CONCENTRATION OF REDUCED, OXIDIZED AND TOTAL OXIDIZED ASCORBIC ACID IN VEGETABLES DURING THE FORTY-EIGHT HOURS AFTER HARVESTING

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BY

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TABLE OF CONTENTS

TABLES

PLATES

INTRODUCTION

INTRODUCTION

Ascorbic acid, more commonly known as Vitamin C, has been the object of considerable study since it was discovered to be an important element in human nutrition. The chemistry of this vitamin and its isolation as a pure substance was the first link in the chain of events leading to our present day knowledge of the material. Early recognition of the water solubility of ascorbic acid and its highly labile chemical nature constituted the second step and opened another area of study. The work done on the retention of Vitamin C in the storage, processing and cooking of food should be considered the third step. The latter not only includes the study of the solubility of the vitamin and its possible extraction during cooking but also the effect of heat, storage and any other type of processing to which a food might be subjected in preparation for the table. The fourth subject of interest in the study of ascorbic acid, and one which has recently come into prominence, is related to the various chemical forms in which the vitamin exists. The problem to be presented in this paper is concerned with the retention of Vitamin C in certain vegetables during the forty-eight hours following harvest, and the chemical forms of the vitamin which are present in freshly harvested green vegetables.

A study of the loss of ascorbic acid in vegetables would seemingly be well worth consideration since vegetables and fruits are the principal contributors of this vitamin to the human diet. It has been suggested that a decrease in ascorbic acid values might be indicative of a general decline in the nutritive value of a food product. Kelley (1946) has shown that there is a tendency for a decrease in palatability as ascorbic acid losses progress. Fenton (1940) proposed that ascorbic acid could be considered as the key vitamin in the study of vitamin retention in vegetable foods. This premise has been followed by several other workers in the interpretation of their results.

Ascorbic acid retention in vegetables has been studied quite extensively in the preservation by freezing, canning and dehydration. There also has been considerable research carried on concerning cooking losses but the information on vitamin retention in fresh vegetables held under refrigeration is quite limited and in many cases contradictory. There apparently is need for more study in this area. This paper will be concerned with the extent of ascorbic acid losses in fresh vegetables held under conditions of mechanical refrigeration over a forty-eight hour period. In the study no attempt was made at comparison of temperatures, i.e., room versus refrigeration temperatures, since previous work has demonstrated that losses in both eating quality and vitamin value are slower at refrigeration temperatures.

Before a vegetable reaches the table for consumption there have been many periods where vitamin losses may have occurred other than possible losses during actual processing. If a food is purchased at the market there have been periods of holding since harvesting, which involve time in transportation as well as time in storage at the market. If the vegetable is canned or frozen by either the homemaker or commercial processer, delays due to the absolute bulk of material to undergo processing at the peak of the harvest season may precipitate vitamin losses. Since holding of food products often is unavoidable there should be some effort applied in obtaining information regarding the best possible conditions for vitamin retention during such periods.

As before mentioned, there is an increasing interest in the chemical forms in which ascorbic acid exists in food materials. It is known that a given vegetable tissue may contain either the reduced or dehydro (oxidized) form or a mixture of both. The tissues of a young fresh vegetable contain a small proportion of the oxidized ascorbic acid. Older, more mature vegetables, and those subjected to storage or processing frequently have lower reduced ascorbic acid values than younger plants but it is possible that there has been no actual decreases in the vitamin but only a conversion to the oxidized form. There is little information in the literature concerning the distribution of the two forms of the vitamin except that obtained under conditions where the food extract has been exposed to an oxidizing agent and the vitamin determined as total oxidized ascorbic acid. The reduced form may

previously have been measured and the dehydro form found by difference. It is questionable whether such values for dehydroascorbic acid are reliable.

Most of the studies of ascorbic acid have measured the reduced form of the vitamin and the decrease in values occuring over a period of time, expressed as losses. If conversion to the oxidized form takes place on storage it is not logical to say that the vitamin is lost, since dehydroascorbic acid has been proved to be biologically active.

It has been suggested that the time and effort spent in determining both forms of the vitamin in a fresh food is unnecessary since by far the greatest proportion of the vitamin exists in the reduced form. A study of the literature reveals no work which has checked this assumption on various foods. Not only is this information of interest to the research person from the nutritional standpoint but it also is of practical value. The method by which total dehydroascorbic acid is determined is more expensive of both time and materials than is the method for determining reduced ascorbic acid. Therefore, information concerning the measurement of reduced ascorbic acid as a measure of total biological value is necessary.

In making this study the author had two points in mind. First, to determine the ratio or proportion of the chemical forms of ascorbic acid present in the fresh product and second, to trace any change in this ratio during the storage period.

Such information once obtained could be treated to prove or disprove the following facts: (1) that it is logical to assume the difference between total oxidized and reduced ascorbic acid values as the amount of the oxidized form present in the plant tissue, (2) that so called losses as determined by measuring the reduced form are losses and not conversion to the oxidized form, (3) that it is safe to assume that a measurement of the reduced form gives a true picture of the antiscorbutic substance available in a fresh food.

LITERATURE CITED

History of The Discovery of Ascorbic Acid and The Methods of Its Analysis

The metabolic disfunction commonly known as scurvy for which ascorbic acid (Vitamin C) is a specific cure has been recognized since the time of the Crusades. It was prevalent in Europe during the fifteenth and sixteenth centuries. All explorers and sea faring men of that period were well acquainted with the disease as it appeared frequently in their crews. During this ara several extracts of plant tissue were found which had the ability to cure scurvy but it wasn't until 1841 that Budd postulated that certain foods owed their remedial powers to some definite substance contained in them. In 1932 King isolated and identified this substance chemically, and his work was quickly confirmed by several others, Harris and Ray (1933 b), and Birch, Harris and Ray (1933). A process for synthesizing the vitamin followed shortly afterward.

In 1922, Sherman, La Mer and Campbell published the description of a biological method for determining the Vitamin C content of food materials quantitatively. Such biological work demanded the use of the guinea pig as the experimental animal since rats are able to synthesize the vitamin. The work was slow and costly but it remained as the only method of assay until some of the chemistry of the vitamin was clarified.

Clark and co-workers (Cohen, Gibbs and Clark, 1924) were the first to report that certain fruit juice and animal tissues were able to decolorize a redox dye of the indophenol class (the 2, 6-dibromo-derivative).

Szent-Gyorgyi (1926) demonstrated the existence in various vegetable and animal tissues of a naturally occurring reducing substance which he succeeded in isolating and named hexuronic acid. He pointed out that this substance seemed to be identical with a reducing substance known to occur in active vitamin C concentrates. He did not produce proof for this hypothesis, perhaps because he was influenced by Zilva (1932, a, b, c) who was opposed to the idea that hexuronic acid could be identified with the vitamin.

Tillmans and co-workers (1928) had noted that natural foods reduced the indophenol dye while artificial foods did not. The same workers in 1932 noted that titration values varied directly with the antiscorbutic activity of foods or vitamin C preparations. They concluded that the reducing substance was the carrier of vitamin C activity and later (1932 a) suggested that Szent-Gyorgyi's hexuronic acid was identical to vitamin C.

Svirbely and Szent-Gyorgyi (1932 b) recorded that hexuronic acid protected guinea pigs from scurvy. Simultaneously, King and Waugh, (1932) announced that they had isolated vitamin C and that it had the same properties as hexuronic acid. Harris and Ray (1932, 1933) and Birch, Harris and Ray (1933) reported experiments which definitely supported the theory of the identity of hexuronic acid and vitamin C.

Tillmans (1932)used a titration method to test the reduction of the indophenol dye on the addition of a food containing ascorbic acid. He observed that the oxidation of

ascorbic acid with hydrogen peroxide or with dichlorophenol-indophenol could be reversed with hydrogen sulphide.

Bessey (1933) worked with the iodine titration and found that the results of this titration on any given food extract checked well with the amount of vitamin found by titration with 2, 6-dichlorophenolindophenol and previous biological studies. He pointed out the presence of interfering substances which also reduced the indophenol dye, and considered the values obtained by titration valuable only when properly interpreted. He also noted that the titration method measured only the reduced form of the vitamin which could account for some of the discrepancies between chemical and biological values.

Karrer (1933) reported on the structure of dehydroascorbic acid (the reversibly oxidized form of the vitamin). Van Eckelen (1935) pointed out the formation of the oxidized form of the vitamin and the fact that titration with indophenol did not necessarily represent the amount of biologically available vitamin present in a food.

Mindlin and Butler (1937-38) developed a method based on principles discussed by Rosen and Evelyn (1937) for measuring the reduction of the indophenol dye with a photelometer. This method eliminated the subjective element in the reading of an end point.

Bessey (1938) offered a modified method to allow for the determination of either reduced or oxidized ascorbic acid in

turbid and colored solutions. He determined the concentration of the vitamin present in a given material before and after treatment with hydrogen sulphide. The difference between the two values was considered to be the amount of dehydroascorbic acid present in those tissues. In 1941, Morell presented a modification of the Mindlin and Butler method. King (1941) pointed out that an error was introduced in the use of hydrogen sulphide for measuring total ascorbic acid from the presence of ketones, aldehydes and other interfering substances in food. Carruthers (1942) added mercuric chloride to extracts to inhibit these interfering substances. Harris and Oliver (1942) were able to demonstrate good agreement between the concentration of vitamin found by indophenol titration and biological assays. They claimed that fresh fruit and vegetables had too little dehydroascorbic acid to be worth determining and if that form of the vitamin were present to any extent, it was due to failure to prevent oxidation during the process of analysis.

Loeffler and Ponting (1942) further modified the method for the determination of reduced ascorbic acid. They worked with turbid and colored solutions and found that, in moderate amounts, color and turbidity did not interfere with the determination since a photoelectric instrument could be calibrated with proper blanks.

Stotz and Pepkowitz (1943) reported another study of the interfering substances which complicate the method for the

determination of reduced ascorbic acid. In 1945, Robinson and Stotz claimed to have increased the specificity of the indophenol method by the extraction of the excess indophenol dye with xylene after reaction with the ascorbic acid, and measurement of the color in this solvent in a photelometer.

The papers mentioned thus far have all been based on the measurement of vitamin concentration by the reduction of an indophenol dye. Other workers were interested in finding methods of analysis based on some other factor and two types of determinations have resulted. Becker and DiGleria (1937) and Kirk and Tressler (1939) described methods of measuring vitamin C potentiometrically. These workers had great difficulty with determining the end points by this method, but Harris, Mapson and Wang (1942) produced a method involving the use of a special mercury platinum double electrode which has given good results. The methods whereby the oxidation-reduction potential is measured are not commonly used but they may have value and are worth mentioning.

In 1936, Roe reported a determination based on the formation of furfurals. He could separate the oxidized and reduced forms of the vitamin by boiling them with hydrochloric acid. The oxidized form did not yield furfural in the process. This was the first direct method for the determination of oxidized ascorbic acid. From that year until 1943, little work is reported on the determination of oxidized ascorbic acid either as it occurs spontaneously in plant tissue or after the tissue

extract has been treated to oxidize all of the reduced ascorbic acid present. Part of the lack of work in this direction may have been due to doubt concerning the biological value of the oxidized form of the vitamin, although Fox and Levy (1936) had confirmed the antiscorbutic activity of dehydroascorbic acid in guinea pigs.

To avoid confusion in the naming of the chemical forms of ascorbic acid, it might be well to define the forms which will be used in the remainder of the paper. The terms dehydroascorbic acid or oxidized ascorbic acid apply to the oxidized form of the vitamin as it occurs naturally in a given product. The terms total dehydro or total oxidized ascorbic acid refer to the vitamin present in a material after treatment with an oxidizing agent. The total vitamin content can then be determined as oxidized ascorbic acid.

Hochberg and Melnick (1943) stressed the necessity of determining both the reduced and dehydro form of the vitamin. Gould (1943) presented a new method for the bioassay of dehydroascorbic acid. Penney (1943)studied the chemical behavior of dehydro-l-ascorbic acid in vitro and in vivo. Fearon (1943) suggested the determination of dehydroascorbic acid by a coupling reaction with 2, 4-dinitrophenylhydrazine which, when treated with sulfuric acid produced a color measurable in a photoelectric colorimeter. This method was proposed for work with animal fluids and tissues.

In 1944, Roe developed the method for the determination of dehydroascorbic acid in plant tissues with the dye as mentioned above. By the former method, the dehydro form could be determined in the presence of the reduced form and, by the addition of treatment with acid-washed norite the reduced material was oxidized and total ascorbic acid could be measured.

