THE UNIVERSITY OF MANITOBA

GAS CHROMATOGRAPHIC ANALYSIS OF VOLATILE COMPOUNDS FROM MAIZE

by

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ABSTRACT

An inexpensive device for rapid packing of gas chromatographic

columns was developed and evaluated. Packing of columns under gas

pressure accompanied by gentle vertical tapping increased the packing

density, reduced the packing irregularities and improved the column

efficiency.

Volatile aroma compounds were isolated from maize varieties by

steam distillation and extraction of the distillate. Gas chromato-

graphic analysis of the essences revealed 91 components. Essences

from five varieties of maize investigated contained largely the same

components, but the quantitative compositions differed significantly.

The characteristic compositions of the aroma essences might be of

use for chemotaxonomic classifications and for quality control in

the food and beverage industries.

INTRODUCTION

Components of flavor fractions of many foodstuffs have been the subject of intensive research in recent years. Of less obvious interest has been the aroma from plant seeds and cereal grains, usually not considered of importance in the food industry. A few studies on cereal grain aroma have been reported, for reasons of taxonomy, quality control and academic interest.

There has been interest in this Department in studying volatile material emanating from cereal grains for two purposes, namely, (1) to use the aroma composition as a possible means for distinguishing between commercial varieties of maize and wheat which may be difficult to distinguish by visual inspection and, (2) to elucidate a possible correlation between the aroma composition of distilled beverages and of the grains from which they were made.

The present study was undertaken with the following objectives:
(1) to isolate volatile material from maize samples, (2) to resolve the volatile material into pure components by gas chromatography, (3) to identify components by gas chromatography - mass spectrometry (GC-MS) in collaboration with the Chemistry Department, and (4) to investigate possible correlations between the compositions of the aroma volatiles from a series of maize varieties and from crude whiskies prepared from these maize samples. The latter part of this work was initially supported by the Distillers Corporation Limited, Canada.

The last two objectives have not been realized. Preliminary experiments with the Finnigan 1015 GC-MS system in the Chemistry Department showed that this instrument did not have sufficient sensitivity to allow analysis of a mixture whose components were present in minute concentrations. This part of the study has been deferred to a later date, when other techniques such as infrared spectroscopy and selective subtraction by gas chromatography will be employed. Precolumn subtraction techniques have been investigated for this purpose. The anticipated study of correlations between the aromas of maize varieties and of crude whiskies was curtailed as a result of an accident. A flood in a seed storage room destroyed the maize samples which had been obtained for this purpose.

In connection with the second objective, i.e., the gas chromatographic fractionation of maize volatiles, it became desirable to investigate possibilities for improving the separations obtained with conventional packed columns. This study led to the development of an efficient device for packing long gas chromatographic columns which gave superior separation.

In accordance with recently adopted practice at this University, completed manuscripts as submitted for publication in scientific journals have been incorporated in the thesis. Two manuscripts appear (MS I and MS II) as an appendix. For continuity, the contents of these papers are briefly referred to in the main body of the thesis.

The two main chapters of the thesis, which are concerned with column technology and odor analysis, review pertinent literature, describe

preliminary experiments, make reference to final results, and terminate

in a discussion. References are numbered consecutively by order of men-

tion in the text and appear in the general bibliography. In some cases,

references which appear in the appended manuscripts are repeated in the

text.

THE GAS CHROMATOGRAPHIC COLUMN

In this study observations were made on the formation of packed beds in a column. It was revealed that packing irregularities could be reduced and gas chromatographic efficiency could be increased by packing beds under gas pressure while simultaneously consolidating the packing by gentle vertical tapping. A Column Packer was developed, as described in MS I, and used to produce long packed columns. Efficiencies and retention characteristics were evaluated for "identical" pairs of columns prepared by this technique.

Column Theory

The column is often referred to as the heart of the gas chromatograph for it is the column which effects separation. Modern technology has provided the scientist with a wide variety of effective materials for the chromatographic column. Theoreticians have attempted with considerable success to explain emperical results concerning the chromatographic process. In spite of these advances, the making of a gas chromatographic column remains an art. Perhaps because of its apparent simplicity, the process of packing a column has remained virtually unchanged. The filling of a tube with fine granular material accompanied by agitation to consolidate the particles seems trivial; during this process, however, two important parameters which influence the chromatographic efficiency are determined, namely, packing density (uniformity of packing structure), and variation of partical size due to some crushing of the particles.

A review of plate height theory combined with experimental observations as they relate to column parameters will serve as a guide to practical techniques for producing efficient gas chromatographic columns.

The term chromatographic "separation" is used in a non-mathematical sense to refer to the degree of separation between two or more peaks; separation is achieved due to differential migration of component zones. This process is accompanied by zone spreading. The term "column efficiency" refers to a single peak and describes, in mathematical terms, the narrowness of the peak relative to its retention time. A high column efficiency implies large migration and limited zone spreading. Efficiency is expressed quantitatively by the number of theoretical plates (N) for a column, or by the height equivalent to a theoretical plate (HETP or simply H). The original equation of van Deemter (1) equates the theoretical plate height to a sum of terms as a function of linear gas velocity:

$$H = \frac{L}{N} = 2\lambda d_p + \frac{2\gamma D_g}{v} + \frac{8}{\pi^2} R(1-R) \frac{d_f^2 v}{D_l} \qquad [1]$$

which, for convenience, is often written

$$H = A + \frac{B}{v} + Cv \qquad [1a]$$

where L is the column length,

N is the number of plates,

d_p is the mean partical diameter,

v is the linear gas velocity,

D_g and D_1 are the diffusion coefficients for the solute in the

gas and liquid phases, respectively,

R is the ratio of peak velocity to gas velocity,

d_f is the effective film thickness, and

λ and γ are empirical constants of the order of unity.

More recent theoretical work (2) and experimental evidence have

resulted in the following equation:

$$H = \sum_i \frac{1}{\frac{1}{2\lambda_i\,d_p} + \frac{D_g}{\omega_i\,d_p{}^2 v}} + \frac{2\gamma\,D_g}{v} + \sum_i q_i \left(\frac{V_i}{V_l}\right) R\,(1-R)\,\frac{d_i{}^2 v}{D_l} + \frac{\omega_i{}'\,d_p{}^2 v}{D_g} + H_{ic} \qquad [2]$$

which, for convenience, is often written,

$$H = \sum \frac{1}{1/A_i + 1/C_{o_i}v} + B/v + \Sigma\,C_{li}v + C_i{}'v + H_{ic} \qquad [2a]$$

where ω_i and ω_i' are coefficients for mass transfer equations,

representing a flow inhomogeneity condition,

V_i / V_1 is the fraction of the total column liquid which

occurs in pools of "type i",

d_i is the liquid depth with geometrical configuration

factor q_i, and

H_{ic} is the trans-column contribution to plate height.

The remaining terms have been defined under equation [1] .

Equation [2] or [2a] contains all the known plate height terms which

contribute more than 1% to the total plate height. The first term accounts for coupling between various eddy diffusion effects and gas-phase mass transfer effects. The second term represents ordinary longitudinal diffusion in the gas phase. The third term represents mass transfer in the liquid phase and accounts for variation in liquid pools or films. The fourth term includes gas-phase mass transfer effects which do not interact with eddy diffusion. The last term is the contribution of trans-column or column width effects. It should be noted that there are eight C_1 and eight C_g terms (cf. equation [2a]) which may contribute to plate height, and that in the practical range of chromatographic operation the magnitude of these coefficients largely determine speed and efficiency.

