CONTROLLED ATMOSPHERE APPLE STORAGE VOLATILES-- EVALUATION BY GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY

Pio Angelini

1963

This is to certify that the

thesis entitled

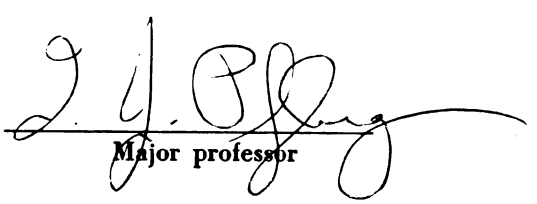
Controlled Atmosphere Apple Storage Volatiles -
Evaluation by Gas Chromatography
and Mass Spectrometry

presented by

Pio Angelini

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Food Science



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Date May 17, 1963

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ABSTRACT

CONTROLLED ATMOSPHERE APPLE STORAGE VOLATILES -
EVALUATION BY GAS CHROMATOGRAPHY
AND MASS SPECTROMETRY

by Pio Angelini

Chemical compounds that diffuse out of apple fruits during storage under controlled atmosphere conditions, described generally as volatiles, were studied using the techniques of low-temperature high-vacuum distillation, gas chromatography, and mass spectrometry. Identification of apple storage volatiles was attempted from storage atmospheres, molecular sieves, cold trap condensates, and activated carbon extracts.

Gas chromatography employing a hydrogen flame ionization detector proved to be a simple, direct, and rapid method for ethylene concentration studies in controlled atmosphere apple storage rooms. The type of detector used was an important feature in this procedure; insensitivity to normal amounts of air gases, carbon dioxide, and water was required plus a high sensitivity to organic compounds. Ethylene concentrations were followed in eleven controlled atmosphere apple storages. In all cases the ethylene concentration increased after sealing the storages until it reached a maximum and then it gradually leveled off at this maximum concentration. The ethylene concentrations then remained constant, with few slight fluctuations until the storage rooms were opened. The air changes of conventional controlled atmosphere storages are directly proportional to the respiration rate of the fruit. From this knowledge and the equilibrium ethylene concentration found in the storages, it was deducted that ethylene production by apples is proportional to respiration rate of the apples. The equilibrium ethylene

concentrations assumed different values in some storages, ranging from concentrations of 1250 ppm to 250 ppm. Storages containing the same variety of apples assumed approximately equal equilibrium ethylene concentrations. These results in conjunction with the knowledge of controlled atmosphere storages suggest a difference in the amount of ethylene produced per unit amount of oxygen used for respiration among apple varieties.

Qualitative analyses of cold trap condensates from storage atmospheres by gas chromatography and mass spectrometry led to the identification of only three compounds: ethylene, ethanol, and acetaldehyde. Extracting activated carbon that was in contact with a controlled atmosphere containing McIntosh apples for one season produced a multitude of components. Gas chromatograms of isopentane extracts of the activated carbon show more than thirty peaks. The high complexity of the compounds in the extract and lack of adequate reference mass spectra prevented the definite identification of the extracted compounds. However, tentative identification of saturated and unsaturated aldehydes containing from four to ten carbon atoms was made from mass spectra of isopentane extracts of the activated carbon.

Gas chromatographic analyses of cold trap condensates of storage atmosphere were used in an attempt to determine the concentrations of other storage volatiles. Ethanol was the only volatile found by this method and was estimated at a concentration between 100 ppb and 2 ppm in one storage room. Further development of techniques using gas chromatography and mass spectrometry appears very promising in apple storage volatile studies.

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A THESIS

Submitted to the College of Agriculture,
Michigan State University of Agriculture and Applied Science
for the degree of

DOCTOR OF PHILOSOPHY

Department of Food Science

1963

ACKNOWLEDGMENTS

Due acknowledgments are hereby expressed by the author to all who knowingly or unknowingly affected the doctoral program undertaken, of which this thesis is a part. The members of the guidance committee -- Chairman, Dr. I. J. Pflug, Food Science Department; Dr. D. H. Dewey, Horticulture Department; Dr. J. L. Fairly, Biochemistry Department; Dr. J. B. Brunner, Food Science Department; Dr. B. S. Schweigert, Chairman, Food Science Department; and Dr. R. J. Evans, Biochemistry Department -- were most essential to the doctoral program and the arrangement of the thesis in its final form. The experimental controlled atmosphere generator used in part of this study was provided and installed by the Whirlpool Corporation. The Analytical Chemistry Section, Pioneering Research Division, at the Quartermaster Research and Engineering Command provided the facilities for the analyses carried out by mass spectrometry.

TABLE OF CONTENTS

	Page
INTRODUCTION	1
REVIEW OF LITERATURE	3
Discussion of Literature Review	12
EXPERIMENTAL	15
Apparatus and Methods	16
Low-Temperature High-Vacuum System	16
Mass Spectrometer	16
Gas Chromatograph	16
Procedures and Results	17
Exploratory Studies	17
Molecular Sieve Adsorption of Volatiles	20
Activated Carbon Extraction	21
Gas Chromatography of Atmosphere Samples	25
Conditions for Direct Analysis	25
Column Selection	26
Identification of Second Peaks in Chromatograms of Rooms 10 and 11 Atmospheres	27
Standard Curve for Ethylene Concentrations in Air	29
Gas Sampling Procedures	29
Results	30
Attempt to Determine Concentration of Other Volatiles in Controlled Atmosphere Storages	32
DISCUSSION	35
Apparatus	35

Page

Mass Spectra of Apple Storage Atmospheres 36

Analysis of Extract from Activated Carbon 37

Ethylene Concentration in Controlled Atmosphere Storages 40

Concentration of Other Volatiles 43

Solubility and Volatility 47

Source of Volatiles 50

Limiting Factors and Suggestions 51

SUMMARY AND CONCLUSIONS 53

BIBLIOGRAPHY 56

INTRODUCTION

The volatile chemical compounds produced by plant tissues are a matter of interest theoretically and practically. All of these volatile compounds are believed to be produced by cell metabolism and there is much evidence showing that at least some are products of the energy producing metabolic process of cell respiration. Identification of these metabolic products would aid in determining their route of biosynthesis and how they affect tissue metabolism.

Identification studies of plant tissue volatiles have been conducted for decades. The more abundant volatiles, ethylene, ethyl alcohol, and acetaldehyde, have been positively identified. Although these compounds account for the majority of the total quantity of volatiles produced by plant tissues, there is sufficient evidence to conclude that the volatiles already identified are only a few of the total number of volatile products produced by plant tissues.

Apple fruit volatiles are important in the storage of apples because the fruit is exposed to the volatiles for long periods of time. Some researchers believe that the storage disorders of scald and premature fruit ripening are due to apple volatiles. Attempts to control these problems by storage practices, such as the use of shredded oiled paper, air purifiers and chemicals have been partially successful in some instances. A higher concentration of volatiles is present in fruit generated controlled atmosphere storages (0.02 air changes per day) than in regular storages (1 to 2 air changes per day). The higher volatile concentration in controlled atmosphere storages may have

a greater effect on apples held in these storages than apples held in regular storages since the volatiles are in contact with susceptible tissues for a longer period of time. A better understanding of apple storage volatiles, particularly their chemical nature and concentrations, is important from both its academic and practical standpoints, and ultimately may be of great utility to the apple storage industry.

The purpose of this study was to increase the knowledge of volatiles in controlled atmosphere apple storages by: (1) qualitative and quantitative identification of apple storage volatiles; (2) establishing a feasible method for following the concentration of one or more apple storage volatiles; and (3) comparing the effects of apple variety, type of carbon dioxide scrubber used, and activated carbon units on the concentration of one or more of these volatiles in controlled atmosphere storages.

REVIEW OF LITERATURE

The effect of storage volatiles on apples, especially with respect to the physiological disorder of storage scald, has been a controversial subject and still is not resolved. Brooks and Cooley (1919), Brooks (1924), and Berl (1951) believe scald is caused by accumulations of certain gases produced by apples in their life processes. On the other hand Gerhardt (1953) presented evidence that these apple volatiles do not cause scald. In reviewing the literature, Smock and Neubert (1950) report that orchard fertilization, spray program, and seasonal factors such as rainfall and temperature all seem to affect the susceptibility of apples to scald. Greater emphasis, however, has been placed on removing the apple volatiles from apple storages and prestorage treatment of the fruit itself.

Gane (1934) identified ethylene (C_2H_4) as the gas in the emanations from ripe apples which has striking physiological effects on plant tissues. Smock (1942) showed that emanations of one lot of apples produced a ripening effect as evidenced by stimulated respiration of a second lot of apples. He believed the ripening effect was largely due to ethylene. Smock and Neubert (1950) point out that Rhode Island Greening and Cortland apples stored in the presence of emanations of McIntosh apples scalded much faster than when stored alone. They also point out that off-flavors and off-odors in storage apples may be absorbed from volatiles produced by surface molds or bacteria, from volatiles from certain types of wood like yellow pine, or from other vegetables or varieties of apples in the storage rooms.

Basically two approaches have been followed toward reducing the problem of apple storage volatiles (Phillips, 1953). The more immediate approach has been to investigate various methods of storage management and fruit handling to reduce storage volatiles. The long-range approach has been to identify the volatile chemical compounds produced by apples in storage.

Brooks and Cooley (1919, 1919 A) utilized paper impregnated with mineral oil either as wrappers for apples or shredded and mixed with the apples for removal of volatiles from apple storages. This method has proved rather effective for controlling scald in many cases; however, Smock and Neubert (1950) cite a number of objections to this method: (1) it is costly and fails to control scald in severe cases; (2) the mineral oil also absorbs foul storage room odors; and (3) fruit buyers object to oily paper in packages of apples.

Phillips (1953) reviewed Smock's modification of Fontanel's idea to use charcoal to remove apple storage volatiles. By impregnating activated charcoal with bromine, Smock attempted to remove, along with other volatiles, the metabolic stimulant ethylene. He found the high corrosiveness of bromine made it impractical for use in storages. Nevertheless, activated carbon has been reported by Gross and Smock (1945) to be an effective means of solving the pine wood volatiles problem; by Uota and Smock (1948) as the most effective means tried for removing food odors and volatiles; and by Southwick and Smock (1948) to control scald more satisfactorily than did shredded oiled paper. Van Doren and Bullock (1950) report significant scald reduction, increase in storage life, and control of off-flavors and storage odors

with high-grade activated coconut shell carbon. Gerhardt (1950) showed that, in commercial apple storages, activated carbon failed to remove ethylene and did not appreciably reduce other volatiles in the storages. However, Gerhardt reports that activated carbon reduced the volatiles to one third in small experimental apple storages as compared to the control storages. Gerhardt, Sainsbury, and Siegelman (1953) report that air purification with activated carbon failed to reduce significantly storage scald of apples and that their study cast serious doubt on the value of air purification of fruit storages. Despite conflicting results concerning the control of volatiles, activated carbon has been widely employed by apple storage operators.

Recently, the treatment of apples with two chemicals, diphenylamine (DPA) and 1, 2-dihydro-6-ethoxy-2, 2, 4-trimethylquinoline (ethoxyquin or Stop-Scald), has been extensively investigated by Mattus (1962) and Hardenburg and Anderson (1962). Both DPA and Stop-Scald dips proved to be more effective as scald inhibitors than oil paper wraps for all apple varieties tested. Tests by Hardenburg and Anderson (1962) showed that DPA was appreciably better for scald control than Stop-Scald. Several tests by Hardenburg and Anderson (1962) using DPA dips have produced 100 per cent scald control. These authors indicate that dip treatments of apples with either DPA or Stop-Scald are feasible methods for controlling scald in commercial apple storages.

Power and Chesnut (1920 and 1922) found that apple fruits contained several organic compounds which are believed to cause the characteristic apple aromas. They used steam distillation to remove and concentrate these compounds from the cortex and epidermal tissues

separately. They identified acetaldehyde and ethyl alcohol and show some evidence for the presence of some methyl alcohol in the cortex. A neutral ester fraction was obtained which upon hydrolysis yielded amyl alcohol and formic, acetic, caproic, and a small amount of caprylic acids. The apple peels contained, in addition, formic and acetic acids in the distillate and from McIntosh apple peels geraniol was identified. They did not determine whether geraniol was esterified. Furfural was also found but believed to be an artifact.

White (1950), modifying the method of Milleville and Eskew (1944), proceeded to concentrate apple juice by 150 fold. This concentrate was fractionated by distillation both at atmospheric pressure and reduced pressures (375 mm Hg absolute). Alcohols composed 92 per cent of the essence which included: methanol, ethanol, propanol, 2-propanol, butanol, isobutanol, d-2-methyl-1-butanol, and hexanol. Carbonyl compounds (6 per cent) included: acetaldehyde, acetone, caproaldehyde, and 2-hexenal. Esters (2 per cent) included ethyl butyrate and ethyl caproate. Components of other esters were identified as methanol, ethanol, 2-propanol, butanol, and formic, acetic, propionic, butyric, and caproic acids. The concentration of these compounds in the original apple juice was approximately 50 ppm.

Attempts to correlate acetaldehyde and ethyl alcohol with storage scald and other storage disorders have been, for the most part, non-conclusive (Smock and Neubert, 1950). Gerhardt (1942) found large accumulations of acetaldehyde in apples showing severe scald. It has not yet been determined whether the scald is a result of the acetaldehyde or vice versa. Off-flavors and physiological disorders may be due to the presence of ethyl alcohol or acetaldehyde or both (Gerhardt, 1942).

Acetaldehyde and ethyl alcohol accumulations have been noted during senescence (Smock and Neubert, 1950) or in low-oxygen atmospheres (Thomas, 1925). Ethyl alcohol seems to act as a poison and depresses the respiration rate when it accumulates in apple tissue.

Walls (1942) achieved some success in both qualitative and quantitative analysis of apple volatiles. Sulphuric acid activated with silver sulphate was used for total volatile adsorption. Quantitative measurements of volatiles were expressed as amounts of carbon dioxide produced by combustion of the absorbed volatiles. Ethylene was shown to form a high proportion of apple volatiles absorbed by silver-activated sulphuric acid. Walls then experimented with three adsorbents for collecting the odorous components of apple volatiles: calcium chloride, silica gel, and adsorbent carbon (Desorex III). Calcium chloride was the best of these three adsorbents, and from it two fractions were obtained by extracting the volatiles by steam distillation. A highly odorous oil, fraction A, composed largely of aliphatic esters, was obtained from all apple varieties studied. A minute amount of small crystals, fraction B, which was less volatile than fraction A, was obtained in an appreciable amount, but only from apple varieties susceptible to scald. Hydrolysis of fraction A yielded formic and acetic acids and an oil having the odor of amyl alcohol. An insufficient quantity of B was obtained to permit further examination.

Thompson (1951) found cold trapping to be very useful for collecting volatiles given off by Granny Smith apples. The volatiles were condensed by passing purified air over the fruit and through a spiral condenser cooled in liquid oxygen. The volatiles were hydrolyzed with

0.2N sodium hydroxide and the alcohols were distilled off while the solution was alkaline; the acids were distilled off after acidifying the solution. Sodium salts of these acids were obtained by bringing the acid distillate to pH 8 with sodium hydroxide and evaporating to dryness. By first converting the sodium salts of the acids to hydroxamic acids, followed by chromatographic separation on paper, the acids were identified as formic, acetic, propionic, butyric (probably normal), valeric, and caproic acids. Formic and acetic acids were shown to be both in the free and esterified form. All other acids were found in the esterified form. Ethanol and n-propanol were identified by paper chromatography after converting them to the respective hydroxamic acids and methanol was identified by a specific color test. The acids obtained were virtually free from carbonyl, hydroxy, or unsaturated groups and the alcohols were predominantly primary and saturated.

Thompson and Huelin (1951) studied the production of esters by Granny Smith apples stored at 0°C and 20°C. The esters were collected by passing purified air over the fruit samples and through three cold traps in series. The first trap consisted of a U-tube cooled in an ice-calcium chloride mixture. The second and third traps each consisted of two spiral absorbers containing 30 ml. of ether and cooled in a dry ice-alcohol mixture. The aqueous portion from the first trap was extracted with ether. The ether extracts from all three traps were dried over calcium chloride and made up to volume in a volumetric flask. Apple ester concentrations were determined colorimetrically by conversion of the esters to hydroxamic acids and then forming ferric hydroxamate complexes. Thompson and Huelin (1951) also show that apples stored at

0°C appeared to increase steadily in ester production while those stored at 20°C increased to a maximum of ester production and then decreased. Early picking reduced whereas higher air flow rates increased ester production. Apples stored in atmospheres of 6 per cent oxygen first increased and then decreased in ester production. Ester production by apples stored in air remained constant or increased during the storage period.

Huelin (1952) continued the study of Thompson and Huelin (1951) and identified the volatile aldehydes and ketones produced by Granny Smith apples. Collection was carried out by passing purified air over the fruit sample and through a spiral absorption trap which was cooled in an ice bath and contained 30 ml. of concentrated metabisulfite solution (25 g. $K_2S_2O_5$ per 100 ml.). The aldehydes were removed by oxidation. Then dinitrophenylhydrazones were prepared, first of the total carbonyl compounds and then of ketones alone. Identification of the dinitrophenylhydrazones was carried out by paper chromatography. Acetaldehyde was found to be the predominating carbonyl compound. Smaller amounts of propionaldehyde and acetone were also found. The presence of acetaldehyde and propionaldehyde was confirmed by conversion to hydroxamic acids which were identified by paper chromatography.

Turk and Smock (1951) used cold traps and activated carbon to collect the volatiles from apples. The volatiles were driven off the activated carbon by heat. Low-temperature high-vacuum distillation procedures were used to fractionate the samples and mass spectrometry and infrared spectroscopy were employed in the analysis of the samples. From the mass spectra data ethanol and acetaldehyde were identified, but most of the mass spectra data could not be resolved to show

specific compounds. Prominent peaks of the mass spectra indicate aldehydes, acetates or methyl ketones, and secondary alcohols. The infrared spectra of the samples is typical for a mixture of esters, aldehydes, and perhaps alcohols, confirming the mass spectra data.

Henze et. al. (1953) used a different approach to recover apple storage volatiles. A quantity of activated carbon was acquired from an air purification unit in a commercial refrigerated apple storage at the close of the storage season. At least twenty extractions of the carbon were made with ether as the solvent. The ether was evaporated off over a steam bath and the residue was analyzed. The neutral portion of the extract, 98 per cent, consisted mainly of esters. By hydrolysis of this fraction and subsequent chromatography on silicic acid columns, methyl, ethyl, isopropyl, n-butyl, and n-hexy alcohols and proprionic, n-butyric, n-caproic, and n-caprylic acids were identified. An impure amyl alcohol and formic, acetic, and impure valeric acids were indicated present. The free acid and free alcohol fractions were found to contain the same alcohols and acids as did the ester fraction with the exception of n-caprylic acid.

Meigh (1956, 1957) conducted a rather extensive study of volatile compounds produced by three varieties of apples: Edward VII, Laxton Superb, and Cox's Orange Pippin. The volatiles were collected by first passing air through air-tight cabinets containing the apples and then through a series of three cold traps where the volatiles were condensed. The first trap was a U-tube emersed in an ice-salt mixture. The other two traps were cooled to dry ice temperature and contained a pure solvent through which the air samples were passed.

The aldehydes and ketones (Meigh, 1956) were converted to 2:4 dinitrophenylhydrazones and were analyzed by paper chromatography, ultraviolet spectroscopy, and melting point determinations. Of the aldehydes and ketones, acetone was in most abundance and smaller amounts of acetaldehyde, n-butanal, propanal, ethyl methyl ketone, and isobutanal were identified. The alcohols were converted to 3:5 dinitrobenzoates, which were separated by paper chromatography and the fractions were estimated with an ultraviolet spectrophotometer (Unicam SP500). The esters were identified by converting them to hydroxamic acids which were estimated colorimetrically as a group, according to the method used by Thompson (1951) and identified by paper chromatography. The alcohols identified were ethanol, D-2-methylbutan-1-ol, 2-methylpropan-1-ol, methanol, isopropanol, and a C_6 alcohol. Esterified acids from C_1 to C_6 inclusive were found.

Quantitative determinations were carried out by Meigh (1956, 1957) for the volatile compounds and their production was followed throughout a storage season. No correlation was found between high rate of evolution of volatile substances and a heavy incidence of scald. Apples stored in normal air produced all the volatile compounds at a greater rate than those stored in controlled atmosphere storage. The air storage rates of volatile production tended to increase, while in gas storage rates of volatile production tended to remain constant or decrease through the storage season. The percentage recovery of these volatiles using this method varied considerably. Recovery tests for acetaldehyde varied from near 100 per cent recovery to as low as 31 per cent.

DISCUSSION OF LITERATURE REVIEW

Control of apple scald and storage volatiles has been attempted in many ways. The use of treated wraps with mineral oil or chemicals, although effective, has serious drawbacks, such as unsightliness, excessive handling, and absorption of foul storage room odors. The use of activated carbon in a so-called air purifier in storages gives inconsistent and questionable scald control when used. The recent experimental results of Mattus (1962) and Hardenburg and Anderson (1962) on prestorage treatment of apples with Stop-Scald or DPA sprays or dips for scald control have been very satisfactory and these chemicals seem feasible for scald control in commercial installations.

Although scald control appears possible through the use of chemical dips or sprays, the cause of scald still remains obscure. Growing seasons and orchard management apparently affect the susceptibility of apples to scald. The results in attempts made by Gerhardt et. al. (1953) support the argument that scald is not caused by apple volatiles. Nevertheless, it has not been shown that apple volatiles or any one apple volatile are not responsible for scald.

The mechanism of scald control by Stop-Scald and DPA is still unknown. In the case of activated carbon and mineral oil impregnated paper, scald control is attributed to the removal of volatiles. Although Phillips (1953) doubts the control of scald by activated carbon air filters, he does state that charcoal dust on the surface of the apple does control scald. This leads to the assumption that removal of volatiles by adsorption or absorption is more effective if the volatile

trapping agent is closer to the apple surface. Meigh (1957) suggests that the less volatile compounds evolved by apples are possibly more directly associated with scald than more volatile compounds. The fact that DPA and Stop-Scald are most effective when applied to the surface of the apple than when applied to paper wraps indicates that the scald-control mechanism of these two chemicals is similar to that of mineral oil or activated carbon next to the apple surface; namely, removal of volatiles from the apple surface. DPA and Stop-Scald are antioxidants. The mechanism by which these antioxidants prevent scald is not known yet. It may be possible that certain metabolic reactions of the fruit are affected by these chemicals. They may either cause or prevent oxidative changes of compounds on the surface of the apple and prevent scald in this way. On the other hand, these are organic chemicals and may be good absorbents for volatiles, thus removing them from the surface of the apple.

