STUDIES ON CONTROL OF RESPIRATION OF APPLES BY PACKAGING METHODS

by

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Cambridge, Massachusetts
May 18, 1962

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Dear Sir:

A thesis entitled, STUDIES ON CONTROL OF RESPIRATION OF APPLES BY PACKAGING METHODS, is hereby submitted in partial fulfillment of the requirements for the degree of Master of Science in Food Technology.

Respectfully Submitted,

Signature redacted

Vatren Jurin S.

A C K N O W L E D G M E N T

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A B S T R A C T

STUDIES ON CONTROL OF RESPIRATION OF APPLES BY PACKAGING METHODS

by

Vatren Jurin

SUBMITTED TO THE DEPARTMENT OF NUTRITION, FOOD SCIENCE AND TECHNOLOGY ON MAY 18, 1962 IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

The storage life of fruit may be extended by lowering the rate of respiratory processes. One of the methods for achieving this aim is the control of the composition of atmosphere surrounding the fruit. This may be done by selection of packaging materials with suitable permeability characteristics on the basis of known respiration characteristics of the fruit.

A static system for the study of respiration rates using gas chromatographic gas analysis was developed and calibrated. Respiration rates of apples of the McIntosh variety were determined as a function of the oxygen and carbon dioxide concentrations in the atmosphere. The critical oxygen concentration for initiation of anaerobic respiration was also determined.

The respiration rates were found to be dependent on both the carbon dioxide concentration and the oxygen concentration. The critical oxygen concentration for the initiation of the anaerobic respiration, however, was found to be independent of the carbon dioxide concentration.

The results of the respiration studies were used to evaluate the package permeability necessary to attain desirable steady state atmospheric conditions within the package. The theoretical evaluation was confirmed by the results of some preliminary experiments on composition of the atmosphere of apple packages subjected to short term storage.

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I. INTRODUCTION

Fruits can ordinarily be held in satisfactory conditions for rather long periods in cold storage. However, there are certain instances where cold storage has not proved entirely adequate. For example, McIntosh apples cannot be held in ordinary cold storage with full assurance that they will be of prime eating quality.

Control of the respiration of fruit is a measure which complements the cold storage advantages of preventing deterioration of the quality of the fruit.

Respiration rates can be retarded by maintaining an atmosphere in which the oxygen content is lower and the carbon dioxide concentration is higher than in normal air.

A very important factor to be taken into account is the fact that the concentration of carbon dioxide and oxygen must be such that physiological disorders not be produced in the fruit. Oxygen concentrations must not be lower than the value at which anaerobic respiration takes place. Excess of carbon dioxide may cause damages also.

Respiration control may be achieved by a suitable method of packaging. The broad variety of plastic films available commercially provides a large range of permeability values for oxygen and carbon dioxide. Through an appropiate selection of these films, the permeability values may afford a way to control the atmospheric composition inside the packages.

In order to evaluate rationally these packaging methods, it is necessary to have information on the respiration processes; the rate of respiration; the influence of the atmosphere composition, especially carbon dioxide and oxygen, on the respiration rates; and the critical oxygen concentration for anaerobic respiration. In addition, it is required to have information about the characteristics of the plastic films used as packaging materials, especially those characteristics concerned with the permeability to carbon dioxide and oxygen, gases which are the decisive factors controlling the respiration processes.

In the present study an attempt was made to evaluate the possibility of predicting the necessary film properties on the basis of respiration studies on fruit.

To evaluate the respiration parameters, a static respiration measurement system was adopted for this study. The system consisted of respiration chambers the atmospheric composition of which could be adjusted to the desired concentrations of oxygen and carbon dioxide. The atmospheric composition could be readjusted at suitable time intervals.

Gas chromatography was used as the analytical method for the determination of oxygen and carbon dioxide concentrations in the atmosphere of the respiration chambers. Using a system of two chromatographic columns, a resolution of carbon dioxide, oxygen, and nitrogen was obtained. In this way the two gases could be determined simultaneously.

For the present study, apples of the McIntosh variety were used because of their relatively long storage life making them available during all the period of experimentation.

II. LITERATURE REVIEW

A. RESPIRATION

1. Chemistry and Physiology

Knowledge of the chemical changes and physiology of apple fruit after harvest is very useful in understanding what happens to the fruit in storage, in the marketing channel, and in the hands of the consumer. Such knowledge may be useful in predicting what happens to a lot of apples if they are stored in a gas-tight package for a period of time. (Smock 1950).

The chemical changes that take place in the detached fruit are directly or indirectly related to the oxidative and fermentative activities collectively referred to as biological oxidation. The cellular physiologist views respiration as the process concerned with the oxidation of predominantly organic substances by the cell or by enzymatic system derived from the cell. Once the fruit is harvested, respiration assumes the dominant role and no longer depends on absorption of water and minerals by the root, on conduction by vascular tissue, and photosynthetic activity of leaves. After harvest the fruit lives an independent life by utilizations of substrates accumulated during growth and maturation. (Biale, J. B. 1960).

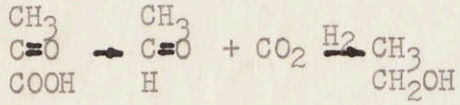
Respiration is a metabolic process, but since it is a mechanism through which energy is mobilized and a variety of carbon skeletons generated, it is basic to the whole metabolic cycle. (Brown, R. 1960).

In its simplest form respiration involves the degradation of sugar to carbon dioxide and water. It occurs in two broad phases. In the first, which may proceed in the absence of oxygen, the six-carbon molecule is degraded into any one of several smaller fragments. In the second, all these are drawn into an oxidation cycle in which they are converted to carbon dioxide and water and which proceeds only in the presence of oxygen. The first phase may take one of two forms. It may involve degradation of the sugar into three-carbon fragments in the process of glycolysis which is similar to fermentation. Or, it may involve the direct oxidation of the sugar.

Two important features of the whole oxidation mechanism are displayed during glycolysis, The process depends on repeated phosphorylation and it yields adenosine triphosphate (ATP). In the initial phase the sugar is esterified to give fructose diphosphate in which phosphate is incorporated in ester linkage. After this stage the hexose is split into three-carbon fragments and further phosphorylation of each fragment occurs. At a late stage in the process pyruvic acid is generated, but before this each phosphate group is transferred to adenosine diphosphate with the formation of adenosine triphosphate, the release of which constitutes a decisively important aspect of respiration.

In glycolysis one mole of sugar is converted to two moles of pyruvic acid. In this process several steps and a corresponding number of enzymes are involved. These enzymes do not require aerobic conditions, and whether oxygen is present or absent pyruvic acid continues to be generated.

In the presence of oxygen the pyruvic acid may be further degraded to two-carbon acetate. In the absence of oxygen pyruvic acid is converted to alcohol with the intermediate formation of acetaldehyde



Many physiologists have considered the anaerobic degradation of carbohydrates to alcohol and carbon dioxide or to lactic acid as a form of respiration. This type of metabolism is often called "anaerobic respiration" or "intramolecular respiration". Some authors prefer to designate this metabolism as fermentation. (Goddard, D. R. 1960). On the assumption that a hexose is the substrate, the summary chemical equation for respiration is

$$C_6H_{12}O_6 + 6O_2 \text{ --- } 6CO_2 + 6H_2O + \text{Energy.}$$

This equation is exactly the reverse of the photosynthetic equation and the same quantity of energy is required in the synthesis of one mole of hexose as is released when one mole of it is oxidized in respiration.

The oxidation of a hexose in plant cells does not take place in a single step as indicated in this convenient summary equation. This equation merely tells us that for the oxidation of one mole of a hexose, six moles of oxygen are required; that six moles of carbon dioxide and six moles of water result from this oxidation. Since equimolecular weights of gases occupy the same volume, the volume of oxygen consumed is equal to the volume of carbon dioxide released.

The water formed as a result of respiration becomes a part of the general mass of water present in the respiratory cell. (Meyer, B. S. 1952).

As has been said "anaerobic respiration" or "fermentation" is characteristic of biological oxidations in an oxygen free environment. "Anaerobic respiration", however, may also take place in an atmosphere abundant in oxygen. (Biale, J. B. 1969).

2. Respiration Rate

The intensity at which respiration proceeds, and the way in which this intensity is modified by altered conditions, is a matter of interest in itself and also because it may shed light on the mechanism of the respiration. There is, however, an initial difficulty met with in dealing with this question, as it is not always a simple matter to find a mode of expressing the intensity of respiration. In the first place, the rate of respiration of a plant or organ might be expressed as the quantity of carbon dioxide evolved by the material in unit of time, or as the quantity of oxygen absorbed, or as the amount of substrate decomposed. If the substrate is always the same, and if the chain of reactions involved remains the same, then any of these three quantities will give a measure of the rate of respiration. But if the substrate and reaction chain alter with time, then the quantity of substrate disappearing, the oxygen absorbed and carbon dioxide evolved, will not bear a constant relation to one another, and it may not always be clear which of the three values will give the best criterion of respiration.

Having these difficulties in mind, the most satisfactory measure of respiration rate is generally regarded as given by the rate of carbon dioxide evolution. Oxygen has been used as a measure of respiration, but the loss in substrate, which might be a better criterion of respiratory activity than either carbon dioxide evolution of oxygen absorption, is not as a rule easily measurable. (Stiles, W. 1950).

3. Respiration Ratios (R. Quotient)

The ratio of the volume of carbon dioxide released to the volume of oxygen absorbed in the respiration process is termed the respiration quotient or ratio. When complete oxidation of a hexose takes place:

$$\frac{\text{Vol } CO_2}{\text{Vol } O_2} = 1$$

The respiration ratio for any plant or plant part can be determined by making parallel measurements of the rates of carbon dioxide release and oxygen consumption.

Factors affecting the respiration ratio:

a. Metabolism of compounds which are relatively poor in oxygen as compared with hexoses (fats).

b. Metabolism of compounds which are relatively rich in oxygen as compared with hexoses.

c. Occurrence of anaerobic respiration.

In this last process, which may occur in the higher green plants under certain conditions, carbon dioxide is released without any corresponding absorption of oxygen.

Sometimes, when the oxygen supply is defficient, both aerobic and anaerobic respiration occur simultaneously in a plant tissue. Some cells may be respiring anaerobically, while others are carrying on aerobic respiration. (Meyers, S. B. 1953).

4. Factors Affecting Respiration Rates After Harvest

a. Age of fruit

Respiration rate is not constant for apples as they ripen. This is true even if they are held at constant temperature. Just prior to the normal time of harvest there is a declining in the rate of respiration.

Following the minimum or low point on the respiration curve, there is a rise in the respiration rate. The rate increases until it reaches a peak which is then followed by a gradual decline. The rise in the rate is called a "climacteric" rise and the peak is called the "climacteric" (Kidd, F. 1930, 1933). While apples are declining in rate following the peak they are said to be in "senesence".

The climacteric rise in respiration is due primarily to an increase in ADP concentration with the change in ATP/ADP ratio and the tissue's capacity for increasing the activity of respiration enzymes. (Pearson, J. 1954).

b. Variety of apples

In general, varieties with a short storage life have a more rapid respiration rate than varieties with a long storage life.

The height of the climacteric curve serves as a basis to compare the respiratory activity of apples. For example, the peak value of Duchess of Oldenburg (a very short storage variety) was 65 mg of carbon dioxide per kilo per hour; that of Wealthy (a short storage life variety) was 46; that of McIntosh (a moderately short storage life) was 34; and that of Rome Beauty (a long storage life) was 25.

Respiration Rates of Different Varieties (Magnes, J. R. et al. 1926)

VARIETY	CO_2mg/kg per hour (16° C)
Grines Golden	29.95
Jonathan	26.48
Baldwin	19.6
Winesap	20.5

c. Effect of temperature

Most chemical reactions, according to Van't Hoff's law, increase two or four times for every 10° C rise in temperature. The exact increase for such temperature has been called the temperature coefficient Q_{10}.

The Q_{10} for respiration of apples varies with variety, temperature of storage, composition of the atmosphere, and age of the fruit. In general, of course, the respiration rates rise with an increase in temperature. In studies of different varieties, the Q_{10} for apple respiration has been shown to vary between 2.1 and 2.9. (Gore, H. C. 1911). Larger Q_{10} as 3.27 was found for English grown apples.

In the following table rates of respiration at different temperatures for apples of the Baldwin variety are tabulated. (Magnes, J. R. 1926).

T °C	CO_2 mg/kilo per hour
4	5.37
16	19.65
30	38.49

d. Effect of carbon dioxide

The presence of carbon dioxide around the apple tends to slow down the respiration rate. In general, the higher the concentration of carbon dioxide the more the respiration rate is depressed. (Kidd, F. 1927).

The effect of carbon dioxide might be explained on the basis of the law of mass action. However, the real reason that carbon dioxide slows down the rate of respiration is not perfectly known. Other authors suggest that there is an effect on the pH of the tissue and hence on respiration. Apples are sensitive to carbon dioxide and their tolerance varies with the temperature of storage and variety. In general, apples are more tolerant of its presence at high temperature than at low. Carbon dioxide injury is evidenced by browning of the flesh in some varieties and/or by rough depressed areas on the skin of other varieties. Susceptibility may depend also upon the growing or climateric conditions in the orchard. (Plagge, H. H. 1942).

