Volatile Components of Bananas

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Methods have been developed for the preparation of a reproducible yield (10 to 20 p.p.m.) of odor concentrate from ripe Gros Michel bananas. Sufficient concentrate was obtained to allow its separation by gas chromatography and collection of fractions for direct investigation. Examination of the fractions resulted in rigorous identification of eight compounds. Probable identification of three compounds was accomplished, as was tentative identification of four substances. The general chemical nature of six substances was determined.

BASIC RESEARCH on the nature of the compounds responsible for the flavor of natural foodstuffs is necessary to provide a foundation for the eventual understanding of the chemistry of flavor, and of flavor changes which occur during ripening, storage, or processing of a food product.

A study of the isolation, separation, and identification of volatile compounds which contribute to the odor of ripe Gros Michel bananas has been undertaken. Such an investigation requires that the mixture of odorous compounds present in bananas be isolated in a reproducible manner and in a yield sufficiently great to allow the subsequent separation and identification of each odor component. The production and isolation of an odor concentrate from ripe bananas is described below and factors influencing the isolation and characterization of the concentrate are discussed.

Early investigations of the volatile components of bananas have been reviewed by Hultin and Proctor (*6*) who identified the following compounds in a vacuum distillate obtained from 150 grams of ripe banana pulp: methanol, ethanol, *i*-amyl alcohol, acetic acid, ethyl acetate, methyl acetate, *i*-amyl acetate, 2-hexenal, 2-pentanone, and 2-octanone. *n*-Butanol was identified in heat processed banana puree, but not in fresh bananas.

Since the above research had been carried out on small quantities of bananas, this investigation has been concerned with the study of relatively large quantities (10 kg.) of banana pulp to obtain odor isolates of high organoleptic quality.

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Part I. Isolation of an Odor Concentrate

Important losses of banana odor components during preparation of an odor concentrate could result from degradative chemical reactions as well as from effects of solubility and volatility inherent in the separation procedures. Although the influence of such changes on the quality of banana odor was judged by subjective organoleptic evaluation, an objective measure of the recovery of volatile components throughout the isolative procedure was required.

On the assumption that the major components of banana odor must be present in the air when bananas are sniffed by an observer, and based on the fact that argon ionization detectors can detect very minute quantities of volatile substances (10^{-12} grams per second for the detector used), gas chromatographic separation and ionization detection of the vapor over a known quantity of the various flavor isolates has served to monitor qualitative and quantitative changes in the odor components present. Similar methods have been employed by Buttery (*2*), Buttery and Teranishi (*3*), and Mackay *et al.* (*9*) for the analysis of vapors from foods.

Experimental Procedure

Removal of Flavor Isolate from Bananas. Batches of approximately 10 kg. (22 pounds) of the pulp from Gros Michel bananas which had been ripened to a peel color index of 6 to 6.5 under normal commercial conditions, without ethylene stimulation, were mixed with an equal weight of water and passed twice through a Hydropulse homogenizer (Scott & Williams, Inc., Laconia, N. H., Model No 1-LB-23) at 2000 p.s.i.g. The resulting homogenate was immediately distilled in a modified falling film evaporator at 25 to 35 mm. pressure and 24° to 30° C. Dilution of the banana pulp was necessary to prevent solidification of the thick homogenate and blockage of the evaporator chamber.

The evaporator was a laboratory size stainless steel Turba-film evaporator (Rodney-Hunt Machine Co., Orange, Mass.). A thin turbulent film of homogenate was maintained in contact with the heated wall of the evaporator portion of the apparatus by a high speed motor driven rotor. A separator section, above the evaporator, acted as a mechanical foam breaker and deaerator and returned liquid which was entrained in the rising vapor to the evaporator. Banana homogenate was introduced at the top of the evaporating section, and the concentrate from which volatile components were stripped was removed at the bottom into a 20-liter carboy, cooled by ice water. The distillate was condensed in a vertical, shell-and-tube-type heat exchanger cooled by tap water circulated in the shell, and collected in a series of receivers maintained at 0°, −78°, and −196° C., respectively. Reduced pressure (25 to 35 mm. of Hg) was maintained in the evaporator with a two-stage mechanical vacuum pump. The major portion of the distillate was collected in a 20-liter Pyrex carboy chilled in an ice-water bath. The rest was distributed in two 1-liter dry ice–ethanol cooled traps and in two 1-liter liquid nitrogen cooled traps. Connecting lines were Tygon, stainless steel, polyethylene, and glass tubing. Polyethylene stoppers were fabricated for the large receivers. Ultimate vacuum obtained in the assembled system was 0.15 mm. of Hg measured at the top of the evaporating section with a McLeod gage.

Preparation of homogenates from 10 kg. of banana pulp required about 1 hour after peeling of the fruit was started. Distillation of the resulting homogenates required 50 to 65 minutes. Three to 9 liters of distillate were produced.

Steam pressure in the heated jacket of the evaporator section of the apparatus was maintained between 2 and 4 p.s.i.g. by manual control of the steam valve. Jacket temperature and the rate at which homogenate was introduced to the evaporator were controlled on the basis of the consistency of the stripped concentrate as observed at the evaporator outlet. Optimum conditions required that a maximum thickness of concentrate be maintained consistent with the prevention of excessive temperature effects, such as browning of the homogenate or clogging of the apparatus.

