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Biochemical investigations on the cranberry

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BIOCHEMICAL INVESTIGATIONS ON THE CRANBERRY

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I. INTRODUCTION AND REVIEW OF LITERATURE

1. Purpose of Investigation

This investigation was carried on to determine the changes in the composition of the internal atmosphere and in the catalase activity of cranberries as affected by variety and temperature while the fruit is in storage. The results obtained are correlated with the keeping quality of cranberries in storage.

Storage is an important factor in the cranberry industry. After being harvested, cranberries are usually stored for at least a short period of time, before they are placed on the market. Any information regarding storage conditions and keeping qualities of cranberries should be of value to the cranberry industry.

The work of Morse and Jones (47) shows that the percentage changes in the chemical composition of cranberries produced by different storage temperatures throw little light on the real change at a given temperature. Studies of the respiration and chemical changes of cranberries in storage are not correlated with the keeping qualities of the fruit, as is shown by the above authors.

Very few researches have been published on the composition of the internal atmosphere of fruit, and in the work that was carried on no attempt was made to

correlate the results obtained with the keeping quality of the fruit. In this investigation the relationship between the keeping quality of cranberries and the composition of the internal atmosphere is noted.

Although considerable work has been done on the study of catalase activity in fruits, the results obtained and conclusions reached are somewhat conflicting and contradictory. It was thought advisable to study the changes in the catalase activity of cranberries as affected by variety, temperature, and storage as related to the corresponding changes in the internal atmosphere and keeping qualities.

2. Internal Atmosphere of Fruit

In 1896 Gerber (21) reported the work of Fremy, published in 1840 and 1860, in which the gas contained in apples was analyzed at intervals during their development and ripening. He found oxygen more abundant in the green fruit, the amount decreasing as the fruit matured on the tree.

Heintz in 1873 (29) analyzed the composition of air inside the sugar beet with the following results:

Oxygen.....0.06- 2.10%
Carbon Dioxide.....11.49-78.90%
Nitrogen.....21.04-86.98%

Magness (40) analyzed the gas in the intercellular spaces in apples, potatoes, and carrots at different

storage temperatures. He suggests the possibility of a marked effect of storage temperature on the quality of fruit as influenced by respiration. He found that the proportion of carbon dioxide is much greater and oxygen much less at the higher temperatures. His results are given in the following table:-

Table 1. Gas Analyses of Fruits and Vegetables (Magness)

Product	Temp. of storage	No. of Detns.	Carbon Dioxide	Oxygen
Apples	2	5	6.7 percent	14.2 percent
"	6	30	8.4 percent	12.9 percent
"	11	27	12.2 percent	10.7 percent
"	20	31	17.2 percent	5.5 percent
"	30	29	21.4 percent	3.2 percent
Potatoes	11	8	19.6 percent	10.9 percent
"	22	8	34.4 percent	5.7 percent
Carrots	11	2	12.2 percent	13.1 percent
"	22	2	28.6 percent	5.2 percent

The removal of the peel from the ends of apples resulted in a marked reduction in the amount of carbon dioxide and a similar increase of oxygen, due to the greater ease of escape and entrance. The chief factors determining the amounts are the rate of respiration, the permeability of the peel or skin, and the difference in the pressure of the two gases within and without the tissue.

A year later Magness (41) reported further studies on the air in the intercellular spaces of apples. He found that the carbon dioxide-oxygen ratios within the tissue has a wide variation, depending upon the temperature

at which the fruit is held. He thinks it probable that the carbon-dioxide-oxygen pressures inside the fruit are relatively much more important than those outside in determining what is going on within the fruit. (6.) Lumia, Negri, Devaux, Malaguin.

Lumia (12) showed that gas samples obtained from unripe figs contained 5.25 percent carbon dioxide and only 17.93 percent oxygen. Negri (12) found 9.88 percent carbon dioxide and 16.59 percent oxygen in the gas from immature fruits of Gomphocarpus, while the gas from the ripe fruits contained 3.48 percent carbon dioxide and 23-15 percent oxygen. Devaux (12) analyzed the air from the hollow inside of a pumpkin (Curcurbita maxima) and found 2.52 percent carbon dioxide, 18.29 percent oxygen, and 79.19 percent nitrogen. Malaquin (12) found in the gas from the pods of Colutea, 6.9 percent carbon dioxide and 14.3 percent oxygen.

Kidd, Franklin, and West (32) reported that they had obtained similar results to those of Magness (40), with Bramley's seedling apple; though the increase in carbon dioxide with increasing temperature was less striking.

In 1923 Clark (11) made an investigation into the amounts and composition of gases in apples and changes during soaking in a warm brine solution.

Davis (14) found in the gas samples from potatoes

a carbon dioxide content of 5-6 percent, and 10-11 percent of oxygen when they were stored at 17-18°C. With increasing storage temperatures the carbon dioxide content and the respiratory ratio enlarged rapidly.

Kohman (36) reported that he had made determinations on certain fruits and found them practically free from oxygen, while in others he found the gases to have practically as high an oxygen content as the atmosphere; and the total volume of gas to be over one quarter the volume of the fruit.

Magness and Diehl (42) reported further studies on apples in storage in 1924. They found that anaerobic respiration was just beginning with Rome Beauty apples coated with oil when the carbon dioxide content in the intercellular atmosphere had reached about 16 percent and that of the oxygen had fallen to about two percent. They believe that it should be the carbon dioxide and oxygen contents of the gas surrounding the cells rather than of the gases surrounding the fruit that would be most important in determining the respiration products and the effect of respiration on the quality of the fruit.

In 1930 Harley and Fisher (27) made a study of the internal atmosphere of apples in relation to soft scald. At 0°C the carbon dioxide content remained about three percent. At 21.°C fruit initially stored showed an

increase in carbon dioxide and a decrease in oxygen, reaching a maximum for carbon dioxide in six days. At the end of this time the carbon dioxide content was nine percent and the oxygen 15.2 percent. With further holding the carbon dioxide values decreased and the oxygen values increased. Dowd (16), in 1933 made some preliminary studies on the internal atmosphere of apples. He showed that the percentage by volume of carbon dioxide tends to decrease during the growing period. The higher percentage of carbon dioxide found in the early stages of growth is undoubtedly due to a higher rate of respiration in the young rapidly growing cells. The gradual increase in the percentage by volume of oxygen in the fruit during the growing season is probably due to a slower rate of respiration. In his report Dowd states "The findings of Harley, Fisher, Magness and Ballard emphasize the need for more study of the internal atmosphere of fruits in relation to certain storage disorders. Knowledge of the progressive changes in the internal atmosphere of growing fruit may constitute a background for further storage studies."

Kertesz (30) studied the gas from pea pods and found that the average carbon dioxide content was 1.6 percent. In pea pods gathered from the vines the carbon dioxide content was doubled during the night, decreasing

again in the morning. The carbon dioxide content was increased to 4.5 percent by freezing the pods.

3. Respiration of Fruit.

The effect of temperature on the respiration of apples was studied by Morse in 1908 (46). From the results of his work, some of which were found to be in substantial agreement with those obtained previously by others, it is evident that apples held at summer temperatures exhale carbon dioxide from four to six times more rapidly than when kept in modern cold storage.

Burroughs (9) reports a study made on the changes in respiration rate of ripening apples. Examination of fruit picked at intervals from August to October shows a general increase in the initial rate of respiration as the season goes on. From a study on the Wagner apple, facts indicate that while the fruit keeps its connection with the tree there is a retardation of the ripening or breaking down processes. Off the tree, low temperature exercises a retarding influence on the ripening process. Apparently the same thing happens when the apples are left on the tree, even with a high outside temperature.

Franklin (20) was probably the first to carry out scientific investigations with the cranberry plant under submergence. He found that mature berries which

had been picked after 15 days flooding exhibited considerably more decay than those picked before flooding. This fact was more pronounced in the Early Black variety than in the Howes variety.

Wakabayashi (58) found that submergence weakened the cranberry fruit and that this weakened condition favored the growth of fungi.

De Villiers (15) studied the respiration of grapes and found that the rate of respiration showed a corresponding decrease as the fruit approached maturity. Varieties possessing good shipping and keeping qualities appeared to have a lower rate of respiration than those of poor keeping quality. Temperatures above 4.40°C temporarily stimulated respiration but ultimately produced harmful effects.

Carrick (10) found that freezing accelerated the rate of respiration of apples. This acceleration lasted for several days and gradually declined.

Kidd (31) studied the carbon dioxide ration in the gaseous exchange of the apple at different stages in its life cycle. The ratio was measured at 22.5°C over the period of climacteric, and his results indicate that the change from values of less than unity to values of greater than unity occurs during the rise in respiratory activity, which occurs at this time.

Blackman and Parija (6) investigated the respiration of a population of senescent ripening apples. Apples stored at a temperature of 2.5°C. for a period of eight months, slowly ripen, and pass through the senescent stage, which lies between the adolescent and the mature stages. This stage is characterized by a lowered organization of resistance fundamental changes in the organization of the tissues, and hydrolysis of reserve and semi-reserve material, more rapidly than during the mature stage. The output of carbon dioxide is increased during the senescent stage, and is decreased when that stage has been completed. During senescence two independent and opposite processes occur: (a) the starvation drift, which tends to lower respiration, and (b) increased hydrolysis, which tends to accelerate respiration.

Harding (26) made respiration determinations on Grimes apples held at 15.56°C., 10°C., 2.2°C., and 1.1°C. The rate of respiration increased with the development and maturity of the fruit under a uniform temperature. At higher temperatures the maturity or stage of development affected the respiration rate. Fruit picked and held at higher temperatures soon reached a very high rate of respiration.

Kidd and West (33) found that the development of internal breakdown in apples in cold storage is

accompanied in its early stages by a considerable increase in the carbon dioxide production rate, but by the time the deep brown condition of the flesh is reached respiration has ceased.

In another paper the above authors (34) studied the changes in the respiration activity of apples during their senescence, at different temperatures. The respiratory history of an apple during ripening is characterized by a rise followed by a fall in activity. The rise is attributed to a change of state, which is associated with a high temperature coefficient, in the protoplasm. At all temperatures, death by fungal diseases was found to intervene after approximately the same total amount of carbon dioxide had been evolved.

Haller, Harding, Lutz, and Rose (23) investigated the respiration of some fruits in relation to temperature. The respiratory ratio (carbon dioxide/oxygen) indicates the type of material being respired. The complete oxidation of hexose sugars gives a ratio of - 1; while complete oxidation of citric or malic acid gives a ratio of 1.33. A ratio greater than 1.33 in fruits would indicate intramolecular respiration.

4. Catalase Activity and Plant Metabolism

The estimation of catalase activity of various plant tissues has been used in the past as a measure of

the relative metabolic activity or phsiological condition resulting from any particular treatment or stage of development of the tissues with which the investigation was concerned. There is some evidence that the activity of this enzyme at least parallels certain metabolic processes.

The suggestion of Loew in 1901 (39) that catalase acts in a protective capacity destroying the hydrogen peroxide formed in the cell, is rather generally accepted. Appleman (2) found that the catalase in potato juice gives a striking correlation with the respiratory activity of the tuber. Reed (50) believes, as a result of his experiments and those of others, that the protective capacity of catalase is small, if any really exists.

It is difficult to show that hydrogen peroxide is produced in the plant tissues, because, if it were, it would be immediatly decomposed if there were any catalase present. Although a number of workers have shown that catalase will not act on organic peroxides, there may be some substance produced to which the action of catalase is related (Knott) (35).

There is considerable indirect evidence that hydrogen peroxide may be formed in the tissues. Dakin (13) thinks that the old idea of catalase activity as a protector has much to recommend it. Although he cannot see any

evidence relating catalase to oxidation, he does think it reasonable to suppose that hydrogen peroxide may be a transitory product formed in the tissues if molecular oxygen acts as an acceptor for the activated hydrogen resulting from the dehydrogenation of the substance being oxidized.

McLeod and Gordon (44) state that all bacteria lacking in catalase produce hydrogen peroxide when a reducing mechanism is operating within them to produce nascent hydrogen in the presence of oxygen. Burnet (8) also reports the formation of traces of hydrogen peroxide sufficient to check growth of isolated staphylococci and other organisms when exposed to light, and believes that the presence of catalase and diffusing substances in a bacterial colony is an indication of a primitive means of keeping the environment such that the most suitable type of growth can take place. Schlunk (53) finds that bacteria which produce the most catalase grow luxuriantly in the presence of hydrogen peroxide, while those producing little or no catalase are injured.

With regard to the effect of light on the production of hydrogen peroxide, Kugelmass and McQuarrie (37) explain the action of the reducing vapors which make a photographic plate developable as being due to the production of hydrogen peroxide as an intermediate product of organic oxidations,

as indicated by Russel (52). All substances capable of producing such fogging have a capacity for absorbing oxygen. Burge and Burge (7) show that Spirogyra poticalis exposed to different temperatures in the light and in the dark gave a much gr ater increase in catalase in the light.

While the evidence is not conclusive, Knott (35) believes that it is strongly suggestive that under conditions of light and oxygen where rapid metabolic activity is going on, the formation of considerable hydrogen peroxide may take place. Ordinarily it is not detectable because under the same conditions a high catalase activity is usually present and the peroxide is destroyed. To say that the plant produces this catalase to protect itself against the peroxide is teleological and merely evades the issue. In vitro, the catalase is decomposed during the reaction with the peroxide. If this happened in the tissue, then a high catalase content might be the balance remaining after a still greater amount produced by the protoplasm has interacted with the peroxide. On the other hand it is not impossible to assume that under the conditions existing in the protoplasm of the plant, the catalase is not decomposed by the peroxide or other substances upon which it acts. The action of the catalase preparation or extract can conceivably be quite different from the reaction

in vivo. If this be assumed the catalase would increase in relation to the peroxide or other condition which causes its production by the protoplasm, disappearing as it underwent autodecomposition. The catalase content at any given time would then be the balance between the amount produced up to the limit of the cell capacity and that lost by decomposition. The rate of decomposition would depend upon the stability of the catalase.

Appleman (2) found that the catalase activity of the expressed juice of sweet corn is almost directly proportional to the respiration of the plant in the milk stage. Drain (17) reported that respiration determinations give very little indication of ripening changes in apples after picking; and also that respiration rates and catalase activity are not closely correlated among apple varieties. Lantz (38) reported contradictory results to those obtained by Appleman (2). The results of Lantz showed no close correlation between catalase activity and respiration. No warrant was found for concluding that catalase is the enzyme chiefly concerned in physiological oxidation. The evidence rather favors the theory that catalase prevents excessive oxidation.

Harding (25) found that with both immediate and deferred storage at 1.11°C. and 2.22°C. and with continued

storage at 10.0°C. Grimes Golden apples are consistently higher in catalase activity. In deferred storage, fruit, just prior to breakdown, high catalase activity was registered with no corresponding increase in respiratory intensity. Respiration was not so sensitive an indicator of the approach of disorder as was catalase activity, which indicated early in the storage season whenever breakdown was to occur.

The work of Neller (48) indicates that catalase activity tends to be higher in apples going through the breakdown process and to be decreased below that of normal fruits in the advanced stages of breakdown. The catalase activity of apples that did not develop breakdown tended to increase during the earlier and to decrease during the later periods of storage corresponding to the youth and senescence of the apple. He concluded that physiological breakdown is associated with, or caused by, an accelerated metabolic rate. There is a general tendency of fruit showing breakdown to be higher in the percentage of dry matter and sucrose.

