AN ABSTRACT OF THE THESIS OF

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ABSTRACT

During certain investigations relating to storage and handling of apples and pears, a need arose for an accurate chemical method of determining ethylene in the small amounts present in fruit tissues. Although methods have been developed for estimation and semiquantitative determination of small amounts of ethylene, the procedures used were considered inadequate either from the standpoint of accuracy or the size of samples required.

The purpose of this research was to perfect a method for the microdetermination of ethylene in its usual concentrations in fruit tissues. This was calculated to be about 1 part in 40,000.

Extensive preliminary investigations show the reaction with bromine to be the most promising for precise determination of ethylene. Accordingly a simple bromination system was devised to treat the gases removed from fruit tissues.

The apparatus consists of three parts:

1. Extractor. This was constructed on the same principle as a Töpler pump. It served to remove the internal gases from the fruit by evacuation.

2. Purification unit. The gases from the evacuated tissues were treated with NH_3 to remove aldehydes, then passed over desicchlora to remove excess ammonia, and finally through a cooling coil immersed in a dry-ice-ether bath.

3. Reaction flask. The ethylene-air mixture from the purification unit was brominated in a partially evacuated reaction flask. Bromine was obtained from the reaction of KBr on acid $KBrO_3$. The excess was converted to iodine and titrated with $Na_2S_2O_3$.

The method has been found suitable for analysis of ethylene in amounts as small as 0.0006ml., in 35-40ml. of air.

The results of several analysis are included.

THE DETERMINATION OF ETHYLENE IN THE INTERNAL ATMOSPHERE OF PLANT TISSUES

by

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TABLE OF CONTENTS

THE DETERMINATION OF ETHYLENE IN THE INTERNAL ATMOSPHERE OF PLANT TISSUES

Introduction

During certain physiological investigations relating to the handling and storage of apples and pears, a need developed for an accurate chemical method of determining the small amounts of ethylene contained within the fruit tissues. Since these fruits produce ethylene (5,7), which is definitely known to affect certain chemical changes (6,8) that are associated with the ripening and storage of fruit, a means of obtaining data of this nature is desirable.

Although ethylene has been identified as a constituent of fruit emanations (5,11) and has been semiquantitatively estimated, the procedures used would not lend themselves to the development of a rapid and accurate method for the determination of the gas contained in the internal atmosphere of the tissue.

The amount of ethylene that occurs in plant tissues is thought to be very small (4). Nelson (10) reports the ethylene content of McIntosh apples as 0.12 mg. per kilogram. Since one kg. of fruit tissues will contain on the average from 300 to 600 ml. of total gas, this represents a dilution of the order of one part in four thousand. If these values are approximately correct, it would appear

that a method which could accurately determine 0.001 ml. of ethylene in a dilution of 40 ml. (1 part in 40,000) might serve suitably for an estimation of this gas in the internal atmosphere of fruit tissue.

A survey of the possible chemical reactions which might serve as a basis for a microdetermination indicated that bromination or oxidation with potassium permanganate would be the most promising. A method using permanganate has been described recently by Nelson (10).

Extensive preliminary experiments conducted in this laboratory indicate that the reaction of ethylene with permanganate, besides being affected by traces of acid, bases, or organic impurities, does not always proceed to a definite quantitative product--viz., glycol--and as a result only the roughest of approximations may be obtained by its use.

Bromination on the other hand has the distinct advantage that the reaction does not proceed rapidly beyond the formation of ethylene dibromide and is not so easily influenced by small amounts of foreign materials. For these reasons a simple method modified from the macrodetermination of Davis, Crandall, and Higbee (3) was adopted.