Studies done by Hulme (1956) showed that carbon dioxide injury is accompanied by an increase in succinic acid in the tissue. It appears that an abnormality of metabolism is induced in the tissue by hypernormal concentration of carbon dioxide in the surrounding atmosphere which results in an accumulation of succinic acid. This in turn, it is suggested, kills the tissue.

e. Effect of oxygen

When the concentration of oxygen present in the atmosphere is reduced, there is a reduction in the rate of apple respiration. This is easily explained by the fact that oxygen is one of the reacting materials in the process of respiration. Experiments made by Kidd and West (1945). With apples under different oxygen concentrations showed that:

i. The concentration of oxygen influenced the time of onset of the climateric rise

ii. Lowering the oxygen concentration to 5% has a marked effect on the rate of carbon dioxide production in the postclimateric phase. Oxygen concentration between 5-100% has relatively little effect on the rate of carbon dioxide production in the preclimateric phase. The experiments were done with apples of Bramley's Seedling varieties.

Blackman and Parija (1928) in respiration studies found that in apples of the Bramley Seedling variety either an increase or decrease in oxygen concentration from about 3.5% increased the intensity of respiration (in terms of carbon dioxide). This particular percentage has been called the "extinction point of N. R." (that is, respiration in nitrogen), when the oxygen available was just sufficient to stop anaerobic respiration. As the percentage was increased the respiration rate also increased simultaneously.

f. Effect of relative humidity

Studies have shown that the relative humidity did not affect the respiration of apples unless the humidity became very low. This reduction could be attributed to physical changes in the character of the skin which accompanied shrivelling. In other words, the resistance to diffusion of gases was increased. Variations in the relative humidity in the higher ranges (70-100%) seemed to be ineffectual in affecting the respiration rate. An explanation of this lack of effect might be the fact that the humidity of intercellular spaces of the fruit stay close to saturation until the apples become somewhat dessicated. In other words, external changes in relative humidity may not markedly affect internal relative humidity until considerably large quantities of water have been lost from the fruit. (Smock, R. M. 1950)

5. Measurement of Respiration Rate

The initial studies on plant respiration were done in 1804 by de Saussene in Paris. He used a closed container in which plants were enclosed. The measurements were made by analysis of oxygen consumed and carbon dioxide liberated. (Goddard 1960). Since then several systems and different analytical methods have been used. Most of them will be reviewed here.

The most widely used system has been the Pettenhoffer method. In this method a stream of air which is carbon dioxide free is passed over the respiring plant parts; the respiratory carbon dioxide is then removed by absorption in sodium hydroxide solution, and the carbon dioxide determined volumetrically. Respiration is determined here in terms of carbon dioxide only; oxygen uptake is not determined.

A modification of this method in which the carbon dioxide is absorbed in 0.050N sodium hydroxide in a tower which contains platinized platium electrodes, and the decrease in conductivity that results from the conversion of sodium hydroxide to sodium carbonate is read on a 1000 cycle Wheatstone bridge. (Wolf, J. N. 1952).
Objections have been made to the Pettenhoffer method in the sense that the method cannot distinguish between carbon dioxide of fermentation and that of respiration.

H. C. Gore (1911) and P. Parija (1928) used a similar method in their studies concerning respiration rates of a wide variety of vegetables and fruits.

Platenius (1942) used a method in which the rate of oxygen uptake and carbon dioxide evolution were measured simultaneously. The method developed by Magness and Diehl(1924) and modified by Haller (1932) consists of a container connected to a Mariotte bottle. The oxygen uptake is measured by the water displacement due to the changes in pressure; and carbon dioxide is absorbed by a potasium hydroxide solution inside the container and determined volumetrically. Using this method, Platenius determined the rate of respiration of peas, snap beans, spinach, lettuce, carrots, peppers, tomatoes, and cucumbers.

S. T. Shaw (1942) used the Warburg's respirometer using the Bancroft differential technique. In this method changes in volume are registered as positive or negative pressure by the manometer. Sodium hydroxide inside the vessel absorbs the carbon dioxide, and changes in pressure will be proportional to the oxygen takeup. A. H. Brown (1952) developed a method of following oxygen uptake, and also carbon dioxide production, using a mass spectrometer and heavy (stable) isotopes.

In this study gas chromatography will be applied as the analytical method to measure respiration rates. Carbon dioxide and oxygen are measured simultaneously. To apply this technique the respiration chambers are held under "static conditions": the atmosphere surrounding the fruits is not changed.

A complete description of the system and method will be given in the EXPERIMENTAL part of this report.

B. CONTROL OF RESPIRATION BY ATMOSPHERIC CONTROL

Apples can ordinarily be held in satisfactory condition for rather long periods in cold storage. There are certain instances, however, where cold storage has not proved entirely adequate. For example, McIntosh apples cannot be held in ordinary cold storage with full assurance that they will be of prime eating quality after February or March.

McIntosh apples frequently develop a cold storage disorder known as brown core when held for extended periods in ordinary cold storage. (Smock 1950). To complement the cold storage the so-called "controlled atmosphere storage" is used. Controlled atmosphere storage was originally developed in England by Kidd and West (1935).

As has been described previously, fruits in general carry on respiration. In respiration sugars are oxidized in the presence of oxygen. Carbon dioxide, water vapor, and heat are produced as a result of the process. The more rapid the respiration takes place, the more quickly the fruit will deteriorate.

Commercial methods of storing fruits and vegetables in modified atmosphere (controlled atmosphere) are based on the fact that respiration can be retarded by maintaining an atmosphere in which the oxygen content is lower and the carbon dioxide concentration is higher than in normal air. Many investigators assume that it is the presence of carbon dioxide rather that the limited oxygen supply which has a depressing effect on the physiological activity of the plant tissue.

There is a reason to believe, however, that the importance of a limited oxygen supply has been underestimated. Studies done with potatoes and onions show that the respiration rates were increased when held in an atmosphere of normal oxygen content to which varying quantities of carbon dioxide had been added. On the other hand, the same treatment had a depression effect on the respiration activity of asparagus. (Platenius, H. 1943).

A very important factor to be taken into account is the fact that the concentration of carbon dioxide and oxygen must be such that physiological disorders not be produced in the fruit. Oxygen concentrations must not be lower than the value at which anaerobic respiration takes place. Excess of carbon dioxide concentration causes browning of the flesh, and excess of oxygen, rough depressed areas on the skin of other varieties. In other varieties there is not only a browning of the flesh but also a corky appearance in the affected portion.

C. PACKAGING OF APPLES

Prepackaging may be defined in various ways, but it is essentially the packaging of produce in a container of size and shape suitable for the consumer without repacking in stores at the time of sale. Its recent rapid growth is largely a result of the growth of self-service stores, especially the large super market chain stores.

When it is given proper care, prepackaged produce has a number of advantages over bulk produce. Some of the advantages of prepackaging are that:

It simplifies self-service in retail markets

It reduces clerical help needed in stores

It gives protection to the packaged products

It reduces wastes

It reduces water losses and wilting

Packages are sanitary (Schommer 1953a).

Fresh fruits and vegetables differ from processed foods in one important aspect; they remain living organisms until they are cooked or consumed. Being living organisms, they undergo some of the normal life processes; they continue to respire, they lose water in perspiration, and they are subject to slow chemical changes. All of these processes contribute to the gradual deterioration of the product.

Any handling procedure, including prepackaging, should retard these life processes without stopping them altogether.

Once the plant cells have been "killed" the product becomes unfit for food within a few hours unless it is frozen, dried, or kept under sterilized conditions. It is for this reason that the requirement of transparent films for prepackaging differ somewhat from those used for wrapping frozen foods and other non-living products. Transparency and low permeability to water vapor are desirable characteristics in either case. In addition, films for prepackaging fruits and vegetables must be permeable to oxygen and carbon dioxide. (Platenius, H. 1946). To visualize better what happens when a fruit or vegetable is packaged in a plastic film container the same author reports in his work the course of the process when asparagus is packaged in an air-tight container. During the first hours after prepackaging, respiration proceeds at a normal rate. During this period carbohydrates, primarily sugars, are oxidized to carbon dioxide and water. Expressed in terms of a simple chemical equation, the process is

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O.$$

Depending on the temperature which controls the rate of respiration, the oxygen supply becomes exhausted more or less rapidly. As the oxygen is depleted, the rate of respiration also declines. Unfortunately, a sudden change in the course of respiration occurs when the oxygen concentration drops to 3% (for asparagus). At this point, "anaerobic respiration" starts. With no free oxygen entering the reaction, carbohydrates are being oxidized to alcohol and carbon dioxide.

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2.$$

Alcohol imparts an undesirable odor and taste, the plant cells decay, and the product becomes unsalable.

An ideal type of film would be one that permits the diffusion of a quantity of oxygen just enough to maintain an oxygen concentration higher than the limit at which "anaerobic respiration" starts. The same applies to carbon dioxide since high concentrations may injure the product. Most of the plastic films used for packaging do not meet these requirements. Because of this, holes are punched in plastics in order to permit a free diffusion of gases.

Hardenburg (1958), Gehard (1951), Scott (1947), Allen (1950), Schommer (1953b), and Stahl ( ) did studies on packaging of different vegetables and fruits. They emphasize the importance of the permeability of the films to oxygen and carbon dioxide and recommend the practice of punching holes in the bags used for prepackaging of these products. Commercially, the practice of punching holes is being used extensively.

III. EXPERIMENTAL

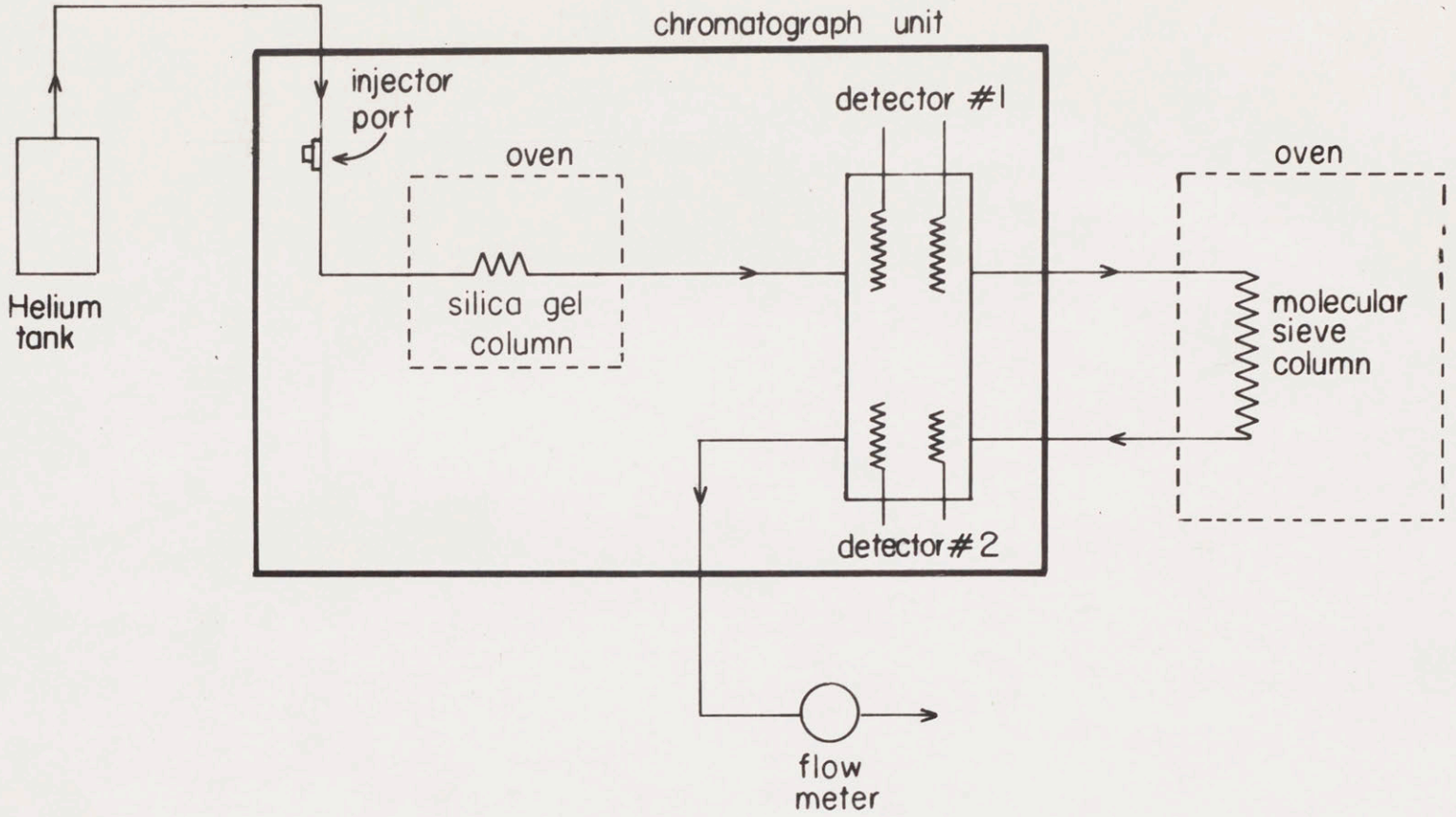
A. CALIBRATION OF EQUIPMENT

An Aerograph Model A-90P gas chromatograph was used in the present work. The following modifications were made in order to adapt the instrument for the requirements of the experiment.

