

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

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SUMMARY

Gas chromatographic and mass spectrometric analyses methods were used for ethylene concentration studies and the identification of other volatiles in controlled atmosphere (CA) storage atmospheres. Direct chromatographing of CA storage atmospheres provided a rapid, simple method for following the ethylene concentrations in 11 CA apple storages. Comparisons of the effects of apple variety, two types of carbon dioxide removal equipment, and the presence or absence of activated carbon on ethylene concentration were made. Extracts of activated carbon from an air purification unit which was used in a CA McIntosh apple storage for one season were analyzed. Identification of many of these compounds was achieved by fast scanning mass spectral analysis of effluents from a temperature programmed gas chromatographic column. Saturated and unsaturated hydrocarbons predominated both in number and quantities. Several esters, primary and secondary alcohols, and a few aldehydes were also identified.

INTRODUCTION

The identity of volatile compounds produced by apple fruits has received considerable attention in past decades (Thompson, 1951; Thompson *et al.*, 1951; Huehn, 1952; and Henze *et al.*, 1953, 1954). The literature on the effect of volatiles on product quality is also quite voluminous and has been recently reviewed by Smock (1961), Meigh (1956, 1957), and Phillips (1959). In general, the conclusions are that no relationship between volatiles and scald has been established and that the number of volatiles analyzed, although lengthy, has been lacking especially in the quantitative analysis of the different components.

Most of the methods and techniques that have been used for the earlier

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studies of apple volatiles although sensitive are cumbersome for complete analysis of such a complex mixture of compounds. The methods and results of studies on apple storage volatiles and similar problems suggest that the use of advanced physical-chemical methods of sampling and analysis should add significant information to the apple volatile picture as shown by Turk *et al.* (1951), MacGregar *et al.* (1961) and Meigh (1960, 1964).

This study stresses the application of low-temperature high-vacuum distillation, mass spectrometry and gas chromatography for qualitative and quantitative analysis of apple storage volatiles. The purpose of this study was the qualitative and quantitative analysis of some controlled atmosphere apple storage volatiles and how they are affected by various storage conditions.

EXPERIMENTAL PROCEDURE

Direct ethylene concentration analyses. Ethylene concentration in controlled atmosphere storages was analyzed directly with an Aerograph model A-600 Hy-Fi chromatograph. A $\frac{1}{8}$ -in. \times 10-ft. stainless-steel column packed with 40% Castorwax on Chromosorb W, an oven temperature of 50°C and a gas sample size of 10 μ l were used for all gas analyses of storage room atmospheres. A management summary of the 11 storage rooms used in the study is shown in Table 1.

The desired levels of oxygen and carbon dioxide were obtained purely by the respiration of the fruit with scrubbers to remove excess carbon dioxide in all storages except room 11. This storage had an experimental controlled atmosphere generator and a carbon dioxide scrubber to produce and maintain the desired concentrations of oxygen and carbon dioxide. The oxygen and carbon dioxide, once obtained, were maintained at optimum concentrations for the varieties of apples present in each storage chamber according to the State of Michigan specifications for CA storage of apples. In all cases the fruit was in very

good to excellent condition with minimal spoilage when it was removed from storage.

A standard curve for ethylene concentrations in air was prepared by obtaining gas chromatograms of concentrations of ethylene in air ranging from 1 to 4000 ppm. Peak heights were plotted against ethylene concentration; the resulting curve (Fig. 1) is within the limits of experimental error a straight line. This curve was used to determine the ethylene concentrations of all storage atmosphere samples. A check on the calibration of the chromatograph was made with known concentrations of ethylene in air each time storage samples were analyzed for ethylene concentration.

Samples of the storage room atmospheres were taken in 4-L polyethylene bags from the storage room gas sampling tubes. The gas sampling tubes were connected to the storage atmosphere recirculating lines between the blowers and the adsorption towers of the respective atmosphere scrubbing systems. The polyethylene bags were filled with the storage room atmosphere being collected and collapsed completely three times to flush out any residual gas. The open end of the polyethylene bag was gathered tightly around a rubber-vaccine-bottle-stopper and held in place by a rubber band. The gas was taken from the polyethylene sample bag with a syringe and injected directly into the gas chromatograph injection port.

