Gas Chromatographic Method for Comparative Analysis of Fruit Flavors

JOHN W. RHOADES and JOHN D. MILLAR

Southwest Research Institute, San Antonio, Tex.

A rapid method for comparing the concentration of volatiles in fruits has been developed. The fruit is stripped with an inert gas at reduced pressure, and the volatiles in the condensate are concentrated further by microextraction before injection into a gas chromatograph. The time required for preparation of the sample for injection is about 30 minutes. High temperatures and the evaporation of large volumes of solvent are not required, so that thermal degradation and loss of sample through volatilization are avoided. Compounds boiling above 220° C. can be measured together with highly volatile components by using a temperature-programmed gas chromatograph equipped with a flame ionization detector. The minimum level of detectability for volatile components which can be determined by this procedure is about 0.005 p.p.m.

MANY investigators in the fruit flavor field have used gas-liquid chromatography (GLC) as an analytical tool. However, in most cases conventional methods of distillation and extraction have been employed to obtain a concentrate for GLC analysis. Large amounts of raw fruits and lengthy procedures have been required. Moreover, the elevated temperatures often used in distillations and the evaporation of large quantities of solvent, in conjunction with the prolonged period of handling during sample preparation, may give rise to chemical changes or losses. To overcome these disadvantages a simple, more rapid preparation technique, designed to take advantage of the high resolution of GLC combined with the sensitivity of ionization detection, has been developed.

Some previous workers (*1, 2, 5–9*) have tried to circumvent the above problems by restricting their analytical examination to the head space vapors. This method is rapid and sensitive to low boiling materials but is less applicable to components boiling above 150° C. In the case of fruits, many of these higher boiling compounds are believed to be important to the flavor.

The work reported herein gives a method for the quantitative comparison of the higher boiling constituents of fruit in addition to the more volatile materials. The method should be useful in quality control and basic flavor studies of other food products. Its principal advantages are:

Small fruit sample (approximately 50 grams).
Moderate temperature (40° C.).
Short preparation time (30 minutes).
No solvent evaporation.
Choice of atmosphere in contact with fruit.

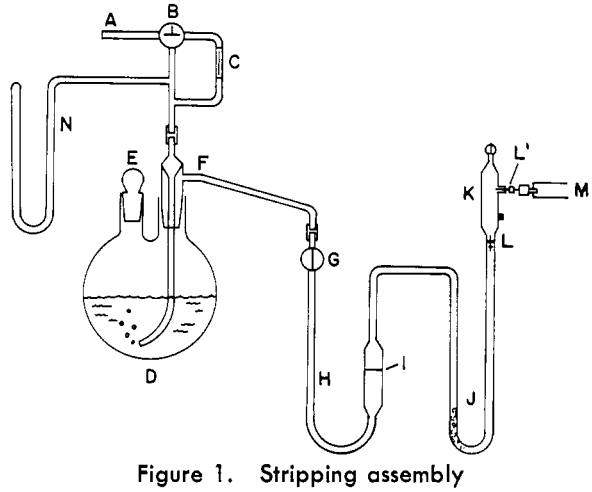


Figure 1. Stripping assembly

A. Entry for sweep gas
B. Stopcock, 3-way
C. Capillary restriction, approx. 3 ml. per min. at atm. pressure
D. Flask, 300-ml. 2-necked, 24/40
E. Gloss stopper
F. Head with gas entry and exit for gas and volatiles
G. Stopcock
H. Condenser, ice water–cooled
I. Calibration mark
J. Condenser, liquid nitrogen–cooled, containing glass wool
K. Condensate holder-extractor
L. Metal connectors, Burrell (greaseless valves)
M. Vacuum tubing to bleed control and vacuum pump
N. Closed-end Hg manometer

Reagents

Dow-Corning Antifoam AF emulsion was used in all stripping operations. Interfering volatile materials in the antifoam agent were removed by stripping an aqueous suspension of 2 grams in 100 ml. of water in the manner prescribed for stripping a fruit sample, continuing stripping in this instance until 80% of the water had been removed. The residue in the stripping pot was retained for use.

Distillation of diisopropyl ether to remove major impurities is desirable.