The preceding pages covering the development of methods for ascorbic acid analysis are not, of necessity, complete, However, they do include the principal steps in the development of the methods which have been in common use.

Discussion of Methods of Ascorbic Acid Analysis

The problems which have appeared in the development of methods for ascorbic acid analysis are not definitely settled at the present time. The original indophenol titration developed by Tillmans (1932) and modified by various other workers involved the titration of the unknown extract with an accurately standardized dye solution. This method measured only the reduced form of the vitamin although Tillmans did note that the dehydro form could be obtained by treatment of the vegetable extract with hydrogen sulphide. Besides the initial difficulty in the subjective determination of end points, the problem was further complicated by the presence of interfering substances which also caused the dye to fade. Also, there was no way of correcting for turbid and colored solutions.

The later methods for determining reduced ascorbic acid involved the same principles as did the titration methods but a photoelectric colorimeter was used. The use of this instrument eliminated subjective color readings and could correct for moderate amounts of color and turbidity in solutions by the use of appropriate blanks. The problem of interfering substances still arises. The use of a 15 second reading period, when testing for the reduction of the indophenol dye by ascorbic acid, was adopted because ascorbic acid completes its reaction with the dye in 15 seconds. Mindlin and Butler (1937-1938) point out that any subsequent reduction of the dye is due to the presence of certain interfering substances.

A recent piece of work reported by McMillan and Todhunter (1946), in which they carried out determinations on cabbage using the reduced method as developed by Loeffler and Ponting (1942) and the dehydro total methods of Roe (1944) have shown good agreement with Roe's work.

However, Pijoan and Gerjovitch (1946) have questioned the use of Roe's method for total dehydroascorbic acid in some instances. They found that a sample of orange juice which had been aerated for ten days, sufficient to oxidize most of the ascorbic acid present, gave values of sixty to seventy milligrams per 100 milliliters as determined by the total dehydroascorbic acid method developed by Roe (1944). Upon feeding the orange juice to guinea pigs, the animals developed scurvy. With the hydrogen sulphide method, the authors found only 4 milligrams of ascorbic acid per 100 grams. They suggested that the phenylhydrazine reaction, which is the basis of Roe's (1944) method for the determination of total dehydroascorbic acid, is not specific for dehydroascorbic acid. They note that mutarotation may occur in the ascorbic acid with a subsequent loss of the lactone structure and the formation of diketogulonic acid. Penney and Zilva (1943) used 2, 4-dinitrophenylhydrazine for the determination of diketogulonic acid. Pijoan (1946) concludes that the 2, 4-dinitrophenylhydrazine method thus far appears to be valid for certain fresh biological materials

but strongly questions the advisability of its use for materials where "unpredictable antecedant oxidation of the vitamin" may have occurred.

Research workers are at this point in their studies of ascorbic acid methods and it is obvious to most of them that there is need for more work in the field of methods before results are entirely satisfactory.

Development of an Extractant for the Determination of Ascorbic Acid

According to Bessey (1933) it was necessary to carry out the analysis of ascorbic acid in some extraction medium, since the amount of ascorbic acid present in any given type of tissue would be too minute to facilitate direct work upon the vitamin. That some type of acid would be chosen as an extracting medium followed, since there was a need to prevent oxidation during extraction.

The extraction process used by early workers involved the grinding of tissues to be analyzed in a mortar in the presence of acid and acid-washed sand. Grinding the tissues ruptured cell walls and insured a more complete extraction of ascorbic acid. To insure complete extraction the tissues were subjected to several periods of grinding and washing with acid.

Tillmans (1932), Bessey (1933) and Birch, Harris and Ray (1933) advocated the use of trichloroacetic acid for extraction purposes. Bessey (1933) noted that this reagent caused a slow fading of the indophenol dye and that allowance for that effect must be made in all titrations. He suggested the use of hot acetic acid when extracting vegetable tissue, reserving the trichloroacetic acid for use with animal tissue. He found that acetic acid did not react with the indophenol dye and that he was able to obtain a sharper end point in titration.

Fujita and Iwatake (1935) suggested the use of metaphosphoric acid in a two percent solution for the extraction of vitamin C from tissues. However, they presented no experimental evidence of its superiority over that of other acids. Musulin and King (1936), basing their work on the suggestion of Fujita and Iwatake (1935), were able to prove that metaphosphoric acid was a satisfactory acid for extraction purposes. They were able to show that trichloroacetic acid, in a four to eight per cent solution, disrupted the cellular structure in some tissues more rapidly than in others. However, they found that in the presence of trichloroacetic acid alone, the vitamin might be oxidized at an appreciable rate thus introducing errors. Metaphosphoric acid (1-5%) exerted a protective effect against both atmospheric and trichloroacetic acid oxidation. Metaphosphoric acid showed no effect on the reaction of the dye. These workers recommended a mixture of trichloroacetic and metaphosphoric acid as an extractant.

Mack and Kertesz (1936) also found metaphosphoric acid to be a good stabilizing agent. Though Fujita and Iwatake (1935) had suggested metaphosphoric acid for extraction of the vitamin, Mack and Kertesz found that better results were obtained if the metaphosphoric acid was combined with other acids suitable for extraction. In their work a two per cent solution of the acid gave satisfactory results.

Lyman, Shultz and King (1937) were able to demonstrate the protective action of metaphosphoric acid. They found that a three per cent solution gave complete extraction and adequate protection from oxidation.

Mack and Tressler (1937) suggested the use of a strongly ionized acid to prevent interfering materials from reaction with the indophenol dye. They found that a combination of sulfuric acid and metaphosphoric acid gave reliable results.

Morell (1941) introduced the use of the Waring Blendor, replacing the grinding of tissues in a mortar. He used three per cent metaphosphoric acid which he buffered to a pH of 3.6.

Reid (1942) pointed out that the final concentration of metaphosphoric acid in the extracting solution was not the most important factor in the extraction process. She felt that the concentration during the process of grinding should have prime consideration. She advocated the use of a five to eight per cent solution for early stages of extraction and a two per cent solution for washing and making up to volume.

Loeffler and Ponting (1942) pointed out that workers using photoelectric methods for the determination of ascorbic acid have noted the fading of the dye in strong acid solution and buffered their extracts to a pH of 3.6 before testing. Keuther and Roe (1941) suggested that the final pH after addition of the dye should be no higher than 3.0 since at higher values the effect of interfering substances was more pronounced. Loeffler and Ponting (1942) found that buffering could be eliminated if one per cent metaphosphoric acid were used in a large proportion. They recommended a seven to one ratio between extractant and solids. Such a large proportion of extractant yielded a pH ranging between 2.4 and 3.1.

Ponting (1943) in studying various acids for the extraction of ascorbic acid states that the problem is to obtain an extractant which is high enough in acid concentration to protect against oxidation and not high enough to cause bleaching of the dye and subsequent drifting of the galvanometer. He experimented with thirteen different acids and found that oxalic and metaphosphoric acids were the only ones with the reaction qualities required. Either one is satisfactory in the laboratory and they may be substituted for each other. He recommended a concentration of 0.3 per cent when using the oxalic acid and 1.0 per cent when using metaphosphoric acid.

Roe (1936) used 1 per cent oxalic acid for extracting plant tissues. For animal tissues he used a 5 per cent sulfasalicylic acid in a 10 percent acetic acid solution. The same author in 1943 recommended the use of 6 per cent trichloroacetic acid for the analysis of total ascorbic acid in blood and urine. The method which he published in 1944 for the determination of dehydroascorbic acid made use of a 5 per cent metaphosphoric acid solution which contained 1 per cent thiourea. The thiourea was added to stablize the ascorbic acid during extraction and subsequent treatment.

In adapting the 1943 method for use with plant tissue, Roe (1944) suggested the use of a solution containing 5 per cent metaphosphoric acid and 10 per cent acetic acid. The high concentration of acetic acid was present to counteract

the adsorption of the vitamin on the norit used in the determination. An equally high concentration of trichloroacetic acid had a tendency to crystallize when sulfuric acid was added later in the process.

The History of Studies Concerning Ascorbic Acid Retention in Vegetables Stored Under Refrigeration

Early workers in the field of ascorbic acid retention were primarily concerned with losses during cooking processes since they knew the degree of solubility of the vitamin in water. As the understanding of the chemical nature of this vitamin grew, the work rapidly expanded to include both a wide sampling of fruits and vegetables and many processing methods. Ascorbic acid retention during cooking and various forms of preservation has been studied extensively and still includes a large bulk of present day research. The National Cooperative Group studying the Conservation of the Nutritive Value of Foods has made particular contributions in this area. Their studies and those of numerous others such as those carried on by Tressler, Mack and King (1936), Mack, Tapley and King (1939), Harris, Wismann and Greelie (1940), Hummel (1942), McIntosh (1942), Porter, Schlaphoff, Wharton, Briant and Baltz (1944), Platinius and Caldwell (1945), Morgan, MacKinney and Cailleau (1945) and Kelley, Jackson, Sheehan and Ohlson (1946) have shown that the final concentration of ascorbic acid in the product which reaches the consumer varies considerably with the type of treatment to which the vegetables have been subjected. All of the studies of this type are too numerous to mention but they all point to the same trends in ascorbic acid loss, namely: increasing losses as the degree of processing increases.

There has been a tendency to overlook the period between harvesting and actual processing. However, within the last few years there has been an increasing amount of interest in the change in vitamin value of vegetables from the time of harvest to the time of consumption or actual processing for preservation. This period involves some type of storage. One could list many possible variables in storage conditions such as humidity, type of atmosphere, etc., but the variable of prime consideration appears to be temperature. Although the studies on this factor are not numerous some work has been done. The earliest work on storage had to do with the keeping qualities of winter vegetables such as cabbage, turnips and parsnips, Wheeler, Tressler and King (1939). It is only recently that there has been more emphasis on the short periods of storage for more perishable products.

Tressler, Mack and King (1936) found no loss of ascorbic acid in freshly picked spinach after three days storage at 1-3° C (33.8-37.4° F.). Olliver (1936) reported rapid loss of vitamin C from some freshly picked vegetables stored at room temperature and slower losses during storage at 32° F. At room temperature, spinach lost 78 per cent of its original vitamin C content in two days and asparagus tips 80 per cent in 4 days. Mack, Tressler and King (1936) stored peas in pods for 6 days after harvesting; they observed practically no loss of ascorbic acid at 1° C. and at 9° C., but at 18° C. and 22° C. there were considerable losses.

Feener, Palmer and Fitzgerald (1937) found little change in the ascorbic acid content of fresh market spinach when it was stored at 1-3° C. (33.6-37.4° F.). After 12 hours at 21-22° C. (69.8-71.6° F.) it had lost 50 to 59 per cent of its original ascorbic acid. Yaroshenko (1938) reported 30 to 50 per cent loss of vitamin C in spinach stored at room temperature for two days, while storage of the same vegetable at 2° C. (35.5° F.) reduced the loss to 10 to 17 per cent.

Fitzgerald and Fellers (1938) noted a 20 per cent loss of ascorbic acid from fresh market asparagus stored at 21.1° C. (70° F.) for 24 hours. They found that market spinach lost 43 per cent of its vitamin C value when stored at 21.1° C. (70° F.) for 24 hours and 47 per cent after 48 hours. Mack, Tapley and King (1939) reported that string beans lost vitamin C rapidly at all temperatures. In refrigerated samples these losses ranged from 11 to 45 milligrams per 100 grams in different varieties after one days storage, and 50 to 77 milligrams per 100 grams after 6 days. The vegetables they used were grown nearby and were analyzed shortly after being harvested. Wheeler, Tressler and King (1939) studied the vitamin C content and retention in various freshly harvested green leafy vegetables. They found that the loss of ascorbic acid in broccoli, cauliflower, and endive during storage at temperatures slightly above freezing, was very slow over a period of two weeks. The loss in these vegetables was very

marked when they were held four days at room temperature. Lettuce lost ascorbic acid much more rapidly than any of the other vegetables even at the lower temperatures.

Harris, Wismann and Greelie (1940) studied the effect of relative humidity on losses of vitamin C from vegetables held for 7 days at 41° F. At 98 and 93 per cent humidity little difference could be shown. There was considerably more loss at 65 per cent humidity.

Hummel (1942) stated that tomatoes lose very little ascorbic acid when held at room temperatures for as long as a week. He found that spinach, lettuce, corn and peas deteriorate very rapidly at room temperature and fairly rapidly at a lower storage temperature with the exception of spinach which retained its ascorbic acid quite well at 2° C.