Experimental

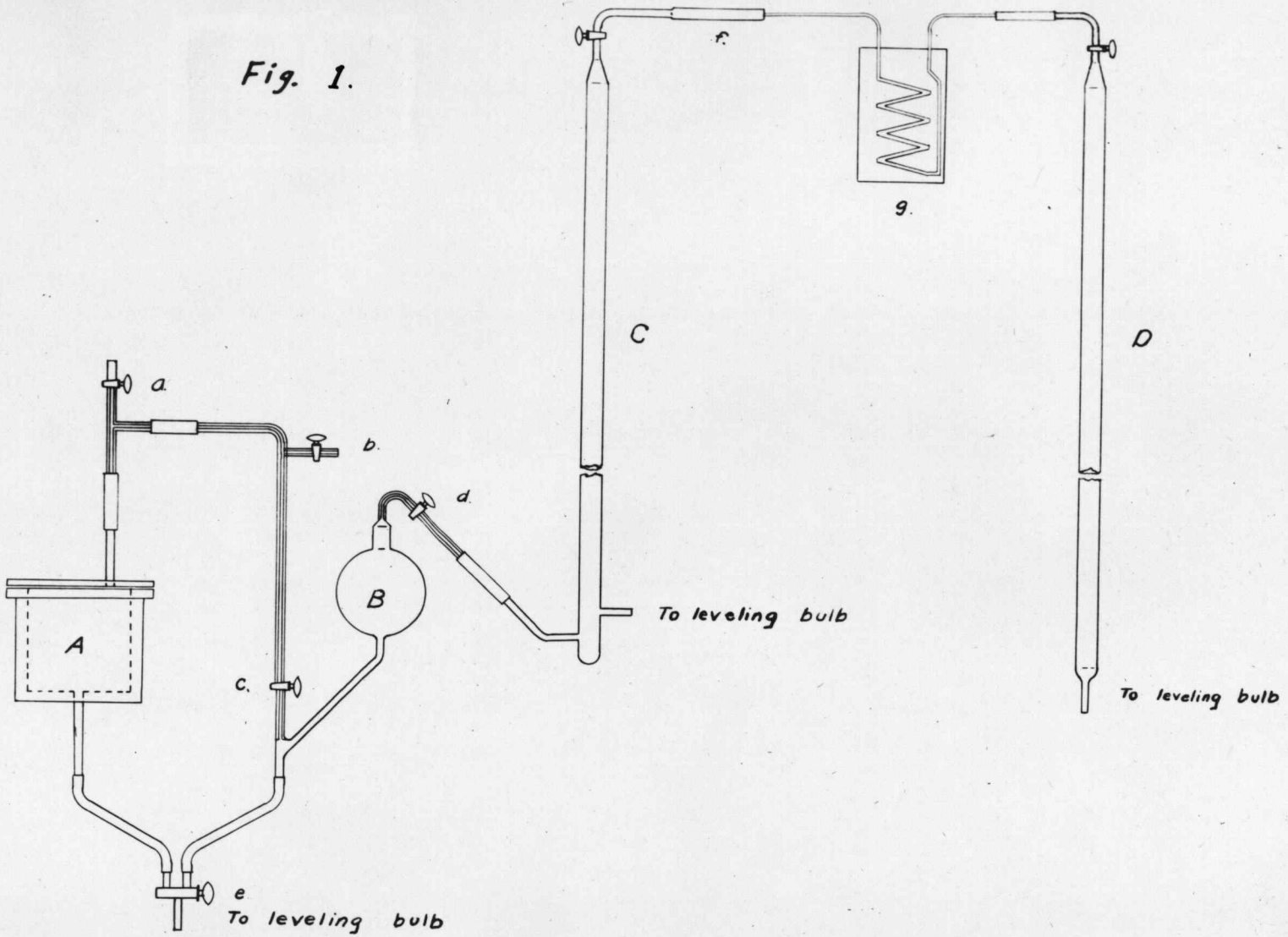
The apparatus consisted of three units: an extractor

a purification train, and a reaction flask.

Extractor. The extractor (Figurel) was constructed on the same principle as a Topler pump and consists of two parts: a chamber A, and a pump, B. The extraction chamber, A, was constructed from an iron pipe 8.9 cm. (3.5 inches) in inside diameter and 7.6 cm. (3 inches) over-all. To the bottom was welded a steel plate fitted with a 0.64 cm. (0.25-inch) steel tube which served as an inlet for the confining fluid. A 1.25 cm. (0.5 inch) iron collar was welded to the opposite end and then carefully machined to give a smooth surface. The cover, equipped with an exit tube, was made from a 0.64 cm. (0.25 inch) steel plate carefully polished and fitted to the collar. A tight connection capable of maintaining vacuum was obtained by the use of a greased rubber gasket. The cover was held in position by means of a heavy screw clamp like those used on specimen jars.