Although over a decade old, the original form of plate height equation [2] remains virtually unaltered. The interdependence or "coupling" of eddy diffusion and gas phase diffusion has been confirmed (3,4) and C_1 terms have been correlated with pore sizes to show that, to good approximation, C_1 is proportional to $R(1-R)/D_1$ as predicted by theory.

Gas phase mass transfer processes contribute significantly to plate height. The C_g coefficients are of particular interest because they are dependent upon column geometry and packing characteristics, such as particle diameters, tortuosity, flow channels, coiling and tube radii, tube shape and flow variations.

In a study of the nature of gas phase mass transfer (5) a comparison of theoretical packing models and empirical results showed that the principal contribution to plate height in the gas phase arises from differences

in velocities in flow channels of unequal size. Differences in inter-particle flow channels were shown between close packed and simple cubic structures. The model used to approximate a chromatographic bed was a small repeating unit of an annulus of spheres forming an inner core void. Results showed that the diffusion process within particles could account for no more than 10% of the observed plate height, and that velocity variation within normal flow channels, often given credit for the entire plate height term, could account for no more than 2% of the observed value. The structure of the model used has been supported by electron micrographs showing that large interconnected voids were distributed through the packing materials (6) and by a study of computer simulated diffusion through cubic packed spheres (7).

It is apparent that the fabrication of a uniform packing structure in which interchannel interactions are eliminated or reduced would sub-stantially increase chromatographic efficiency. The causes of non-uniformity as related to packing technique may be divided into three catagories, namely, "particle fractionation," static electricity, and bed formation.

Particle fractionation due to variation in velocity with particle size has been observed for packings descending down tubes (8), an effect which is accentuated by vibration (4). This effect may be reduced by using vacuum packing (9) or closely graded support particles (10).

Static charges on packing particles are induced by rotary evapora-tion in the coating procedure and by the sieving of coated support.

In situ coating of the support material, or freezing of the support, alleviate this problem.

The most difficult problem in the packing process arises from the dynamics of the formation of packed beds. The formation of voids or channels occur, however uniform the particles are. Falling particles hit the accumulating bed and at impact must make a suitably shaped hole by shifting near neighbours which it will tend to compact. If a particle does not have sufficient momentum it may just form a cover over a particle-sized space which will then quickly become "fossilized" by subsequent layers. This effect becomes most pronounced for particles whose diameter is less than about 100 μ. The terminal velocities of 100-μ particles in air are only 25% of those in vacuo; consequently, porous beds packed in air give inferior chromatographic performance (9). Vibration or tapping may improve the bed characteristics but should be used with moderation. During vibration, a space persists between the column wall and the pack-ing; consolidation should follow, using veritcal tapping (11). Caution must be exercised because excessive tapping is known to produce fines, high back-pressure, and reduced efficiency (12).

Experimental

In the investigation of gas chromatographic column packing techniques, 4 ft x 5 mm o.d. glass tubes were employed in observing packed bed forma-tion. The packing material used was a mixture of Chromosorb W-NAW, 60/80 mesh, and dyed Chromosorb W-AW 100/120 mesh supports (6:1 by wt.).

The mixture of packings was poured into vertically aligned glass tubes using a funnel, to allow observation of possible separation of the particles during their descent. This process was observed for undisturbed, vibrated, and tapped tubes. The effects of subsequent vibration and tapping on the consolidation of these beds were also noted. For all vibration and tapping in this work, a hand-operated vibrator was applied along the entire column, always moving the vibrator in a downward direction. Tapping was performed by repeatedly letting the vertical column drop a distance of approximately 5 cm on to the floor.

Glass tubes were filled with the mixture of packings using the Column Packer as described in MS I. The Column Packer consists essentially of a reservoir tube to hold packing material which is rapidly blown into the column by pressurized gas. The packing was consolidated by gentle vertical tapping. The dispersion of the colored particles and the effect of tapping on the bed were observed.

To test the reproducibility and efficiency of columns produced with the Column Packer, two duplicate pairs of "identical" SE 30 and OV 17 columns were prepared. The liquid phases for these columns were coated on Anakrom ABS, 80/90 mesh (1:20 by wt.). Examination of the Anakrom ABS 80/90 mesh, showed that pre-sieving was unnecessary; the support was free of dust and fines. The support was "pan coated", i.e., a solution of the liquid phase was mixed with the support in an open container and the solvent evaporated without stirring. The packing material was then vacuum-

oven dried, and preconditioned, prior to packing, with nitrogen gas flow

for 24 hr at 250°C.

Column performance was evaluated with n-octane by preparing plots

of HETP versus flow rate and plots of the reciprocal of retention time

versus flow rate. A Varian Aerograph Series 1800 instrument was used,

with flame ionisation detector and the columns described. The following

temperatures were used: column, 92°C; injector, 94°C; and detector,

126°C. Triplicate on-column injections of 0.050 μl n-octane were made

at each flow rate used. A Honeywell Electronic 194 recorder was used

with chart speed 1.5 in/min. For calculation of plate numbers, peak

widths were determined with a Nikon Profile Projector, Model 6C, used in

the transmission mode with 10x magnification.

Results and Discussion

Simple pouring of the mixture of supports into a vertically aligned

glass tube produced a bed in which agglomerates of colored particles were

visible on the outer walls. The non-colored particles had a tendency to

fall down the center of the tube, the colored particles down along the

walls. This effect did not seem to be influenced by vibration or tapping

during the filling of the columns.

The bed was subsequently consolidated by vibration. Although the

bed was compressed considerably, the following irregularities were noted.

As the vibrator was passed from the top to the bottom of the column,

particles below the point of contact were compacted while a vibratory

node above the contact point disturbed and expanded the bed. Vibration also caused the bed to rotate horizontally, often shearing the bed, with different portions rotating in opposite directions. No matter how intensive the previous vibration had been, additional compaction of the bed was always achieved by subsequent vertical tapping.

As described in MS I, a Column Packer was devised for rapid filling of a column and compacting of the packing. It is believed that rapid filling tends to minimize the mentioned separation effect, and that a high momentum of the support particles gives rise to a more regular bed. In addition, the use of continued gas pressure might minimize any recoil of particles during the consolidation by vertical tapping.

When glass tubes were filled with the Column Packer, the tubing was filled instantly by a plug-like flow. The bed was easily consolidated by gentle vertical tapping without apparent distortion or breaks. Columns filled with the Column Packer gave a more uniform dispersion of colored particles than those filled by simple pouring with a funnel. In addition, the packing density was increased approximately 25% with use of the Column Packer and vertical tapping as compared to simple pouring and vibration. This increase in packing density should be considered a tentative value only, as the columns compared were packed by different individuals.

The reproducibility and efficiency of two pairs of "identical" columns, packed with the Column Packer, were determined by measuring the following parameters; packing density, HETP minimum, and the associated

optimum flow rate, v_{opt}. As expected, plots of the reciprocal of reten-

tion time versus flow rate were linear. The correlation coefficients

and the slopes of the regression lines were calculated. Results are

given in Figures 1-4 and in Table 1.

Fig. 1. Variation in HETP with flow rate for pairs of "identical" OV 17
columns.

Calculated for n-octane; nitrogen carrier gas; column temperature

92°C.