Olliver (1943) stated that as a general rule the majority of green vegetables and peas in the pod lose 10 per cent of their initial vitamin C for each day that elapses after harvesting, whereas root vegetables lose vitamin C at a slower rate. Jones and Fenton (1943) found that fresh asparagus retained 97 per cent of its ascorbic acid after 24 hours of storage in the hydrator tray of a mechanical refrigerator held at 1.7° C. (35° F.).

Gleim, Tressler and Fenton (1944) reported that asparagus lost 40 per cent of its ascorbic acid after storage in an open wooden crate at room temperature, 18.8 to 25.5° C. (66-78° F.), while spinach lost 29 per cent. After a weeks

storage at 0 to 4.4° C. (32-40° F.) the ascorbic acid loss in asparagus was 57 per cent and in spinach 35 per cent. The vegetables were freshly harvested for this study.

Poole, Heinze, Welch and Grimbail (1944) found that uncooked cabbage stored under refrigeration for two weeks lost only 11 per cent of its ascorbic acid content. Zepplin and Elvehjem (1944) reported that lettuce, after storage in either a mechanical or ice refrigerator lost an average of less than 40 per cent in 48 to 72 hours increasing to 60 per cent after 96 hours. Lettuce lost 50 per cent of its ascorbic acid value after 24 hours at room temperature. The findings which Zepplin and Elvehjem (1944) reported concerning the loss of ascorbic acid in spinach differ with the findings reported by Tressler, Mack and King (1936). Zepplin and Elvehjem found that spinach could be stored for 72 hours in either a mechanical or ice refrigerator with a loss of less than 50 per cent of vitamin C. Tressler, Mack and King reported that after 3 days storage at 1 to 3° C. (33.8-37.4° F.), spinach retained practically all of its ascorbic acid. Zepplin and Elvehjem pointed out that this difference might be attributed to differences in temperature and humidity in refrigeration. Also, the condition of the vegetable at the time of storage would be a contributing factor.

Zepplin and Elvehjem also disagreed with Mack, Tapley and King (1939) on the ascorbic acid losses in green snap beans.

The latter found losses rapidly at all temperatures while Zepplin and Elvehjem found that losses ranged from 11 to 45 milligrams per 100 grams according to variety after one day and losses of 50 to 77 per cent after 6 days.

These same workers found big losses of ascorbic acid in swiss chard for the first 24 hour period, the losses ranging from 20 to 40 per cent. However, the losses remained at 40 per cent after three days storage. Their studies on broccoli revealed a loss of only 40 per cent after five days of refrigeration. All vegetables for this study were freshly harvested.

Lampitt, Baker and Parkinson (1945) found the following in their studies on freshly harvested vegetables. Lettuce showed a very rapid loss of vitamin at room temperature, while at 3° C. there was a rapid increase for 2 days followed by an equally rapid fall in values. Spinach and broad beans decreased rapidly in ascorbic acid value at room temperature. Spinach showed a very slow decrease at 3° C. Broccoli lost ascorbic acid slowly at room temperature and showed a slight increase at 3° C. Cabbage (quarters) retained original ascorbic acid values for one day at room temperature and then lost it rapidly. At 3o C. cabbage retained ascorbic acid to a high degree for 4 days.

Lampitt, Baker and Parkinson (1945) found that storage at 3° C. was much more favorable to retention (or even increase) of vitamin C than was storage at room temperature (20° C.).

They also pointed out that there was a rough correlation between "conditions" or degree to which the vegetable had deteriorated, and the retention of vitamin C in the vegetable. They concluded that easily perishable vegetables such as lettuce and spinach tend to lose vitamin C more rapidly than other vegetables. Under conditions of refrigeration storage, retention of this vitamin was often found to be very satisfactory after four days. In some cases there was an increase in ascorbic acid values.

A table follows which summarizes the studies presented in the literature which have been cited in this paper. As far as the author knows these are the only studies which have been carried on in regard to ascorbic acid retention in perishable vegetables under conditions of storage at refrigeration temperatures.

TABLE I

SUMMARY OF RESULTS OF STUDIES
CITED FROM THE LITERATURE ON ASCORBIC ACID RETENTION
IN VEGETABLES STORED AT ROOM AND REFRIGERATION TEMPERATURES

Vegetable	Condition	Held at Room Temperatures	Condition	Held at Refrigeration Temperatures
Asparagus	Fresh Market Fresh	1. Loss of 80% in four days 2. Loss of 20% in 24 hours 3. Loss of 40% in 24 hours	Fresh Fresh	1. Loss of 3% in 24 hours 2. Loss of 57% in 1 week
Broccoli	Fresh	1. Marked loss after 4 days	Fresh Fresh	1. Very slow loss during 2 weeks 2. Loss of 40% after 5 days
Cabbage	Fresh	1. Good retention for 1 day followed by rapid loss	--- Fresh	1. Loss of 11% in 2 weeks 2. High retention for 4 days
Lettuce	--- --- Fresh	1. Rapid deterioration 2. Loss of 50% after 24 hours 3. Very rapid loss	Fresh Fresh Fresh	1. Rapid loss 2. Loss of 40% in 48-72 hours increasing to 60% after 96 hours 3. Rapid increase for 2 days, followed by rapid fall

TABLE I (CONT.)

Vegetable	Condition	Held at Room Temperatures	Condition	Held at Refrigeration Temperatures
Peas	Fresh	1. Considerable loss after 6 days	Fresh	1. Practically no loss
	---	2. Rapid deterioriation	---	2. Fairly rapid loss
		3. Loss of 10% for each day after harvest.		
Spinach	Fresh	1. Loss of 78% in 2 days	Fresh	1. No loss after 3 days
	Market	2. Loss of 50-59% after 12 hours	Market	2. Little change
	---	3. Loss of 30-50% in 2 days	---	3. Loss of 10-17% in 2 days
	Market	4. Loss of 43% in 24 hours	Fresh	4. Loss of 35% in 1 week
	Market	5. Loss of 47% in 48 hours	Fresh	5. Loss of less than 50% after 3 days
	Fresh	6. Loss of 59% in 24 hours	Fresh	6. Very slow decrease
	Fresh	7. Rapid decrease		
String beans	Fresh	1. Rapid loss at all temperatures	Fresh	1. Rapid loss at all temperatures
			Fresh	2. Loss of 11-45% after 1 day
			Fresh	3. Loss of 50-77% after 6 days

TABLE I (CONT.)

Vegetable	Condition	Held at Room Temperatures	Condition	Held at Refreigeration Temperatures
Swiss chard			Fresh	1. Loss of 20-40% in 24 hours but losses remained at 40% after 3 days
Tomatoes	---	1. Very little loss in 1 week		

1. "Fresh" indicates freshly harvested.

EXPERIMENTAL PROCEDURE

EXPERIMENTAL PROCEDURE

Sample and Preparation

The vegetables analyzed for this study were peas, spinach, asparagus, tomatoes and wax beans. They were obtained from the college experimental gardens as they were ready for harvesting. Each vegetable was harvested for three consecutive days. The samples were picked at random since no attempt was made to represent the entire crop. The samples were collected early in the morning and within one-half hour were in the laboratory. The sample to be analyzed and recorded as the zero hour sample was placed directly into a 5 per cent solution of metaphosphoric acid. The remainder of the vegetable was stored in a hydrator on the bottom shelf of a mechanical refrigerator. The internal temperature of the refrigerator ranged from 4 to 8° C. during the experimental period.

Samples for the analysis of reduced, dehydro and total dehydroascorbic acid were withdrawn from the refrigerator at 1 hour, 3, 24 and 48 hours. In an attempt to get a representative sample from the refrigerator, the well filled hydrator was sampled from each side, in the middle and from both the top and bottom portions of the pan.

Spinach leaves were washed and patted dry between folds of a clean cotton dishtowel. Asparagus was treated in the same manner, then the stalks were cut with a plastic knife into 1 inch lengths, put into a large beaker and thoroughly mixed

before weighing the sample to be analyzed. Peas were shelled, placed in a beaker and well stirred before weighing the sample. Wax beans were cut with a plastic knife into suitable lengths and a sample taken as for the peas. Tomatoes presented a more difficult sampling problem. It was recognized that a variation between holding periods would appear, due to sampling, if there was no attempt made to keep the proportions of solids and liquid constant. The five or six tomatoes which were withdrawn from the hydrator were quartered with a plastic knife, placed in a beaker and stirred gently to avoid as little loss of juice as possible. The sample to be weighed was transferred from the beaker to the weighing dish with a small porcelain spoon.

After the preliminary treatments stated above, the sample withdrawn from the refrigerator for analysis was weighed on a Tripp Balance and placed in a five per cent metaphosphoric solution. Wherever possible the analysis of the sample was started immediately. However, it was necessary to hold some samples to be analyzed later. The samples to be held were extracted in the Waring Blender and made to volume with 5 per cent metaphosphoric acid solution. These blended samples were placed in ground glass-stoppered sampling bottles and held in the refrigerator until the analyses could be made. The dilutions on which the three chemical analyses were done were all made from the same blended sample. The temporary storage of blended samples was justified on the basis of previous experimental work which indicated the stability of ascorbic acid under

these conditions. Mapson and Mawson (1943) found that the ascorbic acid in cabbage extract (2 per cent solution of metaphosphoric acid) was stable at 20° C. in the dark for two days. Prolonged stability was apparent at 0° C. The temperatures of storage for blended samples used in this study was 5-8° C. The higher percentage of metaphosphoric acid also added to the stability of the extract since Ponting (1943) demonstrated that increased acidity aided in preventing oxidation. No samples were held for a period exceeding 18 hours before being analyzed.

Discussion of Methods Used in the Analysis of Ascorbic Acid in this Study

In an attempt to avoid as many variables as possible, all of the determinations for the various forms of ascorbic acid were made on one extraction of vegetables. This extraction was made in a 5 per cent metaphosphoric acid solution which was filtered and adjustments made for extractants and dilutions demanded by the individual methods.

Roe's (1944) method for the determination of dehydroascorbic acid is carried out in a five per cent metaphosphoric acid and one per cent thiourea solution. Since dehydroascorbic acid exists in a small amount in most fresh vegetables, no further dilution of the original extract was necessary. One gram of thiourea was weighed and placed in a 100 milliliter volumetric flask and the original extract was transferred to the flask to a total of 100 milliliters of solution.

The extract was then filtered through a 15 centimeter hand folded filter paper. Following filtration four milliliters of the filtrate were pipetted into each of four test tubes. Three of the test tubes had one milliliter of two per cent 2, 4-dinitrophenlyhydrazine in approximately nine N sulfuric acid added to them. The remaining test tube, held as a blank, was allowed to stand at room temperature. The test tubes containing the dye were incubated in a

constant temperature oven at 37° C. for three hours. At the end of that period all four of the test tubes were cooled in a pan of ice water and 1 cc. of the 2, 4-dinitrophenylhydrazine was added to the blank. To each of the four test tubes, five milliliters of 85 per cent sulfuric acid wereadded a drop at a time. During this addition the tubes were suspended in an ice bath and shaken constantly to insure complete mixing. The tubes were removed from the water bath and after 30 minutes, the depth of color was read in a Coleman Spectrophotometer set at a wave length of 540 millimicrons. The blank tube was used to obtain a 100 setting and at the same time to correct for color and turbidity in the extract.

The method for the determination of total dehydroascorbic acid as developed by Roe (1944) demands a five per cent metaphosphoric acid and 10 per cent acetic acid diluting medium. The acetic acid was added and suitable dilution of the sample made by the following procedure. If the sample being analyzed needed a further dilution of one to four, a 100 milliliter volumetric flask was used containing 50 milliliters of 10 per cent acetic acid; to it was added 25 milliliters of the original extract. The flask was then diluted to volume with five per cent metaphosphoric acid.

The method for the analysis of total dehydroascorbic acid follows the same procedure as the method for the determination of dehydroascorbic acid with two exceptions. Before filtering, the extract to be analyzed for total ascorbic acid was treated with norite. This step in the procedure is necessary for the

oxidation of the vitamin and for decolorization of pigmented extracts. Secondly, one drop of a ten per cent thiourea solution was added to each test tube before incubation. The readings for this determination were carried out as for the analysis of dehydroascorbic acid.