5. Chemistry of Catalase

The exact biological significance of the relation of catalase to respiratory activity is unknown. It is believed by many that the function of catalase is to

destroy the celltoxin, hydrogen peroxide, which if formed during the metabolic processes of the cell.

a. Oxidation by catalase

Stern (53) reported that the catalase system is composed of catalase, anticatalase, philocatalase and an activator of philocatalase, and is developed only in the aerobic organs. There is no relation between this system and the mode of oxidation. The catalase system exists only where there is active oxidase activity and is absent where there is oxydone activity. The latter differs from the oxidases in that in oxidase reactions hydrogen peroxide is produced from the interaction of nascent hydrogen and molecular oxygen; whereas in oxydone reactions water is formed. The role of the catalase system is the splitting of the hydrogen peroxide and the further promotion of oxidation by the liberation of active oxygen.

Euler, Runehjdm, and Steffenburg (19) found that in the oxidation of phenolphthalein by means of hydrogen peroxide, copper sulphate was found to have a catalytic acti n similar to that of the catalase of blood, tissues, and other biological materials. The trace of copper found in most tissues may explain the catalase activity of the latter. The copper is believed to be in the form of the anion, HOCuO.

According to Shibata (54) the catalase activate the hydrogen peroxide simultaneously to H··O¯O··H and HO··OH; the hydrogen peroxide decomposition is due to the mutual dehydrogenation and hydrogenation of these activated forms. Activation occurs in the following manner: the respective enzymes combine loosely with the water or hydrogen peroxide respectively. Thus the mechanism of the action of these enzymes is the same in all cases. Evidence is presented in support of the view that the activating action of catalase and peroxidase on the hydrogen peroxide is essentially the same, but it is different only in quantitative respects. The mechanism of the activation of hydrogen peroxide by complex metal salts and by the natural peroxidases and catalases is identical, as was proved by the fact that the mutual interference may occur in solutions containing the complex metal salt together with three natural enzyme.

A review of Thurnberg's (57) contributions on cellular oxidations shows that the oxygen consumed during the respiration is transformed into water. Hydrogen peroxide must be the first or at least the most important product of this reaction between hydrogen and oxygen. Through the action of catalase half the oxygen of the hydrogen peroxide is liberated and can thus function as a hydrogen acceptor

once more. Thus catalase is an enzyme which renders possible a more economic use of oxygen.

Biletzky (5) believes that catalase activity is dependent, in plants, upon conditions of nutrition and growth. The native colloidal carriers of catalase are the plant proteins. Their transformation into products of smaller complexity brings about an exchange of carriers with an increase in the amount of zymolabile carrier which results in increased enzymatic activity. Conversely, a synthesis of proteins from these lower products would result in increased zymostability of the system and hence a decrease in activity. Plants cultivated on glass sand without nutrients show a higher catalase activity and values. This fact is attributed to the greater loss of potassium and phosphorus as compared to nitrogen, the synthesis of the proteins being thus retarded, with a consequent accumulation of nitrogenous non-protein substances which constitute the most labile carriers of the active substance of the catalase system.

b. The Catalase - Hydrogen Peroxide Reaction

Maximovick and Avotonomova (43) state that the reaction between catalase and hydrogen peroxide consists of two simultaneous independent processes, viz. and enzymatic decomposition of hydrogen peroxide by the catalase and an inactivation of the catalase by the hydrogen peroxide.

The enzymic process follows the laws of monomolecular reaction, the extent of the deviation depending on the intensity of the accompanying inactivation.

Haber and Willstatter (22) in a discussion of the action of catalase, suggest that the contact material (enzyme) is univalently reduced while the substrate is univalently oxidized. The dehydrogenation products is, therefore, a radical with one valence space, which can link either to carbon or to oxygen, and which links more readily to carbon. The course of the two main reactions, which are continually repeated, gives reaction chains that are broken when two like radicals disappear as a result of dimerization or of disproportionation or two unlike radicals by the formation of additional compounds.

Haldane (24) in a study of the molecular statistics of an enzyme reaction found that one molecule of catalase acting at a temperature of $0^{o}C$ catalyzes the decomposition of 2×10^{5} molecules of hydrogen peroxide per second. The mean life of the active catalase hydrogen peroxide molecule is approximately 10^{-7} seconds. The velocity constant for the combination of catalase and hydrogen exceeds 7×10^{6}.

According to Morgulis (45) the destruction of catalase by hydrogen peroxide, which has been found to increase with the temperature, has now been shown also to depend even more

markedly upon the degree of buffering. The effect is not due to a low salt concentration, as it can be reproduced even when the salt concentration is kept constant. In the presence of sufficient buffering the catalase reaction becomes directly proportional to the concentration of the enzyme and independent of the hydrogen peroxide concentration. If the buffering is insufficient, the reaction decreases with increasing hydrogen peroxide concentration and, furthermore, with greater concentration of hydrogen peroxide the proportionality between the enzyme and the oxygen set free also tends to become less general.

Richter (51) believes that the comparison of the photochemical decomposition of hydrogen peroxide with the enzyme reaction indicates that the latter may be a chain reaction.

Albers (1) has reported on intermediate products formed during the catalytic decomposition of hydrogen peroxide. He finds that the hydrogen peroxide forms two kinds of compounds with the catalase molecule: a reactive intermediate compound, catalase - $(H_2O_2)_2$, and in the presence of excess substrate unreactive compound, probably catalase $(H_2O_2)_6$. This is in agreement with Wieland's conception of catalase as a dehydrogenase with a specific hydrogen acceptor.

c. The Active Group in Catalase

Wolff (60) reported that colloidal ferric ferrocyanide, formed by mixing dilute solutions of potassium ferrocyanide and ferric salts, gives all the reactions of catalases.

Reproductions of spectographs, according to Euler, Zeile, and Hellstrom (19) show catalase and hemin as identical in (C_5H_5N) solutions and almost identical in 0.002 N sodium hydroxide solutions. This fact suggests that the active group is the same in both substances.

Zeile (61) in further studies on the active group in catalase, shows that the close relationship between porphyrin iron content and catalase activity of horse liver extract is further shown by the constancy of this ratio in purified preparations. The same relationship is now shown to hold for catalase preparations from plant sources. The ratio of enzyme to porphyrin-iron, in the cotyledon of germinating pumpkin seeds, as determined by the hemochromogen spectrum is approximately 8,000, about three times as high as that of liver extract. The dissociation constant of the HCN compound of liver catalase is about 1/3 that of liver catalase. In both enzymes the active group is probably of similar constitution.

Stern (56) has conducted a series of experiments on

the properties of animal catalase. His results support the view that catalase of the liver is identical with that of the red blood corpuscles; from the diffusion capacity a molecular weight of 69,000, similar to that of hemoglobin was deduced. But the bearer of the catalase, protein in nature, to which a special fluoresence is ascribed, seems to be different from the protein of hemoglobin, the iso-electric point being different.

At the present time very little is actually known about catalase and its functions. Catalase is found in nearly all the tissues and secretions of plant and animal organisms. The only known action of this enzyme is the decomposition of hydrogen peroxide into oxygen and water. Catalase is not capable of decomposing other peroxides such as the organic derivatives of hydrogen peroxide. The oxygen liberated appears to be in the inactive molecular form. The wide distribution of catalase in living organisms suggests that this enzyme may possess an important physiological function in the facilitation of biological oxidation processes. Catalase is inactivated at high temperatures and its optimum reaction has been found to take place at a pH of 7.00. The active group of catalase appears to be a porphyrin-iron complex.

Michaelis and Pechstein, Morgulis and Roma, and

Damboviceanu (59) have reported that concentrated catalase solutions from animal origin possess a relatively stronger activity than dilute solutions of the enzyme. The hydrogen peroxide quantities decomposed in a given time are directly proportional to the concentration of the catalase. It is apparent from such observations that catalase is not capable of acting as a true catalyzer, but that a given quantity of catalase is capable of transforming only a given amount of hydrogen peroxide.

II. EXPERIMENTAL WORK

1. Equipment for Collecting and Analyzing Gas

Since the methods used by previous workers were not suitable for this particular investigation it was necessary to devise a method forcollecting the gas contained in the voids of cranberries. The following method for collecting gas from small fruit is quite rapid and sufficiently accurate for this work.

The gas collecting apparatus consists of a glass funnel inverted in a one liter beaker, containing approximately 500 cc. of water, (see Plate I) freshly boiled, to expel any dissolved gases. The sample of cranberries, approximately 70 grams, is placed in the beaker of water and the inverted funnel is placed over the fruit. A

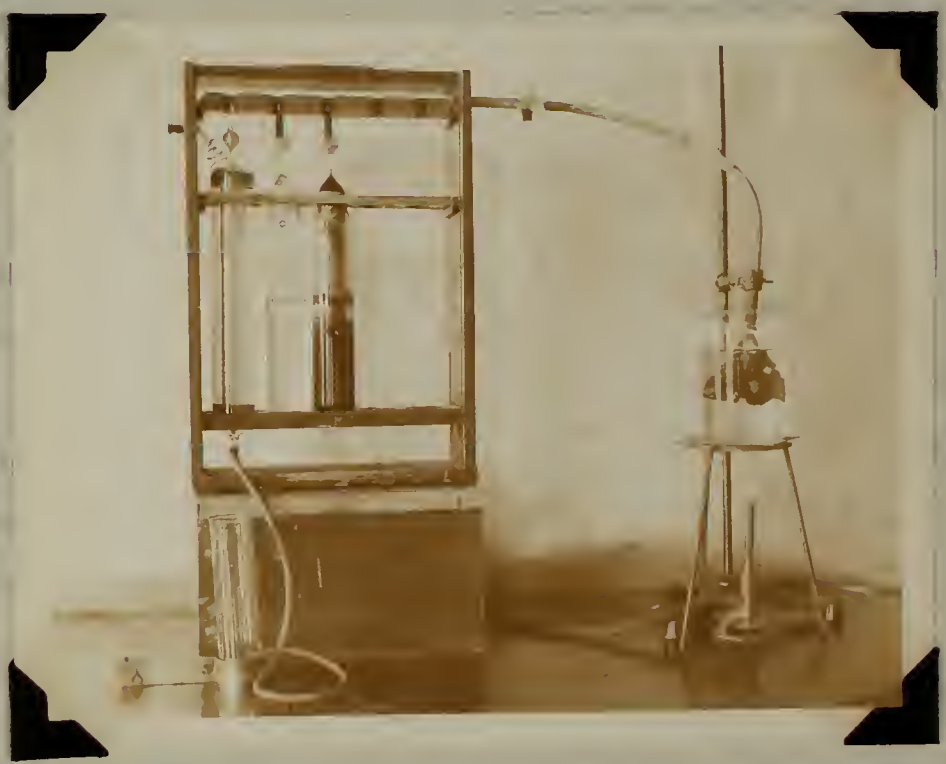
length of rubber tubing connects the stem of the funnel with the gas analysis apparatus.

For the actual analysis of the gas a modification of the original Orsat apparatus was used. This apparatus consisted of a gas measuring burette, and two "bubbling absorption pipettes." The first pipette, used to absorb carbon dioxide, contains a 12-molar solution of sodium hydroxide, while the second pipette, used to absorb oxygen, contains an alkaline pyrogallic acid solution.

The gas collecting and analyzing apparatus is set up as shown in Plate I. By means of the water level bottle the air in the funnel is removed, and the funnel and tube connecting the funnel with the Orsat apparatus, are filled with "gas free water" from the beaker. The water in the beaker is heated until all of the gas is expelled from the voids and intercellular spaces of the cranberries. This gas collects in the upper part of the funnel. As soon as the gas is collected it is drawn into the gas measuring burette by means of the water level bottle. The gas is allowed to stand until it has come to the temperature of the apparatus, and then its volume is determined by holding the water level bottle so that the water level is the same in the bottle as in the burette. The sample of gas is passed into the absorption pipette containing sodium hydroxide, and then passed back into the measuring burette. The

Plate I

APPARATUS FOR COLLECTING AND ANALYZING GAS



absorptions are continued until a constant reading is obtained in the measuring burette. The volume is read and the difference from the original volume represents the volume of carbon dioxide contained in the sample. After the volume of carbon dioxide is determined, the volume of oxygen is determined in a similar manner by bubbling the gas in to the absorption pipette containing the alkaline pyrogallic acid solution. The residual gas after the absorption of carbon dioxide and oxygen is calculated as nitrogen. The volume of each of the gases is expressed in terms of percentage of total volume of gas.

A uniform sample was taken from each lot of test cranberries, and for each analysis a portion of approximately 70 grams of cranberries were used.

There was some variation in each portion due to individual differences in the cranberries. Several portions from each sample were analyzed in order to obtain more accurate results.

2. Affect of Freezing and Submergence on the Composition of the Internal Atmosphere of Cranberries

In order to determine the affect of various environmental factors on the composition of the internal atmosphere of cranberries, several samples of fruit were collected from a natural cranberry bog located at Millis, Massachusetts.

The cranberries were of the Howes variety. The composition of the internal gas, and the carbon dioxide oxygen ration for each of the samples of cranberries was determined.

Sample I.

Cranberries were raked between September 4th to 10th and stored in a ventilated barn until October 13th, when they were cleaned, graded, and placed into standard crab-berry boxes. The cranberries then remained in storage in the barn until October 30th. The sample was analyzed on October 31st.

Sample II.

This sample consisted of cranberries which had been dropped on the bog when the berries were previously raked on September 4th to 10th. The bog was flooded about September 15th to 20th. When the berries were gathered on October 30th, there was on-quarter inch of ice on the bog and the cranberries were floating in the water, under the ice. None of the cranberries were frozen and the fruit was ap arently in very good condition. The sample was analyzed on October 31st.

Sample III.

The cranberries which compose this sample were left on the vines until October 25th and were under water at the time they were picked. All of the fruit was in good condition. The sample was analyzed on October 25th.

Sample IV.

These cranberries were left on the vines until October 25th and were all above the water on the bog and had been frosted at the time of picking. Most of them were quite soft. The sample was analyzed on October 29th.

Sample V.

These cranberries remained on the vines until October 30th. At this time the bog was frozen over and the cranberries were above the ice. About one third of the sample were frosted and the other two thirds were in apparently good condition. The sample was stored at 3°C. from October 31st to November 3rd, when the analysis was made.

The results of the analyses of the above samples of cranberries are given in Table II.

Discussion of Results

Considering the carbon dioxide / oxygen ratio as indicative of the rate of respiration, the cranberries which were left on the vines had a much higher rate of respiration than those which had been harvested about a month earlier and placed in storage. (Samples I and V). Since a high rate of respiration is detrimental to fruit, it would seem advisable not to leave the cranberries on the vines too long after they are ripe.

Of the cranberries which were raked early in September, the fruit which was left on the bog and submerged

in water had a much higher rate of respiration after a month had elapsed after picking, than those cranberries which had been stored in a barn during this time. (Samples I and II).

Of the cranberries which were left on the vines, those which were submerged in water showed a higher rate of respiration than those which were above water and in the air. (Samples III and V). It is possible to correlate these results with those obtained by Franklin (20) and Wakabayashi (58), who found that submergence weakened cranberries and makes them more susceptable to disease and breakdown. A correlation of results indicates that the weakened condition of cranberries, which have been submerged in water is due to the increased rate of respiration.

In berries which have been frosted the rate of respiration is materially reduced. (Samples IV and V).

3. Composition of Internal Atmosphere as Affected by Variety, Storage Time, and Temperature of Storage.

A. Early Blacks and Howes Stored at 24°C. and 3° C.

In order to determine the variations in the composition of the internal atmosphere of cranberries throughout a definite storage period, and at different temperatures, two varieties of cranberries, Early Blacks and Howes, were stored in a cold storage room at 3°C. and in a warm room at 24°C. Samples of the fruit were taken at weekly intervals and the internal gas was analyzed. In order to have a representative sample, cranberries were taken from various parts of the storage container and mixed together to make a uniform sample.

During this experiment, from October 1934 to May 1935, five different lots of cranberries were studied, consisting of three lots of Howes and two lots of Early Blacks. Each lot of cranberries was divided into two portions at the start of the experiment, one portion being stored in the cold room and the other in the warm room. In this manner it was possible to note the effect of different temperatures on the cranberries during storage. All of the cranberries used in this experimental work came from Wareham, Massachusetts, and were kept in cold storage until the tests were

begun.