OV 17a - - - - □ - - - -   OV 17b _____ o _____

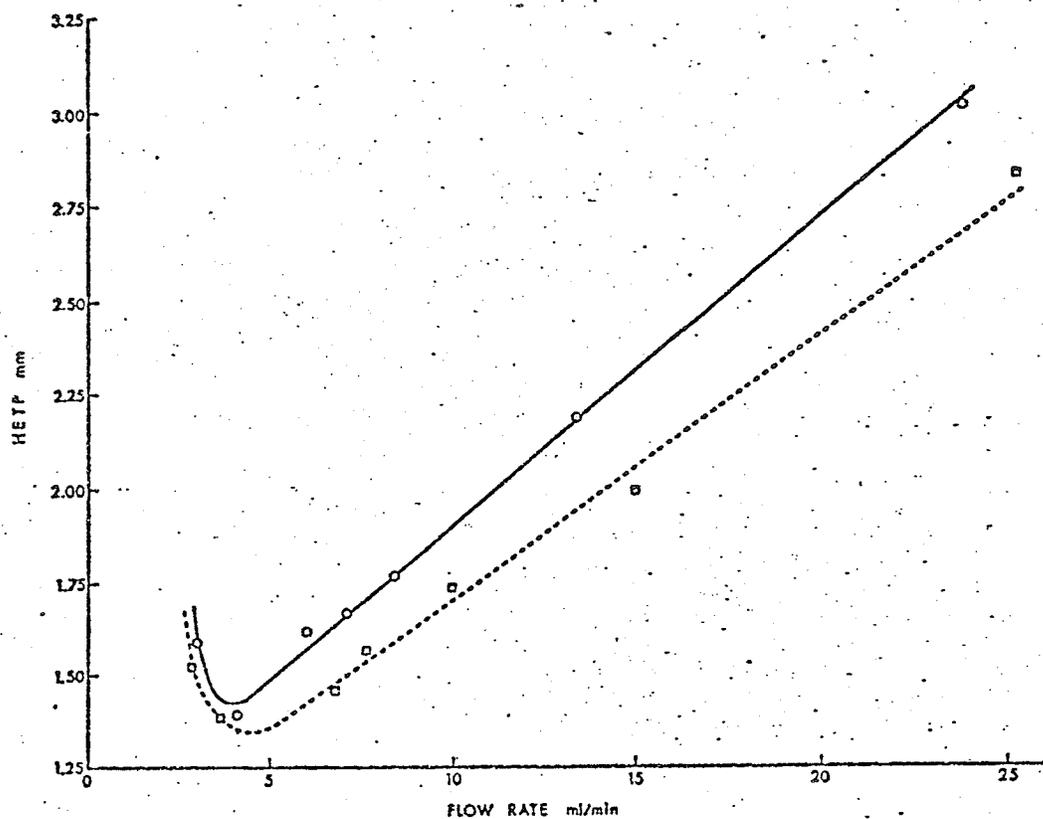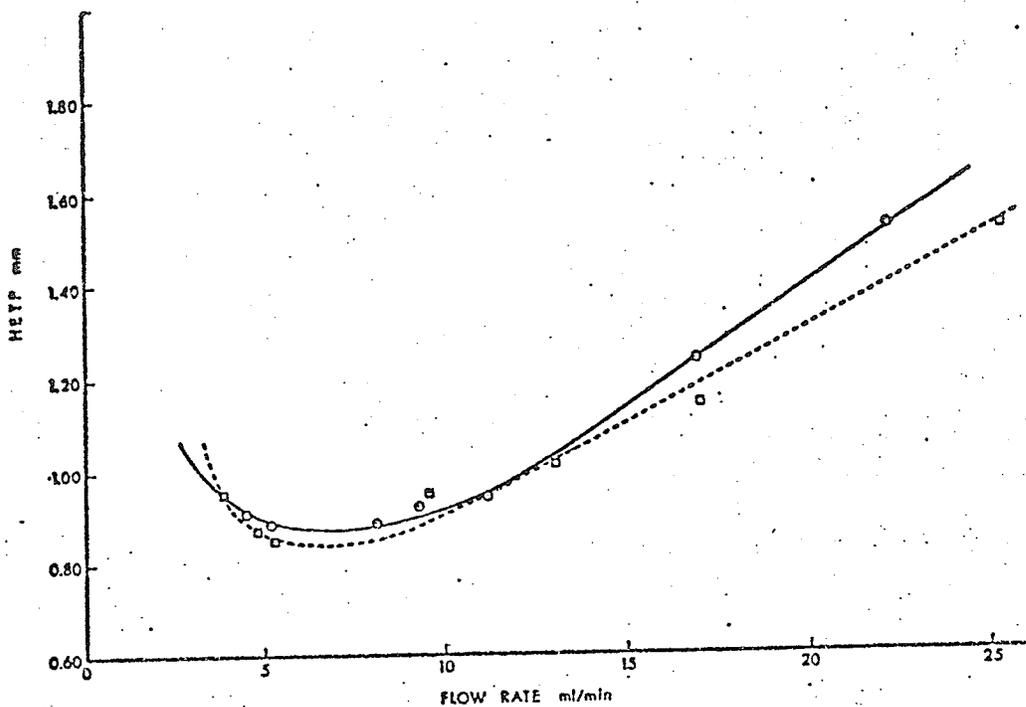Fig. 2. Variation in HETP with flow rate for pairs of "identical" SE 30
columns.

Calculated for n-octane; nitrogen carrier gas; column temperature

92°C.

SE 30a - - - - □ - - - -   SE 30b ———— o ————
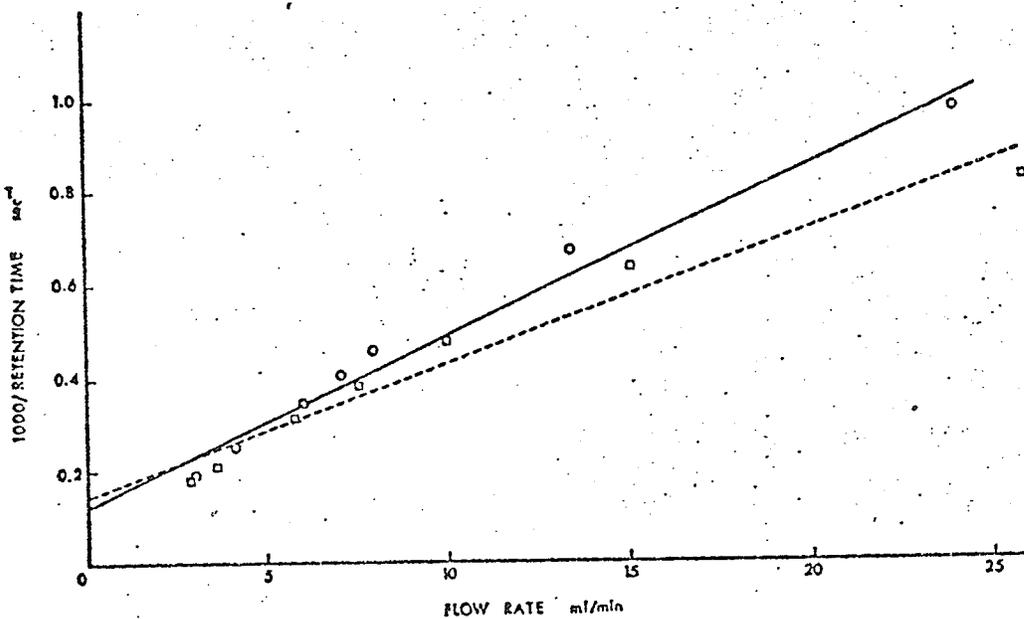
Fig. 3. Variation of retention time with flow rate for pairs of "identical"
OV 17 columns. Elution of n-octane by nitrogen at 92°C.