The adjustment of the diluting solutions necessary for the determination of reduced ascorbic acid were accomplished in a manner similar to that described above. It was required that the final extract be a one per cent solution of metaphosphoric acid. If a one to five dilution of the original extract was needed for reading, 20 milliliters of the extract were pipetted into a 100 milliliter volumetric flask. The flask was then made to volume with distilled water to give not only the correct concentration of ascorbic acid for reading but also the desired concentration of metaphosphoric acid solution.

Reduced ascorbic acid was determined by a modified method used in the departmental laboratories. This method is based on the directions developed by Morell (1941) and Loeffler and Ponting (1942). The method proceeds as follows. The one per cent metaphosphoric extract was filtered through a 15 centimeter hand folded filter paper. The filtrate was diluted as needed to provide an ascorbic acid concentration which would read within the approximate range of three to six micrograms per milliliter of extract.

Test tubes which previously had been calibrated with distilled water, were used for reading. To these test tubes were

added five milliliters of indophenol dye which was made up each day from a concentrated stock solution stored in the refrigerator. The final concentration of dye was in a one to 52 dilution or contained 0.02 milligrams of dye per milliliter.

A Coleman Spectrophotometer set at a wave length of 540 was employed. A 100 setting was obtained by pipetting five milliliters of one per cent metaphosphoric acid solution into one of the test tubes containing dye and then completely decolorizing the solution with ascorbic acid crystals. A blank reading on the dye was made by the same method omitting the ascorbic acid crystals.

Each sample was read in triplicate by transferring five milliliters of the unknown wxtract into the test tubes containing the dye, by means of a quick delivery pipette. The tube was shaken and inserted into the instrument and read at the end of 15 seconds. If the extract contained any trace of pigment or turbidity, a 100 setting was made with five milliliters of the extract and ascorbic acid crystals to correct for these factors.

Recovery Studies

Recovery studies were done on all of the methods used. Table II shows the results obtained on the method for the determination of dehydroascorbic acid. The percentage recoveries ranged from 94.7 to 101.0 and are in good agreement with those reported by Roe (1944).

Table III shows the percentage recoveries obtained on the total dehydroascorbic acid method and Table IV lists the results obtained on the reduced method. The range of percentage recoveries for the reduced ascorbic acid method was 94.7 to 101.0 and for the total dehydroascorbic acid method the percentage range was 92.0 to 105.7. In both instances, the high recoveries are indicative of the reliability of the methods.

Since the plan of this experiment demanded the determination of the two chemical forms of the vitamin from one extract, a fourth recovery study was initiated. Solutions of known concentrations of pure ascorbic acid were added to a series of unknown extracts of grapefruit juice and determinations of the two chemical forms of the vitamin were made on each extract plus ascorbic acid solution. Table V reports the results of this work. The table shows that the percentage recoveries for the three methods cover a wide range. It is probable that this variation is due to errors in the adjustments necessary for making all three determinations on one extract. The figures on the lower part of the table are more consistent and represent data collected at a later date when the technique for adjustment was perfected. The range of percentage recoveries for the dehydroascorbic acid method is 89.6 to 115.9. The range for the reduced ascorbic acid method is 73.3 to 116.6 and for the total dehydroascorbic

acid method, 33.9 to 101.2. The majority of the values are sufficiently near to 100 per cent recovery to conclude that the individual methods are not seriously affected by the adjustment necessary for doing all three chemical tests on one extraction of vegetable.

Calibration of the Reading Instrument

It was necessary to calibrate the reading instrument for each of the three methods of ascorbic acid determination. The calibration of the instrument for the reading of dehydroascorbic acid was made as follows. The dehydroascorbic acid standard was prepared by weighing 100 milligrams of ascorbic acid and making it to volume in a 100 milliliter volumetric flask with five per cent metaphosphoric acid. The resulting solution was treated with three of four drops of bromine, mixed until yellow, and separated from the undissolved bromine. The solution was aerated until colorless. Standard solutions ranging from 0.25 to 15 micrograms per milliliter of ascorbic acid were made from the bromine treated solution and diluted to volume with five per cent metaphosphoric acid containing one per cent thiourea. Test tubes containing four milliliters of the standard solution and one milliliter of the dye were incubated, as described, and read on the spectrophotometer.

The standard solution for the calibration of the instrument for the reading of the total dehydroascorbic acid determinations was prepared by weighing 100 milligrams of pure

TABLE II

RECOVERIES OF PURE ASCORBIC ACID ADDED
TO GRAPEFRUIT JUICE:
The reduced ascorbic acid method

Micrograms Ascorbic Acid				Percent Recovery
Grapefruit Juice	Added	Total Calculated	Total Determined	
175.0	129.6	304.6	305.0	100.0
175.0	90.7	265.7	260.8	98.0
175.0	86.4	261.4	257.9	98.7
253.5	86.4	339.9	333.0	97.9
253.5	90.7	344.2	328.0	95.3
253.5	172.8	426.3	404.0	94.7
362.5	172.8	535.3	540.0	101.0
362.5	90.7	453.2	440.0	97.1
362.5	129.6	492.1	480.0	97.5

TABLE III

RECOVERIES OF PURE ASCORBIC ACID
FROM FOOD EXTRACTS:
Roe's total dehydroascorbic
acid method

Food Extract	Micrograms Ascorbic Acid				Percent Recovery
	Food	Added	Total Calculated	Total Determined	
Lemon Juice	106.00	46.00	152.50	155.25	101.80
	182.50	46.00	228.50	235.50	103.30
Cabbage	403.12	260.63	663.75	637.50	96.10
	241.88	260.63	502.51	462.00	92.00
	275.00	260.63	535.63	566.25	105.70
	165.00	260.63	425.63	440.00	103.37
	136.25	260.63	396.88	406.25	102.37
	81.75	260.63	342.38	325.00	95.00
Spinach	156.25	481.25	637.5	596.25	93.6
	111.25	481.25	592.5	565.00	95.4

TABLE IV

RECOVERIES OF PURE ASCORBIC ACID[1]
ADDED TO GRAPEFRUIT JUICE:
Roe's dehydroascorbic acid method

Micrograms Ascorbic Acid				
Grapefruit Juice	Added	Total Calculated	Total Determined	Percent Recovery
105.00	35.00	140.00	137.20	98.0
171.75	35.00	206.75	208.82	101.0
133.05	94.37	227.42	217.19	95.5
123.12	94.37	217.49	226.19	104.0
151.63	57.08	209.71	203.84	97.2
110.88	57.08	167.96	140.11	98.9

1. Oxidized by treatment with bromine.

TABLE V

PERCENTAGE RECOVERIES OF PURE ASCORBIC ACID ADDED TO SUCCESSIVE SAMPLES OF GRAPEFRUIT JUICE: Dehydro, Total Dehydro and Reduced Ascorbic Acid Methods[1]

Dehydro	Total Dehydro	Reduced
93.9	-	73.3
94.3	-	82.0
89.6	-	93.6
76.3	33.9	90.1
108.8	72.5	81.9
115.9	87.4	91.5
99.9	101.2	116.6
103.4	86.9	87.3
108.6	96.6	100.9
101.8	98.9	103.4
96.5	97.4	96.3
104.5	97.5	98.2
76.9	62.4	91.9
91.8	85.9	96.5
99.7	97.2	92.3

ascorbic acid, transferring the vitamin to a liter volumetric flask and making up to volume with five per cent metaphosphoric acid and 10 per cent acetic acid in a one to one ratio as used for unknown solutions. Standards ranging in concentration from 0.25 to 15 micrograms per milliliter were prepared. These standards were treated with norite, filtered, and pipetted in four milliliter portions into test tubes. The incubation period and the reading of the material proceeded as for the usual determination.

Standard solutions containing the same concentrations of ascorbic acid as those used in the calibration of the photoelectric instrument for the other two methods, were used to calibrate the instruments for the determination of reduced ascorbic acid. However, the pure ascorbic acid was made to volume and diluted with one per cent metaphosphoric acid. Matched test tubes containing five milliliters of 2, 6-dichlorophenolindophenol dye were used for the readings. The instrument was set at 100 by adding five milliliters of one per cent metaphosphoric acid to a tube of the dye and completely decolorizing the solution by the addition of pure ascorbic acid crystals. A blank reading on the dye solution was accomplished by adding five milliliters of one per cent metaphosphoric acid to a test tube of dye and reading at the end of fifteen seconds. The reading of the standard solutions of ascorbic acid proceeded in the same manner as for the blank. Because of a gradual change in the titre of the concentrated dye solution it was

necessary to make blank readings with each set of determinations.

Two instruments have been used in this laboratory for reading concentrations of ascorbic acid. The instruments give results differing in degree but comparable for the interpretation of the concentration of vitamin in any given solution by both chemical methods used. Both instruments were standardized by the author with solutions of pure ascorbic acid.

Semi-logarithmic paper was used for plotting the readings obtained on standard solutions.

Plate I shows the results of five sets of determinations of standard solutions of dehydroascorbic acid read on the Coleman spectrophotometer. The data were treated statistically to determine the slope of the line expressing the trend of readings. The standard error of the line was found to be .0875 which is large but which would be expected from the scattering of points about the predicting line. Upon studying Plate I it is apparent that for concentrations of the vitamin above 25 micrograms per four milliliters of solutions the scattering of points around the line is much greater than for lower concentrations. The conclusion to be drawn from such an observation is that solutions containing one to 25 micrograms of ascorbic acid per four milliliters of extract are more accurately determined than those with a higher concentration of the vitamin. The readings for this study were made in these recommended concentrations.

The study of standard solutions by the total dehydroascorbic acid method was made on the Cenco Photelometer and represents the work of two people[1] in addition to the author. Plate II shows the results of statistical analysis of the combined data to determine the slope of the line and the standard error. The figure for the standard error was 0.015, indicating a small scattering of points about the predicting line. Any error introduced in a set of determinations for total dehydroascorbic acid would not be attributable to this method of determination. There can be little question of the reproducibility of the method since the standard error and the scatter of points are in such good proximity to the prediction line. Again, as in the case of work with dehydroascorbic acid, the range for reliable readings lies between 70 and 100 on the galvanometer scale.

The spectrophotometer was again used for the study of known solutions of the vitamin by the reduced ascorbic acid method. The problem of calibration of the spectrophotometer for reading of reduced ascrobic acid presented a slightly different problem than that of the other two methods. The dye solution used for the determination of dehydro and total dehydroascorbic acid varies only slightly from day to day and variation is corrected in the 100 setting of the instrument. The titre of the stock solution of indophenol dye used for the determination of reduced ascorbic acid gradually falls on storage.

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1. Date were supplied through the courtesy of Dr. Paul and Miss Kelley.

This fact accounts for the variation in the dye even though it is made in the same concentration each day. This change is not corrected in the 100 setting but in the blank reading. There are two ways in which the correction of the blank could be handled. The dye could be standardized each day to give the same blank readings or the blank could be allowed to vary and calibration lines to fit the various blanks calculated. The latter method was the one adopted in this study. Plate III shows four calibration lines made from predicting equations.[1] It will be noted that the standard error and scattering of points are not plotted since deviations were too small to graph without confusion. Listed below are the standard errors of the lines for the four blank settings reproduced (Plate III).

Blank	Standard Error
45	.0247
47	.0086
49	.0071
50	.0064

Plate III indicates an intersection of the calibration lines. The lines constructed from blank readings of 49 and 50 intersect those for the blank readings at 45 and 47. A possible explanation for this occurence is the change in the titre of the dye solution upon storage and the subsequent effect on the reactivity of the dye. It has been noted in the laboratory that the blank reading tends to increase

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1. Some data contributed through the courtesy of Miss Kelley

numerically as the dye is held in storage, and it is these higher blanks which produce predicting lines which intersect those constructed from the lower blank readings.

Method of Calculation

The method of calculation of ascorbic acid concentration in unknown extracts was essentially the same for each of the three chemical methods. The micrograms of ascorbic acid contained in the amount of unknown extract read in the spectrophometer was read from the calibration line constructed for the individual method. The following equation was used to convert the reading from the calibration line to milligrams of ascorbic acid per 100 grams of vegetable, fresh weight.

$$\text{Milligrams ascorbic acid per 100 grams fresh vegetable} = \frac{\text{Ascorbic acid Equivalent of Reading of Unknown}}{\frac{\text{Volume to which sample was made}}{\text{Grams vegetable in sample}} \times 0.1^*} \times \text{Milliliters of extract used in reading}$$

* 0.1 is a constant figure which converts micrograms to milligrams.