The following table indicates the time at which each of the above lots of cranberries were placed under experimental conditions.

Lot of Cranberries		Date Expt. started	Date Fruit Broke Down	Temp. of Storage, Deg. C.
1. Early Blacks	(a)	10/24/34	11/8/34	24°
	(b)	10/24/34	2/2/35	3
2. Howes	(a)	10/31/34	11/21/34	24
	(b)	10/31/34	5/17/35	3
3. Early Blacks	(a)	12/8/34	12/20/34	24
	(b)	12/8/34	2/2/35	3
4. Howes	(a)	12/8/34	12/29/34	24
	(b)	12/8/34	5/17/35	3
5. Howes	(a)	3/30/35	4/16/35	24
	(b)	3/30/35	5/17/35	3

The cranberries stored at 24°C. deteriorated in a relatively short time; whereas cranberries stored at the lower temperature remained in good condition for several months.

The results of the weekly analyses of the samples of cranberries are given in tables III - XII. Results are figured as percentage by volume of carbon dioxide, oxygen, and nitrogen, in addition the carbon dioxide/ oxygen ratio is given. The above results are portrayed by graphs I-VIII.

B. Discussion of Results

The temperature of storage has a marked effect on the keeping quality of cranberries. The Early Blacks stored at 24°C. broke down in approximately twenty days, while those stored at 3°C. remained in good condition for over three months. At a temperature of 24°C. the Howes also broke down in approximately twenty days; and at this warm temperature their keeping quality was no better than that of the Early Blacks. However, at a temperature of 3°C. the Howes remained in good condition for seven months.

Throughout all of the tests the internal atmosphere of the Howes had a much higher carbon dioxide content and a correspondingly lower oxygen content than did the Early Blacks. Hence the carbon dioxide/oxygen ratio of the Howes was considerably greater than that of the Early Blacks. These results indicate that the keeping quality of cranberries may vary with the carbon dioxide content and the carbon dioxide/oxygen ratio.

The nitrogen content of the internal gas of the cranberries is similar to that of the atmosphere; about 79 percent, except when the fruit is respiring at a very rapid rate. When the respiration rate is high the increased carbon dioxide content apparently replaces some of the nitrogen. But in general it may be said that the percent

of nitrogen in the internal atmosphere of cranberries is quite stable. When the cranberries have stopped respiring, or the cells are dead, the composition of the internal atmosphere approximates that of the outside atmosphere, e.g. 79 percent nitrogen and 20 percent oxygen.

When stored at a warm temperature the Howes showed a very rapid increase in the carbon dioxide content and a correspondingly rapid decrease after the peak was reached. Under similar conditions the carbon dioxide content of the Early Blacks showed a more gradual increase in decrease and the peak was lower than in the Howes variety. Magness (41), Kidd et al (32), and Harley and Fisher (27) obtained similar results from their studies on the internal atmosphere of apples.

A study of the graphs of the carbon dioxide/oxygen ratios of both the Howes and Early Blacks indicates that the lots of cranberries started in October showed a greater amount of respiration before breaking down, than the lots of cranberries which were tested later in the season, when the test was made at 24°C.

The most interesting and important feature of this work is shown by the graphs of the carbon dioxide/oxygen ratios. In the case of the Early Blacks, the area covered by the graph of this ratio up to the time that the fruit

began to break down in storage at 24°C. is equal to the area covered by the graph of this ratio of the Early Blacks stored at 3°C. up to the time that internal breakdown set in. Similar results obtained by making graphs of the carbon dioxide/oxygen ratios of the Howes. These facts indicate that in the cranberry there is a definite amount of respirable material which must be used up during respiration, before internal breakdown sets in. If the fruit is stored at a high temperature this material is used up more rapidly, as is shown by the carbon dioxide/oxygen ratios. In a similar manner it is shown that when cranberries are stored at low temperatures this respirable material is used up very gradually, with the result that the life of the cranberry is greatly prolonged.

It is quite interesting to note that two of the samples of Howes and one of the samples of Early Blacks stored at 24°C. showed a secondary peak in the carbon dioxide content and in the carbon dioxide/oxygen ratio. In all three cases these secondary peaks occurred after the fruit was very soft and decomposed. The odor of the cranberries made it quite evident that fermentation was taking place within the fruit. In all probability this increase in the carbon dioxide content of the internal gas was due to the action of microorganisms on the decomposed fruit.

The data obtained from these tests indicate that by a study of the carbon dioxide/oxygen ratios, based on the composition of the internal atmosphere of cranberries, it is possible to determine the length of time, with in a few days, that cranberries may be kept in cold storage before internal breakdown sets in, and the fruit is in an unmarketable condition. This information may be obtained by running a test on cranberries, stored at a high temperature for about ten days in the fall when the fruit is first placed in storage. By comparing the carbon dioxide/oxygen ratios obtained it is possible to forecast ahead just how long cranberries may be kept in storage. Such a forecast of the keeping quality of cranberries should be of value to canneries and people using cold storages.

4. Methods of Measuring Catalase Activity

a. Volumetric Measurement of Oxygen

The determination of catalase activity in cranberries by the volumetric measurement of oxygen liberated from hydrogen peroxide, as used in this work, is a modification of the method proposed by Hawk and Bergeim (28). Forty grams of cranberries are thoroughly ground in a mortar with clean sharp sand, and 20 cc. of water are added. The resulting pulp is pressed through four layers of cheesecloth to obtain the enzyme extract.

The apparatus (see Plate II) consists of a 500 cc. wide mouthed bottle fitted with a two hole rubber stopper carrying two glass tubes. One is a straight glass tube fitted with a piece of pressure tubing closed with a screw clamp, which is used to keep the air in the bottle at atmospheric pressure until the determination is started. The second glass tube connects by means of pressure tubing and a bent glass tube to a eudiometer filled with water.

The determination is made by introducing 10 cc. of three percent hydrogen peroxide and 5 cc. of a mixture of equal parts M/15 Na_2HPO_4 and M/15 KH_2PO_4 into the 500 cc. bottle. Five cc. of the enzyme extract, contained in the base of a test tube is also placed in the bottle. The bottle is placed in a water bath at 21°C. The screw clamp on the vent tube is closed, and the enzyme extract dumped out of the test tube, and the bottle is shaken for ten minutes. The oxygen liberated from the hydrogen peroxide is collected in the eudiometer and measured. The volume of oxygen obtained indicates the relative catalase activity of that particular sample of cranberries. In these determinations no correction has been made for temperature and pressure because previous workers (Neller (49)/ Balls and Hall (4)) have found that the greatest correction for gas volumes obtained was considerably less than the variations

Plate II

APPARATUS FOR MEASURING CATALASE ACTIVITY

between samples.

b. Potassium Permanganate Titration Method

The potassium permanganate titration method for the determination of catalase activity is carried on as follows: The catalase or enzyme extract is prepared as in the preceding method. Five cc. of this extract plus 50 cc. of water are placed in a 150 cc. Erlenmeyer flask, and 0.5 cc. of three percent hydrogen peroxide are added. The mixture is allowed to stand for 30 minutes at room temperature. At the end of this time 5 cc. of 10 percent sulphuric acid are added to the contents of the flask, and the mixture is immediately titrated with 0.1 N potassium permanganate solution. By this method the hydrogen peroxide not decomposed by the catalase, is determined. The amount of potassium permanganate solution used in the titration varies inversely with the catalase activity.

b.1. Effect of Reducing Substances Present in the Cranberry on Potassium Permanganate

In order to check the accuracy of this method of determining catalase activity in cranberries the following tests were made.

1. Titration with cranberry extract as per standard procedure given above;

$KMnO_4$ solution required..11.79cc.

2. Titration with 5 cc. of ten percent sulphuric acid plus 50 cc. water produced on end point appeared upon the addition of one drop of $KMnO_4$.

3. Titration according to the standard procedure but omitting the cranberry extract

$KMnO_4$.....8.92 cc.

4. Titration with cranberry extract which was heated at 80°C. for 5 minutes to inactivate the catalase. Standard procedure used.

$KMnO_4$.....14.93 cc.

5. Titration with cranberry extract as per standard procedure, but omitting the hydrogen peroxide.

$KMnO_4$..... 3.17 cc.

5. Catalase Activity as Affected by Variety and Storage Conditions

The catalase activity in the samples of cranberries used in the study of the changes in the composition of the internal atmosphere of cranberries was determined at weekly intervals. Determinations were made on both Early Blacks and Howe cranberries which were stored at 3°C. and 24°C. Throughout this work the catalase activity was determined by the volumetric measurement of the oxygen liberated from hydrogen peroxide. (Method a). The results of these

determinations are given in Tables XIII-XXIV and are shown by Graphs IX-XII. The results are given as the volume of oxygen liberated, as well as the ratio of the catalase activity of the fruit stored at 24°C. to the fruit stored at 3°C.

The potassium permanganate method of determining catalase activity was carried out on the second lots of Early Black and Howe cranberries which were studied. The results are given in Tables XXV-XXVI and Graphs XIII-XIV. All the results are given in terms of the number of cubic centimeter of potassium permanganate solution necessary to oxidize the residual hydrogen peroxide. This method was discontinued, however, because as is shown on page 36 there are other substances, tannins and pigments, present in the fruit which also reduce the permanganate. Hence results obtained by the use of this method are not a true indication of the actual catalase activity.

1. Discussion of Results

In both the Early Blacks and the Howes cranberries stored at 3°C. there is a fluctuation in the catalase activity during the period of storage. In both varieties of berries stored at this temperature there is no correlation between catalase activity and the percent of carbon dioxide in the internal atmosphere of the fruit.

In both varieties of cranberries stored at 24°C. the catalase activity increased directly with the increase of carbon dioxide in the internal atmosphere of the fruit. The catalase also decreased directly with the carbon dioxide content of the internal gas, after its peak was reached. Also the results obtained indicate that the catalase activity precedes the increase in carbon dioxide. The catalase activity reached its peak in all cases a little in advance of the maximum carbon dioxide content of the fruit.

The ratio of catalase activity (24°C./3°C.) varies more directly with the carbon dioxide content and the carbon dioxide/oxygen ratio of the cranberries held at 24°C. than does the direct index of catalase activity (the volume of oxygen liberated).

In the Early Black cranberries held at 3°C. there was a sharp increase in the catalase activity a short time before the berries began to break down.

Throughout the tests the Early Black variety tended to exhibit a higher catalase activity than did the Howes variety; which may indicate that a higher catalase activity is correlated with a poorer keeping quality in cranberries.

The results obtained from the potassium permanganate determinations of catalase activity cannot be

correlated with the results obtained by the first method.

III. GENERAL SUMMARY

1. Submergence causes an increased rate of respiration in cranberries and an increase in the carbon dioxide content of the internal atmosphere. It is probable that the increased rate of respiration weakens the cranberry and makes it more susceptible to disease; as it has been observed by Franklin (20) and also Wakabayashi (51) that submergence weakens cranberries and makes them more susceptible to attacks by fungi.

2. Frosting reduces the rate of respiration in cranberries.

3. Evidence obtained indicates that there is an increase in the respiration of cranberries if they are left on the vines after the usual picking time.

4. The temperature of storage has a marked affect on the keeping quality of cranberries. Cranberries stored at 24° C. became soft and partially decayed in 20 days.

5. The carbon dioxide content and the carbon dioxide/oxygen ratio vary directly with the keeping quality of the cranberries.

6. The nitrogen content of the internal gas of cranberries is relatively stable and approximates that of the at-

mosphere. The carbon dioxide and oxygen contents of the internal gas vary with the rate of respiration.

7. Cranberries contain a definite amount of respirable material which must be used up before the cranberries spoil in storage. When there is a high rate of respiration this material is used up more rapidly and the length of life of the cranberry in storage is correspondingly decreased.

8. By means of the carbon dioxide/oxygen ratio it is possible to forecast with fairly good accuracy several months in advance the keeping qualities of cranberries in cold storage.

9. There is no correlation between catalase activity and respiration of cranberries in cold storage.

10. The catalase activity varies directly with the carbon dioxide content of the internal atmosphere and the carbon dioxide/ oxygen ratio in cranberries stored at 24° C.

11. With the two varieties of cranberries examined, a high catalase activity is correlated with poor keeping quality.

12. Cranberries show a sharp increase in catalase activity a short time before they begin to show structural

break down in cold storage.

13. The potassium permanganate titration method for the determination of catalase activity in cranberries is inaccurate because of interfering substances which are present in the cranberry.

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TABLE II. AFFECT OF FREEZING AND SUBMERGENCE ON THE COMPOSITION OF THE INTERNAL ATMOSPHERE OF CRANBERRIES. SAMPLES FROM MILLIS, MASSACHUSETTS

Date Harvested	Sample	Portion		Percent Composition Carbon Dioxide	Oxygen	Nitrogen	Carbon Dioxide / Oxygen Ratio
Sept. 4-10	I	1		7.78	15.56	76.66	
		2		7.43	17.56	75.01	
		3		7.37	15.06	77.57	
			Mean	7.52	16.06	76.41	0.468
Oct. 30	II	1		9.89	12.76	77.35	
		2		7.06	8.82	84.12	
		3		15.30	8.33	76.37	
			Mean	10.75	9.97	79.28	1.075
Oct. 25	III	1		28.64	7.02	64.33	
		2		10.75	9.75	79.50	
		3		6.41	13.36	80.23	
		4		12.27	11.36	76.37	
		5		9.44	8.89	81.67	
		6		14.02	11.31	74.67	
		7		11.20	10.34	78.46	
			Mean	13.24	10.57	76.19	1.252
Oct. 25	IV	1		6.66	13.33	80.01	
		2		4.23	10.38	85.39	
		3		4.66	12.00	83.33	
			Mean	5.18	11.90	82.92	0.435
Oct. 30	V Sound Fruit	1		11.56	13.29	75.15	
		2		11.38	10.24	78.38	
		3		11.23	12.36	76.41	
		4		10.30	10.30	79.40	
			Mean	11.12	11.54	77.33	0.972

TABLE II. (Continued)

Oct. 30	V	1		5.32	10.35	84.33	
	Frosted	2		4.76	14.62	80.62	
	Fruit		Mean	5.02	12.48	82.47	0.404

TABLE III. LOT 1a. EARLY BLACKS STORED AT 24°C. COMPOSITION OF INTERNAL ATMOSPHERE

Date	Portion	Percent Composition			Carbon Dioxide Oxygen Ratio	Condition of Fruit
		Carbon Dioxide	Oxygen	Nitrogen		
Oct. 24	1	4.68	13.28	82.04		
	2	4.76	10.47	84.77		
	3	3.89	10.71	85.40		
	4	4.68	12.50	82.82		
	5	5.34	12.26	82.40		
	6	6.83	13.66	79.51		
	7	6.81	12.87	80.32		
	8	8.33	13.19	78.48		
	Mean	5.62	12.22	82.16	0.460	Very Good
Nov. 1	1	9.09	14.20	76.71		
	2	6.99	13.28	79.73		
	3	9.49	13.92	76.59		
	4	7.81	12.50	79.69		
	Mean	8.44	13.47	78.18	0.626	Slightly Soft
Nov. 8	1	11.53	12.08	76.39		
	2	14.78	9.15	76.07		
	3	17.77	4.44	77.79		
	4	9.04	11.17	79.79		
	Mean	13.28	9.21	77.51	1.442	Soft and badly spotted.
Nov. 15	1	11.07	5.68	83.25		
	2	14.02	5.48	80.50		
	3	13.35	5.90	80.75		Early rot increasing
	4	15.09	4.40	80.51		Fruit soft and
	Mean	13.38	5.36	81.25	2.490	shriveled.

TABLE III. (continued.)