The volumes of distillates collected during distillation of four 10-kg. batches of ripe banana pulp are given in Table I. The quantity of oxidizable carbon present in these distillates, as determined by the method of Gertner and Ivecovic (*5*), is also given.

The contents of all the traps were combined. Aliquots (2 liters) of the total distillate were saturated with 1 kg. of sodium chloride and then extracted, while cold, with 300-, 150-, and 50-ml. portions of ethyl ether (Mallinckrodt, analytical reagent). The resulting ether extracts were combined and dried over anhydrous sodium sulfate. Total volumes of ether extracts varied from 1.2 to 1.6 liters.

The dry ether extracts were concentrated to about 0.2 ml. by careful distillation at approximately 20° C. in an atmosphere of nitrogen. Removal of the last several milliliters of ether was carried out in 0.5-dram vials in which the concentrates were stored. The vials were sealed with rubber serum caps which had been exhaustively extracted with ether. The yields of banana odor concentrates thus obtained are shown in Table II. Concentrates were stored in a laboratory freezer until investigated.

Gas Chromatographic Analysis of Vapor over Distillates and Concentrates. The GC apparatus was constructed in the authors' laboratory. An argon diode ionization detector and associated amplifier-power supply (Jarrell-Ash Co., Newtonville, Mass.) was used. The detector was maintained at 200° C. during all analyses. Its limit of detection (*8*) for *i*-amyl acetate when operated at 1600 v. was approximately 3×10^{-12} grams per second, determined at twice the noise level. Peak-to-peak noise level was approximately 6×10^{-11} amp. at 1600 v. Standing current, with argon flowing, was 6×10^{-9} amp. An argon purge flow of 60 ml. per minute was maintained.

Separation of vapor samples was carried out on a 2-meter, 2-mm. i.d., glass column packed with 1% Ucon 50 HB-2000 oil on 100- to 120-mesh Gas-Chrom A (Applied Science Lab., Inc., State College, Pa.). The column was operated at 23 p.s.i.g. back pressure and an argon flow of 17.5 ml. per minute. Column temperatures were between 32° and 36° C.

Vapor samples (1.2 ml.) were taken with a 5-ml., gas-tight, Hamilton syringe from known weights of banana or from known volumes of distillates in 250-ml. Erlenmeyer flasks sealed with amber rubber caps (Sani-Tab caps, Davol Rubber Co., Providence, R. I.). An interval of 5 minutes between closure of the flasks and removal of sample permitted enrichment of the vapor space while minimizing chemical and enzymatic changes.

Separation of 1.2 ml. of vapor over the following samples was carried out: 50 grams of banana pulp from each of two bananas selected from the batch being processed, 50 grams of banana homogenate, 50 grams of homogenate from which volatile components had been removed, 50 ml. of the contents of the main distillate receiver (ice-water bath), 25 ml. of the combined contents of the dry ice–ethanol traps, 25 ml. of the combined contents of the liquid nitrogen traps, 50 ml. of total distillate (combined contents of all traps and the main receiver), and 50 ml. of the total distillate after it had been ether extracted. Analysis of 50 µl. of the vapor over 130 mg. of banana concentrate was also carried out.

Typical chromatograms obtained as described above are shown in Figures 1, 2, and 3. Consistent results were obtained from all four 10-kg. batches of banana pulp investigated.

Organoleptic Evaluation of the Total Banana Distillate. Immediately after collection of the banana distillates from batch 3, the odor of 10-ml. aliquots of the combined total distillate was compared with the odor of 10-gram aliquots of banana homogenate which had been held under refrigeration during distillate preparation (2 hours). Each panelist was given two samples of homogenate and one sample of distillate in 60-ml. glass-stoppered bottles covered with aluminum foil so that the contents were not visible. The samples were at room temperature and were identified by two-digit numbers selected at random. Evaluations were carried out under red light to minimize the effect of color differences between the samples.

Panelists were asked to indicate which samples were identical, which sample differed, and which sample appeared to have the most desirable banana odor. Of the 18 judges who participated in the triangle test, 13 matched the samples correctly. An odor difference between the banana homogenate and the distillate was noted which was significant at the 1% level (*1*). Only seven of the 13 judges who had matched the samples correctly preferred the odor of the homogenate to that of the distillate. This difference in preference was insignificant.

Table I. Oxidizable Carbon Content and Volume of Banana Distillates

Batch	Distillate Collected in	Distillate Volume (ml.)	Mg. Carbon per Kg. Banana Pulp
1	Ice-water bath	~7000	78.6
	Dry ice–ethanol traps	200	...[a]
	Liquid nitrogen traps	120	...[a]
	Total	~7300	...[a]
2	Ice-water bath	3500	61.9
	Dry ice–ethanol traps, Liquid nitrogen traps	300	17.2
	Total	3800	79.1
3	Ice-water bath	8250	...[a]
	Dry ice–ethanol traps	365	22.8
	Liquid nitrogen traps	120	3.1
	Total	8735	213
4	Ice-water bath	4010	64.1
	Dry ice–ethanol traps	250	22.8
	Liquid nitrogen traps	95	3.1
	Total	4355	90.0

[a] Value not determined.

