

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 chromatographic 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 mention 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:

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

which, for convenience, is often written

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

where L is the column length,

N is the number of plates,

d_p is the mean particle diameter,

v is the linear gas velocity,

D_g and D_l 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:

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

which, for convenience, is often written,

$$H = \sum \frac{1}{1/A_i + 1/C_i v} + B/v + \Sigma C_i v + C_i' v + H_{ic} \quad [2a]$$

where ω_i and ω_i' are coefficients for mass transfer equations, representing a flow inhomogeneity condition,

V_i / V_l 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 substantially increase chromatographic efficiency. The causes of non-uniformity as related to packing technique may be divided into three categories, 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 evaporation 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 packing; consolidation should follow, using vertical 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 formation. 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 retention 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.

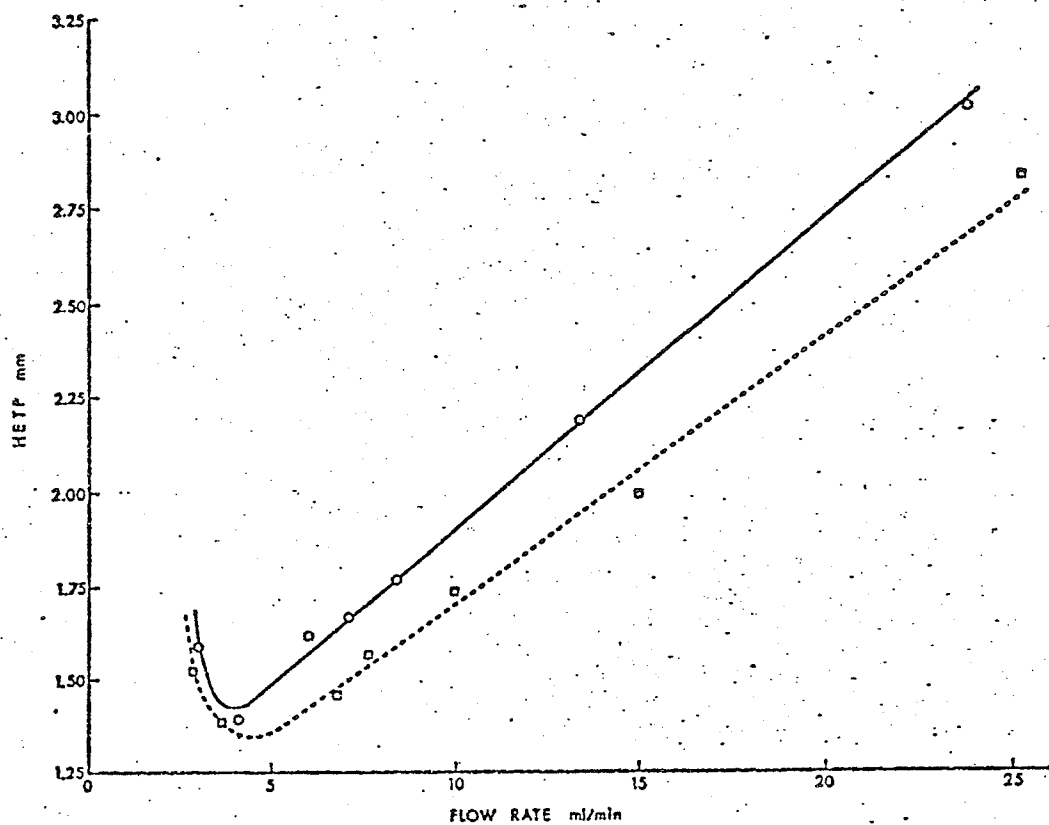


Fig. 1. Variation in HETP with flow rate for pairs of "identical" OV 17 columns.

Calculated for n-octane; nitrogen carrier gas; column temperature 92°C .