1. A thermostat was installed to control the temperature of the inside column.
2. A constant temperature oven was installed and used to maintain the outside column at constant temperature.
3. A polarity reversal switch was installed so that the recorder deflection could always be maintained in the same direction.

To detect simultaneously CO_2, O_2, and N_2, two different kinds of columns were used. The first column, silica gel, performs the separation of carbon dioxide from oxygen and nitrogen. The second column, "molecular sieve" (a synthetic zeolite) absorbs carbon dioxide and separates oxygen and nitrogen. A diagram of the gas chromatograph appears in Figure No. 1.

Working with silica gel and molecular sieve columns, and under the following conditions, a satisfactory CO_2, O_2, N_2 resolution was obtained.



SCHEMATIC DIAGRAM OF GAS CHROMATOGRAPHIC APPARATUS FOR GAS ANALYSIS.

FIG. 1

Column temperature	100° F
Filament current	200 m.a.
Carrier gas	Helium
Flow of carrier gas	100 cc/min.
Silica gel column (30/60 mesh)	6 inches length
Molecular sieve column (90% 13x, 10% 5-A)	20 inches length

The signals from the detectors were recorded with a Leeds-Northrup recorder.

B. CALIBRATION CURVES

Using the conditions enumerated before, satisfactory retention times for carbon dioxide, oxygen, and nitrogen were obtained. A typical chromatogram appears in Figure No. 2, in which the retention time for each one of these gases is shown. By retention time is meant the time required from the beginning of the sample injection to reach the maximum peak of the test gas. For a given system of columns, under specified conditions, it designates the order of the component gases coming out of the column. Under the present experimental conditions, the following retention times were found:

Carbon dioxide	76.4 seconds
Oxygen	148.9 seconds
Nitrogen	193.5 seconds

The quantitative interpretation of the chromatograms were based on the peak area. The area under the peak was calculated by multiplying the peak height by the width at half height. A special device was designed to obtain these dimensions, and using a cathetometer the peak height was measured and the half height determined. In the device the strip chart is maintained in a vertical position parallel to the

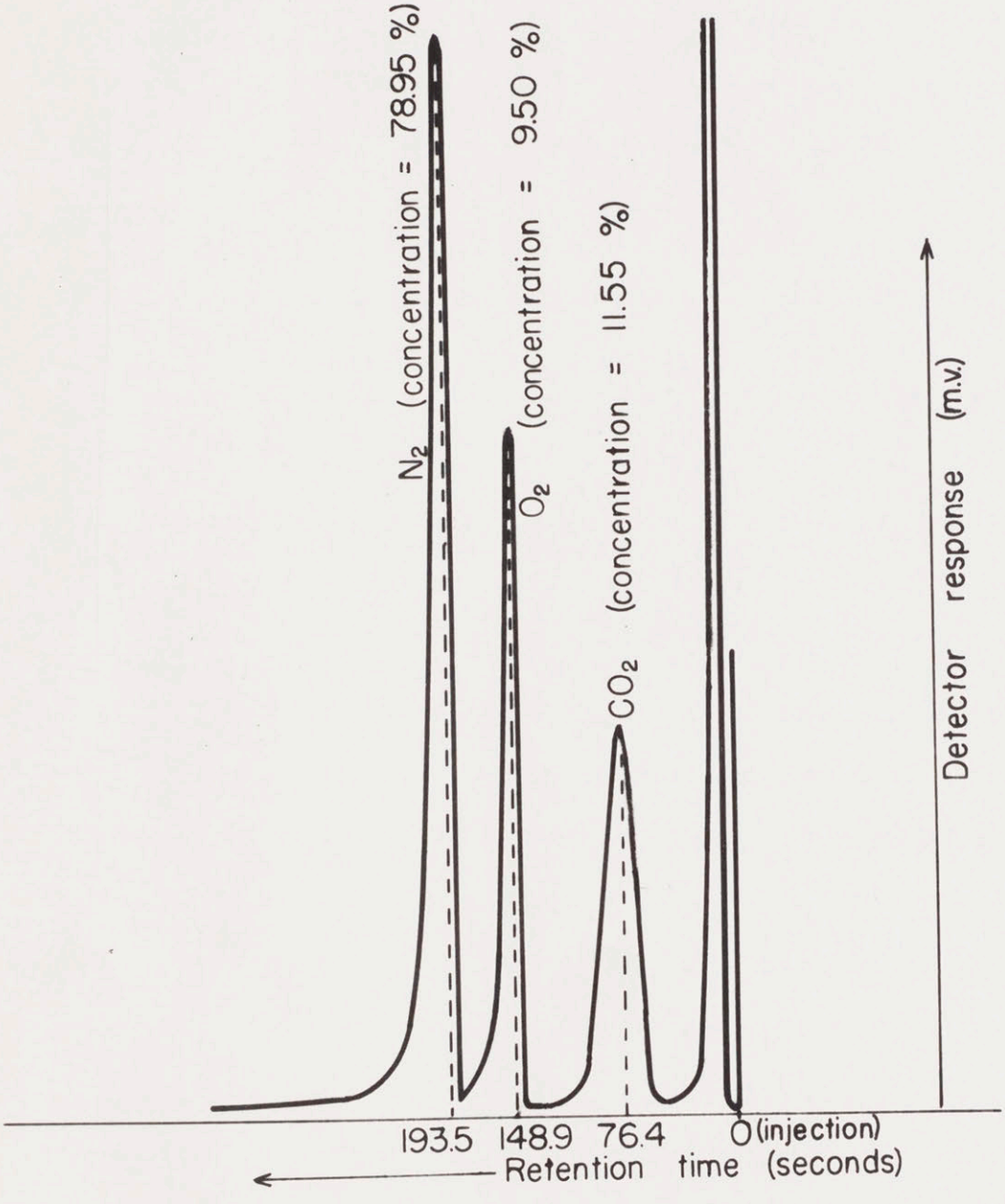


FIG. 2

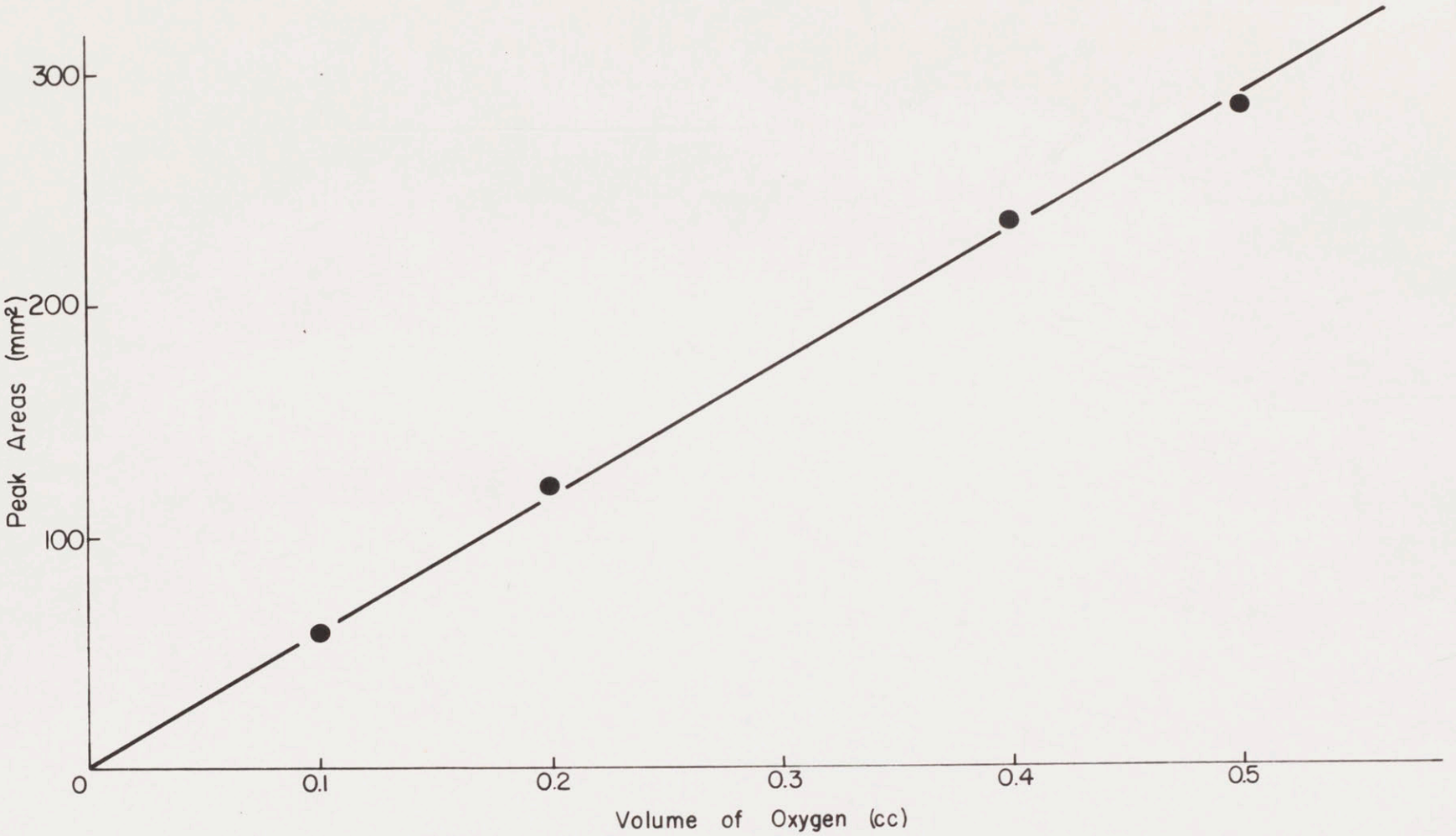
TYPICAL CHROMATOGRAM OF A GAS SAMPLE.

cathetometer, and moved over a flat surface by means of rollers in such a manner as to maintain the individual peaks parallel to the cathetometer scale. The width at half height is measured with a calibrated micrometer placed in the cathetometer eyepiece. The calibration curves were made by plotting the peak areas against the gas volume. Calibration curves for carbon dioxide and oxygen with different sensitivities were made. The gas samples were measured and injected with calibrated 10 cc and 1.0 cc gas-tight syringes. Figures No. 3 and No. 4 show typical calibration curves for oxygen and carbon dioxide at a sensitivity of 1/12. The area of the peak was found to be proportional to the volume of gas analyzed. In order to convert the peak area into gas volume, a conversion factor was calculated for each gas at each sensitivity. This conversion factor is the inverse of the slope of the calibration curve and represents the volume of gas per unit of area. The results of calibrations are shown in Table I.