No change in ethylene concentration was detected over a period of 6 hr in samples collected and analyzed in the manner described previously. All

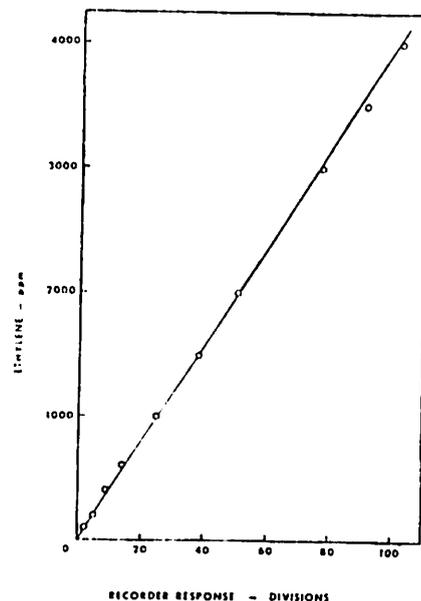


Fig. 1. Standard curve for ethylene concentration in air.

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Table 1. Storage room management summary.

Room no.	Variety	Date sealed	Temperature °F	Desired gas concentrations		Date obtained	Type scrubbing unit used	Activated carbon used	Date opened
				% O ₂	% CO ₂				
1	McIntosh	10/2/61	37	3	5	10/28/61	Water	Yes	1/15/62
2	McIntosh	10/10/61	37	3	5	11/8/61	Water	Yes	3/4/62
3	Red Delicious	10/21/61	32	3	3	11/21/61	Water	Yes	3/4/62
4	Jonathan	10/16/61	32	3	5	11/22/61	Water	Yes	2/11/62
5	Northern Spy	11/15/61	37	5	8	12/1/61	Water	Yes	After 5/1/62
6	McIntosh	10/10/61	37	3	5	11/5/61	Water	Yes	4/27/62
7	Jonathan and Golden and Red Delicious	10/28/61	32	3	3	11/28/61	Water	Yes	3/22/62
8	Jonathan and Golden and Red Delicious	10/30/61	32	3	3	12/9/61	Water	Yes	4/21/62
9	McIntosh	10/8/61	37	3	5	11/10/61	Caustic Soda	Yes	4/28/62
10	McIntosh	10/12/61	37	3	5	11/29/61	Caustic Soda	No	5/18/62
11	Mixture ¹	10/15/61	32	3	3	10/20/61	Water	No	5/4/62

¹ McIntosh, Jonathan, Rome Beauty, and Golden and Red Delicious.

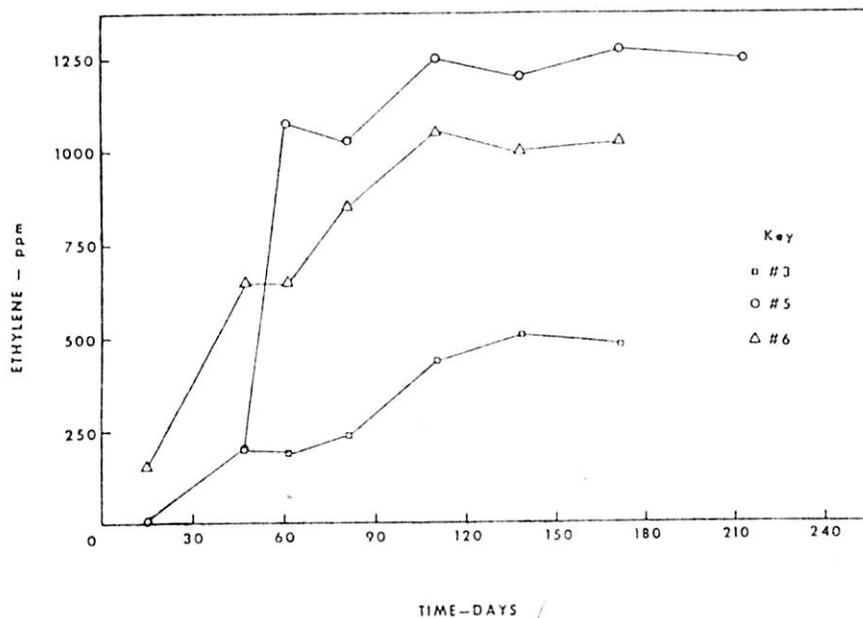


Fig. 2. Ethylene concentration in atmosphere of CA storage rooms 3, 5, and 6 versus time.