The pumping compartment, B, was constructed from a 250 ml. Pyrex bulb and was connected to the extraction chamber with one mm. capillary tube. Stopcock c was used to control the flow of gas between these two units. Stopcock a provided a means of releasing the vacuum, while b led to a manometer for the measuring of the pressure in the extraction chamber. In order to insure flexibility a rubber connection was placed between stopcocks a and b. By means of stopcock e, the same leveling bulb was

Fig. 1.


a
b
c
d
e
f
g
A
B
C
D
To leveling bulb
To leveling bulb
To leveling bulb

used for forcing mercury into either A or B.

A nitrometer, C, which served to trap the extracted gases, was connected to B through stopcock d.

Purification. The purification train consisted of a small desicchlora tube, f, and a copper coil, g (2 mm. inside diameter by 100 cm.), immersed in solid carbon dioxide-ether mixture, was connected between nitrometer C and gas buret B. The total volume of the tube and coil was 5.3 ml. Buret D, equipped with a leveling bulb containing mercury, served to store and measure the purified gas prior to analysis.

Reaction Flask. The reaction flask (Figure II) was constructed from a 50 ml. Erlenmeyer to which was sealed a 12/30 standard taper and a capillary stopcock.

Procedure

A weighed amount of tissue (either whole or cut) was placed in chamber A. The cover was then clamped into position and connected to the pumping compartment. With stopcock a open, A was filled with mercury from the leveling bulb; a was then closed and the mercury was allowed to drain away, leaving the tissue in a Torricellian vacuum. By means of stopcock e and the same leveling bulb, chamber B was filled with mercury and evacuated. Nitrometer C was then filled with mercury over which was

placed 1 ml. of 2.5 per cent ammonium hydroxide solution.

The gas in the storage flask was now completely removed and transferred to nitrometer C by merely raising and lowering the leveling bulb and operating stopcock c and d. This process was continued until no further gas could be extracted.

After standing for 15 minutes in nitrometer C, the gas was passed slowly (approximately 4 ml. per minute) through the purification train to the measuring buret, D, where it remained until ready for analysis.

The reaction flask (Figure 2) was then charged with 5.00ml. of 0.0025 N potassium bromate (measured with a microburet) and 0.5 ml. of 6 N sulfuric acid, and then partially evacuated. Buret D was detached and approximately 40 ml. of sample were transferred to the reaction flask. One milliliter of 0.1 N potassium bromide was finally introduced without releasing all the vacuum. This mixture was shaken vigorously for 15 minutes on a mechanical shaker and then 1 ml. of 0.1 N potassium iodide was introduced by means of the residual vacuum. The iodine liberated was titrated with 0.0025 N sodium thiosulfate from a microburet. The amount of potassium bromate used for bromination was determined by the difference between blank run (using air) and the actual determination. Duplicate blank runs checked consistently within 0.2 ml.