TABLE I

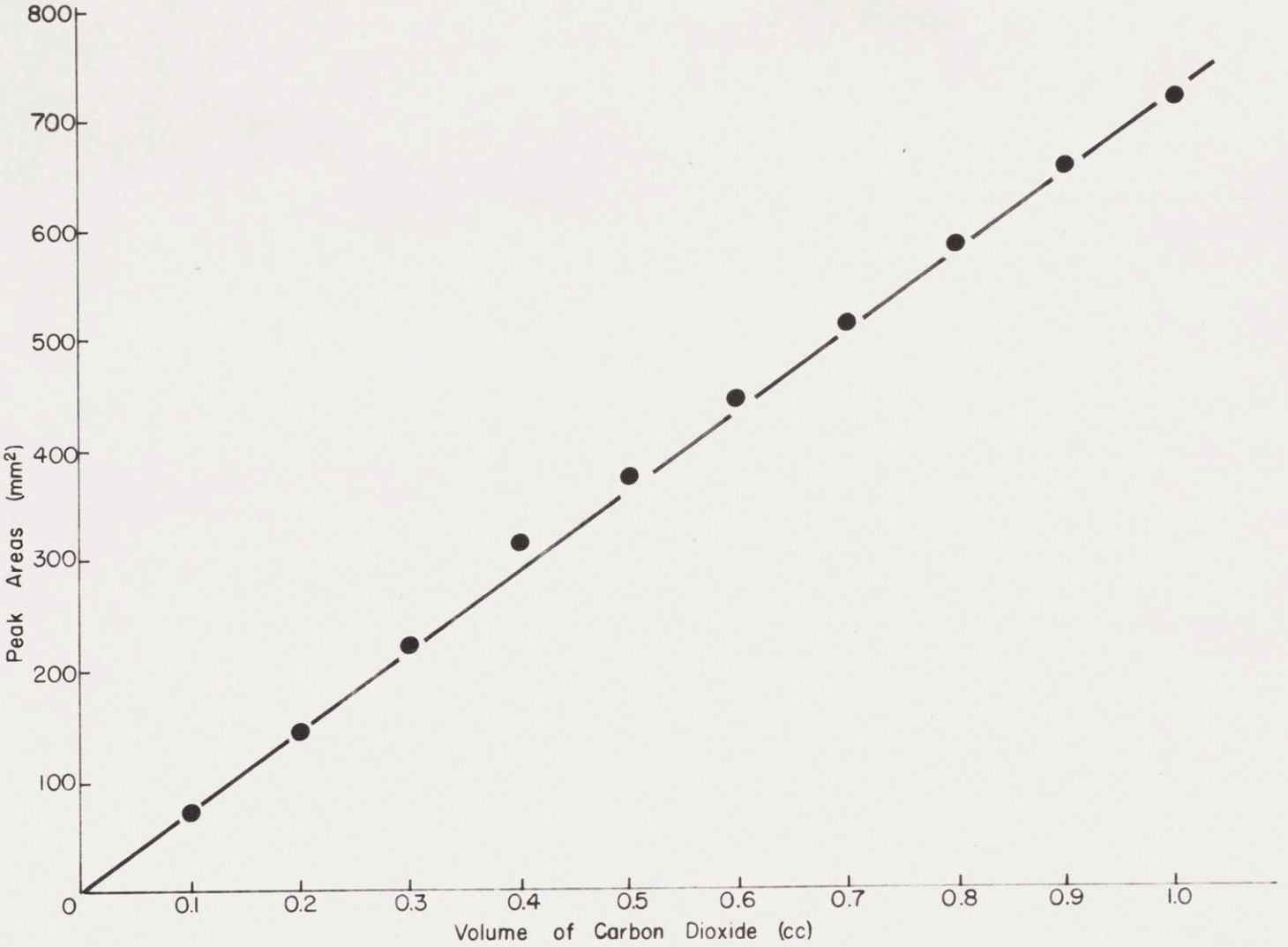
Oxygen

Sensitivity (s)	a	$b \times 10^4$	$b \times s \times 10^4$
1	-	1.44	1.44
1/2	-	2.69	1.35
1/4	-	5.55	1.39
1/12	-	17.5	1.46
1/20	-	28.8	1.44
1/69	-	101.0	1.46



TYPICAL CALIBRATION CURVE FOR OXYGEN (SENSITIVITY 1/12).

FIG. 3



TYPICAL CALIBRATION CURVE FOR CARBON DIOXIDE (SENSITIVITY 1/12).

FIG. 4

Carbon Dioxide

Sensitivity (s)	a	b	b x s 10^4
1	-1.45×10^{-3}	1.415	1.415
1/2	-	2.18	1.09
1/4	-	4.43	1.11
1/12	-	13.9	1.16
1/69	-3.6×10^{-1}	83.0	1.20

s = sensitivity
a = a constant
b = conversion factor $\frac{\text{cubic centimeters}}{\text{square millimeters}}$

To apply the factors tabulated in Table I the following formula is used:

$$V = a + bA$$

where V = volume of gas present in the sample (cubic centimeters)

A = area under the curve in square millimeters

b = conversion factor

a = a constant

C. RESPIRATION CHAMBERS

For the present respiration studies, respiration chambers were constructed adapting one-gallon glass jars. The lids of these chambers have two copper tubing lines used to evacuate, fill or replace the atmosphere inside the chambers. Besides, they have a sampling system for gas-tight syringes.

The injection ports consisted of 3/16" copper compression unions fitted with gas-tight, self-sealing injector gaskets and soldered to the chamber lids. With this system it is possible to take the samples directly without disturbing or changing the inside atmosphere. In order to avoid changes in the inside atmosphere due to gas interchange between inside and outside of the chamber, these chambers must be gas-tight. To check this, the chambers were evacuated and filled with nitrogen. Samples were taken immediately after filling with nitrogen and after 24, 48, and 72 hours. No changes in the gas composition were detected. The chambers were also found to maintain a vacuum. A diagram of a respiration chamber appears in Figure No. 5.

D. SYSTEM TO CHARGE THE CHAMBERS WITH GASES

A system was devised to charge the chambers with known concentrations of oxygen, nitrogen, and carbon dioxide. The chambers were evacuated and the absolute pressure determined with a mercury manometer. The individual gases were then admitted until the desired partial pressure was reached. Since in the range of pressures used, the partial pressure may be considered proportional to volume, the concentration of each gas could be controlled accurately. The final pressure inside the chambers was equal to the atmospheric pressure. Samples were analyzed to check the final composition. A complete diagram of this system appears in Figure No. 6.

The following example illustrates the way this system was applied:

Oxygen introduced until total pressure was 85 mm Hg.

Nitrogen introduced until inside pressure was equal to atmospheric pressure.

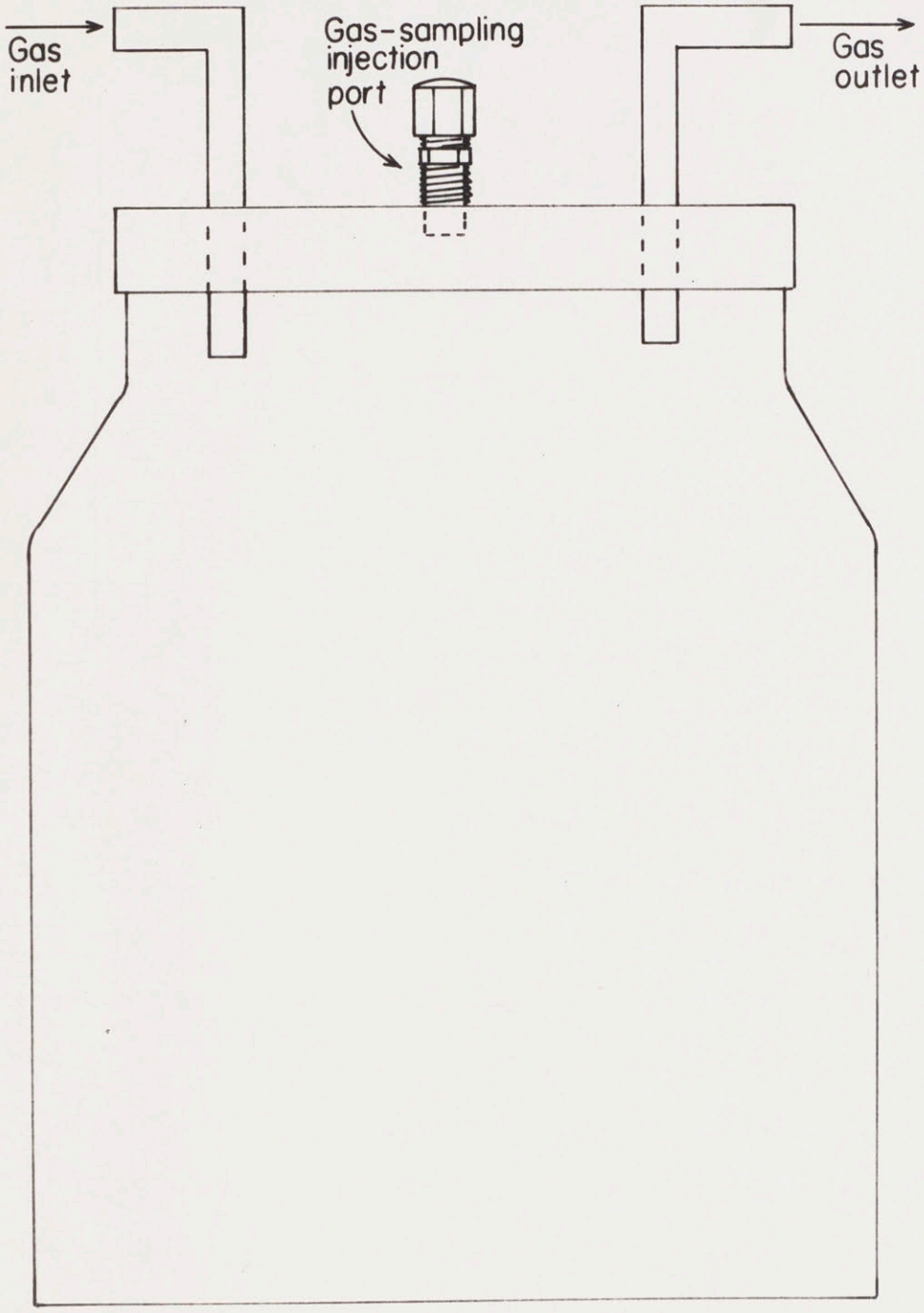
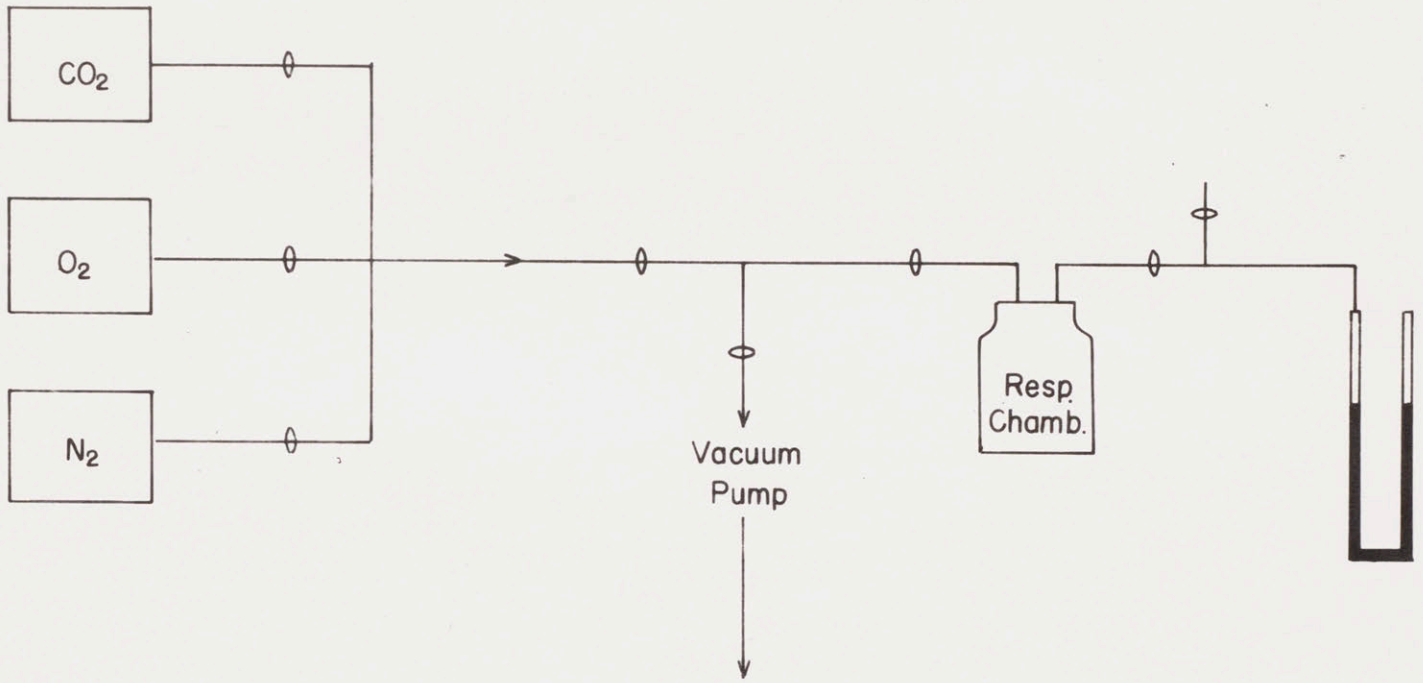


DIAGRAM OF A RESPIRATION CHAMBER.

FIG. 5



SCHEMATIC DIAGRAM OF SYSTEM FOR ADJUSTMENT OF ATMOSPHERES IN RESPIRATION CHAMBERS.

FIG. 6

In this way the atmosphere inside the chamber will be expected to have a concentration of approximately 10% oxygen and 90% nitrogen. When these concentrations were checked with the gas chromatograph, the oxygen concentration was found to be always a little higher than the concentration expected, due to residual air left in the chamber after the evacuation.

E. DEVELOPMENT OF PROCEDURE AND EVALUATION OF RESULTS

The general procedure was as follows: Apples were placed inside the chamber and the chamber was checked for gas-tightness. The chambers containing the apples were then conditioned at the desired temperature for a period of twenty-four hours. After this time the atmosphere in the chamber was replaced by a new atmosphere containing the desired concentration of oxygen, nitrogen, and carbon dioxide. This step was done using the system described before. Samples were taken and analyzed to check the initial concentrations. The chambers were then stored at the desired temperature, and samples were taken at known intervals of time and analyzed for oxygen and carbon dioxide.