OV 17a - - - - □ - - - -   OV 17b ——— : — o ————
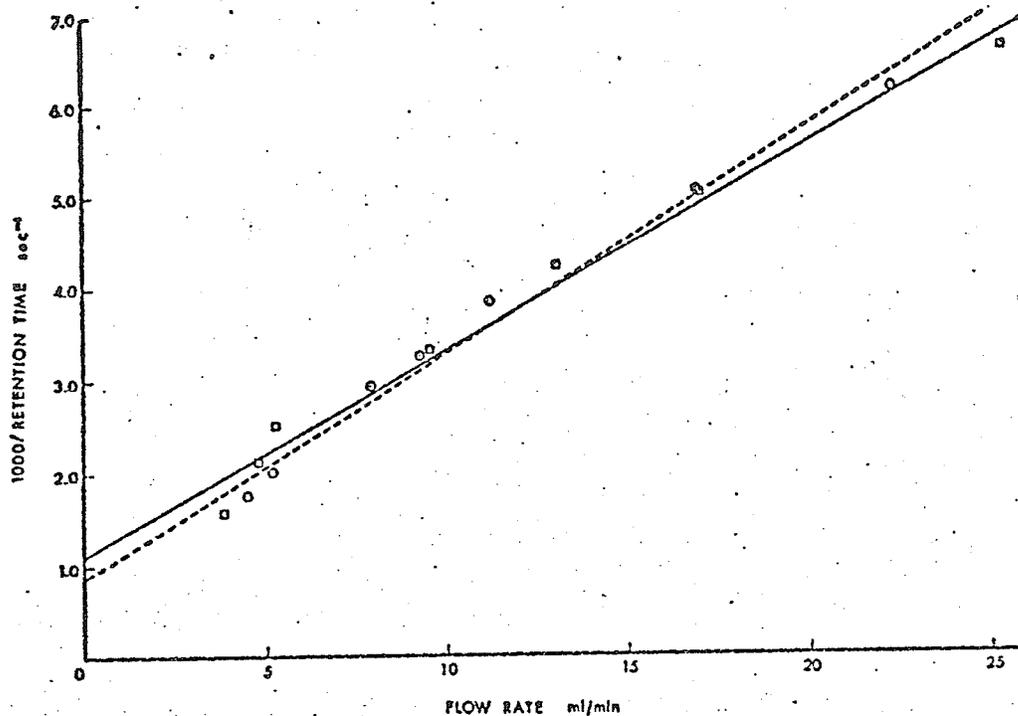
Fig. 4. Variation of retention time with flow rate for pairs of "identical" SE 30 columns. Elution of n-octane by nitrogen at 92°C.

SE 30a - - - - □ - - - -   SE 30b ———— o ————

TABLE 1.   COLUMN CHARACTERISTICS

Column	Packing Density (g/ft)	$HETP_{min}$ (mm)	Flow Rate V_{opt} (ml/min)	Slope	Corr. Coeff.
OV 17a	0.349	1.34	4.65	0.000284	0.982
OV 17b	0.373	1.41	3.98	0.000365	0.992
SE 30a	0.355	0.84	6.75	0.000244	0.995
SE 30b	0.346	0.87	7.00	0.000223	0.993

HETP is influenced by many parameters such as column permeability, substance chromatographed, liquid phase, carrier gas, dead-space, injector and column temperatures, and carrier gas pressure. For a quantitative evaluation of packing procedures through the resulting efficiency of prepared columns, all the mentioned parameters, with exception perhaps of permeability, should be identical. For this reason, it was difficult in the present work to make direct comparison of the prepared columns with others reported in the literature. As a general rule, however, well prepared analytical columns have HETP minima of about 1 mm or less (13). Using this criterion for comparison, the SE 30 columns with average HETP of 0.86 mm may be considered to be efficient, while the OV 17 columns with average HETP of 1.38 mm were less efficient. It is generally recognized that efficiency measurements with a non-polar substance will be higher for a non-polar column than a polar column. The large difference in efficiency between the SE 30 and OV 17 columns may be attributed to the differences in polarities of the liquid phases and the corresponding effect on the retention time of n-octane. At identical flow rates, the moderately polar phase, OV 17, gives much shorter retention times for n-octane than does the non-polar phase SE 30. This effect may accentuate peak broadening compared to elution time for the OV 17 columns.

Increasing the packing densities will tend to decrease both the HETP minimum and optimum flow rate (14). An increase in packing density has been shown to increase column efficiency until a limit is reached (15);

a further increase in the packing density results in either no additional gain or in a loss in efficiency, possibly due to particle fracture. This effect is evident in Fig. 1: the more densely packed OV 17b column has a reduced optimum flow rate but an increased HETP minimum.

The plots of the reciprocal of retention time versus flow rate resulted in non-parallel regression lines for the "identical" columns of each pair. The large differences in the slopes of the regression lines in Figs. 3 and 4 reveal the sensitivity of the retention time to packing density. The slope differences may also be attributed to packing non-uniformity. For dual column gas chromatography, with programmed temperature, the regression lines for the two columns should preferably be super-imposed or parallel. This would probably have been achieved with the present column pairs if identical amounts of packing had been used for each column.

VOLATILE COMPOUNDS FROM MAIZE

This study reports the isolation of a concentrate of volatile maize

compounds (aroma essence) having reproducible composition. Essences from

different maize varieties were analysed by gas chromatography. It was

shown that five varieties of maize could be distinguished from one another

by the composition of their aroma essences.

Formation of Aroma Compounds

Many different types of organic reactions lead to the production of

aroma volatiles. The most comprehensive reviews, summarizing existing

knowledge about browning reactions and food flavor have been made by

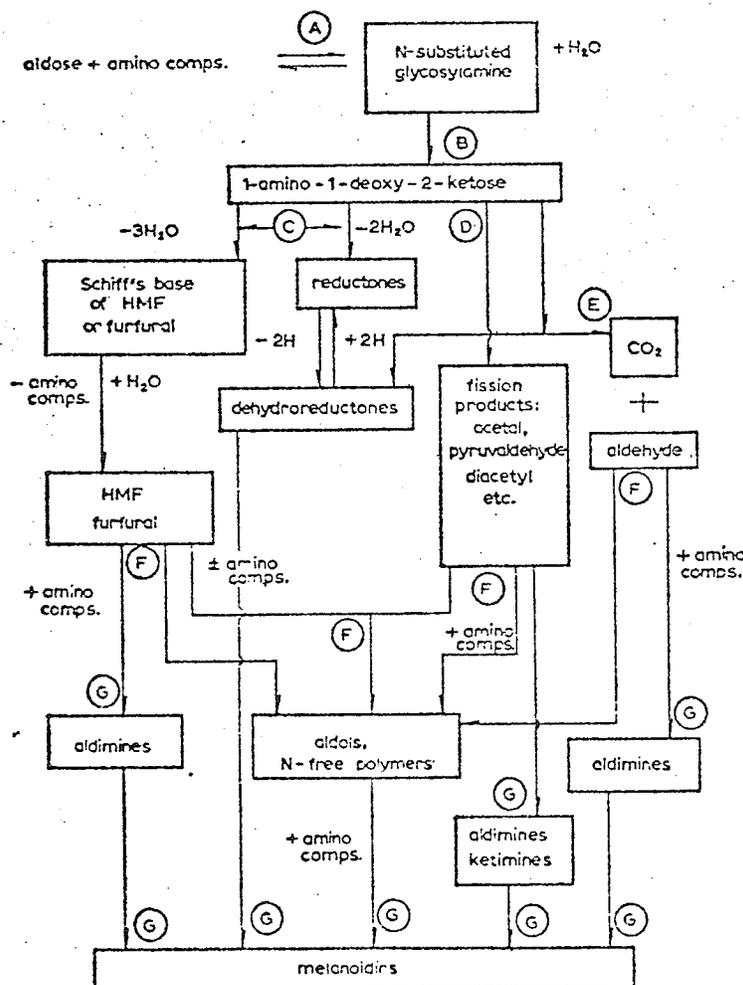Hodge (16,17) as indicated schematically in Figure 5.

