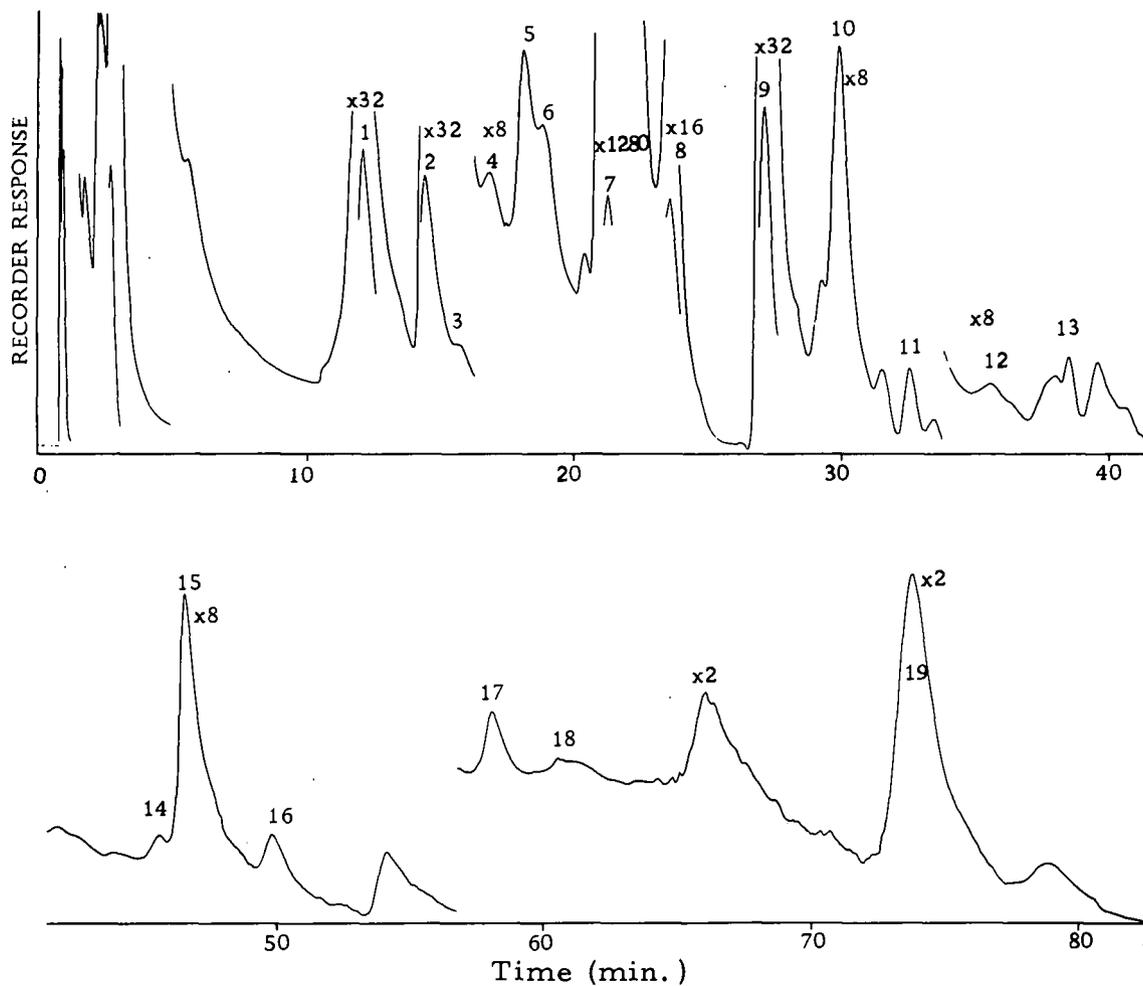


Figure 3. Chromatogram of ether extract of aqueous vacuum distillate from microwave popped corn on the DEGS packed column.

temperature programming over a very wide range was employed, the relative retention time values are subject to some variation from one run to another and from one instrument to another. The most variation is encountered in very early peaks and very late peaks and is caused largely by small variations in the initial and final temperatures attained by the gas chromatograph. Because of this variation, elution order, odor, and quantitative differences among the peaks were used along with relative retention times to substantiate the mass spectral identifications.

Tentative mass spectral identifications are given where the spectra were either very weak or were of a mixture of several compounds so that a positive match with reference spectra was not possible. Some spectra were not interpretable or no reference spectra were available for them and in these cases the probable molecular weight of the compound is given. A positive mass spectral identification indicates good agreement with reference spectra. When comparing with reference spectra, some allowances necessarily must be made for background interferences and small variations in the intensities of the various mass peaks due to differing instruments and conditions. When the relative retention time of a reference compound (synthesized by published methods or obtained from commercial sources) is in agreement with that of the sample compound on which a positive mass spectral identification was made, the compound is

considered to be positively identified. Chloroform was determined to be a contaminant in the lot of diethyl ether used for the three samples in this phase of the work.

Infrared Spectra. Since the sample requirement for good infrared spectra is considerably higher than that needed for GC or mass spectral analysis, an insufficient amount of most compounds was eluted from the column for infrared spectra. This is especially true if the components are impure and must be rechromatographed on a different column and trapped again. Such a procedure was not used successfully in this study. Usable infrared spectra were obtained for 5-hydroxymethylfurfural, and two later eluting components thought to be long chain fatty acids based on the characteristic broad OH-stretching region, carbonyl band, and remaining spectral characteristics (12).