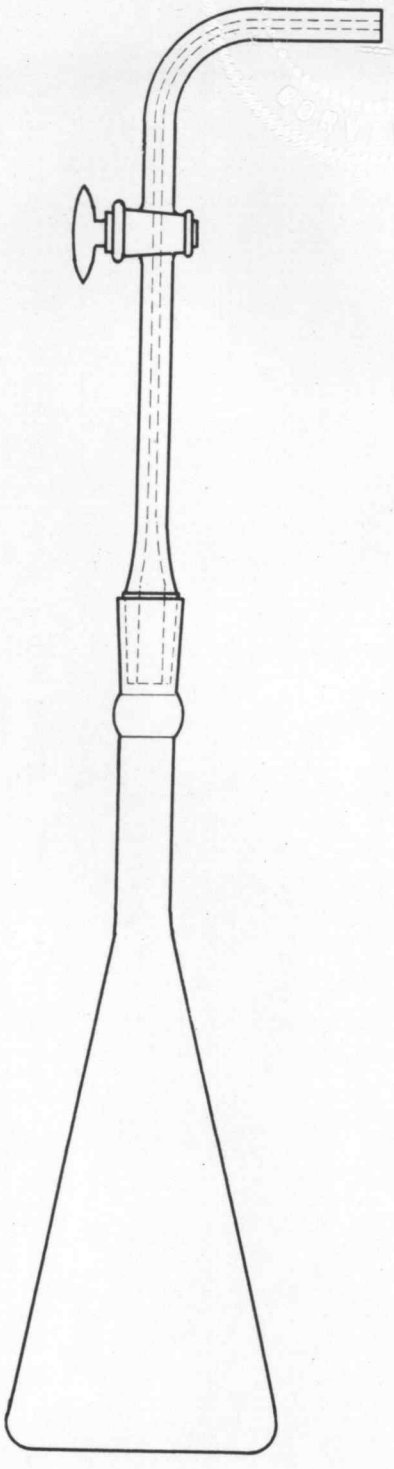


FIG. 2.

One-tenth milliliter of 0.0025 N potassium bromate is equivalent to 0.0028 ml. of ethylene at normal temperature and pressure. In all determinations a correction was made for t he gases remaining in the purification train.

Discussion and Results

Efficiency of Extraction. To determine the efficiency of the apparatus for extracting ethylene from fruit tissues, blank determinations were made using ethylene-air mixtures. Approximately 100 grams of sliced apples were placed in the storage compartment and all the free ethylene present was removed by extraction. Small known quantities of ethylene were introduced into the chamber and sufficient time was allowed to ensure sufficient diffusion throughout the fruit and container. Forty milliliters of air were introduced (that amount being the approximate volume of gas taken for analysis). This gaseous mixture was then removed and analyzed in the manner previously described.

From the results of a number of determinations, shown in Table 1, it is apparent that small quantities of ethylene can be recovered quantitatively when added to apple tissue. Furthermore, consecutive tests on fresh samples have failed to show any trace of ethylene after the first extraction. Ethylene did not appear to be

given off in a definite pressure range.

The advantages of this type of extraction are: It attains a practically complete removal of gas; it permits the study of either whole or cut tissue; and it is equipped to measure the pressures at which the gases are extracted. Although several methods (1,2,9,10) for the removal of internal gases from fruit tissues are described in the literature, none attain all these objectives.

TABLE 1.

Recovery of Ethylene Added to Apple Tissue

Ethylene Taken Ml.	Ethylene Recovered Ml.
0.058	0.051
0.057	0.059
0.058	0.056
0.058	0.062
0.061	0.061

Effectiveness of Purification Train. In order to determine the ethylene content in the vapors derived from fruit tissues it is necessary to remove the other components, such as aldehydes, esters, and alcohols.

Preliminary experiments carried out under the conditions specified for analysis showed (1) no bromination of ethyl acetate, (2) slight bromination of ethyl alcohol, and (3) considerable bromination of acetaldehyde.

To eliminate these substances, 1 ml. of 2.5 per cent of ammonium hydroxide was placed in nitrometer C over which the vapors were permitted to stand for fifteen minutes. Blank determinations showed that this treatment completely removed both acetaldehyde and alcohol vapors, even when present in great excess over that found in fruit vapors. In using this procedure, however, care must be taken to prevent any of the ammonia from entering the analytical flask, since it would alter the acidity of the reaction mixture. To ensure the removal of ammonia and possibly other active agents, the extracted gas was finally passed through a Desicchlora tube and a cold trap.

Since it appears that ethylene is the only unsaturated gas present in apple (5), pear (7), and banana (11) vapors, no special precautions were taken to remove possible traces of acetylene or propylene.

Accuracy of the Analytical Method. To determine the accuracy of the analytical procedure, a number of typical runs were made with pure ethylene and tabulated in table II.