PLATE I

The Slope and the Standard Error of the Line Expressing the Trend of the Data on the Calibration of the Spectrophotometer for the Determination of Dehydroascorbic Acid.

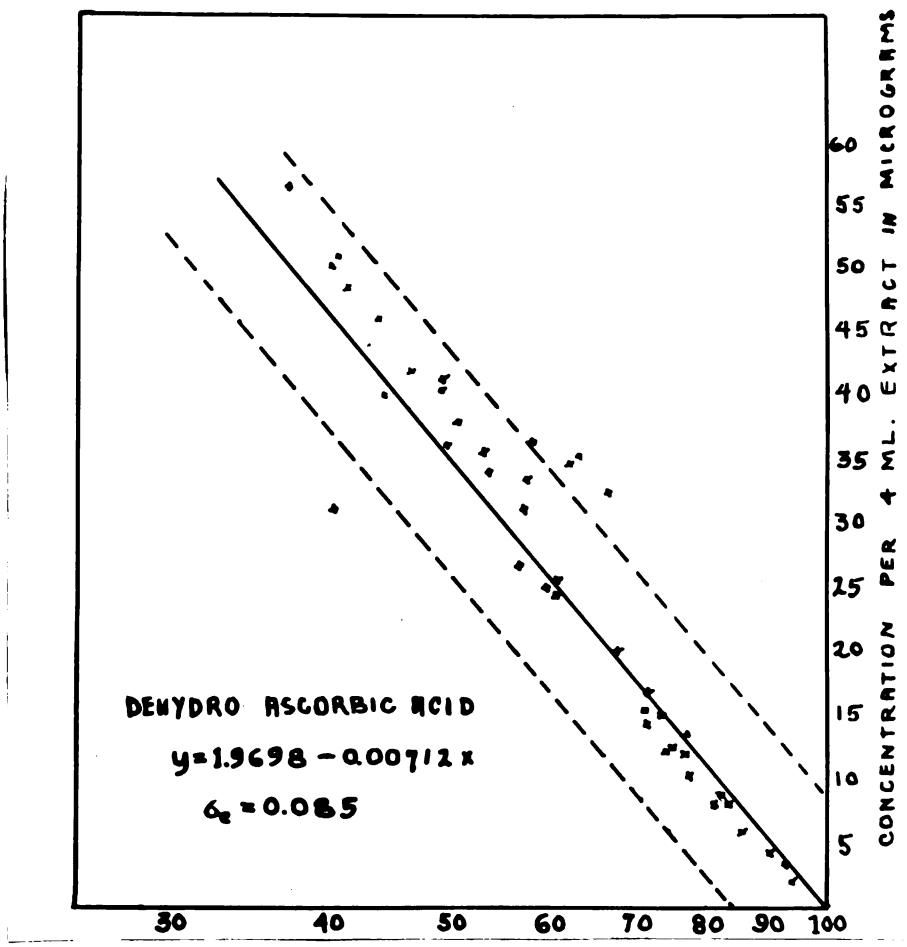

DEHYDRO ASCORBIC ACID
y=1.9698 − 0.00712 x
σ_e = 0.085
60
55
50
45
40
35
30
25
20
15
10
5
CONCENTRATION PER 4 ML. EXTRACT IN MICROGRAMS
30
40
50
60
70
80
90
100
Galvanometer Reading

PLATE II

The Slope and the Standard Error of the Line Expressing the Trend of the Data on the Calibration of the Spectrophotometer for the Determination of Total Dehydroascorbic Acid.

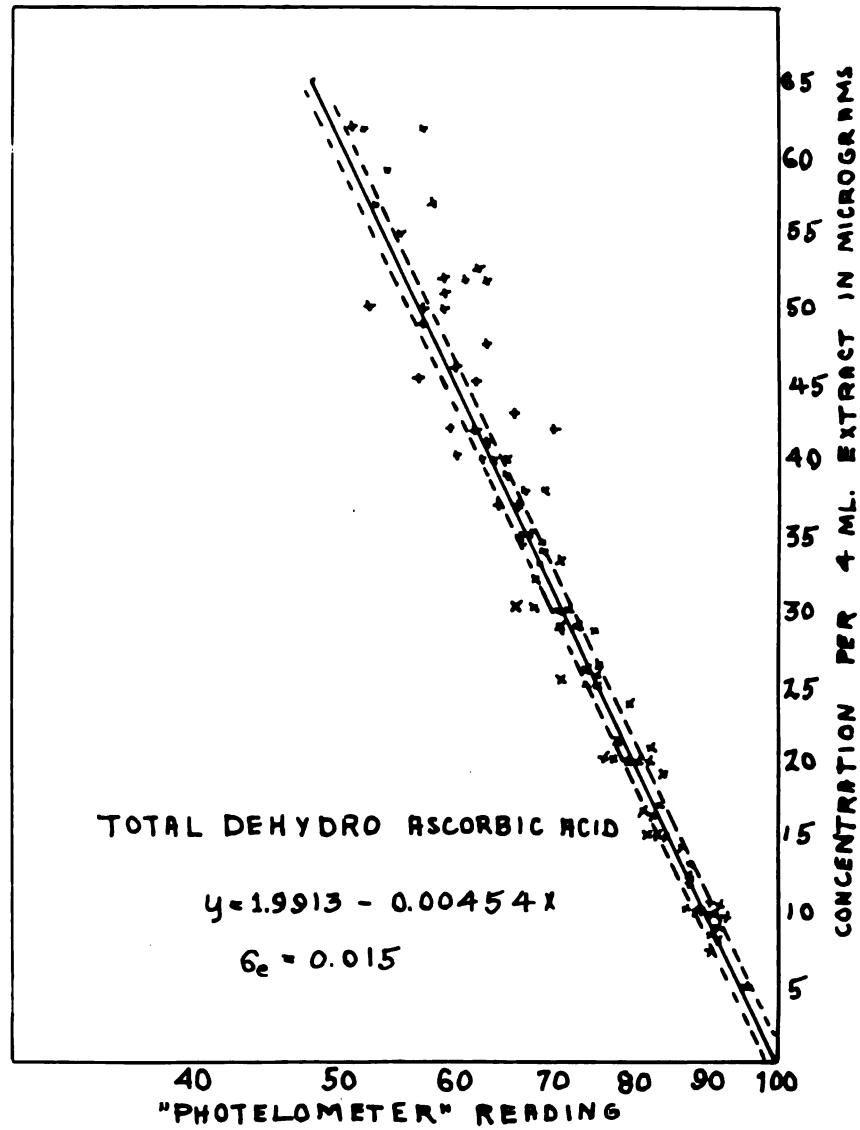
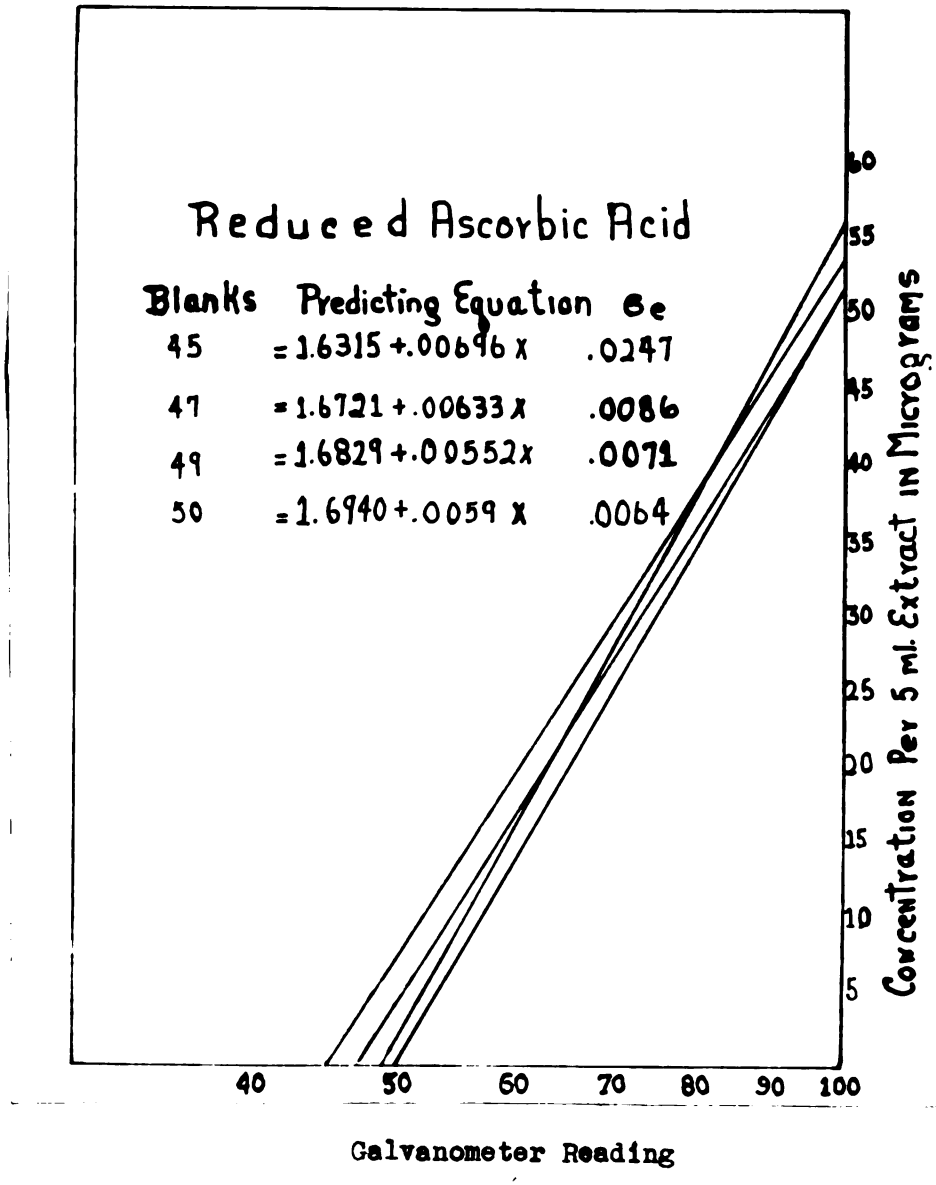

TOTAL DEHYDRO ASCORBIC ACID
y = 1.9913 - 0.00454 x
σ_e = 0.015
65
60
55
50
45
40
35
30
25
20
15
10
5
CONCENTRATION PER 4 ML. EXTRACT IN MICROGRAMS
40
50
60
70
80
90
100
"PHOTELOMETER" READING

PLATE III

The Predicting Lines for the Reduced Ascorbic Acid Method Based on Four Blank Readings.


Reduced Ascorbic Acid
Blanks Predicting Equation se
45 = 1.6315 + .00696 X .0247
47 = 1.6721 + .00633 X .0086
49 = 1.6829 + .00552 X .0071
50 = 1.6940 + .0059 X .0064
60
55
50
45
40
35
30
25
20
15
10
5
Concentration Per 5 ml. Extract in Micrograms
40
50
60
70
80
90
100
Galvanometer Reading

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Vitamin Retention

The results of this study on the Vitamin C retention in asparagus, peas, spinach, tomatoes and wax beans stored at refrigeration temperatures for 48 hours are recorded in Plates 4, 5, 6, 7 and 8 and in Tables 6, 7, 8, 9, 10, 11 and 12. Details of the data are presented in the figures. For clarity and ease of discussion each vegetable will be considered separately. All of the percentage changes which will be discussed are in comparison with the zero hour reading.

Asparagus: Dehydroascorbic acid concentrations increased over 50 per cent during the first hour of holding and then showed a rapid and consistent decline. At the end of the 48 hour holding period there was found only 50 per cent of the dehydroascorbic acid present in the freshly harvested vegetable. The pattern of change of reduced ascorbic acid was similar but of a lower magnitude with a reduction of 23.8 per cent in 48 hours. The ascorbic acid values determined by the total dehydroascorbic acid method showed practically no change after one hour of holding, a slight decrease after three hours and a 36.0 per cent decrease at the end of 48 hours. The sum of the 36.0 per cent reduction of total dehydroascorbic acid and the 23.8 per cent reduction of reduced ascorbic acid after 48 hours, compare favorably with the 57.0 per cent loss of reduced ascorbic acid reported by Gleim, Tressler and Fenton (1944)

after one week at refrigeration temperatures since losses of vitamin content tend to level off after two or three days storage. The fact that the figures of this study are based on moist weight of vegetables would tend to make them somewhat lower if there were moisture losses during storage.