OV 17a - - - - □ - - - - OV 17b - - - - ○ - - - -

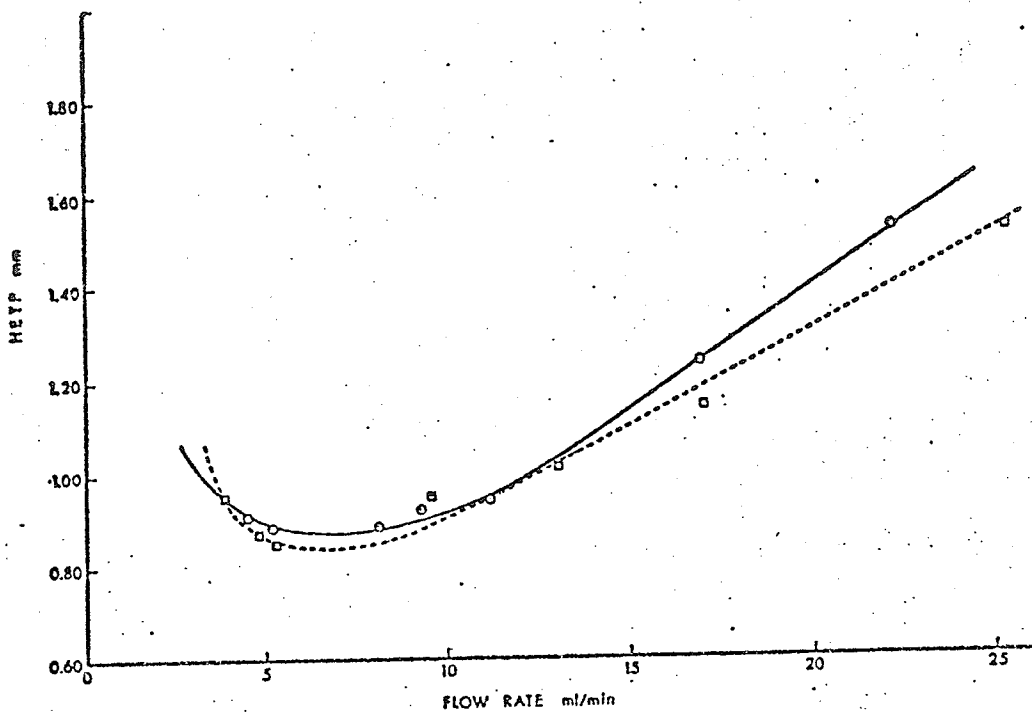


Fig. 2. Variation in HETP with flow rate for pairs of "identical" SE 30 columns.

Calculated for n-octane; nitrogen carrier gas; column temperature 92°C .