Many organic compounds given off by apples have already been identified, as seen in the literature review. Several compounds, such as ethylene, methanol, ethanol, acetaldehyde, and esters as a group, have been found by many investigators. Nevertheless, most of these investigators have identified compounds which are not common with the general findings. Experimental procedures and apple differences (variety, storage conditions, growing conditions, and seasons) are believed to be responsible for the differences. The differences in odor and taste of different apples indicate a difference in volatile composition. Artifacts or destruction of compounds may occur with different methods of analysis. It is also quite possible that some methods of analysis do

not detect some of the volatiles due to the low concentration of most of the volatiles. Furthermore, the ester fractions separated have been hydrolyzed and the component alcohols and acids identified but little has been done to identify the esters as such. It is evident, therefore, that more qualitative and quantitative information would be very useful in order to determine the role of apple volatiles in storage scald.

A great deal of work has gone into obtaining the available information on apple storage volatiles. The acquiring of more information by most of the methods and techniques already used is hampered by the lack of sensitivity, lack of specificity, and/or great laboriousness. The determination of ethylene by absorption in a mercuric perchloric solution followed by reduction of cerate sulfate provides a specific and sensitive analysis for ethylene in apple storages but is rather cumbersome for frequent analysis. The sulfuric acid ceric sulfate method of analysis for the non-ethylenic volatiles has provided useful information but it is not specific and is cumbersome. More work with mass spectrometry would appear to be a good way to further resolve the work started by Turk and Smock (1950). Meigh (1957) also points out that gas chromatography, with properly developed techniques, provides a potential method to further the work on apple storage volatiles.

EXPERIMENTAL

The Review of Literature cites a rather extensive effort and considerable success in the identification of apple storage volatiles. Most of the methods and techniques used in previous studies give evidence for the low concentration of most of these volatiles in storage room atmospheres. The sensitivity of most of the above-cited methods is great but the limitations of these methods make them difficult to use. The many different kinds of compounds make the specificity of most of these methods a hindrance to complete identification of all the compounds in a sample by any one method. In forming derivatives of certain types of compounds, reactions with other types of compounds present may destroy existing volatiles and/or produce artifacts. Most of the methods used thus far are also so cumbersome that a great deal of work is necessary for any complete analysis of apple storage volatiles.

The methods and results of studies on apple storage volatiles and similar problems indicate that the use of advanced physical-chemical methods of sampling and analysis will solve many of the problems encountered by most previously used procedures. Low-temperature high-vacuum distillation used in handling the volatiles will keep to a minimum the destruction of the compounds and/or formation of artifacts. Mass spectrometry eliminates the formation of derivatives in identification and lends itself well to analyzing samples prepared by low-temperature high-vacuum distillation. Gas chromatography can be a rapid method for concentration studies of previously identified volatiles.

APPARATUS AND METHODS

Low-Temperature High-Vacuum Distillation

A low-temperature high-vacuum distillation apparatus was designed and constructed using a Cenco Hyvac 2 vacuum pump and a single stage mercury diffusion pump. All joints in the apparatus were ground glass of standard taper; glass vacuum-type stopcocks were used throughout. Apiezon N stopcock grease was used on all stopcocks and small ground glass joints. The pressure in the system was measured with a Pirani gauge, type GP-110, manufactured by Consolidated Electrodynamics Corporation. Pressures of less than 1 μ Hg absolute were obtainable in this system. At pressures of 0-5 μ Hg absolute, the system gained 4 μ Hg pressure per day over a period of 4 days with all stopcocks closed and vacuum pump off.

Mass Spectrometry

A Model 21-103C Mass Spectrometer, having a vibrating reed amplifier, manufactured by the Consolidated Electrodynamics Corporation was used in this study. The vibrating reed amplifier increases the sensitivity of the instrument by ten-fold over the amplifier normally installed in the instrument.

Gas Chromatography

An Aerograph Model A-600 Hy-FI gas chromatograph with the hydrogen flame ionization detector was used in this study. A one-millivolt, Leeds and Northrup Speedomax H, Model S recorder was used to record the

output potential of the chromatograph amplifier. An Aerograph hydrogen generator, Model A-650, was used to generate and meter both the hydrogen and the air used for combustion by the hydrogen flame detection assembly.

A 1/8"-5' stainless steel column packed with SE-30 on acid-washed chromosorb W was purchased with the chromatograph. Two 1/8"-10' stainless steel columns were obtained from Wilkins Instrument and Research, Incorporated. One of these was packed with 15 per cent Carbowax-15-40 on Chromosorb W; the other, with 40 per cent Castorwax on Chromosorb W. Column selection was based on the nature of compounds anticipated to be present in apple storage volatiles, that is, ethylene and oxygenated organic compounds. The operating conditions for the desired analyses were determined by trial and error.

PROCEDURES AND RESULTS

Exploratory Studies

Two bushels of yellow transparent apples were placed in an air-tight metal container and held at refrigerated temperatures, 34 to 38°F. The CO_2 in the container was maintained constant by controlled ventilation to prevent CO_2 injury to the fruit. The mass spectrum of the atmosphere within the container was obtained and is shown in Figure 1. Subtracting the mass spectra due to air gas from the mass spectrum of the total sample, Figure 1, results in the mass spectrum shown in Figure 2. From this resulting spectrum no positive identifications were made. The concentrations of the compounds giving the spectrum in

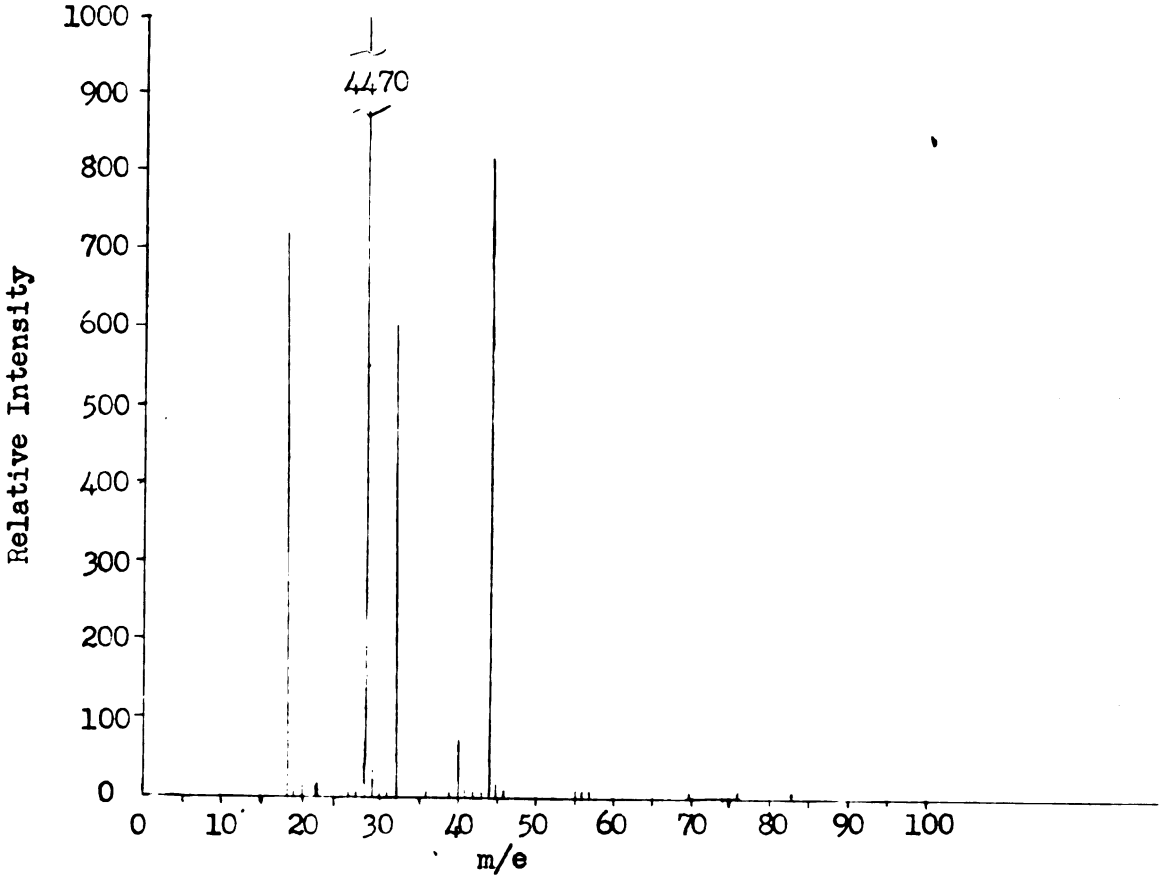

Fig. 1. Mass spectrum of CA storage atmosphere

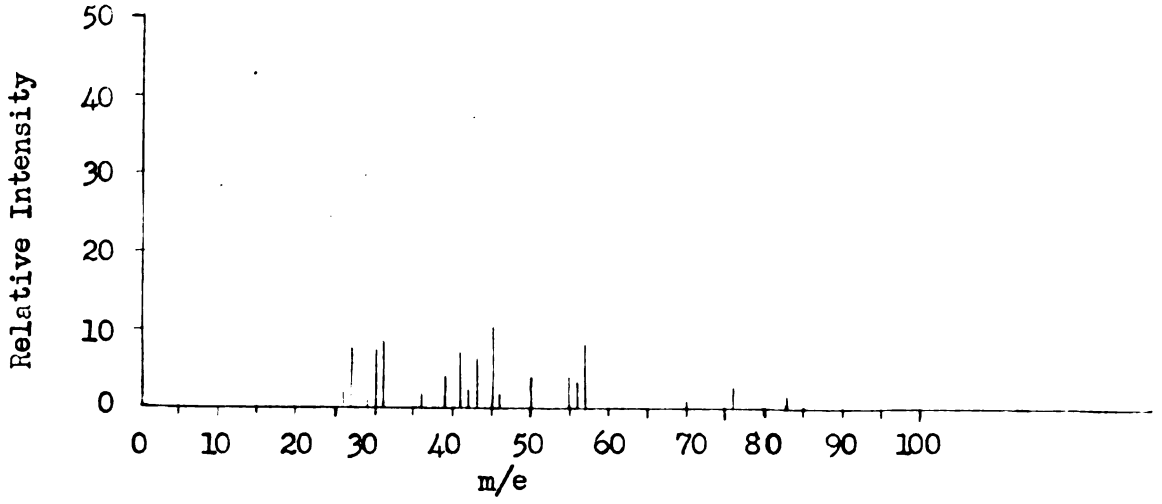

Fig. 2. Mass spectrum of CA storage minus mass spectra of air gases

Figure 2 were too small to give sufficient spectra for identification, but short chained alcohols and aldehydes are indicated by peaks at m/e 45, 43, 31, and 29. Peak m/e 57 may indicate the possibility of esters of propionic acid and is an interesting peak to observe.

Low-temperature high-vacuum (LTHV) distillation was used to remove the highly volatile air gases that interfered with analysis of the apple volatiles. Further fractionation of the samples was carried out by LTHV distillation after the removal of air in an attempt to separate the apple volatiles according to their volatility.

One sample was taken by filling an evacuated bottle with the atmosphere from the container. The sample was held at -196°C and all gases volatile at this temperature were removed by pulling a high vacuum on the sample. The compounds remaining were fractionated into three parts. This was accomplished by holding the remaining compounds at -155°C, -100°C, and at room temperature, respectively. Collection of the volatile compounds at these three temperatures was accomplished by allowing them to condense in a liquid nitrogen trap. Compounds exerting vapor pressures greater than 5 μ Hg between the temperatures -196°C and -155°C, -155°C and -100°C, and -100°C and room temperature were collected in the three fractions prepared from this sample. Mass spectra of these three fractions are shown in Figures 3, 4, and 5.

After allowing for CO_2 in the -155°C to -100°C fraction and water and some CO_2 in the -100°C to room temperature fraction, no identifications can be made since only a few peaks with very low intensities remain. Ethylene is indicated as present in the -155°C to -196°C fraction by m/e's 26, 27, and 28. Peaks seen at m/e's greater than 44

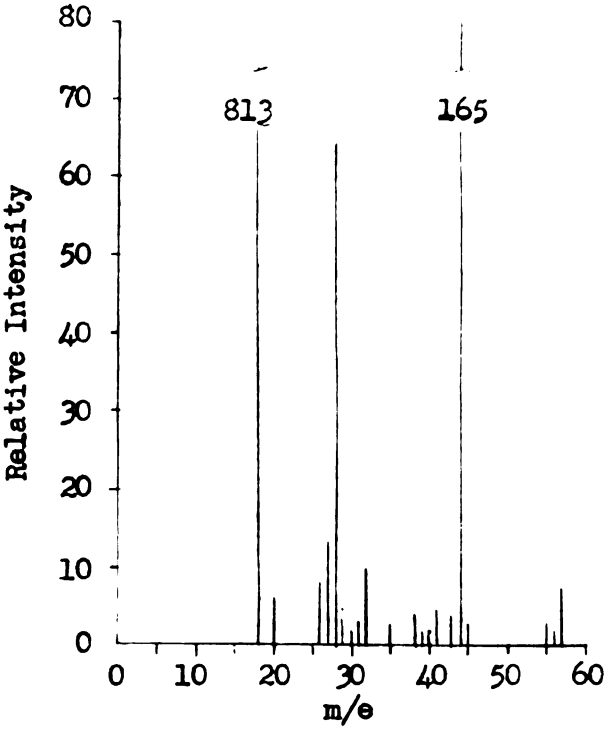

Fig. 3. Mass spectrum of CA storage atmosphere -155°C to -196°C fraction

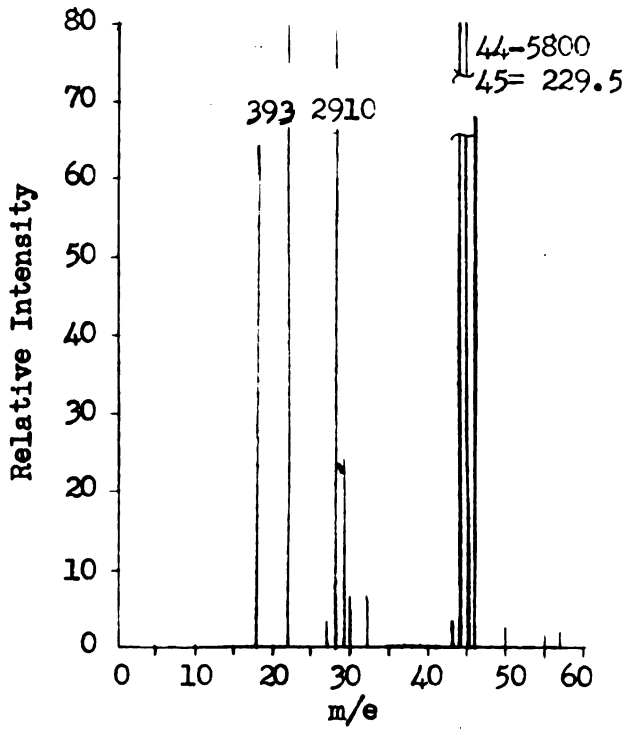

Fig. 4. Mass spectrum of CA storage atmosphere -100°C to -155°C fraction

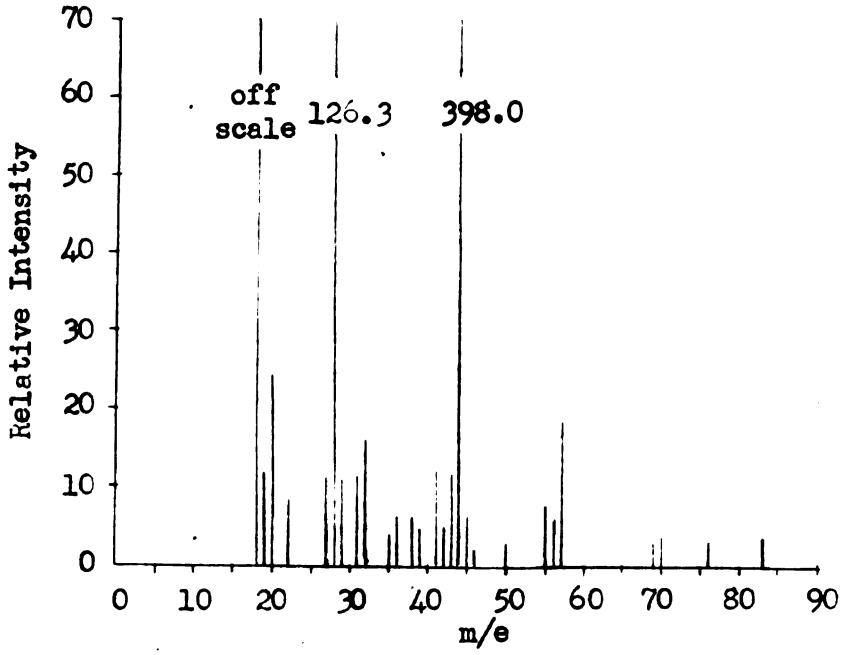

Fig. 5. Mass spectrum of CA storage atmosphere room temperature to -100°C fraction

would not be expected in the fractions collected at temperatures lower than -100°C but are present. This is believed to be due to contamination from previous samples by absorption of compounds by the stopcock grease used in the high vacuum system. Disregarding the peaks contributed by air, CO_2, and H_2O, m/e's 57, 43, and 41 are of greatest significance. These peaks indicate the same families of compounds, aliphatic alcohols, aldehydes, and ketones, indicated by the scan in Figure 2.

Sampling procedures used thus far were not suitable for collecting sufficient volatiles for identification. In the next test, samples were collected by passing ten cubic feet of the chamber atmosphere through a liquid air cold trap. This sample was also fractionated using LTHV distillation. Vacuum pumping was not as complete at -196°C as it was for the previous sample. Fractions prepared from this sample were between the temperatures of -196°C to -155°C, -155°C to -125°C, -125°C to -100°C, -100°C to -78°C, and -78°C to room temperature. Mass spectra of these fractions are illustrated in Figures 6-10.

Figure 6 shows the presence of ethylene. Acetaldehyde was identified from the -125°C to -100°C fraction, Figure 8, and both acetaldehyde and ethyl alcohol were identified from Figure 9. No other positive identifications could be made from these spectra. The m/e's above 44 show up in greater intensities in the higher temperature fraction, -78°C to room temperature, than in the other fractions, as is expected from the types of compounds, aliphatic alcohols, aldehydes, and ketones, believed present and indicated by these m/e values. The LTHV distillation techniques, therefore, are effective in separating these compounds

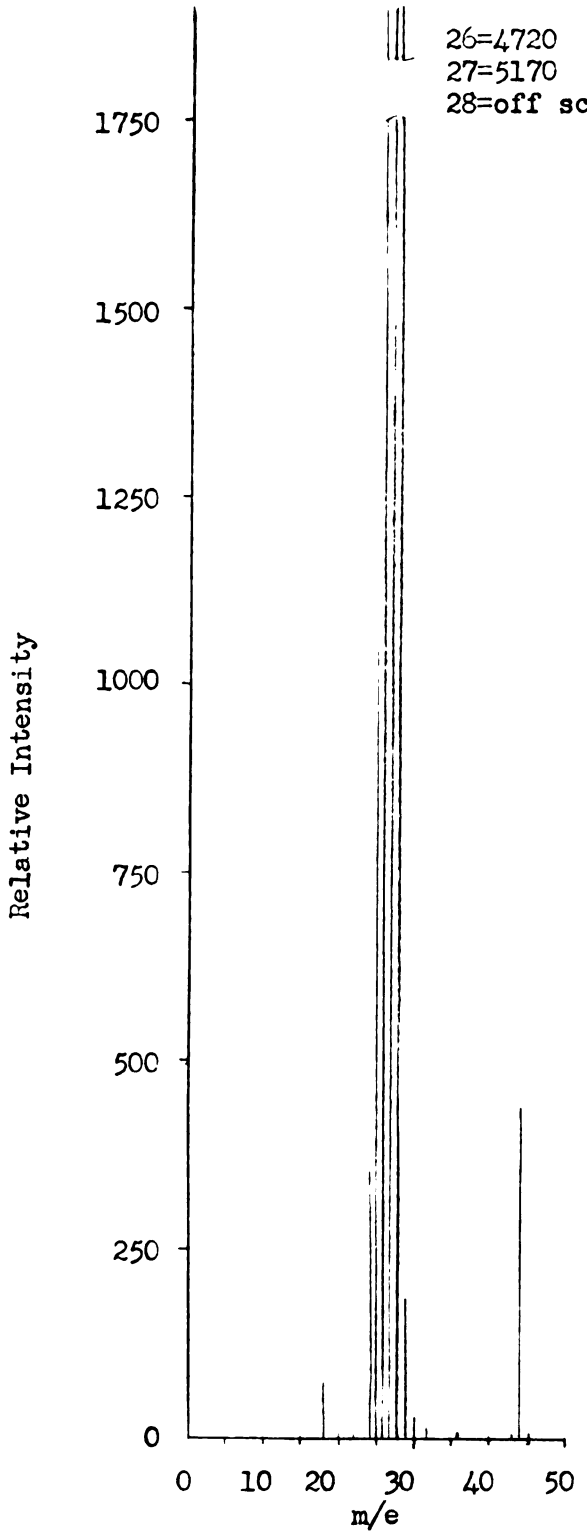

Fig. 6. Mass spectrum of condensed CA storage atmosphere -155°C to -196°C. fraction