Equipment

The chromatographic work was performed with an F&M Model 1609 flame ionization attachment on an F&M Model 500 gas chromatograph. The column was an 8-foot by 1/4-inch length of copper tubing packed with 3% Carbowax 20M on Chromosorb P (60- to 80-mesh). Although this column was satisfactory for the evaluation of the sample preparation technique, a stainless steel or glass column is recommended for most food flavor work. Water was added to the nitrogen carrier gas by

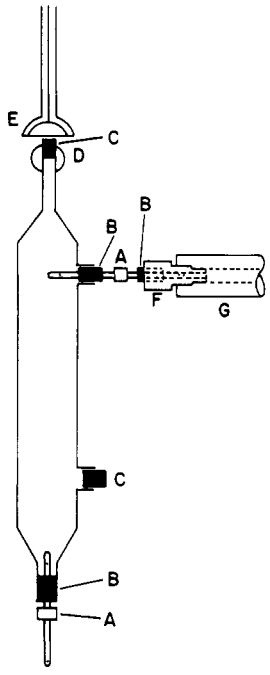
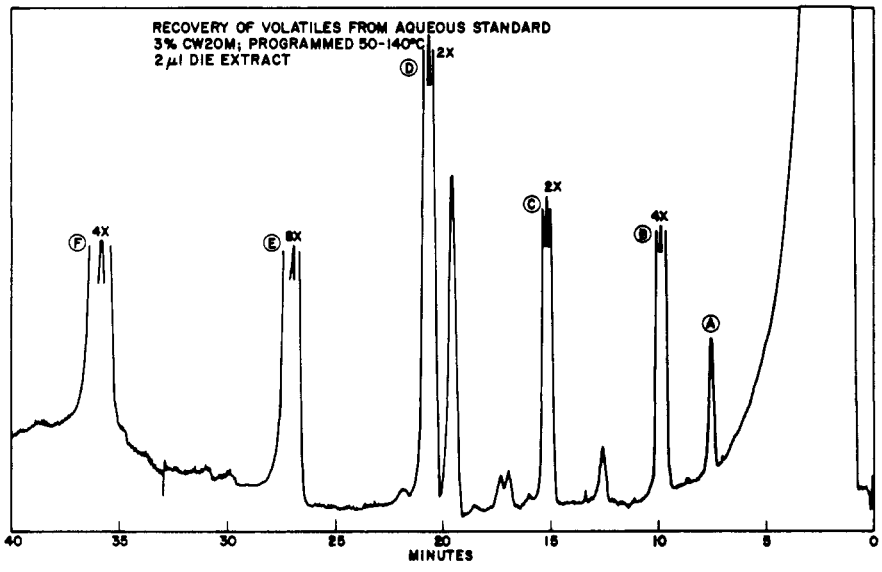


Figure 2. Condensate holder-extractor

A. Metal connectors, Burrell
B. Silicone rubber plug, 1-hole, Burrell
C. Silicone rubber plug, solid, Burrell
D. Semiball joint, 12/5
E. Semiball joint, 12/2
F. Metal socket, Burrell
G. Vacuum hose