Figure 4 compares the infrared spectra of authentic 5-hydroxymethylfurfural with that isolated from the microwave popped corn aqueous condensate ether extract. On the basis of the closely matching infrared spectra and corresponding relative retention times (t_R/t_R) Authentic = 1.541, (t_R/t_R) unknown = 1.541, based on acetyl pyrrole = 1.000) this compound was positively identified as 5-hydroxymethylfurfural.

Table 4 is the summary of all the compounds identified in this study. An asterisk denotes compounds for which insufficient data is

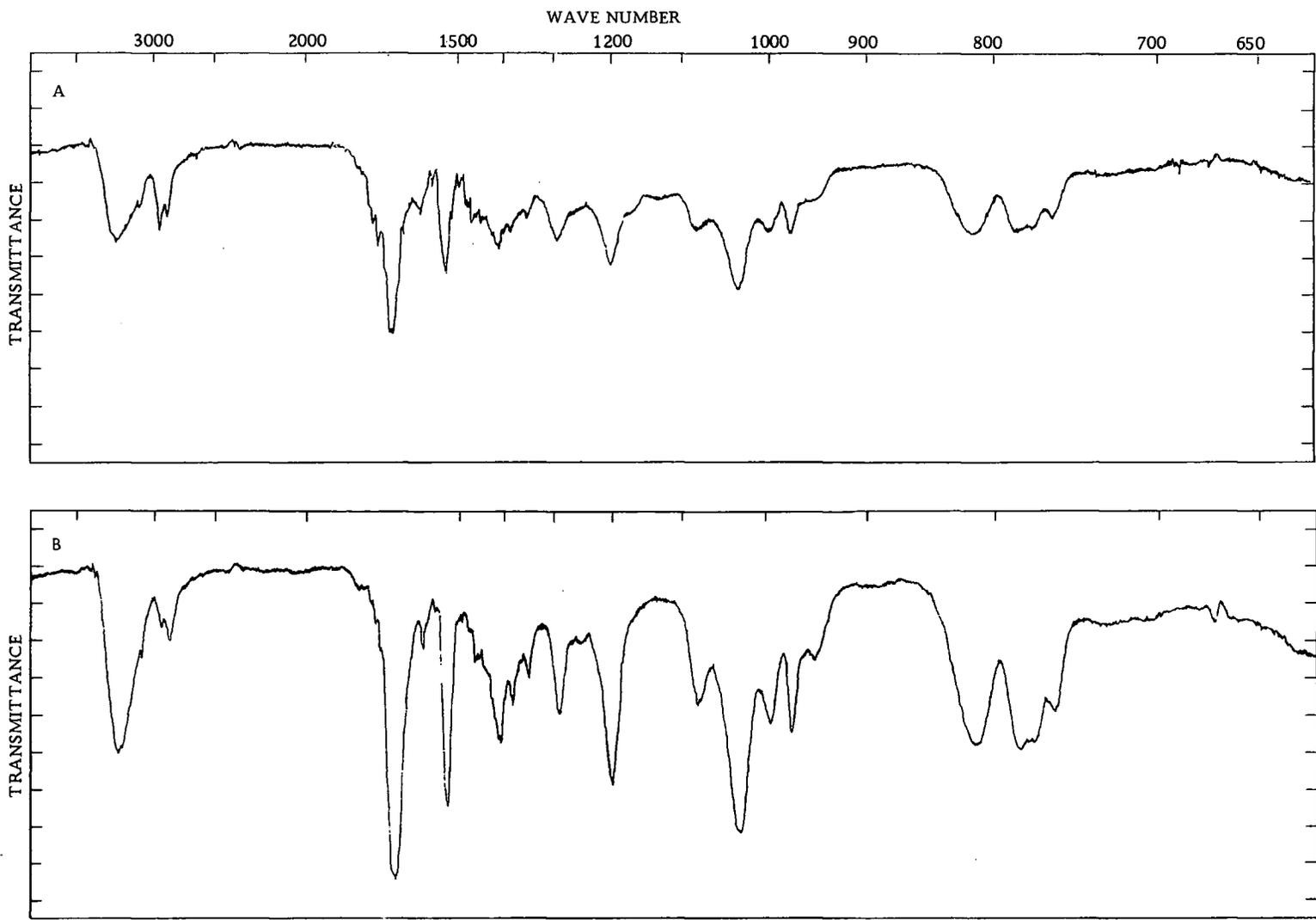


Figure 4. Infrared spectra of isolated (A) and authentic (B) 5-hydroxymethyl furfural (neat).