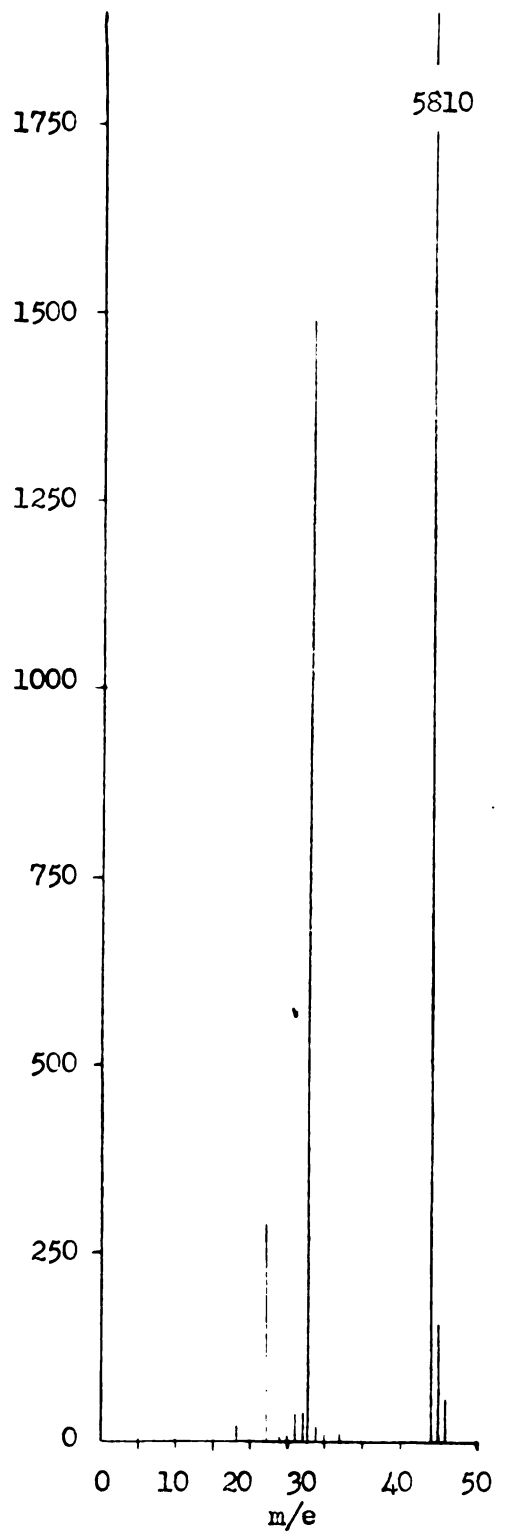

Fig. 7. Mass spectrum of condensed CA storage atmosphere -125°C to -155°C fraction

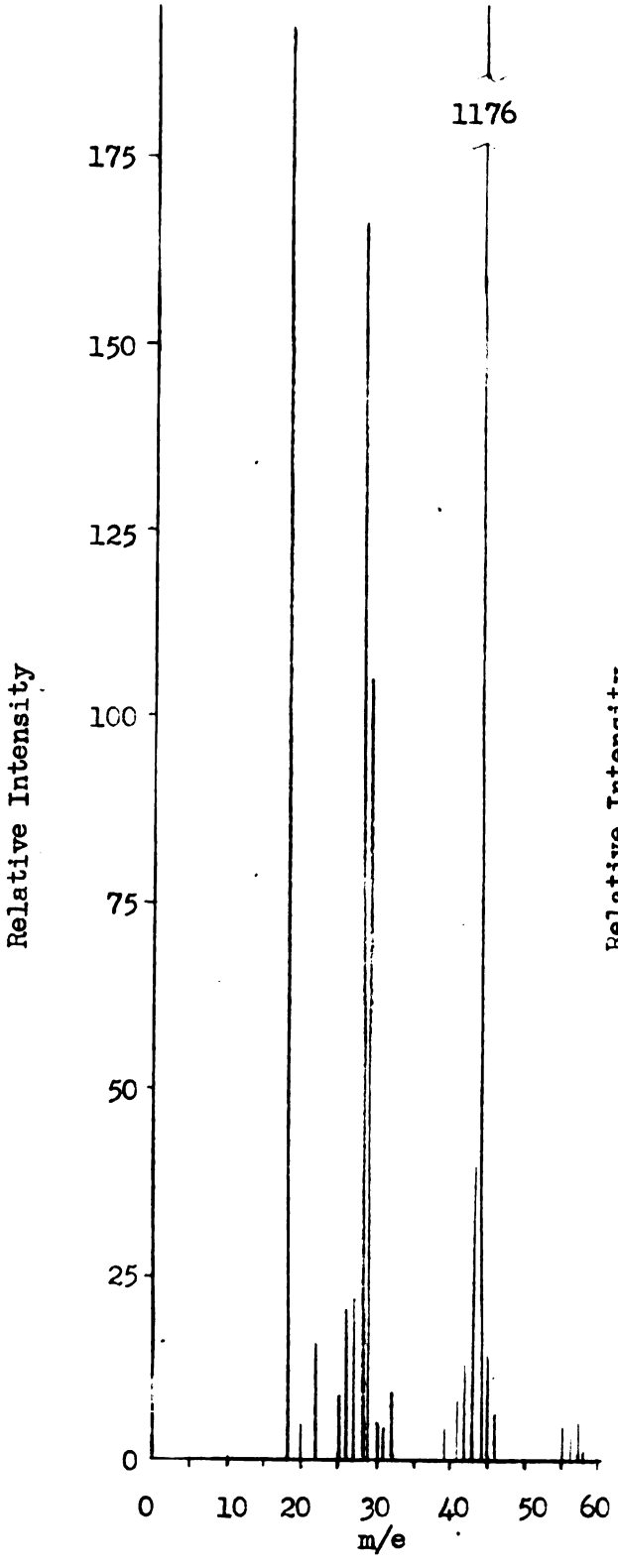

Fig. 8. Mass spectrum of condensed CA storage atmosphere -100 C to -125 C fraction

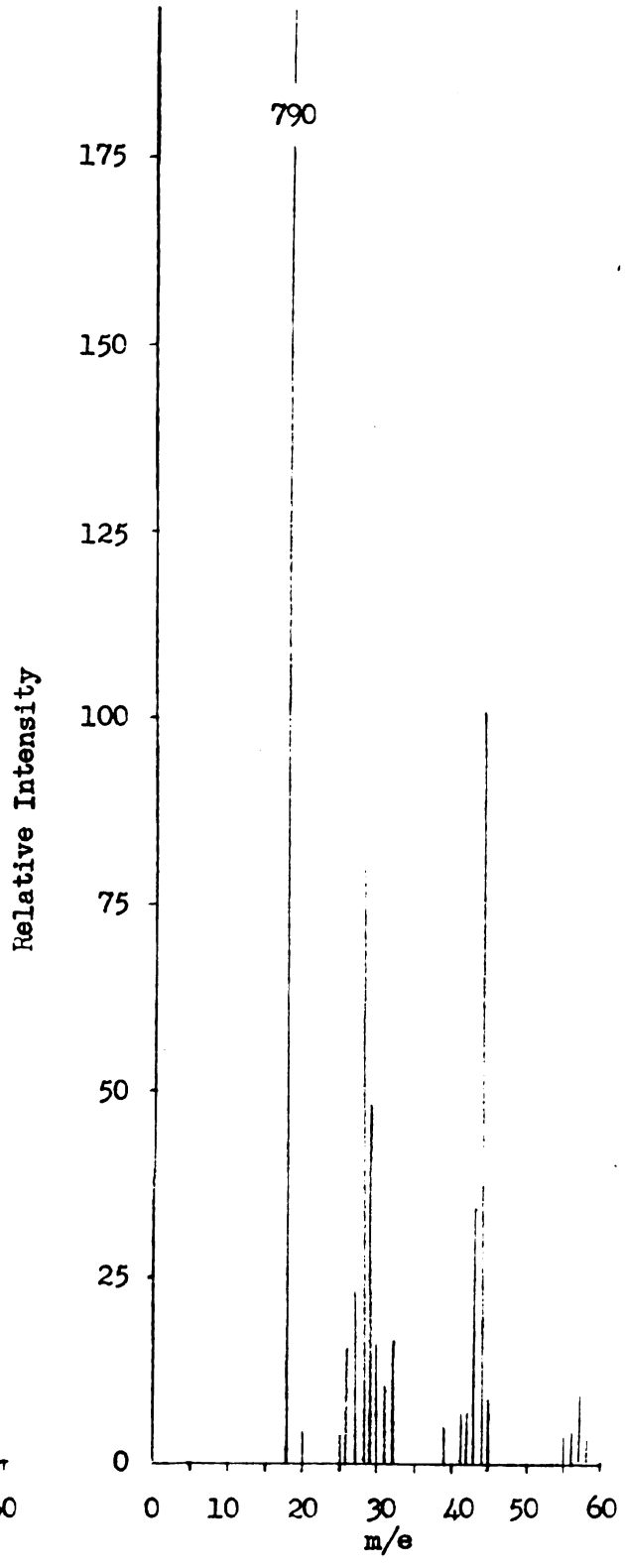

Fig. 9. Mass spectrum of condensed CA storage atmosphere -78 C to -100 C fraction

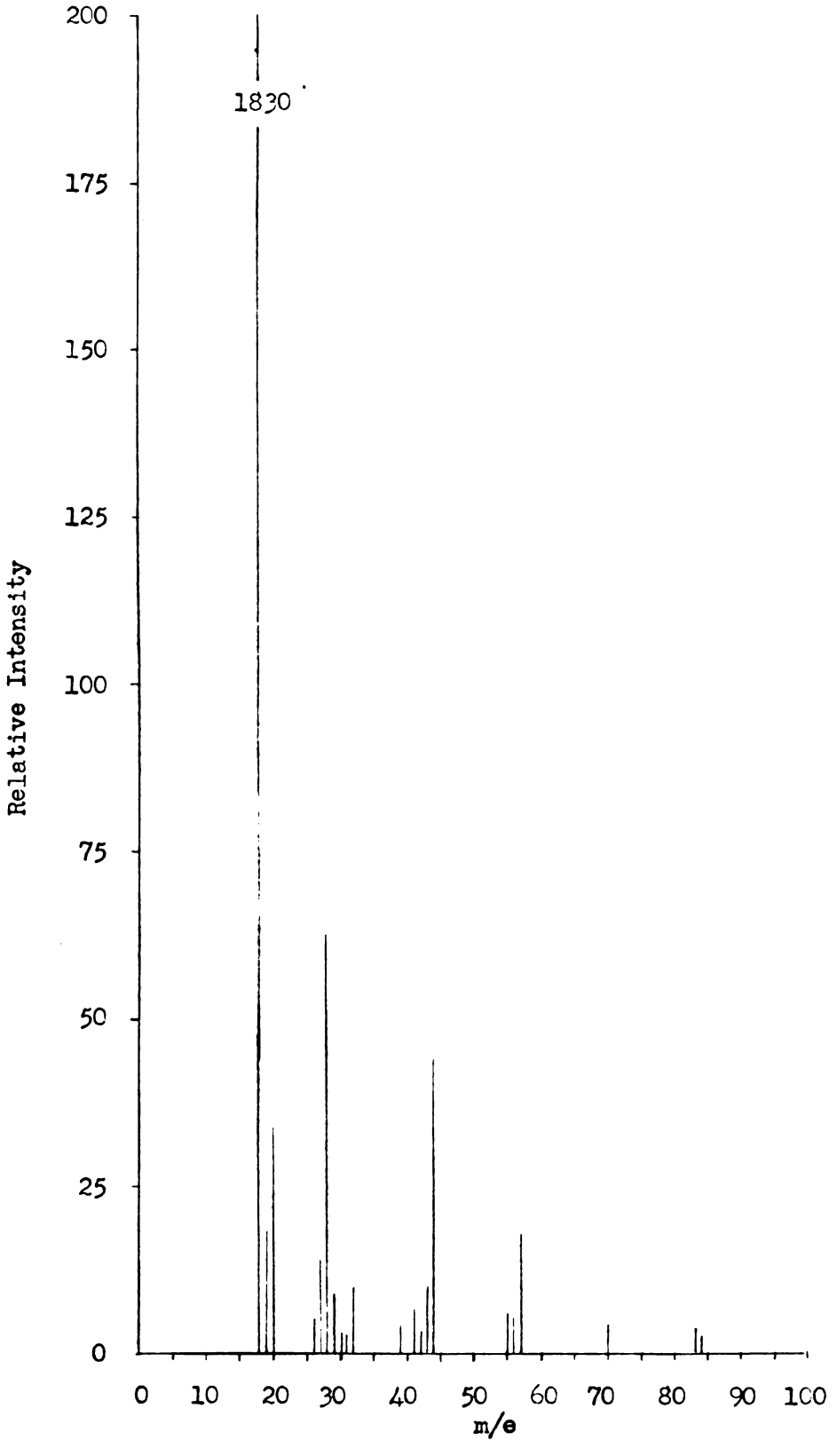

Fig. 10. Mass spectrum of condensed CA storage atmosphere
room temperature to -78°C fraction

from the more volatile air gases but not from water since water exerts a significant vapor pressure above -78°C. Water vapor is present in far greater amounts in apple storage atmospheres than are these other compounds. This fact is quite evident when the mass spectrum of -78°C to room temperature fraction is considered.

Molecular Sieve Adsorption of Volatiles

An attempt was made to separate the volatiles from water by employing molecular sieves. One-sixteenth-inch pellets of Linde Molecular sieves, type 5A, were activated by heating to 180°C and evacuating under high vacuum (1-10 μ Hg) each time before using. Atmosphere samples of various sizes were taken from a 35-bushel controlled atmosphere storage chamber filled with McIntosh apples. The atmosphere samples were held at -135°C and all materials volatile at this temperature were pumped off. The remaining volatiles from each sample were adsorbed onto 15g. of activated molecular sieves. The sieves were then heated slowly until the materials were driven off. There was no increase in vapor pressure above the sieves for any samples until the temperature of the sieves approached 180°C. The materials given off at this temperature were collected in a liquid nitrogen trap and its vapor pressure was observed at various temperatures from 0°C to -78°C. The vapor pressures at these temperatures coincided well with those of water.

Activated Carbon Extractions

As a result of the work with molecular sieves, an attempt was made to remove apple storage volatiles from the activated carbon of air purification units that had been in a controlled atmosphere McIntosh apple storage during one season of approximately 200 days. The precise procedure for activation of the carbon used was not obtainable due to company policies. The Condensed Chemical Dictionary (1956) states that activation of carbon is generally achieved by heating to 800°C to 900°C in an atmosphere of steam or carbon dioxide. Procedures for activation by commercial companies are expected to meet defined standards. After activation, the carbon is placed in sealed containers until ready for use.

The carbon was removed from two 6"x10" units from a controlled atmosphere storage room. The carbon was held in a polyethylene bag until ready to use. A 10 g. sample of the carbon was placed in a gas bottle. The bottle was cooled to -196°C and all materials volatile at this temperature were pumped off on the LTHV system. The volatiles were then driven off the carbon by heating it to 180°C. Condensation of the volatiles took place in a cold trap at -196°C under high vacuum. Approximately one-fourth milliliter of clear, colorless liquid was obtained which had a strong acrid odor not resembling apple odor in the least.

Solvent-extraction of volatiles from carbon was also attempted. Diethyl ether and isopentane were the two solvents used for extraction. Identical procedures were carried out for extraction by each solvent. Ten grams of carbon were extracted with three ten-milliliter portions

of solvent. The solvent extract was placed in a gas bottle and the solvent was distilled off at -78°C under high vacuum. The solvent was condensed in a gas bottle at -196°C, leaving behind the apple storage volatiles. Extractions using both diethyl ether and isopentane yielded approximately one-half milliliter of clear, colorless liquid. Unlike the volatiles removed by heat, the volatiles removed by solvent extraction in both cases had a sweet, apple-like odor combined with a foul, musty odor.

Gas chromatograms of the head space from each of these samples were obtained and are presented in Figures 11, 12, and 13. Similar chromatograms were obtained from all three columns available. The silicone column was chosen to be used in further investigations because it gave the best resolution of the extracts. The chromatograms are correlated with the observations made by smelling the samples. A radically different chromatogram was obtained from the volatiles removed from the carbon by heat as compared to the chromatograms from the volatiles removed from carbon by solvent extraction. Little difference is noticed from the chromatograms obtained from the solvent-extracted volatiles. Degradation of the volatiles due to heat appears to have taken place while attempting to drive them off the carbon by heating. Extraction by isopentane was the method used in obtaining more volatile samples from carbon because isopentane is more volatile than diethyl ether and easier to distill off at low temperatures. The extraction method rather than heat was used in obtaining other samples because of the degradation believed due to heat. An unused sample from the same lot of activated carbon used was not available for a control. However, the defined

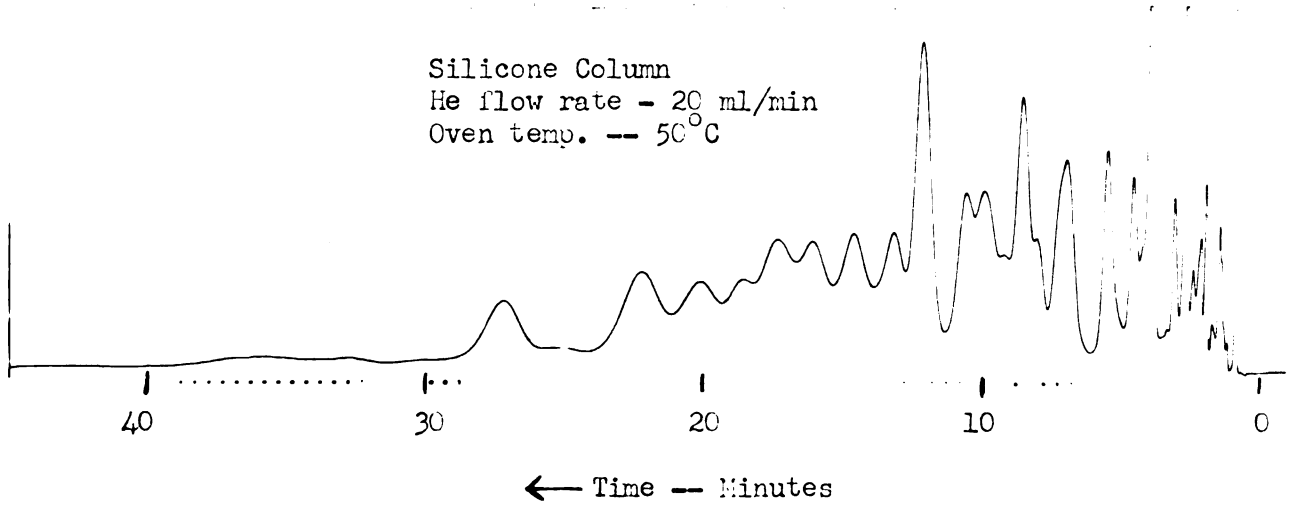

Fig. 11. Gas chromatogram of heat extract of activated carbon

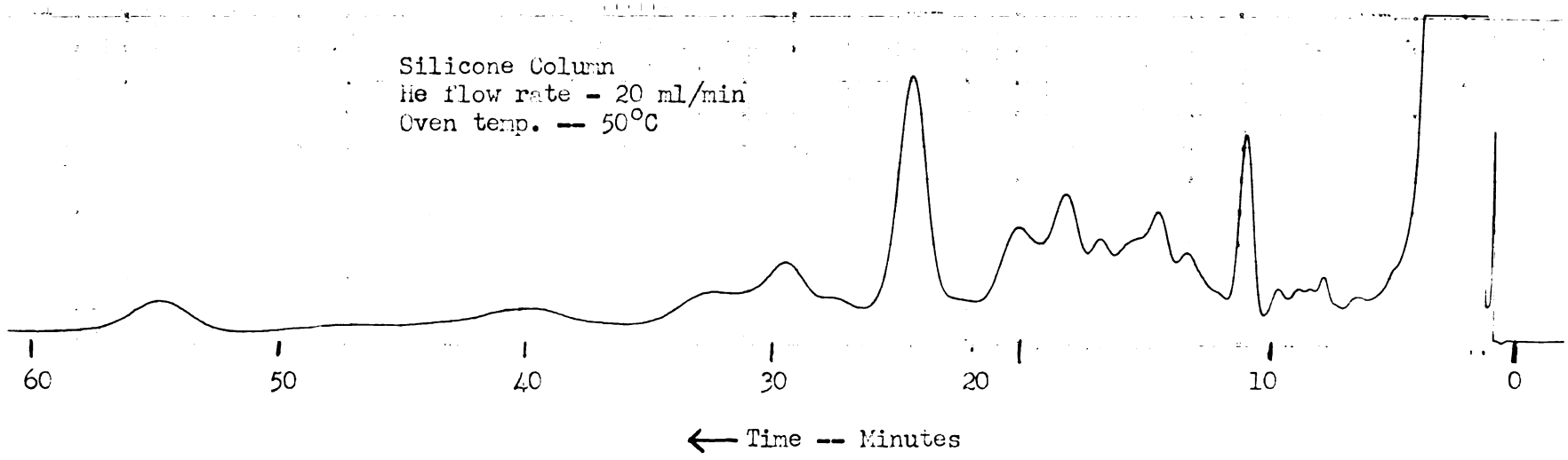

Fig. 12. Gas chromatogram of diethyl ether extract of activated carbon

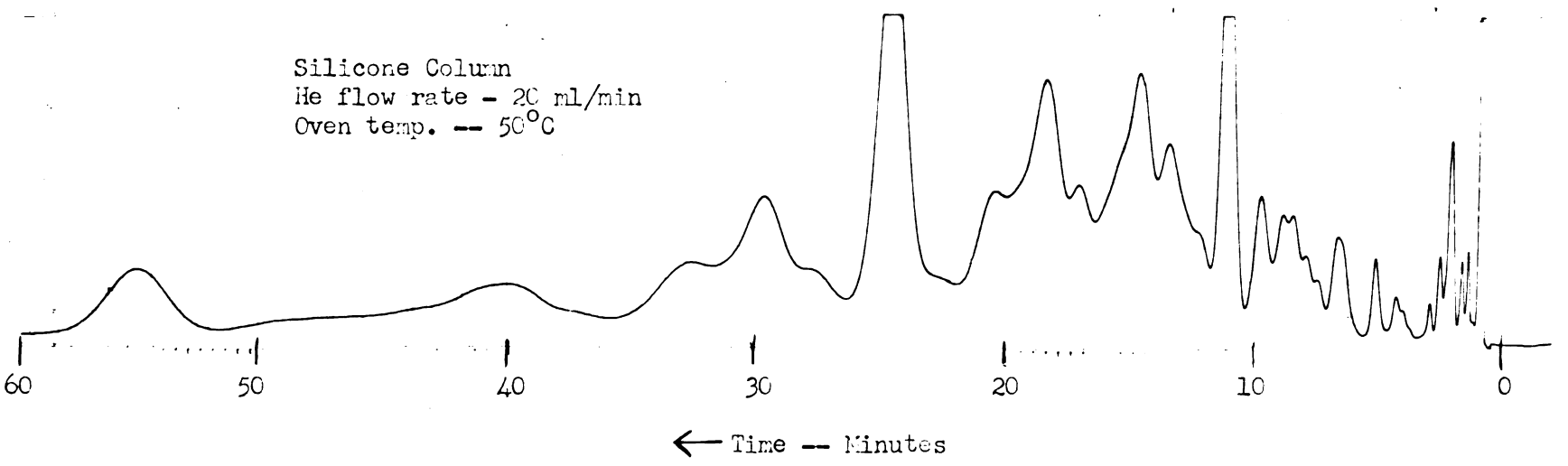

Fig. 13. Gas chromatogram of isopentane extract of activated carbon

activation treatment is severe enough to destroy all oxygenated hydrocarbon compounds (Royals, 1958).