Table II. Yield of Banana Odor Concentrates

Batch	Weight of Concentrate (Ether-Free Basis, Mg.)	Yield from Banana Pulp (P.P.M.)
1	121	12.1
2	121	12.1
3	180	18.0
4	150	15.0

Results and Discussion

A reproducible yield of odor isolate which exhibited the characteristic odor of bananas was obtained. Distillation

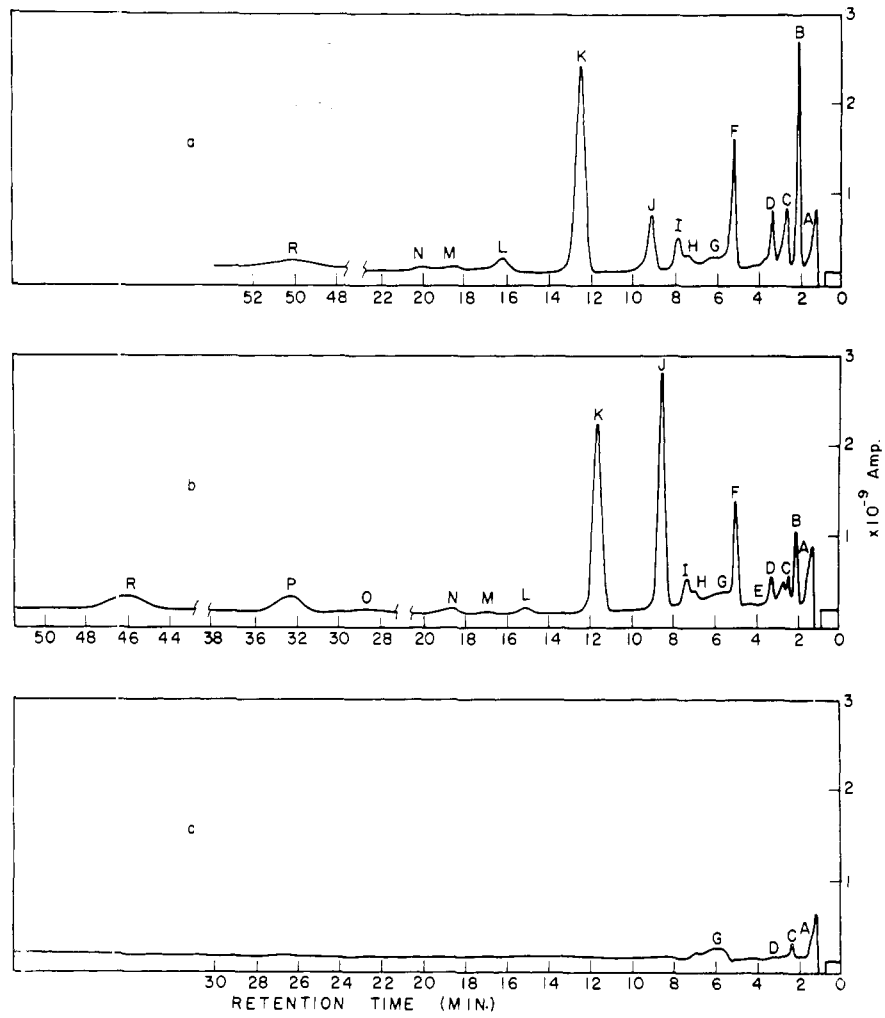

Figure 1. Chromatograms showing distribution of volatile compounds in preparation of concentrate

(a) Crushed banana (b) Banana homogenate (c) Stripped homogenate

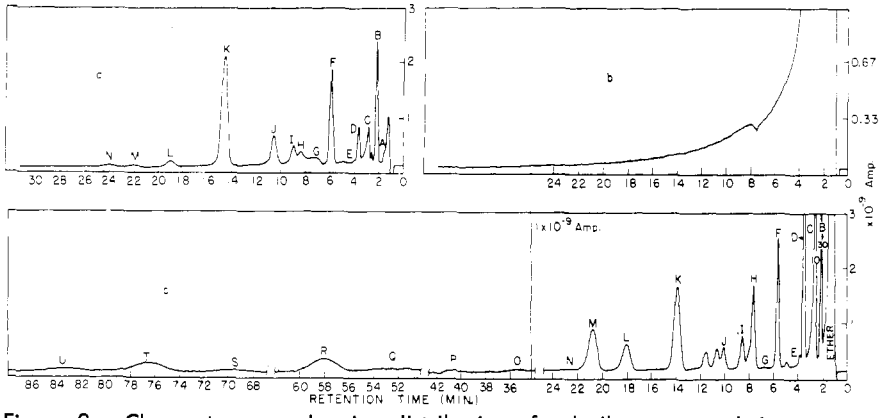

Figure 2. Chromatograms showing distribution of volatile compounds in preparation of concentrate

(a) Total distillate. (b) Aqueous layer from ether extraction. (c) Concentrate

of homogenates yielded a total volume of 3.8 to 8.7 liters of clear aqueous distillate. The volume of distillates and the oxidizable carbon content of each are given in Table I. The major portion of oxidizable material was collected at 0° C.

The total amount of oxidizable carbon obtained from each of the four batches varied from 79.1 to 213.0 mg. of carbon per kilogram of banana pulp (Table I). This range of carbon content probably is the result of natural variability in the amount of distillable substances in the bananas. Dimick and Makower (*4*) have reported a range of carbon recovery from fruit purees of 30 to 150 mg. per kg. after a single pass through a continuous vacuum flash evaporator. This is comparable to the carbon recovery obtained from bananas. Three of the four batches (1, 2, and 4) of bananas were from Ecuador. Batch 3 was fruit from Panama. It is not known whether the differences in oxidizable carbon in the distillates and in yields of concentrate are related to the country of origin of the fruit or to differences in ripeness.