Nov. 23	1	9.39	4.12	86.51		A large percent of fruit soft, shriveled, or infected with early rot.
	2	11.45	3.10	85.45		
	3	12.13	3.66	84.21		
	4	11.76	3.23	85.01		
	Mean	11.18	3.52	85.24	3.170	
Nov. 27	1	9.73	11.56	78.71		
	2	6.71	13.10	80.19		
	3	9.06	12.50	78.44		
	4	10.48	11.73	77.79		
	Mean	8.99	12.22	78.77	0.736	Fruit badly decomposed.

TABLE IV. LOT 1b. EARLY BLACKS STORED AT 3°C. COMPOSITION OF INTERNAL ATMOSPHERE

Date	Portion	Percent Composition: Carbon Dioxide	Oxygen	Nitrogen	Carbon Dioxide / Oxygen Ratio	Condition of Fruit
Oct. 24	See table I.					
Nov. 1	1	2.53	18.42	79.05		
	2	1.46	21.16	77.38		
	3	4.11	19.41	76.48		
	4	2.90	18.60	78.50		
	Mean	2.75	19.49	77.85	0.141	Good
Nov. 8	1	3.98	19.20	76.82		
	2	3.60	17.26	79.14		
	3	3.03	18.18	78.79		
	4	2.21	12.39	85.40		
	Mean	3.20	16.75	80.04	0.191	Good
Nov. 23	1	2.49	3.49	94.02		
	2	2.58	6.45	90.97		New sample taken from cold storage Nov. 20.
	3	4.76	9.52	85.72		
	4	2.73	12.26	85.01		
	Mean	3.14	7.93	88.93	0.396	
Nov. 27	1	2.66	17.55	79.79		
	2	5.12	16.55	78.33		
	3	5.75	17.81	76.44		
	4	7.14	14.26	78.60		
	Mean	5.16	16.54	78.29	0.312	Good

TABLE V. LOT 2a. HOWES CRANBERRIES STORED AT 24°C. COMPOSITION OF INTERNAL ATMOSPHERE

Date	Portion	Percent Composition			Carbon Dioxide Oxygen Ratio	Condition of Fruit
		Carbon Dioxide	Oxygen	Nitrogen		
Oct. 31	1	10.88	14.85	74.27		
	2	8.61	14.72	76.67		
	3	7.30	13.69	79.01		
	4	8.96	14.57	76.47		
	5	10.14	13.04	76.82		
	6	7.90	16.27	75.83		
	7	8.94	14.21	76.85		
	Mean	8.94	14.47	76.56	0.618	Very Good
Nov. 7	1	13.85	9.03	77.12		
	2	16.12	5.80	78.08		
	3	18.40	5.31	75.99		
	4	17.07	6.09	76.84		
	Mean	16.43	6.55	77.00	2.510	Slightly soft
Nov. 14	1	15.38	6.15	78.47		
	2	22.85	1.90	75.25		
	3	15.10	3.26	81.64		
	4	24.64	3.07	72.32		
	Mean	19.48	3.59	76.92	5.420	
Nov. 21	1	17.68	4.57	77.75		Berries becoming soft, early rot setting in.
	2	21.87	2.50	75.63		
	3	19.46	2.68	77.86		
	4	21.56	1.96	76.48		
	Mean	20.14	2.92	76.93	6.900	

TABLE V. (continued)

Nov. 27	1	18.17	3.27	78.56		
	2	11.55	12.70	75.75		Softness and
	3	17.45	5.52	77.03		early rot increase.
	4	18.20	5.84	75.96		
	Mean	16.36	6.83	76.82	2.390	
Dec. 6	1	17.67	6.98	75.35		
	2	19.71	4.97	75.32		Large percent of fruit
	3	8.18	5.45	86.37		soft. Softness more
	4	18.78	4.78	76.44		prevalent than early
	Mean	18.72	5.57	75.70	3.360	rot.
Dec. 13	1	21.10	2.40	76.50		
	2	16.43	7.24	76.33		
	3	19.33	4.27	76.40		Most of fruit
	4	19.99	4.00	76.01		very soft.
	Mean	19.21	4.47	76.31	4.290	
Dec. 19	1	16.55	4.92	78.53		
	2	19.93	4.57	75.50		Fruit very soft.
	3	17.93	3.47	78.60		
	Mean	18.13	4.32	77.54	4.190	
Dec. 27	1	16.80	4.01	79.09		
	2	12.43	9.70	77.87		
	3	16.66	4.02	79.32		
	Mean	15.33	5.91	78.76	2.590	

TABLE VI. LOT 2b. HOWES CRANBERRIES STORED AT 3°C. COMPOSITION OF INTERNAL ATMOSPHERE

Date	Portion	Percent Composition			Carbon Dioxide Oxygen Ratio	Condition of Fruit
		Carbon Dioxide	Oxygen	Nitrogen		
Oct. 3.	See Table III.					
Nov. 7	1	7.02	14.60	78.38		
	2	9.57	16.75	73.68		Good
	3	11.04	12.88	76.08		
	4	7.58	11.55	80.81		
	Mean	8.80	13.94	77.25	0.631	
Nov. 14	1	7.83	15.66	76.51	0.499	Good
Nov. 24	1	7.69	14.86	77.45		A new sample of
	2	9.10	12.40	78.50		fruit taken from
	3	7.15	10.00	83.20		cold storage.
	Mean	7.98	12.42	79.71	0. 643	
Nov. 27	1	5.81	17.02	77.17		
	2	6.78	16.05	77.17		
	3	5.16	15.46	79.38		
	4	8.40	14.75	76.85		
	Mean	6.53	15.92	77.64	0.410	Good
Dec. 6	1	9.90	12.93	77.17		
	2	7.30	15.23	77.47		
	3	9.38	11.87	78.75		
	4	12.40	11.11	76.49		
	Mean	9.74	12.78	77.47	0.762	Good

TABLE VI. (continued)

Dec. 13	1		10.22	9.60	80.18		
	2		7.00	17.06	75.94		
	3		9.20	13.82	76.98		
		Mean	8.80	13.49	77.70	0.653	Good
Dec. 19	1		5.50	17.80	76.70		
	2		8.29	15.00	76.71		
	3		7.95	13.91	78.14		
		Mean	7.24	15.57	77.18	0.465	Good
Dec. 27	1		5.59	12.73	81.68		
	2		4.93	14.79	80.82		
	3		5.32	12.97	81.71		
		Mean	5.28	13.47	81.22	0.394	Good

TABLE VII. LOT 3a. EARLY BLACKS STORED AT 24°C. COMPOSITION OF INTERNAL ATMOSPHERE

Date	Portion		Percent Composition Carbon Dioxide	Oxygen	Nitrogen	Carbon Dioxide Oxygen Ratio	Condition of Fruit
Dec. 8	1		7.62	13.95	77.43		
	2		8.33	14.42	77.25		
		Mean	7.97	14.18	77.34	0.562	Very Good
Dec. 15	1		9.46	9.66	80.88		
	2		5.50	11.00	83.50		
	3		6.94	13.20	76.86		
		Mean	7.30	11.28	80.41	0.647	Good
Dec. 20	1		11.14	10.37	78.49		
	2		11.11	8.32	80.57		Early rot setting in.
	3		9.76	8.97	81.27		
		Mean	10.67	9.22	80.11	1.156	Fruit becoming soft
Dec. 29	1		13.64	6.65	79.71		
	2		9.69	11.07	79.24		
	3		13.37	7.48	79.15		
		Mean	12.23	8.40	79.36	1.453	
Jan. 5	1		13.75	7.50	78.75		
	2		10.70	9.52	79.78		Early rot very
	3		15.00	4.63	80.37		prevalent.
		Mean	13.15	7.21	79.63	1.824	
Jan. 15	1		10.64	9.30	80.06		Fruit very soft and
	2		12.49	6.75	80.76		in poor condition.
	3		14.21	5.98	79.81		
		Mean	12.44	7.34	80.21	1.694	

TABLE VII. (continued).

Jan. 19	1	7.61	11.85	80.54		Fruit very soft and shriveled.
	2	6.03	14.78	79.19		
	3	9.75	8.29	81.96		
	Mean	7.79	11.64	80.56	0.670	
Jan. 25	1	9.90	11.13	78.97		
	2	12.30	8.21	79.49		
	3	11.64	9.13	79.23		
	Mean	11.28	9.49	79.23	1.139	
Feb. 1	1	9.36	9.59	81.05		
	2	9.44	8.81	81.75		Fruit very soft.
	3	9.18	9.42	81.40		
	Mean	9.32	9.27	81.40	1.005	
Feb. 9	1	6.41	12.55	81.04		
	2	9.19	8.11	82.70		
	Mean	7.80	10.33	81.87	0.755	
Feb. 16	1	10.00	7.33	82.67	1.364	Fruit shriveled and dried.
Feb. 26	1	8.59	8.59	82.82	1.000	Fruit 100 percent soft, rotten, shriveled, or dried.

TABLE VIII. LOT 3b. EARLY BLACKS STORED AT 3°C. COMPOSITION OF INTERNAL ATMOSPHERE

Date	Portion	Percent Composition			Carbon Dioxide Ratio	Condition of Fruit
		Carbon Dioxide	Oxygen	Nitrogen	Oxygen Ratio	
Dec. 8	See Table V.					
Dec. 31	1	2.54	17.52	79.94		
	2	1.99	17.20	80.81		
	3	3.19	14.47	82.34		
	Mean	2.57	16.39	81.03	0.156	
Jan. 5	1	2.58	15.98	81.44		
	2	1.53	16.15	82.32		
	3	3.59	15.56	80.85		
	Mean	2.56	15.89	81.53	0.161	
Jan. 19	1	3.25	18.37	78.38		Fruit in good condition
	2	1.28	18.30	80.42		
	Mean	2.26	18.33	79.40	0.123	
Jan. 25	1	1.94	17.96	80.10		
	2	1.49	17.60	80.91		
	Mean	1.71	17.78	80.50	0.096	
Feb. 16	1	1.86	15.81	82.33	0.117	50 percent of fruit decayed.
Feb. 26	1	5.59	15.37	79.04	0.367	

TABLE IX. LOT 4a. HOWES STORED AT 24°C. COMPOSITION OF INTERNAL ATMOSPHERE

Date	Portion	Percent Composition			Carbon Dioxide Oxygen Ratio	Condition of Fruit
		Carbon Dioxide	Oxygen	Nitrogen		
Dec. 8	1	8.49	15.50	76.01		
	2	8.49	15.28	76.23	0.552	Very Good
	Mean	8.49	15.39	76.12		
Dec. 15	1	8.23	10.76	81.01		
	2	12.55	6.09	81.36		
	3	12.40	6. 21	81.39		
	Mean	11.06	7.68	81.25	1.440	
Dec. 20	1	16.00	9.43	74.57		Fruit becoming soft.
	2	24.21	4.12	71.67		
	3	17.27	5.55	77.18		
	Mean	19.16	6.36	74.47	3.030	
Dec. 29	1	18.42	5.26	76.32		
	2	20.66	2.75	76.59		
	3	21.45	5.93	72.62		
	Mean	20.17	4.64	75.14	4.340	
Jan. 5	1	25.27	2.02	72.71		
	2	16.56	4.14	79.30		
	3	22.25	2.53	74.22		
	Mean	21.36	2.89	75.41	7.390	
Jan. 14	1	16.84	5.79	77.37		
	2	18.70	2.63	78.67		
	3	20.81	1.89	77.30		
	Mean	18.78	3.43	77.78	5.360	

TABLE IX. (continued).

Jan. 19	1	17.87	7.26	74.87		
	2	16.03	6.10	77.87		
	3	14.57	5.73	79.70		
	Mean	16.22	6.36	77.48	2.630	
Jan. 25	1	14.92	9.46	75.62		
	2	15.29	5.58	79.13		
	3	17.84	5.07	76.49		
	Mean	16.01	6.90	77.08	2.320	
Feb. 1	1	18.16	5.14	76.70		
	2	17.35	4.74	77.91		Fruit very soft.
	3	15.65	5.60	78.75		
	Mean	17.05	5.16	77.75	3.305	
Feb. 9	1	11.83	9.19	78.98		
	2	14.11	6.64	79.25		
	3	12.87	6.76	80.37		
	Mean	12.93	7.52	79.53	1.716	
Feb. 16	1	12.83	6.15	81.52		Fruit badly
	2	9.68	6.13	84.19		shriveled.
	Mean	11.25	6.14	82.85	1.830	
Feb. 23	1	11.46	5.73	82.81	2.000	Fruit in a decayed condition.

TABLE X. LOT 4b. HOWES STORED AT 3°C. COMPOSITION OF INTERNAL ATMOSPHERE

Date	Portion		Percent Composition: Carbon Dioxide	Oxygen	Nitrogen	Carbon Dioxide/ Oxygen Ratio	Condition of Fruit
Dec. 8	See Table VII						
Jan. 14	1		6.54	9.81	83.65		
	2		7.11	11.00	81.89	0.656	
		Mean	6.82	10.40	82.77		
Jan. 19	1		3.79	18.73	77.48		
	2		6.42	14.81	78.77		
	3		7.04	14.32	78.64		
		Mean	5.75	15.95	78.29	0.360	Fruit slighly soft.
Jan. 25	1		5.97	15.41	78.62		
	2		5.58	17.14	77.28		
	3		6.69	13.36	79.95		
		Mean	6.08	15.30	78.61	0.397	
Feb. 16	1		7.35	15.10	77.55		
	2		5.91	14.79	79.30		
		Mean	6.63	14.94	78.42	0.441	Fruit slightly soft, otherwise in good condition.
Feb. 23	1		5.97	13.43	80.60	0.444	

TABLE X CONTINUED

Apr. 7	1		4.94	15.94	79.12		
	2		4.50	15.17	80.33		
		Mean	4.72	15.55	79.72	0.303	
May 15	1		4.43	15.20	80.37		
	2		5.31	15.72	78.97		Fruit 50-70 percent soft and unmarketable.
	3		4.65	15.55	79.80		
		Mean	4.79	15.49	79.71		

TABLE XI. LOT 5a. HOWES STORED AT 24°C. COMPOSITION OF INTERNAL ATMOSPHERE

Date	Portion	Percent Composition			Carbon Dioxide Oxygen Ratio	Condition of Fruit
		Carbon Dioxide	Oxygen	Nitrogen		
Mar. 30	1	4.92	10.38	84.70		
	2	6.93	18.01	75.06		
	Mean	5.92	14.19	79.88	0.417	Good
Apr. 2	1	12.50	6.77	80.73		
	2	11.56	8.85	80.41		
	Mean	12.03	7.81	80.57	1.541	
Apr. 7	1	12.04	8.17	79.79		Fruit about 70 per-
	2	12.25	8.59	79.16		cent soft.
	3	9.30	12.23	77.97		
	Mean	11.36	9.66	78.97	1.136	
Apr. 16	1	10.20	12.25	77.55		Fruit quite soft and
	2	11.37	7.48	81.15		unmarketable.
	3	13.53	8 .59	77.88		
	Mean	11.70	9.44	78.86	1.240	

TABLE XIII. LOT 1a. CATALASE ACTIVITY IN EARLY BLACKS STORED AT 24°C.

Date	Portion	Cc. Oxygen Liberated	Mean
Nov. 13	1	1.75	
	2	1.59	1.67
Nov. 20	1	3.30	
		3.64	3.47
Nov. 26	1	4.04	
	2	4.75	
	3	4.32	4.37
Dec. 4	1	6.83	
	2	6.65	6.74
Dec. 6	1	2.43	
	2	4.10	3.26
Dec. 11	1	5.59	
	2	5.60	5.59

TABLE XIV. LOT 1b. CATALASE ACTIVITY IN EARLY BLACKS STORED AT 3°C.