A sample of volatiles was obtained from 10 grams of carbon by extraction with isopentane followed by subsequent distillation at -78°C under high vacuum. LHTV distillation was used to fractionate the sample. Two samples were collected at -78°C. The first reduced the vapor pressure of the sample from 800 μ to 400 μ Hg. pressure; the second, from 400 μ to 0 μ Hg. pressure. Other fractions were collected of materials volatile between temperatures of -60°C to -78°C, -40°C to -60°C, -30°C to -40°C, -20°C to -30°C, -10°C to -20°C, 0°C to -10°C, and room temperature to 0°C. All samples were condensed at -196°C under high vacuum. Mass spectra of some of these fractions are shown in Figures 14, 15, 16, 17, and 18.

A comparison of Figure 14, the mass spectrum of the fraction collected at -78°C between 800 μ and 400 μ pressure, with Figure 15, the mass spectrum of isopentane, shows this fraction of the sample to be composed almost entirely of isopentane, the solvent used for extraction. Inspection of Figures 16, 17, and 18 confirms similarities in these three sample fractions, indicating likeness in composition. The prominent peaks in these spectra occur at the same m/e values in each case. Some variations of proportions in intensity between these major m/e values is noticed between different sample fraction spectra due to varying proportions of the compounds in the different samples. This is expected when the techniques of fractionation are considered.

The groupings of m/e values which are prominent and of interest in these spectra are 55, 56, 57; 69, 70, 71; 82, 83, 84, 85, 86; 95, 96,

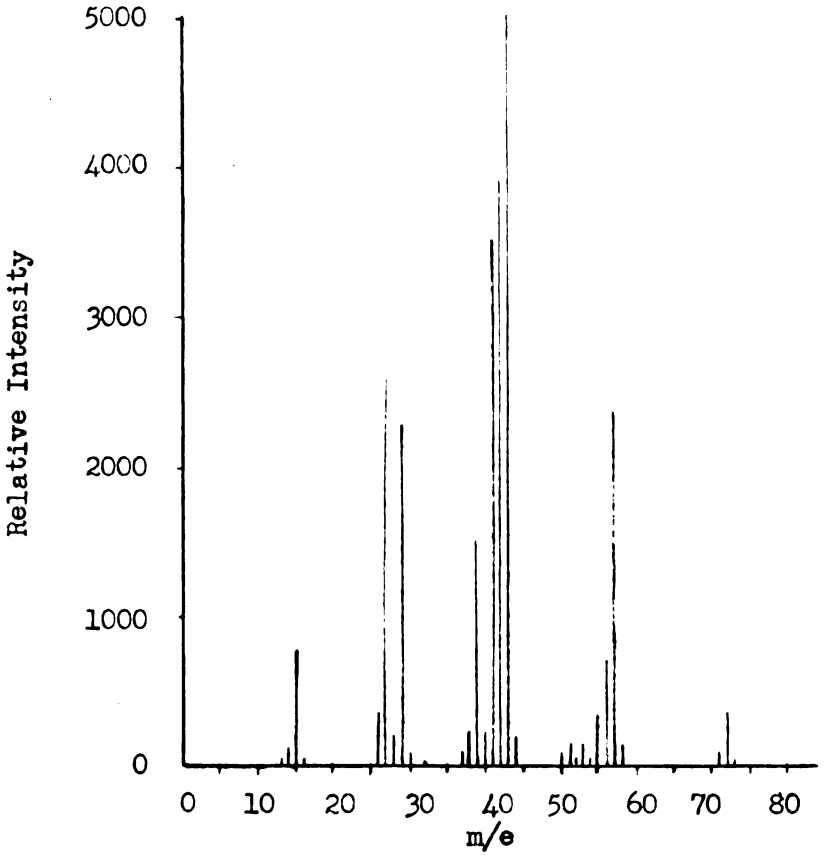

Fig. 14. Mass spectrum of isopentane extract of activated carbon
-78°C; 800 μ Hg to 400 μ Hg pressure fraction

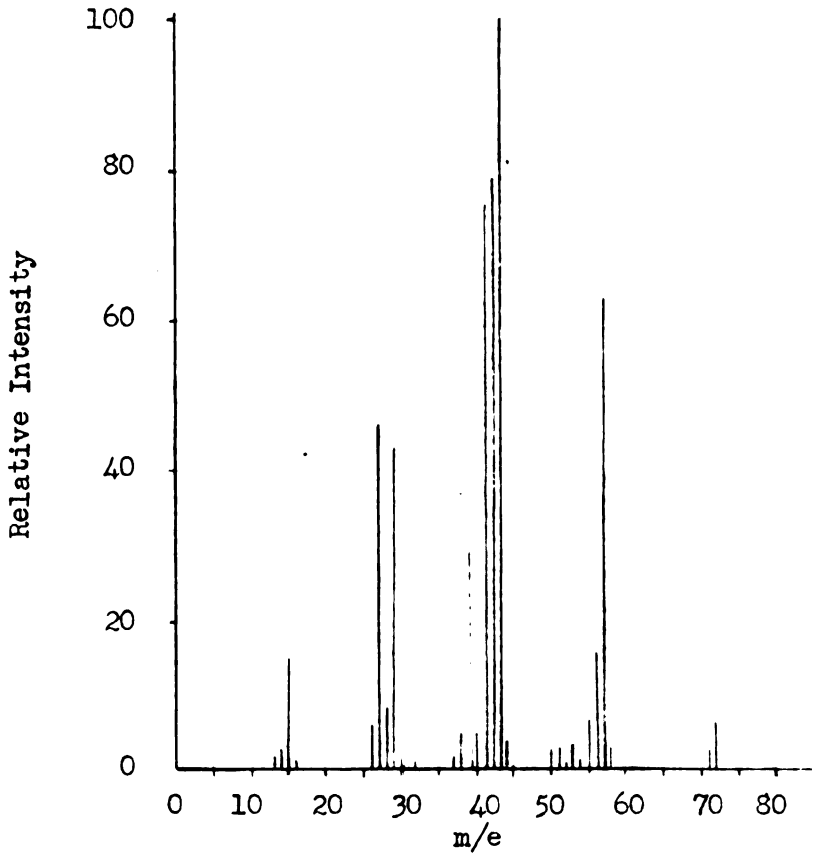

Fig. 15. Mass spectrum of isopentane

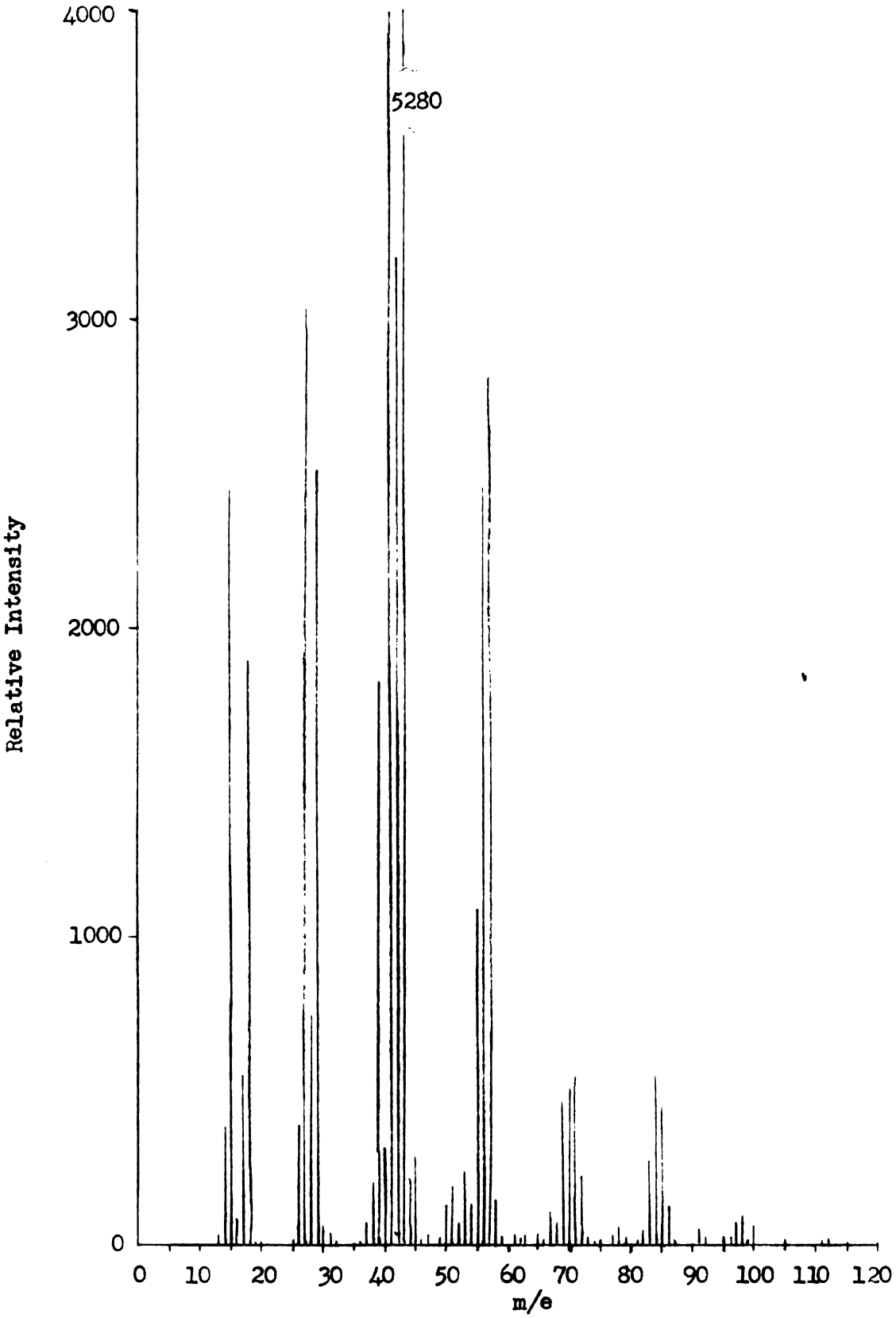

Fig. 16. Mass spectrum of isopentane extract of activated carbon -60°C to -78°C fraction

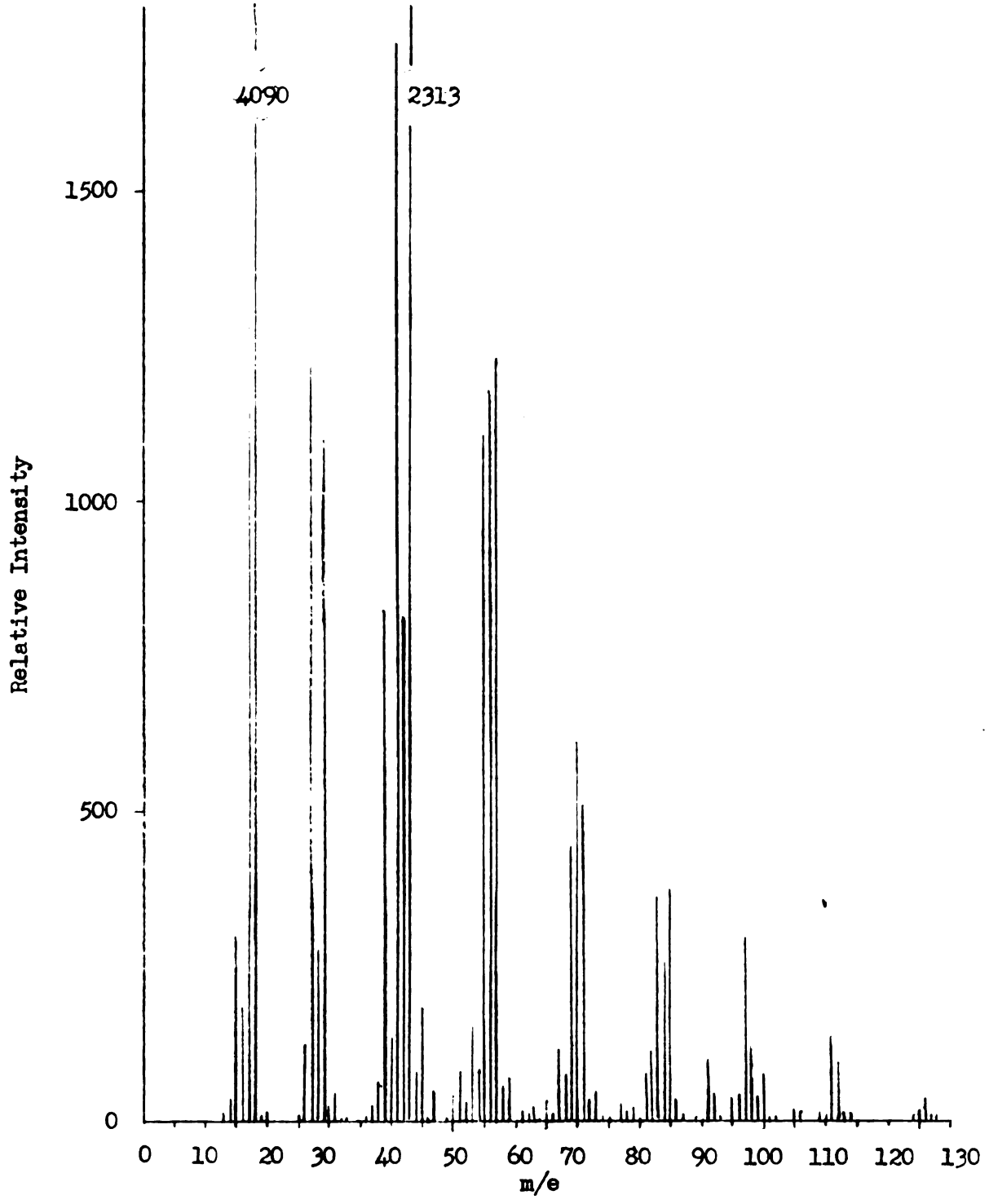

Fig. 17. Mass spectrum of isopentane extract of activated carbon -40°C to -60°C fraction

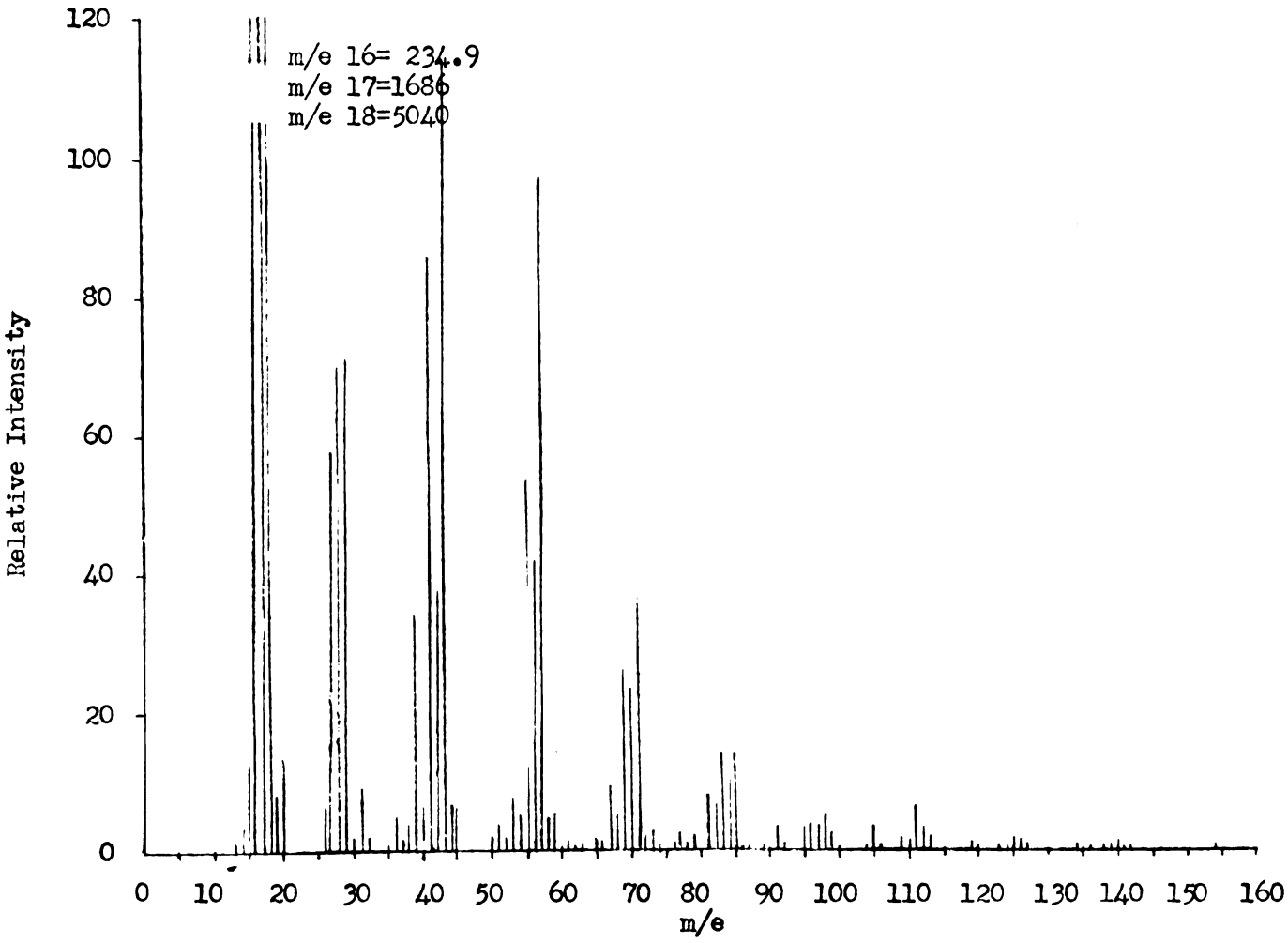

Fig. 18. Mass spectrum of isopentane extract of activated carbon -20°C to -30°C fraction

97, 98, 99, 100; 111; 112; 125, 126; 140; and 154. These m/e values indicate a series of singly oxygenated aliphatic hydrocarbons. The lack of any significant intensity of m/e 31 and 45 eliminates the possibility of any significant amounts of alcohols in these fractions. Ketones are very unlikely in these fractions. Ketones of 5 or less carbon atoms may be possible, but the spectra show no possibility of any significant amounts of ketones with more than 5 carbons. A comparison of figures 20, 21, and 22 emphasizes the differences in mass spectra between aldehydes and ketones. The parent peak (m/e indicating the molecular weight of the compound) for ketones stands alone and the m/e for the parent peak minus 1 is very small compared to the parent peak. For aldehydes, on the other hand, the parent peak minus 1 is not insignificant in comparison with the parent peak. This is due to the fact that the aldehyde hydrogen is much more easily removed by electron bombardment than are any of the hydrogens on the ketone molecule. Furthermore, the saturated and unsaturated aldehydes show large peaks at m/e 29 and 27 respectively, also present in the spectra of the sample fraction, whereas m/e 43 and 45 are expected to be of considerable size for ketones. Therefore, m/e 154, 140 and 139, 126 and 125, 112 and 111, 100 and 99, and 98 and 97 are very probably due to decenal, nonenal, octenal, heptenal, hexanal, and hexenal respectively. In order to prove the presence of these and other compounds present in these fractions, mass spectra of these compounds are necessary but were not all available.

The series of peaks in these spectra at m/e 57, 89, and 117 indicate the possibility of a small amount of esters in these fractions. The inability to obtain mass spectra of the more prominently suspected

compounds in these fractions made it impossible to verify the presence of esters. Water, of course, is the compound in greatest abundance in all of these fractions, as indicated by the size of m/e 18.

The mass spectrum in Figure 19 shows the room temperature to 0°C fraction is composed largely of air and isopentane. Peaks present in the spectrum of this fraction other than those due to air and isopentane are similar to the ones noted and discussed in the spectra of foregoing fractions.

Gas Chromatography of Atmosphere Samples

Conditions for Direct Analysis

Various operating conditions for gas chromatographic procedures were tried and the following conditions appeared to be best suited for direct analysis in controlled atmosphere storages. An oven temperature of 50°C was selected for operation. At this temperature any thermal destruction would be very unlikely and, since the compounds in question are volatile at 0°C, this temperature would be high enough for volatilization of the compounds in question. The hydrogen flow rate and carrier gas flow rate were set at 20 ml. per minute as suggested by the operating manual. The recorder chart rate of $\frac{1}{2}$" per minute was very satisfactory for this study. By trial and error a gas sample size of 10 μl was found most appropriate. These operating conditions were used for all gas analyses of storage atmospheres. A summary of the storage rooms used in this study is shown in Table I.