In general, five different types of experiments were run:

1. Respiration Rates Without Readjustment of Chamber Atmosphere

The variables studied in these experiments were initial respiration rates, influence of the oxygen pressure decrease on the respiration rates, influence of the carbon dioxide build-up on the respiration rates, critical oxygen concentration for anaerobic respiration.

2. Respiration Rates at Approximately Constant Oxygen Concentrations

The atmosphere in these experiments was replaced every twenty-four hours by a new atmosphere containing the same initial oxygen concentration. In these experiments the following variables were studies: influence of oxygen concentration on respiration rates and critical oxygen concentration for anaerobic respiration.

3. Respiration Rates with Continuous Removal of Carbon Dioxide

This method served to evaluate the accuracy and convenience of the static method used to determine respiration rates.

4. Respiration Rates Starting with Atmospheres Containing Carbon Dioxide

This provided a system to observe the influence of carbon dioxide on the respiration rate of apples.

5. Respiration Rates of Apples at Different Storage Temperatures

The influence of temperature on respiration rates and the Q_{10} value were studied in this experiment.

The following data were required to evaluate the results:

a. Atmosphere volumes of the respiration chambers
b. Volume of apples
c. Weight of apples
d. Initial oxygen concentrations
e. Final oxygen concentrations
f. Initial carbon dioxide concentrations
g. Final carbon dioxide concentrations
h. Volume of carbon dioxide produced
i. Volume of oxygen produced
j. Time of storage between samplings
k. Temperature of storage

6. Packaging Experiment

In these experiments, the changes in the composition of the atmosphere inside plastic film bags containing apples were evaluated.

F. SERIES OF EXPERIMENTS AND RESULTS

Series No. 1: Respiration Studies Without Readjustment of Chamber Atmosphere

Apples weighing 524 grams were placed in the respiration chamber containing air and maintained at a temperature of 20° C. Two runs were made. Their duration was twelve days and thirteen days, respectively. During each run, samples of the chamber atmosphere were taken every twenty-four hours, and analyzed for oxygen and carbon dioxide. The results of these analyses were used to determine the respiration rates as a function of oxygen consumption and carbon dioxide evolution.

The results of the runs are presented in Tables II and III, and in Figures 7 and 8.

Tables II and III show the following variables as a function of time:

Average oxygen concentration

Average carbon dioxide concentration

Average rate of oxygen consumption

Average rate of carbon dioxide evolution

Average ratio of carbon dioxide evolution to oxygen consumption

Figures 7 and 8 show a graphical representation of the results. Figure 7 shows average carbon dioxide and oxygen concentrations as a function of time, and Figure 8 shows the effect of oxygen concentration on the rate of oxygen consumption, and on the respiration ratio.

The rates of respiration are expressed in terms of cubic centimeters of gas per kilogram of apples per hour (cc/Kg.Hr.).

TABLE II

Respiration Studies on Apples

Experimental Series 1

Run 1

Time (Hours)	Average O_2 concentration (%)	Average CO_2 concentration	CO_2 evolution rate cc/Kg.Hr.	O_2 consumption rate cc/Kg.Hr.	Respiration ratio (CO_2/O_2)
12	19.95	1.40	7.1	5.6	1.2
36	17.5	4.25	7.2	6.4	1.12
60	14.5	6.75	6.3	7.5	0.85
84	11.7	9.1	6.5	7.2	0.88
108	9.44	11.7	6.5	5.6	1.15
132	7.55	13.95	5.03	4.88	1.03
156	6.02	16.02	5.3	3.89	1.3
180	4.73	17.9	5.2	3.11	1.0
204	3.35	19.4	3.96	3.4	1.16
228	2.4	20.8	3.24	2.4	1.3
253	1.8	22.1	3.3	1.55	2.13
278	1.35	23.7	4.4	1.10	4.0

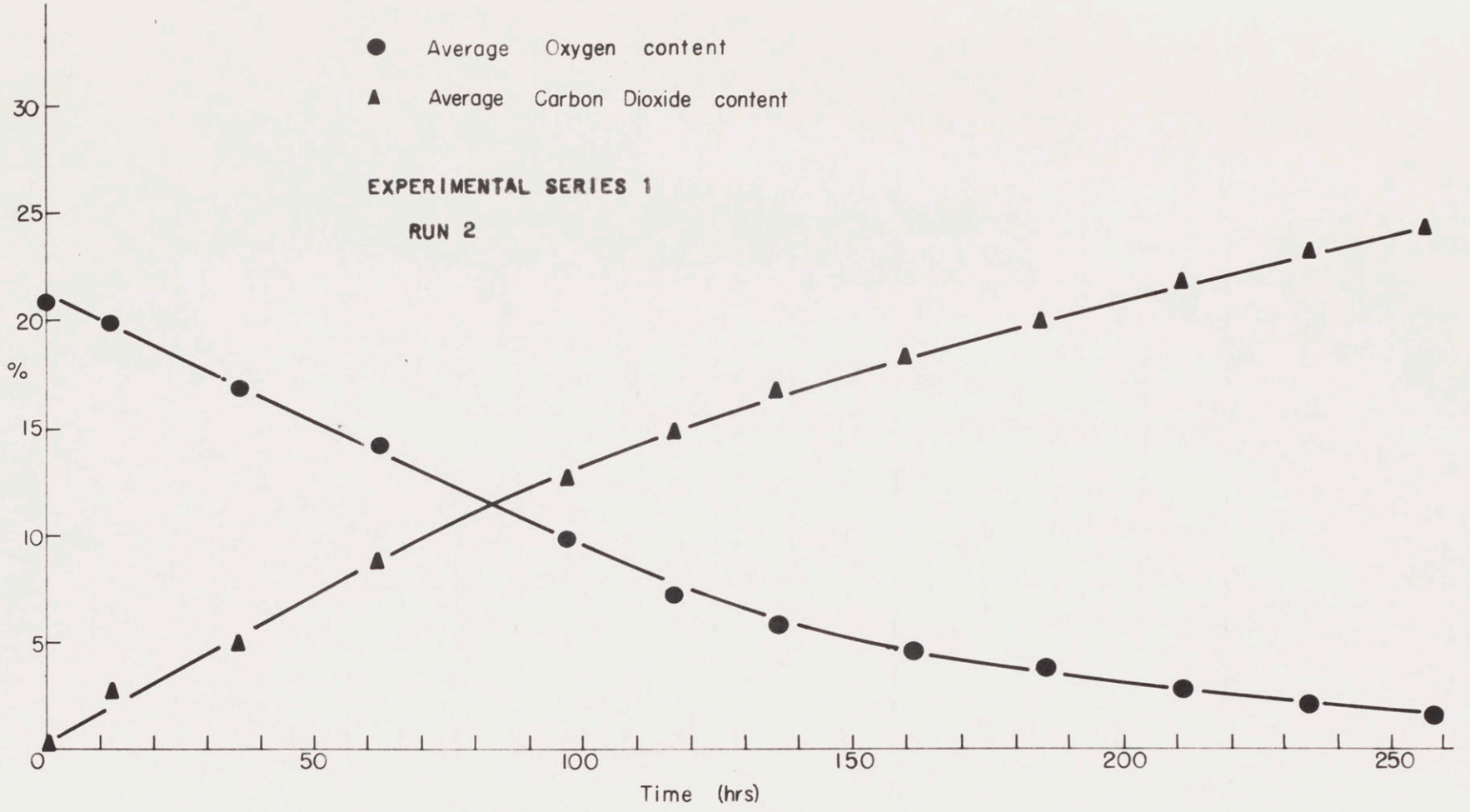
TABLE III

Respiration Studies on Apples

Experimental Series 1

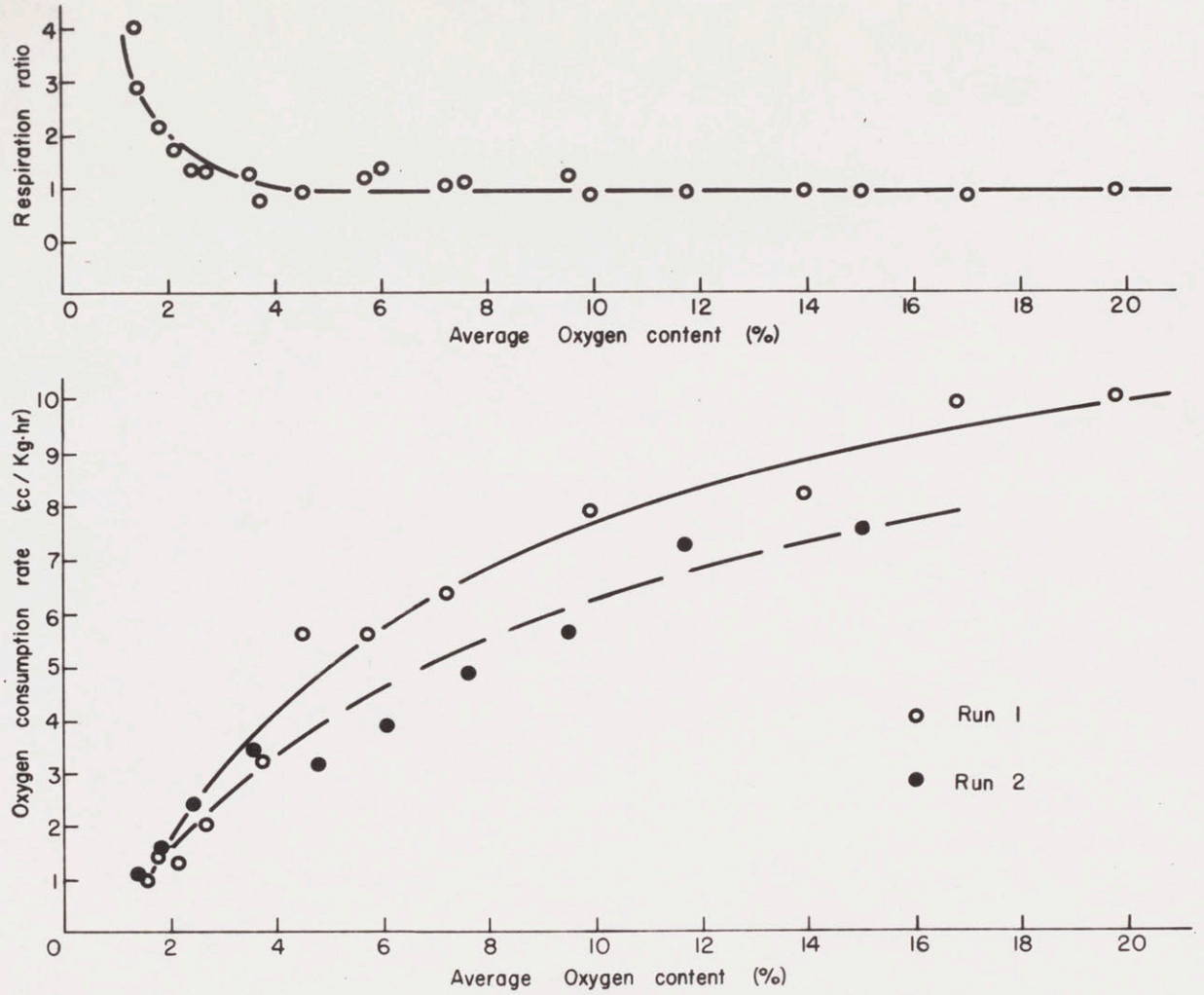
Run 2

Time (Hours)	Average O_2 concentration (%)	Average CO_2 concentration	CO_2 evolution rate cc/KgHr.	O_2 consumption rate cc/KgHr	Respiration ratio (CO_2/O_2)
12	19.8	2.71	8.44	10.	0.85
36	16.85	5.1	7.45	9.9	0.75
62	13.9	5.7	7.2	8.2	0.88
97	9.9	12.05	6.4	7.9	0.81
117	7.2	14.65	6.2	6.35	0.98
136	5.7	16.6	6.5	5.6	1.16
161	4.5	18.3	5.2	5.6	0.93
186	3.7	19.8	2.47	3.2	0.78
211	2.7	21.6	2.6	2.0	1.3
235	2.1	23.0	2.15	1.3	1.6
258	1.75	23.9	1.9	1.45	1.3
294	1.4	25.7	2.9	1.0	2.9



CHANGES IN GAS COMPOSITION IN RESPIRATION CHAMBERS.

FIG. 7



EFFECT OF OXYGEN CONCENTRATION ON RESPIRATION RATE AND RESPIRATION RATIO. (EXPERIMENTAL SERIES 1.)

FIG. 8

Series No. 2: Respiration Rates at Approximately Constant Oxygen Concentrations

Apples weighing between 514 and 538 grams were placed in the respiration chambers and maintained at a temperature of 20° C. Samples of the chamber atmosphere were taken every twenty-four hours and analyzed for oxygen and carbon dioxide.

In this experimental series the atmosphere was replaced every twenty-four hours by a new atmosphere containing the same initial oxygen concentration, so that the concentration was held approximately constant for each run. Also, the carbon dioxide build-up was prevented, and in no case did the carbon dioxide exceed 4% in the runs.