Date	Portion	Cc. Oxygen Liberated	Mean
Nov. 13	1	2.00	
	2	1.04	1.52
Nov. 20	1	2.13	
	2	4.46	3.29
Nov. 26	1	3.25	
	2	3.20	3.22
Dec. 4	1	7.12	
	2	6.14	6.63
Dec. 6	1	2.62	
	2	2.70	2.66
Dec. 11	1	5.35	
	2	6.00	
	3	5.56	5.45

TABLE XV. LOT 1a. and 1b. CATALASE ACTIVITY IN EARLY BLACKS STORED AT 24°C. and 3°C.

RATIO OF CATALASE ACTIVITY OF FRUIT STORED AT 24°C. TO THAT STORED AT 3°C.

Date	Ratio
Nov. 13	1.11
Nov. 20	0 .816
Nov. 26	1.35
Dec. 4	1.08
Dec. 6	1.52
Dec. 11	1.02

TABLE XVI. LOT 2a. CATALASE ACTIVITY IN HOMES STORED AT 24°C.

Date	Portion	Cc. Oxygen Liberated	Mean
Nov. 13	1	2.53	
	2	1.80	2.17
Nov. 20	1	3.85	
	2	3.86	3.85
Nov. 26	1	2.95	
	2	2.56	2.90
Dec. 6	1	1.57	
	2	1.93	1.75
Dec. 11	1	4.75	
	2	4.88	4.81
Dec. 19	1	2.78	
	2	2.89	2.85
Dec. 28	1	3.76	
	2	3.69	3.72
Jan. 2	1	2.74	
	2	2.79	2.76
Jan. 8	1	2.30	
	2	2.72	2.51

TABLE XVII. LOT 2b. CATALASE ACTIVITY STORED AT 3°C. , in HOWES

Date	Portion	Cc. Oxygen Liberated	Mean
Nov. 13	1	1.64	
	2	2.00	1.82
Nov. 20	1	1.74	
	2	1.67	1.71
Nov. 26	1	2.87	
	2	2.90	2.88
Dec. 6	1	2.56	
	2	1.69	
	3	1.96	2.07
Dec. 11	1	4.05	
	2	3.91	3.98
Dec. 19	1	2.26	
	2	3.10	2.68
Dec. 28	1	4.20	
	2	4.74	4.47
Jan. 2	1	3.02	
	2	2.78	2.90
Jan 8	1	2.60	
	2	2.94	2.77

TABLE XVIII. LOTS 2a. and 2b. RATIO OF CATALASE ACTIVITY OF HOWES STORED AT 24°C. and 3°C.

Date	Ratio
Nov. 13	1.19
Nov. 20	2.25
Nov. 26	1.00
Dec. 6	0.84
Dec. 11	1.21
Dec. 19	1.06
Dec. 28	0.83
Jan. 2	0.95
Jan. 8	0.90

TABLE XIX. LOT 3a. CATALASE ACTIVITY IN EARLY BLACKS STORED AT 24°C.

Date	Portion	Cc. Oxygen Liberated	Mean
Dec. 8	1	2.40	
	2	2.23	
	3	2.62	2.41
Dec. 15	1	2.10	
	2	2.95	2.52
Dec. 21	1	6.27	
	2	5.32	
	3	6.01	5.86
Dec. 29	1	3.33	
	2	3.51	3.42
Jan. 5	1	4.99	
	2	4.79	4.89
Jan. 12	1	1.92	
	2	2.51	2.21
Jan. 19	1	2.30	
	2	2.04	2.17
Jan. 26	1	3.05	
	2	3.05	3.05
Feb. 3	1	2.78	
	2	2.75	2.76
Feb. 10	1	2.54	
	2	1.90	2.22
Feb. 26	1	4.17	
	2	4.12	4.14

TABLE XX. LOT 3b. CATALASE ACTIVITY IN EARLY BLACKS STORED AT 3°C.

Date	Portion	Cc. Oxygen Liberated	Mean
Dec. 8	1	2.40	
	2	2.23	
	3	2.62	2.41
Dec. 15	1	3.02	
	2	2.43	2.72
Dec. 21	1	3.93	
	2	3.93	3.93
Dec. 29	1	2.75	
	2	2.54	2.64
Jan. 5	1	3.96	
	2	3.45	3.70
Jan. 12	1	2.89	2.89
Jan. 19	1	2.25	
	2	2.20	2.22
Jan 26	1	2.73	
	2	2.79	2.76
Feb. 3	1	3.10	3.10
Feb. 10	1	3.76	
	2	3.50	3.63
Feb. 26	1	2.81	
	2	3.11	2.96

TABLE XXI. LOTS 3a and 3b. RATIO OF CATALASE ACTIVITY OF EARLY BLACKS STORED AT 24°C. and 3°C.

Date	Ratio
Dec. 8	1.00
Dec. 15	0.926
Dec. 21	1.492
Dec. 29	1.295
Jan. 5	1.321
Jan. 12	0.765
Jan. 19	0.977
Jan. 26	1.100
Feb. 3	0.890
Feb. 10	0.612
Feb. 26	1.397

TABLE XXII. LOT 4a. CATALASE ACTIVITY IN HOWES STORED AT 24°C.

Date	Portion	Cc. Oxygen Liberated	Mean
Dec. 8	1	2.04	
	2	2.51	
	3	2.30	2.28
Dec. 15	1	3.44	
	2	4.24	3.84
Dec. 21	1	3.17	
	2	3.46	3.31
Dec. 29	1	2.93	
	2	2.86	2.89
Jan. 8	1	3.77	
	2	3.43	3.60
Jan. 12	1	2.75	
	2	3.38	3.06
Jan. 19	1	3.05	
	2	2.86	2.95
Jan. 26	1	1.39	
	2	1.22	1.30
Feb. 2	1	2.78	
	2	2.83	2.80
Feb. 9	1	2.42	
	2	2.19	2.30

TABLE XXIII. Lot 4b. CATALASE ACTIVITY IN HOWES STORED AT 3°C.

Date	Portion	Cc. Oxygen Liberated	Mean
Dec. 8	1	2.04	
	2	2.51	
	3	2.30	2.28
Dec. 15	1	2.15	
	2	2.64	2.39
Dec. 29	1	2.60	
	2	2.48	2.54
Jan. 8	1	2.60	
	2	2.94	2.77
Jan. 12	1	3.04	
	2	3.09	3.06
Jan. 19	1	2.30	
	2	2.74	2.52
Jan. 26	1	1.62	
	2	1.30	1.46
Feb. 2	1	3.64	
	2	3.58	3.61
Feb. 9	1	2.03	
	2	1.64	1.83

TABLE XXIV. LOTS 4a and 4b. RATIO OF CATALASE ACTIVITY OF HOWES, STORED AT 24° C. and 3° C.

Date	Ratio
Dec. 8	1.000
Dec. 15	1.605
Dec. 29	1.137
Jan. 8	1.300
Jan. 12	1.000
Jan. 19	1.171
Jan. 26	0.864
Feb. 2	0.776
Feb. 9	1.256

TABLE XXVI. CATALASE ACTIVITY IN EARLY BLACKS, LOTS 3a and 3b, AS DETERMINED BY POTASSIUM PERMANGANATE TITRATION

	Cc. 0.1 N $KMnO_4$ to oxidize residual hydrogen peroxide	
Date	Lot 3a (24°C.)	Lot 3b (3°C.)
Jan. 8	11.76	12.34
Jan. 12	11.07	-----
Jan. 19	11.01	10.43
Jan. 26	10.56	11.62
Feb. 2	11.06	11.39
Feb. 9	10.35	11.54

TABLE XXVI CATALASE ACTIVITY IN HOWES, LOTS 4a and 4b, AS DETERMINED BY POTASSIUM PERMANGANATE TITRATION

	Cc. 0.1 N $KMnO_4$ to oxidize residual hydrogen peroxide	
Date	Lot 4a (24°C.)	Lot 4b (3°C.)
Jan. 8	11.12	11.47
Jan. 12	11.48	13.60
Jan. 19	11.59	12.46
Jan. 26	11.92	11.87
Feb. 2	11.29	12.30
Feb. 9	10.83	11.70

Fig. I. EARLY BLACKS- CARBON DIOXIDE CONTENT AS AFFECTED BY TEMPERATURE AND TIME IN STORAGE.