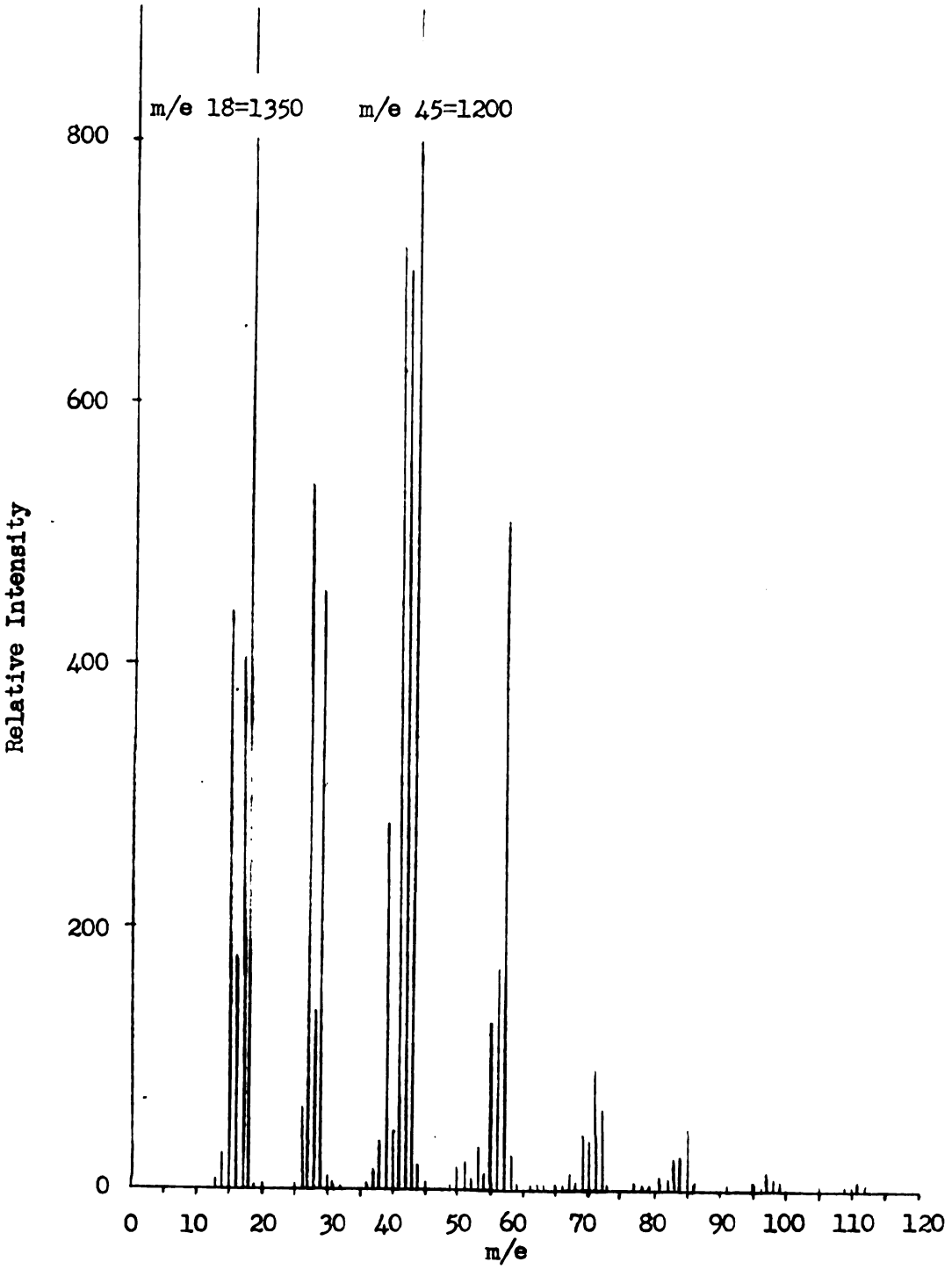

Fig. 19 Mass spectrum of isopentane extract of activated carbon room temperature to 0°C fraction

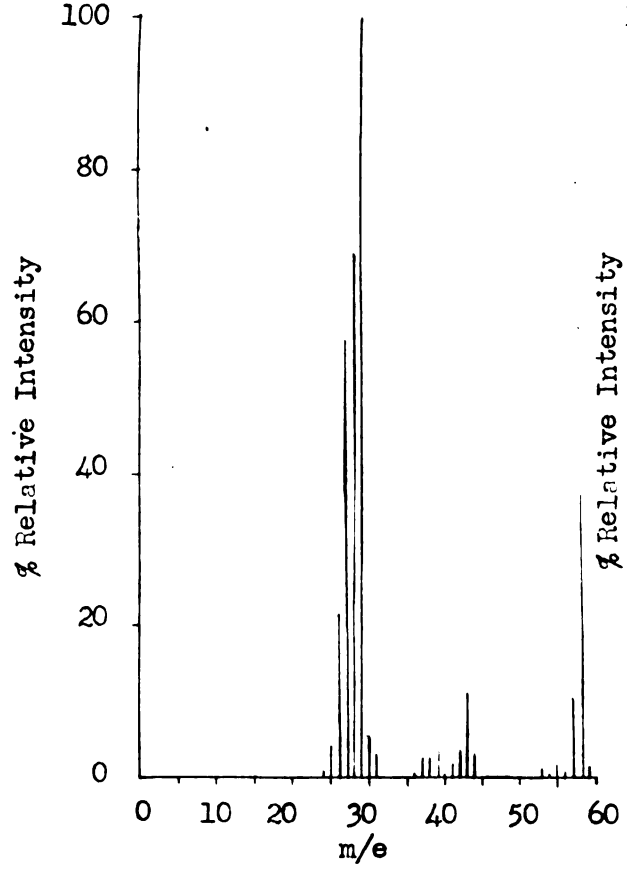

Fig. 20. Mass spectrum of propanal

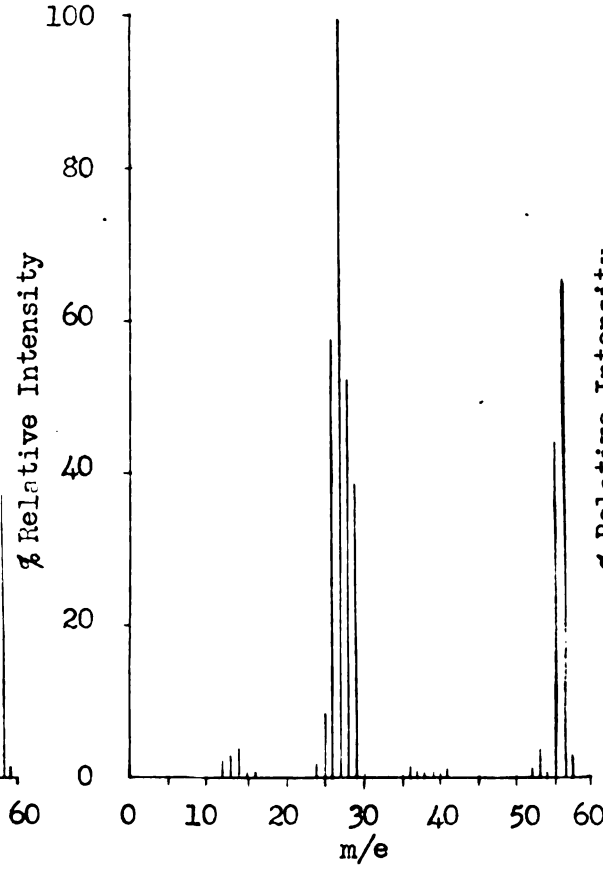

Fig. 21. Mass spectrum of propenal

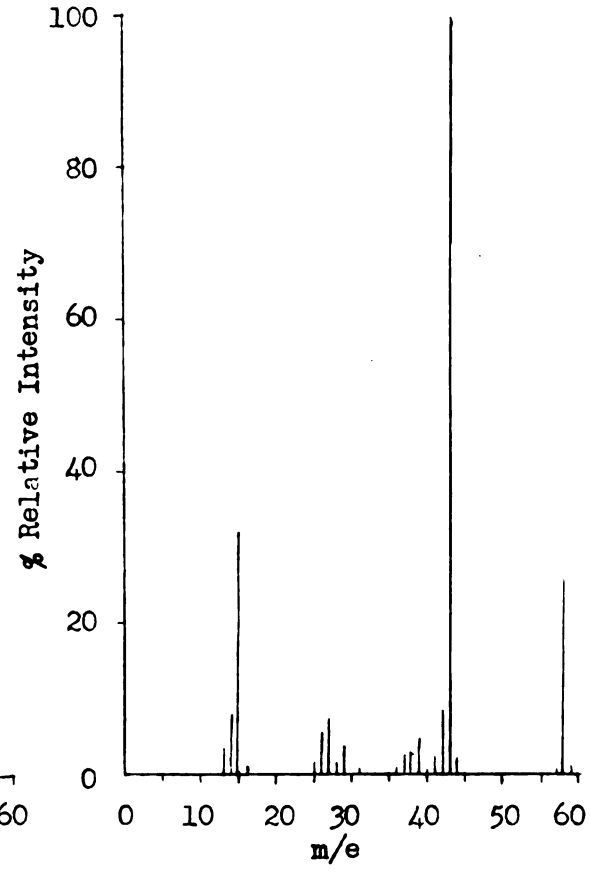

Fig. 22. Mass spectrum of acetone

TABLE I

STORAGE ROOM MANAGEMENT SUMMARY

ROOM NO.	VARIETY	DATE SEALED	DESIRED GAS CONCENTRATIONS OBTAINED	TYPE SCRUBBING UNIT USED	ACTIVATED CARBON USED	DATE OPENED
1	McIntosh	10/2/61	10/28/61	Water	Yes	1/15/62
2	McIntosh	10/10/61	11/8/61	Water	Yes	3/4/62
3	Red Delicious	10/21/61	11/21/61	Water	Yes	3/4/62
4	Jonathan	10/16/61	11/22/61	Water	Yes	2/14/62
5	Northern Spy	11/15/61	12/1/61	Water	Yes	After 5/1/62
6	McIntosh	10/10/61	11/5/61	Water	Yes	4/27/62
7	Jonathan and Golden and Red Delicious	10/28/61	11/28/61	Water	Yes	3/22/62
8	Jonathan and Golden and Red Delicious	10/30/61	12/9/61	Water	Yes	4/21/62
9	McIntosh	10/8/61	11/10/61	Caustic Soda	Yes	4/28/62
10	McIntosh	10/12/61	11/29/61	Caustic Soda	No	5/18/62
11	McIntosh, Jonathan, Rome Beauty, and Golden and Red Delicious	10/15/61	10/20/61	Water	No	5/4/62

Column Selection

Samples from three of the storage rooms were analyzed on the three different columns available. The three rooms from which these samples were taken were on the Michigan State University campus and readily accessible. These results are shown in Figure 23. The silicone column was eliminated due to its inability to resolve ethylene from the other peaks found in the atmospheres of rooms number 10 and 11. The castorwax column was selected for routine analysis of ethylene in controlled atmosphere storages because it gave better resolution of the peaks in Figure 23 than did the carbowax column.

A small amount of ethylene was added to the atmosphere samples from rooms number 10 and 11. Chromatograms were obtained from these samples before and after the addition of ethylene using the castorwax column. These results are shown in Figure 24. From the increased height of the first of the two peaks from each of the samples from rooms number 10 and 11 after ethylene addition, it was concluded that the first of the two peaks in the chromatograms from rooms number 10 and 11 are due to ethylene.

Chromatograms of samples from each of the eight storage rooms, numbers 1 through 8, at Belding Fruit Sales, Belding, Michigan, using the castorwax column are shown in Figure 25. A chromatogram of a sample of the same size, 10 μl, of air is shown in the same Figure, showing that air makes no contribution to the ethylene peak or anything else for samples of this size. All eight of the Belding storages show a single peak, as did room number 9, Figure 23, when chromatograms were obtained using the castorwax column.

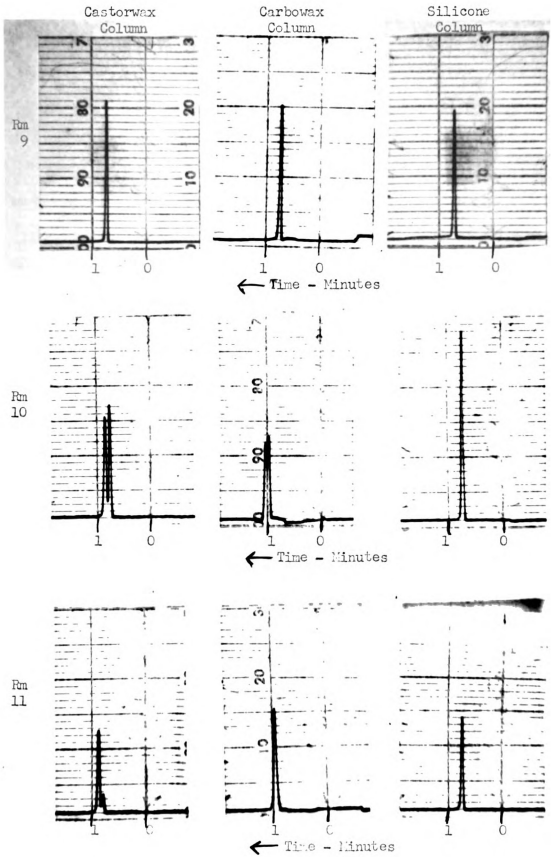

Fig. 23. Gas chromatograms of CA storage atmospheres

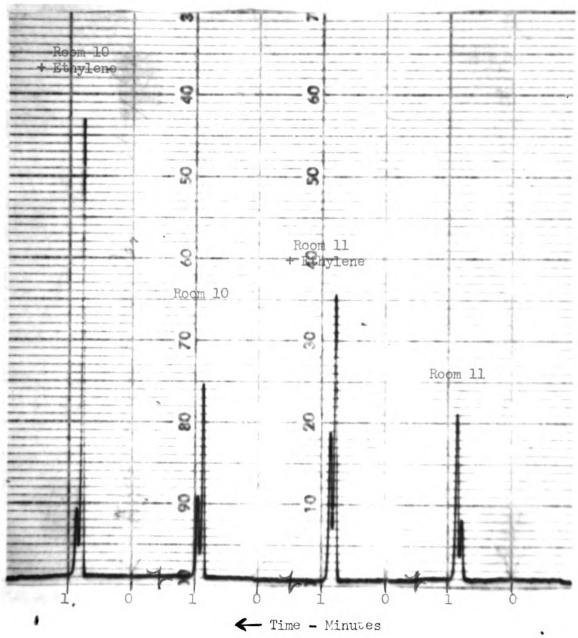

Fig. 24. Gas chromatograms of CA storage atmospheres and CA storage atmospheres plus ethylene

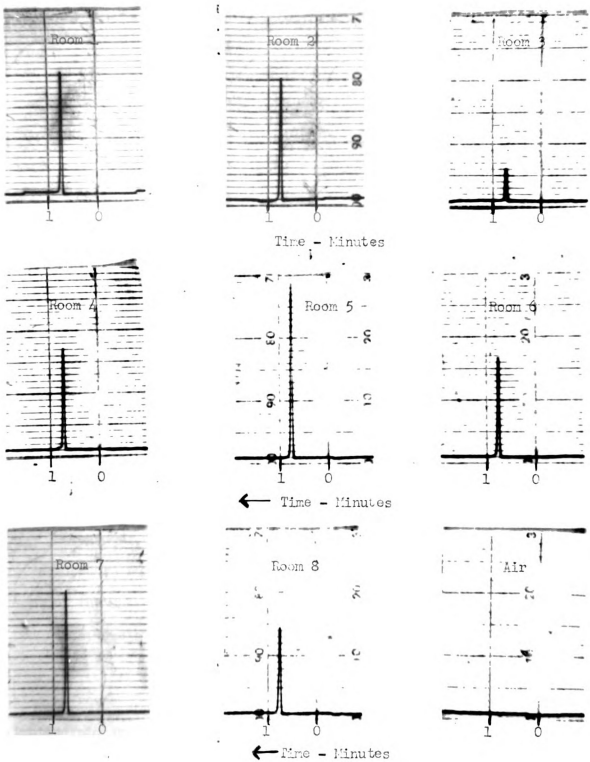

Fig. 25. Gas chromatograms of CA storage atmospheres and air

The gas chromatographic technique was found to be an excellent method for routine analysis of ethylene concentration in controlled atmosphere apple storages. Two characteristics of the method as described make it especially advantageous: first, a sharp peak is obtained for ethylene; and, second, the retention time for ethylene is less than one minute, making possible the analysis of many samples in a relatively short period of time. The sharpness of the ethylene peak makes it possible to use peak height as a measure of ethylene concentration rather than resorting to the area measurement method.

Identification of Second Peaks in Chromatograms of Rooms 10 and 11 Atmospheres

The chromatograms of atmospheres from rooms 10 and 11 both show a second peak. This characteristic is peculiar to these two rooms alone. In all the other nine rooms, ethylene alone is detected by the analysis method described above. It would be helpful to identify the compound or compounds responsible for the second peak in the atmosphere chromatograms of rooms 10 and 11.

Compounds possibly producing the second peak in the chromatograms of atmosphere samples from rooms 10 and 11 were considered. Reinforcement of the unknown peaks was achieved by adding a small amount of known compounds, one at a time, to the unknown sample. Ethylene was found to reinforce the first of the two peaks found in the atmospheres from storage rooms 10 and 11. Three compounds, propane, used in the controlled atmosphere gas generator, propylene, a homolog of ethylene, and Refrigerant 12 (dichlorodifluoromethane), the refrigerant used, were

found to enforce the second peak detected from the atmospheres of storage rooms 10 and 11, Figures 26 and 27.

By bubbling the atmosphere from storage room 10 through a solution of $HgClO_4$ (mercuric perchlorate), also used for ethylene analysis from apples, it was possible to obtain a sample of all unsaturated hydrocarbons present in the atmosphere. The addition of LiCl (lithium chloride) to the $HgClO_4$ solution causes the release of all absorbed unsaturated hydrocarbons. The gas chromatogram of the gases released from the $HgClO_4$ solution shows a single peak. This gas reinforced the first of the two peaks obtained by chromatographing the atmosphere of room number 10, Figure 28. From these observations it was concluded that the single peak seen in the gas chromatograms of the atmospheres from rooms 1 through 9 and the first of the two peaks found in rooms 10 and 11 are due to ethylene present in the atmosphere of the storage rooms. The second peaks (the ones not due to ethylene) detected from the atmospheres of rooms 10 and 11 consequently are not due to the presence of propylene in the storage room atmospheres.

An acetylene halide detection torch was used to determine the presence of Refrigerant 12 in storage room 10. The positive halide test lead to the conclusion that the second peak in the chromatogram was due to Freon 12 in storage room 10. In the case of room 11, the second peak in the chromatogram is believed due to a compound from the experimental CA storage atmosphere generator which was used with this storage room. The gas fed into the storage room from this generator produced this second peak when chromatogramed.

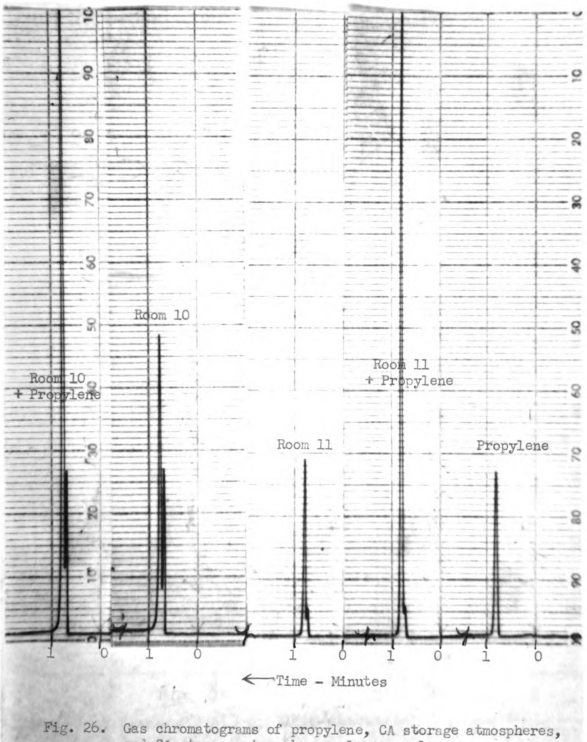

Fig. 26. Gas chromatograms of propylene, CA storage atmospheres, and CA storage atmospheres plus propylene

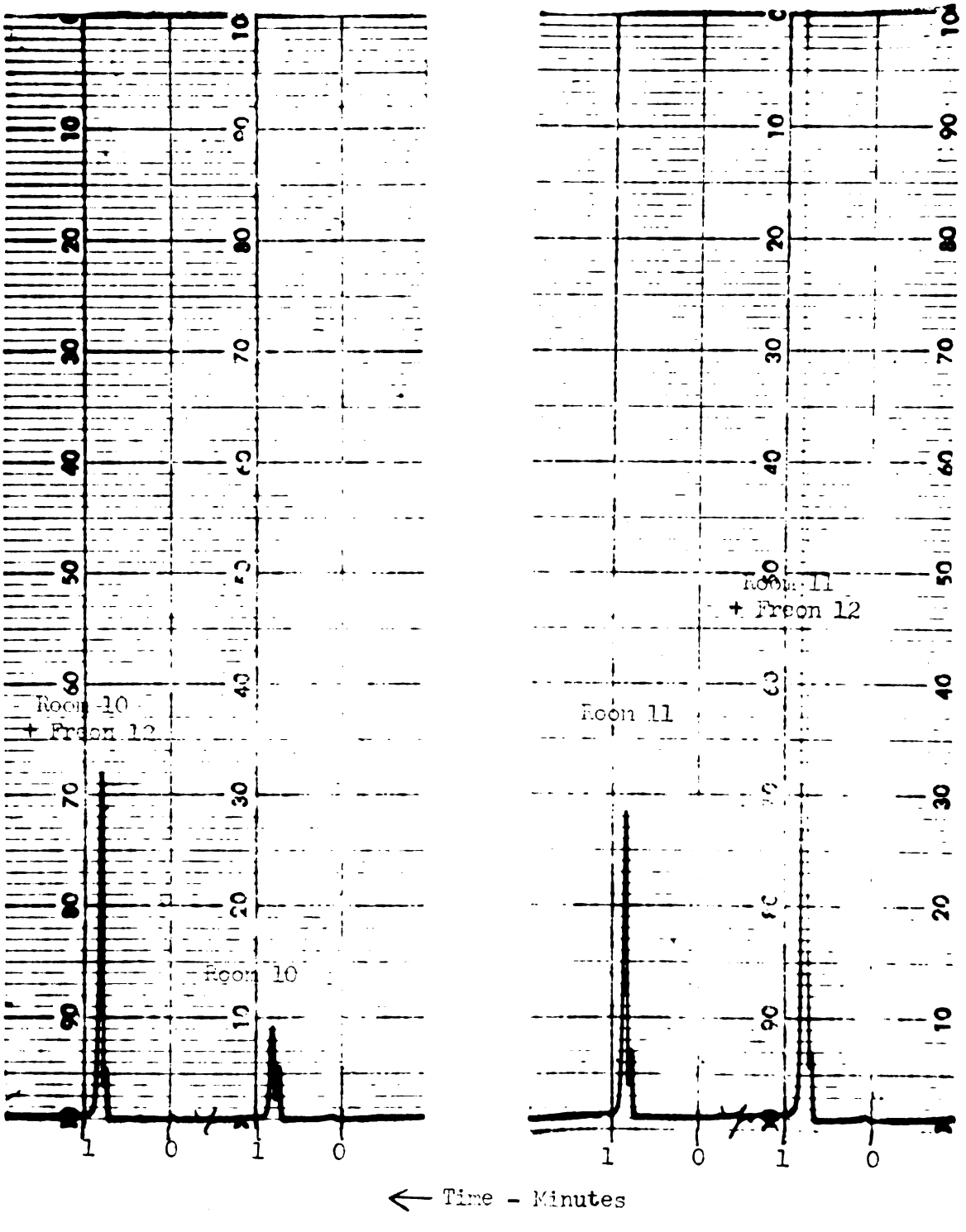

Fig. 27. Gas chromatograms of CA storage atmospheres and CA storage atmospheres plus Freon 12

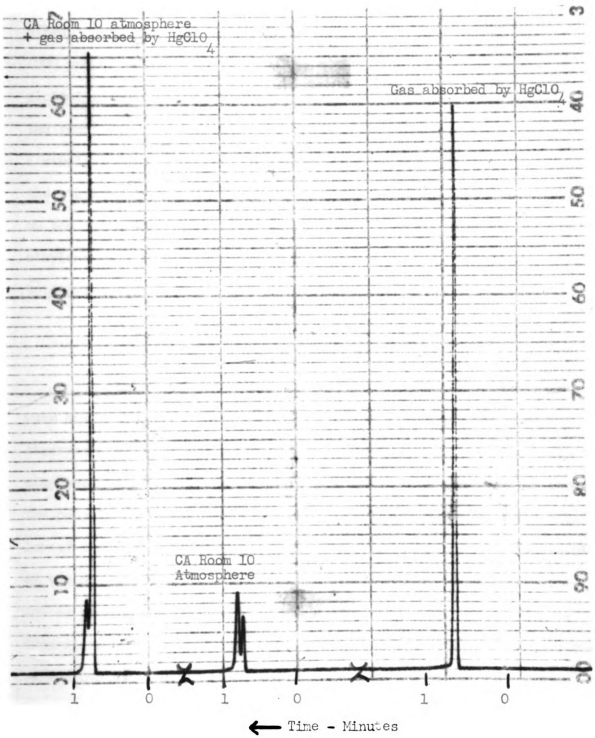

Fig. 28. Gas chromatograms of gas absorbed by $HgClO_4$, CA storage atmosphere, and CA storage atmosphere plus gas absorbed by $HgClO_4$

Standard Curve for Ethylene Concentrations in Air

A standard curve for ethylene concentrations in air was determined. Gas chromatograms of concentrations ranging from 100 ppm to 4000 ppm of ethylene in air were made at random. Peak heights were plotted against ethylene concentration to establish the standard curve. The resulting standard curve, Figure 29, is for all practical purposes a straight line. This straight line standard curve was used to determine the ethylene concentration of all storage atmosphere samples. A check on the chromatograph was made each month with known concentrations of ethylene in air for assurance that the sensitivity of the chromatograph to ethylene was not changing with time.