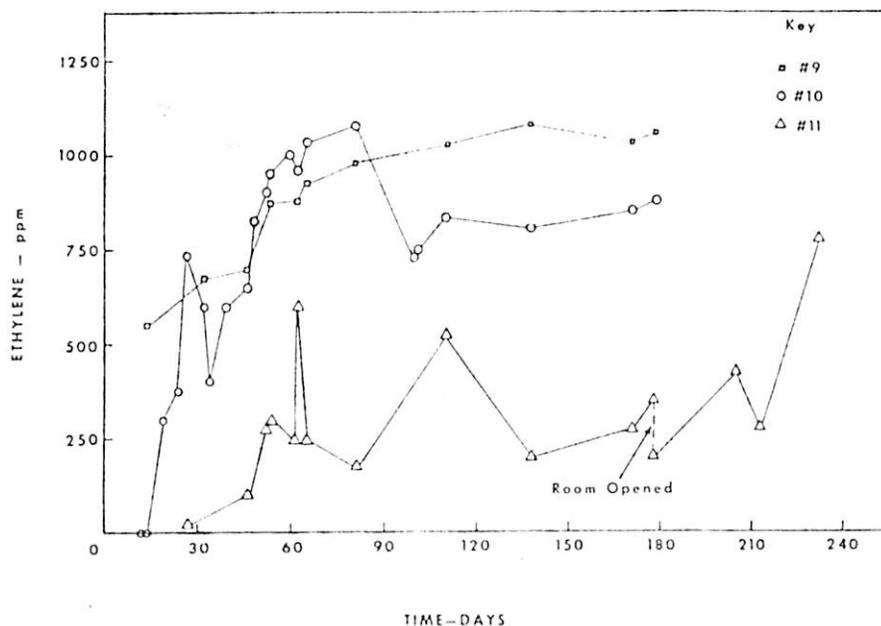


Fig. 3. Ethylene concentration in atmospheres of CA storage rooms 9, 10, and 11 vs. time.

samples used for ethylene concentration determinations were analyzed within 4 hr after collection.

Cold trap samples. Cold trap samples were collected by passing 10 ft³ of storage chamber atmosphere through a liquid nitrogen cold trap. The samples collected in this manner were held at -196°C and evacuated to remove all gases at this temperature with a low temperature-high vacuum (LTHV) apparatus similar to that described by Merritt *et al.* (1959). The compounds remaining were selectively fractionated into five parts by collecting compounds that were volatile at -155 , -125 , -100 , -78°C , and at room temperature. The volatile compounds were collected in a liquid nitrogen trap. The five fractions contained compounds exerting vapor pressures greater than $5 \mu\text{Hg}$ between the temperatures -196 and -155°C , -155 and -125°C , -125 and -100°C , -100 and -78°C , -78°C and room temperature and were analyzed with a consolidated model 21-103C mass spectrometer.

Activated carbon extracts. Concentrates of apple storage volatiles were obtained from activated carbon that had been in a controlled atmosphere McIntosh apple storage during one season of approximately 200 days. Three methods for removing volatiles from activated carbon were tried and compared. The first method consisted of placing a 10-g carbon sample in a gas bottle and cooling it to -196°C . All materials volatile at -196°C were pumped off using the LTHV system. Subsequently, the carbon was heated to 180°C and the remaining compounds driven off and condensed in a -196°C cold trap under high vacuum.

Compounds adsorbed on the activated carbon were also removed by two solvent-extraction techniques using diethyl ether and methyl butane (isopentane). Identical procedures were carried out for extraction by each sol.

vent. Ten g of carbon were extracted with three 10-ml portions of solvent. The solvent extract was placed in a gas bottle and the solvent was distilled off at -78°C under high vacuum, leaving behind the apple storage volatiles that were not volatile at -78°C .

The isopentane extract of activated carbon was analyzed by wide range temperature programmed gas chromatography combined with fast scanning mass spectrometry. A $\frac{1}{8}$ -in. \times 10-ft stainless-steel column packed with 5% Carbowax 20M on firebrick was temperature programmed from -50 to 150°C at 2°C per min. The effluent of the column was directed into the ionization source of modified Bendix model 14 time-of-flight mass spectrometer as described by Merritt *et al.* (1964). Mass spectra of the sample compounds separated on the column were obtained as the compounds were eluted from the column.

RESULTS AND DISCUSSION

The ethylene concentration of eleven CA storage rooms was checked at intervals throughout the 1961-62 storage season by sampling and analyzing the storage atmospheres as described above. The results obtained from six of the storages are shown in Figs. 2 and 3. Rooms 1 through 8 were commercial storages remote to the laboratory and were sampled less frequently.