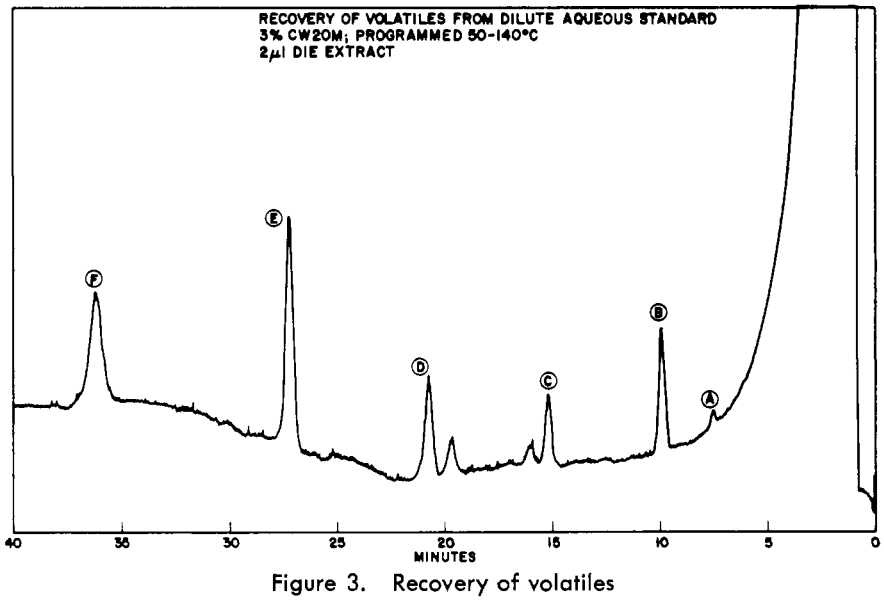


Figure 3. Recovery of volatiles

A. Hexanol
B. Isopentyl acetate
C. Ethyl hexanoate
D. *cis*-3-Hexen-1-ol
E. 2-Ethyl-1-hexanol
F. α-Terpineol

the method of Knight (*4*). The carrier gas flow through the column as measured at the exit was 72 ml. per minute. The starting column temperature was 50° C. and was programmed to 140° C. at a rate of 3° per minute.

Procedure

First strip the volatiles and about 10% of the water, at reduced pressure, from the fruit specimen. Collect the stripped vapor in specially constructed dual traps, so that a total collection is accomplished. Then transfer the entire condensate to an evacuated condensate holder by a procedure which precludes the loss of volatile materials. Perform a microextraction by injection of 0.25 ml. of solvent through a silicone septum placed in the side of the condensate holder. After shaking the condensate holder, inject salt water, forcing the solvent layer into a capillary tube to facilitate removal by a microsyringe for injection into the chromatographic instrument. If desired, a head space or aqueous sample may be taken and injected into the instrument prior to the extraction procedure.

The stripping assembly and the condensate holder-extractor are shown in Figures 1 and 2.

All of the glass connecting tubing is 6 mm. in diameter. The capillary, *C*, is of such diameter and length that the atmospheric exit flow is approximately 3 ml. per minute when the gas pressure at the opposite end is 10 p.s.i.g. This pressure is for determining capillary flow only.

Procedure for Stripping Specimen, Collecting Condensate, and Transferring Condensate to Holder-Extractor. Assemble the apparatus as shown in Figure 1, after thorough cleaning and equipping with new silicone rubber seals. Set the sweep gas flow at approximately 200 ml. per minute by adjusting the tank regulator (approximately 1 p.s.i.g.). Sweep the air from the apparatus by proper positioning of stopcocks *B* and *G* and the metal connectors at *L* and *L'*, so that the flow of gas is *A*, *B*, *F*, *D*, *F*, *G*, *H*, *J*, *L*, *L'*, *M*. Shut off the sweep gas at *B* and evacuate the system. Move the metal connector at *L'* to isolate the rest of the system from the vacuum pump. Turn stopcock *B* so that sweep gas fills the system from *A* to *L'*. Place an ice water bath around *H* and a liquid nitrogen bath around *J*. Close stopcock *G*. Turn stopcock *B* so that flow is through *A*, *B*, *C*, to *D*. Remove stopper *E* and introduce 50 grams of macerated fruit, 50 ml. of distilled water, and a few drops of antifoam agent. Replace stopper *E* and open vacuum bleed so that only a slight vacuum is being pulled on tubing *M*. Reposition the connector at *L'* and stopcock *G* so that the flow of gas is *A*, *B*, *C*, *F*, *D*, *F*, *G*, *H*, *J*, *L*, *L'*, *M*. Gradually close bleed control valve