Gas Sampling Procedures

The atmospheres of several controlled atmosphere storage rooms were analyzed at intervals throughout the 1961-1962 storage season. Atmosphere samples were taken from the storage rooms in 4-liter polyethylene bags. Samples were taken from the storage gas-sampling tubes. The polyethylene bags were filled with the sample atmosphere and collapsed completely three times to flush out any residual air. The mouth of the polyethylene bag was gathered tightly around a rubber vaccine-bottle stopper and held in place by a stretched rubber band. A predetermined amount of gas was removed from the polyethylene sampling bag with a syringe and injected directly onto the gas chromatographic column for analysis.

Figure 30 illustrates the stable ethylene concentration of an atmosphere sample in a polyethylene bag obtained as described above

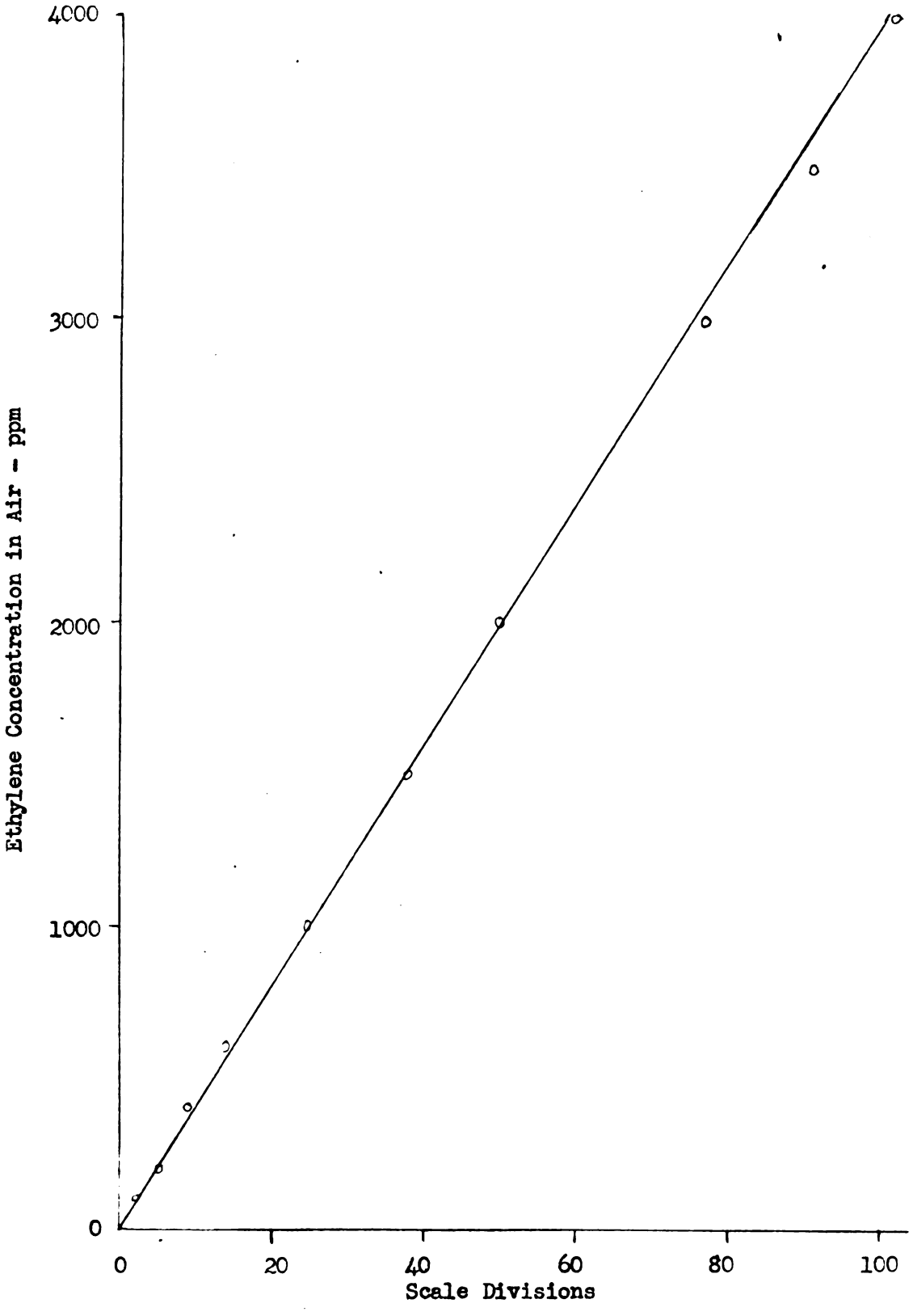

Fig. 29. Standard curve of ethylene concentration in air

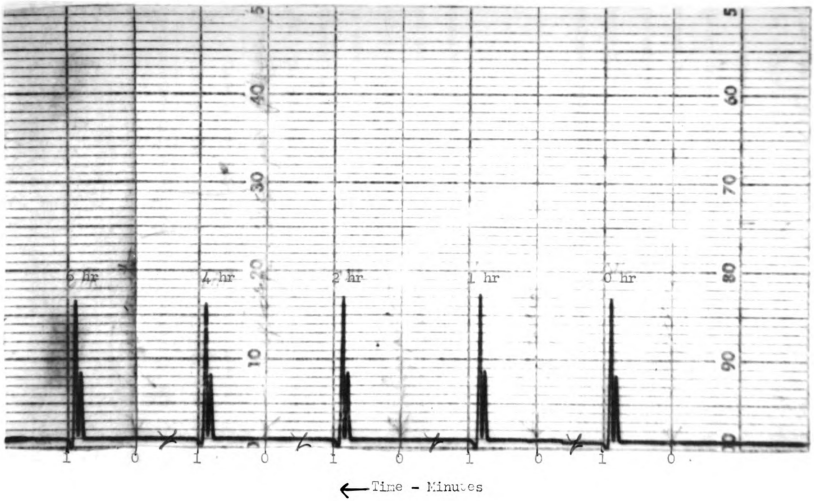

Fig. 30. Gas chromatograms of a sample of CA storage atmosphere at different times after collection

during a period of six hours. Ten μl portions were analyzed from this sample 0, 1, 2, 4, and 6 hours after collection. The constant height of the ethylene peak (first peak) can readily be seen from the figure. Since ethylene concentration is determined by peak height, no detectable change in ethylene concentration takes place in a sample collected in the manner described and held for a period of six hours. All samples used for ethylene concentration determinations were analyzed within four hours after collection. It is, therefore, evident that the samples when analyzed contained the same ethylene concentration as when collected.

Results

The ethylene concentration of eleven controlled atmosphere storage rooms was followed throughout the 1961-1962 storage season by sampling and analyzing the storage atmospheres as described above. The sampling dates are indicated by the points on the graphs. Since rooms 9, 10, and 11 were on the Michigan State University campus, they were sampled more frequently than rooms 1 through 8 which were located at Belding, Michigan. The management conditions of all eleven storage rooms are summarized in Table I.

Plots of ethylene concentration versus time were made for the eleven storage rooms studied (Figures 31, 32, and 33). The ethylene concentration increased steadily after sealing the room until it leveled off at a constant maximum level. After reaching constant concentration, no significant persisting change took place but remained the same until the storage room was opened. Although equilibrium ethylene concentrations were observed, not all the rooms leveled off at the same

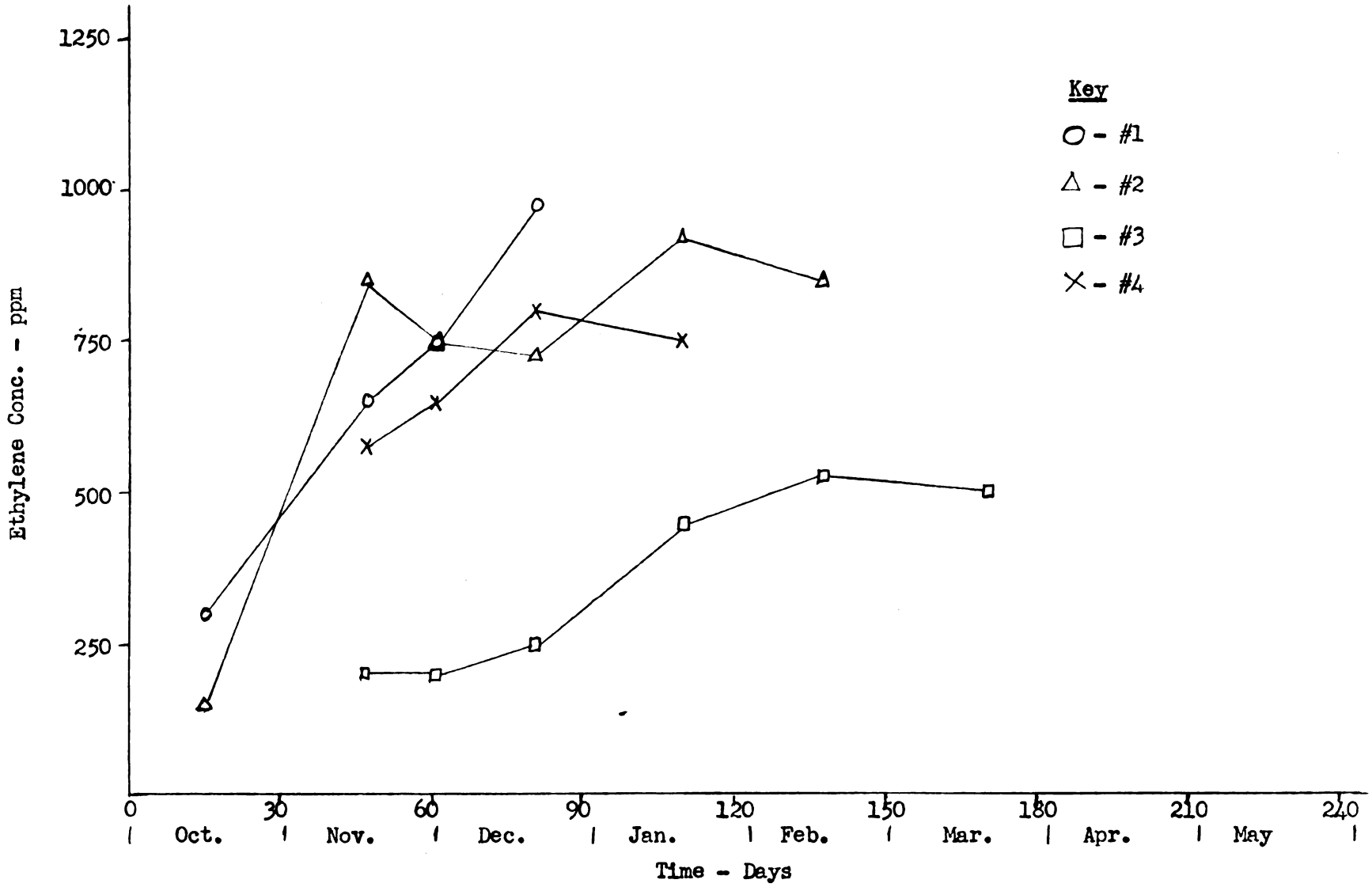

Fig. 31. Ethylene Conc. vs. Time — Storage Rooms 1-4

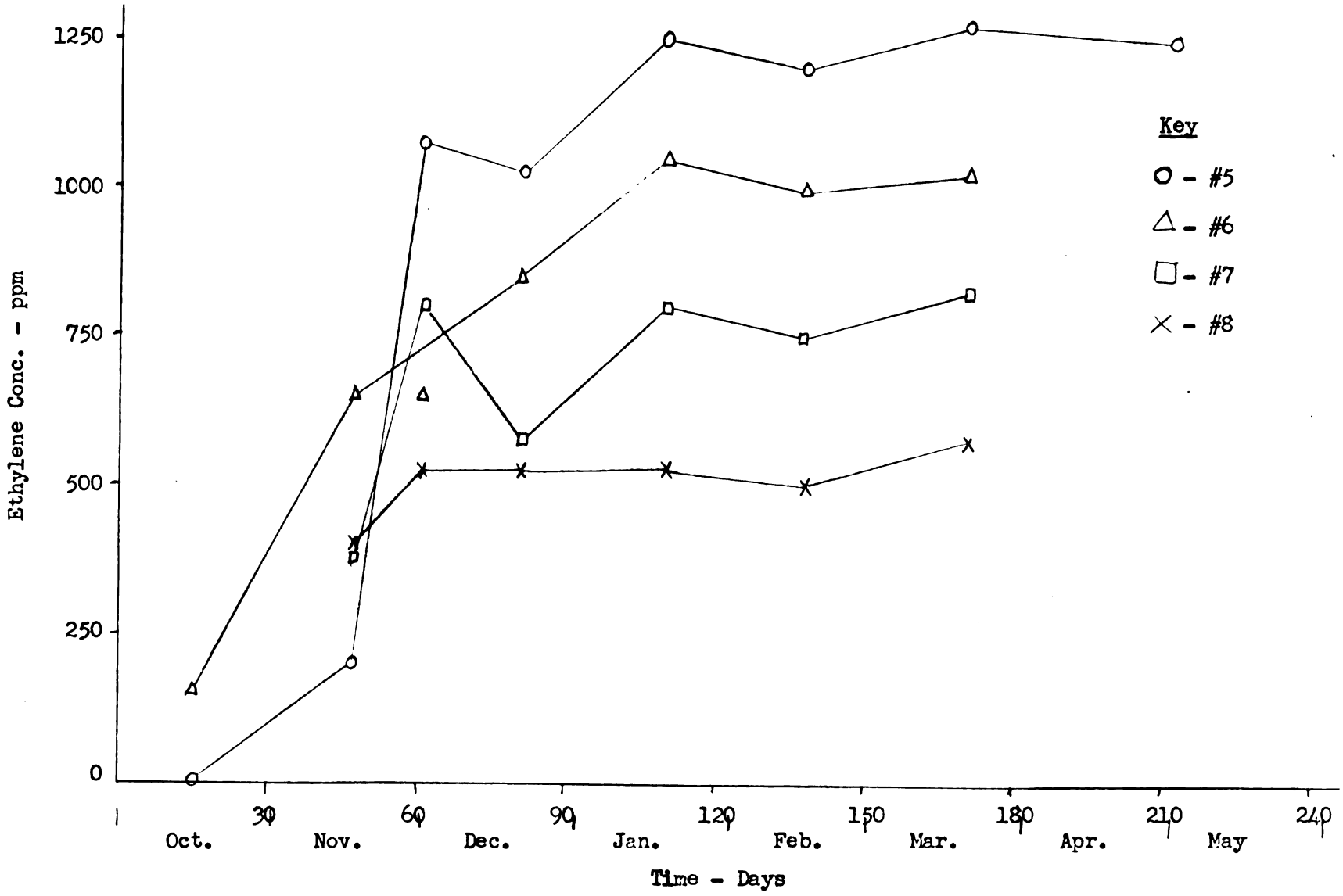

Fig. 32. Ethylene Conc. vs. Time -- Storage Rooms 5-8

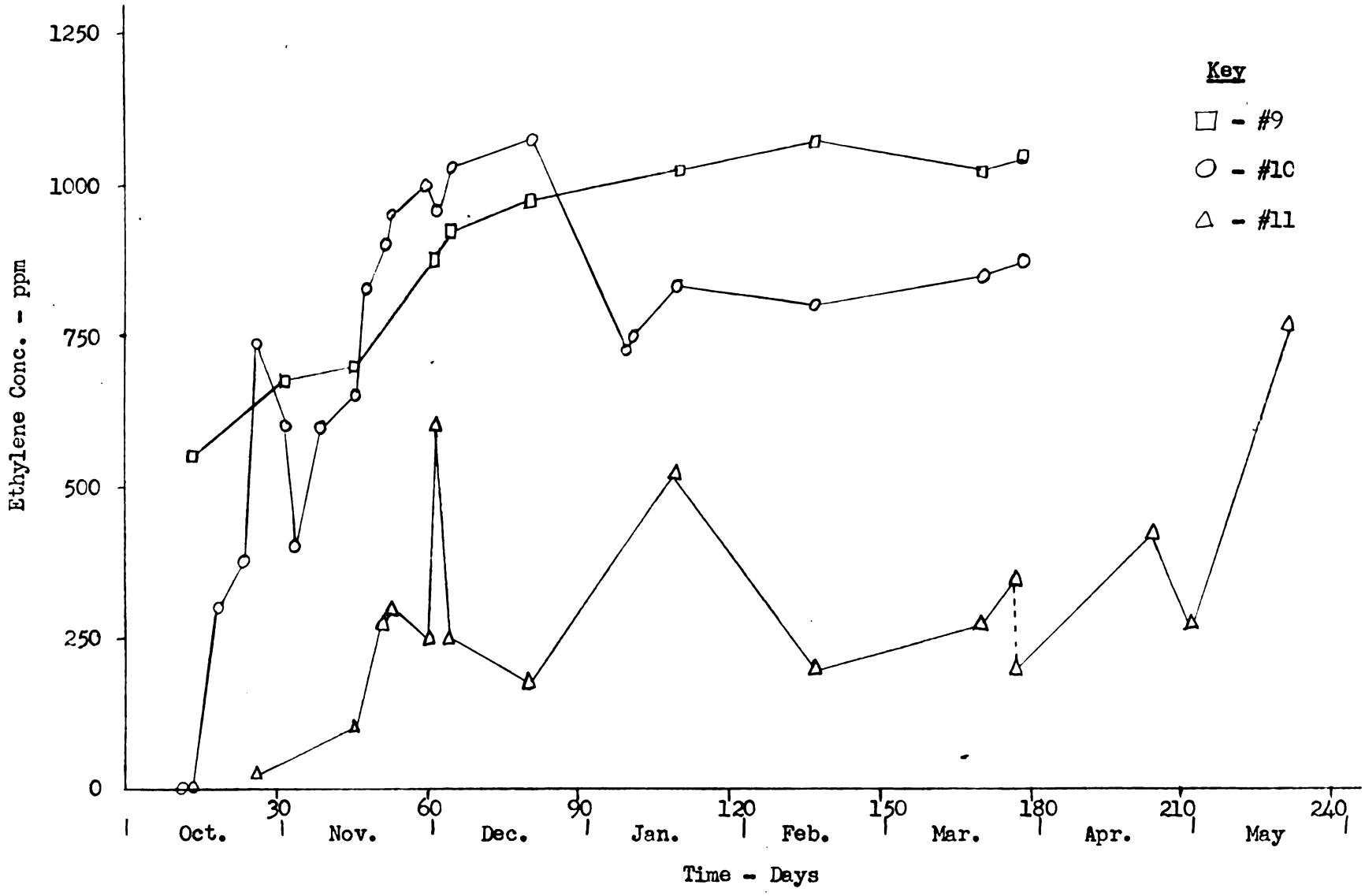

Fig. 33. Ethylene Conc. vs. Time — Storage Rooms 9-11

concentration. . The equilibrium ethylene concentration level reached in a storage room appears to depend on the variety of apples stored in the room. The lower ethylene concentration, approximately 250 ppm, found in room 11 is believed due to dilution of the atmosphere by the controlled atmosphere generator. The high equilibrium concentration, approximately 1250 ppm, was reached in room 5, containing Northern Spy apples, and the low, approximately 500 ppm, was in room 3, containing Red Delicious apples. Rooms with McIntosh apples reached an equilibrium concentration of approximately 1000 ppm and the room with Jonathan apples had an ethylene concentration of approximately 750 ppm. Rooms 7 and 8, containing mixtures of apple varieties, produced equilibrium ethylene concentrations between those found for the rooms containing the single varieties of apples.