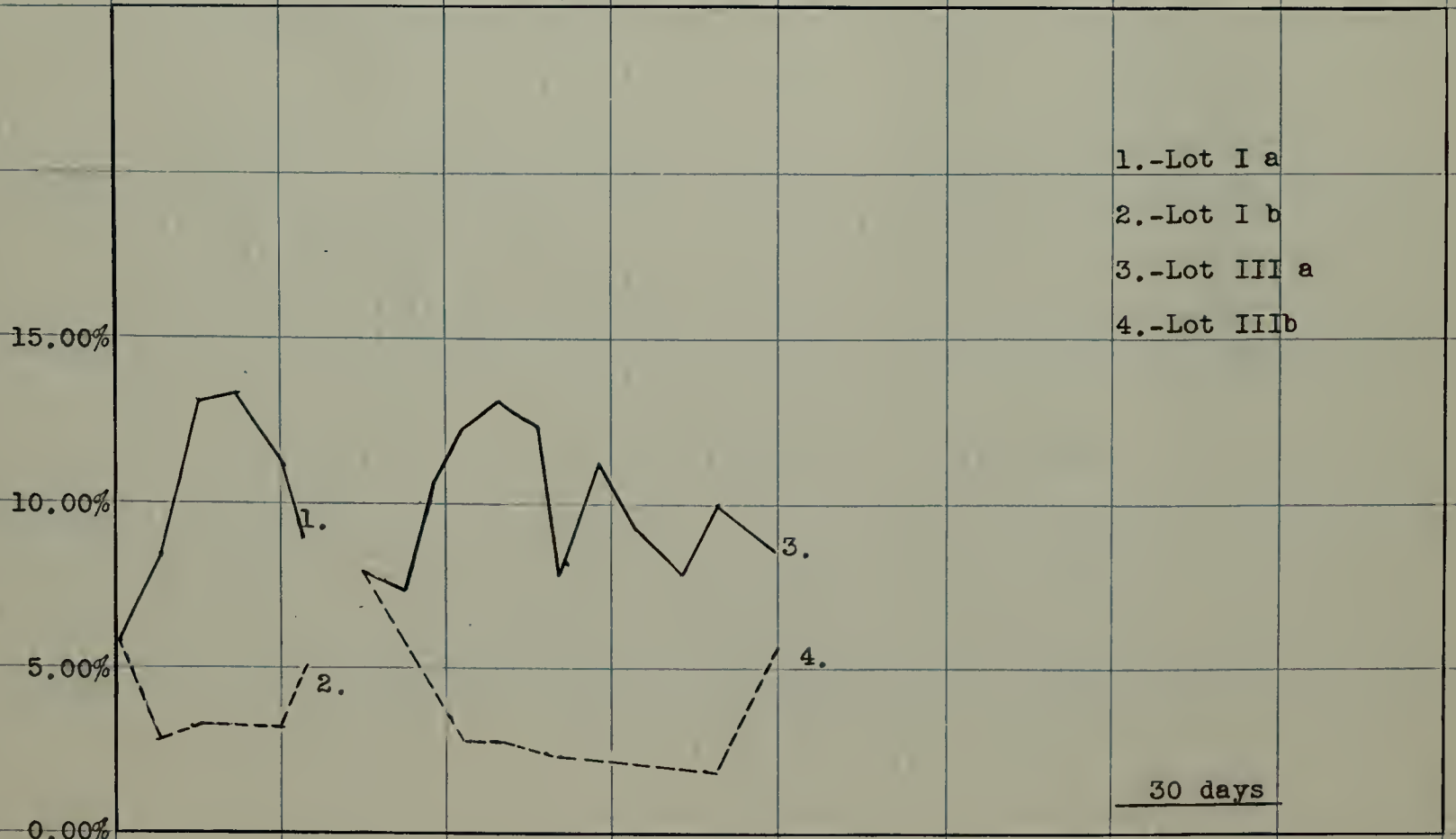


Fig.II. HOWES- CARBON DIOXIDE CONTENT AS AFFECTED BY TEMPERATURE AND TIME IN STORAGE

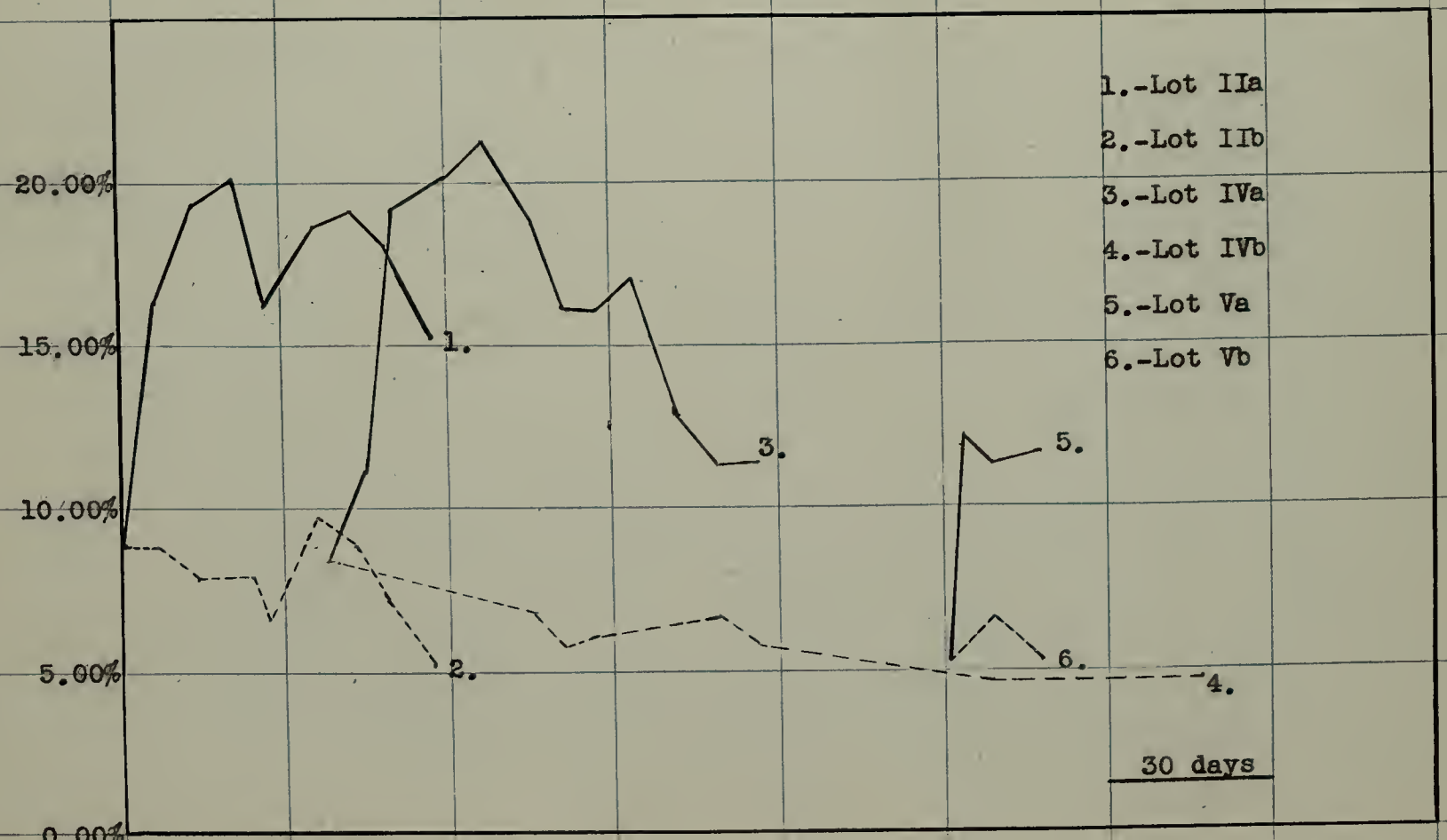


Fig. III. EARLY BLACKS- OXYGEN CONTENT AS AFFECTED BY TEMPERATURE AND TIME IN STORAGE

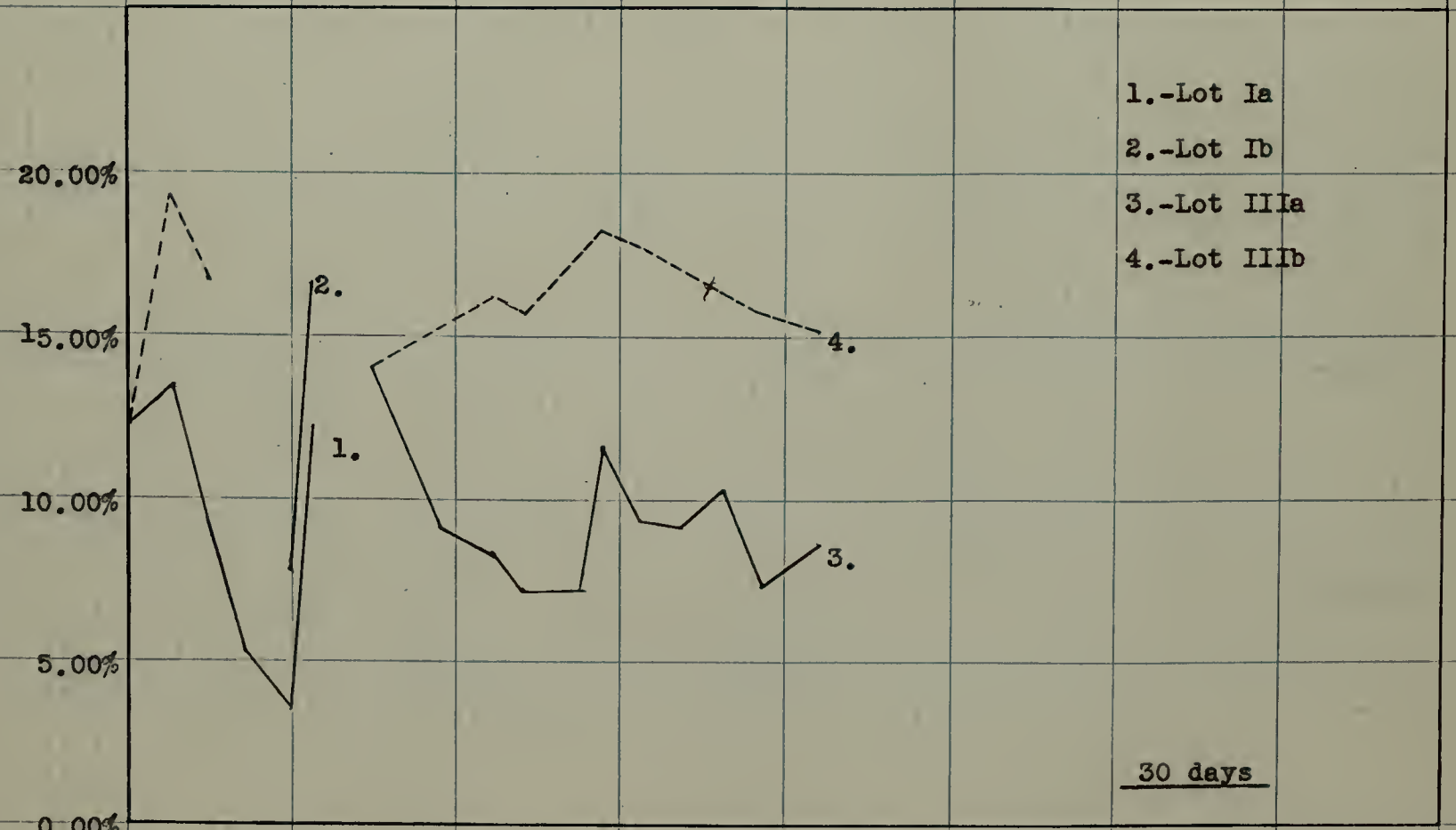


Fig.IV. HOWES-OXYGEN CONTENT AS AFFECTED BY TEMPERATURE AND TIME IN STORAGE

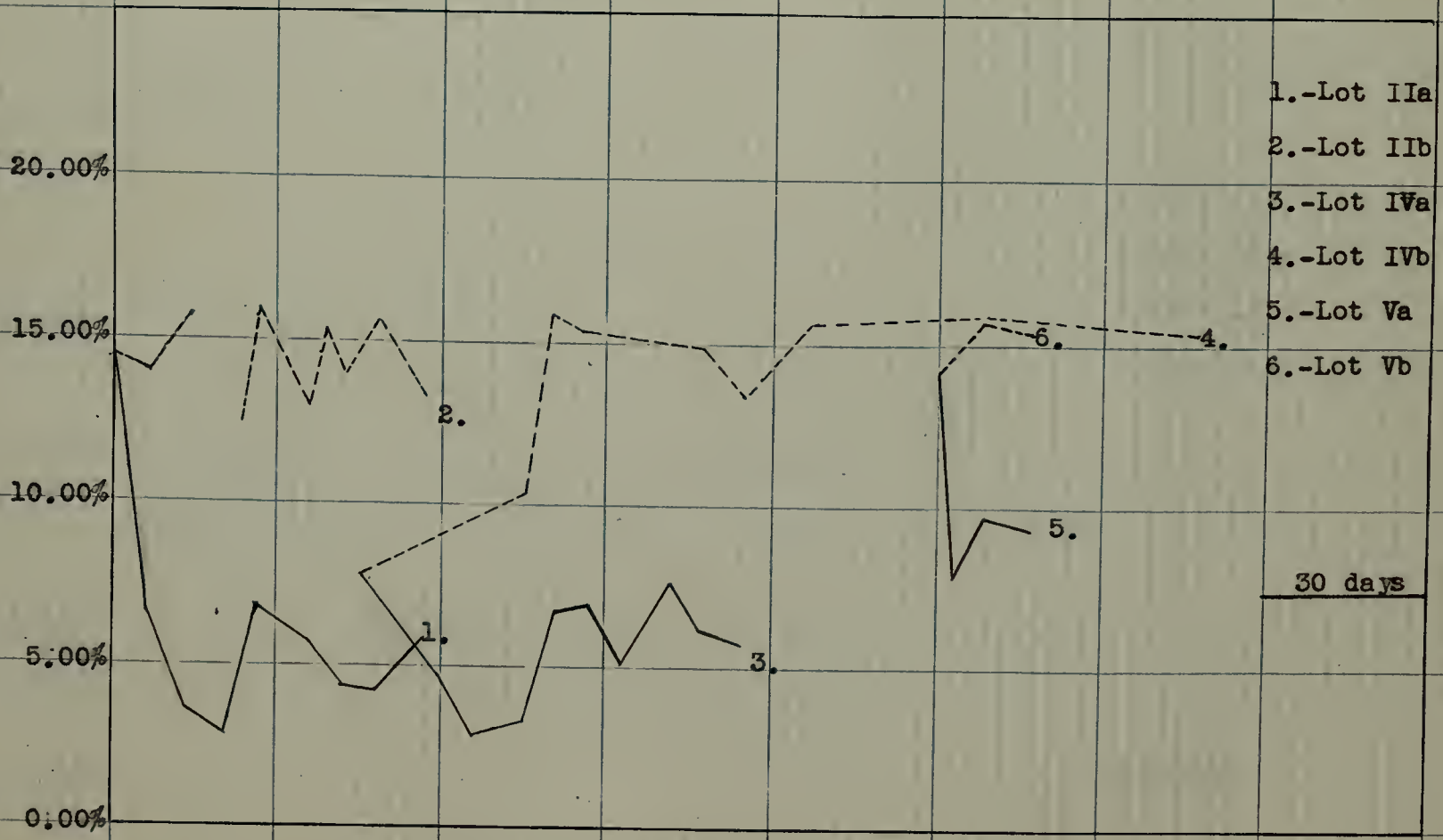


Fig.V. EARLY BLACKS-NITROGEN CONTENT AS AFFECTED BY TEMPERATURE AND TIME IN STORAGE

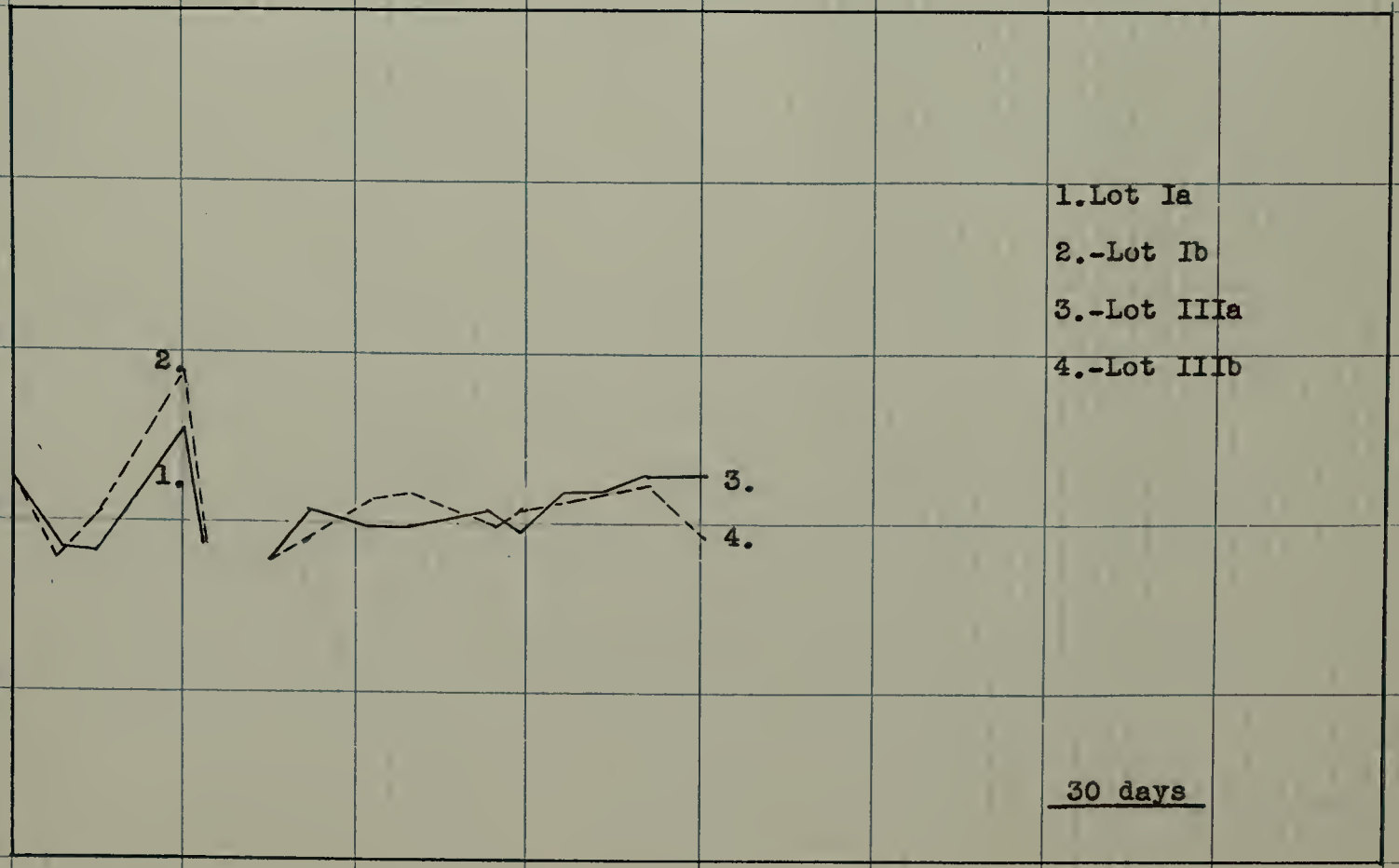