Fig. 5. The Hodge scheme for browning reactions.

In this Figure the encircled letters denote the following reactions:

(A)   The Maillard reaction

(B)   The Amadori rearrangement

(C)   Dehydration of sugar addition products

(D)   Fission products

(E)   Strecker degradation

(F)   Recombination of nitrogenous residues of amino acids with carbonyl

groups forming aldols or N-free polymers

(G)   Reactions leading to pigments, not associated with aroma volatiles

A combination of the Maillard condensation and the Amadori rearrangement, as well as fission of sugar addition products, provides dicarbonyl compounds for Strecker degradation yielding aldehydes or ketones. In addition, the dehydration of sugar addition products yields furfural and also hydroxymethylfurfural (HMF) which may react with amino compounds forming other aroma compounds.

Thermal degradation of carbohydrates is known to change or develop flavor in many foodstuffs. There have been 96 volatile compounds identified from heated glucose (18). Pyrolysis temperatures ranged from 150° to 500°C. Reaction mechanisms of carbohydrate pyrolysis included enolization, dehydration, hydrolytic fission, dismutation, self- and cross-condensation, dimerization and polymerization. A study of the products from pyrolysis of glucose in air and nitrogen at 250°C revealed

that oxidation was not involved in the degradation (19).

Proteins have been found to emit volatile products when heated (20). Roasting of casein in air at 250°C resulted in a foul odor and produced 36 volatile components. In this process, amino acid residues were suggested as precursors of aldehydes through oxidative degradation. Radical cleavage and recombination of the amino acids were thought to produce amines. The lipids bound to casein produced aldehydes when roasted in air.

Aroma Compounds from Cereal Grains

Johns and Oldham (21) first attempted to isolate the compound responsible for the odor of yellow corn. Aroma volatiles were isolated via steam distillation of pressed or extracted oils. Repeated fractional distillations of the steam distillate gave two fractions, namely, a clear odorous liquid and a waxy solid melting slightly above 20°C. Small yields made further fractionation impractical. Analysis of the fragrant liquid suggested that the compound responsible for corn odor had a molecular weight of less than 150, contained one atom of oxygen and had a carbon-hydrogen ratio of 1:2. The waxy solid was thought to be a hydrocarbon. This technique of steam distillation and fractional distillation failed when applied to ground corn kernels.

Geockner (22) attempted to isolate and identify steam volatiles from corn starch, germ and oil. Volatile materials obtained from starch were of minute concentration, making isolation impractical. The addition of

alkali to an aqueous suspension of corn germ destroyed the odor. Acidic steam distillation, followed by continuous methylene chloride extraction produced an odorous extract containing furfural and free fatty acids. Corn germ steam distilled in "conductivity water" was found to be the best source of aroma constituents. An extract obtained by steam distillation and methylene chloride extraction revealed 29 components, 14 of which were identified by gas chromatography and mass spectrometry. The aroma extract was estimated to constitute 0.12% of the crude oil or 2 ppm of the intact kernel.

Steam distillation of microwave popped corn followed by ether extraction revealed 58 components when the volatile concentrate was examined by gas chromatography and mass spectrometry (23). Compounds believed to make important contributions to the flavor and aroma of popcorn included pyrazines, furans, pyrroles, carbonyls and substituted phenols.

Studies on volatiles from steam distillates of ground wheat (24) and wheat gluten (25) resulted in the identification of 18 and 15 compounds, respectively.

Quantitative differences between species were reported in a study of the composition of fatty acids, carbonyls and hydrocarbons from triticale and wheat flour (26). Each group of compounds was isolated by direct extraction of flour and analysed by gas chromatography. In this work, only some of the reported components were sufficiently volatile to be considered to contribute to aroma. The only varietal

difference noted was that Scout, a winter wheat, had a higher short chain n-hydrocarbon content than the other wheats examined. In hydrocarbon composition, Scout resembled triticale more than wheat.

Headspace vapor from maize, wheat, rye and triticale were found to be qualitatively similar but quantitatively different in composition (27). Gas chromatography of the headspace vapors over maize at $120^{\circ}C$ revealed 39 components, 13 of which were tentatively identified. Varietal differences in composition were reported among four maize samples and among four out of five wheat samples.

A tabulation of the organic volatiles identified from grains and grain by-products is shown in Table 2. For popped corn, only those volatiles listed for the other products are included in the Table. In addition, a large number of complex heterocyclic compounds were identified from popcorn (23), which suggests that substantial thermal degradation was induced by the microwave popping. It appears from the Table that aliphatic carbonyl compounds largely predominate in all the samples.

TABLE 2. VOLATILES OBTAINED FROM GRAINS AND GRAIN BY-PRODUCTS

Compound	Corn (27)[a]	Corn Oil (22)	Popped Corn (23)	Wheat (24)	Wheat Gluten (25)
Methanol	x				x
Ethanol	x		x		x
Propanol					x
2-Methylpropanol					x
Butanol					x
3-Methylbutanol				x	x
Pentanol				x	x
Methanal					x
Ethanal	x		x	x	x
Phenylethanal				x	
Propanal	x				x
2-Methylpropanal	x	x	x	x	
Butanal	x			x	x
3-Methylbutanal	x	x	x	x	x
2-Butenal		x		x	
Furfural		x	x		
Pentanal	x		x	x	x
Hexanal	x	x	x	x	x
Heptanal	x		x	x	
Octanal				x	
Acetone					x
Propanone	x	x			
Butanone	x			x	
3-Methyl-2-butanone				x	
2,3-Butanedione			x	x	
2,2-Dimethyl-3-pentanone				x	
Cyclopentanone				x	
Furan		x			
2-Acetylfuran		x	x		
Pyridine		x	x		
Butanoic acid		x			
2-Methyl pentanoic acid		x			
Pentanoic acid		x			
3-Methyl pentanoic acid		x			
Hexanoic acid		x	x		
Ethyl ethanoate			x	x	

[a] Reference 27.

Experimental

Volatile compounds from ground maize were isolated by conventional steam distillation at atmospheric pressure. Steam was directed through a slurry of ground maize in distilled water in a 12-litre thermal insulated flask. Steam was condensed with the aid of a water cooled condenser and collected in a 6-litre capacity ice-cooled trap containing methylene chloride.