SE 30a - - - - □ - - - - SE 30b - - - - ○ - - - -

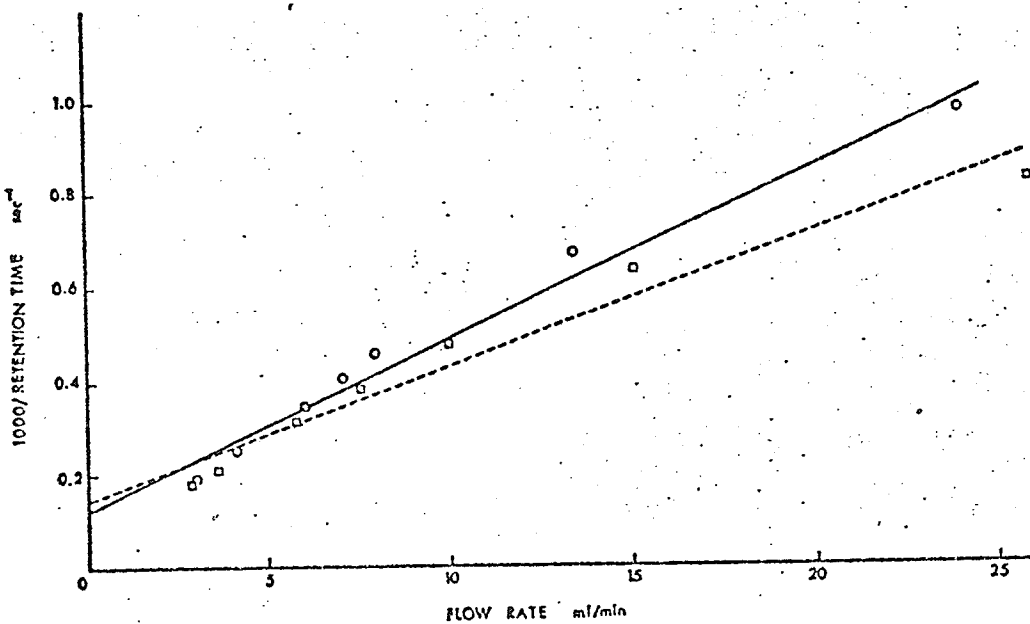


Fig. 3. Variation of retention time with flow rate for pairs of "identical" OV 17 columns. Elution of n-octane by nitrogen at 92°C .

OV 17a - - - - □ - - - - OV 17b - - - - ○ - - - -

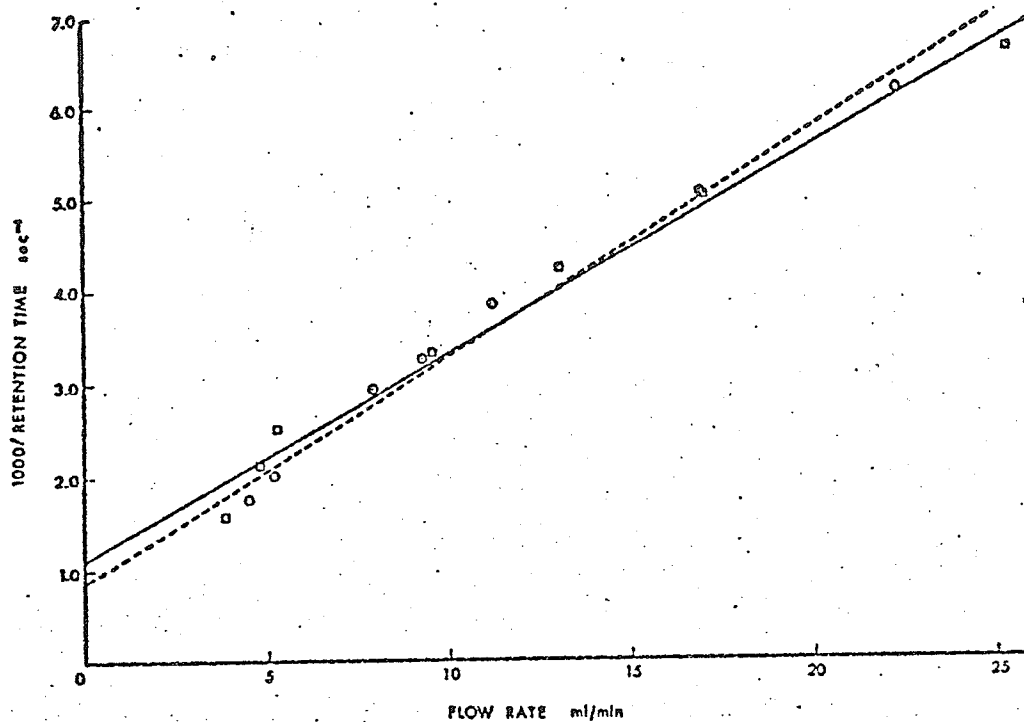


Fig. 4. Variation of retention time with flow rate for pairs of "identical" SE 30 columns. Elution of n-octane by nitrogen at 92°C.

SE 30a - - - - □ - - - - SE 30b ———— ○ ————

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 superimposed or parallel. This would probably have been achieved with the present column pairs if identical amounts of packing had been used for each column.

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°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.