Fig. VI. HOWES-NITROGEN CONTENT AS AFFECTED BY TEMPERATURE AND TIME IN STORAGE

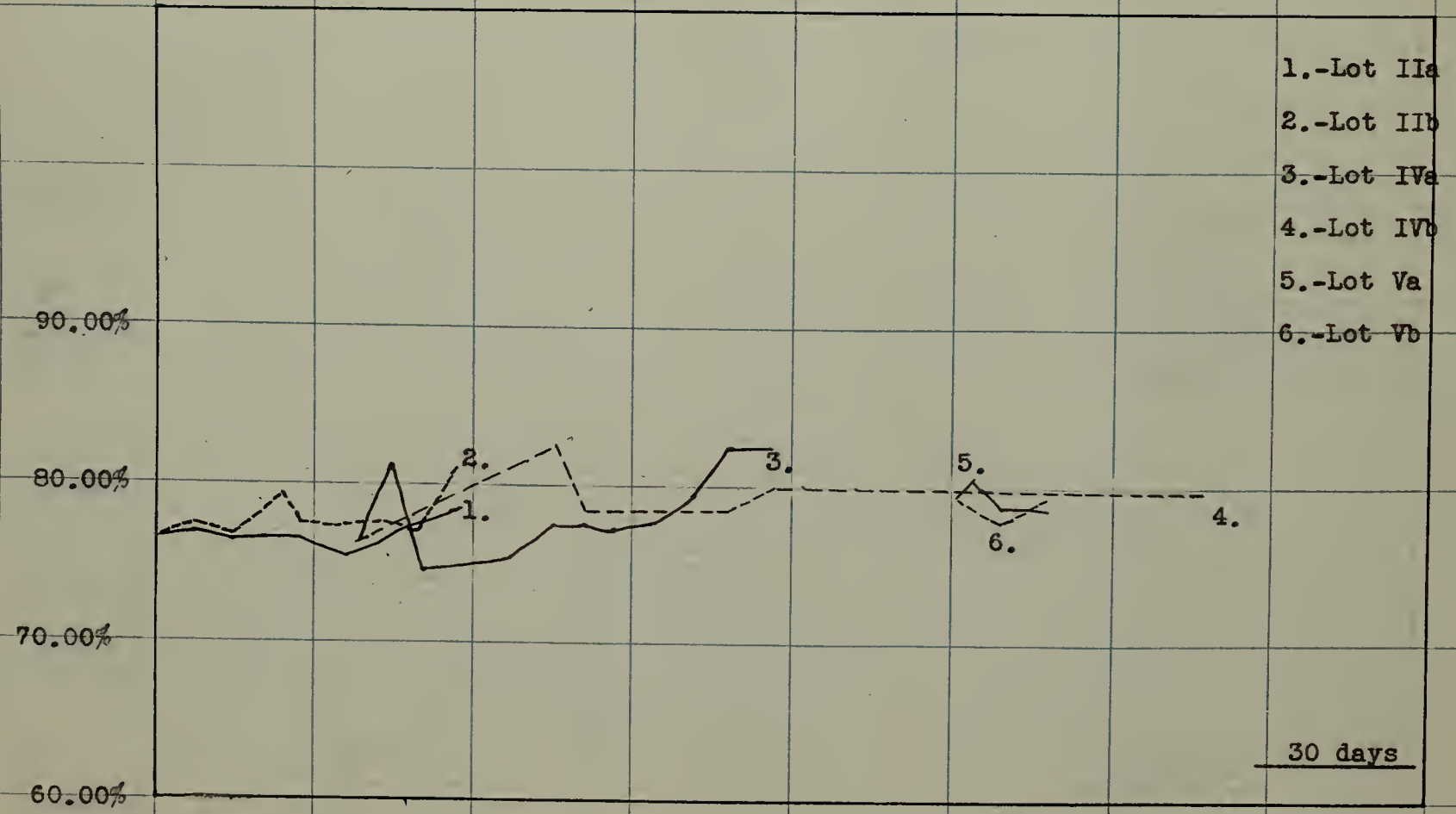


Fig. VII. EARLY BLACKS-CARBON DIOXIDE/OXYGEN RATIO AS AFFECTED BY TEMPERATURE AND TIME IN STORAGE

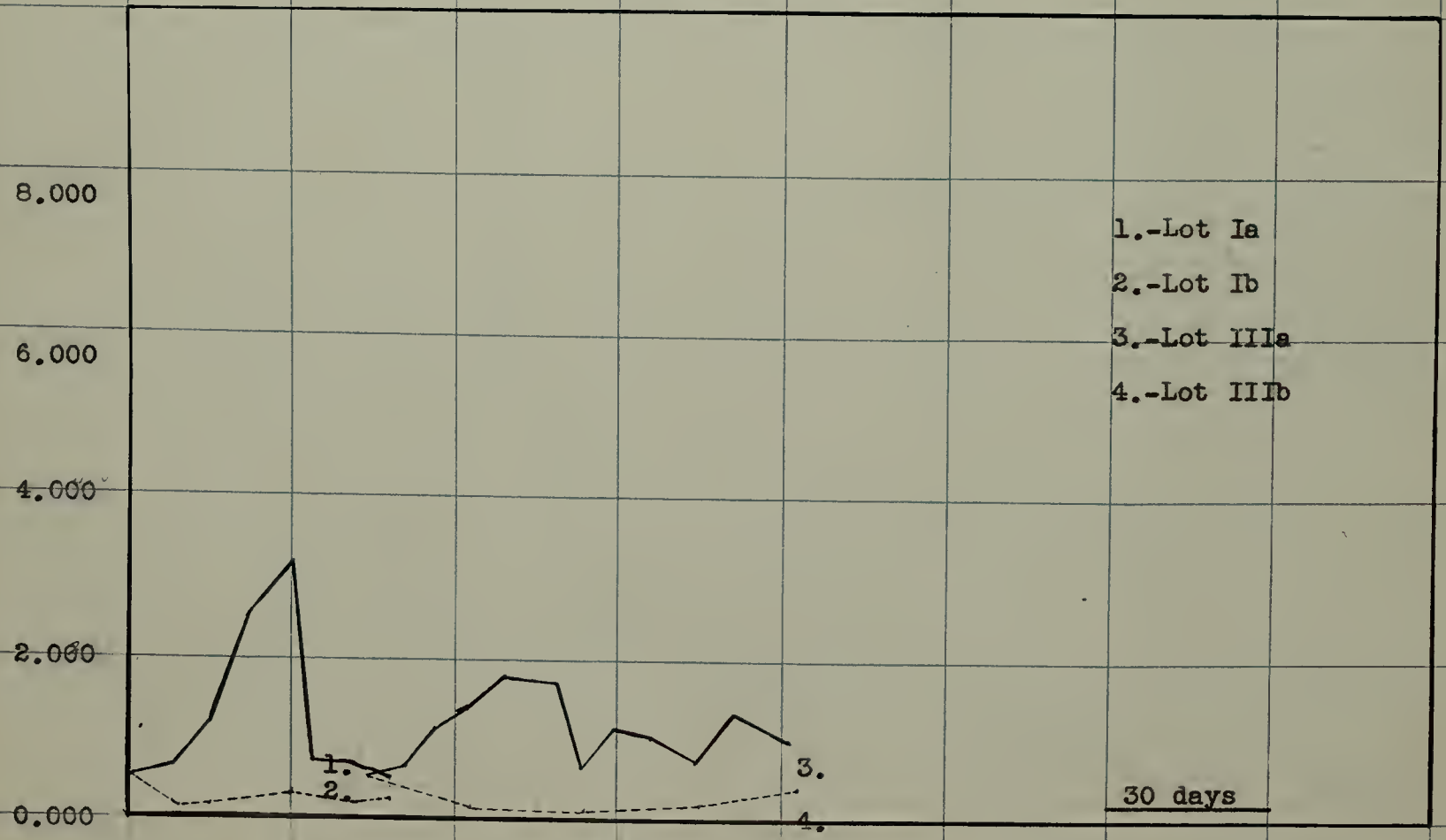


Fig. VIII. HOWES-CARBON DIOXIDE/OXYGEN RATIO AS AFFECTED BY TEMPERATURE AND TIME IN STORAGE

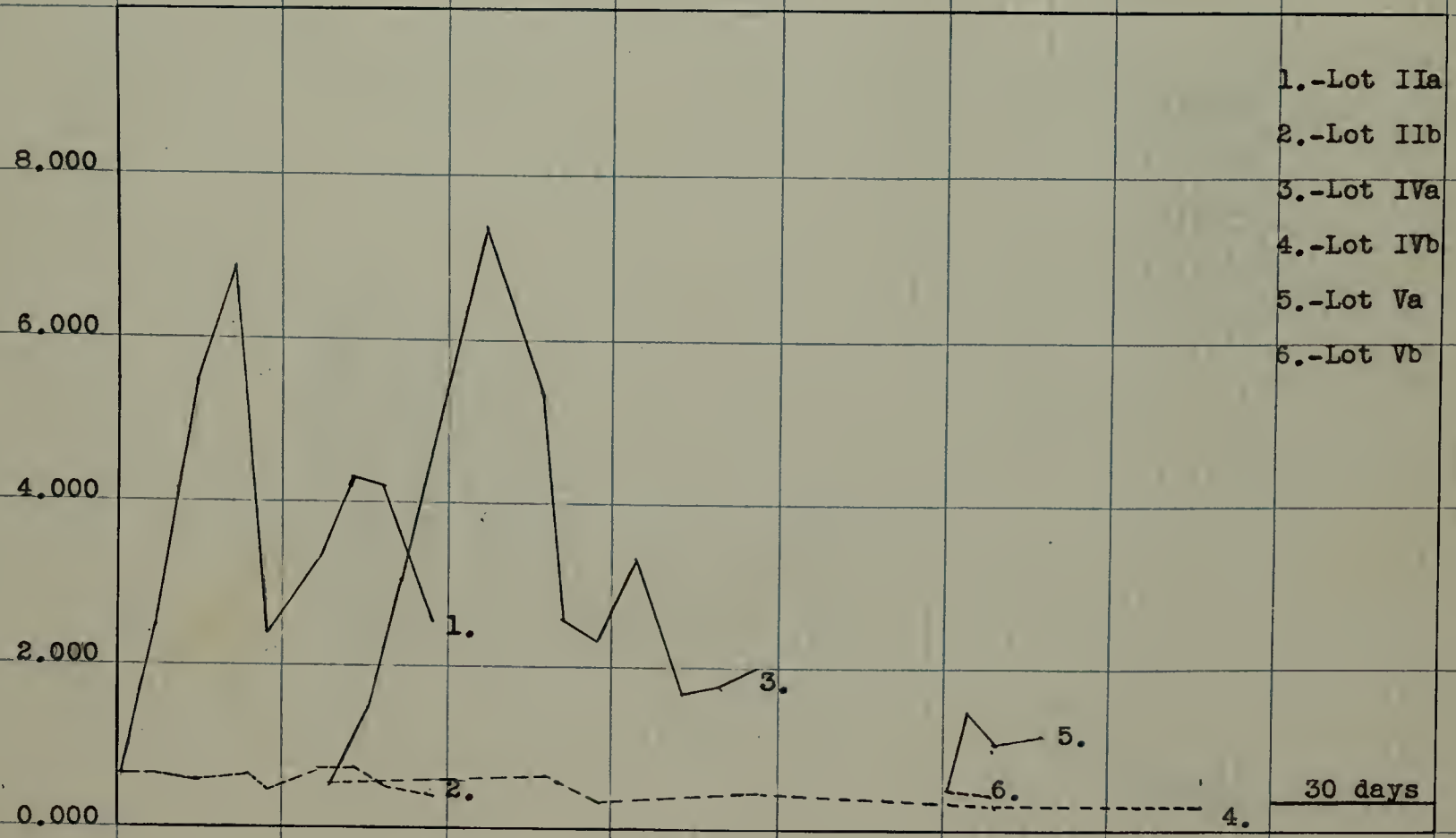


Fig. IX. EARLY BLACKS-CATALASE ACTIVITY AS AFFECTED BY TEMPERATURE AND TIME IN STORAGE

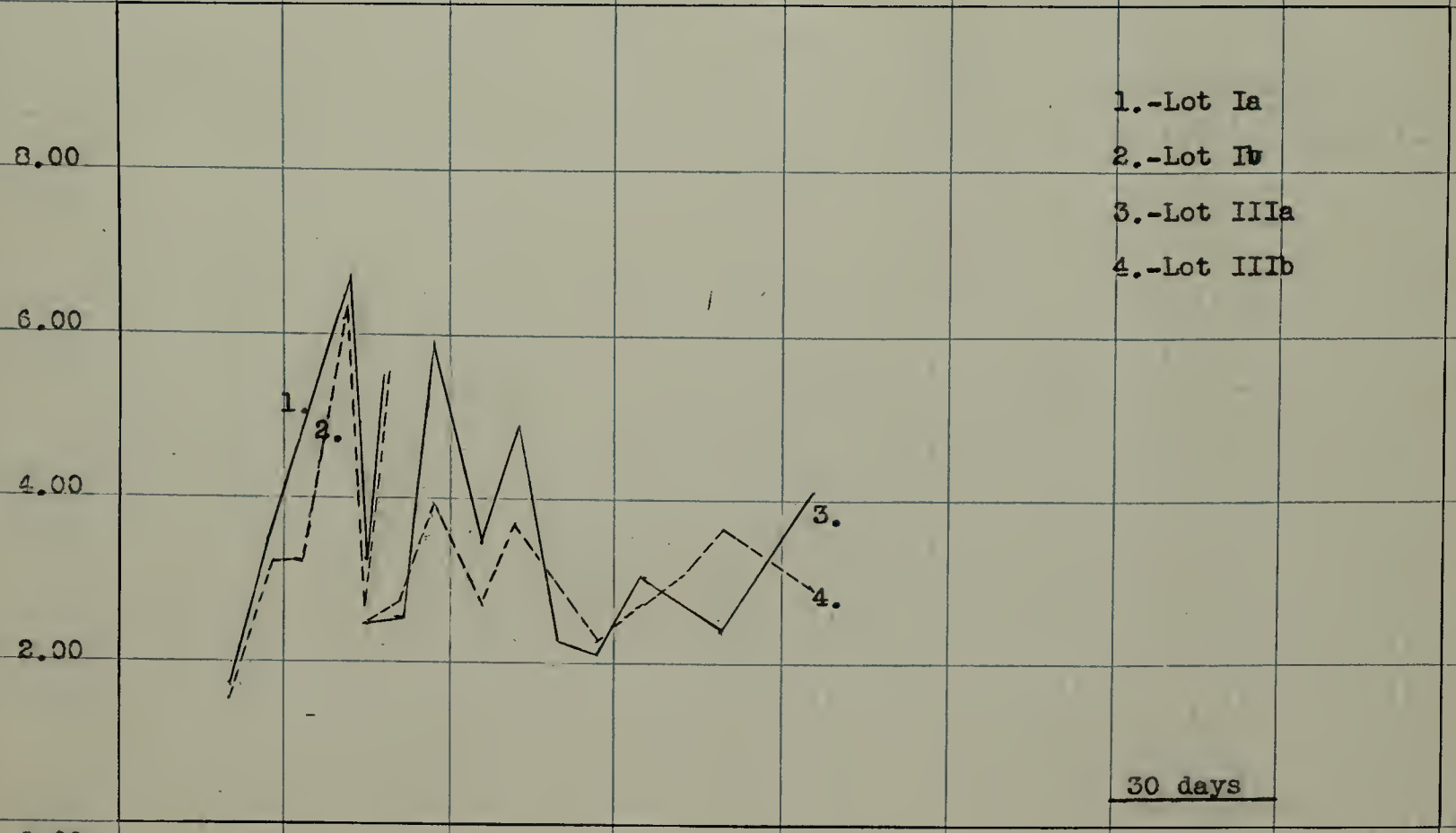


Fig.X. EARLY BLACKS-RATIO OF CATALASE ACTIVITY OF FRUIT STORED AT 24°C. TO THAT STORED AT 3°C.