Table 4. Summary of compounds identified from popcorn.

| Compound | MS identification | GC confirmation | | | | Aroma |
|--------------------------------|----------------------|------------------|---------------|---------------------|----------------|-------|
| | | BDS capillary | BDS packed | Apiezon L packed | DEGS packed | |
| Acetaldehyde | + | + | | | | |
| *Ethyl formate | tentative | | | | | |
| Ethyl acetate | + | + | | | + | + |
| 2-Methyl propanal | + | | | | | |
| Diacetyl | + | + | | | | + |
| Ethanol | + | + | | | | |
| 3-Methyl butanal | + | + | | | | + |
| Pentanal | + | + | + | | | + |
| Hexanal | + | + | + | | | + |
| 2-Pentyl furan | + | + | + | + | | |
| *Heptanal | tentative | | | | | |
| Hexane | + | | | | | |
| *Pyridine | tentative | | | | + | |
| Pyrazine | + | | | | + | + |
| *Thiazole | tentative | | | | + | |
| *Ethyl benzene | tentative | | | | | |
| *A dichlorobenzene | tentative | | | | | |
| 2-Methyl pyrazine | + | | | | + | + |
| 2, 5-Dimethyl pyrazine | + | + | | | + | + |
| 2, 3-Dimethyl pyrazine | + | | | | + | + |
| 2-Ethyl-5-methyl pyrazine | + | | | | + | |
| *2, 3, 5-Trimethyl pyrazine | tentative | | | | + | + |
| Acetic acid | + | | | | + | + |
| 2-Ethyl-3, 6-dimethyl pyrazine | + | | | | + | + |
| Furfural | + | + | + | + | + | + |
| 2-Acetyl furan | + | + | | | + | |
| Benzaldehyde | + | | | | + | |
| 5-Methyl furfural | + | + | | | + | + |
| *2-Pentyl thiophene | tentative | | | | | |
| *n-Dodecane | tentative | | | | | |

Table 4. Continued

| Compound | MS identification | GC confirmation | | | | Aroma |
|--|----------------------|------------------|---------------|---------------------|----------------|-------|
| | | BDS capillary | BDS packed | Apiezon L packed | DEGS packed | |
| *2-Acetyl pyrazine | tentative | | | + | + | + |
| *N-Ethyl pyrrole-2-aldehyde | tentative | | | | | |
| Phenyl acetaldehyde | + | | | | + | + |
| *(5-Methyl-2-furyl)-(2-furyl)-methane | + | | | | | |
| Furfuryl alcohol | + | + | | | + | + |
| *2-Methoxy phenol | tentative | | | | | |
| γ-Butyrolactone | + | + | | | + | + |
| *5-Methyl-2-acetyl furan | tentative | | | | | |
| *Bi-(5-methyl-2-furyl)-methane | + | | | | | |
| *2-Formyl-5-methyl thiophene | tentative | | | | | |
| Hexanoic acid | + | | | | + | + |
| *Phenol | tentative | | | | | |
| *N-Isoamyl pyrrole-2-aldehyde | tentative | | | | | |
| N-Furfuryl pyrrole | + | | | | + | |
| *2-Ethyl-5-butyl thiophene | tentative | | | | | |
| *Benzothiazole | tentative | | | | + | |
| 2-Acetyl pyrrole | + | + | | | + | + |
| Pyrrole-2-carboxaldehyde | + | + | | | + | + |
| *4-Hydroxy-2,5-dimethyl-3(2H)-furanone | tentative | | | | + | |
| *5-Methyl-pyrrole-2-aldehyde | tentative | | | | | |
| 4-Vinyl-2-methoxy phenol | + | | | | + | + |
| N-furfurylpyrrole-2-aldehyde | + | | | | + | |
| 4-Vinyl phenol | + | | | | + | + |
| Vanillin | + | | + | + | + | + |
| ‡5-Hydroxymethyl furfural | | | | | + | |
| Methyl palmitate | + | + | | | | |
| Diethyl phthalate | + | | | | + | |
| Palmitic acid | + | | | | + | |

* Indicates compounds tentatively identified

‡ Indicates compound identified by matching infrared spectra

available for a positive identification.

Reference Compounds

Many of the compounds indicated by the mass spectral data were not commercially available. Some of the pyrazine derivatives needed for retention time verification and odor assessment were kindly supplied by Dr. Michael E. Mason of International Flavors and Fragrances, Union Beach, New Jersey. Included were 2,3-dimethyl pyrazine, 2,3,5-trimethyl pyrazine, 2-ethyl-5-methyl pyrazine, 2-ethyl-3,6-dimethyl pyrazine, 2-ethyl-3,5-dimethyl pyrazine, and acetyl pyrazine. Seven other compounds were synthesized by the methods cited from the literature.

Furan-2-glyoxal

This compound was first suspected as being a product from a glucose-glycine model browning system. Several weak mass spectra from the popcorn extracts had also shown compounds of molecular weight 124. Relative retention times ($t_R/t_R = 1.720$ relative to furfural on the DEGS column) later ruled out the possible identification of furan-2-glyoxal in popcorn volatiles.

The mass spectrum of the furan-2-glyoxal obtained by BDS

capillary column GC--MS under conditions similar to those previously described were as follows:

m/e 95 (100%), 39 (70%), 38 (20%), 37 (15%), 67 (9%),

124 (molecular ion, 7%), 96 (7%), 51 (5%), 68 (3%), 66 (3%)

The compound is a viscous yellow oil with a weak aroma similar in character to the acetyl furan from which it was prepared. The final product after vacuum distillation consisted of 91.2% furan-2-glyoxal and 8.7% acetyl furan. The infrared spectrum is shown in Figure 5.