The organoleptic quality of the aqueous banana distillates was judged by comparison in triangle tests of the odor of freshly prepared total distillate with the odor of banana homogenate. An odor difference was noted which was significant at the 1% level (*1*). However, preference for either the distillate or the homogenate was insignificant. Comments of panelists who had made correct judgments indicated that the odor of the distillate was less intense but fresher than the homogenate odor. A loss of fresh odor from the homogenate may have been caused by chemical or enzymatic changes occurring during the period (2 hours) between homogenate preparation and organoleptic evaluation.

Since the odor of the total distillate was similar to that of fresh bananas, the essential components of banana odor appeared to be present and detailed investigation was warranted. It is not surprising that the odor of homogenate and distillate were differentiated if one considers that distillation would necessarily cause the natural balance of odor and flavor components to be altered. Even if recovery of volatile constituents were quantitative, the partial pressures of the components would probably be modified by the nonvolatile components of the substrate. Thus, identical concentrations of volatile compounds could result in qualitatively different odors if they were dissolved in water rather than in banana homogenate.

Comparison of the yield of banana concentrates (Table II) with the quantity of oxidizable carbon in the total distillates (Table I) showed that either the distillates contained large quantities of compounds not extracted by ether, or that losses of volatile components had occurred during extraction and concentration of the ether extract. The concentrates weighed only 121 to 180 mg., while at least 791 mg. of carbon was present in the distillate obtained from 10 kg. of pulp. However, the concentrates possessed an intense characteristic banana odor.

Possible Changes in or Loss of Odor Components during Isolative Procedures. Important losses of banana odor components could result from degradative chemical reactions and from separation procedures. Although organoleptic evaluations had shown that losses of essential odor components during distillation had apparently not been great, an objective measure of the recovery of volatile components throughout the

isolative procedures was obtained by gas chromatographic analysis of vapor over the various odor isolates.

Figure 1 shows that preparation of an aqueous banana homogenate caused no major changes in the characteristic chromatogram of the vapor over crushed banana pulp. Although the relative quantity of certain components (as indicated by peak areas) appeared altered these differences are no greater than those observed between chromatograms obtained from two different bananas taken from the same batch.

Comparison of Figures 1*a* and 1*b* with Figure 1*c* showed that most volatile components had been removed. The relatively large quantity of peak C and of peak A (shoulder on positive part of air peak) in the stripped homogenate suggested that these substances may be formed in the stripped material after distillation is completed. Only traces of D and H remained in the homogenate. Peak G has been attributed to water vapor since it appears in the vapor of aqueous samples as well as in the chromatogram of distilled water vapor. Since the early portion of peak A is observed in chromatograms of room air, it probably has no analytical significance.

The distribution of odor components in the total distillate, in the aqueous layer remaining after ether extraction, and in the banana concentrate are shown in Figure 2. Comparison of the vapor over the total distillate (Figure 2*a*) with the vapor over crushed banana pulp (Figure 1*a*) and over the homogenate (Figure 1*b*) showed that the same volatile components were present in each at approximately similar concentrations. This evidence provided objective support for the organoleptic judgment that the total distillate possessed characteristic banana odor.

The effectiveness of ether extraction in removing odor components from the distillate is illustrated by Figures 2*b* and 2*c*. Vapor over the extracted distillate contained primarily ether while vapor from the banana concentrate was qualitatively similar to vapor from the original crushed bananas (Figure 1*a*). All the odor components noted in crushed banana were detected in the concentrate as well as several not previously detected. Little quantitative significance could be attached to relative peak areas since Figure 1*a* was obtained from vapor over banana pulp, and Figure 2*c* from vapor over concentrate. Two small peaks appear between peaks J and K in the chromatogram of concentrate vapor (Figure 2*c*) which are not present in the distillate or banana vapor chromatograms. To determine whether these peaks were caused by the production of artifacts during concentration, the concentrate was diluted with water to approximately that of the distillate. The chromatogram of the vapor over the