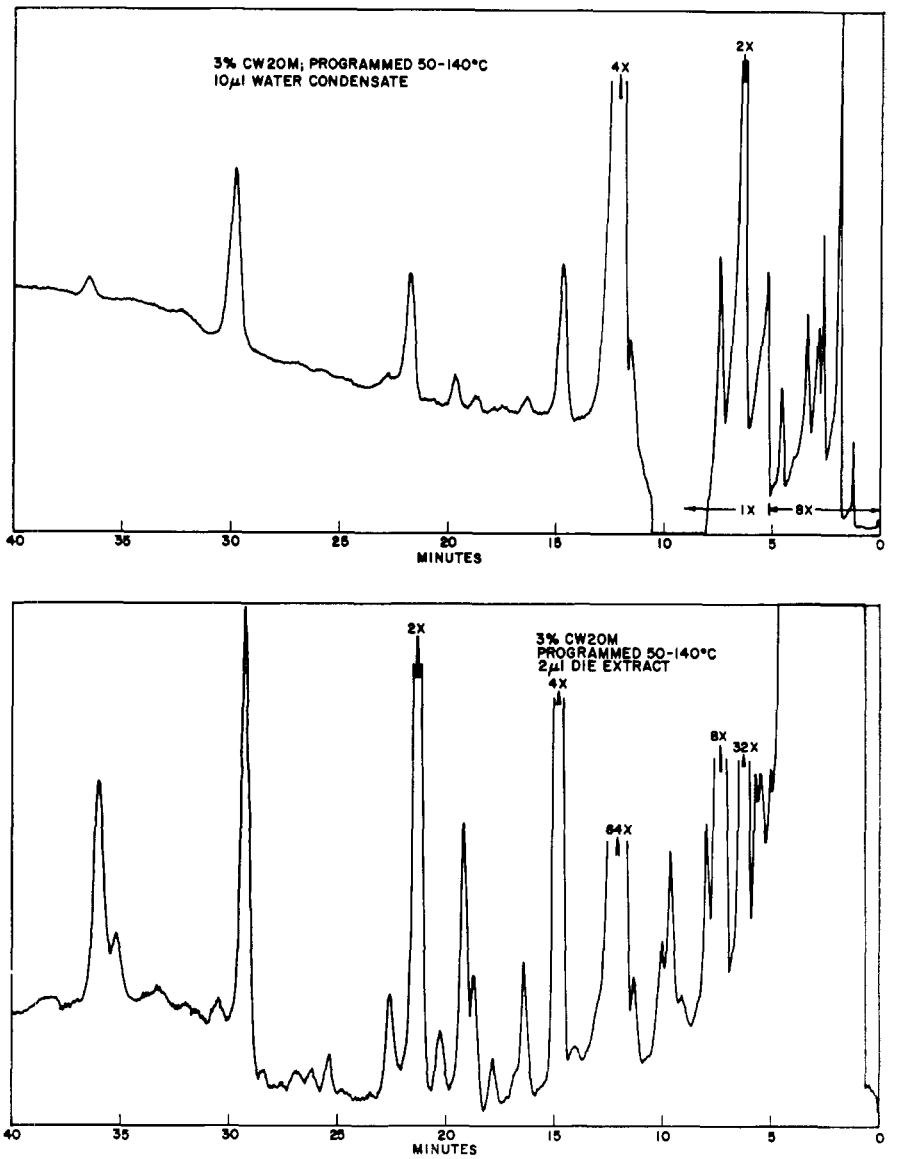


Figure 4. Chromatograms of strawberries

until the system pressure is approximately 40 mm. of Hg as indicated at *N*. Supply heat to *D* by means of an electric heating mantle, maintaining a gentle boil within the flask. Close attention is often required to maintain a careful balance between system pressure and heat applied during the early minutes of a run while degassing is occurring. Once a true boil is reached, equilibrium is easily maintained.

Allow the stripping to proceed until 10 ml. of condensate have been collected in *H*, as indicated by the calibration mark, *I* (approximately 20 minutes). Isolate the system from the vacuum pump by moving the connector at *L'*, and immediately close stopcock *G*. Turn stopcock *B* so that the sweep gas enters *D* by route *A*, *B*, *F*, *D*. When atmospheric pressure is reached in *D*, turn off sweep gas entirely by repositioning *B*. Remove the total condensate collector, *H-J*, and the condensate holder-extractor, *K*, as a single unit by disconnecting at the rubber tubing connection slightly above *G* and the vacuum tubing, *M*. Warm *J* by submerging briefly in tap water. Invert assembly *H-J-K*, and slowly open stopcock *G* to allow air or inert gas to push the condensate slowly from *H* through *J* into *K*. When atmospheric pressure is reached in *K*, isolate *K* by moving the connector at *L*. Disconnect *K* from *H-J* at *L*. The condensate from the stripping operation is now contained in *K*.