N-furfuryl pyrrole

The mass spectrum of N-furfuryl pyrrole in popcorn volatiles was the key to its identity. The unknown spectrum was simple and agreed well with the published spectrum (79). The mass spectrum of the synthesized compound is compared with that from the popcorn sample and the published spectrum in Table 5:

Table 5. Mass spectra of N-furfuryl pyrrole.

| m/e | % Relative intensity | | |
|---------------------|----------------------|-----------|---------|
| | Synthetic | Ref. (79) | Popcorn |
| 81 | 100 | 100 | 100 |
| 147 (molecular ion) | 33 | 42 | 38 |
| 53 | 29 | 22 | 53 |
| 27 | 17 | 9 | 30 |
| 39 | 10 | 5 | 7 |
| 58.5 | 3 | 2 | 5 |
| 148 | 5 | | 6 |

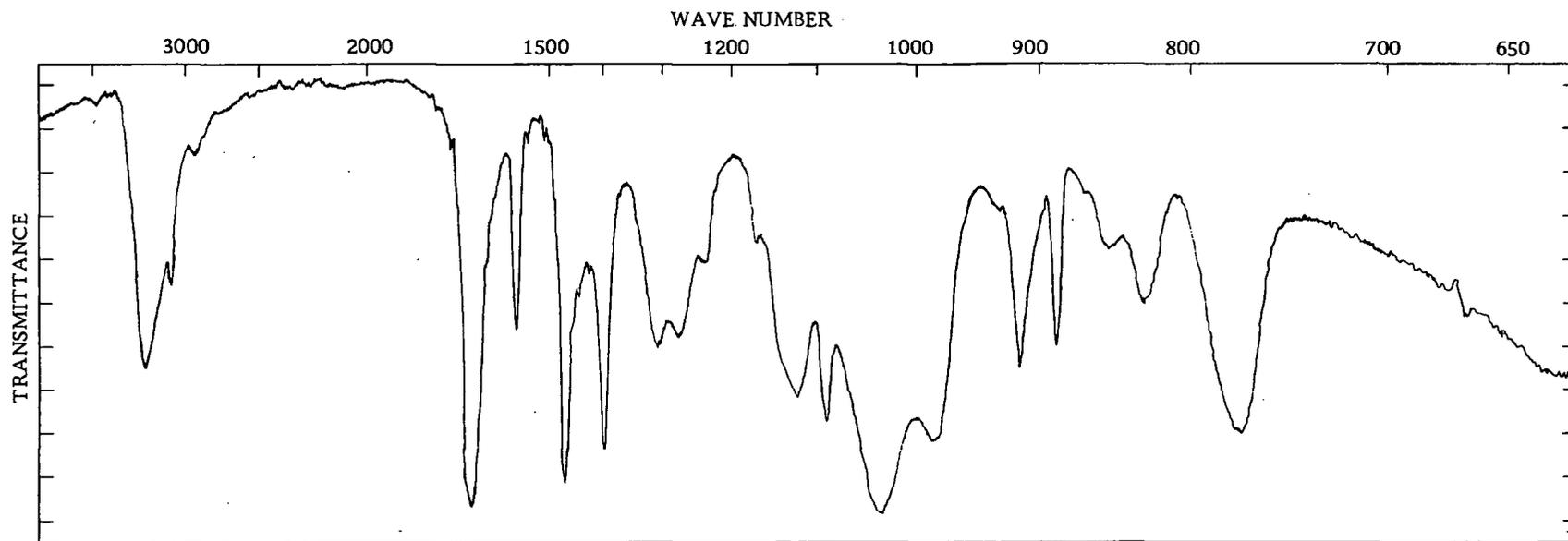


Figure 5. Infrared spectrum of furan-2-glyoxal.

The refractive index was determined to be 1.5311 which compared closely to the literature (26) value of 1.5317. The infrared spectrum of synthesized N-furfuryl pyrrole was virtually identical to the published spectrum (26).

N-furfuryl pyrrole-2-carboxaldehyde

The synthesis of this compound was accomplished, but the predominant product of the formylation reaction was one with a molecular weight of 203 which appeared to be the dialdehyde derivative. The two products were separated by GC, and mass and infrared spectra were obtained on the pure fractions. The mass spectrum for synthesized N-furfuryl pyrrole-2-carboxaldehyde is compared to that from popcorn and from the literature (79) in Table 6.

Table 6. Mass spectra of N-furfuryl pyrrole-2-carboxaldehyde.

| m/e | % Relative intensity | | |
|---------------------|----------------------|---------------|---------|
| | Synthetic | Reference(79) | Popcorn |
| 81 | 100 | 100 | 100 |
| 175 (molecular ion) | 27 | 32 | 15 |
| 53 | 27 | 25 | 37 |
| 27 | 14 | 14 | 22 |
| 39 | 10 | 8 | 14 |
| 146 | 1 | 2 | 2 |
| 94 | 2 | 2 | 3 |
| 118 | 1 | 2 | 2 |
| 176 | 3 | | 2 |

The mass spectrum of the other reaction product, tentatively identified as an N-furfuryl pyrrole dicarboxaldehyde, is as follows:

m/e 109 (100%), 53 (55%), 203 (molecular ion, 50%), 94 (49%),
28 (37%), 110 (35%), 81 (32%), 27 (31%), 39 (25%), 51 (24%),
52 (20%), 27 (20%), 43 (18%), 174 (10%), 95 (10%), 79 (10%),
and 38 (10%).