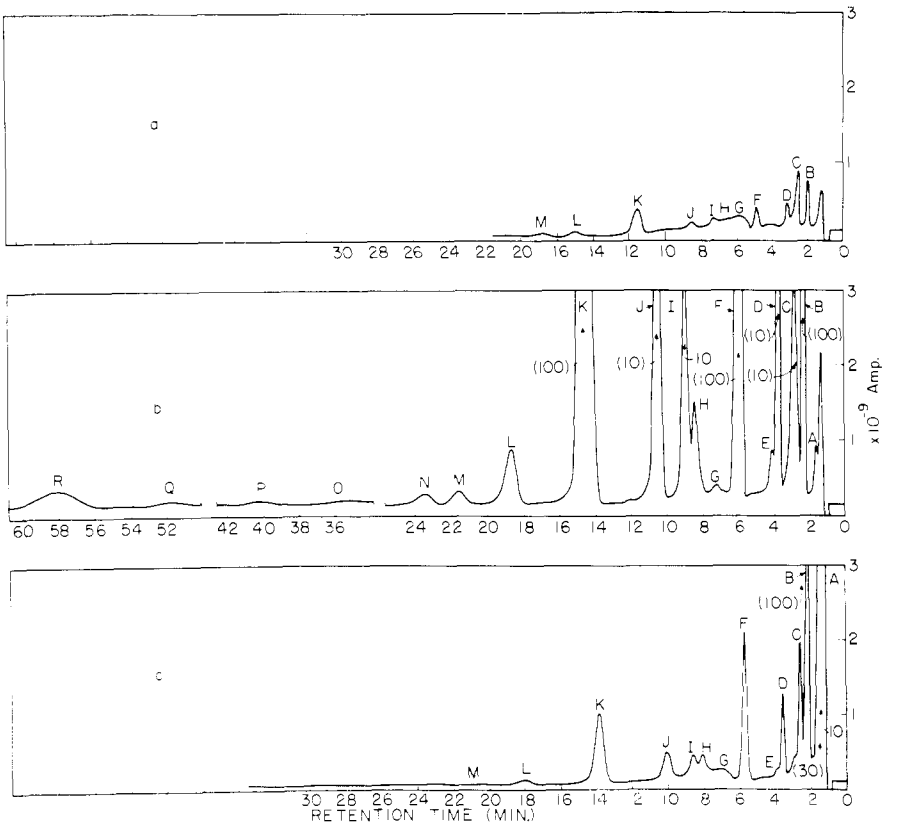

Figure 3. Chromatograms showing distribution of volatile compounds in preparation of concentrate

(*a*) Ice-water trap. (*b*) Dry ice–ethanol trap. (*c*) Liquid N_2 trap

dilute solution showed a reduction in the areas of peaks B, C, D, and M relative to peak K and almost complete disappearance of the peaks which were possible artifacts. These differences in relative concentration in the vapor over aqueous and nonaqueous samples were probably due to association of some of the components with water. There is no direct evidence that artifacts were produced in significant quantities by the preparative procedures.

The distribution of odor components in the cold traps is indicated by Figure 3. Although the dry ice–ethanol traps appeared (Figure 3*b*) to contain the highest concentration, quantitative comparisons cannot be made directly from the chromatograms. Not only does the concentration of components in any trap differ, but also the dry ice–ethanol and liquid nitrogen traps would contain larger quantities of substances of greater volatility than would the ice-water trap. Thus chromatograms in Figures 3*b* and 3*c* reflect the presence of components having high vapor pressures. The condensate collected in the ice-water traps may have contained a higher concentration of less volatile substances which were not detected by the analytical method employed. The ice-water trap contained 10 to 20 times the combined volume of the other four traps. Thus, even though concentrations appear lower in the ice-water trap, it may contain a greater amount of the volatile components.

Since some of the components shown in Figure 3*b* had not been observed in the chromatogram of banana pulp (Figure 1*a*) or of homogenate (Figure 1*b*), the possibility existed that they were artifacts resulting from processing procedure, or that they had not been present in sufficient quantity to be detected. The fact that a vapor sample taken from a whole crushed banana (126.8 grams) rather than a 50-gram portion contained all the components shown in Figure 3*b* indicated that no artifacts were present in the condensate collected in the dry ice–ethanol traps.

On the basis of the chromatograms shown in Figures 1, 2, and 3, and on the basis of odor, the preparation of banana odor concentrates was considered to be successful. Undue losses of essential odor components had apparently not occurred.

Part II. Separation and Identification of Odor Components

Positive identification of the components detected in the vapor over banana concentrates required that each be isolated in as large a quantity as possible and be subjected to direct investigation of its purity and chemical nature.