In all cases the ethylene concentration increased steadily after sealing the rooms. When the room remained sealed for a long enough period of time, as was the case for rooms 3, 5, 6, 7, 8, and 9, the ethylene concentration leveled off and varied about an equilibrium concentration until the room was opened. Room 1 was opened before the ethylene concentration reached equilibrium and rooms 2 and 4 were opened before sufficient data could be collected to establish equilibrium ethylene concentrations. The ethylene concentrations in rooms 10 and 11 show more fluctuations than in other rooms. The irregularities are due to the fact that other relatively large atmosphere samples were removed from room 10 for analysis and that the experimental controlled atmosphere generator used on room 11 was very erratic in its operation.

The results suggest that the equilibrium ethylene concentration level reached in a storage room depends on the variety of apples stored in the room if oxygen concentration is maintained at a constant level. Room 5 contained Northern Spy apples and reached an equilibrium ethylene concentration of approximately 1250 ppm. Rooms with McIntosh apples reached

an equilibrium ethylene concentration of 1000 ppm, while the room with Red Delicious apples achieved an equilibrium in ethylene concentration of 500 ppm. Rooms containing mixtures of apple varieties produced equilibrium ethylene concentration between those found for the rooms containing the respective single varieties of apples.

No great difference in equilibrium ethylene concentration was observed between rooms using water or caustic soda absorption systems and/or the presence or absence of activated carbon. Rooms 6, 9 and 10 all contained McIntosh apples and all reached an equilibrium ethylene concentration of approximately 1000 ppm. Room 6 had a water absorption system, and rooms 9 and 10 had caustic soda absorbers. Rooms 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.

Mass spectra of the cold trap condensate fractions enabled the identification of ethylene from the -196 to -155°C fraction and acetaldehyde and ethyl alcohol from the -125 to -100°C fraction. No other positive identifications could be made from the cold trap condensates. Nevertheless, mass spectra of the -78°C to room temperature fraction shows m/e 's characteristic of organic compounds along with a large amount of water. The LTHV distillation techniques, therefore, are effective in separating these

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

Gas chromatograms of the headspace from each of the activated carbon extracts are shown in Fig. 4. The chromatograms are correlated with the observations made by smelling the samples. Both solvent extracts had a sweet apple-like odor combined with a musty storage room odor. Except for size of the solvent peak, the first large peak in each chromatogram as compared with the other peaks; both chromatograms from solvent extracted samples are quite similar. A radically different chromatogram and odor were produced by samples removed from activated carbon by heat. It is believed that these differences are due to heat degradation of the components during removal from the carbon. Isopentane proved a more satisfactory solvent for this extraction than diethyl ether because it is more volatile and thus easier to distill off at low temperatures.

Compounds identified from isopentane extracts of activated carbon by combined gas chromatography and fast scanning mass spectrometry are listed in Table 2 according to class. These compounds were identified by comparing their respective retention times and