No attempt was made to determine the position of substitution of the two aldehyde groups on the molecule. The infrared spectra of N-furfuryl pyrrole-2-carboxaldehyde and the dialdehyde derivative are shown in Figure 6. The infrared spectrum of N-furfuryl pyrrole-2-carboxaldehyde is in agreement with published data (79). No evidence for the presence of the N-furfuryl pyrrole dicarboxaldehyde in popcorn was found.

2, 3-Dihydrobenzofuran

On the basis of a mass spectrum, 2, 3-dihydrobenzofuran was suspected as a possible constituent of the popcorn volatiles. The compound was prepared by catalytic hydrogenation, and, although the yield achieved was only about 2%, sufficient material was purified by preparative GC for mass spectral analysis and retention times. The mass spectrum matched the literature data (79) for 2, 3 dihydrobenzofuran found in coffee volatiles. The retention time (relative to furfural) on the DEGS column was 1.220 compared to

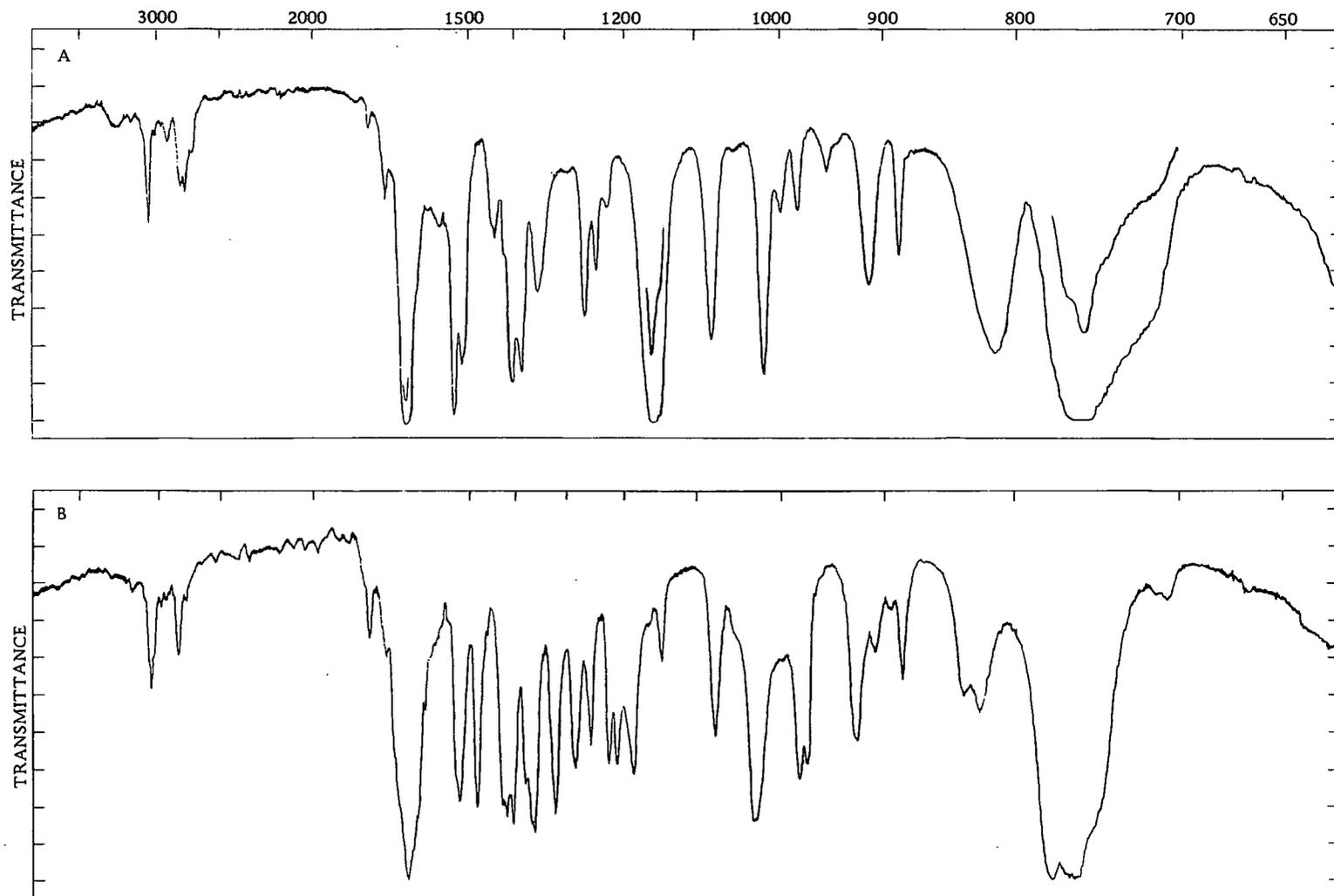


Figure 6. Infrared spectra of (A) N-furfuryl pyrrole-2-carboxaldehyde and (B) N-furfuryl pyrrole dicarboxaldehyde (neat).

2.650 for the suspected unknown compound. Thus, 2,3-dihydrobenzofuran was excluded as a possible popcorn flavor component.