The aqueous distillate was separated from the methylene chloride and saturated with NaCl, and a portion was continuously extracted with methylene chloride. A continuous liquid-liquid extractor, having capacity 1500 ml, was made in the Chemistry Department Glass Blowing Shop and is shown in Figure 6. After drying the combined methylene chloride extracts with Na_2SO_4, the extracts were concentrated to a small volume by slow distillation followed by evaporation at room temperature.
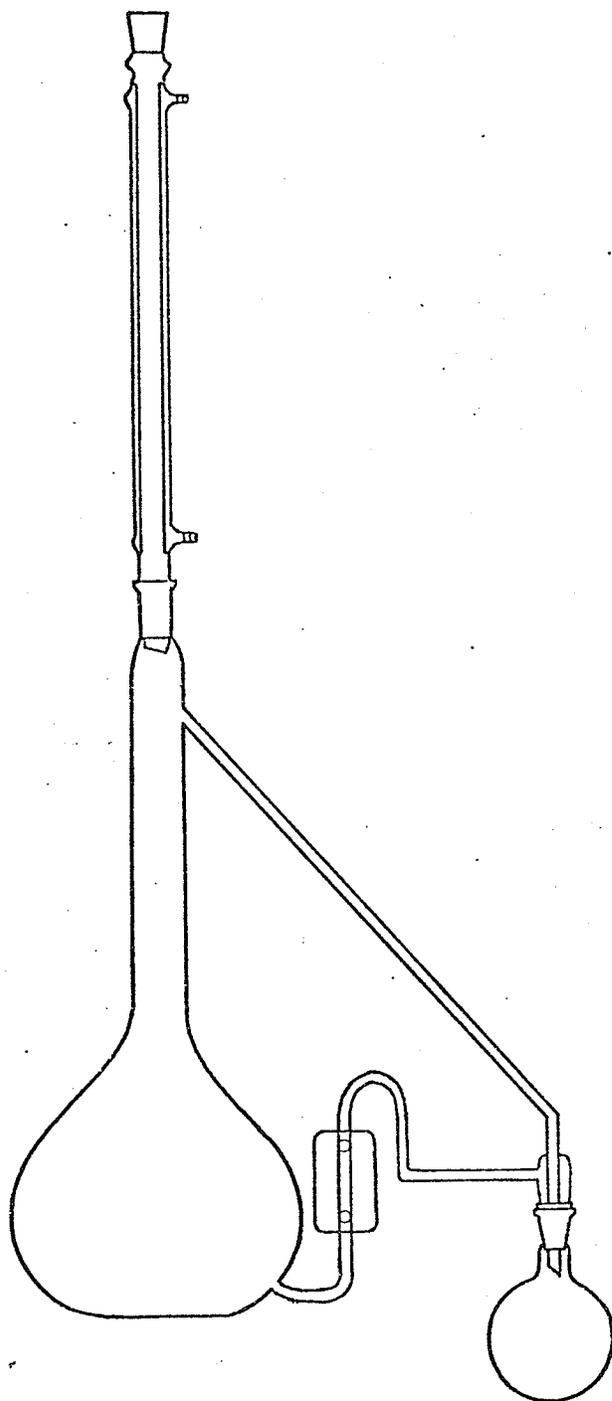
Figure 6. Continuous liquid-liquid extractor.

The concentrated essence was transferred to a small glass tube, mixed with an internal standard, and immediately analysed by gas chromatography. Other details of the procedure used for isolation and analysis of the maize volatiles are given in MS II.

Results and Discussion

As described in MS II, volatile materials from five maize varieties have been isolated and analysed by gas chromatography.

In a preliminary investigation of isolation procedures for the maize volatiles, a method by Redshaw (28) was used. A tube containing ground maize was placed in a gas chromatographic oven, held at elevated temperatures, and purged with inert gas; the effluent was condensed in an ice-cooled trap. The aqueous condensate obtained by gas purging had a burnt acrid odor if the temperature of the maize exceeded $150^{\circ}C$. At lower temperatures, water vapor interacted with the ground maize, plugging the system.

This technique was abandoned in favor of steam distillation, partly to reduce the possible formation of pyrolysis products. Steam distillation may be regarded as a relatively mild technique; the only reported pyrolysis at $100^{\circ}C$ is that of cellulose, yielding CO, CO_2 and H_2O (29).

Continuous extraction of the steam distillates were first performed with ethyl ether. Ether extracts proved to be difficult to dry and, when injected for analysis, often resulted in baseline shifts which made quantitation of the peaks difficult. Aqueous samples are known to produce

"memory" or "ghost" peaks (30).

Methylene chloride was finally chosen for extraction of the aqueous distillate. It was an efficient extraction solvent, easily obtained in high purity and easily dried. In addition, methylene chloride offered the following advantages over ether: it is less toxic, self extinguishing, and free from potentially explosive peroxides.

Concentration of the extracts was attempted by vacuum distillation. The small volumes and "bumping" induced by vacuum made this technique impractical. Gentle distillation followed by slow evaporation at $20^{o}C$ resulted in an extract of reproducible composition. The final volume of the extract, 15 μl, represented 12 ppm of the original maize sample. If the extract was allowed to evaporate to "dryness", a waxy yellow-brown residue remained.

Chromatographic separation of the aroma essence was attempted using various temperature programs, three liquid phases and two Durapak column packing materials. FFAP, Carbowax 20M, Carbowax 400/Porasil S and Carbowax 4000/Porasil S were rejected because of excessive bleed during temperature programming. The liquid phase OV 225 was found to give the best performance; its thermostability allowed temperature programming without baseline shift; it gave superior separation, and it allowed direct silanization of the packed column to reduce tailing and improve resolution. The maize essence was separated into 91 components by gas chromatography; some of the smaller components were seen only in some

of the samples investigated.

The volatiles from five maize varieties were isolated and analysed in duplicate. Gas chromatographic analysis was limited to one injection per aroma essence. Repeated injections of the same essence at short time intervals of 3 hr resulted in chromatograms with irregular baselines. Quantitation under these circumstances was difficult. Although injections at longer time intervals of 2 days overcame this difficulty with the baseline, the composition of the aroma essence changed considerably during this interval, presumably due to evaporation.

Of the 91 peaks, only 27 which appeared to differ substantially among the 5 varieties were included in the statistical analysis. Each of the 27 peaks was chosen on the criterion that both of the peak areas obtained by duplicate analysis for one variety were either larger, or smaller, than the peak areas for all the duplicate analyses of the other four varieties. Analysis of variance revealed that 18 of these peaks differed significantly among the 5 varieties investigated. It has thus been established that the different maize varieties studied could be distinguished from one another by the composition of their aroma essences.

The analytical error associated with the isolation and analysis of the volatile material, expressed as the mean difference between corresponding peak areas for duplicate analyses was $\pm$ 11.7%. The error associated with the extraction of only a 1500 ml portion of a total of 1600 $\pm$ 30 ml of aqueous distillate was estimated to be a maximum of $\pm$ 2%. The experimental error from other sources are more difficult to assess. Caking of

the maize slurry on the slides of the container during steam distillation might have changed the composition of the volatile essence. The resolution of chromatographic peaks and, consequently, the areas as measured by the electronic integrator might have varied as a result of unavoidable differences in the technique of injection for the different samples.

CONTRIBUTIONS TO KNOWLEDGE

1. An inexpensive apparatus and rapid method for preparation of efficient gas chromatographic columns was developed and evaluated.

2. Gas chromatographic analysis of a steam volatile aroma essence from maize revealed 91 components; only 39 had been earlier reported (27).

3. The quantitative composition of the aroma essences from five maize varieties studied were significantly different.

SUGGESTED FUTURE INVESTIGATIONS

1. High speed motion picture studies of the formation of packed chromatographic beds to better observe the effects of different packing techniques.

2. Gas chromatography of volatile compounds from cereal grains with glass capillary columns for better resolution and quantitation.

3. Organoleptic evaluation of volatile compounds from maize to determine which compounds are responsible for the characteristic odor.