Extraction. Open the sealed holder-extractor by removing the solid silicone plug, *C*, in the semiball joint, *D*. Add 4 grams of sodium chloride to the condensate. Reseal the holder-extractor by replacing the solid plug and shake gently until salt no longer appears to be dissolving. Inject 0.25 ml. of diisopropyl ether through the solid plug, *C*, at the side of the holder-extractor when in an upright position. Shake vigorously for 30 to 60 seconds and clamp the holder-extractor with the semiball joint, *D*, pointing upward. Remove the solid plug, *C*, at *D*, and attach *E*, an outer semiball joint with a 2-mm. capillary, with a semiball joint clamp. Force the solvent phase upward into the capillary in *E* by injecting saturated aqueous sodium chloride solution with a hypodermic syringe through plug *C* at the side of the holder-extractor. As the solvent phase passes by *B*, tilt the holder-extractor slightly toward *B* to prevent trapping small amounts of the solvent phase. When the solvent phase reaches to within 1 to 2 cm. of the open end of the capillary in *E*, stop the injection of salt solution. Remove the desired quantity of solvent phase for injection into the chromatograph by inserting the needle of a microsyringe into the open end of the capillary.

In some instances it may be desirable

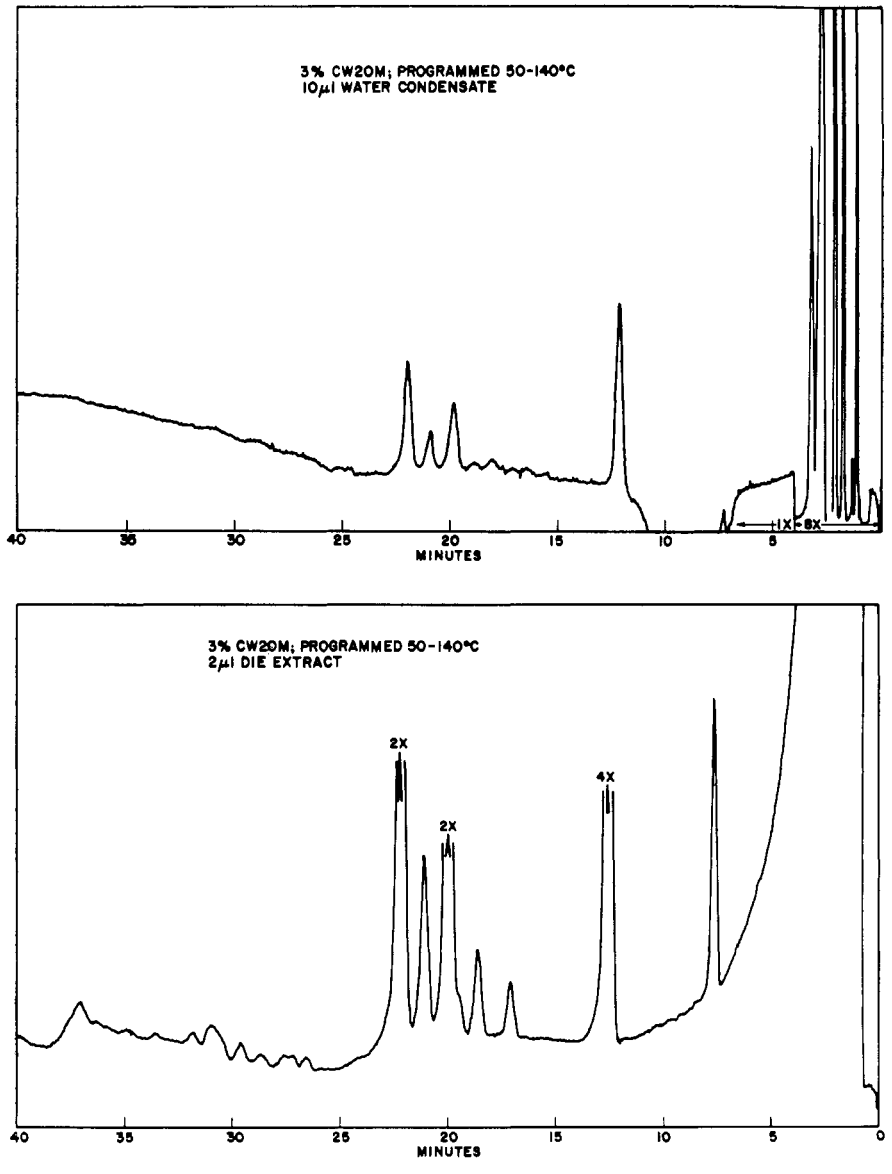


Figure 5. Chromatograms of peaches

to perform the extraction without exposure of the condensate to the atmosphere. This may be done by placing 4 grams of sodium chloride in the condensate holder-extractor at the time the apparatus is assembled. To prevent the salt from plugging the hole in the connector, a piece of Teflon tubing larger than the connector and long enough to extend upwards above the bed of salt should be placed in position before the salt is added. Injection of condensate saturated with salt produces poor chromatograms and is not recommended.