4-Vinyl-2-methoxyphenol

Pure 4-vinyl-2-methoxyphenol was easily prepared by thermal decarboxylation of ferulic acid. Its relative retention time of 2.207 (furfural = 1.000) on the DEGS column matched that of a compound previously tentatively identified in popcorn volatiles by mass spectrometry. The mass spectrum of the synthesized 4-vinyl-2-methoxyphenol is compared with the spectrum of the compound from popcorn and literature data in Table 7.

Table 7. Mass spectra of 4-vinyl-2-methoxyphenol.

| m/e | % Relative intensity | | |
|---------------------|----------------------|----------------|---------|
| | Synthetic | Reference (79) | Popcorn |
| 150 (molecular ion) | 100 | 100 | 100 |
| 135 | 81 | 70 | 91 |
| 107 | 43 | 24 | 56 |
| 77 | 38 | 21 | 65 |
| 51 | 31 | 9 | 40 |
| 39 | 22 | 8 | 39 |
| 63 | 12 | 5 | 20 |
| 89 | 7 | 4 | 11 |
| 27 | 12 | 4 | 23 |

The infrared spectrum of the synthesized 4-vinyl-2-methoxyphenol is shown in Figure 7(A). The odor of 4-vinyl-2-methoxyphenol is intensely clove-like as it elutes from the GC column. On standing when exposed to room air, the purified compound was

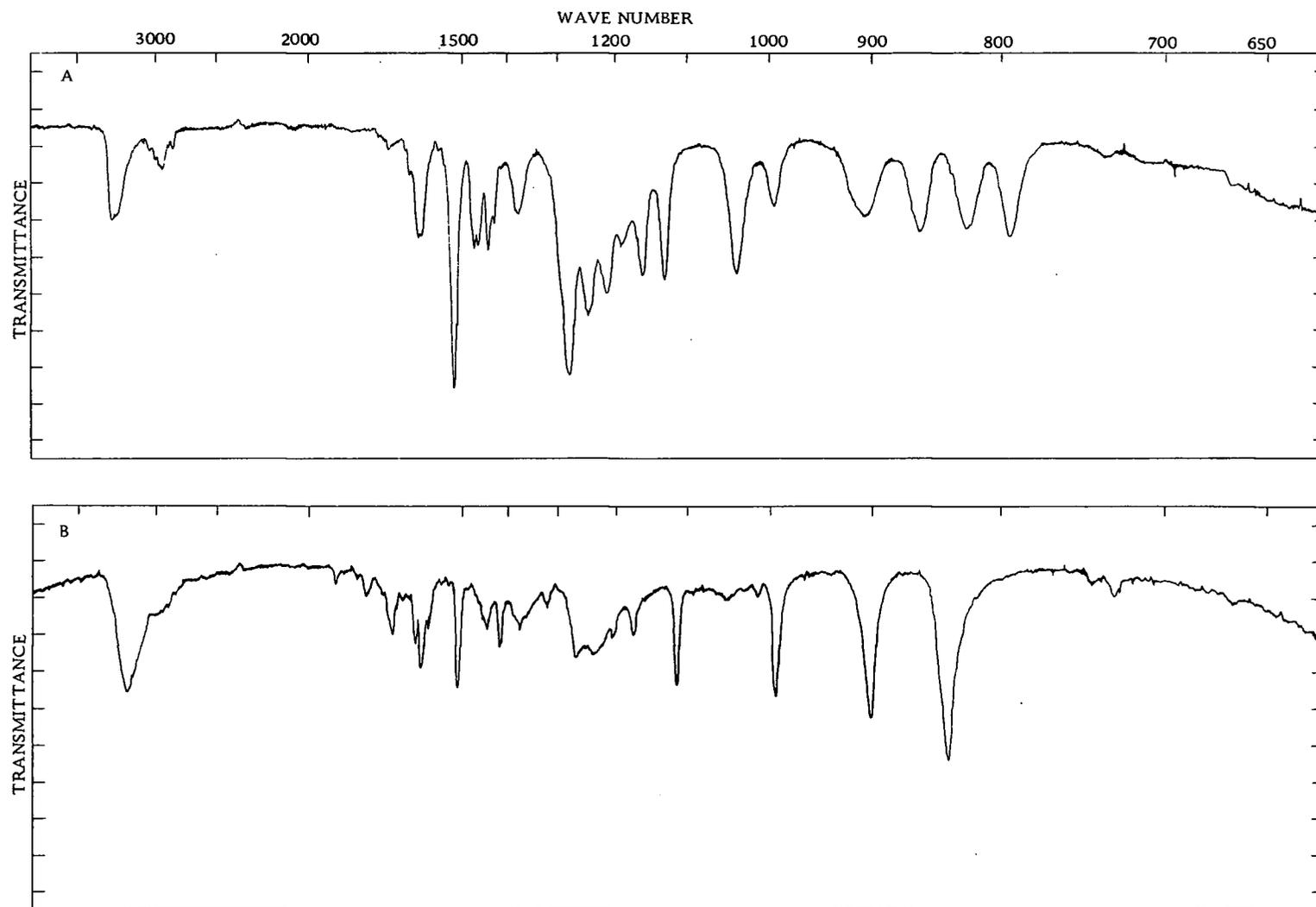


Figure 7. Infrared spectra of (A) 4-vinyl-2-methoxyphenol and (B) 4-vinylphenol (neat).

observed to be slowly oxidized to vanillin. This observation, based on aroma, was verified by GC retention times.