4. Identification of the odorous compounds of maize by gas chromatography-mass spectrometry, infrared spectroscopy, column subtraction techniques and retention parameters.

5. Develop a rapid, simple and reproducible method of identification of cereal varieties through gas chromatographic analysis of volatiles, possibly by headspace or pyrolysis techniques.

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APPENDIX

MANUSCRIPT I

AN INEXPENSIVE COLUMN PACKER FOR GAS CHROMATOGRAPHY

to be submitted for publication to

ANALYTICAL CHEMISTRY

AN INEXPENSIVE COLUMN PACKER FOR GAS CHROMATOGRAPHY

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Mechanized methods employed for packing gas chromatographic columns include tapping of the column (1,2), tapping followed by gas pressure (3), and vibration with simultaneous gas pressure (4). A major problem, particularly with long columns, is to obtain a high packing density without crushing the support particles. As Giddings (5) has shown, the principle contribution to plate height in the gas phase arises from irregularities in the packed bed. The present paper describes an inexpensive device for rapid packing of columns with high packing density and with a minimum formation of "fines".

The column packer (Figure 1) is constructed from commonly available materials. It consists essentially of a tube reservoir (C) from which the column packing is blown by pressurized gas into the column. Two modifications are made to the Swagelok fittings. Part B is reamed with a 1/4" bit, and a fine-mesh steel screen is inserted to prevent blow-back of packing material. Part G is drilled completely through with a 1/8" bit to allow free passage of packing material.
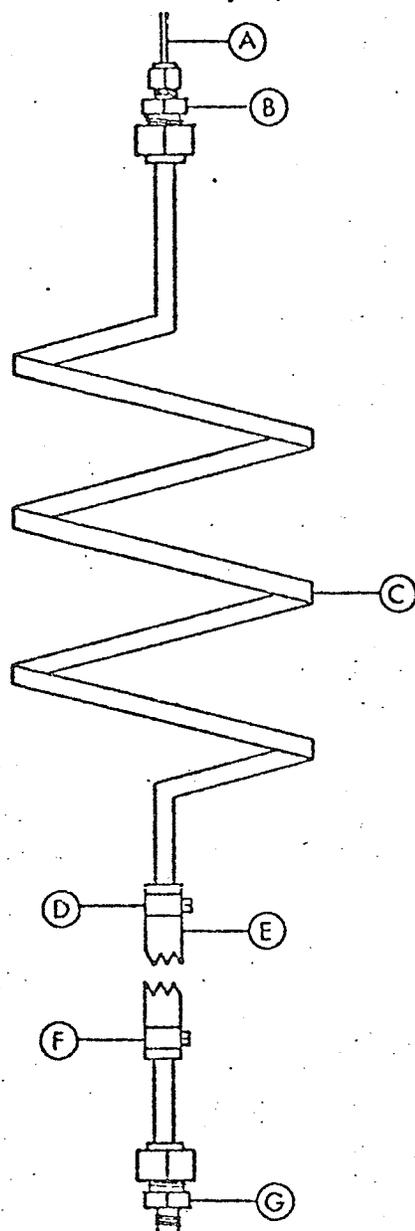
Figure 1. Assembled column packer

A   Teflon or other tubing to nitrogen supply

B, G   Swagelok reducing union (cat. No. 400-6-2)

C   Stainless steel tubing (40" x 1/4" o.d.)

D, F   Hose clamp

E   Polyethylene tubing (12" x 5/8" o.d.)

To pack a column, the column tubing (with the usual Swagelock fittings and glass wool plug) is supported vertically in a groove in a long narrow board. An excess amount of column packing is poured (through G) into the reservoir of the inverted column packer. The packer, still inverted, is then connected to a source of dry nitrogen (at A) and to the column (at G). The packing is blown into the column by again inverting the packer and immediately applying a suitable gas pressure (50 psi for a 15' x 1/8" o.d. column) while gently tapping the column-board vertically against the floor. The gas pressure and tapping are maintained until no additional packing material enters the column, as observed by the level in the transparent tube (E). Finally, the gas pressure is reduced to atmospheric; this must be done gradually to prevent blow-back of material from the column.

Use of the column packer in our laboratory has provided columns of higher efficiencies than corresponding columns packed by conventional vibration. Also, an increase in packing density of approximately 25% has been obtained, as measured by the weight of packing material contained in the prepared columns. The packing of a 15' x 1/8" o.d. column usually is completed in 20 minutes.

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MANUSCRIPT II

VARIETAL DIFFERENCES IN AROMA ESSENCE FROM MAIZE KERNELS (Zea mays L.)

as submitted for publication to

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

VARIETAL DIFFERENCES IN AROMA ESSENCE FROM MAIZE KERNELS (Zea mays L.)

Aroma essences of reproducible composition were isolated from maize kernels (Zea mays L.) by steam distillation, extraction of the distillate, and slow evaporation of the extract. Gas chromatography revealed 91 components in the aroma essences. The quantitative composition of the essences differed significantly among five varieties of maize investigated.

The composition of the volatile aroma materials of some vegetable foodstuffs has been shown to be dependent upon plant variety and geographical location, as reported for cranberries (Anjou and von Sydow, 1969), onions (Chua et al., 1968), cocoa beans (Bailey et al., 1962), tea (Bondarovich et al., 1967), coffee, bananas, oranges, tomatoes, potatoes, peppermint oils, spearmint oils, and peanuts (reviewed by Pattee and Singleton, 1972). Only recently have the aroma compounds of cereal grains come under investigation, with identification of compounds from corn (Goeckner, 1958), rice (Yasumatsu et al., 1966), and wheat (McWilliams and Mackey, 1969). Species differences have been reported in the composition of the carbonyl compounds and hydrocarbons extracted from wheat and triticale flours (Lorenz and Maga, 1972). Varietal differences have been reported in the composition of the headspace vapors from maize and wheat (Hougen et al., 1971).

In connection with a study on the quality of maize for production

of distilled beverages, the present investigation was undertaken to de-

termine whether a reproducible technique could be developed to differen-

tiate maize varieties by the composition of their steam-volatile materials.

Such differences could aid in taxonomic studies and in quality control in

the food and fermentation industries.

MATERIALS AND METHODS

Materials. Five varieties of maize (Zea mays L.), grown at Morden,

Manitoba, were obtained for investigation (Table I). The cleaned kernels

were frozen at -20°C, ground to 20-40 mesh with an Allis Chalmer mill,

and stored at -20°C.

Preparation of Aroma Essence. The procedure of Heinz et al. (1966)

was used with some modifications. A sample of frozen, ground maize

(1250 g) was added to boiling, distilled water (7.5 l). Steam was passed

through the slurry for 4 hr. The distillate (1600 ml $\pm$ 30 ml) was col-

lected in an ice-cooled trap containing methylene chloride (200 ml). The

aqueous distillate was separated from the solvent extract and saturated

with NaCl; a 1500-ml portion was continuously extracted with methylene

chloride for 6 hr. After drying the combined extracts with Na_2SO_4, the

solvent was removed by slow distillation until approximately 25 ml re-

mained. This solution was transferred to a 50-ml pear-shaped flask,

warmed with gentle swirling in a 60°C waterbath until 2-3 ml remained,

and further allowed to evaporate at 20°C until approximately 15 μl

remained, as indicated by a mark on the flask. Without delay, this aroma

essence was transferred with a 10-μl syringe to a Pyrex tube (30 mm x 2 mm

o.d.), 0.30 μl of 2,2,4-trimethylpentane was added as an internal standard

(using a 1-μl syringe), the tube was capped with Teflon tape and inverted

twice to ensure mixing, and 1.0 μl of the mixture was injected for gas

chromatographic analysis.