Several experiments were conducted using different average oxygen concentrations ranging from 1.4 to 18.6%.

The purpose of this experiment was to study the influence of oxygen concentration on respiration rate and the oxygen concentration at which anaerobic respiration starts under conditions of frequent adjustment of chamber atmospheres.

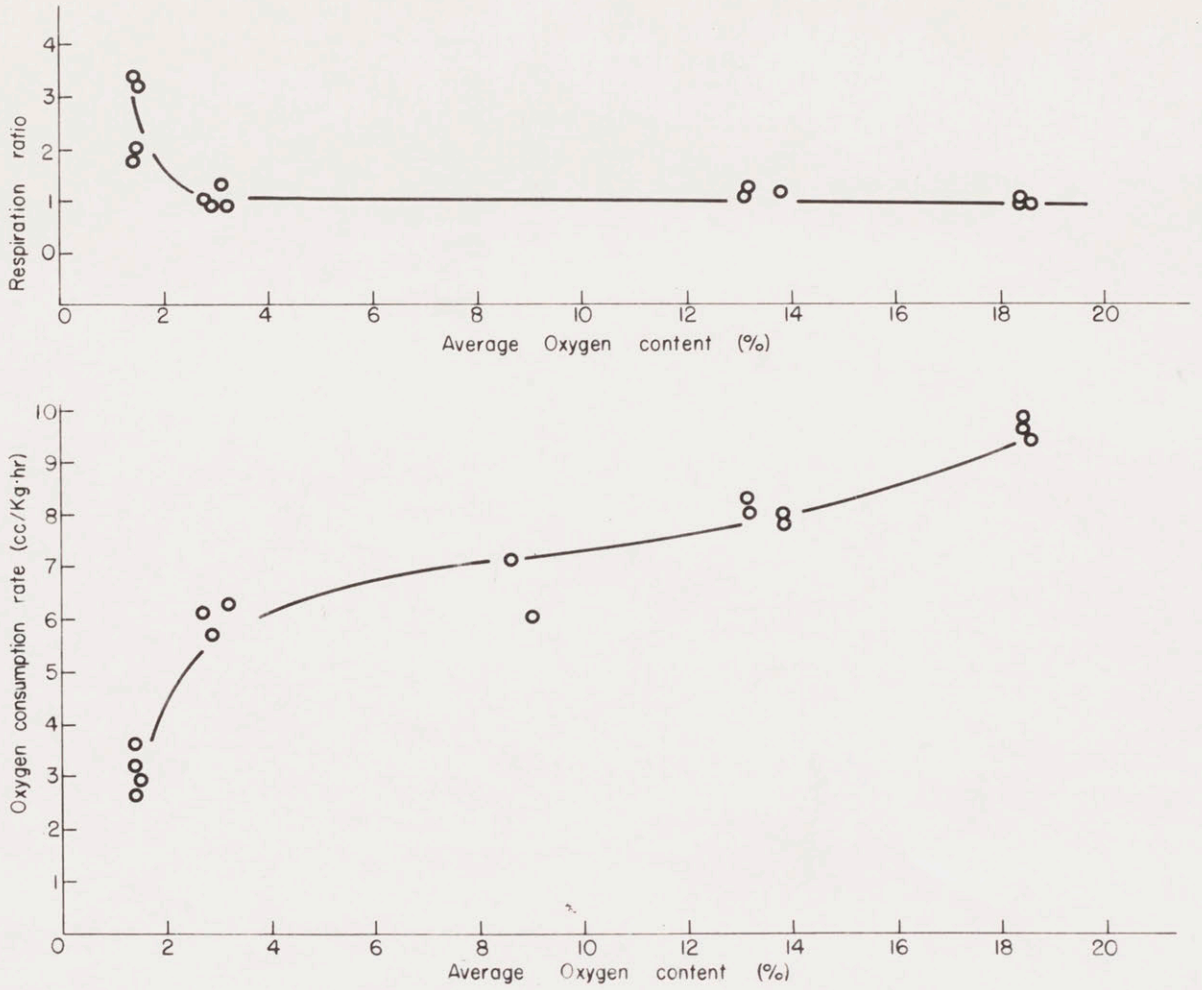
The results obtained in this experimental series are presented in Table IV and Figure 9. Table IV shows the respiration parameters: average oxygen concentration, average carbon dioxide concentration, rate of oxygen consumption, rate of carbon dioxide evolution as a function of time. Figure 9 presents the rate of oxygen consumption and the respiration ratio as a function of the average oxygen concentration.

TABLE IV

Respiration Studies on Apples

Experimental Series 2

Time (Hours)	Average O_2 concentration (%)	Average CO_2 concentration	CO_2 evolution rate cc/KgHr	O_2 consumption rate cc/KgHr	Respiration ratio (CO_2/O_2)
12	18.6	3.54	8.5	9.4	0.92
36	18.4	3.6	9.7	9.6	1.0
12	18.4	3.4	9.1	9.8	0.93
36	18.4	3.5	9.1	9.8	0.93
12	13.1	3.2	8.7	8.3	1.04
36	13.8	3.5	9.1	7.8	1.1
12	13.2	3.6	9.6	8.0	1.2
36	13.8	3.7	9.3	8.0	1.1
12	9.05	3.4	--	6.0	--
12	8.6	3.6	--	7.1	--
12	2.87	2.27	5.1	5.7	0.9
36	2.76	2.4	6.2	6.1	1.0
12	3.12	2.3	5.85	4.3	1.3
36	3.18	3.36	5.9	6.3	0.93
12	1.39	3.69	9.3	2.63	3.4
36	1.5	3.68	9.28	2.94	3.2
12	1.43	2.64	6.6	3.23	2.0
36	1.40	2.69	6.7	3.6	1.8



EFFECT OF OXYGEN CONCENTRATION ON RESPIRATION RATE AND RESPIRATION RATIO. (EXPERIMENTAL SERIES 2)

FIG. 9

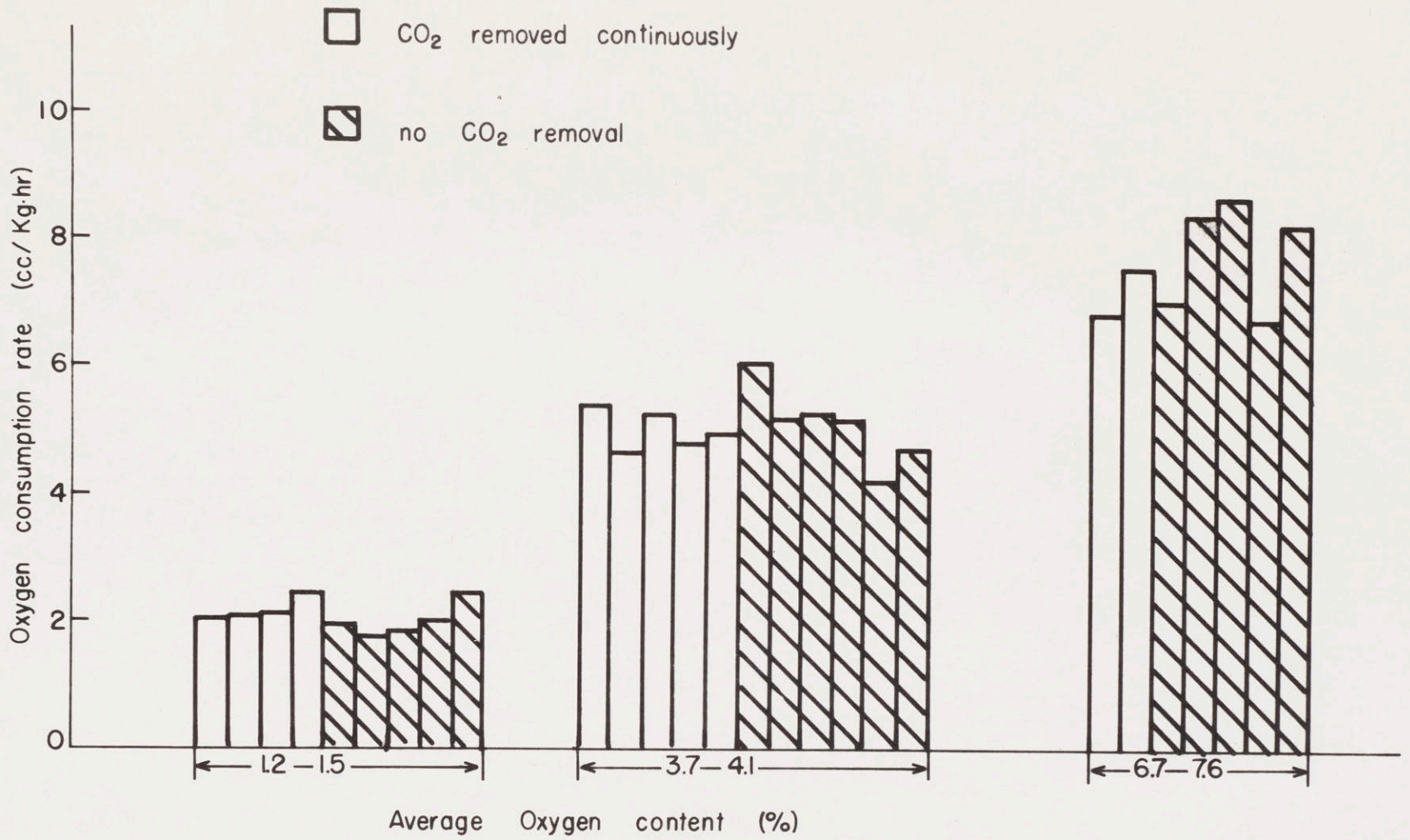
Series No. 3: Respiration Studies with Continuous Removal of Carbon Dioxide

Apples weighing between 500 and 540 grams were placed in the respiration chambers and maintained at a temperature of 20° C. Carbon dioxide was removed by absorption in a 1N solution of sodium hydroxide and 1M barium chloride. A crystallizer containing the absorber solution was placed in the bottom of the chamber. The solution was continuously stirred by a magnetic stirrer in order to prevent formation of a layer of sodium carbonate which had caused error due to retardation of diffusion. Control experiments without absorbing the produced carbon dioxide were conducted simultaneously. Every twenty-four hours, before changing the atmospheres, samples were taken for analysis of carbon dioxide and oxygen. In the chambers with absorber solution, the total amount of carbon dioxide produced was determined by analysis of the amount of carbon dioxide absorbed by the sodium hydroxide-barium chloride solution. The amount absorbed was determined by titration of the remaining sodium hydroxide with 1N hydrochloric acid using phenolphthalein as an indicator. The carbon dioxide produced was completely absorbed since the gas chromatograph showed complete absence of carbon dioxide.

The purpose of this series of experiments was to determine whether the build-up of carbon dioxide during the twenty-four hour intervals used in the preceding series of experiments (experiments at approximately constant oxygen concentration) had an effect on the respiration rates.

The results are presented in Tables V and VI, which show the respiration parameters determined at different oxygen concentrations,

under the conditions described below. Table V shows the results obtained with removal of carbon dioxide and Table VI shows the results obtained without removing the carbon dioxide. Figure 10 compares the results from the two different methods.



EFFECT OF CONTINUOUS CARBON DIOXIDE REMOVAL ON RESPIRATION RATES.

FIG. 10

TABLE V

Respiration Studies on Apples

Experimental Series 3

Run with Continuous Removal of CO_2 Produced

Average O_2 content	CO_2 evolution rate cc/Kg.Hr.	O_2 consumption rate cc/Kg.Hr.	Respiration ratio CO_2/O_2
8.9	9.7	7.3	1.3
7.6	7.27	6.8	1.07
6.8	8.26	7.5	1.1
3.89	7.3	5.3	1.4
4.02	7.7	4.6	1.7
3.91	7.8	5.2	1.5
3.91	7.28	4.25	1.5
3.8	8.4	4.9	1.7
1.44	6.97	2.02	3.4
1.5	6.3	2.01	3.1
1.3	6.4	2.0	3.2
1.29	6.45	2.4	2.7

TABLE VI

Respiration Studies on Apples

Experimental Series 3

Run Without Removal of CO_2

Average O_2 content (%)	Average CO_2 content	CO_2 evolution rate cc/Kg.Hr.	O_2 consumption rate cc/Kg.Hr.	Respiration ratio CO_2/O_2
7.1	2.4	11.8	8.2	1.4
7.1	2.3	11.3	6.7	1.6
6.7	2.1	10.3	8.65	1.2
6.9	2.1	10.3	8.35	1.2
6.9	1.9	9.2	7.0	1.2
3.74	1.5	7.3	6.0	1.2
4.0	1.04	5.2	5.13	1.0
3.9	1.19	5.9	5.2	1.1
4.0	1.5	7.3	5.1	1.4
4.1	1.0	4.9	4.2	1.1
4.1	1.1	5.6	4.7	1.2
1.47	2.1	9.4	1.8	5.1
1.43	1.5	6.3	1.65	3.8
1.42	1.4	5.85	1.77	3.2
1.2	2.1	8.94	1.95	4.5
1.3	1.5	6.3	2.3	2.8
1.3	1.4	5.9	1.4	4.1

Series No. 4: Respiration Rates at Constant Oxygen Concentration and Variable Carbon Dioxide Concentration

Apples weighing between 540 and 556 grams were placed in the respiration chambers and maintained at a temperature of 20° C. The atmosphere in the chambers contained known concentrations of carbon dioxide and oxygen. Samples of the gas were taken every twenty-four hours before replacing the atmosphere by a new one having approximately the same composition. In these experiments the oxygen concentrations were maintained at an approximately constant level and the carbon dioxide concentration was varied from 3.15% to 14.3%.