4-Vinylphenol

The 4-vinylphenol, prepared from p-hydroxycinnamic acid, had a retention time on the DEGS column of 2.434 (furfural = 1.000) which compared favorably with the value of 2.650 for the previously mentioned unknown of molecular weight 120. Comparison of the mass spectra, shown in Table 8, confirms the identity of the unknown as 4-vinylphenol. Observation of the characteristic smoky, phenolic odor of the compound from popcorn volatiles, eluting from the GC column at the appropriate retention time, served as further confirmation of its identity.

Table 8. Mass spectra of 4-vinylphenol.

| m/e | % Relative intensity | |
|---------------------|----------------------|---------|
| | Synthetic | Popcorn |
| 120 (molecular ion) | 100 | 100 |
| 91 | 43 | 54 |
| 39 | 23 | 44 |
| 65 | 22 | 34 |
| 119 | 22 | 24 |
| 107 (impurity) | 13 | |
| 51 | 11 | 20 |
| 63 | 11 | 20 |
| 94 | 10 | 13 |
| 121 | 10 | 10 |

The peak at m/e 107 is probably due to a small amount of 4-ethyl phenol (MW 122) present in the sample as an impurity from synthesis since a loss of 13 from the molecular ion is not likely. The spectra of *o*-ethyl phenol and *m*-ethyl phenol (13) show m/e 107 as the base peak with the molecular ion at m/e 122 about 30 to 40% as strong. Assuming that 4-ethyl phenol also follows that pattern, its presence as a contaminant is verified and accounts for the m/e 107 in the spectrum of 4-vinylphenol. Figure 7(B) shows the infrared spectrum of GC purified 4-vinylphenol taken as a thin film on a salt plate.

2, 3-Dimethyl-5, 6-dihydropyrazine

Since Bondarovich et al. (7) had observed that some of the intermediates in the synthesis of pyrazines had "popcorn-like" aromas, the synthesis of one of the intermediate dihydropyrazines was attempted. The white crystalline product obtained from a condensation of diacetyl and ethylene diamine was estimated to be about 40% 2, 3-dimethyl pyrazine and 60% 2, 3-dimethyl-5, 6-dihydropyrazine based on the relative intensity of the molecular ion peaks from the mass spectrum. The observed odor of the product was variable. An odor described as popcorny or unpleasant, amine-like was perceived. This observation appeared to depend on the concentration and the degree of olfactory fatigue of the observer. The crystalline material,

on standing at room temperature, readily darkened and appeared to slowly form a brown, viscous polymer. The popcorn-like aroma was no longer evident after about two weeks. The dihydro pyrazines are quite unstable and, therefore, would be difficult to utilize in a flavoring application unless some method of stabilization could be employed.

2-Acetyl pyrazine

A patent has been issued (61) covering the use of 2-acetyl pyrazine, 5-methyl-2-acetyl pyrazine, and 6-methyl-2-acetyl pyrazine in food products and tobacco to impart a popcorn-like flavor and aroma. The 2-acetyl pyrazine obtained from Dr. Mason was found to have a very pleasing aroma much like fresh buttered popcorn. When higher concentrations of the material were present (produced by heating some of the crystals or sniffing the GC effluent), the odor became harsh and even unpleasant. While acetyl pyrazine was not positively identified in this study as a constituent of popcorn volatiles, some indications of its possible presence were noted: (1) a compound with a weak mass spectrum (mixed with other compounds) which indicated the possible presence of acetyl pyrazine was obtained from the "popcorny" smelling fraction trapped from the Apiezon L packed column, (2) the relative retention time of acetyl pyrazine, 1.352 (furfural = 1.000) on the DEGS column was in agreement with the

repeatedly observed "popcorn-like" aroma emerging with a relative retention time of 1.338 on the same column. No discreet peak could be associated with the popcorn-like aroma, so it may be due to either a very low concentration of a highly odorous compound (possible 2-acetyl pyrazine) or a mixture of two or more incompletely resolved components.

The mass spectrum determined for known acetyl pyrazine is as follows:

m/e 43 (100%), 80 (52%), 122 (molecular ion, 46%), 28 (44%),
52 (43%), 53 (36%), 79 (28%), 94 (21%), 26 (18%), 42 (12%),
39 (8%), 81 (7%), 38 (7%), and 107 (6%).

Origin and Significance of Compounds

Furans

The concentration of furfural found was at least ten fold greater than that of any of the other compounds isolated from popcorn. Plant materials such as corn bran and popcorn cobs are reported to produce furfural in yields of 19.6% and 23.0%, respectively, as estimated by the A. O. A. C. method (17). The pentosans are considered to be the chief precursors of furfural from these materials. It is probable that the furan compounds arise largely from the carbohydrate material contained in the corn kernel or the hull material as a result of the

applied heat during popping. The various model system studies which have been conducted bear this out (32, 86, 59, 30, 29, 69, 46). The Maillard browning pathways also allow for formation of various furan derivatives (32), with an amino compound participating in the sugar degradation.