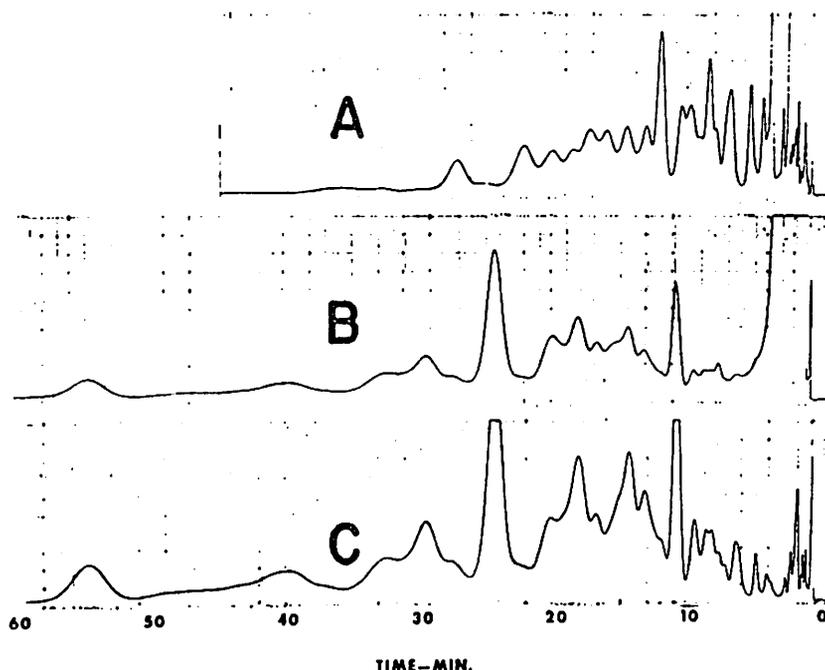


Fig. 4. Gas chromatograms of apple storage volatiles removed from activated carbon by A, heat; B, diethyl ether extraction; C, isopentane extraction. Aerograph Model A-600 My-F1 gas chromatograph; Column: $\frac{1}{8}$ " \times 5' Stainless steel column packed with 5% SE-30; on acid-washed chromosorb W; Carrier Gas: Helium at 20 ml/min; Oven Temperature: Isothermal at 50°C .

Table 2. Compounds identified from activated carbon extracts.

Hydrocarbons	Alcohols	Esters	Aldehydes
2-methylbutane	2-propanol	n-butyl formate	2-methylpropenal
n-hexane	2-methyl-1-propanol	sec-butyl formate	isovaleraldehyde
2-methylpentane	2-methyl-2-propanol	butyl acetate	heptanal
cyclohexane	2-pentanol	octyl acetate	octanal
n-heptane	3-pentanol	ethyl butyrate	nonanal
n-octane	2-methyl-1-butanol	butyl butyrate	decanal
n-nonane	3-methyl-1-butanol	hexyl butyrate	
methyl octane	2-methyl-2-butanol	hexyl pentanoate	
n-decane	3-methyl-2-butanol		
methyl nonane	1-hexanol		
n-undecane	3-methyl-1-pentanol		
1-heptene			
1-octene			
1-nonene			
1-decene			
1-undecene			
1-octyne			
1-nonyne			
1-decyne			
methyl benzene			
ethyl benzene			
n-propyl benzene			
i-propyl benzene			
n-butyl benzene			
tert-butyl benzene			
n-pentyl benzene			

mass spectra with those of known compounds using the same instrument system and identical operating parameters. Several other compounds could not be positively identified because of one or more of the following reasons: insufficient separation by gas chromatography; unavailable matching mass spectra; amounts of compounds too small to obtain identifying mass spectra.

Hydrocarbons were found in much greater abundance both in number and in amounts than any of the other classes of compounds. Solvent and unexposed activated carbon blanks yielded the presence of isopentane, n-hexane and 2-methyl pentane only. It was concluded, therefore, that the rest of the compounds listed in Table 2 were absorbed onto the activated carbon from the apple storage atmosphere.

The origin of the identified compounds could be from several sources. The combined apple-like and musty odor of the solvent extracts of the exposed activated carbon suggests two of the more obvious sources: namely, from the apples and from microorganisms growing within the storage chamber. Other sources, some not quite so obvious, must also be considered. Volatiles may be contributed to storage atmospheres by solvents and impurities in the building materials used to construct the chamber. The wooden container used to hold fruit may contain volatile resins which would add to storage atmosphere volatiles. The refrigeration equipment within the storage chamber would be expected to contribute volatile substances from lubricants and from minute refrigerant leaks if present. Storage room ventilation and/or at-

mospheric washing (CA storages) would lower the concentration of volatiles produced within the storage chamber but would in turn add volatiles from outside the chamber. If air for ventilation is taken from a service room, this operation could add considerable volatile materials to the storage room atmosphere.

Most of the alcohols, esters, and aldehydes identified are produced by the apple fruits (MacGregor *et al.*, 1964 and Meigh, 1956, 1957). Some of the oxygenated volatiles are likely to be products of microorganisms within the storage chamber. The hydrocarbons, however, could originate from any of these sources. Ethylene and other short chained hydrocarbons are known to be present in the emanation from apples (Meigh, 1959, 1960), and recently long chain hydrocarbons containing from 10 to 30 carbons have been isolated and identified from apples (Meigh, 1964). In view of this, the apples could be responsible for many of the aliphatic hydrocarbons identified in this study. The aromatic hydrocarbons are more probably derived from building materials and/or lubricants used on storage room equipment.

The theory that apple scald is produced by volatile emanations from some apple varieties has not yet been fully established, even though there is much evidence supporting this theory. Meigh's (1964) results indicate that neither the fatty acids nor the hydrocarbons found in his study are involved in producing scald but further investigations would be necessary to prove this. Nevertheless, analytical techniques presently available and in use on the problem show great promise

of determining the validity of this theory.

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