Gas Chromatography. A Varian Aerograph Series 1800 instrument was

used, with flame ionization detector and a single column (4.57 m x 1.8 mm

i.d., stainless steel, packed with OV 225 on Chromosorb W, AW-DMCS, 80-

100 mesh, 1:20 by wt). The column oven was held at 60°C for 20 min after

injection of a sample, then increased 2°C per min for 10 min, held at

80°C for 15 min, increased 2°C per min for 50 min, held at 180°C until

the end of the analysis, and further held at 180°C overnight for equili-

bration of the column before analysis of the next sample. The injection

port and detector temperatures were 246° and 251°C, respectively. The

flow of nitrogen carrier gas was 19.6 ml per min. Peak areas were mea-

sured with an electronic digital integrator (Infotronics CRS 100).

Statistical Analysis. All peak areas were standardized with respect

to the internal standard, i.e., each area was divided by that of the stand-

ard in the same chromatogram. In some cases where peaks were not suffi-

ciently resolved, their combined area was used in the computations. To

compare the standardized peak areas for the five maize varieties, a nested

analysis of variance was performed; where significant F ratios occurred,

the least significant difference was calculated to ascertain whether

significant differences existed between corresponding peaks.

RESULTS AND DISCUSSION

An aroma essence from maize was prepared by steam distillation of

the ground kernels followed by extraction of the distillate and evapora-

tion of the extract to a small volume. In order to ensure a high degree

of precision in the preparation of aroma essences, these operations were

carefully standardized for the different samples studied. The essences

had an aroma typical of ground maize kernels.

Gas chromatographic analyses of the aroma essences revealed 91

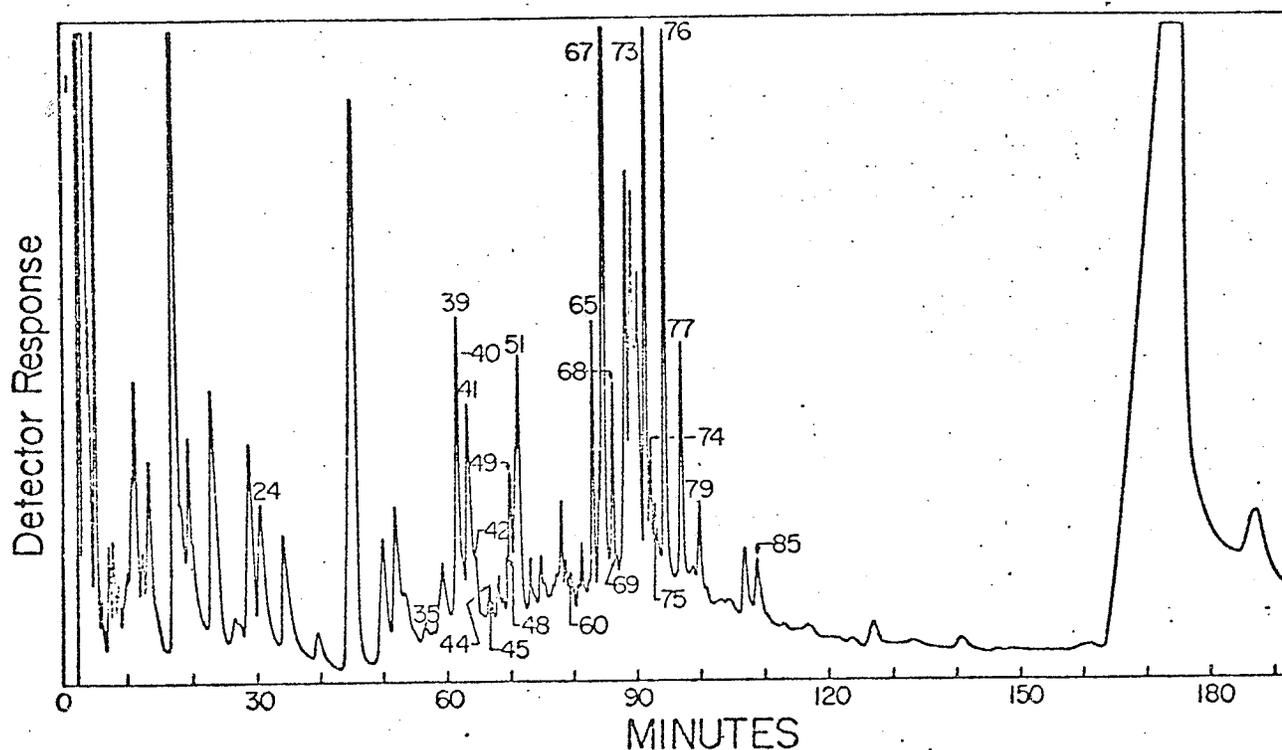components (Figure 1). On evidence of retention times and peak areas,

Figure 1. Typical gas chromatogram of aroma essense from maize kernels

different maize varieties appeared to yield the same 91 components, but in different amounts. Some of the components might possibly have been artifacts formed by the effect of heat on carbohydrates (Fagerson, 1969) or other substances in the maize. The earlier analyses of headspace vapors from maize kernels revealed 39 components (Hougen et al., 1971).

For a quantitative comparison of the aroma essences from five maize varieties, duplicate samples of each variety were processed and analyzed. Only one chromatographic analysis was used for each essence preparation, however, as its composition tended to change, presumably as a result of evaporation, before a second analysis could be made. These analyses gave reproducible results, provided the prepared aroma essences were analyzed without delay as described; the standardized peak areas for corresponding peaks for duplicate samples differed on the average by $\pm$ 11.7% relative to the mean area.

An internal standard was added to the essence preparations to ensure that the analyses would be independent of the volumes of essence prepared and sample injected. The precision of adding a constant volume of standard to each sample was assessed by injecting replicate samples of the standard substance into the gas chromatograph. A high precision was obtained, as indicated by a relative standard deviation of 0.65% for the peak areas for ten 0.20-μl injections.

The five varieties were clearly distinguished from one another on the basis of the analyzed composition of the aroma essences. Twenty-

seven peaks which appeared to vary substantially among the samples were selected for statistical analysis. For each variety, one or more of these peaks (or double peaks), as enumerated in Table I, differed

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Table I. Peaks Distinguishing Any One Variety From the Other Four

Variety	Peak-number
CM17 x CM7	73
CM36 x CM46	76
CO103 x ND203	(44+45)
Rainbow Flint	60
Minnesota 13	24, 35*, (39+40), (41+42), (48+49), (50+51), 65, 67, (68+69), 74, 75, 77, 79, 85

* Peak 35 (standardized area) for Minnesota 13 differs significantly, at the 95-% level, from the corresponding peaks for the other four varieties. For all other peak-numbers in the Table, the corresponding differences are significant at the 99-% level.

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significantly from the corresponding peaks for the other four varieties. In addition to these simple criteria for differences, other peaks (not included in Table I) showed significant differences between various combinations of two varieties and the remaining three varieties. None of

the earlier examined maize varieties (Hougen et al., 1971) were included in the present study.

This investigation has shown that a relatively large quantity of steam-volatile aroma essence from maize kernels can be isolated with reproducible composition, and that the composition is dependent on the plant variety. The application of this technique to a larger number of varieties and a study of possible environmental effects remain to be done.

ACKNOWLEDGEMENT

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