In relation to popcorn flavor, furfural is definitely an important contributor, both from the standpoint of its concentration and flavor properties. The nutty, rich aroma of 5-methyl furfural must surely be significant in the overall "smooth" desirable aroma of popcorn. The 4-hydroxy-2,5-dimethyl-3(2H)-furanone has been isolated from pineapple (73), beef broth (83), and as a decomposition product from rhamnose (32). It has a pleasant caramel odor and could contribute similarly to popcorn aroma.

Pyrazines

The various pyrazine derivatives are probably responsible for the characteristic background nutty aroma of popcorn. It is possible that acetyl pyrazine, or some other yet unidentified derivative, actually contributes directly to the unique "popcorny" aroma.

Pyrazines have been shown to arise from non-enzymatic browning reactions in model system studies (15, 85, 43) from amino acids and sugars. One of the mechanisms proposed for this formation (57) suggests that the final formation of methyl substituted pyrazines is by

condensation of two small (2-4 carbon) compounds previously formed by cleavage of unsaturated ketoamines. This is in agreement with the suggestions of Dawes and Edwards (15) and seems reasonable in light of some of the condensation reactions employed in the laboratory synthesis of pyrazines (35, 48).

Pyrroles

The extent of the contribution of the identified pyrrole derivatives to popcorn flavor is not clear. N-furfuryl pyrrole has a strong, green or hay-like aroma, and could easily make a contribution.

Pyrrole derivatives have been isolated from model sugar-amino acid browning systems (46, 37, 38). A mechanism has been proposed (38) for the formation of N-substituted pyrrole-2-aldehydes which is analogous to the formation of furfural in carbohydrate-amine browning reactions. A specific route for formation of N-furfuryl pyrrole derivatives has not been established.

Aldehydes

The most important source of acetaldehyde, 2-methyl propanal, 2-methyl butanal, and phenylacetaldehyde is probably the Strecker degradation of alanine, valine, leucine, and phenylalanine, respectively. This oxidative degradation of α -amino acids can be catalyzed by a compound containing a $-C:O-C:O-$ or a $-C:O-(CH:CH)_n-C:O$

grouping. These groupings are found in the sugar-derived intermediates in non-enzymatic browning and caramelization reactions and in furfural (32).

The 2-methyl propanal and 2-methyl butanal probably make important contributions to "malty" notes in popcorn aroma. Phenylacetaldehyde possesses a very heavy floral scent which, in the small amounts present, probably blends into the background flavor. Straight chain aldehydes including pentanal, hexanal, and heptanal probably contribute to the flavor of corn that has been popped in oil. Their origin is primarily from the thermal oxidation of unsaturated fatty acids (40). Upon standing after popping, the level of such fat oxidation products would normally increase. This may be partially responsible for the eventual onset of a "stale" flavor.

Phenols

Three phenolic compounds were identified including 4-vinyl-2-methoxyphenol, 4-vinylphenol and vanillin. All three of these would be expected to contribute to the flavor and aroma. The 4-vinyl-2-methoxyphenol has a very pleasant clove-like aroma while 4-vinylphenol possesses an unpleasant smoky and phenolic aroma.

As was demonstrated by the methods of synthesis, 4-vinyl-2-methoxyphenol can be produced by the action of heat on ferulic acid and 4-vinylphenol can be produced analogously from p-hydroxycinnamic

acid (76, 20). Exposure to air was observed to cause the oxidation of 4-vinyl-2-methoxyphenol to vanillin. The presence of ferulic acid and p-hydroxycinnamic acid in corn grain has been shown by Steinke and Paulson (76).

Acids

Three fatty acids, acetic, hexanoic, and hexadecanoic (palmitic) were identified. Acetic and hexanoic acids are believed to contribute to the flavor and aroma of popcorn while the contribution of palmitic acid is probably small.

Acetic acid is known to occur as a product of carbohydrate browning reactions (69, 80, 86, 46). While Sugisawa (80) reported identifying acids from formic through n-valeric from decomposition of glucose caramel, no mention could be found of the identification of hexanoic acid from model systems. From these results, it would seem reasonable that the six carbon acid could also be formed in these systems. Palmitic acid probably arises from triglyceride hydrolysis (55) since corn oil usually contains about 10% palmitic acid (5).

Gamma-butyrolactone probably contributes "richness" to the overall flavor and aroma of popcorn. It has been identified as a product from heated glucose (69) and sucrose pyrolysis (36), so its formation by nonenzymatic browning reactions during popping seems reasonable.

SUMMARY AND CONCLUSIONS

Thirty-six compounds were positively identified and another 20 compounds tentatively identified by coupled gas chromatography-mass spectrometry in the study of popcorn volatiles.

It is believed that compounds containing the pyrazine ring are of primary importance in the flavor of popcorn. Furfural is the most abundant single compound and, along with other furan compounds, constitutes an important contribution to popcorn flavor. The origin of the pyrazines, furans, and pyrrole compounds is probably Maillard browning which involves the carbohydrate and protein or amino acid constituents of the corn kernel. Phenolic compounds including 4-vinyl-2-methoxyphenol, 4-vinylphenol, and vanillin which probably arise from the decomposition of lignin or lignin precursors in the kernel are also important in the overall flavor.

There remains unidentified a considerable number of compounds for which inadequate data was obtained or reference spectra or chemicals were not available to verify possible identities. Among those incompletely characterized compounds are undoubtedly some which are important contributors to popcorn flavor.

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