Experimental and Results

An aqueous solution containing known amounts of six compounds previously reported to be in ripe strawberries was prepared for use in recovery and detection experiments. These compounds and their initial concentrations (parts per million) in solution were:

Hexanal	0.15
Isopentyl acetate	0.25
Ethyl hexanoate	0.25
cis-3-Hexen-1-ol	0.40
2-Ethyl-1-hexanol	0.40
α-Terpineol	0.40

A 100-ml. portion of the aqueous standard solution was stripped and extracted by the procedure given. A 10-ml. portion of the aqueous standard solution, plus 90 ml. of distilled water, was treated likewise. Injections of 2 μl. of each of the extracts obtained produced the chromatograms shown in Figure 3. The ratios of heights of corresponding peaks on the two chromatograms were: hexanal, 0.089; isopentyl acetate, 0.130; ethyl hexanoate, 0.112; *cis*-3-hexen-1-ol, 0.104; 2-ethyl-1-hexanol, 0.100; and α-terpineol, 0.103. These ratios are in good agreement with the theoretical ratio of 0.1, indicating that the relationship between peak height and initial concentration is reasonably linear. On the basis of these experiments, the calculated detection limit is around 0.005 p.p.m. Recovery of these compounds was 80% or better.

A sample of 50 grams each of fresh strawberries, peaches, and apricots was stripped, and the condensate was transferred to the holder-extractor. A chromatogram of 10 μl. of each aqueous condensate was obtained. The aqueous condensate of each fruit was extracted and the chromatogram of 2 μl. of the extract was obtained (Figures 4, 5, and 6).