Jones and Fenton (1943) reported a reduced ascorbic acid loss of three per cent after 24 hours under conditions of refrigeration. This study showed a 9.1 per cent gain of reduced ascorbic acid during the same period of time. However the total dehydroascorbic acid value decreased 23.3 per cent in 24 hours. If one can assume biological availability of the dehydroascorbic acid, this is a total loss in 24 hours of 14.2 per cent. The reader is referred to Table VI and VII and Plate IV. Table VI reports the percentage losses of ascorbic acid during 48 hours as determined by the three chemical methods, and Plate IV shows the same material graphically.

Peas: Table VI and VIII and Plate V show that dehydroascorbic acid values decreased 67.8 per cent during the first hour and increased 16.9 per cent at the end of the third hour. An increase of 28.8 per cent is reported at the end of 48 hours.

There was a slight loss of reduced ascorbic acid at the end of one hour but an increase of 15.1 per cent at the end of the three hours of holding. At the end of 48 hours a small decrease of 1.4 per cent is apparent.

The values for total dehydroascorbic acid show much the same pattern, with a decrease of 6.9 per cent in one hour,.

a slight gain in three hours and a loss of 5.1 per cent in 48 hours. These results are not in agreement with those reported by Hummel (1942), who found that peas lost ascorbic acid rapidly even at low storage temperature. However, Mack, Tressler and King (1936) found very little loss after six days storage at 1° C. and 9° C.

It is interesting to note that all three of the chemical methods show some gain of ascorbic acid at the end of three hours following a slight fall after one hour. The literature on the subject gives no clues to the reason for this change. There are no studies reported where analyses were made at the end of one hour of storage. There is always the possibility that there was an error in sampling but upon studying the graphs showing ascorbic acid changes during the first hour of holding, it would seem that the first hour is a critical one. Most vegetables show either a marked decrease or increase during that period.

Spinach: From Tables VI and IX and Plate VI it is evident that the change during the first hour of holding involves only the oxidized form of the vitamin. Dehydroascorbic acid increased 22.5 per cent while reduced ascorbic acid remained the same and total dehydroascorbic acid values increased only 1.9 per cent.

There was no loss of dehydroascorbic acid until the end of the 48 hour period when a 25.8 per cent loss was found. At the end of 24 hours, reduced ascorbic acid showed a decrease of 16.6

per cent. Losses as determined by the total dehydroascorbic acid method begin at the three hour period with a 19.6 per cent decrease. At the end of the 48 hour period there was a 23.1 per cent loss as determined by the total dehydrascorbic acid method, a 7.2 per cent loss by the reduced method and 25.8 per cent loss of preformed dehydroascorbic acid. Such figures would indicate that the reduced ascorbic acid is little affected by the storage process but that the dehydro form may be quite labile.

In comparing these results with those reported in the literature on reduced ascorbic acid losses, good agreement is evident. Tressler, Mack and King (1936) found no loss after three days storage at 1 to 3° C. Feener, Palmer and Fitzgerald (1937) found little change in ascorbic acid content of fresh market spinach stored at 1 to 3° C., in 12 hours. Yaroshenko found 10 to 17 per cent loss after two days at 2°C. Gleim, Tressler and Fenton (1940) reported a loss of 35.0 per cent after one weeks storage at 0 to 4.4° C. Lampitt, Baker and Parkinson (1945) found a slow decrease in ascorbic acid values at 3° C. However, Zepplin and Elvehjem found that there was a loss of less then 50 per cent after three days storage at 1 to 3° C.

Tomatoes: Tables VI and X and Plate VII show the results obtained on the study of tomatoes held under refrigeration storage. Dehydroascorbic acid concentration increased 5.7 per cent during the first hour, reaching a peak of 88.6 per

cent more vitamin at the end of the 24 hour holding period. At the end of 48 hours, there was still over 50 per cent as much dehydroascorbic acid present as when the vegetable was harvested.

Reduced ascorbic acid showed a sharp increase of 33.8 per cent during the first hour and a decline to 5.6 per cent increase at the end of 48 hours.

The values obtained by the total dehydroascorbic acid method follow the same pattern with a 33.7 per cent increase in one hour and a 21.1 per cent increase at 48 hours.

The only finding cited from the literature on ascorbic acid retention in tomatoes is the statement by Hummel (1942) that tomatoes lose very little ascorbic acid when held at room temperature for a week.

Wax Beans: Tables VI and XI and Plate VIII are presented for the results of the study on wax beans. This vegetable shows a consistent loss of ascorbic acid during the 48 hours of storage. The losses of dehydroascorbic acid fluctuate considerably during the 48 hour period with a final loss of 27.5 per cent at 48 hours of storage.

Reduced ascorbic acid remained at approximately the same concentration during the first hour. It decreased 6.3 per cent at three hours, 17.9 per cent at 24 hours and 46 per cent at 48 hours.

Total dehydroascorbic acid losses are similar to the losses of reduced ascorbic acid except in the last 24 hour period when they were 17.6 per cent.

No work is reported on ascorbic acid retention in wax beans but Mack, Tapley and King (1939) reported that string beans lost 11 to 45 per cent after two days storage and 50 to 77 per cent after six days storage at refrigeration temperatures. These figures are in good agreement with those obtained in this study.

From the results of this study on the ascorbic acid retention in fresh vegetables held at refrigeration temperatures one is able to conclude that asparagus, peas, and spinach increase in ascorbic acid value for the first three hours of the holding period. The first appreciable losses in ascorbic acid concentrations appear after 24 hours. Neither the losses after 24 hours or 48 hours of storage are large, none of the losses for any form of the vitamin exceeding 36 per cent.

Tomatoes show no loss of ascorbic acid value after 48 hours of storage, but rather a gain in ascorbic acid concentration. Lampitt, Baker and Parkinson (1945) in surveying the studies reported on ascorbic acid retention in fresh vegetables, noted that the majority of studies reported only losses and no gains in ascorbic acid concentration. They pointed out that an increase in ascorbic acid values are not only possible but also probable, since metabolic processes

within plant cells are not inactivated by harvesting a product. The results of this study appear to emphasize this point.

Wax beans are the only vegetable in this study which showed a consistent decrease in ascorbic acid values throughout the holding period. At no time was there any appreciable gain in ascorbic acid concentration.

The above findings have certain practical aspects related to the harvesting of vegetables for home consumption and preservation. It is apparent from this study that a holding period of three hours at refrigeration temperatures does not appreciably affect the ascorbic acid concentration of a vegetable. The homemaker who finds it necessary to delay the processing of vegetables for preservation or who wishes to crisp vegetables in the refrigerator before serving can be assured that the ascorbic acid content of the vegetable is approximately as great as at the time of harvesting. Even a longer holding period of 24 hours at refrigerator temperature does not greatly reduce the ascorbic acid potency of any good source of the vitamin.

TABLE VI

PERCENTAGE CHANGES IN ASCORBIC ACID CONCENTRATION OF VEGETABLES DURING THE HOLDING PERIOD.[1]

Vegetable	Method	Hours of Holding			
		1	3	24	48
Asparagus	Dehydro	51.6	32.0	-12.9	-45.2
	Reduced	3.9	9.1	4.4	-23.8
	Total Dehydro	-0.7	-6.1	-23.3	-36.0
Peas	Dehydro	-67.8	16.9	-1.7	28.8
	Reduced	- 3.4	15.1	-4.6	-1.4
	Total Dehydro	- 6.9	1.8	1.8	-5.1
Spinach	Dehydro	22.5	5.6	10.1	-25.8
	Reduced	0.0	0.8	-16.6	- 7.2
	Total Dehydro	1.9	-19.6	-28.8	-23.1
Tomatoes	Dehydro	5.7	14.3	88.6	57.1
	Reduced	33.8	28.8	21.9	5.6
	Total Dehydro	33.7	32.2	21.1	21.1
Wax Beans	Dehydro	-37.5	0.0	-42.5	-27.5
	Reduced	0.4	-6.3	-17.9	-46.0
	Total Dehydro	1.4	-7.1	-18.4	-36.0

- = A Decrease In Ascorbic Acid Concentration

1. Percentages based on Averages of Three Day's Replications

The Methods Study of the Analysis of Ascorbic Acid in Fresh Vegetables

The discussion of the results is based on the three hypotheses presented in the introduction to this paper. They were: (1) that is is logical to assume the difference between total oxidized and reduced ascorbic acid values as the amount of the oxidized form present in the plant tissues, (2) that so called losses as determined by measuring the reduced form of the vitamin are losses and not conversion to the oxidized form, (3) that it is safe to assume that a measurement of the reduced form gives a true picture of the antiscorbutic substance available in a fresh food.

Tables 12, 13 and 14 and Plates 4, 5, 6, 7 and 8, show more clearly than a description in words, the points which can be made regarding the results of the study

From the tables and the graphs it is easy to see that the difference between the values obtained by the reduced method and the total dehydro method is not the value obtained for dehydroascorbic acid. Peas are the only vegetable in this study where the concentrations of preformed dehydroascorbic acid and reduced ascorbic acic total to equal the total dehydroascorbic acid values. Tests from other laboratories and this study show the apparent reliability of all of the methods when used independently. McMillan and Todhunter (1946), working with cabbage found that the sum of the dehydroascorbic acid and reduced ascorbic acid equalled the total oxidized ascorbic

acid when each was measured independently. Roe (1941) also was able to confirm this relationship on some food products such as asparagus, peaches and green peas. However, in many other products such as rhubarb, potatoes, squash, turnips, citrus juices and cauliflower the amount of dehydroascorbic acid determined by difference and the amount of the oxidized form determined in the laboratory did not agree. Table XIV shows the lack of agreement between calculated and determined dehydroascorbic acid which is apparent in this study. Therefore, it does not seem safe to assume that the differences between reduced and total dehydroascorbic acid values is preformed dehydroascorbic acid.

This study also suggests the fact that the percentage losses of ascorbic acid as determined by the reduced method, do not indicate the entire loss of the vitamin. Table XIII shows the percentage changes of ascorbic acid concentrations in vegetables held at refrigeration temperature for 48 hours, as determined by the reduced and total dehydroascorbic acid methods. In all cases, with the exception of string beans, the changes as determined by the total dehydroascorbic acid method are greater than those determined by the reduced method.

In studying the graphs of ascorbic acid changes over a 48 hour period it is apparent that peas are the only vegetable where the total dehydroascorbic and reduced ascorbic acid values approximate each other over the entire storage period. The difference in concentrations are large for the other four

vegetables which were studied. The measurement of reduced ascorbic acid,does not necessarily give a true picture of the antiscorbutic potency of a given food product.

It is apparent that in vegetables, the amoung of dehydro-ascorbic acid as determined chemically, does not make up the difference between the total and reduced values. There are two possible explanations for this result: (1) that the total dehydroascorbic acid method measures something other than ascorbic acid or (2) that the amount of dehydro or reduced ascorbic acid present is not measured quantitatively. The small amount of dehydroascorbic acid present in fresh vegetable tissues may have influenced the effectiveness of the method for its determination. High concentrations of vegetable tissue were required for the analysis of dehydroascorbic acid in order to obtain a reading, in the spectrophometer, which was in the desired range. A high concentration of vegetable tissue may have introduced more interfering substances which would effect the results of the analysis. The method for the determination of dehydroascorbic acid also has the highest probability of standard error of the three methods used.

In any case, there is need for more information on the methods for the measurement of ascorbic acid concentrations before continuing study on vitamin retentions.