The results are shown in Table VII and Figure 11. Table VII presents the respiration parameters determined in these experiments.

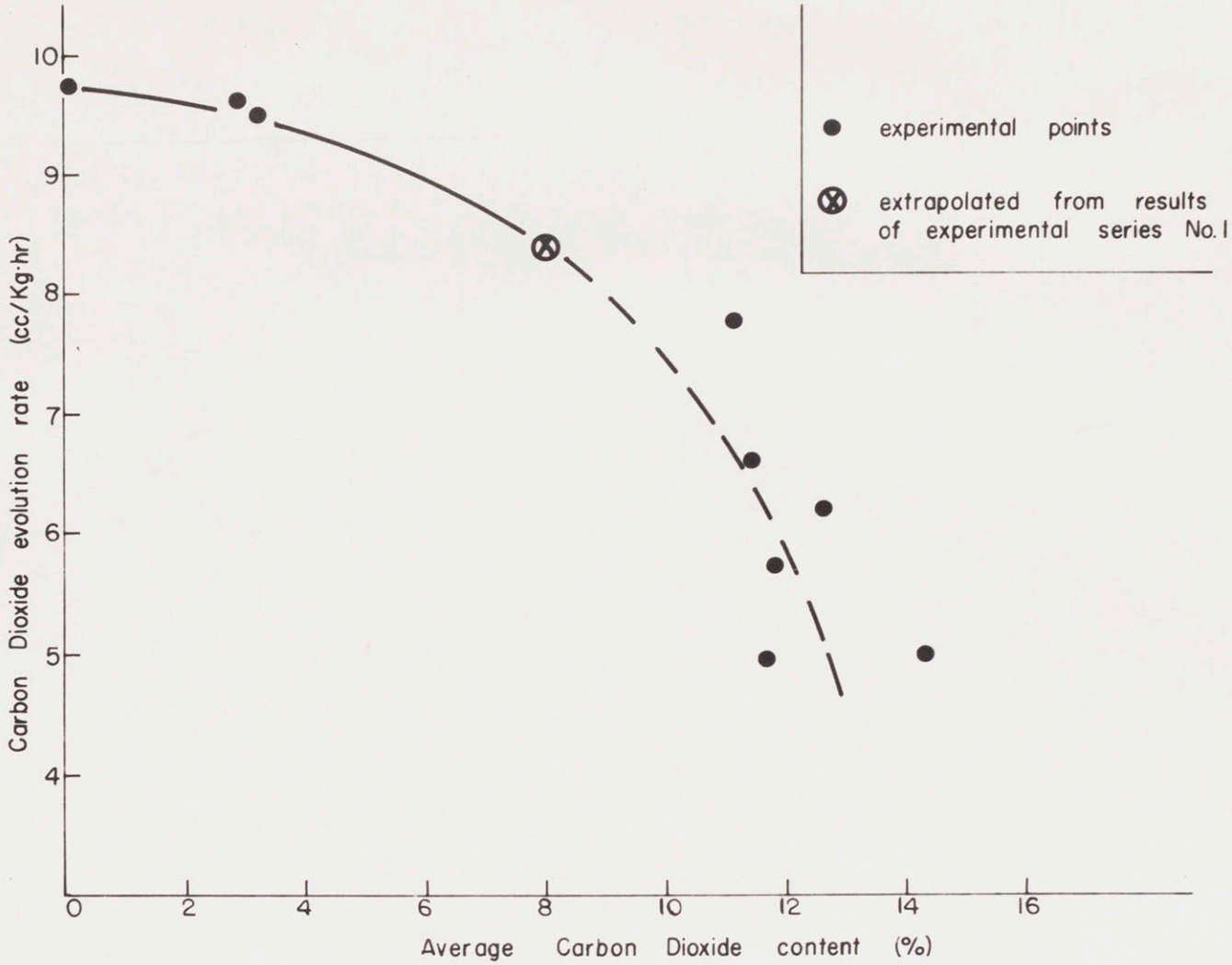
Figure 11 shows the effect of carbon dioxide concentration at approximately constant oxygen level on the rate of carbon dioxide production.

TABLE VII

Respiration Studies on Apples

Experimental Series 4

Average O_2 concentration (%)	Average CO_2 concentration (%)	CO_2 evolution rate cc/Kg.Hr.	O_2 consumption rate cc/Kg.Hr.	Respiration ratio CO_2/O_2
17.3	12.6	6.2	6.1	1.0
17.5	11.7	4.97	6.4	0.8
16.5	14.3	4.85	5.0	0.97
15.7	11.8	5.73	5.46	1.05
16.7	11.1	7.8	6.3	1.2
17.0	11.4	6.6	4.5	1.4
15.6	2.85	9.6	9.1	1.0
16.1	3.28	7.85	7.85	1.0
17.3	3.15	9.5	9.1	1.0



EFFECT OF CARBON DIOXIDE CONCENTRATION ON RESPIRATION RATE.

FIG. 11

Series No. 5: Respiration Rates of Apples at Different Storage Temperatures

Apples weighing 546 grams were placed in the respiration chambers and maintained at different temperatures: 0° C, 30° C, 37° C. The oxygen concentration was atmospheric, and the chambers' atmospheres were replaced periodically to prevent carbon dioxide build-up. Samples were taken every forty-eight hours for apples stored at 0° C, every 12 hours for samples stored at 30° and 37° C.

The results are presented in Table VIII and in Figure 12. Figure 12 presents a semi-logarithmic plot of the oxygen consumption rate against the reciprocal of the absolute temperature. The Q_{10} for respiration rates was calculated on the basis of this plot.

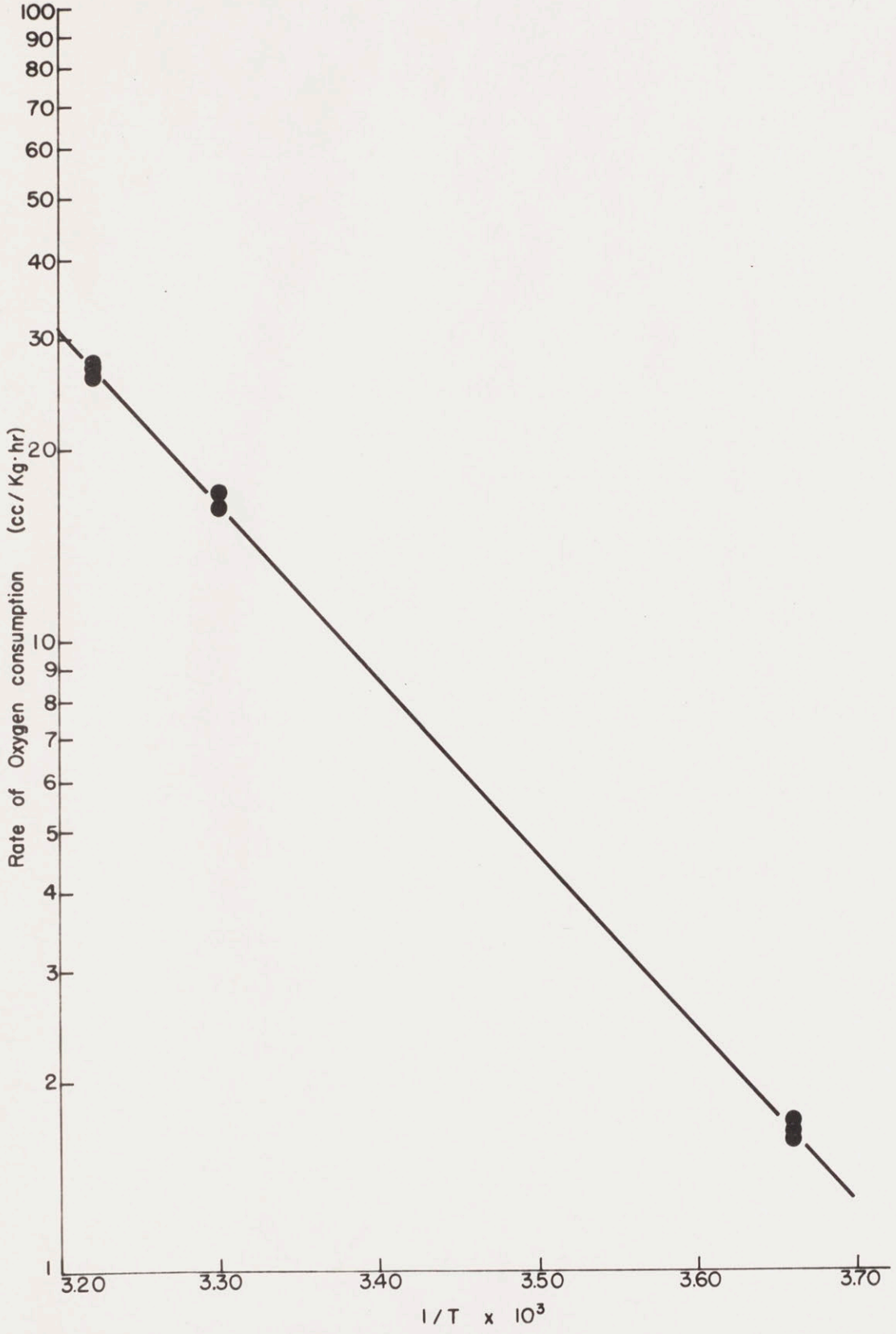
TABLE VIII

Respiration Studies on Apples

Experimental Series 5

Temperature Effect on Rate of Respiration

Temperature °Centigrade	Temperature °Absolute	$1/T \times 10^3$	CO_2 consumption rate cc/Kg.Hr.
0	273	3.66	1.68
0	273	3.66	1.63
0	273	3.66	1.73
30	303	3.30	17.4
30	303	3.30	16.3
37	310	3.22	27.4
37	310	3.22	27.6
37	310	3.22	26.4



EFFECT OF TEMPERATURE ON RATE OF RESPIRATION.

FIG. 12

Packaging Experiments: Evaluation of Atmospheric Changes in Plastic Bags

The results of the respiration studies obtained in the preceeding experiments were used to evaluate expected changes in the atmospheric composition of packaging containing apples.

This evaluation was checked experimentally by an experiment in which bags containing apples were stored at a temperature of 20° C.

The experimental procedure was as follows:

Two apples weighing approximately 250 grams were placed in plastic bags and stored at 20° C.

The plastic bags were made of polyethylene film, 1.5 mil thichness, having a total area of 600 square centimeters. The bags were heat sealed. Every one or two days, the atmosphere in the bags was analyzed and its concentrations of carbon dioxide and oxygen were determined. Each bag was used for only one analysis, and the bags which had been analyzed were discarded.

The only parameters considered in this experiment were time and atmospheric composition in the bags.

A total of fourteen bags were stored and the experiment was continued during eleven days.

Table IX shows the results obtained in the experiments.

TABLE IX

Respiration Studies on Apples

Packaging Experiments

Days of Storage	Composition of the Atmosphere inside the bag	
	% CO_2	% O_2
1	4.1	10.1
2	5.1	6.5
2	4.1	9.8
3	4.52	8.75
3	3.52	10.8
5	3.38	10.0
7	3.39	8.06
9	3.53	7.33
9	3.27	10.3
11	3.11	9.05
11	2.76	10.03

IV. DISCUSSION OF RESULTS

The storage life of fresh fruits and vegetables depends considerably on the composition of the atmosphere in which they are stored. This atmospheric composition may be controlled to a large extent by controlling the rate of diffusion through the package. The proper package design for fruits and vegetables must take into consideration the following characteristics of the product to be packaged:

A. Respiration rate as a function of atmospheric composition

B. The critical oxygen concentration below which anaerobic respiration takes place, since this type of respiration is known to result in impairment of the product quality. (Platenius, H. 1946).

The experiments described in the preceding sections were concerned with the evaluation of a gas chromatographic static system for the determination of the above chracteristics using as the fruit apples of the McIntosh variety.

The initial series of experiments (Experimental Series No. 1) were conducted under conditinns in which carbon dioxide build-up and oxygen depletion were allowed to take place simultaneously, since no readjustment of chamber atmosphere was practiced.

Under these conditions it was found (Tables II and III and Figure 8) that the rate of respiration was dropping continuously with time. The evaluation of the respiration ratio showed that as long as the oxygen

concentrations in the respiration chambers remained between 21% and 3.5%, the respiration ratio was approximately equal to one. Below oxygen concentrations of 3.5%, the respiration ratio has values greater than one, showing that at these concentrations anaerobic respiration is taking place.