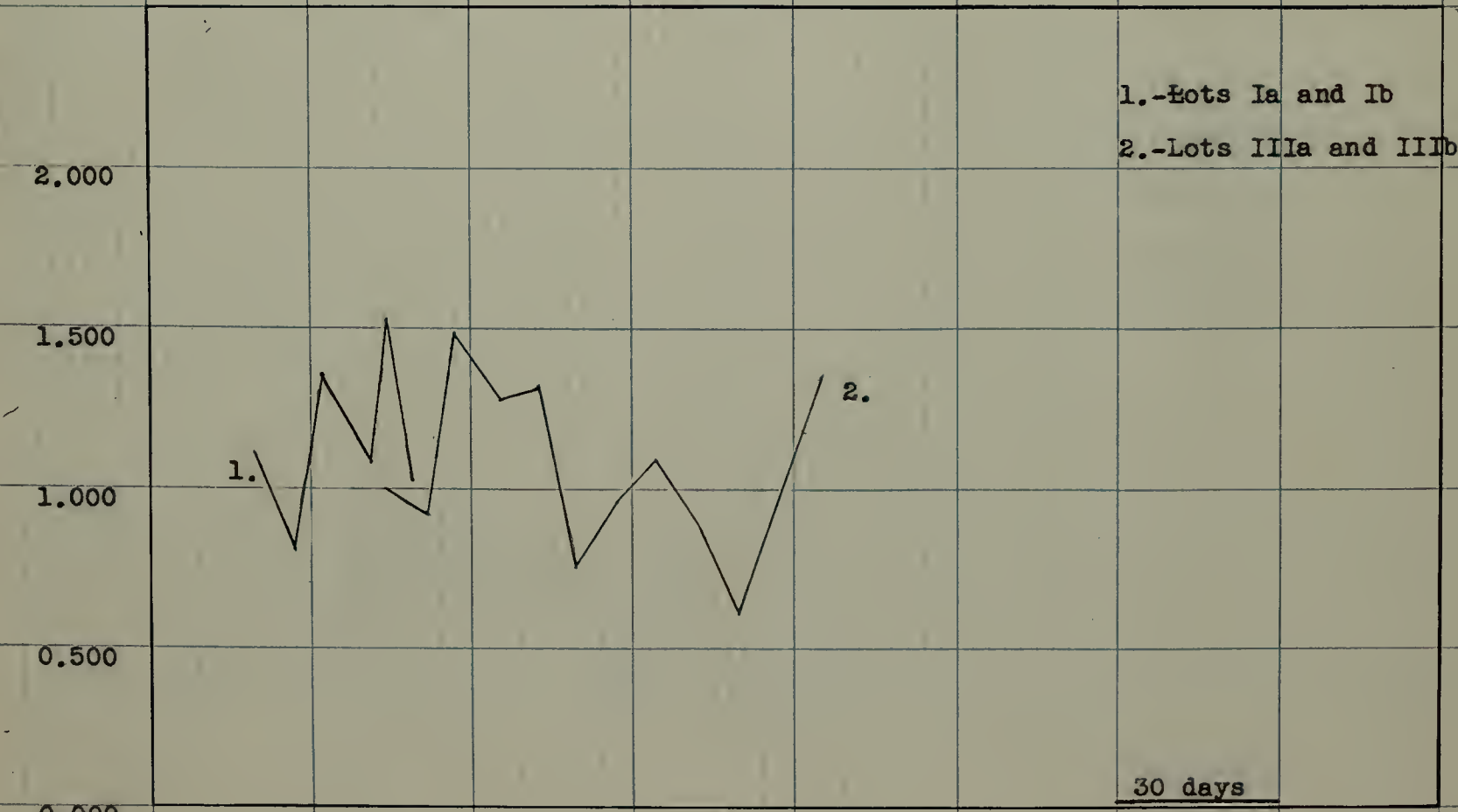


Fig.XI. HOWES-CATALASE ACTIVITY AS AFFECTED BY TEMPERATURE AND TIME IN STORAGE

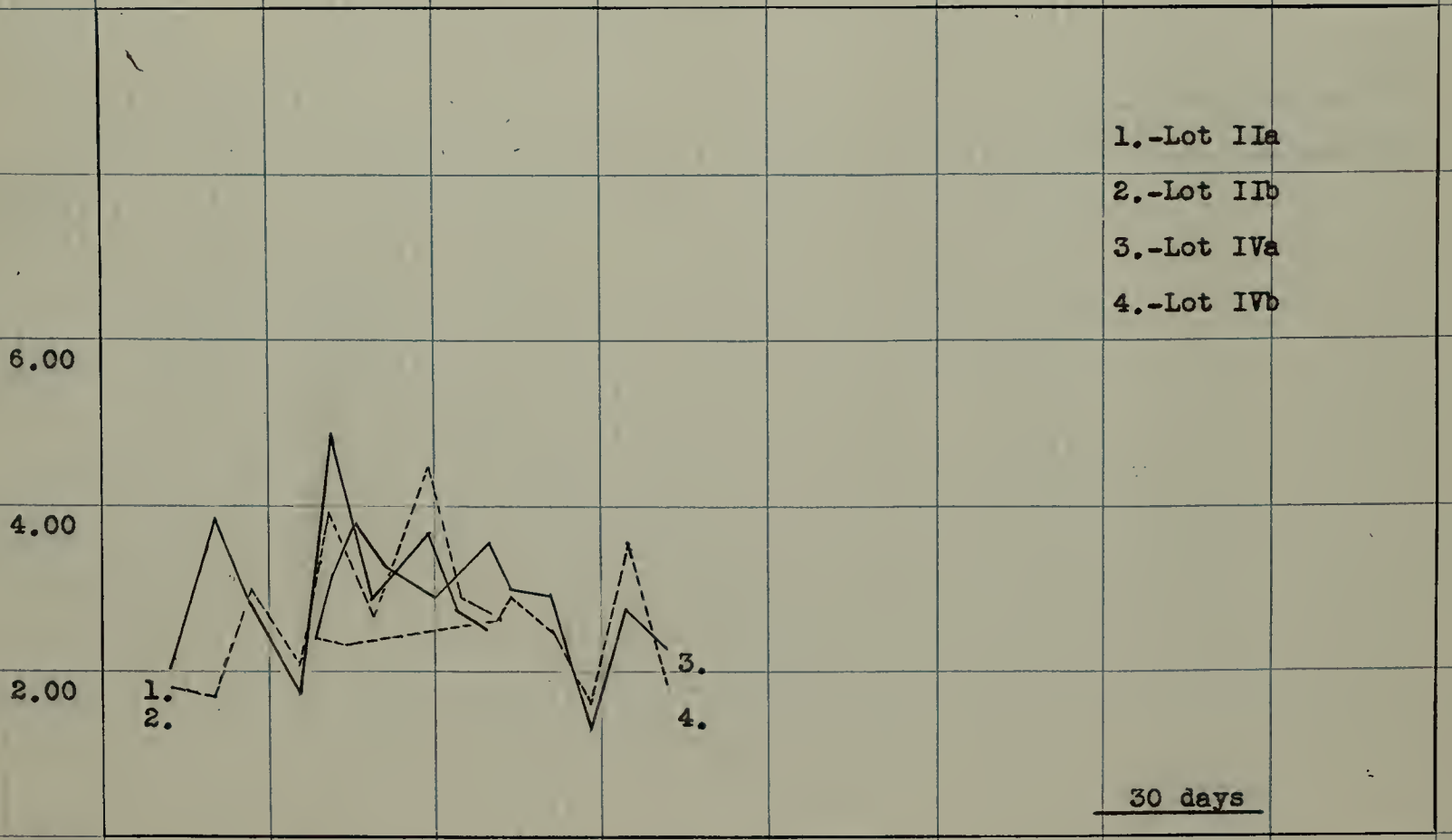


Fig XII. HOWES-RATIO OF CATALASE ACTIVITY OF FRUIT STORED AT 24°C. TO THAT STORED AT 3°C.

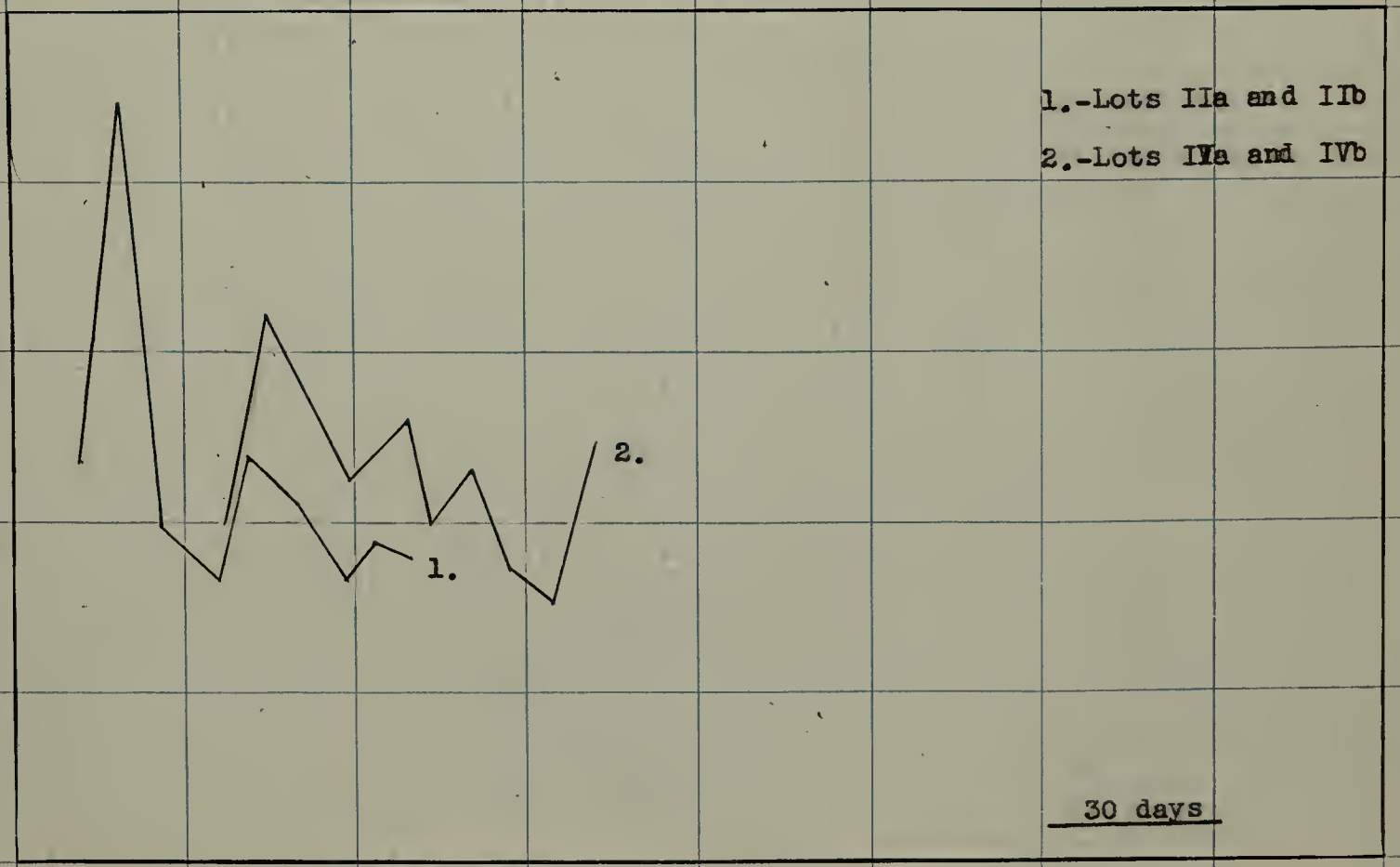


Fig.XIII. EARLY BLACKS-CATALASE ACTIVITY AS DETERMINED BY POTASSIUM PERMANGANATE TITRATION

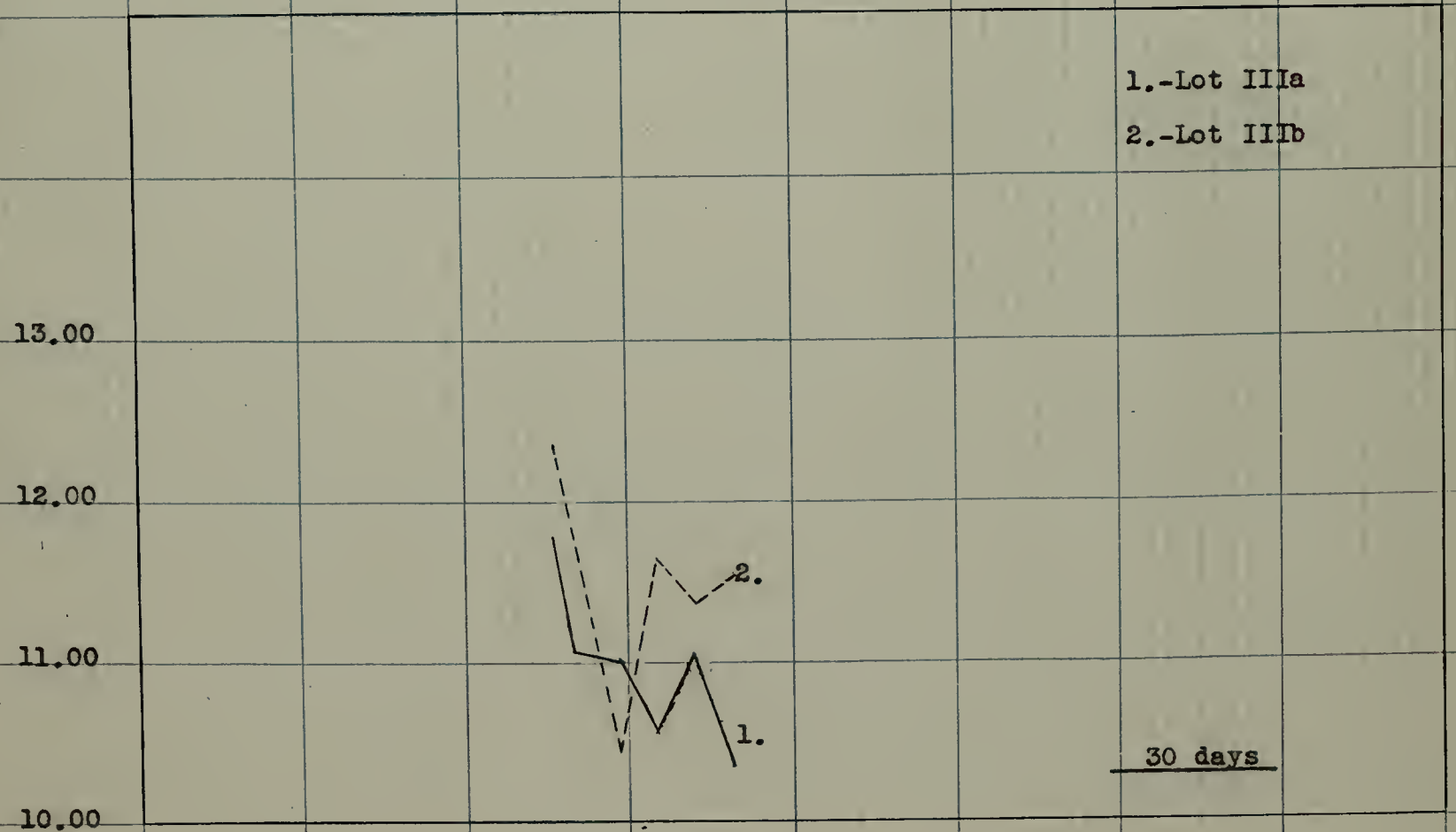
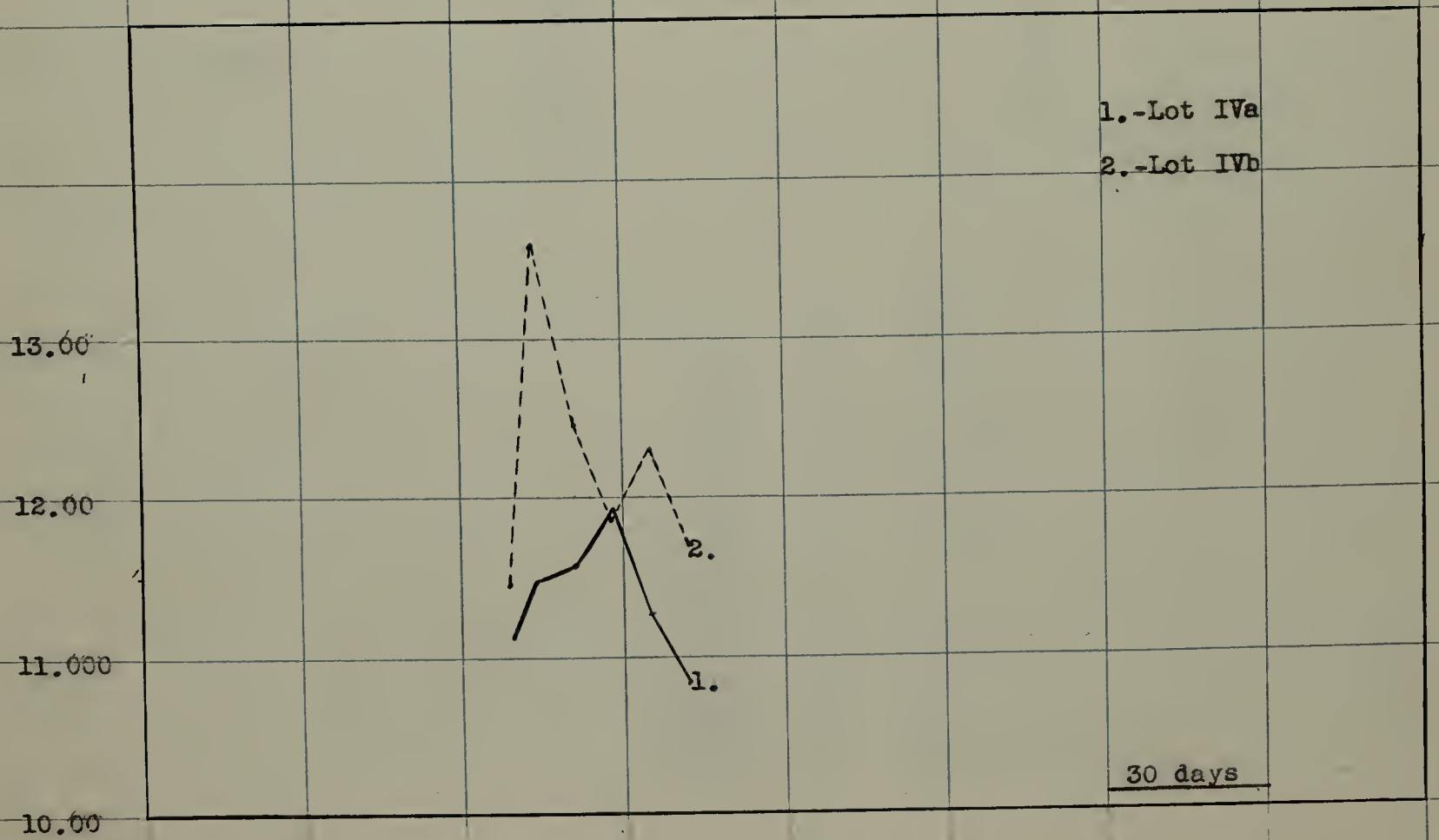


Fig.XIV. HOWES-CATALASE ACTIVITY AS DETERMINED BY POTASSIUM PERMANGANATE TITRATION



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Date May 27, 1935