PLATE IV

The Concentration of Dehydro, Total Dehydro and Reduced Ascorbic Acid in Asparagus Held Under Conditions of Refrigerator Storage for Forty-eight Hours

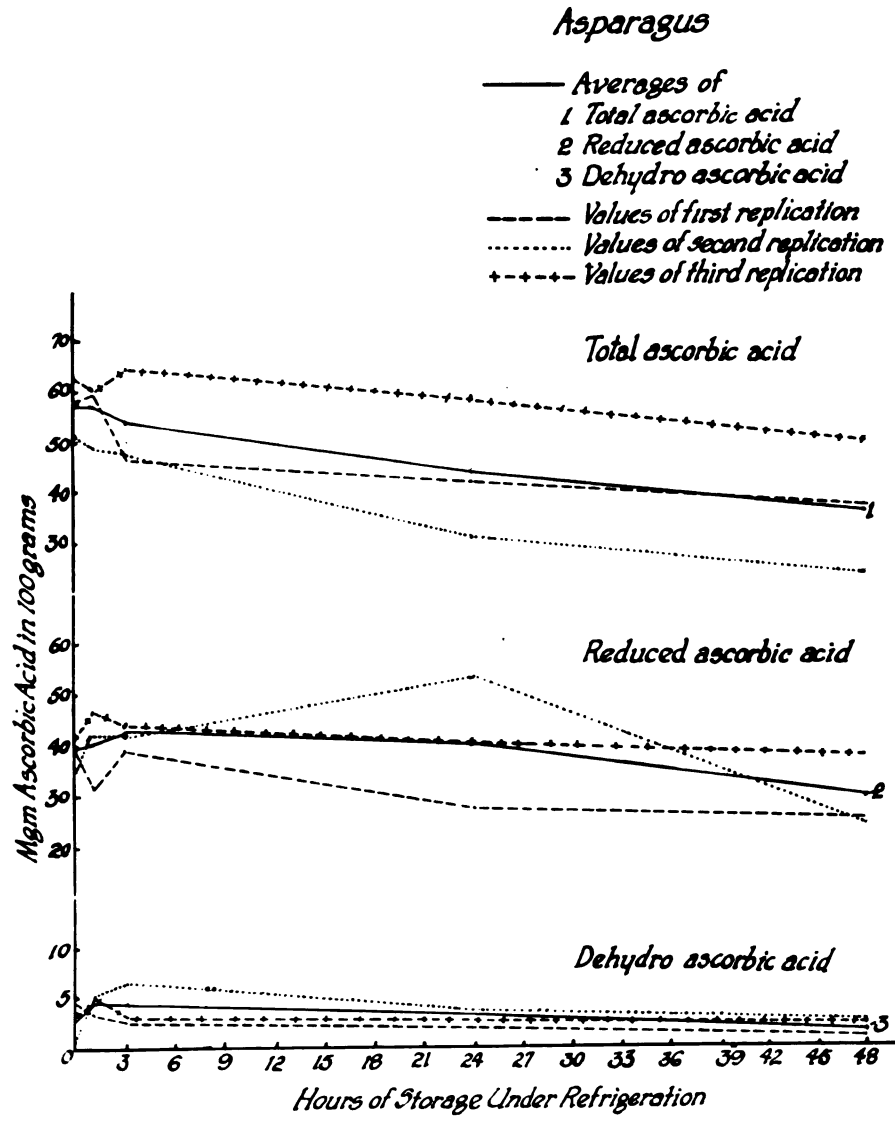

Asparagus
Averages of
1 Total ascorbic acid
2 Reduced ascorbic acid
3 Dehydro ascorbic acid
Values of first replication
Values of second replication
Values of third replication
Total ascorbic acid
Reduced ascorbic acid
Dehydro ascorbic acid
Mgm Ascorbic Acid in 100 grams
Hours of Storage Under Refrigeration
0
3
6
9
12
15
18
21
24
27
30
33
36
39
42
46
48
10
20
30
40
50
60
70
1
2
3

PLATE V

The Concentration of Dehydro, Total Dehydro and Reduced Ascorbic Acid in Peas Held Under Conditions of Refrigerator Storage for Forty-eight Hours

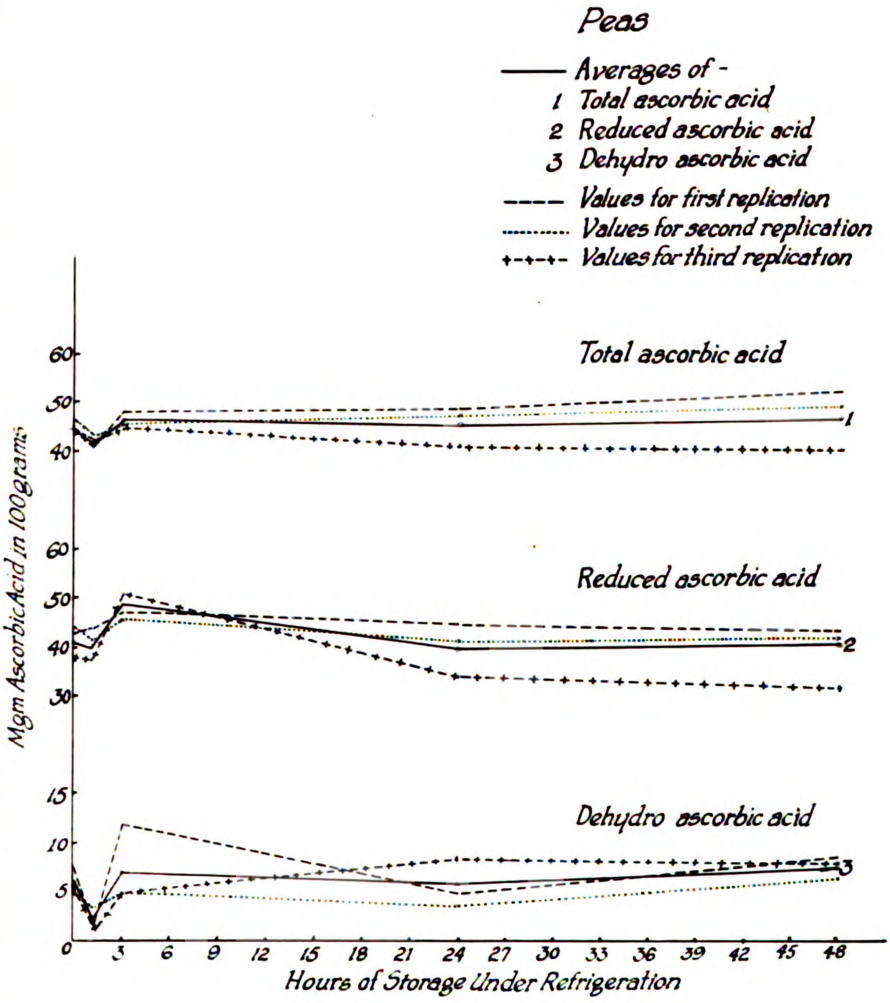

Peas
Averages of -
1 Total ascorbic acid
2 Reduced ascorbic acid
3 Dehydro ascorbic acid
Values for first replication
Values for second replication
Values for third replication
Total ascorbic acid
Reduced ascorbic acid
Dehydro ascorbic acid
Mgm Ascorbic Acid in 100 grams
Hours of Storage Under Refrigeration
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48
60 50 40 30 15 10 5

PLATE VI

The Concentration of Dehydro, Total Dehydro and Reduced Ascorbic Acid in Spinach Held Under Conditions of Refrigerator Storage for Forty-eight Hours

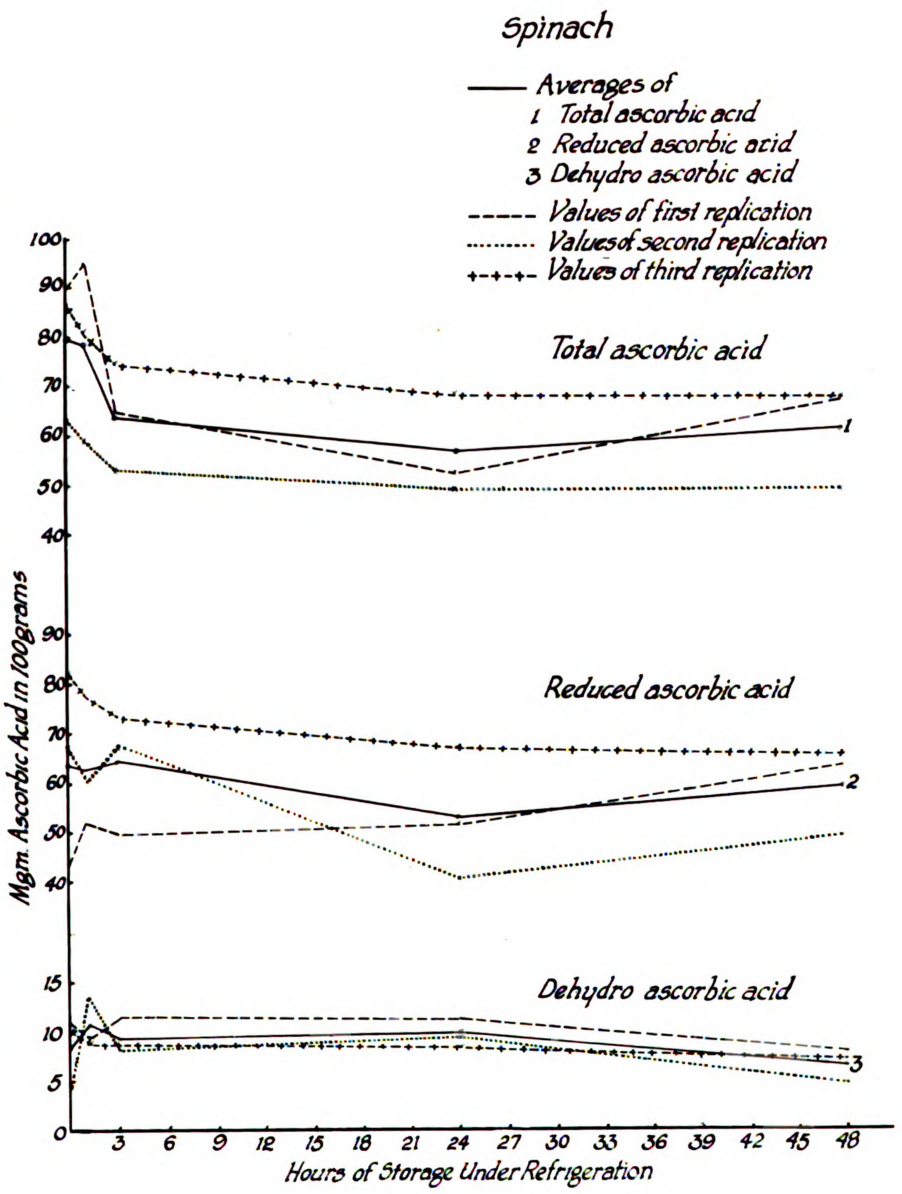

Spinach
Averages of
1 Total ascorbic acid
2 Reduced ascorbic acid
3 Dehydro ascorbic acid
Values of first replication
Values of second replication
Values of third replication
Total ascorbic acid
Reduced ascorbic acid
Dehydro ascorbic acid
Mgm. Ascorbic Acid in 100 grams
Hours of Storage Under Refrigeration
100
90
80
70
60
50
40
15
10
5
0
3
6
9
12
15
18
21
24
27
30
33
36
39
42
45
48
1
2
3

PLATE VII

The Concentration of Dehydro, Total Dehydro and Reduced Ascorbic Acid in Tomatoes Held Under Conditions of Refrigerator Storage for Forty-eight hours