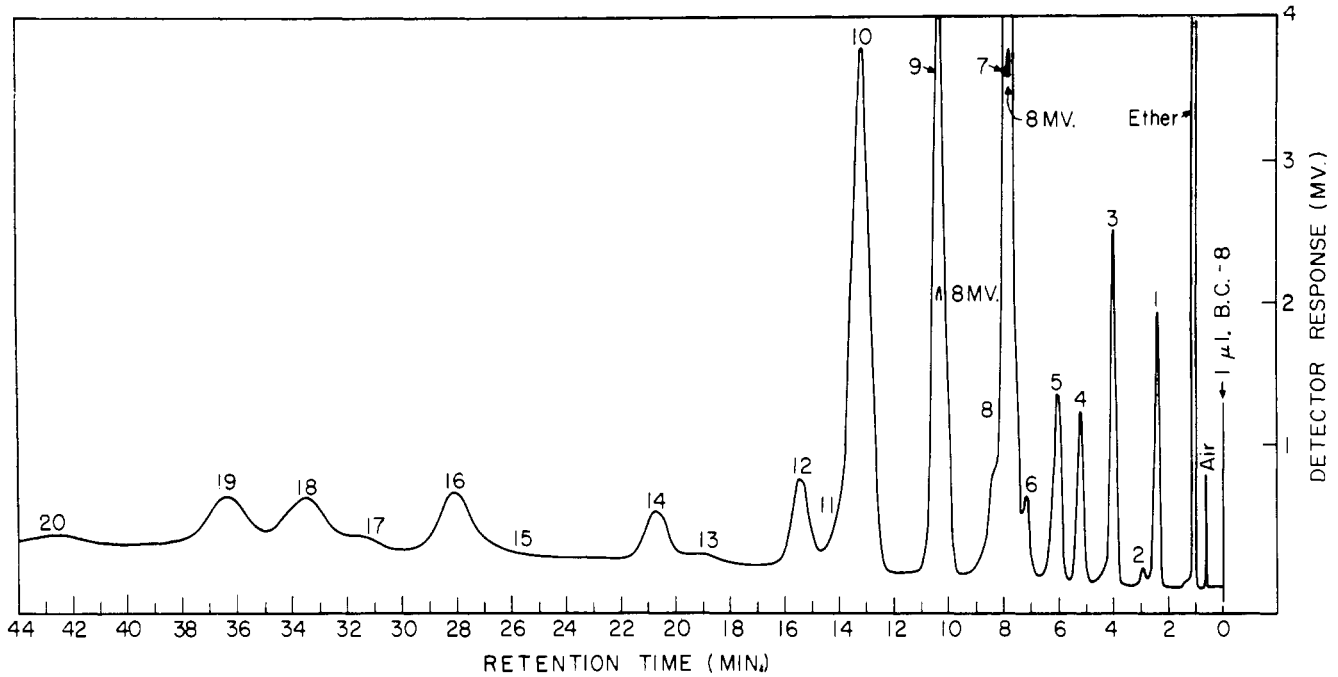

Figure 4. Analytical gas chromatogram of banana concentrate

Sufficient concentrate (121 to 180 mg.) was obtained from each 10-kg. batch of banana pulp to allow its separation by gas chromatography and the collection of fractions for direct investigation.

Experimental Procedure

Separation. The GC apparatus was constructed in this laboratory. The detector was a thermal conductivity cell employing a matched pair of 100,000-ohm thermistors (Type A-177, Victory Engineering Corp., Union, N. J.). It was operated at 12-ma. bridge current and maintained at 170° C.

Analytical chromatograms of 1-μl. samples (Figure 4) were obtained on a 2-meter, 4-mm. i.d., glass column packed with 20% Ucon 50 HB-2000 oil on 30- to 60-mesh untreated firebrick (Coast Engineering Co.). It was operated at 12 p.s.i.g. pressure, and a helium flow of 50 ml. per minute.

Preparative separations of 50-μl. samples (Figure 5) were obtained at 85° C. on a 2-meter, 8-mm. i.d., column packed with 20% Ucon 50 HB-2000 oil on 48- to 60-mesh Johns-Manville Chromosorb. It was operated at 22 p.s.i.g. and a helium flow of 200 ml. per minute.

Rechromatography of certain fractions collected from the preparative column was carried out at 85° C. on a 2-meter, 4-mm. i.d., glass column containing 20% diethyleneglycol succinate (DEGS) on 30- to 60-mesh untreated firebrick, operated at 7.5 p.s.i.g. and a flow of 50 ml. per minute.

Homogeneity of all fractions collected from the preparative column was evaluated by separation of 200- to 400-μl. samples of their vapor, on a 1% Ucon 50 HB-2000 column under the conditions described in Part I.

Retention volumes relative to *i*-amyl acetate were determined on the 20% Ucon, 20% DEGS, and 1% Ucon columns for the following series of reference compounds: normal aliphatic alcohols (C_1 to C_6) and their acetates and *n*-butyrates (methyl through pentyl); *i*-alcohols (C_3, C_4, and C_5) and their acetates; normal aliphatic propionates (methyl, ethyl, *n*-propyl, *n*-butyl, *n*-amyl, *i*-butyl, and *i*-amyl); 2-alkanones (C_3 to C_8); normal aliphatic aldehydes (C_1 to C_8) and *trans*-2-hexenal. Values determined on the 20% Ucon and DEGS columns were reproducible to better than 2%. Values determined at different times on the 1% Ucon column agree within 8%. This relatively poor reproducibility of retention times on the 1% Ucon column was due to difficulty in controlling temperatures close to room temperature.

Fractions were trapped at the detector outlet in glass U-tubes chilled in liquid nitrogen. The traps were packed with sand for collection of the dilute fractions following peak 12 (Figure 5). Trapping efficiency was approximately 98% with the sand-packed traps.

Contents of traps were transferred on a vacuum manifold to 4 × 80 mm. glass tubes which were filled with nitrogen, sealed, and stored in a freezer until infrared spectra could be determined.

Infrared spectra were determined by means of a Beckman IR-5 spectrophotometer equipped with a 5× KBr lens-type beam condenser. Fractions were transferred to a type D sodium chloride cavity cell (Connecticut Instrument Co., Wilton, Conn.) of nominal path length 0.05 mm. When quantities permitted, pure liquid as well as carbon tetrachloride solution spectra were obtained. Infrared spectra of selected reference compounds were obtained from samples which had been purified by chromatography on the preparative column.

Results and Discussion

Separation of Odor Components. A typical analytical chromatogram obtained from a 1-μl. sample is shown in Figure 4. All the banana concentrates gave qualitatively the same chromatogram. No significant change in composition was noted chromatographically during a storage period of 6 months, although organoleptically the development of a slight, haylike, off-odor was noted. It thus appeared that the components shown on the chromatogram could be collected and investigated with a minimum of loss due to instability of the concentrates.