These results do not allow the differentiation between the effects of decreasing oxygen concentration and the increasing carbon dioxide concentration.

For instance, it was observed that the most rapid decrease in respiration rates occurred when oxygen concentration decreased to a level of approximately 8%. However, at that time the carbon dioxide had increased to a level of 15% (Figure 7), and it is impossible to determine which of the above factors was responsible for the decrease in the respiration rate.

A separation of these effects is possible, however, on the basis of results of Experimental Series 2, 3, and 4.

In Experimental Series No. 2, the oxygen concentration was maintained approximately constant by replacing the chamber atmosphere every twenty-four hours. Respiration rates at a range of oxygen concentrations between 1.4 to 18.6% were studied.

By replacing the chamber atmosphere every twenty-four hours, the carbon dioxide build-up was prevented, and in no case did the concentration exceed 4% in the runs.

These experiments, therefore, should allow the evaluation of the oxygen concentration effect without any influence of changes in carbon

dioxide concentration provided that the slight carbon dioxide build-up occurring during the periods between readjustment of the chamber atmosphere had no significant effect.

This last assumption was tested in Experimental Series No. 3. To achieve this purpose, two simultaneous runs were made; in one of them the carbon dioxide was permitted to accumulate in the respiration chambers, and in the second one the carbon dioxide was absorbed by means of a solution of 1N sodium hydroxide and 1M barium chloride. The efficiency of carbon dioxide absorption was checked by the analysis of headspace composition in the chambers, and it was found that the absorber removed the carbon dioxide at a sufficient rate to prevent any measurable accumulation. Also, since the possibility existed that the slight carbon dioxide build-up during the twenty-four hour periods might have an effect at some average oxygen concentrations but not at others, these experiments were conducted at three different levels of oxygen, chosen particularly in the range of oxygen concentrations where respiration rates are changing rapidly, (1.29% to 7.6%).

The results of these experiments are tabulated in Tables V and VI, and represented graphically in Figure 10. These results show that the carbon dioxide build-up in the respiration chambers during the twenty-four hour periods between readjustment of chamber atmospheres had no significant effect on the respiration rates compared with results obtained in the experiments where the carbon dioxide produced was removed by absorption. It is important to point out that this effect was studied only for average carbon dioxide concentrations as high as 2.4% which is the maximum level attained for periods of twenty-four hours. This was the period of time between sampling adopted in the present work.

Having established the validity of ignoring the slight carbon dioxide build-up effect, it was possible to evaluate the effect of oxygen concentration on respiration rate on the basis of the experiments with chamber readjustment every twenty-four hours (Experimental Series No. 2).

The results of the Experimental Series No. 2 are presented in Table IV and Figure 9. These results show that the respiration rate decreased with decrease in the oxygen concentration. When the oxygen concentration remained approximately between 18.5% and 5% the drop in respiration rates was relatively slight. Below oxygen concentrations of 4-5% the drop in the respiration rate became rapid. In the range of 5-19% of oxygen concentration the respiration rate remained in the range of 6.5 to 9.0 cubic centimeters of oxygen per kilogram per hour. In the range of 4 to 2% the respiration rates dropped from 6 to 3 cubic centimeters of oxygen per kilogram of fruit per hour. In the first case a decrement of 14% in the oxygen concentration caused a drop of 27.8% in the respiration rate while in the second case a drop of only 2% in the oxygen concentration caused a drop of 50% in the respiration rate.

In Figure 9 the respiration ratio values appear plotted against the oxygen concentration in the chambers. It is apparent that the values of this ratio remained approximately equal to one when the oxygen concentrations were between 18.5% and 3.5%. Below concentrations of approximately 3.5% the respiration ratios started to have values larger than one. This fact indicates that the critical point for anaerobic respiration is approximately 3.5%.

A comparison of results of Experimental Series No. 1 and No. 2, that is, respiration studies with and without significant carbon dioxide build-up, shows that the rapid drop in the respiration rates in the Experimental Series No. 2 appeared when the oxygen concentration in the respiration chamber was around 5 to 6%. In the Experimental Series No. 1 the same effect appeared when the oxygen concentration was between 8% and 10%. This difference was due probably to the effect of carbon dioxide build-up allowed in the Experimental Series No. 1. At this stage the carbon dioxide concentration was around 15% in the Experimental Series No. 1. It seems justified to conclude that the drop in respiration rates at approximately 5 - 6% of oxygen concentration represents a true effect of oxygen concentration, since the level of carbon dioxide concentration could not cause that effect.

It is interesting to note that the respiration ratios observed in the two experimental series coincide. In the experiments in which carbon dioxide build-up was prevented, anaerobic respiration appears to start when oxygen concentration dropped to 3.5% approximately, at which time the carbon dioxide concentration was in the range of 3 to 4%. In the Experimental Series No. 1 it was observed that anaerobic respiration also started at 3.5% oxygen concentration, but in this experiment the carbon dioxide concentration was 22.5% (Figures 7, 8, 9).

These results appear to indicate while high carbon dioxide concentrations have a significant effect on depression of respiration rate, there is no such effect with respect to respiration ratio. The results seem to indicate, therefore, that the only factor controlling initiation of anaerobic respiration is the oxygen concentration in the respiration chambers (Table II, III and Figure 8 and 9).

It is interesting to note that oxygen concentration has been found to be the controlling factor for initiation of anaerobic respiration of fresh produce. For instance, Platenius working with a carbon dioxide-free system found that oxygen concentrations in the range of 1 - 2% are responsible for initiation of anaerobic respiration. The fact that different critical oxygen concentrations have been found for different produce is not surprising in view of metabolic differences, and, in fact, different critical concentrations have been found for various plants by other investigators (Platenius 1943).

It appeared of interest also, to determine the effect of higher carbon dioxide concentrations on both the respiration rate and the respiration ratios, under conditions in which oxygen concentration was maintained at a sufficiently high level to have relatively little effect on respiration rates. In these experiments oxygen concentration was maintained in the range of 16 to 17%, but carbon dioxide was varied in a range of 3.2% to 14.3%. This effect was studied in Experimental Series No. 4. The results of this series of experiments are tabulated in Table VII, and Figure 11 shows them graphically. In the curve plotted in Figure 11, it may be observed that carbon dioxide build-up starts to have a significant effect on the respiration rates when its concentration is higher than approximately 5 to 6%. The results indicate also that at the relatively high oxygen concentrations used in these experiments, increasing the carbon dioxide concentration up to 15% had no effect on the respiration ratio. Thus, the conclusions drawn from the preceding experimental series,

namely, that the onset of anaerobic respiration is controlled by oxygen concentration, and not by carbon dioxide, are confirmed. The comprehensive investigations of the effects of carbon dioxide and oxygen concentrations were carried out at a single temperature, namely, 20° C. It would have been of interest to observe all these factors at different temperatures, but this was not possible because of time limitations. However, respiration rates at different temperatures were studied in Experimental Series No. 5. Table VIII and Figure 12 show the results of experiments on respiration rates which conducted at 0° C, 30° C, and 37° C. If it is assumed that the only effect of temperature is that of changing the respiration rates, the results from the experiments carried at 20° C can be extrapolated to other temperatures, taking into account the effect of the temperature. This effect can be included using Q_{10} values for respiration rates.

The Q_{10} values were calculated from the curve plotted in Figure 12 and were found to be between 1.98 and 2.22.

Q_{10} values between 2.2 and 2.8 have been reported in the literature (Gore, H. C. 1911).

The packaging experiment was conducted by placing apples inside plastic bags. The bags were sealed and stored at a temperature of 20° C. Periodically, bags were removed from the constant temperature room, and the atmosphere inside them was analyzed for oxygen and carbon dioxide content. The experiment was carried on during eleven days. The results appear in Table IX.

The purpose of this experiment was to evaluate the steady conditions resulting in a concentration of approximately 6% oxygen with a carbon dioxide concentration not exceeding 4 - 5%. The assumption was made that the approach to steady state conditions will be relatively rapid.

Knowing the rate of respiration, the weight of the apples, and assuming a desired oxygen concentration in the bag, (6% for oxygen concentration was assumed because, according to the results obtained experimentally, this concentration is well above the critical oxygen concentration for anaerobic respiration) the following formula can be applied in order to determine the desired permeability for an "ideal" packaging material:

$$V/t = AP_{O_2}x^{-1}(\rho_{1O_2} - \rho_{2O_2})$$

where

V/t = volume of oxygen consumed by the apples in the bag per unit time (c.c./day)

A = area of bag (m^2)

P_{O_2} = permeability of bag material (cc · mil · m^{-2} · $days^{-1}$ · atm^{-1})

x = thickness of material (mils)

ρ_{1O_2} = partial pressure of oxygen outside the bag (atm)

ρ_{2O_2} = steady state partial pressure of oxygen in the bag (atm).

Assuming a weight of 250 grams of apples per bag, a bag area of 0.06 m^2, a steady state partial pressure of oxygen in the bag of 0.06 atm., and obtaining from the experiments the maximum oxygen consumption rate for the given conditions as 7 cc O_2/hr. kg., the desired permeance (P/x) may be calculated as follows:

$$P/x = \frac{R_{O_2} \cdot m}{t(p_1 - p_2)A} = \frac{(7)(cc \cdot hr^{-1} \cdot kg^{-1})(0.25)(kg)}{(1/24)(days/m)(.21 - .06)(atm)(.06)(m^2)} = 4300$$

Among the readily available commercial films 1.5 mil thick, low density polyethylene films showed the closest permeance value to the one calculated, namely, a P/x value of 6000 $cc/m^2 \cdot day \cdot atm$.

It was calculated that with this material the expected steady state concentration of oxygen would be approximately 9%. It was decided to check this experimentally. A check of the expected steady state concentration of carbon dioxide was calculated from the following equation:

$$p_{1CO_2} = \frac{(V/t) \cdot (x)}{P_{CO_2} \cdot A}$$

where

$p_{1_{CO_2}}$ = steady state partial pressure of CO_2 (atm)

V/t = rate of CO_2 evolution (cc/day . bag)

x = thickness of film (mils)

A = area of bag (m^2)

P_{CO_2} = film permeability to CO_2 $(cc \cdot mil)(m^2 \cdot day \cdot atm)$

Assuming atmospheric carbon dioxide concentration to be zero and the polyethylene permeability to carbon dioxide 35,000 (Taylor 1960) then we obtain:

$$p_{CO_2} = \frac{(7)(24)(0.25)(1.5)}{35{,}000 \cdot 0.06} = 0.030 \text{ atm.}$$

0.030 atmospheres of partial pressure corresponds to a concentration of 3.0%. This value represents the carbon dioxide concentration inside the bag when the steady state conditions were attained. However, due to the many variables involved in the experiment such as changes in respiration rates, differences in the area of the bags, differences in the weight of the apples, this calculated value should be considered only as an approximation.

The results obtained in this preliminary experiment are presented in Table IX. It is evident from these results that steady state concentration of oxygen and of carbon dioxide were approached very rapidly. At the end of twenty-four hours the oxygen concentration was at the approximate steady state value of ten per cent. The carbon dioxide concentration was approximately 4%, and, therefore, relatively close to the ultimate steady value of approximately 3%.

The fact that only one material was studied and the fact that its oxygen permeability was only approximately equal to that desired theoretically oxygen permeability, make it impossible to make definite statements with respect to the precision of the theoretical prediction. However, the following conclusions appear justified;

A. It is possible to control the atmospheric composition inside plastic bags containing apples by a suitable selection of the plastic film characteristics such as permeability to carbon dioxide and to oxygen.

B. The experimental steady state values for oxygen and carbon dioxide have been in reasonable agreement with the theoretical steady state values considering the many variables involved in the experiment.

V. CONCLUSIONS

A. Gas chromatographic analysis was found to be suitable for the simultaneous determination of carbon dioxide and oxygen in samples from respiration chambers containing samples of fruit.

B. The static system was found suitable for the determination of respiration rates. The influence of oxygen concentration and of carbon dioxide on the respiration rates and on initiation of respiration rates could be studied provided the respective concentrations were kept in a range in which the effects were independent.

C. The effect of oxygen on respiration rate was found to be independent of carbon dioxide when that concentration was maintained below 4%.

D. The effect of carbon dioxide on respiration rate was found to be independent of oxygen concentration, provided the oxygen concentration was maintained above 18 per cent.

E. The critical oxygen concentration for initiation of anaerobic respiration was found to range between 3.0 and 3.5%. Carbon dioxide concentration between 0% and 24% was found not to influence the critical oxygen concentration for anaerobic respiration.

F. The temperature coefficient of the respiration rate was found to be in general agreement with values reported in the literature for respiration of apples.

G. The results of the respiration studies were applied to the evaluation of packaging conditions resulting in steady concentrations of oxygen and carbon dioxide in packages of apples.
Experimental determination of the atmospheric composition in apple packages confirmed, in general, the validity of the theoretical approach to the prediction of these conditions.

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