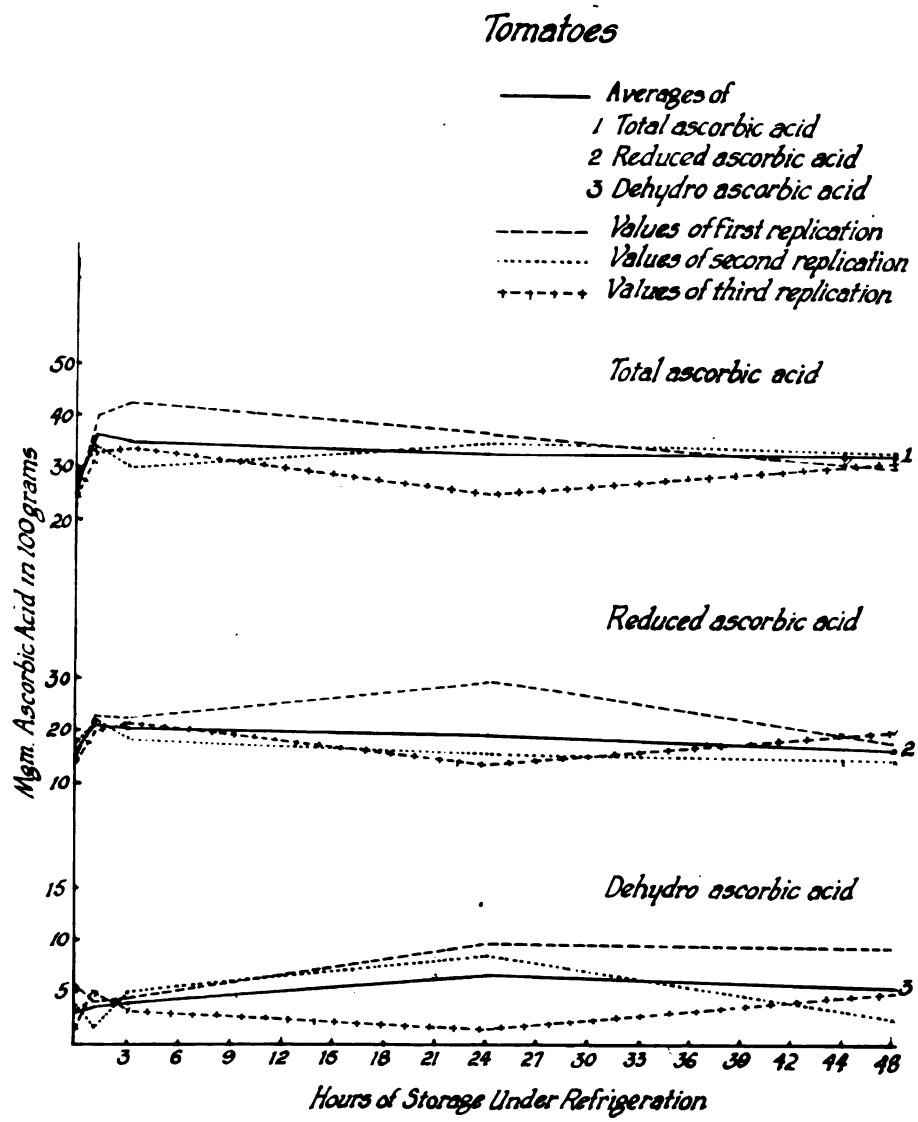
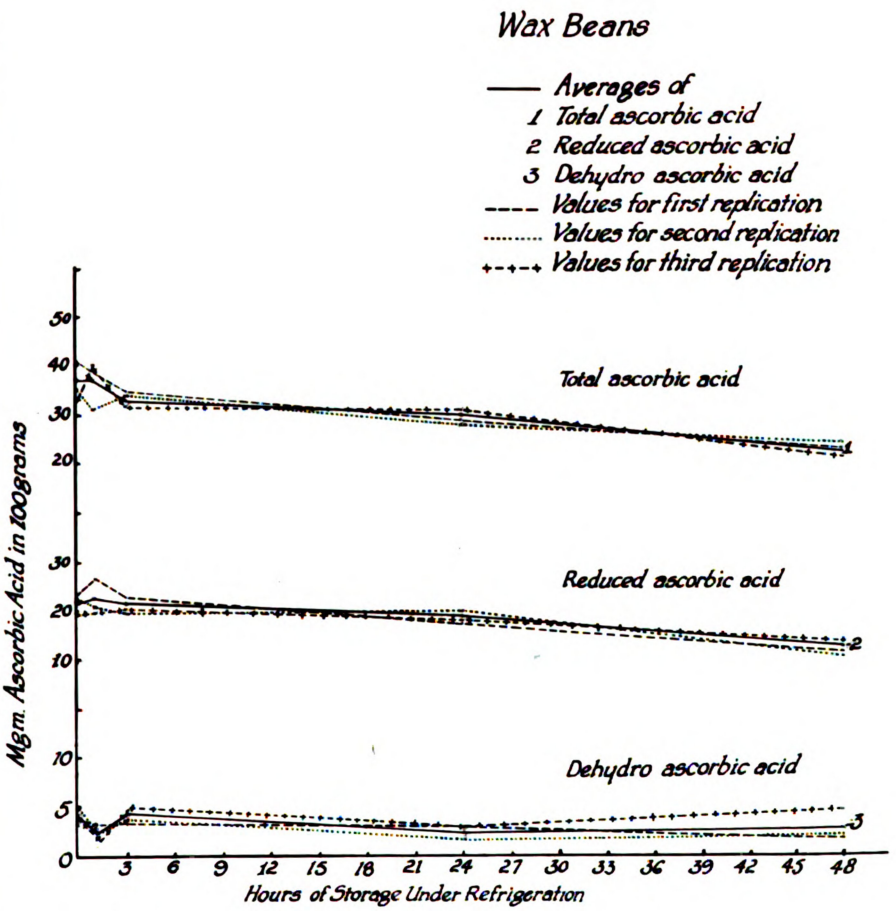

Tomatoes
Averages of
1 Total ascorbic acid
2 Reduced ascorbic acid
3 Dehydro ascorbic acid
Values of first replication
Values of second replication
Values of third replication
Total ascorbic acid
Reduced ascorbic acid
Dehydro ascorbic acid
Mgm. Ascorbic Acid in 100 grams
50
40
30
20
30
20
10
15
10
5
1
2
3
3 6 9 12 16 18 21 24 27 30 33 36 39 42 44 48
Hours of Storage Under Refrigeration

PLATE VIII

The Concentration of Dehydro, Total Dehydro and Reduced Ascorbic Acid in Wax Beans Held Under Conditions of Refrigerator Storage for Forty-eight Hours


Wax Beans
Averages of
1 Total ascorbic acid
2 Reduced ascorbic acid
3 Dehydro ascorbic acid
Values for first replication
Values for second replication
Values for third replication
Total ascorbic acid
Reduced ascorbic acid
Dehydro ascorbic acid
Mgm. Ascorbic Acid in 100grams
Hours of Storage Under Refrigeration
0
3
6
9
12
15
18
21
24
27
30
33
36
39
42
45
48
5
10
20
30
40
50

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

1. The reduced, dehydro and total dehydroascorbic acid concentrations of asparagus, peas, spinach and tomatoes were studied during a 48 hour period of refrigerator storage.

2. The percentage changes of reduced ascorbic acid in asparagus, peas, spinach, tomatoes and wax beans held for 48 hours were, respectively, -23.8, -11.6, -7.2, 5.6 and -12.2. The percentage changes of total dehydroascorbic acid in the above vegetables were -36.6, -5.1, -23.1, 21.1 and -23.3.

3. The data obtained from this study indicates that the difference between total dehydroascorbic acid and reduced ascorbic acid values is not the true dehydroascrobic acid concentration, as determined, except for peas.

4. Percentage changes of vitamin concentration, as determined by the reduced method, are not indicative of actual losses of anti-scorbutic potency. Therefore, the measurement of the reduced ascorbic acid contained in a food produce does not constitute a measure of actual anti-scorbutic activity.

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APPENDIX

TABLE VII

ASPARAGUS: Milligrams of ascorbic acid per 100 grams of vegetable

Method	Fresh			Held 1 hour			Held 3 hours			Held 24 hours			Held 48 hours		
	Days of Study														
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Dehydro	4.7	0.8	3.8	3.6	5.2	5.2	2.6	6.5	3.2	2.0	3.5	2.7	0.6	2.5	2.0
Reduced	38.3	35.8	41.7	31.5	41.7	47.0	39.2	42.3	44.8	27.8	52.7	40.3	25.3	25.2	37.6
Total Dehydro	58.1	51.6	62.8	59.7	49.9	61.7	47.9	48.8	65.3	42.1	31.7	58.6	37.0	23.2	50.2

TABLE VIII

PEAS: Milligrams of ascorbic acid per 100 grams of vegetable

Method	Fresh			Held 1 hour			Held 3 hours			Held 24 hours			Held 48 hours		
	Days of Study														
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Dehydro	7.4	4.8	5.6	1.3	3.3	1.1	11.6	4.6	4.4	5.0	3.8	8.7	8.2	6.5	8.1
Reduced	43.0	43.8	38.0	43.4	40.6	36.6	46.6	45.6	51.6	44.6	40.6	34.0	43.4	42.6	32.0
Total Dehydro	44.8	46.0	44.3	40.3	43.5	41.8	48.0	45.0	44.5	48.5	47.5	41.3	52.3	49.5	40.1

TABLE IX

SPINACH: Milligrams of ascorbic acid per 100 grams of vegetable

Method	Fresh			Held 1 hour			Held 3 hours			Held 24 hours			Held 48 hours		
	Days of Study														
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Dehydro	11.0	4.9	10.9	9.6	13.6	9.4	11.5	8.2	8.6	11.2	9.5	8.8	7.7	4.6	7.4
Reduced	42.0	67.7	82.3	52.5	60.7	78.3	50.0	67.7	74.3	52.0	41.0	67.2	63.3	49.7	65.4
Total Dehydro	90.0	63.7	86.3	95.0	59.2	81.4	64.6	53.3	75.0	53.0	49.6	68.4	67.5	49.2	67.9

TABLE X

TOMATO: Milligrams of ascorbic acid per 100 grams of vegetable

Method	Fresh			Held 1 hour			Held 3 hours			Held 24 hours			Held 48 hours		
	Days of Study														
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Dehydro	5.5	3.6	1.4	3.9	1.9	5.2	4.2	4.6	3.3	9.6	8.6	1.7	9.2	2.3	5.1
Reduced	15.8	18.0	14.3	21.8	21.3	21.1	22.5	18.3	21.1	29.5	15.8	13.1	16.4	14.3	20.0
Total Dehydro	26.3	29.4	25.4	40.1	34.6	34.6	33.6	42.1	30.6	37.0	35.1	26.1	32.1	33.4	32.6

TABLE XI

WAX BEANS: Milligrams of ascorbic acid per 100 grams of vegetable

Method	Fresh			Held 1 hour			Held 3 hours			Held 24 hours			Held 48 hours		
	Days of Study														
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Dehydro	3.5	4.9	3.6	3.0	2.5	2.0	3.7	3.9	4.3	2.6	1.8	2.6	1.8	2.2	4.8
Reduced	23.9	23.8	19.5	26.9	20.5	20.0	22.8	19.8	20.3	18.0	18.9	18.3	11.6	11.5	13.1
Total Dehydro	40.3	35.3	33.5	39.3	31.4	40.0	34.8	34.3	32.3	29.2	28.2	31.7	22.6	24.8	22.5

TABLE XII

AVERAGE CONCENTRATIONS OF ASCORBIC ACID BASED ON THREE REPLICATIONS

Micrograms Ascorbic Acid					
Method	Fresh	Held 1 hour	Held 3 hours	Held 24 hours	Held 48 hours
	Asparagus				
Dehydro	3.1	8.7	4.1	2.7	1.7
Reduced	38.6	40.1	42.1	40.3	29.4
Total Dehydro	57.5	57.1	54.0	44.1	36.8
	Peas				
Dehydro	5.9	1.9	6.9	5.8	7.6
Reduced	46.6	40.2	47.9	39.7	41.0
Total Dehydro	45.0	41.9	45.8	45.8	47.3
	Spinach				
Dehydro	8.9	10.9	9.4	9.8	6.6
Reduced	64.0	63.8	64.0	53.4	59.4
Total Dehydro	80.0	78.5	64.3	57.0	61.5

TABLE XII (CONT.)

Micrograms Ascorbic Acid					
Method	Fresh	Held 1 hour	Held 3 hours	Held 24 hours	Held 48 hours
	Tomatoes				
Dehydro	3.5	3.7	4.0	6.6	5.5
Reduced	16.0	21.4	20.6	19.5	16.9
Total Dehydro	27.0	36.1	35.7	32.7	32.7
	Wax Beans				
Dehydro	4.0	2.5	4.0	2.3	2.9
Reduced	22.4	22.5	21.0	18.4	12.1
Total Dehydro	36.4	36.9	33.8	29.7	23.3

TABLE XIII

THE DIFFERENCE FOUND BETWEEN CHANGES IN ASCORBIC ACID CONCENTRATION OVER A STORAGE PERIOD OF 48 HOURS AS DETERMINED BY THE TOTAL DEHYDRO AND AND REDUCED ASCORBIC ACID METHOD

Vegetable	Difference	
	in Percentage Values	in Micrograms
Asparagus	13.2	7.4
Peas	3.7	5.7
Spinach	15.9	2.1
Tomatoes	15.5	15.8
Wax Beans	10.0	11.2

TABLE XlV

CONCENTRATION OF DEHYDROASCORBIC ACID IN FRESHLY HARVESTED VEGETABLES AS CALCULATED BY THE DIFFERENCE IN VALUES OBTAINED BY THE REDUCED AND TOTAL DEHYDROASCORBIC ACID METHODS AND THE VALUES DETERMINED BY THE DEHYDROASCORBIC ACID METHOD

Vegetable	Dehydroascorbic Acid Calculated	Dehydroascorbic Acid Determined
Asparagus	18.9	3.1
Peas	1.6	5.9
Spinach	16.0	8.9
Tomatoes	11.0	3.5
Wax Beans	14.0	4.0

Aug 2 '48

Mar 11, 1949

Nov 11 '50