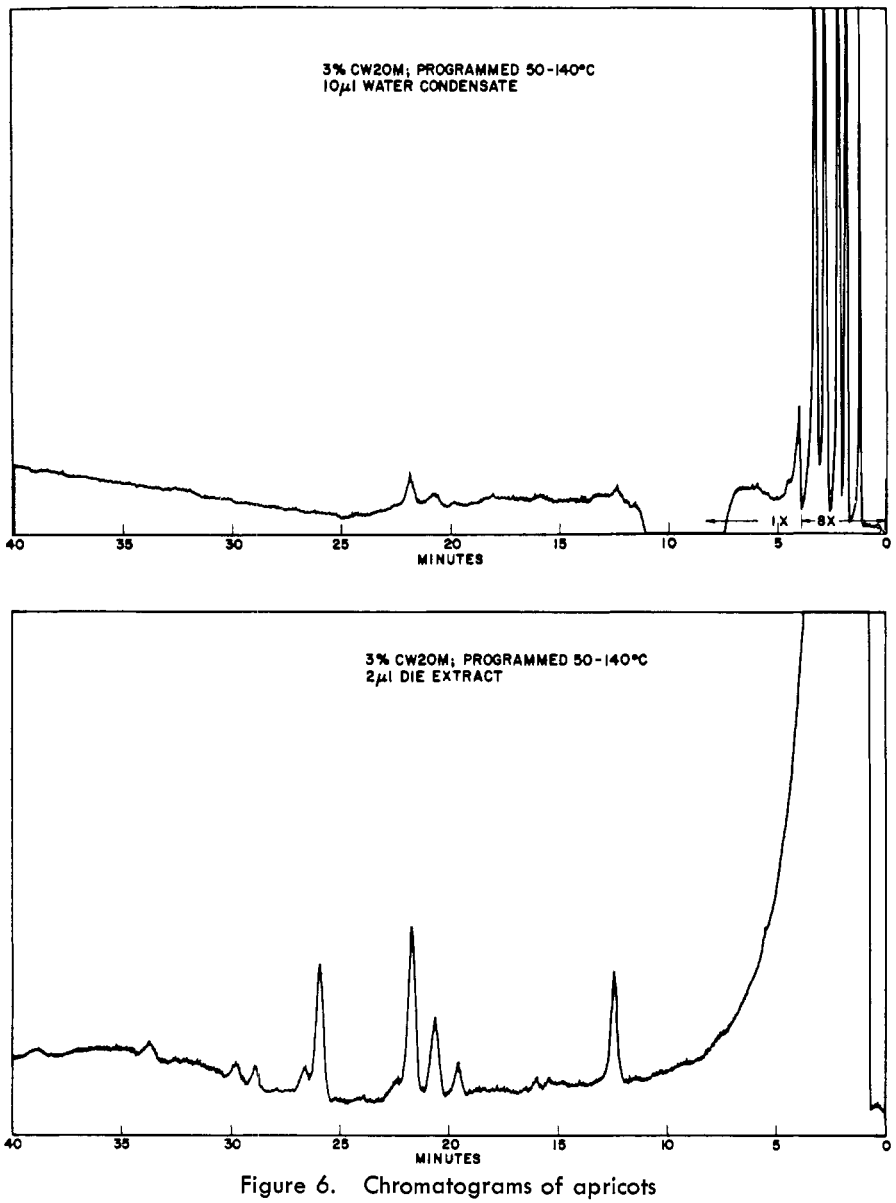


Figure 6. Chromatograms of apricots

Discussion

Although it is possible to inject the aqueous condensate, injection of the extract of the aqueous condensate in order to obtain enhanced instrument response is desirable for most fruits, as illustrated by chromatograms of fruits studied.

Hexane, diethyl ether, and diisopropyl ether were investigated as extraction solvents, using the aqueous standard solution. Hexane was eliminated because of inadequate solvent power. Diethyl ether and diisopropyl ether gave comparable recoveries of the compounds from the aqueous standard solution. Diisopropyl ether was chosen as the solvent for use because of its lower water solubility and lower volatility. Minor impurities in the solvent do not present a problem, since concentration by evaporation is not employed.

A rapidly disappearing but characteristic odor of the fruit was noted in all cases when a small amount of solvent extract was vaporized.

Corse and Dimick (*3*) have reported that the total concentration of some 35 strawberry flavor volatiles was in the range of 1 to 7 p.p.m. The results of the experiment performed with the standard aqueous solution show a lower detection limit for this method, of the order of 0.005 p.p.m. This gives an analytical capability within the concentration range likely to be encountered in fresh fruits. Since the amount of an unknown material which would be carried over into the condensate and its partition coefficient between water and diisopropyl ether would not be known, it would not be possible to determine th actual concentration in the originale fruit. However, the linear relationship between initial concentrations in the standard solutions and the resulting peak heights from the diisopropyl ether injections indicate that the relative amounts of the same material in different samples can readily be determined. This is the principal requirement to be satisfied when one is interested in determining the magnitude of variations in the volatile constituents of foods. Upon subsequent identification, the absolute amount could then be determined from the experimentally obtained relationship between initial concentration and the resulting peak height.

Preliminary experiments performed by using a reduced pressure version of the method reported by Rhoades (*7*) in which the highly volatile materials were removed from strawberries, did not destroy the fresh aroma of the residue. Similar findings were reported by Corse and Dimick (*3*), who found that the characteristic fresh fruit aroma of strawberries was present in the fraction boiling above 85° C. Therefore, the work reported here was restricted to the examination of higher boiling constituents of the condensates and extracts of condensates. However, lower boiling constituents could be determined by substituting suitable columns and solvents.

Acknowledgment

The authors are grateful to the U. S. Army Quartermaster Research and Engineering Center for sponsoring this work under Contract No. DA19-129-AMC-68(N) and for permission to publish this paper.

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Received for review March 16, 1964. Accepted June 22, 1964. 19th Southwest Regional Meeting, ACS, Houston, Tex., December 1963.