A total of 15 fractions was collected from two 50-μl. samples at the positions indicated in the preparative chromatogram shown in Figure 5. Resolution of components was good in spite of the large sample separated. Every effort was made to obtain pure fractions of components which had not been completely resolved by trapping portions of peaks. Since the analytical chromatogram (Figure 4) and infrared spectra indicated that Fraction 7 contained more than one component, this fraction was rechromatographed on the DEGS column and three well-resolved fractions were collected (Figure 6). Peak 7 was separated into two large fractions,

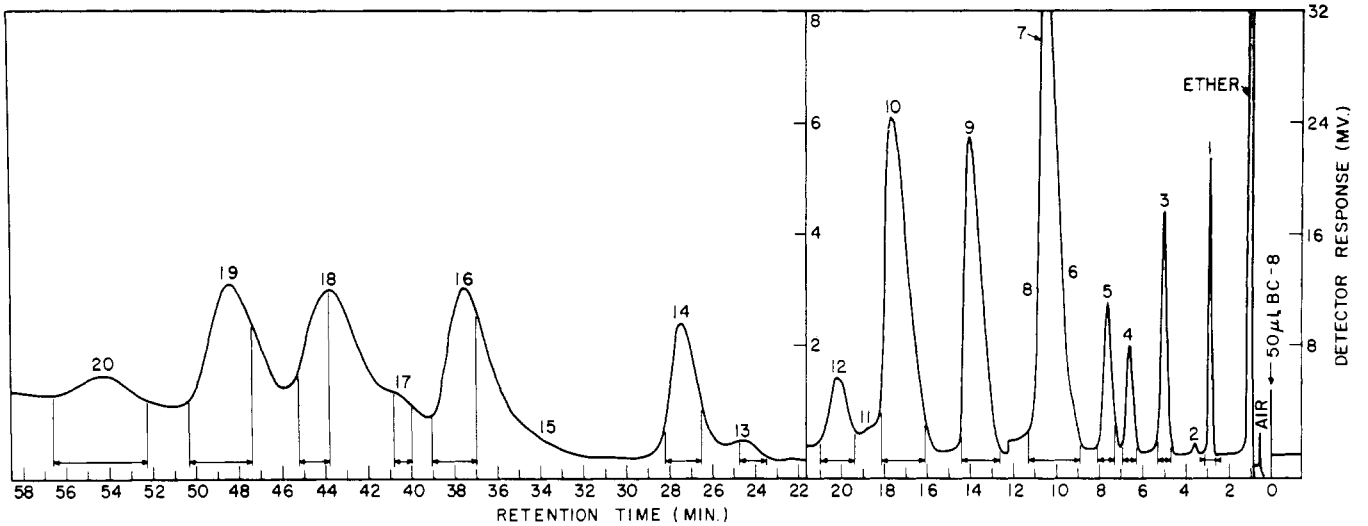

Figure 5. Preparative gas chromatogram of banana concentrate

Arrows indicate fractions collected for infrared analysis

Table III. Identification of Gas Chromatography Fractions

Fraction (In Figures 4, 5, 6)	Fraction (In Figures 1, 2, 3)	Identification
1	B	Ethyl acetate[a,b]
...	C	Ethanol[a,b]
2	...	Unknown
3	D	2-Pentanone
...	...	*n*-Propanol[b]
4	F	*i*-Butyl acetate
5	H	*i*-Butanol
6	I	*n*-Butyl acetate[b]
7-A	J	An acetate
7-B	Between J and K	2-Pentanol[b]
8	Between J and K	*n*-Butanol
9	K	*i*-Amyl acetate
10	N	*i*-Amyl alcohol
11	M	*n*-Amyl acetate[b]
12	L	*trans*-2-Hexenal
13	...	Unsaturated ketone
14	P	Ester
15	...	*n*-Hexyl acetate[b]
16	R	*i*-Amyl butyrate
17	...	Unsaturated alcohol
18	S	Mixture of an alcohol and acetate (approximately equal proportions)
19	...	Unknown
20	...	Unknown

[a] Infrared spectrum consistent with a mixture of ethanol and ethyl acetate.
[b] Identification not rigorous.

Table IV. Retention Volumes, Relative to *i*-Amyl Acetate, of Reference Compounds and of Fractions Tentatively Identified on the Basis of Gas Chromatography Data

Fraction and Reference Compound	Relative Retention Volume: 20% Ucon (85° C.)	20% DEGS (85° C.)	1% Ucon (32–36° C.)
3 (minor component)	0.384	...	0.308
n-Propanol	0.404	...	0.293
6	0.676	0.705	0.597
n-Butyl acetate	0.683	0.767	0.580
11	1.38	...	...
M	...	...	1.53
n-Amyl acetate	1.34	...	1.55
15[a]	2.62	...	4.04
n-Hexyl acetate	2.64	...	4.00

[a] Infrared spectrum indicated presence of an acetate (strong bands at 5.75 μ and 8.03 μ), but solution was too dilute for positive identification.

both of which were resolved from peak 8. However, peak 6 was not resolved from the first major peak (7-A) on the DEGS column.

To determine what contribution the components of the fractions made to banana odor, all of the peaks from a 50-μl. sample of concentrate were collected in a single sand-packed trap. Informal organoleptic evaluation of the trap's contents indicated that it contained most of the basic notes of banana odor. The substances which provide the full-bodied, mellow odor in bananas were not present.

In an attempt to determine whether higher boiling compounds could be eluted from the 20% Ucon column, the column temperature was increased. Between 190° and 230° C., a series of odors resembling cloves, cinnamon, and mint were eluted. Samples were not collected for analysis due to contamination with column liquid. However, definite indication of the presence of high boiling components which might contribute significantly to banana odor was obtained. The nature of these components is under investigation.

Identification of Odor Components. Evidence of the identity of the components of the fractions isolated was obtained from the infrared spectra of the components present in relatively large quantity (greater than 0.5 μl.) and from their relative retention volumes. The purity of each fraction was evaluated by its separation on the 1% Ucon column. The results obtained are summarized in Table III.