Ethylene Content of the Internal Atmosphere of Plant Tissue. Using the procedure outlined, the ethylene content of various kinds of ripe fruit material was determined. In some of these analyses, gas samples were taken from whole fruits; in others, from slices of 8 or more selected specimens. In the case of the cantaloupe, the gas sample

from the cavity was taken by inserting a glass tube and extracting the gas directly into the buret. The results are tabulated in Table III.

TABLE II.

Microdetermination of Ethylene

Ethylene Taken Ml.	Ethylene Found Ml.
0.0026	0.0025
0.006	0.006
0.011	0.011
0.029	0.028
0.052	0.049
0.066	0.066
0.063	0.061

Table lll shows that there is considerable variation in the ethylene content of individual fruits taken from the same lot. Ripe Gravenstein apples showed a variation of 0.022 to 0.063 ml. per 100 grams of tissue. Bartlett pears showed a variation of from 0.008 to 0.029 ml. Some of the data indicate that gas samples taken from the whole fruits gave higher ethylene values than similar samples taken from cut fruit. Whether this difference is due to loss of gas in cutting or to variations in ethylene content in different parts of the fruit is not known at the present time. It is apparent however, that some gas is lost from cut tissue, since more ethylene was found in cut Gravenstein tissue analyzed immediately after sampling than in similar tissue analyzed three hours later.

There is considerable variation in the amount of ethylene found in different kinds of fruits and vegetables. Since the ethylene content of a given variety of fruit is dependent on a number of factors, these values are merely indicative of its presence. The variation in each type of material is a study in itself, and beyond the scope of this paper.

TABLE III.

Ethylene Content of Internal Atmosphere of Fruit and Vegetable Tissues

Kind of Fruit	Method of Sampling	Ethylene Found Ml./100g.
Apple		
Gravenstein	Longitudinal sectors	0.019
		0.016 a
	Whole apple	0.026
		0.040
		0.063
		0.039
		0.022
	Average of 6 whole fruits	0.038
Red June	Longitudinal sectors	0.008
	Average of 6 whole fruits	0.012
Bartlett Pear	Whole fruit	0.015
		0.029
		0.010
		0.008
		0.020
Peaches		0.012
Hale	Whole fruit	0.037
Crawford		0.012
Tomatoes	Whole fruit	0.010
Cantaloupe	Gas from cavity	0.006
	Longitudinal sectors	0.002
Potatoes	Long sectors	0.001*
Bananas	Long sectors	0.001*

a Sectors taken from same apples three hours later
* Less than this amount

Summary

A bromination micromethod for the accurate determination of ethylene within a range of 0.001 to 0.06 ml. at normal temperature and pressure in a volume of 35 to 40 ml. has been developed, and a new apparatus devised for the complete removal of internal gases from plant tissues.

A number of analysis have been carried out to show the presence of unsaturates (ethylene) in various kinds of tissue in quantities measurable by this method.

BIBLIOGRAPHY

(1) Brooks, Charles, Proc. Am. Soc. Hort. Sci., 35, 202 (1938).

(2) Culpepper, C.W., Moon, H.M, and Lutz, J.M., Science, 84, 398 (1936).

(3) Davis, H.S., Crandall, G.S., and Higbee, W.E., Jr., Ind. Eng. Chem. Anal. Ed., 3, 108 (1931).

(4) Denny, F.E., and Miller, L.P., Contrib. Boyce Thompson Inst., 7, 97 (1937).

(5) Gane, R., J. Pomology Hort. Sci., 13, 351 (1935).

(6) Hansen, Elmer, Science, 86, 272 (1937).

(7) Hansen, Elmer, and Hartman, Henry, Ore. Agr. Expt. Sta., Bull. 342 (1935).

(8) Hansen, Elmer, and Hartman, Henry, Plant Physiol., 12, 441 (1937).

(9) Magness, J.R., Botan. Gaz., 70, 308 (1920).

(10) Nelson, R.C., Plant Physiol., 12, 1004 (1937).

(11) Niederl, J.B., and Brenner, M.W., Mikrochemie, 24, 134 (1938).