No great difference in equilibrium concentration was observed between rooms using water or caustic soda absorption systems and/or the presence or absence of activated carbon. Rooms 1, 2, 6, 9, and 10 all contained McIntosh apples and all reached an equilibrium concentration of approximately 1000 ppm. Rooms 1, 2, and 6 had water absorption systems and rooms 9 and 10 had caustic soda absorbers. Rooms 1, 2, 6, and 9 contained activated carbon air purifiers while room 10 did not. Therefore, these two factors did not appear to affect the equilibrium ethylene concentration in controlled atmosphere storage rooms.

Room 11 was very irregular in its ethylene concentration. This is believed due to the irregular operation of this storage room. The storage room was opened from time to time to remove samples. The experimental controlled atmosphere generator was very erratic in its operation

and at times was not in operation. Although it was not determined, the irregularities observed could all be due to the irregular operation of this particular storage room.

Attempt to Determine Concentrations of Other Volatiles in Controlled Atmosphere Storage

The concentration of other storage volatiles during the storage season holds a great deal of interest in this investigation. Atmosphere samples were taken directly from storage room number 10 and injected onto the castorwax column. Figure 34 shows a chromatogram of a 20 ml gas sample from room number 10. Other than the large peak including both ethylene and Refrigerant 12, no other peak is detected. One hundred ppm of ethylene are easily detected from a 10 μl sample. Assuming equal sensitivity for other compounds as for ethylene, any other volatiles present must, therefore, be in concentrations of less than 0.05 ppm.

By recirculating the storage room atmosphere through a liquid nitrogen cold trap, the volatiles from various volumes of atmosphere from room number 10 were concentrated. After collection, the concentrate was held at -155°C and air gases, ethylene, and CO_2 were removed by vacuum pumping. Samples of the remaining water solution of volatiles and equal-sized samples of water and a 0.01 per cent solution of ethanol in water were chromatogrammed, Figure 35. One μl of the concentrate would be equivalent to 295 ml of atmosphere. This is based on the assumption that 100 per cent of the volatiles of interest was condensed in the concentrate, since, at the temperature and relative humidity of

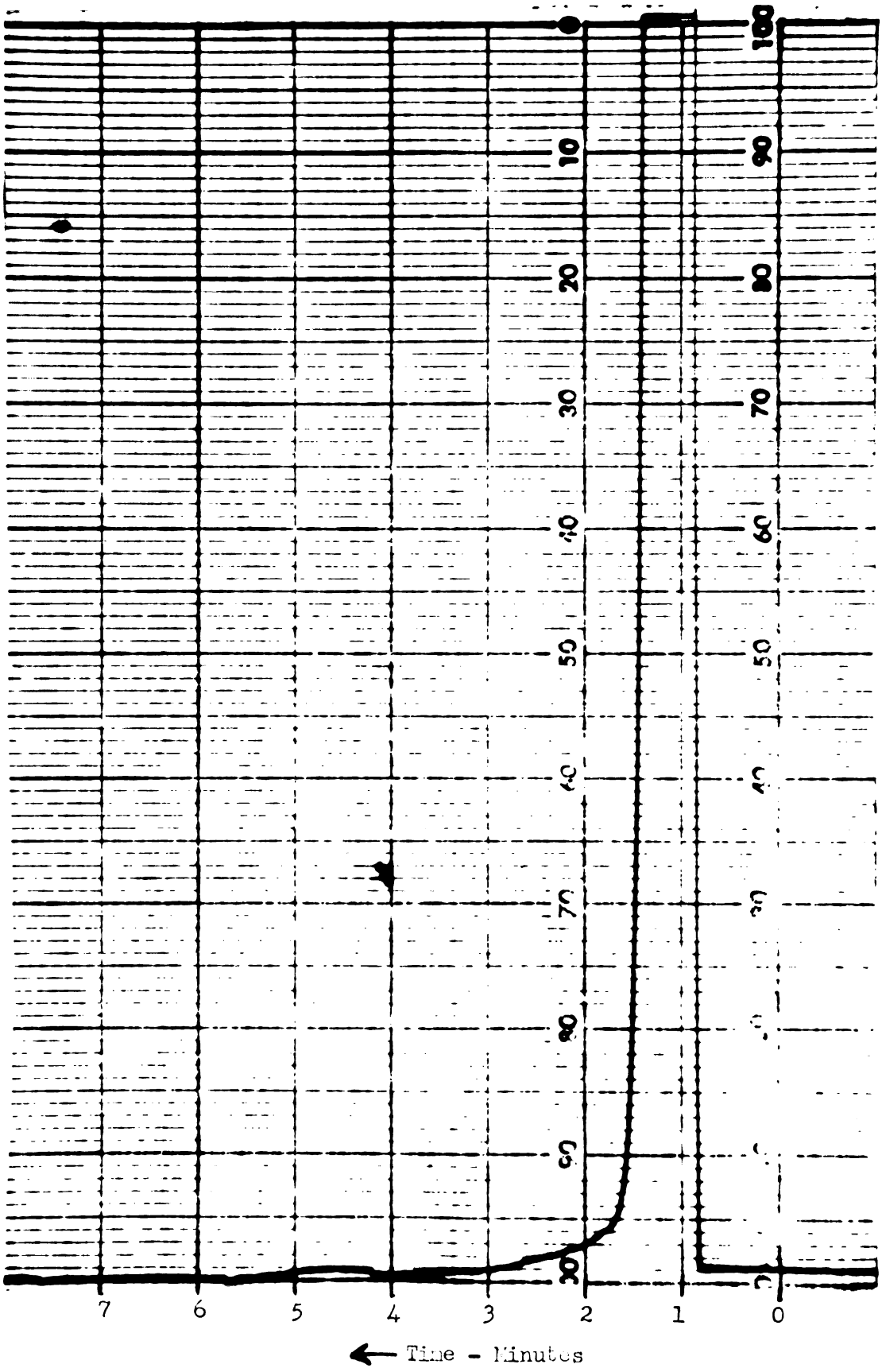

Fig. 34. Gas chromatogram of a 20 ml gas sample from storage room 10

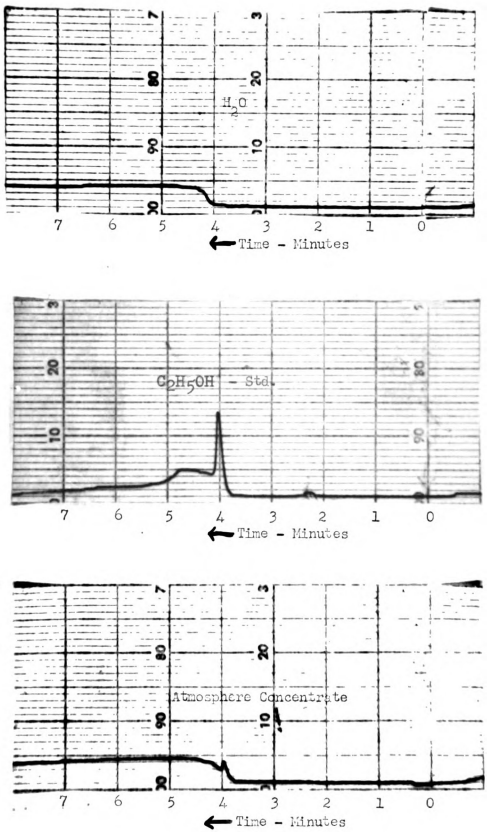

Fig. 35. Gas chromatograms of 2 μl of water, 2 μl of 0.01 per cent ethanol in water standard, and 2 μl of CA storage atmosphere

the storage room (100 per cent at 35°F), each 295 ml of atmosphere would contain approximately 1 μl of water.

A study of Figure 35 shows the 0.01 per cent standard ethanol solution produced four times the response as an equal amount of the storage room atmosphere concentrate. It was also noted that the single sharp peak produced by the concentrate has the same retention time as does ethanol and responds in direct proportion to the sample size. It was, therefore, concluded that the atmosphere concentrate contained ethanol in one-fourth the concentration of the 0.01 per cent standard, which would be 25 ppm. Air saturated with water vapor at 35°F contains 0.43 per cent water by weight. This is equivalent to a concentration of .107 ppm or 107 ppb of ethanol in the atmosphere of storage room 10.

Let it be assumed that it is possible to detect an ethanol peak using a 1 μl sample of atmosphere concentrate. This would mean that 295 ml of atmosphere would be necessary for ethanol detection at a level of approximately 100 ppb. Therefore, it can no longer be assumed that the gas chromatograph used is as sensitive for ethanol as it is for ethylene. One hundred ppm of ethylene in a 10 μl air sample, which is equal to 1.25×10^{-3} μg of ethylene, is easily detected. On the other hand, 25 ppm of ethanol in a 1 μl water sample, which is equal to 5×10^{-2} μg of ethanol, is necessary for detection. This indicates that, under the conditions employed, this chromatograph is more than 40 times as sensitive to ethylene than it is to ethanol. Also, under these conditions, a concentration of 2 ppm ethanol is necessary for detection in a 20 ml atmosphere sample.

Extreme difficulty encountered in reproducing these results made it infeasible to pursue this method for routine analysis of ethanol concentration in storage room atmospheres.

DISCUSSION

APPARATUS

The low-temperature high-vacuum system was used to obtain initial separations of complex mixtures of volatiles. Considering the complex nature of apple volatiles, low-temperature handling should minimize the possibility of chemical reactions among the compounds during concentration and separation procedures. Fewer chemical reactions, if any, would be expected from low-temperature handling as compared to handling the volatiles at higher temperatures. Low-temperature high-vacuum distillation also lends itself well to working with small quantities of materials and to preparation for analysis by mass spectrometry.

Mass spectrometry is one of the methods used to identify volatiles in this study. This method of identification is sensitive, rapid, simple, and direct. Merritt et al. (1958) identified ten compounds in a sample preparation from irradiated beef from a total of approximately $1x10^{-5}$ moles of material. The unknown volatiles are discharged directly into the instrument for analysis without any previous chemical alterations. This feature minimizes the possibility of chemical changes or destruction of the unknown compounds. Mass spectrometry makes possible the identification of the individual volatile compounds in a mixture provided the mixture is not too complex, in which case only a few of the total compounds may be identified due to interfering mass spectra of the compounds. In such a case, separation of the complex mixture into less complex fractions would be necessary for complete identification. Positive identification by mass spectrometry requires the mass spectrum of

the compounds being identified. Tentative identifications of compounds can be made from mass spectra from the physical and chemical knowledge of compounds without the known spectra of the compounds. Specific types of compounds, such as alcohols, sulfur, or nitrogen compounds, can be deduced present from characteristic peaks of mass spectra.

Gas chromatrography was quite useful in obtaining a pattern of compounds present in a sample using the three extraction methods. Differences in patterns indicate different compositions of the samples, Figures 11, 12, and 13. This technique was very useful for following the ethylene concentration in storage atmospheres. The hydrogen flame ionization detector was an important feature in this work due to its insensitivity to normal amounts of air gases, CO_2, and water and high sensitivity to organic compounds of interest in this study.

MASS SPECTRA OF APPLE STORAGE ATMOSPHERE

Identification of apple volatiles from mass spectra of storage atmosphere without previous concentration was unsuccessful. This indicates the concentration of any single volatile in apple storage atmospheres below an estimated 0.1 per cent. Concentration of the volatiles, followed by fractionation by LTHV distillation, was necessary in order to identify ethylene, ethanol, and acetaldehyde by mass spectrometry. The vapor pressure characteristics of these compounds made it possible to separate them from the air gases, carbon dioxide, and water present in the atmosphere by LTHV distillation and thus permitted their identification by mass spectrometry.

A greater percentage of acetaldehyde was expected to be separated from water by LTHV distillation than ethanol, due to the greater volatility of acetaldehyde. The fact is also shown by the fractions in which these two compounds are most prevalent, acetaldehyde being in greater concentration in the -125°C to -100°C fraction and ethanol in a greater amount in the -100°C to -78°C fraction. Any other volatiles with similar vapor pressure characteristics are either not present or present in lower concentrations than either of these two compounds.

ANALYSIS OF EXTRACT FROM ACTIVATED CARBON

Solvent extraction appears to be a better method of recovering volatiles from activated carbon than removal by heating. The solvent extract had a characteristic apple odor, while the compounds removed by heat had a strong, unpleasant acrid odor not resembling apple odor at all. It is probable that the heat necessary to drive the volatiles off the activated carbon causes a degradation of the apple volatiles. This theory is supported by the gas chromatograms obtained from the extracts. The chromatograms of the solvent extracted volatiles show compounds being eluted from the column as much as an hour after injection. For exactly the same chromatographing conditions, all compounds were eluted from the column after 35 minutes for the volatiles removed by heat. This suggests that the compounds of higher molecular weight obtained by solvent extraction are not present in the extraction obtained by heating the activated carbon. The chromatograms show little similarity even among the lower molecular weight compounds. This is a good indication that much of the material shown in the lower molecular weight region of

the chromatogram of the volatiles extracted by heating is due to degradation products of higher molecular weight compounds.

Two differences were observed between the chromatograms of the volatiles extracted by isopentane and diethyl ether. The large peak near the beginning of the chromatogram from the diethyl ether extracted volatiles is due to diethyl ether. The higher volatility and greater physical inertness of isopentane enabled better separation of this solvent from the extracted volatiles than did diethyl ether. With the exception of the peaks obscured by the diethyl ether peak, the peaks in both chromatograms exhibit identical retention times, indicating identical compounds. A second difference is that the relative ratios of amounts of the different compounds differ between the two chromatograms. This fact cannot be used as a criterion for concluding that one of the two solvents is better in this extraction than the other because the relative ratios of the compounds as they exist on the activated carbon were not known. By the same token, it cannot be concluded that all of the compounds are at least partially extracted from the activated carbon. To arrive at such a conclusion would require extraction by many different types of solvents, chromatographing by many different types of columns and column systems, and identification of all the compounds obtained and artifacts formed from this long, involved investigation.

The validity of the assumption that the compounds adsorbed by activated carbon are a valid representation of volatiles produced by apples can be attacked from several standpoints. Firstly, storage room odors produced by microorganisms in the storage room can be adsorbed on activated carbon. Secondly, all compounds do not have the same affinity

for activated carbon, or any adsorbent. It is probable that the compounds present in apple storages are not adsorbed in the same proportions in which they are present in the storage room and possible that some compounds are not adsorbed in any detectable amount. Thirdly, there is evidence that reactions of some compounds can take place while adsorbed on activated carbon. The actual determination of the effects of these possible sources of error could be determined by recovery experiments using compounds both identified as apple volatiles and believed to be produced by apples.

The combination of apple-like and foul, musty, or moldy odor of the extract indicates the presence of compounds produced by both apples and molds present in apple storages. The musty odor was probably responsible for some of the peaks of the chromatograms. Chemical reactions are not likely to any great extent, considering the low-temperature conditions used in the handling of the activated carbon and treatments of the extract. Therefore, it is quite conceivable that most of the peaks on the chromatograms of the solvent-extracted volatiles are due to volatiles produced by apples.

Effective separation of the isopentane extract of apple storage volatiles was not achieved by LTHV distillation. Mass spectra of the fractions obtained by LTHV distillation, by and large, show the same characteristic peaks. Much of this similarity in mass spectra is believed due to absorption of the volatiles by the stopcock grease used in the LTHV system. This would tend to redistribute the volatiles into all of the fractions after separation of the volatiles by LTHV distillation. The mass spectra from these fractions were, nevertheless, very

useful in the tentative identification of some volatiles. The lack of mass spectral patterns of the more abundant, greater molecular weight compounds believed present prevented definite mass spectrometric identification of these and other more abundant lower molecular weight compounds present.

The identification of the less abundant volatiles extracted from activated carbon by mass spectrometry requires effective separation of these from the more abundant volatiles. This can probably be accomplished by chromatographic separation of a large sample of extracted volatiles and collection of the fractions as they are eluted from the column. Mass spectra of the less abundant volatiles could then be obtained since there would be no interfering spectra from the more abundant volatiles. The use of a time-of-flight mass spectrometer would eliminate the necessity of collection of the fractions after elution. Elution of the column directly into a time-of-flight mass spectrometer would enable the instantaneous acquisition of the mass spectrum of each fraction as it was eluted from the column.

ETHYLENE CONCENTRATION IN CONTROLLED ATMOSPHERE STORAGES

In the eleven controlled atmosphere storage rooms studied, the ethylene concentration increased rapidly at first and then leveled off to a relatively constant concentration. This relatively constant ethylene concentration was called the equilibrium ethylene concentration. At this equilibrium concentration, the amount of ethylene being produced by the apples is equal to the amount being removed from the storage room.

Removal of ethylene from the conventionally operated controlled atmosphere storage room could have been done in only two ways. The first way, and perhaps the most important, was by limited ventilation, which is necessary to replace the oxygen used up in respiration. The other possible way was by scrubbing, necessary in removing the excess carbon dioxide produced by respiration. The rates of oxygen consumption and carbon dioxide production in conventional controlled atmosphere storages are a result of respiration. Therefore, the amount of ventilation and scrubbing necessary to keep the oxygen and carbon dioxide concentrations constant in a storage room are directly proportional to the respiration rate of the apples. The possible ways of removing ethylene from the storage and the constant concentration of ethylene found in the storage room indicate that ethylene production must be proportional to respiration rate. This is in agreement with post climacteric studies on apples (Smock and Neubert, 1950).

The ethylene concentration did not assume the same concentration in all of the storage rooms. Room 5 came to equilibrium at an ethylene concentration of about 1250 ppm; rooms 1, 2, 6, and 9, at a concentration of about 1000 ppm; rooms 4, 7, and 10, at a concentration of about 750 ppm; rooms 3 and 8, at a concentration of about 500 ppm; and room 11, at a concentration of about 250 ppm. These equilibrium concentrations are correlated with the varieties of apples stored in the storage rooms. Room 5 contained Northern Spy apples; rooms 1, 2, 6, and 9 contained McIntosh apples; room 4 contained Jonathan apples; room 3 contained Red Delicious apples; rooms 7 and 8 contained a mixture of Jonathan, Red Delicious, and Golden Delicious apples.

In room 2, containing McIntosh apples, the equilibrium ethylene concentration is slightly less than the other rooms which contained McIntosh apples. This is believed to be due to the fact that the scrubbing units on rooms 3 and 4 were not operating properly, thus forcing the part-time use of the scrubbing unit on room 2 on these rooms. As a result, some mixing of the atmospheres of these three rooms took place. This would not only tend to lower the ethylene concentration in room 2 but also increase the ethylene concentration in rooms 3 and 4.

A greater proportion of Jonathan apples in room 7 than in room 8 could account for the difference in the equilibrium ethylene concentrations in these rooms. The sharp irregularities in ethylene concentration in rooms 1 through 10 is assumed to be the result of sampling before or after limited ventilation of the storage room. No reasonable explanation could be found for the lower equilibrium ethylene concentration in room 10 as compared with the other storage rooms containing McIntosh apples. These observations strongly support studies (reported by Smock and Neubert (1950)) showing that a variation of the amount of ethylene produced per unit amount of oxygen used in respiration exists among apple varieties. No difference in equilibrium ethylene concentration was observed between storage rooms employing water scrubbers and those employing caustic soda scrubbers. Therefore, if these carbon dioxide absorbers have any effect on ethylene concentration, the effect is very similar for each system.

Storage room 11 exhibits the lowest equilibrium ethylene concentration of the eleven rooms studied. This is due to the controlled

atmosphere gas generator used with this room causing a constant dilution of the room atmosphere. The two unusually high concentrations of ethylene found in this room occurred when the generator was not in operation. Since lowering of ethylene concentration is believed due to dilution of the controlled atmosphere generator, the same is believed to hold true for all of the volatiles produced by the apples in the storage room.

CONCENTRATION OF OTHER VOLATILES

Perhaps the most convincing argument that volatiles are present is the odor that is perceived from the apple storage atmospheres. Although proving the presence of volatiles, odor tells little about the concentration of apple storage volatiles. Volatile concentration studies using sensory methods are based on odor thresholds. Odor thresholds vary greatly for different substances. Some substances may be detected by odor in concentrations of fractions of parts per billion, whereas some are odorless at all concentrations. Nevertheless, most of the types of compounds believed present in apple storage atmospheres (acids, alcohols, esters, aldehydes, and ketones) would be expected to have their individual thresholds of concentrations in parts per million or parts per billion. Odor thresholds of combinations of volatiles bear no relationship to odor thresholds of the individual components. For this reason and the fact that the composition of apple storage volatiles has not yet been resolved, it is still impossible to use odor thresholds as a study of apple storage volatile concentrations.

A more meaningful indication of apple storage volatile concentrations may be derived from a consideration of sampling and concentration

procedures and the results obtained from these procedures. By the reduction of ceric sulphate after proper absorption (Gerhardt, 1950), the concentration of volatiles has been expressed as milligrams ceric sulphate reduced per cubic foot of storage atmosphere. By separative absorption techniques, ethylene has been separated from other storage volatiles in this type of analysis. In the case of ethylene, the ceric sulphate reduction method furnishes data from which ethylene concentration may be calculated, since the reducing power of ethylene can be determined for the system; but the non-ethylenic volatiles have not all been identified, and the reducing power of this mixture is not known. Consequently, the concentration of any one compound of the mixture or of the whole mixture cannot be precisely determined from the ceric sulphate reduction method.

Concentration methods using absorbers, adsorbers, and cold trapping have been used to collect apple storage volatiles. These methods have been used chiefly for identification rather than concentration determination because of the question of the quantitative recovery of the volatiles. Gas chromatography has been shown to be very useful in ethylene concentration studies, but more appropriate techniques must be developed before this method can, by itself, provide a complete study of apple storage volatile concentrations.

By combining cold trapping and gas chromatography, the only volatile found in this study was ethanol. This was the only compound determined by chromatographing the cold trap condensate of apple storage atmospheres. The low concentration of ethanol in the storage room atmospheres is supported by the mass spectra of the storage room atmospheres.

The actual value of 100 ppb of ethanol in the storage room atmosphere is subject to question. Inconsistent results were obtained when duplicate determinations were made. Although recovery of the water vapor from the atmosphere appears to be quantitative, the same does not necessarily hold true for ethanol. Recovery experiments using these methods yield results as low as 20 per cent recovery. Loss of volatiles could also take place when the more volatile air gases and CO_2 were being pumped out of the bottle.