On the basis of the identity of their infrared spectra with the spectra of authentic reference compounds, and of agreement of relative retention volumes with the reference compounds, the following substances were proved to be present in the banana concentrates: 2-pentanone, *i*-butyl acetate, *i*-butanol, *n*-butanol, *i*-amyl acetate (*6, 7, 10, 11*), *i*-amyl alcohol (*6*), *trans*-2-hexenal (*6*), and *i*-amyl butyrate. *i*-Butyl acetate, *i*-butanol, and *i*-amyl butyrate had not previously been shown to be components of bananas.

The similarity of their infrared spectra and agreement of retention data with those of authentic reference compounds indicated that ethanol (*6, 11*), ethyl acetate (*6*), and 2-pentanol were present in bananas. The presence of 2-pentanol had not been reported previously in fresh banana.

Tentative evidence based on agreement of retention data with those of

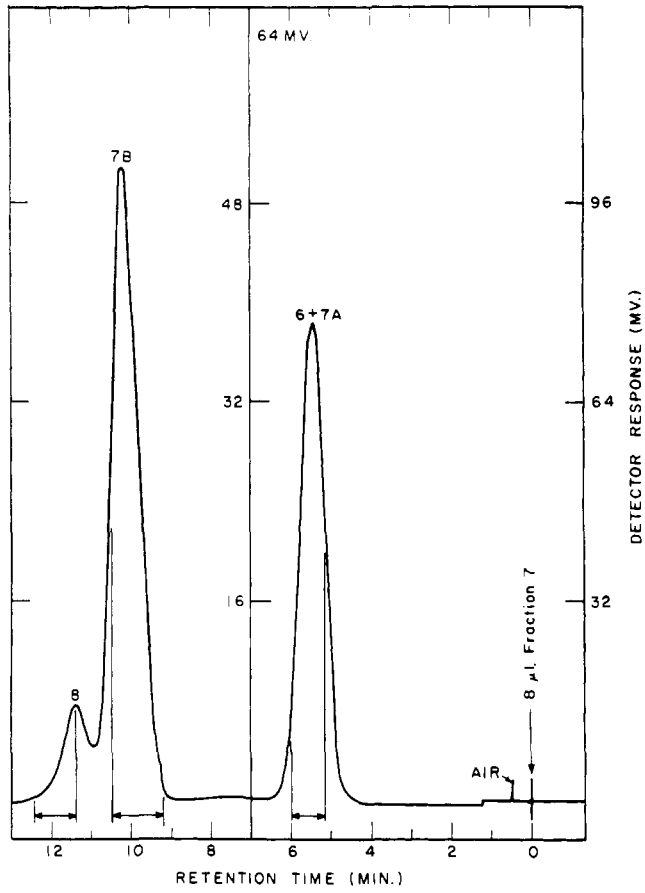

Figure 6. Fraction 7 chromatographed on 20% DEGS column

reference compounds (Table IV) suggested the presence of *n*-propanol, *n*-amyl acetate, *n*-hexyl acetate, and methyl acetate (*6*). Only the latter compound had been reported previously.

A number of fractions could be only generally characterized by their spectra as containing an acetate, an unsaturated ketone, an ester, an unsaturated alcohol, and a mixture of an alcohol and an acetate. The components of four fractions were unknown.

The compounds and fractions described are only partially responsible for the typical odor of bananas. Further investigations are necessary to elucidate their individual contributions to banana odor and their origin in the fruit.

Acknowledgment

The authors express their appreciation to United Fruit Co. for the grant-in-aid which supported this research, and to Jane Schachter, S. Chang, and A. Pyne for their assistance. The advice and assistance of Amidee Gauthier in the construction of the GC apparatus is gratefully acknowledged.

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Received for review January 5, 1962. Accepted April 16, 1962. Contribution No. 437 Department of Nutrition, Food Science and Technology, Massachusetts Institute of Technology, Cambridge, Mass. From the thesis submitted by Phillip Issenberg in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the Massachusetts Institute of Technology, September 1961. Division of Agricultural and Food Chemistry, 140th Meeting, ACS, Chicago, September 1961.

FOODSTUFFS ANALYSIS

Nonvolatile Acids of Blueberries

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PRIOR TO THE DEVELOPMENT of chromatographic methods of analysis, qualitative and quantitative determination of acids present in small proportions in biological materials was a time-consuming and exacting operation. Minor acids were, therefore, studied only in cases of special interest. Foods were usually analyzed for two or three major acids (*9*), such as citric, malic, tartaric, or oxalic (*11*), and, of course, ascorbic acid (*17*).

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There is both academic interest and practical importance in the study of the individual acids of fruits. Acids are known to participate actively in the metabolism of the fruit (*16, 7*). They also definitively affect the flavor or off-flavor (*15*) of fruit and fruit products, and they may be involved in discolorations (*10, 12*).

A technique originally developed by Busch *et al.* (*4*) for the separation and determination of the Krebs cycle acids and further improved by Palmer (*14*) and Hulme and Wooltorton (*8*) appeared suitable for the study of the nonvolatile acids of blueberries, a fruit of rapidly increasing economic importance (*2*). Previously, Nelson (*13*) identified citric and malic acids in blueberries, and Kohman (*11*) determined the oxalic acid content of this fruit. Recently, Dewey and coworkers (*3, 19, 20*) studied, among other characteristics, the change of titratable acidity of blueberries during ripening.

The purpose of the present study has been to detect and quantitatively estimate as many as possible of the non-