Since 100 ppb of ethanol was detected in storage room 1C by chromatographing cold trap concentrates of the atmosphere, at least this concentration is reached. Since ethanol was not detected by direct chromatographing of the atmosphere in storage room 10, it was concluded that the ethanol concentration in room 10 was less than 2 ppm. It must be borne in mind that storage room 10 contained no activated charcoal. Due to the great affinity of activated charcoal for ethanol, lower concentrations of ethanol would be expected in storage rooms containing sufficient activated charcoal. It would, therefore, appear that the non-ethylenic volatiles exist in concentrations of a few ppm or in ppb each.

Practices and equipment used on controlled atmosphere storages will undoubtedly affect the concentrations and pattern of volatiles in storages. The effect produced by the presence of activated carbon is demonstrated by Gerhardt (1950). Although activated carbon caused no significant change in ethylene concentration, it greatly lowered the ceric sulphate reducing power of the storage atmosphere. This is a good indication of the removal of volatiles from the storage atmosphere.

The differences in affinity of activated carbon, or of any other adsorbent or absorbent, for the volatiles present in apple storage atmospheres cannot be determined until the volatiles have been identified. Recommendations for minimum amounts of adsorbents or absorbents used to obtain desired effects in the storage rooms have been made. These minimum recommendations indicate saturation of the adsorbents or absorbents with storage volatiles or an equilibrium condition set up between the "scavengers" (such as activated carbon and oiled paper) and the storage volatiles when insufficient amounts of the scavengers are used.

Volatiles may also be removed from controlled atmosphere storages by equipment used for this type of apple storage. Any volatile compound, such as an acid, that could form a non-volatile compound with sodium hydroxide will be removed, at least in part, by a caustic soda absorption system used for carbon dioxide control. The detectable apple odor of the gas desorbed from the water carbon dioxide removal equipment indicates at least partial removal of volatiles from controlled atmosphere apple storages by water absorption systems. Where controlled atmosphere generators or bottled nitrogen are used to acquire and maintain controlled atmosphere conditions, dilution of the storage volatiles would be inevitable. The extent of dilution would depend upon the amount of nitrogen or generator gas being fed into the storage room.

Volatile differences due to apple variety have been shown to exist by the effects of one lot of fruit on another. The different aromas of apple varieties also indicate differences of volatiles. Apple volatile analyses have shown good evidence for these differences. The effect of storage practices and equipment on concentrations of

apple storage volatiles has not yet been fully shown. Failure of previously used methods to accomplish this feat emphasizes the need for more appropriate methods of analysis. Mass spectrometry appears to be the ideal method for qualitative analysis of apple storage volatiles. Collection and preparation of the volatiles for mass spectral analysis are critical steps in this procedure. The steps in this procedure must exclude the presence of large quantities of water with respect to other apple volatiles. Preliminary separations with gas chromatography would be helpful in obtaining a more complete qualitative analysis by mass spectrometry. Quantitative analysis of apple storage volatiles requires more investigation into techniques. Multiple column gas chromatography techniques with detectors sensitive to organic compounds in the range of parts per million to fractions of parts per billion would prove very useful. Quantitative analysis by less sensitive methods would require quantitative concentration of volatiles before analysis. Recovery experiments should be carried out on any of these concentration procedures before using them. This would permit quantitative analysis with less sensitive gas chromatographic detectors or mass spectra used in conjunction with volume-pressure data.

SOLUBILITY AND VOLATILITY

Two physical properties of the compounds which determine their activity are solubility and volatility. Solubility is the ability of the molecules of a substance to become dispersed in among the molecules of another liquid or solid substance. Volatility is the tendency of the molecules of a substance to escape from the liquid or solid state

into the gaseous state. Generally, water solubility of an organic compound is proportional to its volatility. The Handbook of Chemistry and Physics (1953) shows that methanol, ethanol, acetaldehyde, propanol, and acetone are all very soluble in water. Butanol, propanal, and amyl acetate are slightly soluble in water, and amyl propionate, hexanal, and geraniol are listed as insoluble in water. The vapor pressures exerted by some of these compounds at 0°C are found to be:

Compound	Vapor Pressure in mm of Hg
acetaldehyde	>200
acetone	85
methanol	38
ethanol	13
propanol	5
butanol	1
geraniol	< 1

Damage caused to the apples by the more volatile compounds could be controlled by reducing the partial pressure of the more volatile compounds in the surrounding atmosphere, thus causing the escape of these compounds from the aqueous intercellular solution of the apple. Damage to apple tissues from the more soluble of the apple volatiles, such as acetaldehyde, acetone, and ethanol, would be expected to appear throughout the apple flesh with the most damage to the flesh most remote to the atmosphere. Such storage disorders as internal breakdown, mealy breakdown, and internal browning, if affected by volatiles, would be affected to a greater extent by the more soluble volatiles than would the surface disorders.

The less water-soluble compounds, such as amyl propionate, hexanol, and geraniol, would have a tendency to leave the aqueous phase of the intercellular spaces of the apple tissues. Since apple fruits respire,

heat is generated by them. This heat of respiration is removed from the surface of the apple by cold air circulation. In order for heat to move from within the apple to the apple surface where it is removed, a temperature gradient is necessary. Therefore, the apple flesh must be at a higher temperature than the apple surface. Generally, since vapor pressure of a material increases with temperature, a concentration gradient of these volatiles could exist between the warmer and cooler portions of the apple fruit. The temperature gradient is indeed small but exists continuously in the same direction. Since mass transfer will proceed with the heat energy transfer, this mechanism could concentrate certain volatiles in the surface layers of the apple tissue.

On reaching the surface of the apple, the non-water-soluble compounds would have to overcome three difficulties (low volatility, the skin barrier, and the wax cuticle barrier) in order to escape from the apple surface into the surrounding atmosphere. Because of their low volatility at cold storage temperatures, these compounds may collect on the apple surface. The apple skin may provide a barrier to slow down the escape of the volatiles. The wax cuticle may be a more difficult barrier to overcome than the skin. The apple cuticle is composed of waxes which may act as an organic solvent for apple volatiles. The wax cuticle may also act as an adsorbent for organic volatiles. Although there is a lack of data for proof, it seems quite plausible from the foregoing argument that a high enough concentration of apple volatiles may be trapped on the apple surface to cause damage to the tissues in that immediate area. Scald, Jonathan spot, and other spot disorders could be effected by these entrapped apple volatiles.

SOURCE OF VOLATILES

Apple storage volatiles may come from any of several sources. Molds or other microbial life are believed to contribute foul odors to apple storages. Building materials, equipment, or containers may also contribute to apple storage volatiles. Nevertheless, the volatiles generated by the apple fruit itself are of greatest interest here.

Apple fruits produce organic acids, alcohols, aldehydes, ketones, and esters. Ethanol and acetaldehyde are undoubtedly the products of anaerobic respiration. The lack of sufficient oxygen would cause a shift in the energy producing reactions of the electron transport system and the Krebs cycle, causing a decrease in the utilization of pyruvate which is produced by glycolysis of carbohydrates. When the rates of the Krebs cycle reactions have been decreased to such an extent that all of the pyruvate produced by glycolysis cannot be utilized, the pyruvate is irreversibly converted to acetaldehyde plus carbon dioxide in plant cells. This conversion produces some energy. More energy may be obtained by the plant cell by the reversible conversion of acetaldehyde to ethanol. This theory is supported by the fact that apples produce more ethanol and acetaldehyde when oxygen is lacking than when oxygen is in abundance (Smock and Neubert, 1950).

Acids and hydrocarbons could be produced from amino acids by deamination and/or decarboxylation. Deamination of amino acids could directly form fatty acids which are observed as apple volatiles either in the free or esterified state. Deamination and decarboxylation of amino acids would produce an aliphatic hydrocarbon that, if oxidized to various extents, could produce the respective alcohols, aldehydes, or

ketones. However, since no correlation has been observed in the initial rapid drop of amino acids and the production of volatiles in grape juice fermentation, some believe that, if amino acids contribute to apple volatiles, they do so indirectly. On the other hand, Meigh (1957) shows that apples produce larger quantities of volatiles other than acetaldehyde and ethanol when oxygen is in abundance than when oxygen is restricted. This would tend to show that apple volatiles are produced by an altogether different system than fermentation, one requiring oxygen.

The pathways for production of volatiles from fatty acids is less obscure. It is conceivable that the carbon chains of these volatiles could be produced by the successive combination of acetyl-co-enzyme A or acetyl-co-enzyme A and propionyl-co-enzyme A molecules (Meigh, 1957). There is little scientific evidence supporting these theories for apple biochemistry but the formation of ketone bodies and many other compounds similar to apple volatiles, such as esters and aldehydes, is part of fatty acid metabolism.

LIMITING FACTORS AND SUGGESTIONS

The feat of showing the biochemical pathways by which volatiles are formed is hampered by two very obvious factors. The direct approach of finding the systems by biochemical studies of all metabolic processes is complicated by the vast number of biochemical reactions already known about the cell and undoubtedly many more unknown biochemical pathways and reactions. The indirect approach consists first of identifying the end products, or volatiles, setting up model biochemical

reactions that could produce these compounds, and then showing the presence of these reactions in the apple fruit cell. The pathways of production of acetaldehyde and ethanol have been successfully determined by the indirect approach. The indirect approach is hindered by the limitations on identification of the products of the reactions. The small amount of volatiles produced by apples, resulting in low concentrations of volatiles in storage atmospheres, make volatile identification difficult. However, advanced present-day equipment and techniques look very promising for overcoming the low volatile concentration drawback. Work is presently in progress with elaborate gas chromatographic procedures which will overcome at least part of the problem. Mass spectrometry has contributed toward the identification of volatiles in this thesis and, in conjunction with gas chromatography, can contribute a great deal more.

Volatile collection methods leave much to be desired. Much of the problem here is due to low volatile concentration. Cuticle removal from apples might cause a greater concentration of volatiles in storage chambers. Such studies could also be used as evidence for or against the theory that the cuticle acts as a barrier for apple volatiles. Placement of good organic absorbers and adsorbers on the surface of apples for collection of volatiles might also be tried. Removal of these collecting agents, along with the cuticle after storage may provide a more appropriate means of concentrating apple volatiles for analysis. A great deal can be added to the present knowledge of apple storage volatiles by keeping in mind what has already been found and by applying new methods to the problems as both the problems and methods develop.

SUMMARY AND CONCLUSIONS

A selected review of literature on apple storage volatiles is presented. Methods used for controlling storage volatiles and their effectiveness are discussed. Identified volatiles and the analysis methods used are presented. The results obtained by previous methods of isolation and identification indicate that apple storage volatiles are organic acids, alcohols, aldehydes, ketones, esters, and hydrocarbons in individual concentrations of a few ppm in the storage atmosphere. The application of gas chromatography and mass spectrometry provided a promising outlook on gaining extensive knowledge of apple storage volatiles.

Ethylene, ethanol, and acetaldehyde were identified in controlled atmosphere apple storage atmospheres by LTHV distillation and mass spectrometry. The other volatiles were too low in concentrations and their vapor pressure characteristics are too similar to those of water to be separated from water by LTHV distillation alone. Molecular sieve adsorption techniques also failed to give satisfactory recovery and separation of volatiles. Recovery of volatiles from activated carbon air purifier units gave some results. The harsh acrid odor, not resembling apple odor, of the heat eluted volatiles from activated carbon gave evidence of degradation of the volatiles when compared with the sweet apple-like odor of solvent extracts of activated carbon. Isopentane extraction combined with LTHV distillation was found to be the best of the methods tried for extracting volatiles from activated carbon. Gas chromatograms indicate at least 30 compounds in the

isopentane extract from activated carbon. Saturated and unsaturated aldehydes containing from 4 to 10 carbon atoms were tentatively identified from mass spectra of the activated carbon extract.

The volatile ethylene was found to be in greater concentration by far than any other apple volatile, except, of course, for carbon dioxide and water vapor. Gas chromatography using the hydrogen flame ionization detector provided a rapid, simple, and very satisfactory way to carry out routine analysis for ethylene concentrations in controlled atmospheres as is shown in this thesis.

The ethylene concentrations in conventional controlled atmosphere storage rooms were studied. After sealing the room, the ethylene concentration builds up and then gradually levels off at a maximum equilibrium concentration which holds rather constant until the storage room is opened. Ethylene production appears to be proportional to the amount of oxygen used for respiration for a particular variety of apples. This is deduced from the constant equilibrium ethylene concentration. No difference in equilibrium ethylene concentration was noticed in storage rooms employing caustic soda scrubbers as compared with those using water scrubbers. The presence of activated carbon had no effect on equilibrium ethylene concentration. The equilibrium ethylene concentration values varied with apple variety. Approximate equilibrium ethylene concentration values for the different varieties studied are: 1250 ppm for Northern Spy, 1000 ppm for McIntosh, 750 ppm for Jonathan, and 500 ppm for Red Delicious. It would appear, therefore, that ethylene production varies per unit amount of oxygen used for respiration among different varieties of apples.

The lowest concentration of 250 ppm was found in the storage room which employed an experimental controlled atmosphere generator. The inverse proportionality noticed between ethylene concentration in the room and amount of generator gases metered into the room shows the lower ethylene concentration to be due to dilution of the storage room atmosphere with the controlled atmosphere generator gases.

Chromatograms of direct sampling of storage room atmosphere and of cold trap condensates of storage room atmosphere show only the presence of ethanol. Its concentration in the atmosphere of storage room 10 was calculated to between 100 ppb and 2 ppm. Therefore, ethanol was the next most abundant volatile to ethylene in storage room 10, which contained McIntosh apples but no activated carbon.

In view of the information obtained, mass spectrometry, with the development of collection techniques after chromatographing and more elaborate gas chromatographic equipment, would make possible more qualitative and quantitative information of apple storage volatiles.

BIBLIOGRAPHY

1. Berl, W. G. 1951. Physical Methods in Chemical Analysis. Academic Press, Inc., New York, 587.

2. Brooks, C. 1924. Oiled Wrappers, Oils and Waxes in the Control of Apple Scald. J. Agr. Res., 26, 513.

3. Brooks, C., J. S. Cooley, and D. F. Fisher. 1919. Apple-Scald. J. Agr. Res., 16, 195.

4. Brooks, C., J. S. Cooley, and D. F. Fisher. 1919 A. Nature and Control of Apple-Scald. J. Agr. Res., 18, 211.

5. Coats, V. J., H. F. Noebels, and Fagerson, ed. 1958. Gas Chromatography. Academic Press, Inc., New York.

6. Dibeler, V. H. 1956. Mass Spectrometry. Analyt. Chem., 28, 610.

7. Gane, R. 1934. Production of Ethylene by Some Ripening Fruits. Nature, 134, 1008.

8. Gerhardt, F. 1942. Simultaneous Measurement of Carbon Dioxide and Organic Volatiles in the Internal Atmosphere of Fruits and Vegetables. J. Agr. Res., 64, 207.

9. Gerhardt, F. 1950. Air Purification in Apple and Pear Storage. Refrig. Engin., 58, 145.

10. Gerhardt, F., G. F. Sainsbury, and H. W. Siegelman. 1953. Air Purification for Fruit. Ice and Refrig., 124 (4), 16.

11. Gilpin, J. A., and F. W. McLafferty. 1957. Mass spectrometric Analysis of Aliphatic Aldehydes. Analyt. Chem., 29, 990.

12. Gross, C. R., and R. M. Smock. 1945. Studies on Odor Elimination in Apple Storage. Refrig. Engin., 50, 535.

13. Happ, G. P., D. W. Stewart, and H. C. Cooper. 1957. Mass Spectrometric Determination of Volatile Solvents in Industrial Waste Water. Analyt. Chem., 29, 68.

14. Hardenburg, R. E., and R. E. Anderson. 1962. Chemical Control of Scald on Apples Grown in Eastern United States. Mkt'g. Res. Report No. 538, Agr. Mkt'g. Serv., U.S.D.A., Washington, D. C.

15. Henze, R. E., C. E. Baker, and F. W. Quackenbush. 1953. The Chemical Composition of Apple Storage Volatiles. Bul. No. 679, Purdue Agr. Ex. Sta., Lafayette, Indiana.

16. Henze, R. E., C. E. Baker, and F. W. Quackenbush. 1954. Carbonyl Compounds in Apple Storage Volatiles. Bul. No. 22, Purdue Agr. Ex. Sta., Lafayette, Indiana, 2, 1118.

17. Heulin, F. E. 1952. Volatile Products of Apples. III. Identification of Aldehydes and Ketones. Austral. J. Sci. Res., Series B, 5, 328.

18. James, A. T., and A. J. P. Martin. 1954. Gas-Liquid Chromatography: A Technique for the Analysis and Identification of Volatile Materials. Brit. Med. Bul., 10, 170.

19. James, A. T., and A. J. P. Martin. 1956. Gas-Liquid Chromatography: The Separation and Identification of the Methyl Esters of Saturated and Unsaturated Acids from Formic to N-Octadecanoic Acid. Biochem. J., 63, 144.

20. Keulmans, A. I. M. 1957. Gas Chromatography. Reinhold Publishing Corp.

21. Kirchner, J. G. 1949. Chemistry of Fruit and Vegetable Flavors. Advances Food Res., 2, 259.

22. Mass Spectrometry - A Report. 1950. The Institute of Petroleum.

23. Mattus, G. E. 1962. Apple Scald Inhibitors for Virginia for 1962. Va. Fruit, 50, No. 7.

24. McLafferty, F. W. 1957. Mass Spectrometric Analysis of Aliphatic Ethers. Analyt. Chem., 29, 1782.

25. Meigh, D. F. 1956. Volatile Compounds Produced by Apples. I. Aldehydes and Ketones. J. Sci. Food Agr., 7, 396.

26. Meigh, D. F. 1957. Volatile Compounds Produced by Apples. II. Alcohols and Esters. J. Sci. Food Agr., 8, 313.

27. Meigh, D. F. 1959. Nature of Olefines Produced by Apples. Nature, 184, 1072.

28. Meigh, D. F. 1960. Use of Gas Chromatography in Measuring the Ethylene Production of Stored Apples. J. Sci. Food Agr., 11, 381.

29. Merritt, C., S. R. Bresnick, M. L. Bazinet, J. F. Walsh, and P. Angelini. 1958. The Determination of Volatile Components of Foodstuffs. I. Techniques and Some Preliminary Studies on Irradiated Beef. Res. Report No. 9, Analyt. Chem. Series, Pioneering Res. Div., U. S. Army QM R & E Ctr., Natick, Mass.

30. Milleville, H. P., and R. K. Eskew. 1944. Recovery and Utilization of Natural Apple Flavors. Fruit Prod. J., 24, 48.

31. Pflug, I. J., P. Angelini, and D. H. Dewey. 1957. Fundamentals of Carbon Dioxide Absorption as They Apply to Controlled-Atmosphere Storages. Bul. No. 1, Mich. Agr. Ex. Sta., Michigan State University, East Lansing, 40, 131.

32. Phillips, W. R. 1953. The Apple Volatile Problem. Canadian Refrig. J., June.

33. Power, F. B., and V. K. Chesnut. 1920. The Odorous Constituents of Apples. Emanation of Acetaldehyde from the Ripe Fruit. J. Am. Chem. Soc., 42, 1509.

34. Power, F. B., and V. K. Chesnut. 1922. The Odorous Constituents of Apples. II. Evidence of the Presence of Geraniol. J. Am. Chem. Soc., 44, 2938.

35. Robertson, A. J. B. 1954. Mass Spectrometry. Methuen and Co., Ltd., London; John Wiley and Sons, Inc., New York.

36. Smock, R. M. 1942. The Influence of One Lot of Apple Fruits on Another. Amer. Soc. Hort. Sci., 40, 187.

37. Smock, R. M. 1943. The Influence of Stored Apples on the Ripening of Other Apples Stored With Them. Bul. No. 799, Cornell Univ. Agr. Ex. Sta., Ithaca, New York.

38. Smock, R. M. 1949. Controlled Atmosphere Storage of Apples. Bul. No. 759, Cornell Univ. Ext. Bul., Ithaca, New York

39. Smock, R. M., and A. M. Neubert, 1950. Apples and Apple Products. Interscience Publishers, Inc., New York.

40. Southwick, F. W., and R. M. Smock. 1948. Further Studies on Air Purification in the Apple Storage. Proceedings Amer. Soc. Hort. Sci., 52, 219.

41. Stahl, W. H. 1957. Gas Chromatography and Mass Spectrometry. Chemistry of Natural Food Flavors - A Symposium. Quartermaster Food and Container Institute.

42. Stoffel, W., F. Chu, and E. H. Aherns. 1959. Analysis of Long-Chain Fatty Acids by Gas-Liquid Chromatography: Micromethod for Preparation of Methyl Esters. Analyt. Chem., 31, 307.

43. Thomas, M. 1925. The Controlling Influence of Carbon Dioxide. V. A Quantitative Study of the Production of Ethyl Alcohol and Acetaldehyde by Cells of the Higher Plants in Relation to Concentration of Oxygen and Carbon Dioxide. Biochem. J., 19, 927.

44. Thompson, A. R. 1951. Volatile Products of Apples. I. Identification of Acids and Alcohols. Austral. J. Sci. Res., Series B, 4, 283.

45. Thompson, A. R., and F. E. Heulin. 1951. Volatile Products of Apples. II. Production of Volatile Esters by Granny Smith Apple. Austral. J. Sci. Res., Series B, 4, 544.

46. Turk, A., R. M. Smock, and T. I. Taylor. 1951. Mass and Infrared Spectra of Apple Vapors. Food Tech., 5, 58.

47. Uota, M., and R. M. Smock. 1948. Odor Removal Studies in Refrigerated Storage. Refrig. Engin., 55, 256.

48. Van Doren, A., and R. M. Bullock. 1950. Air Purification Studies in Pacific Northwest Refrigerated Storage Rooms. Amer. Soc. Hort. Sci., 55, 239.

49. Walls, L. P. 1942. The Nature of the Volatile Products from Apples. J. Hort. Sci., 20, 59.

50. Wheelco Instruments Division. 1959. Gas Chromatograph Ionization Detection Systems. Barber-Coleman Co., Rockford, Ilinois.

51. White, J. W. 1950. Composition of a Volatile Fraction of Apples. Food Res., 15, 68.

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